



US 20190241629A1

(19) **United States**(12) **Patent Application Publication**
MIZUMURA et al.(10) **Pub. No.: US 2019/0241629 A1**(43) **Pub. Date: Aug. 8, 2019**(54) **RECOMBINANT PROTEINS DERIVED
FROM GENUS LIMULUS, AND DNA
MOLECULES ENCODING SAME**(71) Applicant: **SEIKAGAKU CORPORATION**,
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Tokyo (JP)(21) Appl. No.: **16/331,810**(22) PCT Filed: **Oct. 18, 2017**(86) PCT No.: **PCT/JP2017/037629**

§ 371 (c)(1),

(2) Date: **Mar. 8, 2019**(30) **Foreign Application Priority Data**

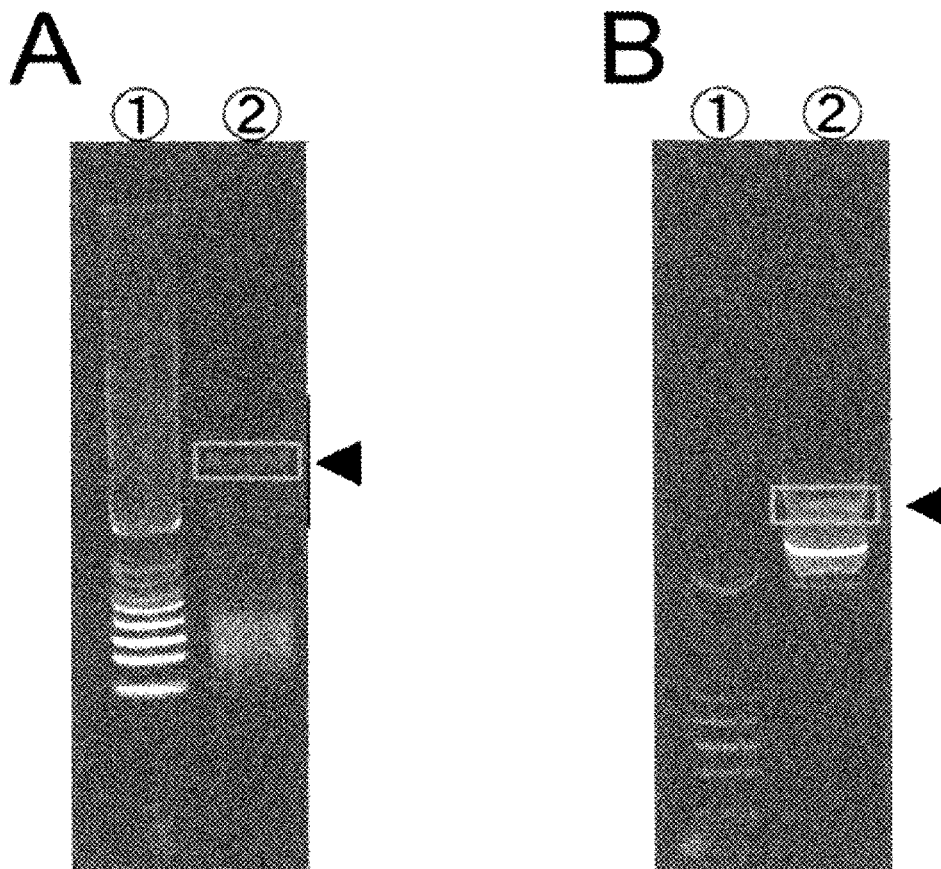
Oct. 18, 2016 (JP) 2016-204729

Publication Classification(51) **Int. Cl.****C07K 14/435** (2006.01)**C12N 9/24** (2006.01)**G01N 33/579** (2006.01)**G01N 33/68** (2006.01)(52) **U.S. Cl.**CPC **C07K 14/43509** (2013.01); **G01N 33/68**
(2013.01); **G01N 33/579** (2013.01); **C12N**
9/2477 (2013.01)

(57)

ABSTRACT

Provided are all full-length recombinant proteins involved in the clotting mechanism of *Limulus polyphemus*, cDNAs encoding the same, and applications thereof. A recombinant protein containing the amino acid sequence represented by SEQ ID NO: 2 or 4, a recombinant protein containing the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12, a recombinant protein containing the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22, variants thereof, cDNAs encoding the same, and utilization thereof.

Specification includes a Sequence Listing.

CRL Exhibit 1052
CRL v. Seikagaku
IPR2025-00440

FIG. 1

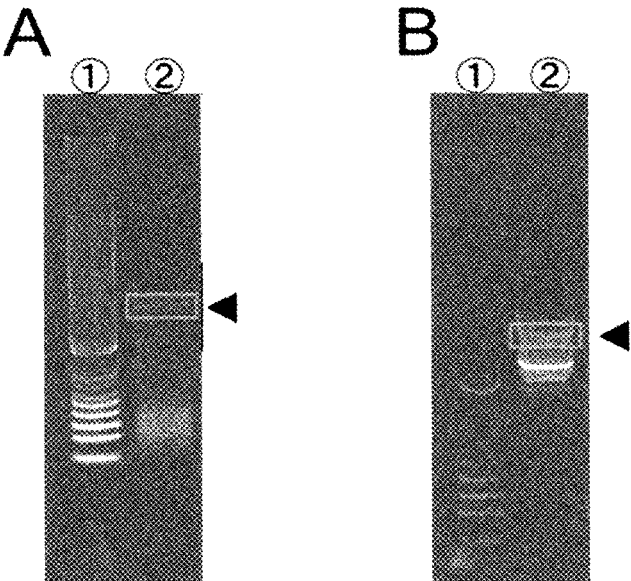


FIG. 2

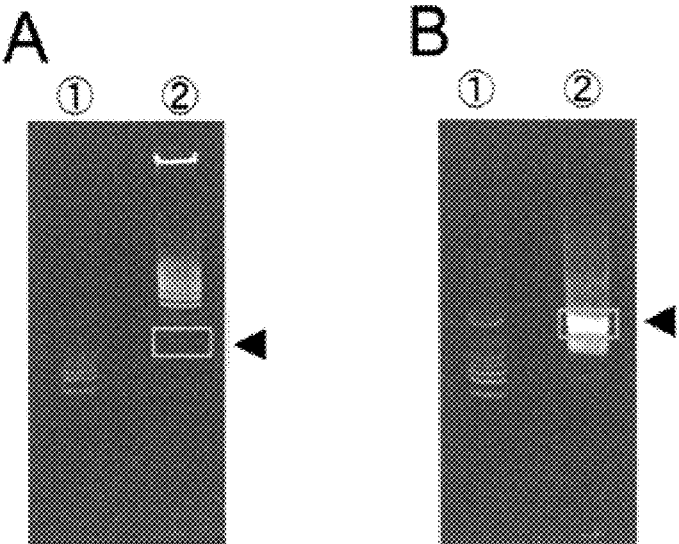
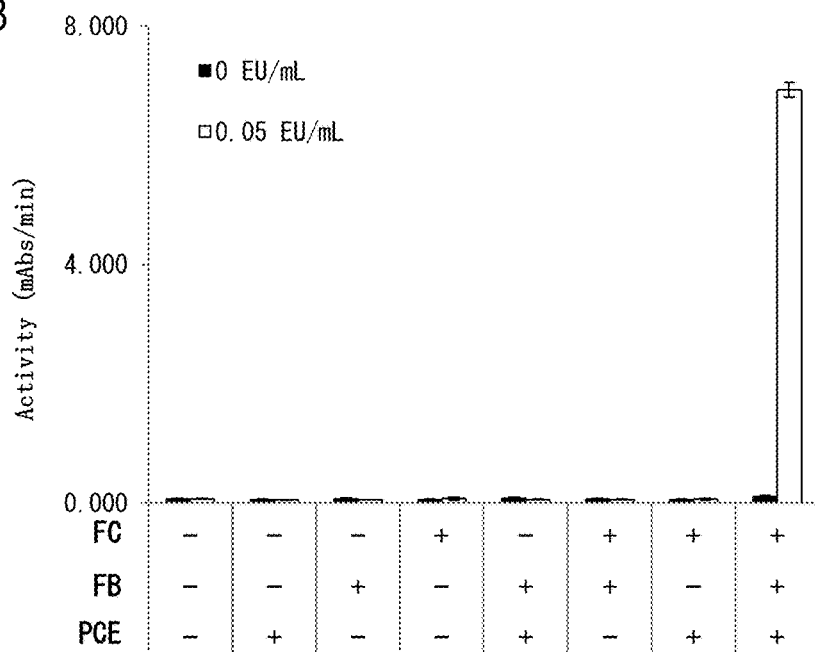


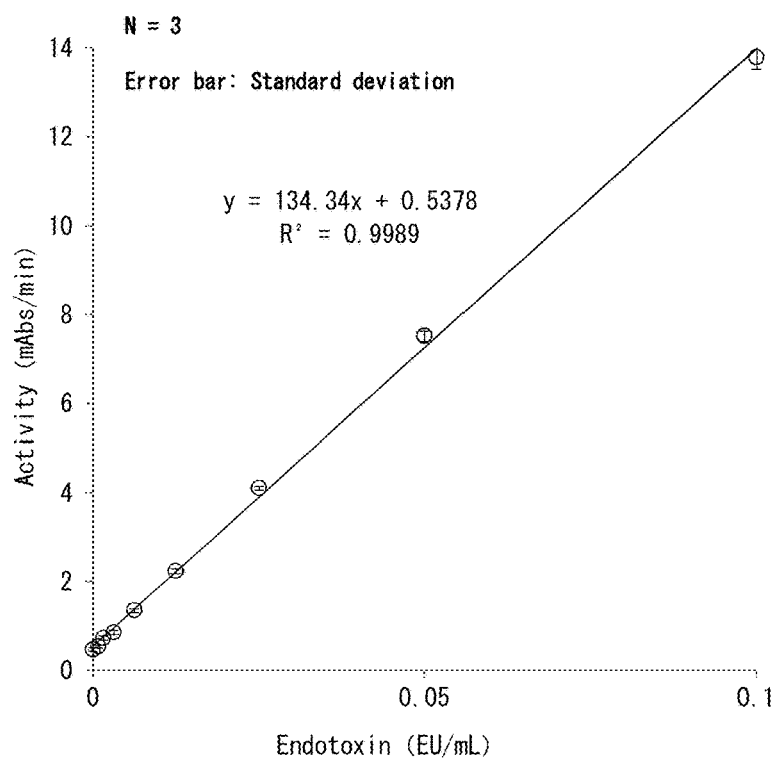
FIG. 3



N = 3

Error bar: Standard deviation

FIG. 4



RECOMBINANT PROTEINS DERIVED FROM GENUS LIMULUS, AND DNA MOLECULES ENCODING SAME

TECHNICAL FIELD

[0001] The present invention relates to recombinant proteins derived from the genus *Limulus*, DNAs encoding the same, and a method for utilizing the same.

BACKGROUND ART

[0002] Horseshoe crabs are called “living fossils” and there exist only two genera and four species on Earth. As the genera, there are only “the genus *Limulus*” living only in the east coast of the North American Continent and “the genus *Tachypleus*” living only in the southeast sea area in Asia.

[0003] A distribution in which one organism group is separately distributed in regions far from each other is called “discontinuous distribution”, and organisms which are separated in two regions on Earth like horseshoe crabs are very rare.

[0004] The organisms belonging to the genus *Limulus* are assigned to only one species: *Limulus polyphemus*, and the organisms belonging to the genus *Tachypleus* are assigned to only three species: *Tachypleus tridentatus*, *Tachypleus gigas*, and *Tachypleus rotundicauda* (also called *Carcinoscorpius rotundicauda*).

[0005] It is known that when an extract of amoebocytes (amoebocyte lysate) present in the blood of this organism comes into contact with an endotoxin, the extract clots. By utilizing this property, the amoebocyte lysate has been widely used for the quality control of pharmaceutical preparations or the like as a reagent for detecting an endotoxin with high sensitivity.

[0006] This reagent is called “lysate reagent”. Further, the property of causing clotting by an endotoxin was found in *Limulus polyphemus* for the first time, and therefore, this reagent is sometimes called “*Limulus* reagent” or “LAL (*Limulus* amoebocyte lysate) reagent”. Even a reagent using an amoebocyte lysate derived from the genus *Tachypleus* is customarily called “*Limulus* reagent” or “LAL reagent”.

[0007] At present, the lysate reagent (*Limulus* reagent or LAL reagent) used by pharmaceutical manufacturers in Japan, U.S. and Europe is derived from *Limulus polyphemus* as a raw material. The number of captures of *Limulus polyphemus* is strictly limited due to extinction concerns, however, some reports also say that the population is still decreasing.

[0008] In order to protect horseshoe crabs, there has been an idea that the reagent is prepared by artificially producing proteins in the amoebocyte lysate using a recombinant technique (PTL 1 to PTL 8). Then, by utilizing recombinant proteins, products such as PyroGene (trademark) (Lonza), EndoZyme (trademark) (Hyglos), and PyroSmart (trademark) (Seikagaku Corporation) have been actually launched in the market.

[0009] However, any of these uses recombinant proteins derived from an organism of the genus *Tachypleus*, and there has been no report of a reagent using recombinant proteins derived from *Limulus polyphemus* which has been widely spread as the lysate reagent.

[0010] The reason for this is because proteins involved in a mechanism of occurrence of clotting by an endotoxin and

genes thereof in *Limulus polyphemus* have not been completely identified structurally and functionally.

[0011] In the genus *Tachypleus*, for example, with respect to factor C which is one of the proteins involved in the clotting mechanism, a full gene sequence and a full amino acid sequence derived from *Tachypleus tridentatus* have been reported in 1991 (NPL 1), and a full gene sequence and a full amino acid sequence derived from *Tachypleus rotundicauda* (*Carcinoscorpius rotundicauda*) have been reported in 1995 (NPL 2).

[0012] However, in *Limulus polyphemus*, complete identification of these proteins and genes from both structural and functional aspects has not been achieved even though 20 years or more has passed since the reports regarding the genus *Tachypleus* described above were published. This is because accurate determination of the full-length sequence structures and elucidation of the expression and functions of these proteins and genes could not be achieved by general techniques due to various differences in biomolecules, diversities, etc. because of differences in genera or species of horseshoe crabs.

[0013] In fact, as part of the invertebrate genome project using a next-generation sequencer, a comprehensive analysis of genes of *Limulus polyphemus* has been carried out (<http://genome.wustl.edu/genomes/detail/limulus-polyphemus/>), however, the full-length sequences of all proteins and genes involved in the clotting mechanism of *Limulus polyphemus* have not been elucidated yet. This fact also supports the difficulty in accurate determination of the full-length sequence structures of these proteins and genes in *Limulus polyphemus*.

CITATION LIST

Patent Literature

- [0014]** PTL 1: U.S. Pat. No. 5,712,144
- [0015]** PTL 2: U.S. Pat. No. 5,716,834
- [0016]** PTL 3: U.S. Pat. No. 5,858,706
- [0017]** PTL 4: U.S. Pat. No. 5,985,590
- [0018]** PTL 5: U.S. Pat. No. 6,645,724
- [0019]** PTL 6: WO 2008/004674
- [0020]** PTL 7: JP-T-2014-510898
- [0021]** PTL 8: WO 2014/092079

Non Patent Literature

- [0022]** NPL 1: Muta T et al., Journal of Biological Chemistry, 266, 6554-6561 (1991)
- [0023]** NPL 2: Ding J L et al., Molecular Marine Biology and Biotechnology, 4(1), 90-103 (1995)

SUMMARY OF INVENTION

Technical Problem

[0024] An object of the present invention is to provide all full-length recombinant proteins involved in the clotting mechanism of *Limulus polyphemus*, cDNAs encoding the same, and applications thereof.

Solution to Problem

[0025] The present inventors were successful in the acquisition of full-length cDNAs encoding all protein factors involved in the clotting mechanism of *Limulus polyphemus* which no one had ever been successful for a long time, and

the expression of all recombinant proteins thereof, and further found that these can be utilized in various applications, and thus completed the present invention.

[0026] That is, the present invention includes the following embodiments.

[0027] [1]

[0028] A recombinant first protein such as a recombinant protein which is any of the following:

[0029] (A) a recombinant protein that contains the amino acid sequence represented by SEQ ID NO: 2 or 4;

[0030] (B) a recombinant protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 2 or 4, and has factor C activity;

[0031] (C) a recombinant protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 1 or 3, and has factor C activity; and

[0032] (D) a recombinant protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 1 or 3 under stringent conditions, and has factor C activity.

[0033] [2]

[0034] A recombinant second protein such as a recombinant protein which is any of the following:

[0035] (A) a recombinant protein that contains the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12;

[0036] (B) a recombinant protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12, and has factor B activity;

[0037] (C) a recombinant protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 5, 7, 9, or 11, and has factor B activity; and

[0038] (D) a recombinant protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 5, 7, 9, or 11 under stringent conditions, and has factor B activity.

[0039] [3]

[0040] A recombinant third protein such as a recombinant protein which is any of the following:

[0041] (A) a recombinant protein that contains the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22; (B) a recombinant protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22, and has proclotting enzyme activity;

[0042] (C) a recombinant protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21, and has proclotting enzyme activity; and

[0043] (D) a recombinant protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21 under stringent conditions, and has proclotting enzyme activity.

[0044] [1-1]

[0045] Use of the recombinant first protein for activating a protein having factor B activity (for example, a second protein such as the following protein):

[0046] (A) a protein that contains the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12;

[0047] (B) a protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12, and has factor B activity;

[0048] (C) a protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 5, 7, 9, or 11, and has factor B activity; or

[0049] (D) a protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 5, 7, 9, or 11 under stringent conditions, and has factor B activity.

[0050] [1-1-1]

[0051] The use according to [1-1], wherein the second protein is the recombinant second protein.

[0052] [2-1]

[0053] Use of the recombinant second protein for activating a protein having proclotting enzyme activity (for example, a third protein such as the following protein):

[0054] (A) a protein that contains the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22;

[0055] (B) a protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22, and has proclotting enzyme activity;

[0056] (C) a protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21, and has proclotting enzyme activity; or

[0057] (D) a protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21 under stringent conditions, and has proclotting enzyme activity.

[0058] [2-1-1]

[0059] The use according to [2-1], wherein the third protein is the recombinant third protein.

[0060] [3-1]

[0061] Use of the recombinant third protein for cleaving a substrate.

[0062] [3-1-1]

[0063] The use according to [3-1], wherein the substrate is a synthetic substrate.

[0064] [1-2]

[0065] A method for detecting an endotoxin in a specimen, including a step of bringing the recombinant first protein into contact with the specimen.

[0066] [1-2-1]

[0067] The method according to [1-2], further including a step of bringing the recombinant first protein having contacted with the specimen into contact with a protein having factor B activity such as the second protein.

[0068] [1-2-1-1]

[0069] The method according to [1-2-1], wherein the second protein is the recombinant second protein.

[0070] [1-2-2]

[0071] The method according to [1-2-1] or [1-2-1-1], further including a step of bringing the protein having factor B activity having contacted with the "recombinant first protein having contacted with the specimen" into contact with a protein having proclotting enzyme activity such as the third protein.

[0072] [1-2-2-1]

[0073] The method according to [1-2-2], wherein the third protein is the recombinant third protein.

[0074] [1-2-3]

[0075] The method according to [1-2-2] or [1-2-2-1], further including a step of bringing the protein having proclotting enzyme activity having contacted with the “protein having factor B activity having contacted with the recombinant first protein having contacted with the specimen” into contact with a substrate for detection.

[0076] [1-3]

[0077] An endotoxin detecting agent, containing the recombinant first protein.

[0078] [1-3-1]

[0079] The agent according to [1-3], further containing a protein having factor B activity such as the second protein.

[0080] [1-3-1-1]

[0081] The agent according to [1-3-1], wherein the second protein is the recombinant second protein.

[0082] [1-3-2]

[0083] The agent according to [1-3-1] or [1-3-1-1], further containing a protein having proclotting enzyme activity such as the third protein.

[0084] [1-3-2-1]

[0085] The agent according to [1-3-2], wherein the third protein is the recombinant third protein.

[0086] [1-4]

[0087] A cDNA which is any of the following (hereinafter also referred to as “first cDNA”):

[0088] (A) a cDNA that contains the base sequence represented by SEQ ID NO: 1 or 3;

[0089] (B) a cDNA that contains a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 1 or 3, and encodes a protein having factor C activity;

[0090] (C) a cDNA that hybridizes to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 1 or 3 under stringent conditions, and encodes a protein having factor C activity;

[0091] (D) a cDNA that encodes a protein containing the amino acid sequence represented by SEQ ID NO: 2 or 4; and

[0092] (E) a cDNA that encodes a protein containing an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 2 or 4, and having factor C activity.

[0093] [1-4-1]

[0094] A DNA construct, containing the first cDNA.

[0095] [1-4-2]

[0096] A cell, expressing the recombinant first protein by being transformed with the DNA construct according to [1-4-1].

[0097] [1-4-3]

[0098] A method for producing the recombinant first protein, including a step of culturing the cell according to [1-4-2].

[0099] [2-4]

[0100] A cDNA which is any of the following (hereinafter also referred to as “second cDNA”):

[0101] (A) a cDNA that contains the base sequence represented by SEQ ID NO: 5, 7, 9, or 11;

[0102] (B) a cDNA that contains a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 5, 7, 9, or 11, and encodes a protein having factor B activity;

[0103] (C) a cDNA that hybridizes to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 5, 7, 9, or 11 under stringent conditions, and encodes a protein having factor B activity;

[0104] (D) a cDNA that encodes a protein containing the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12; and

[0105] (E) a cDNA that encodes a protein containing an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12, and having factor B activity.

[0106] [2-4-1]

[0107] A DNA construct, containing the second cDNA.

[0108] [2-4-2]

[0109] A cell, expressing the recombinant second protein by being transformed with the DNA construct according to [2-4-1].

[0110] [2-4-3]

[0111] A method for producing the recombinant second protein, including a step of culturing the cell according to [2-4-2].

[0112] [3-4]

[0113] A cDNA which is any of the following (hereinafter also referred to as “third cDNA”):

[0114] (A) a cDNA that contains the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21;

[0115] (B) a cDNA that contains a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21, and encodes a protein having proclotting enzyme activity;

[0116] (C) a cDNA that hybridizes to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21 under stringent conditions, and encodes a protein having proclotting enzyme activity;

[0117] (D) a cDNA that encodes a protein containing the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22; and

[0118] (E) a cDNA that encodes a protein containing an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22, and having proclotting enzyme activity.

[0119] [3-4-1]

[0120] A DNA construct, containing the third cDNA.

[0121] [3-4-2]

[0122] A cell, expressing the recombinant third protein by being transformed with the DNA construct according to [3-4-1].

[0123] [3-4-3]

[0124] A method for producing the recombinant third protein, including a step of culturing the cell according to [3-4-2].

[0125] [1-5]

[0126] A method for producing an endotoxin detecting agent, including a step of artificially expressing a protein using the first cDNA.

[0127] [1-5-1]

[0128] The production method according to [1-5], further including a step of artificially expressing a protein using the second cDNA.

[0129] [1-5-2]

[0130] The production method according to [1-5-1], further including a step of artificially expressing a protein using the third cDNA.

BRIEF DESCRIPTION OF DRAWINGS

[0131] FIG. 1 is an electrophoresis photograph of a PCR product in a process for acquiring a cDNA of a protein having factor C activity of *Limulus polyphemus*.

[0132] FIG. 2 is an electrophoresis photograph of a PCR product in a process for acquiring a cDNA of a protein having factor C activity of *Limulus polyphemus*.

[0133] FIG. 3 is a figure showing the activity of cleaving a synthetic substrate by contact of each recombinant protein alone or various mixtures of recombinant proteins with an endotoxin.

[0134] FIG. 4 is a figure showing a calibration curve of an endotoxin using a mixture of three types of recombinant proteins.

DESCRIPTION OF EMBODIMENTS

<1> Recombinant First Protein and Production Method Therefor

(1) Recombinant First Protein

[0135] A recombinant first protein is a recombinant protein having factor C activity.

[0136] The recombinant first protein is specifically a recombinant protein which is either of the following:

[0137] (I) a recombinant protein that contains an amino acid sequence of the protein of *Limulus polyphemus* having factor C activity; and

[0138] (II) a recombinant protein that is a variant of the above (I) and has factor C activity.

[0139] Examples of the amino acid sequence of the protein of *Limulus polyphemus* having factor C activity include the amino acid sequence represented by SEQ ID NO: 2 or 4. Further, examples of a base sequence encoding such an amino acid sequence include the base sequence represented by SEQ ID NO: 1 or 3.

[0140] The recombinant first protein may be more specifically a recombinant protein which is any of the following:

[0141] (A) a recombinant protein that contains the amino acid sequence represented by SEQ ID NO: 2 or 4;

[0142] (B) a recombinant protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 2 or 4, and has factor C activity;

[0143] (C) a recombinant protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 1 or 3, and has factor C activity; and

[0144] (D) a recombinant protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 1 or 3 under stringent conditions, and has factor C activity.

[0145] Note that the expression of “containing an amino acid sequence” or “containing a base sequence” also includes a case of “being composed of the amino acid sequence” or a case of “being composed of the base sequence”.

[0146] This protein was acquired for the first time in the world by the method disclosed in Examples of this description, and the full-length sequence structure and function thereof were elucidated for the first time. The function of the protein is a function of turning into an active form in the

presence of an endotoxin and converting a protein having factor B activity such as the below-mentioned second protein into an active form (referred to as “factor C activity” in this application document). The recombinant first protein may be a protein that shows the factor C activity in combination with at least the second protein such as a recombinant second protein.

[0147] Whether having the factor C activity can be confirmed by detecting the progress of a cascade reaction in the presence of an endotoxin when combining a target recombinant protein with a protein (which is not in an active form, hereinafter referred to as “inactive form”) having factor B activity such as the second protein (inactive form), a protein (inactive form) having proclotting enzyme activity such as a third protein (inactive form), and a substrate for detection.

[0148] The “cascade reaction” refers to a series of reactions in which a protein (inactive form) having factor C activity is activated by an endotoxin, a protein (inactive form) having factor B activity is activated by the protein (active form) having factor C activity, and a protein (inactive form) having proclotting enzyme activity is activated by the protein (active form) having factor B activity. The progress of the cascade reaction can be detected by cleavage of a substrate for detection.

[0149] Among the recombinant first proteins, the most preferred is the above-mentioned recombinant protein (I) such as the above-mentioned recombinant protein (A), but the recombinant protein may be a variant thereof as long as it has factor C activity.

[0150] The variant may be, for example, a recombinant protein that contains an amino acid sequence including a substitution, a deletion, an insertion, and/or an addition of one or several amino acids at one or several sites in an amino acid sequence (for example, the amino acid sequence represented by SEQ ID NO: 2 or 4) of the recombinant first protein as described above, and has factor C activity. The term “one or several” may be, for example, 1 to 20, 1 to 10, 1 to 5, or 1 to 3. The substitution, deletion, insertion, and/or addition of one or several amino acids is a conservative mutation that maintains the function of the protein normal. A representative conservative mutation is a conservative substitution. The conservative substitution is, for example, a mutation in which a substitution takes place mutually among Phe, Trp, and Tyr when the substitution site is an aromatic amino acid, among Leu, Ile, and Val when the substitution site is a hydrophobic amino acid, between Gln and Asn when the substitution site is a polar amino acid, among Lys, Arg, and His when the substitution site is a basic amino acid, between Asp and Glu when the substitution site is an acidic amino acid, and between Ser and Thr when the substitution site is an amino acid having a hydroxyl group.

[0151] Specific examples of the substitution regarded as a conservative substitution include a substitution from Ala to Ser or Thr, a substitution from Arg to Gln, His or Lys, a substitution from Asn to Glu, Gln, Lys, His, or Asp, a substitution from Asp to Asn, Glu, or Gln, a substitution from Cys to Ser or Ala, a substitution from Gln to Asn, Glu, Lys, His, Asp, or Arg, a substitution from Glu to Gly, Asn, Gln, Lys, or Asp, a substitution from Gly to Pro, a substitution from His to Asn, Lys, Gln, Arg, or Tyr, a substitution from Ile to Leu, Met, Val, or Phe, a substitution from Leu to Ile, Met, Val, or Phe, a substitution from Lys to Asn, Glu, Gln, His, or Arg, a substitution from Met to Ile, Leu, Val, or Phe, a substitution from Phe to Trp, Tyr, Met, Ile, or Leu, a

substitution from Ser to Thr or Ala, a substitution from Thr to Ser or Ala, a substitution from Trp to Phe or Tyr, a substitution from Tyr to His, Phe, or Trp, and a substitution from Val to Met, Ile, or Leu.

[0152] Further, the variant may be, for example, a recombinant protein that contains an amino acid sequence having an 80% or more, 90% or more, 95% or more, 96, or more, 97% or more, 98% or more, or 99% or more identity with an amino acid sequence (for example, the amino acid sequence represented by SEQ ID NO: 2 or 4) of the recombinant first protein as described above, and has factor C activity.

[0153] Further, the variant may be, for example, a recombinant protein that is encoded by a DNA containing a base sequence having an 80% or more, 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more identity with a base sequence (for example, the base sequence represented by SEQ ID NO: 1 or 3) encoding the amino acid sequence of the recombinant first protein as described above, and has factor C activity.

[0154] Further, the variant may be, for example, a recombinant protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to a base sequence (for example, the base sequence represented by SEQ ID NO: 1 or 3) encoding the amino acid sequence of the recombinant first protein as described above under stringent conditions, and has factor C activity. The term “stringent conditions” refers to conditions under which a so-called specific hybrid is formed and no nonspecific hybrid is formed by a general hybridization operation described in *Molecular Cloning: A Laboratory Manual* 2nd ed. (1989) Cold Spring Harbor Laboratory edited by T. Maniatis et al., or the like. The term “stringent conditions” specifically refers to conditions in which a sodium salt concentration is from 15 to 750 mM, preferably from 50 to 750 mM, more preferably from 300 to 750 mM, the temperature is from 25 to 70° C., preferably from 50 to 70° C., more preferably from 55 to 65° C., and a formamide concentration is from 0 to 50%, preferably from 20 to 50%, more preferably from 35 to 45%. Further, in the case of stringent conditions, washing conditions for a filter after hybridization are such that a sodium salt concentration is generally from 15 to 600 mM, preferably from 50 to 600 mM, more preferably from 300 to 600 mM, and the temperature is from 50 to 70° C., preferably from 55 to 70° C., more preferably from 60 to 65° C.

[0155] As the variant, specifically, the above-mentioned recombinant proteins (B) to (D) are preferred. The term “95% or more” in the above (B) and (C) is preferably 96% or more, more preferably 97% or more, more preferably 98% or more, and more preferably 99% or more.

[0156] Further, to the recombinant first protein, an arbitrary peptide such as a His tag or a V tag, or the like may be added as long as it has factor C activity.

[0157] Further, the term “recombinant protein” in this application document refers to a protein obtained by artificially introducing a gene encoding a protein into a host cell other than a horseshoe crab and expressing the protein. Therefore, the protein acquired from a natural horseshoe crab itself does not correspond to the “recombinant protein” in this application document.

(2) Method for Producing Recombinant First Protein

[0158] The full-length amino acid sequence of the recombinant first protein has been disclosed for the first time by the

present invention, and therefore, the recombinant first protein can be produced by a genetic engineering technique using, for example, a DNA encoding this sequence. A method for producing the recombinant first protein by a genetic engineering technique is not particularly limited, and for example, a general method for producing a protein by a genetic engineering technique can be adopted. The recombinant first protein can be produced by, for example, utilizing a heterologous expression system or a cell-free protein synthesis system.

[0159] For example, the recombinant first protein can be produced by artificially introducing the below-mentioned first cDNA into a host cell other than a horseshoe crab and expressing the protein from this cDNA. The present invention also provides such a method for producing the recombinant first protein.

[0160] When artificially introducing a DNA into a host cell, a DNA construct (such as a vector) into which the DNA has been artificially integrated may be used. As such an artificial DNA construct, for example, a DNA construct into which the first cDNA has been integrated can be exemplified. Such an artificial DNA construct will be described later.

[0161] The host cell other than a horseshoe crab is not particularly limited as long as it can functionally express the protein from the first cDNA. Examples of the host cell other than a horseshoe crab include mammalian cells and insect cells. Examples of mammals include rodents and primates. Examples of the rodents include Chinese hamsters, hamsters, mice, rats, and guinea pigs. Examples of the Chinese hamster cells include a Chinese hamster ovary-derived cell line (CHO). Examples of the CHO include CHO DG44, CHO S, and CHO K1. The primates are not particularly limited, however, examples thereof include humans, monkeys, and chimpanzees. Examples of the human cells include a human embryonic kidney cell-derived cell line (HEK). Examples of the HEK include HEK 293.

[0162] The phrase “functionally expressed” refers to that the expressed recombinant first protein shows the factor C activity. Whether a protein shows the factor C activity can be confirmed by the method described in the below-mentioned Example. Further, whether the expressed protein contains a target amino acid sequence of any of the above-mentioned structures (A) to (D) or the like can be confirmed by analyzing the amino acid sequence of the expressed protein and comparing this with the target amino acid sequence.

[0163] A technique for artificially introducing the first cDNA into a host cell is also not particularly limited as long as the cDNA is in a state of being expressibly retained in the host cell. By growing the host cell into which the first cDNA has been artificially introduced, the recombinant first protein can be expressed.

[0164] The growth of the transformed host cell can be carried out by, for example, culturing the cell. The culturing conditions at this time are also not particularly limited as long as the cell can be grown or proliferated, and conditions generally used for culturing the cell can be used by being appropriately modified as needed. For example, when the host cell is a mammalian cell, a culture medium generally used for culturing the mammalian cell can be used. As such a culture medium, for example, RPMI-1640 medium (Sigma Aldrich Co. LLC.), DMEM medium (Sigma Aldrich Co. LLC.), ExpiCHO™ Expression Medium (Thermo Fisher Scientific), or the like can be used. The culturing can be carried out by, for example, static culture, suspension cul-

ture, or the like at 36° C. to 38° C. while supplying 5% to 8% CO₂. A kit dedicated to protein expression may be used.

[0165] The expressed recombinant first protein is collected as a solution fraction containing this and can be utilized in various applications described below.

[0166] The solution fraction containing the recombinant first protein can be, for example, a culture solution, a culture supernatant, a cell homogenate extract, a mixture thereof, or the like. The recombinant first protein may be used by being purified to a desired extent or may be used as such without purification.

[0167] The purification can be carried out by a known method used for purifying a protein. Examples of such a method include ammonium sulfate precipitation, gel filtration chromatography, ion exchange chromatography, hydrophobic interaction chromatography, and hydroxyapatite chromatography.

[0168] According to the present invention, it has become possible for the first time to artificially and functionally express the recombinant first protein. The thus produced recombinant first protein can be used for, for example, the application described below.

<2> Recombinant Second Protein and Production Method Therefor

[0169] The recombinant second protein is a recombinant protein having factor B activity.

[0170] The recombinant second protein is specifically a recombinant protein which is either of the following:

[0171] (I) a recombinant protein that contains an amino acid sequence of the protein of *Limulus polyphemus* having factor B activity; and

[0172] (II) a recombinant protein that is a variant of the above (I) and has factor B activity.

[0173] Examples of the amino acid sequence of the protein of *Limulus polyphemus* having factor B activity include the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12. Further, examples of a base sequence encoding such an amino acid sequence include the base sequence represented by SEQ ID NO: 5, 7, 9, or 11.

[0174] The recombinant second protein may be more specifically a recombinant protein which is any of the following:

[0175] (A) a recombinant protein that contains the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12;

[0176] (B) a recombinant protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12, and has factor B activity;

[0177] (C) a recombinant protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 5, 7, 9, or 11, and has factor B activity; and

[0178] (D) a recombinant protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 5, 7, 9, or 11 under stringent conditions, and has factor B activity.

[0179] The function of this protein is a function of turning into an active form by a protein (active form) having factor C activity such as the first protein having turned into an active form and converting a protein having proclotting enzyme activity such as the below-mentioned third protein into an active form (referred to as “factor B activity” in this

application document). The recombinant second protein may be a protein that shows the factor B activity in combination with at least the first protein such as the recombinant first protein and the third protein such as a recombinant third protein.

[0180] Whether having the factor B activity can be confirmed by detecting the progress of a cascade reaction when combining a target recombinant protein with a protein (active form) having factor C activity such as the first protein (active form), a protein (inactive form) having proclotting enzyme activity such as the third protein (inactive form), and a substrate for detection. In this system, an endotoxin for converting a protein (inactive form) having factor C activity into an active form may further exist.

[0181] To the other explanation, the explanation of the above <1> can apply. That is, for example, “SEQ ID NO. 1 or 3”, “SEQ ID NO. 2 or 4”, “(recombinant) first protein”, “first cDNA”, and “factor C” in the above <1> may be replaced by “SEQ ID NO. 5, 7, 9, or 11”, “SEQ ID NO. 6, 8, 10, or 12”, “(recombinant) second protein”, “second cDNA”, and “factor B”, respectively, and so on.

<3> Recombinant Third Protein and Production Method Therefor

[0182] The recombinant third protein is a recombinant protein having proclotting enzyme activity.

[0183] The recombinant third protein is specifically a recombinant protein which is either of the following:

[0184] (I) a recombinant protein that contains an amino acid sequence of the protein of *Limulus polyphemus* having proclotting enzyme activity; and

[0185] (II) a recombinant protein that is a variant of the above (I) and has proclotting enzyme activity.

[0186] Examples of the amino acid sequence of the protein of *Limulus polyphemus* having proclotting enzyme activity include the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22. Further, examples of a base sequence encoding such an amino acid sequence include the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21.

[0187] The recombinant third protein may be more specifically a recombinant protein which is any of the following:

[0188] (A) a recombinant protein that contains the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22;

[0189] (B) a recombinant protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22, and has proclotting enzyme activity;

[0190] (C) a recombinant protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21, and has proclotting enzyme activity; and

[0191] (D) a recombinant protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21 under stringent conditions, and has proclotting enzyme activity.

[0192] The function of this protein is a function of turning into an active form by a protein (active form) having factor B activity such as the second protein having turned into an active form and cleaving the below-mentioned substrate (a

protein or a peptide) (referred to as “proclotting enzyme activity” in this application document). The recombinant third protein may be a protein that shows the proclotting enzyme activity in combination with at least the second protein such as the recombinant second protein.

[0193] Whether a protein has the proclotting enzyme activity can be confirmed by detecting the progress of a cascade reaction when combining a target recombinant protein with a protein (active form) having factor B activity such as the second protein (active form) and a substrate for detection. In this system, a protein (active form) having factor C activity for converting a protein (inactive form) having factor B activity into an active form may further exist. Further, in this system, an endotoxin for converting a protein (inactive form) having factor C activity into an active form may further exist.

[0194] To the other explanation, the explanation of the above <1> can apply. That is, for example, “SEQ ID NO. 1 or 3”, “SEQ ID NO. 2 or 4”, “(recombinant) first protein”, “first cDNA”, and “factor C” in the above <1> may be replaced by “SEQ ID NO. 13, 15, 17, 19, or 21”, “SEQ ID NO. 14, 16, 18, 20, or 22”, “(recombinant) third protein”, “third cDNA”, and “proclotting enzyme”, respectively, and so on.

<4> Use of Recombinant First Protein

[0195] The recombinant first protein can be used for activating a protein having factor B activity such as the second protein. In other words, the present invention includes a method for activating a protein having factor B activity including a step of bringing the recombinant first protein into contact with the protein having factor B activity.

[0196] Examples of the protein having factor B activity include the second protein.

[0197] The second protein is specifically a protein which is either of the following:

[0198] (I) a protein that contains an amino acid sequence of the protein of *Limulus polyphemus* having factor B activity; and

[0199] (II) a protein that is a variant of the above (I) and has factor B activity.

[0200] The second protein may be more specifically a protein which is any of the following:

[0201] (A) a protein that contains the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12;

[0202] (B) a protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12, and has factor B activity;

[0203] (C) a protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 5, 7, 9, or 11, and has factor B activity; and

[0204] (D) a protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 5, 7, 9, or 11 under stringent conditions, and has factor B activity.

[0205] Further, examples of the protein having factor B activity also include the following proteins:

[0206] (III) a protein that contains an amino acid sequence of a protein of a horseshoe crab other than *Limulus polyphemus* having factor B activity; and

[0207] (IV) a protein that is a variant of the above (III) and has factor B activity.

[0208] To the explanation of the protein having factor B activity, the explanation of the above <2> can apply.

[0209] According to the present invention, it has been revealed for the first time that the protein (A) of the recombinant first protein has factor C activity. The protein (A) having such a function and the variant thereof are applied to the activation of the protein having factor B activity.

[0210] The protein having factor B activity used here may be a protein acquired from a natural substance or a protein produced artificially (for example, the recombinant second protein) as long as it has the above-mentioned structure and function. According to the present invention, it has become possible for the first time to artificially and functionally express the recombinant second protein and also it has become possible to stably produce the second protein with constant quality without acquiring it from native *Limulus polyphemus*. Therefore, the protein having factor B activity is preferably the recombinant second protein.

[0211] The activation of the protein having factor B activity using the recombinant first protein can be carried out by bringing a molecule of the recombinant first protein (active form) and a molecule of the protein (inactive form) having factor B activity into contact with each other. Incidentally, in order to convert the recombinant first protein into an active form, the recombinant first protein (inactive form) and the protein (inactive form) having factor B activity may be brought into contact with each other in the presence of an endotoxin.

[0212] The conditions during this contact are not particularly limited as long as the conditions allow the recombinant first protein (active form) to exhibit the factor C activity so as to be able to activate the protein having factor B activity (when the operation is carried out in the presence of an endotoxin, the conditions further allow the endotoxin to be able to activate the recombinant first protein). For example, reaction conditions for a known lysate reagent can be adopted.

[0213] Whether the protein having factor B activity has been activated can be confirmed by the method described in the below-mentioned Example.

<5> Use of Recombinant Second Protein

[0214] The recombinant second protein can be used for activating a protein having proclotting enzyme activity such as the third protein. In other words, the present invention includes a method for activating a protein having proclotting enzyme activity including a step of bringing the recombinant second protein into contact with the protein having proclotting enzyme activity.

[0215] Examples of the protein having proclotting enzyme activity include the third protein.

[0216] The third protein is specifically a protein which is either of the following:

[0217] (I) a protein that contains an amino acid sequence of the protein of *Limulus polyphemus* having proclotting enzyme activity; and

[0218] (II) a protein that is a variant of the above (I) and has proclotting enzyme activity.

[0219] The third protein may be more specifically a protein which is any of the following:

[0220] (A) a protein that contains the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22;

[0221] (B) a protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22, and has proclotting enzyme activity;

[0222] (C) a protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21, and has proclotting enzyme activity; and

[0223] (D) a protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21 under stringent conditions, and has proclotting enzyme activity.

[0224] Further, examples of the protein having proclotting enzyme activity also include the following proteins:

[0225] (III) a protein that contains an amino acid sequence of a protein of a horseshoe crab other than *Limulus polyphemus* having proclotting enzyme activity; and

[0226] (IV) a protein that is a variant of the above (III) and has proclotting enzyme activity.

[0227] To the explanation of the protein having proclotting enzyme activity, the explanation of the above <3> can apply.

[0228] The protein having proclotting enzyme activity used here may be a protein acquired from a natural substance or a protein produced artificially (for example, the recombinant third protein) as long as it has the above-mentioned structure and function.

[0229] The activation of the protein having proclotting enzyme activity using the recombinant second protein can be carried out by bringing a molecule of the recombinant second protein (active form) and a molecule of the protein (inactive form) having proclotting enzyme activity into contact with each other. Incidentally, in order to convert the recombinant second protein into an active form, the recombinant second protein (inactive form) and the protein (inactive form) having proclotting enzyme activity may be brought into contact with each other in the presence of a protein (active form) having factor C activity. Further, in order to activate this protein having factor C activity, the protein (inactive form) having factor C activity, the recombinant second protein (inactive form), and the protein (inactive form) having proclotting enzyme activity may be brought into contact with one another in the presence of an endotoxin.

[0230] The conditions during this contact are not particularly limited as long as the conditions allow the recombinant second protein (active form) to exhibit the factor B activity so as to be able to activate the protein having proclotting enzyme activity (when the operation is carried out in the presence of the protein (active form) having factor C activity, the conditions further allow the protein (active form) having factor C activity to exhibit the factor C activity so as to be able to activate the recombinant second protein, and when the operation is carried out in the presence of an endotoxin, the conditions further allow the endotoxin to be able to activate the protein having factor C activity). For example, reaction conditions for a known lysate reagent can be adopted.

[0231] Whether the third protein has been activated can be confirmed by the method described in the below-mentioned Example.

[0232] Examples of the protein having factor C activity include the first protein.

[0233] The first protein is specifically a protein which is either of the following:

[0234] (I) a protein that contains an amino acid sequence of the protein of *Limulus polyphemus* having factor C activity; and (II) a protein that is a variant of the above (I) and has factor C activity.

[0235] The first protein may be more specifically a protein which is any of the following:

[0236] (A) a protein that contains the amino acid sequence represented by SEQ ID NO: 2 or 4;

[0237] (B) a protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 2 or 4, and has factor C activity;

[0238] (C) a protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 1 or 3, and has factor C activity; and

[0239] (D) a protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 1 or 3 under stringent conditions, and has factor C activity.

[0240] Further, examples of the protein having factor C activity also include the following proteins:

[0241] (III) a protein that contains an amino acid sequence of a protein of a horseshoe crab other than *Limulus polyphemus* having factor C activity; and

[0242] (IV) a protein that is a variant of the above (III) and has factor C activity.

[0243] To the explanation of the protein having factor C activity, the explanation of the above <1> can apply.

[0244] The protein having factor C activity used here may be a protein acquired from a natural substance or a protein produced artificially (for example, the recombinant first protein) as long as it has the above-mentioned structure and function.

[0245] To the other explanation, the explanation of the above <4> can apply.

<6> Use of Recombinant Third Protein

[0246] The recombinant third protein can be used for cleaving a substrate. In other words, the present invention includes a method for cleaving a substrate including a step of bringing the recombinant third protein into contact with the substrate.

[0247] This substrate can also be used as the above-mentioned "substrate for detection" and may be a naturally derived substrate or a synthetic substrate. Such a substrate is not particularly limited as long as it is cleaved by the proclotting enzyme activity of the protein (active form) having proclotting enzyme activity such as the third protein (active form).

[0248] Examples of the substrate to be cleaved by the proclotting enzyme activity include a protein such as coagulogen and a synthetic substrate represented by a general formula: X—Y—Z (wherein X— is a protecting group bonded to Y through a covalent bond, Y is a peptide residue, and —Z is a signal substance bonded to Y through an amide bond).

[0249] The protecting group X is not particularly limited, and a known protecting group for a peptide can be used. Examples of such a protecting group include a t-butoxycarbonyl group and a benzoyl group.

[0250] The peptide residue Y is also not particularly limited, and examples thereof include Leu-Gly-Arg (LGR) and Ile-Glu-Gly-Arg (IEGR) (SEQ ID NO: 23).

[0251] The signal substance Z is also not particularly limited, and examples thereof include a dye that is detected under visible light and a fluorescent dye. Examples of such a dye include pNA (p-nitroaniline), MCA (7-methoxycoumarin-4-acetic acid), DNP (2,4-dinitroaniline), and a dansyl-type dye.

[0252] Among these, Boc-Leu-Gly-Arg-pNA (t-butoxycarbonyl-leucyl-glycyl-arginyl-p-nitroaniline, Boc-LGR-pNA) is preferred.

[0253] Whether the substrate has been cleaved by the proclotting enzyme activity can be detected by, for example, detecting “gelation” when coagulogen is used as the substrate. The gelation can be detected by detecting a decrease in fluidity by visual observation or the like.

[0254] Further, for example, when a synthetic substrate as described above is used, the signal Z is released by cleaving the amide bond between Y and Z and a signal such as a developed color or fluorescence is emitted, and therefore, by detecting this signal, whether the substrate has been cleaved by the proclotting enzyme activity can be detected. The detection of the signal may be carried out by a technique according to the type of the signal. For example, the signal of pNA can be detected by an absorbance (405 nm).

[0255] The cleavage of the substrate using the recombinant third protein can be carried out by bringing a molecule of the recombinant third protein (active form) and a molecule of the substrate into contact with each other. Incidentally, in order to convert the recombinant third protein into an active form, the recombinant third protein (inactive form) and the substrate may be brought into contact with each other in the presence of the protein (active form) having factor B activity. Further, in order to convert this protein having factor B activity into an active form, the protein (inactive form) having factor B activity, the recombinant third protein (inactive form), and the substrate may be brought into contact with one another in the presence of the protein (active form) having factor C activity. Further, in order to convert this protein having factor C activity into an active form, the protein (inactive form) having factor C activity, the protein (inactive form) having factor B activity, the recombinant third protein (inactive form), and the substrate may be brought into contact with one another in the presence of an endotoxin.

[0256] The conditions during this contact are not particularly limited as long as the conditions allow the recombinant third protein (active form) to exhibit the proclotting enzyme activity so as to be able to cleave the substrate (when the operation is carried out in the presence of the protein (active form) having factor B activity, the conditions further allow the protein (active form) having factor B activity to exhibit the factor B activity so as to be able to activate the recombinant third protein; when the operation is carried out in the presence of the protein (active form) having factor C activity, the conditions further allow the protein (active form) having factor C activity to exhibit the factor C activity so as to be able to activate the second protein; and when the operation is carried out in the presence of an endotoxin, the conditions further allow the endotoxin to be able to activate the protein having factor C activity). For example, reaction conditions for a known lysate reagent can be adopted.

[0257] To the other explanation, the explanation of the above <4> can apply.

<7> Method for Detecting Endotoxin

[0258] The method for detecting an endotoxin of the present invention is a method for detecting an endotoxin in a specimen including a step of bringing the recombinant first protein into contact with the specimen.

[0259] This method may further include a step of bringing the recombinant first protein having contacted with the specimen into contact with a protein having factor B activity such as the second protein. This protein having factor B activity may be the recombinant second protein.

[0260] Further, this method may further include a step of bringing the protein having factor B activity having contacted with the “recombinant first protein having contacted with the specimen” into contact with a protein having proclotting enzyme activity such as the third protein. This protein having proclotting enzyme activity may be the recombinant third protein.

[0261] The detection of an endotoxin in a specimen can be carried out by detecting the activation of the recombinant first protein having contacted with the specimen. Whether the recombinant first protein has been activated can be confirmed by the method described in the below-mentioned Example. Further, the activation of the recombinant first protein can also be directly detected by utilizing a substrate (substrate for detection). That is, by utilizing a substrate that emits a signal by being cleaved by the factor C activity of the activated recombinant first protein as the substrate for detection, the activation of the recombinant first protein can be directly detected. Therefore, this method may further include a step of bringing the “recombinant first protein having contacted with the specimen” into contact with the substrate for detection.

[0262] When the method further includes the step of bringing into contact with the protein having factor B activity, the detection can be carried out by detecting the activation of the protein having factor B activity by the method described in the above <4>. Further, the activation of the protein having factor B activity can also be directly detected by utilizing a substrate (substrate for detection). That is, by utilizing a substrate that emits a signal by being cleaved by the factor B activity of the activated protein having factor B activity as the substrate for detection, the activation of the protein having factor B activity can be directly detected. Therefore, this method may further include a step of bringing the “protein having factor B activity having contacted with the recombinant first protein” into contact with the substrate for detection.

[0263] When the method further includes the step of bringing into contact with the protein having proclotting enzyme activity, the detection can be carried out by detecting the activation of the protein having proclotting enzyme activity by the method described in the above <5> (the cleavage of the substrate by the method described in the above <6>). Therefore, this method may further include a step of bringing the “protein having proclotting enzyme activity having contacted with the protein having factor B activity” into contact with the substrate (substrate for detection). Examples of the substrate for detection capable of detecting the activation of the protein having proclotting enzyme activity include those exemplified in the above <6>.

[0264] These steps may be carried out sequentially or simultaneously. For example, when these steps are carried out simultaneously, the specimen, the recombinant first protein (inactive form), the protein (inactive form) having factor B activity, the protein (inactive form) having proclotting enzyme activity, and the substrate for detection may be brought into contact with one another simultaneously in the same system.

[0265] The conditions for the contact of these are the same as the conditions described in the above <4> to <6>. For example, the contact of the recombinant first protein with the specimen is not particularly limited as long as the conditions allow the endotoxin in the specimen to be able to activate the recombinant first protein. As the conditions for the contact, for example, reaction conditions for a known lysate reagent can be adopted. For example, as a pH of a reaction solution, a pH of 5 to 10, preferably 7 to 8.5 can be adopted. As a reaction temperature, a temperature of 10° C. to 80° C., preferably 20° C. to 50° C., more preferably 30° C. to 40° C. can be adopted. As the reaction temperature, for example, 37° C. is exemplified. A reaction time is also not particularly limited, and may be appropriately set according to various conditions. The reaction time may be, for example, from 5 minutes to 2 hours, preferably from 15 to 90 minutes, more preferably from 30 to 40 minutes.

[0266] The specimen is also not particularly limited as long as it is a sample requiring detection of an endotoxin. Examples of the specimen include medical water, pharmaceutical preparations, infusions, blood preparations, medical devices, medical instruments, cosmetics, foods and drinks, environmental samples, biological components, natural proteins, recombinant proteins, nucleic acids, and saccharides. The specimen can be subjected to the detection of an endotoxin by mixing, dispersing, or dissolving the specimen itself or an extract thereof or a washing solution thereof in a reaction system.

[0267] The detection of an endotoxin may be qualitative detection or quantitative detection. The quantitative detection can be carried out, for example, as follows: a plurality of reference standards having different endotoxin amounts (concentrations) are used, a relationship between the amount (concentration) of the endotoxin and the detection level according to the type of the substrate (for example, the degree of "gelation" when coagulogen is used as the substrate, and the degree of a signal when a synthetic substrate is used) is associated in advance using, for example, a calibration curve or a relational formula, and the amount (concentration) of the endotoxin is calculated by conversion from the detection level when using the actual specimen.

<8> Endotoxin Detecting Agent

[0268] The endotoxin detecting agent of the present invention is an endotoxin detecting agent containing the recombinant first protein.

[0269] This detecting agent may further contain a protein having factor B activity such as the second protein. This protein having factor B activity may be the recombinant second protein.

[0270] This detecting agent may further contain a protein having proclotting enzyme activity such as the third protein. This protein having proclotting enzyme activity may be the recombinant third protein.

[0271] These proteins can be in the form of, for example, being present in a culture solution, a culture supernatant, a

cell homogenate extract, a mixture thereof, or the like as described in the above <1> to <3>. These proteins may be used by being purified to a desired extent or may be used as such without purification. For example, when these proteins are contained in a culture supernatant, the culture supernatant may be used while containing the culture medium components.

[0272] The respective amounts of the recombinant first protein, the protein having factor B activity, and the protein having proclotting enzyme activity in this detecting agent can be appropriately set according to the activity or the like of each protein, and, for example, as the final concentration of each protein in the reaction solution in a state of being mixed with the specimen, 15 to 30 µg/mL can be exemplified.

[0273] This detecting agent may further contain a substrate (substrate for detection).

[0274] This detecting agent can be produced by incorporating these materials as the constituent components. This detecting agent may further contain an additive such as an excipient, a stabilizing agent, or a buffer other than these. Further, the form of this detecting agent is also not particularly limited, and the agent can be formulated into an arbitrary form such as a solid form (for example, a lyophilized form) or a liquid form.

[0275] In this detecting agent, these materials may be collectively put in a one-unit container as the constituent components or may be put in separate containers.

[0276] Further, this detecting agent may further contain a buffer solution for dissolution, an endotoxin reference standard, a container for a reaction (for example, a tube or a microplate), or the like. This detecting agent also includes a form of a kit.

[0277] This detecting agent can be used in, for example, the method described in the above <7>.

<9> First cDNA and Utilization Thereof

(1) First cDNA

[0278] The first cDNA is a cDNA which is any of the following:

[0279] (A) a cDNA that contains the base sequence represented by SEQ ID NO: 1 or 3;

[0280] (B) a cDNA that contains a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 1 or 3, and encodes a protein having factor C activity;

[0281] (C) a cDNA that hybridizes to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 1 or 3 under stringent conditions, and encodes a protein having factor C activity;

[0282] (D) a cDNA that encodes a protein containing the amino acid sequence represented by SEQ ID NO: 2 or 4; and

[0283] (E) a cDNA that encodes a protein containing an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 2 or 4, and having factor C activity.

[0284] This cDNA was acquired for the first time in the world by the method disclosed in Examples of this description, and the full-length sequence structure thereof and the function of a protein encoded by the cDNA were elucidated for the first time. The function is "factor C activity".

[0285] Among the first cDNAs, the most preferred is the above-mentioned cDNA (A), but the cDNA may be a variant thereof as long as a protein encoded by the cDNA has factor C activity.

[0286] As the variant, specifically, the above-mentioned cDNAs (B) to (E) are preferred. The term “95% or more” in the above (B), (C), and (E) is preferably 96% or more, more preferably 97% or more, more preferably 98% or more, and more preferably 99% or more.

[0287] Further, to the meaning of the “stringent conditions” and the other explanation, the explanation of the above <1> can apply.

[0288] Further, the first cDNA may be a cDNA in which an arbitrary codon is replaced by a codon equivalent thereto. For example, a cDNA in which an arbitrary codon in the above-mentioned cDNA (A) is replaced by a codon equivalent thereto is included in the above (D).

[0289] The full-length base sequence of the first cDNA is disclosed by the present invention, and therefore, by using this sequence information, the first cDNA can be produced by a known DNA synthesis technique or a genetic engineering technique. The first cDNA was acquired for the first time by the method described in the below-mentioned Example, however, since the base sequence is disclosed in the present invention, the first cDNA can also be produced by a method other than the method described in Example.

(2) DNA Construct Containing First cDNA, Transformant, and Method for Producing Recombinant First Protein Using the Same

[0290] The present invention provides a DNA construct containing the first cDNA. As such an artificial DNA construct, for example, a vector can be exemplified. As the vector, a plasmid, a virus, or another known vector can be appropriately selected according to the purpose such as amplification, maintenance, or introduction of a recombinant DNA, library preparation, cloning, protein translation (protein expression), or the like. By introducing the first cDNA into such a vector or the like, the DNA construct containing the first cDNA can be produced.

[0291] Further, the present invention provides a cell containing the first cDNA. Such a cell may be, for example, a cell that is transformed with such a DNA construct so as to express the recombinant first protein. Further, the present invention provides a method for producing the recombinant first protein including a step of culturing such a cell.

[0292] To the method for transforming a cell (host cell) with the DNA construct and the method for producing the recombinant first protein by culturing the cell, the description of the above <1> (2) can apply.

<10> Second cDNA

(1) Second cDNA

[0293] The second cDNA is a cDNA which is any of the following:

[0294] (A) a cDNA that contains the base sequence represented by SEQ ID NO: 5, 7, 9, or 11;

[0295] (B) a cDNA that contains a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 5, 7, 9, or 11, and encodes a protein having factor B activity;

[0296] (C) a cDNA that hybridizes to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 5, 7, 9, or 11 under stringent conditions, and encodes a protein having factor B activity;

[0297] (D) a cDNA that encodes a protein containing the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12; and

[0298] (E) a cDNA that encodes a protein containing an amino acid sequence having a 95% or more identity with the

amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12, and having factor B activity.

[0299] To the other explanation, the explanation of the above <9> (1) can apply. That is, for example, “factor C” and “first cDNA” in the above <9> (1) may be replaced by “factor B” and “second cDNA”, respectively, and so on.

(2) DNA Construct Containing Second cDNA, Transformant, and Method for Producing Recombinant Second Protein Using the Same

[0300] The present invention provides a DNA construct containing the second cDNA. Further, the present invention provides a cell containing the second cDNA such as a cell that is transformed with such a DNA construct so as to express the recombinant second protein. Further, the present invention provides a method for producing the recombinant second protein including a step of culturing such a cell.

[0301] To the other explanation, the explanation of the above <9> (2) can apply.

<11> Third cDNA

(1) Third cDNA

[0302] The third cDNA is a third cDNA which is any of the following:

[0303] (A) a cDNA that contains the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21;

[0304] (B) a cDNA that contains a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21, and encodes a protein having proclotting enzyme activity;

[0305] (C) a cDNA that hybridizes to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 13, 15, 17, 19, or 21 under stringent conditions, and encodes a protein having proclotting enzyme activity;

[0306] (D) a cDNA that encodes a protein containing the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22; and

[0307] (E) a cDNA that encodes a protein containing an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 14, 16, 18, 20, or 22, and having proclotting enzyme activity.

[0308] To the other explanation, the explanation of the above <9> (1) can apply. That is, for example, “factor C” and “first cDNA” in the above <9> (1) may be replaced by “proclotting enzyme” and “third cDNA”, respectively, and so on.

(2) DNA Construct Containing Third cDNA, Transformant, and Method for Producing Recombinant Third Protein Using the Same

[0309] The present invention provides a DNA construct containing the third cDNA. Further, the present invention provides a cell containing the third cDNA such as a cell that is transformed with such a DNA construct so as to express the recombinant third protein. Further, the present invention provides a method for producing the recombinant third protein including a step of culturing such a cell.

[0310] To the other explanation, the explanation of the above <9> (2) can apply.

<12> Method for Producing Endotoxin Detecting Agent

[0311] The present invention also provides a method for producing an endotoxin detecting agent including a step of artificially expressing a protein using the first cDNA.

[0312] This method may further include a step of artificially expressing a protein using the second cDNA. In

addition, the method may further include a step of artificially expressing a protein using the third cDNA.

[0313] A method for artificially expressing a protein using such a cDNA is also not particularly limited, however, for example, the method described in the above <9> (2), <10> (2), or <11>(2) can be used.

[0314] The recombinant first protein is expressed by being artificially expressed using the first cDNA according to such a method. Similarly, the recombinant second protein is expressed by being artificially expressed using the second cDNA, and the recombinant third protein is expressed by being artificially expressed using the third cDNA.

[0315] The expressed protein can be in the form of, for example, being present in a culture solution, a culture supernatant, a cell homogenate extract, a mixture thereof, or the like as described in the above <1> to <3>. These proteins may be used by being purified to a desired extent or may be used as such without purification. For example, when these proteins are contained in a culture supernatant, the culture supernatant may be used while containing the culture medium components.

[0316] To the other explanation, the explanation of the above <8> can apply. Therefore, the method for producing an endotoxin detecting agent of the present invention also includes the method for producing a kit.

[0317] Incidentally, in the present invention, either one or both of the second protein (such as the recombinant second protein) and the third protein (such as the recombinant third protein) may be replaced by their equivalents derived from a horseshoe crab other than *Limulus polyphemus*. The same also apply to the second cDNA and the third cDNA. Examples of the horseshoe crab other than *Limulus polyphemus* include organisms belonging to the genus *Tachypleus* such as *Tachypleus tridentatus*, *Tachypleus gigas*, and *Tachypleus rotundicauda*.

[0318] That is, in place of the second protein (such as the recombinant second protein), factor B (such as recombinant factor B) derived from a horseshoe crab (such as *Tachypleus tridentatus*) other than *Limulus polyphemus*, in place of the third protein (such as the recombinant third protein), a proclotting enzyme (such as a recombinant proclotting enzyme) derived from the same organism, in place of the second cDNA, a factor B gene (such as a cDNA) derived from the same organism, and in place of the third cDNA, a proclotting enzyme gene (such as a cDNA) derived from the same organism may be used, respectively.

[0319] These proteins and genes may all be those containing an amino acid sequence or a base sequence actually found in a horseshoe crab other than *Limulus polyphemus*, or may be variants thereof. To the variants, the explanation of the variant in the above <1> to <11> can apply. Further, these proteins and genes may be or may not be derived from the same horseshoe crab other than *Limulus polyphemus*.

[0320] Then, as apparent also from the above-mentioned description, in the method for producing an endotoxin detecting agent of the present invention, an embodiment in which each of the first cDNA, the second cDNA, and the third cDNA (with respect to the second cDNA and the third cDNA, either one or both may be replaced by a gene derived from a horseshoe crab (such as *Tachypleus tridentatus*) other than *Limulus polyphemus*) is artificially introduced into a host cell other than a horseshoe crab, the respective recombinant proteins (three types in total) are expressed from these respective cDNAs, and then, the expressed respective

recombinant proteins are incorporated as the constituent components as described in the above <8> is also included.

EXAMPLES

[0321] Hereinafter, the present invention will be specifically described by way of Examples, however, these are merely examples of the present invention, and the scope of the present invention is not limited thereto.

(1) Synthesis of Primers

[0322] In order to attempt cloning of a protein having factor C activity in *Limulus polyphemus*, the following respective primers were synthesized.

<Primers Used in Cloning of Protein Having Factor C Activity>

[0323]

```
SK15-dT20: (SEQ ID NO: 24)
CTGCAGGAATCGATTTTTTTTTTTTTTTTTTTT

SK15-FC-S: (SEQ ID NO: 25)
ATCGATAAGCTTGATGATCTGGCTTGTGTGATGA

SK15-As: (SEQ ID NO: 26)
CTGCAGGAATCGAT

LFC-5'race: (SEQ ID NO: 27)
CTACACCAAGTTCCA

LFC-1st-S: (SEQ ID NO: 28)
GTAAACCATGTGACAACTGGAGGC

LFC-1st-As: (SEQ ID NO: 29)
AATAAGGCCTCCATCGATAGAAGTA

LFC-EcoRI-kozak-S: (SEQ ID NO: 30)
GGGGAATTCAAGCTTGCCACCACCATGGTACTAGCGTCGTTT

LFC-XhoI-stop-As: (SEQ ID NO: 31)
GGGCTCGAGTCAAATGAACTGCCGAATCCACGATA
```

[0324] Further, in order to attempt cloning of each of a protein having factor B activity and a protein having proclotting enzyme activity, the following respective primers were synthesized.

<Primers Used in Cloning of Protein Having Factor B Activity and Protein Having Proclotting Enzyme Activity>

[0325]

```
SK15-FB-S: (SEQ ID NO: 32)
ATCGATAAGCTTGATCACATGCAAGGAAAAGTTCT

SK15-FB-As: (SEQ ID NO: 33)
CTGCAGGAATTCGATCACTGTTTAAACAACTGAA
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-continued

SK15-PCE-S: (SEQ ID NO: 34)
 ATCGATAAGCTTGATAGACCAGAGTGGTCTTTCTG

SK15-As: (SEQ ID NO: 26)
 CTC CAGGAATTCGAT

(2) Preparation of Total RNA from Blood Cells of *Limulus polyphemus*

[0326] In the preparation of total RNA, PureLink (registered trademark) RNA Mini Kit and PureLink (registered trademark) DNase kit (Thermo Fisher Scientific) were used, and the basic operation was carried out according to the accompanying instructions. A TRIzol solution was added to 2.23 g of a blood cell pool of *Limulus polyphemus*, and the cells were homogenized. Thereafter, chloroform was added thereto, followed by centrifugation, whereby an aqueous layer was obtained. After ethanol was added to the obtained aqueous layer, the resulting mixture was allowed to pass through a silica membrane cartridge included in the kit, whereby a nucleic acid component was bound to the silica membrane. To this silica membrane, DNase I included in the kit was added so as to degrade the DNA, and after the cartridge was washed, an elution buffer was added thereto, whereby total RNA (1.3 mg) was collected.

(3) Acquisition of cDNA (First cDNA) of Protein Having Factor C Activity of *Limulus polyphemus*

(3-1) First Step

[0327] Reverse transcription was carried out using the total RNA collected in the above (2) as a template and SK15-d20 (SEQ ID NO: 24) as a primer, whereby a cDNA was obtained. This primer was designed so as to selectively bind to the poly(A) sequence of mRNA and further has an addition sequence that can be utilized in a polymerase chain reaction (PCR) on the outside thereof. In this reaction, SuperScript III Reverse Transcriptase (Thermo Fisher Scientific) was used as a reverse transcriptase, and the reaction conditions were set according to the accompanying instructions.

(3-2) Second Step

[0328] PCR was carried out using the obtained cDNA as a template, SK15-FC-S (SEQ ID NO: 25) as a sense primer, SK15-As (SEQ ID NO: 26) as an antisense primer, and Tks Gflex DNA polymerase (Takara Bio Inc.) as a polymerase.

[0329] When the PCR product was confirmed by agarose gel electrophoresis, a target DNA (LpFC1) almost could not be detected, and nonspecific DNA amplification was detected (A of FIG. 1). Therefore, the following step was attempted. Incidentally, in A of FIG. 1, the lane "1" is a size marker and the lane "2" is the PCR product. Further, the triangle mark indicates a portion where the presence of LpFC1 is expected.

(3-3) Third Step

[0330] The portion where the presence of LpFC1 was expected was cut out from the gel, and a DNA was extracted and purified. PCR was carried out using the obtained DNA as a template, SK15-FC-S (SEQ ID NO: 25) as a sense

primer, SK15-As (SEQ ID NO: 26) as an antisense primer, and Tks Gflex DNA polymerase (Takara Bio Inc.) as a polymerase.

[0331] As a result of subjecting the PCR product to agarose gel electrophoresis, surprisingly, the target DNA (LpFC1) was remarkably amplified (B of FIG. 1). This LpFC1 was cut out from the gel and extracted and purified. By doing this, the acquisition of a partial fragment (LpFC1) of a DNA that may encode the protein having factor C activity of *Limulus polyphemus* was achieved. Incidentally, in B of FIG. 1, the lane "1" is a size marker and the lane "2" is the PCR product. Further, the triangle mark indicates the position of LpFC1.

(3-4) Fourth Step

[0332] LpFC1 was mixed with pBlueScript II SK (+) treated with EcoRV enzyme and introduced into a vector by recombination using In-Fusion HD Enzyme premix (Takara Bio Inc.). The reaction product was introduced into *E. coli* DH5a competent cells, and the cells were applied to an ampicillin LB medium plate containing X-Gal and IPTG, and colony selection was carried out by blue white screening.

[0333] Thereafter, DNA amplification was carried out by colony PCR for a candidate colony. At this time, M13-20 Primer was used as a sense primer, M13 Reverse Primer was used as an antisense primer, and Tks Gflex DNA polymerase (Takara Bio Inc.) was used as a polymerase. After the obtained PCR product was subjected to agarose gel electrophoresis, a DNA (LpFC1) obtained by cutting out from the gel, extraction, and purification was used as a template, and the sequence thereof was confirmed by sequence PCR. By doing this, the DNA sequence of LpFC1 was identified.

(3-5) Fifth Step

[0334] The 5' end of synthetic DNA LFC-5race (SEQ ID NO: 27) prepared based on the sequence information of LpFC1 was phosphorylated with T4 Polynucleotide Kinase (Takara Bio Inc.) in the presence of ATP. A target DNA (LpFC2) was obtained by reverse transcription using the total RNA as a template and also using the obtained phosphorylated primer and SuperScript III Reverse Transcriptase (Thermo Fisher Scientific) as a reverse transcriptase. By doing this, a partial fragment (LpFC2) of a DNA that may encode an amino acid sequence containing a portion of the protein of *Limulus polyphemus* having factor C activity was obtained.

[0335] LpFC2 was subjected to a high-temperature treatment (95° C., 2 minutes) in the presence of RNaseA, followed by ethanol precipitation. Thereafter, the resulting material was treated with T4 RNA Ligase (New England Biolabs), and as a result, a concatenated DNA (LpFC3) in which LpFC2 is linked in series was obtained. By doing this, a partial fragment (LpFC3) of a DNA that may encode an amino acid sequence containing a portion of the protein of *Limulus polyphemus* having factor C activity was obtained.

[0336] PCR was carried out using LpFC3 as a template, LFC-1st-S (SEQ ID NO: 28) as a sense primer, LFC-1st-As (SEQ ID NO: 29) as an antisense primer, and Tks Gflex DNA polymerase (Takara Bio Inc.), whereby a target DNA (LpFC4) was amplified.

[0337] When the PCR product was confirmed by agarose gel electrophoresis, the target DNA (LpFC4) almost could

not be detected, and nonspecific DNA amplification was detected (A of FIG. 2). Therefore, the following step was attempted. Incidentally, in A of FIG. 2, the lane "1" is a size marker and the lane "2" is the PCR product. Further, the triangle mark indicates the position of LpFC4.

(3-6) Sixth Step

[0338] The portion where the presence of LpFC4 was expected was cut out from the gel, and a DNA was extracted and purified. PCR was carried out using the obtained DNA as a template, LFC-1st-S (SEQ ID NO: 28) as a sense primer, LFC-1st-As (SEQ ID NO: 29) as an antisense primer, and Tks Gflex DNA polymerase (Takara Bio Inc.) as a polymerase.

[0339] As a result of subjecting the PCR product to agarose gel electrophoresis, surprisingly, the target DNA (LpFC4) was remarkably amplified and detected at a very high concentration (B of FIG. 2). This LpFC4 was cut out from the gel and extracted and purified. By doing this, the acquisition of a partial fragment (LpFC4) of a DNA that may encode an amino acid sequence containing a portion of the protein having factor C activity of *Limulus polyphemus* was achieved. Incidentally, in B of FIG. 2, the lane "1" is a size marker and the lane "2" is the PCR product. Further, the triangle mark indicates a portion where the presence of LpFC4 is expected.

[0340] The sequence of LpFC4 was analyzed by sequence PCR using LpFC4 as a template. By doing this, the DNA sequence on the 5' side of the target protein was identified.

(3-7) Seventh Step

[0341] PCR was carried out using the cDNA obtained in the above (3-1) as a template, LFC-EcoRI-kozak-S (SEQ ID NO: 30) as a sense primer, LFC-XhoI-stop-As (SEQ ID NO: 31) as an antisense primer, and Tks Gflex DNA polymerase (Takara Bio Inc.) as a polymerase.

[0342] The PCR product was purified by phenol-chloroform extraction and ethanol precipitation, and then, treated with EcoRI enzyme and XhoI enzyme. A restriction enzyme-treated product was subjected to agarose gel electrophoresis and cut out from the gel, and a DNA was extracted and purified, whereby a target DNA (LpFC5) was obtained.

[0343] LpFC5 was mixed with an expression vector pCA7 (Takeda et al., 2005, J virol 79: 14346-14354) treated with EcoRI enzyme and XhoI enzyme, and a ligation reaction was carried out using Ligation Mix (Takara Bio Inc.). The reaction product was introduced into *E. coli* DH5a competent cells, and the cells were applied to an ampicillin-containing LB medium plate. From the obtained colonies, selection was carried out by colony PCR, and the selected colony was inoculated into an ampicillin-containing LB medium.

[0344] After culturing, plasmid purification was carried out by NucleoSpin (registered trademark) Plasmid Easy Pure (MACHEREY-NAGEL GmbH & Co. KG). The obtained two types of clones of plasmids were used as templates, and the base sequence of each clone was analyzed by sequence PCR.

[0345] By doing this, the elucidation of the full-length sequences (SEQ ID NO: 1 and SEQ ID NO: 3) of cDNAs encoding a protein that may have the factor C activity of *Limulus polyphemus*, and the full-length sequences (SEQ ID

NO: 2 and SEQ ID NO: 4) of amino acids of the protein was achieved for the first time. SEQ ID NOS: 1 and 3 (SEQ ID NOS: 2 and 4) have a variant relationship and are considered to be based on polymorphism between individuals. The expression and the analysis of activity of this protein will be described later.

(4) Acquisition of cDNA (Second cDNA) of Protein Having Factor B Activity of *Limulus polyphemus*

[0346] PCR was carried out using the cDNA obtained in the above (3-1) as a template, SK15-FB-S (SEQ ID NO: 32) as a sense primer, SK15-FB-As (SEQ ID NO: 33) as an antisense primer, and Tks Gflex DNA polymerase (Takara Bio Inc.) as a polymerase. The amplified fragments were subjected to cloning according to the same procedure as in the above (3-4), and with respect to the obtained four types of clones, the base sequences thereof were analyzed.

[0347] By doing this, the full-length sequences (SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11) of cDNAs encoding a protein that may have the factor B activity of *Limulus polyphemus*, and the full-length sequences (SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12) of amino acids of the protein were elucidated. SEQ ID NOS: 5, 7, 9, and 11 (SEQ ID NOS: 6, 8, 10, and 12) have a variant relationship and are considered to be based on polymorphism among individuals. The expression and the analysis of activity of this protein will be described later.

(5) Acquisition of cDNA (Third cDNA) of Protein Having Proclotting Enzyme Activity of *Limulus polyphemus*

[0348] PCR was carried out using the cDNA obtained in the above (3-1) as a template, SK15-PCE-S (SEQ ID NO: 34) as a sense primer, SK15-As (SEQ ID NO: 26) as an antisense primer, and Tks Gflex DNA polymerase (Takara Bio Inc.) as a polymerase. The amplified fragments were subjected to cloning according to the same procedure as in the above (3-4), and with respect to the obtained five types of clones, the base sequences thereof were analyzed.

[0349] By doing this, the full-length sequences (SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 21) of cDNAs encoding a protein that may have the proclotting enzyme activity of *Limulus polyphemus*, and the full-length sequences (SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, and SEQ ID NO: 22) of amino acids of the protein were elucidated. SEQ ID NOS: 13, 15, 17, 19, and 21 (SEQ ID NOS: 14, 16, 18, 20, and 22) have a variant relationship and are considered to be based on polymorphism among individuals. The expression and the analysis of activity of this protein will be described later.

(6) Expression of Recombinant Protein

[0350] The cDNA (SEQ ID NO: 13) obtained in the above (5) was ligated to a vector pCA7 for protein expression in mammalian cells, whereby an expression plasmid was obtained. The cDNA (SEQ ID NO: 5) obtained in the above (4) was ligated to the vector pCA7 for protein expression according to a literature (Kobayashi et al., 2015, J Biol Chem, 290: 19379-19386), whereby an expression plasmid was obtained. By using these expression plasmids and the expression plasmid (in which the cDNA of SEQ ID NO: 1 was integrated) obtained in the above (3-7), and ExpiCHO™ Expression System (Thermo Fisher Scientific), recombinant proteins thereof were obtained as culture supernatant fractions.

[0351] The transfection of the expression plasmids into cells and collection of the recombinant proteins were carried out according to the accompanying instructions.

[0352] By doing this, the expression and acquisition of the protein (SEQ ID NO: 2) that may have the factor C activity of *Limulus polyphemus*, the protein (SEQ ID NO: 6) that may have the factor B activity thereof, and the protein (SEQ ID NO: 14) that may have the proclotting enzyme activity thereof were achieved for the first time. The analysis of the activity of these proteins will be described below.

(7) Measurement of Activity

[0353] In the presence of 50 mM Tris buffer solution (pH 8.0) and a synthetic substrate (Boc-LGR-pNA), with respect to various combinations of the three types of recombinant proteins obtained in the above (6), activation of the three types of recombinant proteins depending on an endotoxin was examined using a United States Pharmacopeia reference standard endotoxin (USP-RSE, Seikagaku Corporation) (0 EU/mL and 0.05 EU/mL, EU is the endotoxin unit) as a specimen. This reaction was carried out at 37° C. for 30 minutes.

[0354] If a cascade reaction proceeds in this assay system, the protein having factor C activity is activated by the endotoxin, the protein having factor B activity is activated by this activated protein having factor C activity, the protein having proclotting enzyme activity is activated by this activated protein having factor B activity, and the synthetic substrate Boc-LGR-pNA is cleaved by this activated protein having proclotting enzyme activity, whereby pNA is released. The release of pNA was detected by measuring a change in the absorbance (A405 nm). As a result, when all the three types of recombinant proteins were included, cleavage of the synthetic substrate was observed (FIG. 3). Incidentally, in FIG. 3, FC denotes the protein expressed from the cDNA obtained in the above (3-7), FB denotes the protein expressed from the cDNA obtained in the above (4), and PCE denotes the protein expressed from the cDNA obtained in the above (5). The final concentrations of the proteins in the reaction solution (100 L) in a state of being mixed with the endotoxin specimen were as follows: FC: 22.8 µg/mL; FB: 20.0 µg/mL; and PCE: 23.5 µg/mL. Each assay was carried out by setting N=3, and a standard deviation was also shown in the drawing as an error bar.

[0355] From this, it was shown for the first time that the expressed protein that may have the factor C activity, the expressed protein that may have the factor B activity, and the expressed protein that may have the proclotting enzyme activity have the factor C activity, the factor B activity, and the proclotting enzyme activity, respectively, and the cascade reaction is caused by contact with an endotoxin. Further, it was shown for the first time that by utilizing these recombinant proteins, an endotoxin can be detected.

[0356] Further, when the concentration dependence of an endotoxin was examined under the conditions and a calibration curve was created, it showed favorable linearity within a concentration range from 0.001 to 0.1 EU/mL (FIG. 4). The assay at each endotoxin concentration was carried out by setting N=3, and a standard deviation was also shown in the drawing as an error bar.

[0357] From the above results, it was shown for the first time that by using the above-mentioned respective three

types of recombinant proteins, an endotoxin can also be quantitatively detected with high sensitivity and high accuracy.

(8) Comparison of Activity with Recombinant Factor C Derived from Genus *Tachypleus*

[0358] The activity was compared between recombinant factor C derived from *Limulus polyphemus* (hereinafter referred to as "LFC") and recombinant factor C derived from the genus *Tachypleus* (hereinafter referred to as "TFC").

[0359] LFC was obtained as a culture supernatant fraction by expressing LFC from the cDNA of SEQ ID NO: 1 in the same manner as in the above (6). TFC was obtained as a culture supernatant fraction by expressing TFC from a factor C gene of *Tachypleus tridentatus* (SEQ ID NO: 1 in WO 2014/092079) in the same manner as in the above (6) by the method described in Example 1. (2) in the same literature. Here, as a vector for protein expression, pCI-neo (Promega) was used for both.

[0360] With respect to LFC and TFC to be subjected to a test, Western blot was carried out using a factor C specific monoclonal antibody (2C12; Yoshiki Miura, et al., J. Biochem. 112: 476-481 (1992)). As a result of applying an equal volume of samples (3 µL each) and optically measuring the intensities of the bands, the intensity of LFC was 18036 and the intensity of TFC was 24516. That is, the relative amount (TFC/LFC) of the recombinant factor C in the equal volume of the samples was 1.4.

[0361] Subsequently, the activity of each recombinant factor C in a cascade reaction system (a system containing factor C, factor B, and a proclotting enzyme) was compared. As both the factor B and the proclotting enzyme, recombinant proteins derived from the genus *Tachypleus* were used. The recombinant proteins obtained as culture supernatant fractions by expressing the former from a factor B gene of *Tachypleus tridentatus* (SEQ ID NO: 5 in WO 2014/092079) and by expressing the latter from a proclotting enzyme gene of the same organism (SEQ ID NO: 7 in the same literature) using the method described in the same literature (Example 2. Preparation of Recombinant Factor B and Recombinant Proclotting Enzyme) were used.

[0362] By using each sample (the same volume for each) of the recombinant factor C (LFC or TFC), and the recombinant factor B (TFB) derived from *Tachypleus tridentatus* described above and the recombinant proclotting enzyme (TPCE) derived from the same organism, the activity was measured by the method described in the above (7). The conditions were set the same except for the recombinant factor C to be used.

[0363] The changes in the absorbance (mAbs/min) at the respective endotoxin concentrations of 0 EU/mL (blank) and 0.05 EU/mL in the respective cascade reaction systems were 0.53 and 14.27 in the system of LFC, and 0.51 and 9.15 in the system of TFC. That is, in this system, LFC showed the activity about 1.6 times (about 2.2 times when considering the difference in the protein amount) higher than TFC.

[0364] The disclosure of Japanese Patent Application No. 2016-204729 (filing date: Oct. 18, 2016) is hereby incorporated by reference herein in its entirety.

[0365] All publications, patent applications, and technical standards described herein are incorporated by reference herein to the same extent as if such individual publications,

patent applications, and technical standards were specifically and individually indicated to be incorporated by reference.

INDUSTRIAL APPLICABILITY

[0366] According to the present invention, all full-length recombinant proteins involved in the clotting mechanism of *Limulus polyphemus*, cDNAs encoding the same, and applications thereof can be provided. The present invention is particularly useful for detecting an endotoxin.

[Description of Sequence Listing]

[0367] SEQ ID NO: 1: base sequence of first cDNA
 [0368] SEQ ID NO: 2: amino acid sequence of first protein
 [0369] SEQ ID NO: 3: base sequence of first cDNA variant 1
 [0370] SEQ ID NO: 4: amino acid sequence of first protein variant 1
 [0371] SEQ ID NO: 5: base sequence of second cDNA
 [0372] SEQ ID NO: 6: amino acid sequence of second protein
 [0373] SEQ ID NO: 7: base sequence of second cDNA variant 1
 [0374] SEQ ID NO: 8: amino acid sequence of second protein variant 1
 [0375] SEQ ID NO: 9: base sequence of second cDNA variant 2

[0376] SEQ ID NO: 10: amino acid sequence of second protein variant 2
 [0377] SEQ ID NO: 11: base sequence of second cDNA-variant 3
 [0378] SEQ ID NO: 12: amino acid sequence of second protein variant 3
 [0379] SEQ ID NO: 13: base sequence of third cDNA
 [0380] SEQ ID NO: 14: amino acid sequence of third protein
 [0381] SEQ ID NO: 15: base sequence of third cDNA variant 1
 [0382] SEQ ID NO: 16: amino acid sequence of third protein variant 1
 [0383] SEQ ID NO: 17: base sequence of third cDNA variant 2
 [0384] SEQ ID NO: 18: amino acid sequence of third protein variant 2
 [0385] SEQ ID NO: 19: base sequence of third cDNA variant 3
 [0386] SEQ ID NO: 20: amino acid sequence of third protein variant 3
 [0387] SEQ ID NO: 21: base sequence of third cDNA variant 4
 [0388] SEQ ID NO: 22: amino acid sequence of third protein variant 4
 [0389] SEQ ID NO: 23: synthetic substrate peptide IEGR
 [0390] SEQ ID NO: 24 to 34: primers

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 34

<210> SEQ ID NO 1

<211> LENGTH: 3063

<212> TYPE: DNA

<213> ORGANISM: *Limulus polyphemus*

<400> SEQUENCE: 1

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aagtgtggag atccaggata cggtgttcaac gtccccgcga agcaatgtac gtacttctat      180
cgatggaggc cttattgtaa accatgtgac aaactggagg ctaaagatgt gtgtcccaag      240
tacaaacgat gtcaagagtg tagggctggt ctgcacagtt gtgtgagttg tccacctaac      300
aaatatggaa ctgtgtgtag cggtgagtg cagtgtgaaga atgggggtat ttgtgatcag      360
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ccacaaacta ttgactacag ctgttcacca ggcttcaagc ttaaggcgt ggcacgtatc      540
acctgtcttc caaatgggca tgggagtagc ttccaccca aatgtattcg agaattgtcc      600
atggtttcat ctctagaaca tggcaaagta aactctcta gtgccgatct gatagaagga      660
gctactttaa ggttctcatg ttagatgtccc tactacttga ttggtcaaga aacattaacc      720
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cctgaccttg accctgtaag ccatgctgaa caccaggtta aaattggcct agaacaaaaa      840
tatggtcaat ttctcaagg cactgaagta acctatacgt gtactggtaa ttacttcttg      900

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gatgcctgca	gtggagacag	tggaggacca	ttagtgtttg	ctgatgatc	ccgcaccgat	2940
aggcggtggg	tcctggaagg	gatcgtcagc	tggggcagcc	ccaatggatg	tggcaagtct	3000
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<211> LENGTH: 1020

<212> TYPE: PRT

<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 2

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Ala Gln Gln Met His Pro Val Gln Ser Arg Gly Val Asp Leu Gly Leu
 20          25          30

Cys Asp Asp Thr Arg Phe Glu Cys Lys Cys Gly Asp Pro Gly Tyr Val
 35          40          45

Phe Asn Val Pro Ala Lys Gln Cys Thr Tyr Phe Tyr Arg Trp Arg Pro
 50          55          60

Tyr Cys Lys Pro Cys Asp Lys Leu Glu Ala Lys Asp Val Cys Pro Lys
 65          70          75          80

Tyr Lys Arg Cys Gln Glu Cys Arg Ala Gly Leu Asp Ser Cys Val Ser
 85          90          95

Cys Pro Pro Asn Lys Tyr Gly Thr Trp Cys Ser Gly Glu Cys Gln Cys
100          105          110

Lys Asn Gly Gly Ile Cys Asp Gln Arg Thr Gly Ala Cys Thr Cys Arg
115          120          125

Asp Arg Tyr Glu Gly Val His Cys Glu Ile Leu Gln Gly Cys Pro Leu
130          135          140

Leu Gln Ser Asp Pro Gln Val Gln Glu Val Lys Asn Pro Pro Asn Asp
145          150          155          160

Pro Gln Thr Ile Asp Tyr Ser Cys Ser Pro Gly Phe Lys Leu Lys Gly
165          170          175

Val Ala Arg Ile Thr Cys Leu Pro Asn Gly Gln Trp Ser Ser Phe Pro
180          185          190

Pro Lys Cys Ile Arg Glu Cys Ser Met Val Ser Ser Leu Glu His Gly
195          200          205

Lys Val Asn Ser Pro Ser Ala Asp Leu Ile Glu Gly Ala Thr Leu Arg
210          215          220

Phe Ser Cys Asp Ser Pro Tyr Tyr Leu Ile Gly Gln Glu Thr Leu Thr
225          230          235          240

Cys Gln Gly Asn Gly Gln Trp Ser Gly Gln Ile Pro Gln Cys Gln Lys
245          250          255

Leu Val Phe Cys Pro Asp Leu Asp Pro Val Ser His Ala Glu His Gln
260          265          270

Val Lys Ile Gly Leu Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr
275          280          285

Glu Val Thr Tyr Thr Cys Thr Gly Asn Tyr Phe Leu Met Gly Leu Asp
290          295          300

Thr Leu Lys Cys Asn Pro Asp Gly Ser Trp Ser Gly Thr Gln Pro Ser
305          310          315          320

Cys Val Lys Val Ala Asp Arg Glu Val Asn Cys Asp Ser Lys Ala Val
325          330          335

Asp Phe Leu Asp Asp Val Gly Glu Pro Val Arg Ile His Cys Pro Ala
340          345          350

Gly Cys Ser Leu Thr Ala Gly Thr Val Trp Gly Thr Ala Ile Tyr His
355          360          365

Glu Leu Ser Ser Val Cys Arg Ala Ala Ile His Ala Gly Lys Val Pro

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370	375	380
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	405	410 415
Leu Ala Gln Ser Phe Arg Phe Asp Tyr Val Ser Ser Ser Thr Ala Gly		
	420	425 430
Arg Lys Ser Gly Cys Pro Asp Gly Trp Phe Glu Ile Glu Glu Asn Cys		
	435	440 445
Val Tyr Val Thr Ser Lys Gln Arg Ala Trp Glu Arg Ala Gln Gly Val		
	450	455 460
Cys Thr Asn Met Ala Ala Arg Leu Ala Val Leu Asp Lys Asp Val Ile		
465	470	475 480
Pro Ser Ser Leu Thr Glu Thr Leu Arg Gly Lys Gly Leu Ala Thr Thr		
	485	490 495
Trp Ile Gly Leu His Arg Leu Asp Ala Asp Asn His Phe Ile Trp Glu		
	500	505 510
Leu Met Asp Arg Ser Ser Val Ala Leu Asn Asp Ser Leu Thr Phe Trp		
	515	520 525
Ala Pro Gly Glu Pro Gly Asn Glu Thr Asn Cys Val Tyr Leu Asp Ile		
	530	535 540
Gln Asp Gln Leu Gln Pro Val Trp Lys Thr Lys Ser Cys Phe Gln Pro		
545	550	555 560
Ser Ser Phe Val Cys Met Met Asp Leu Ser Asp Lys Asn Lys Ala Lys		
	565	570 575
Cys Lys Asp Pro Gly Pro Leu Glu Asn Gly His Ala Lys Leu His Gly		
	580	585 590
Gln Ser Ile Asp Gly Phe Tyr Ala Gly Ser Ser Val Arg Tyr Ser Cys		
	595	600 605
Glu Val Leu His Tyr Leu Ser Gly Thr Glu Thr Val Ser Cys Thr Ser		
	610	615 620
Asn Gly Thr Trp Ser Ala Pro Lys Pro Arg Cys Ile Lys Val Ile Thr		
625	630	635 640
Cys Gln Thr Pro Pro Val Pro Ser Tyr Gly Ser Val Asp Ile Lys Pro		
	645	650 655
Pro Ser Arg Thr Asn Ser Ile Ser Arg Val Gly Ser Pro Phe Leu Arg		
	660	665 670
Leu Pro Arg Leu Pro Leu Pro Leu Ala Arg Ala Ala Gly Pro Pro Pro		
	675	680 685
Lys Pro Arg Ser Ala Pro Pro Ser Thr Val Asp Leu Ser Ser Lys Val		
	690	695 700
Lys Leu Pro Glu Gly His Tyr Arg Val Gly Ser Gln Ala Ile Tyr Thr		
705	710	715 720
Cys Glu Ser Arg Tyr Tyr Glu Leu Leu Gly Ser Gln Gly Arg Arg Cys		
	725	730 735
Asp Ser Asn Gly Lys Trp Ser Gly Arg Pro Ala Ser Cys Ile Pro Val		
	740	745 750
Cys Gly Arg Ser Asp Ser Pro Arg Ser Pro Phe Ile Val Asn Gly Asn		
	755	760 765
Ser Thr Glu Ile Gly Gln Trp Pro Trp Gln Ala Gly Ile Ser Arg Trp		
	770	775 780

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Leu Ala Asp His Asn Met Trp Phe Leu Gln Cys Gly Gly Ala Leu Leu
 785 790 795 800
 Asn Glu Lys Trp Ile Ile Thr Ala Ala His Cys Val Thr Tyr Ser Ala
 805 810 815
 Thr Ala Glu Ile Ile Asp Pro Ser Gln Phe Lys Phe Tyr Leu Gly Lys
 820 825 830
 Tyr Tyr Arg Asp Asp Ser Lys Asp Asp Asp Tyr Val Gln Val Arg Glu
 835 840 845
 Ala Ile Glu Ile His Val Asn Pro Asn Tyr Asp Pro Gly Asn Leu Asn
 850 855 860
 Phe Asp Ile Ala Leu Ile Gln Leu Lys Thr Ser Val Ala Leu Thr Thr
 865 870 875 880
 Arg Val Gln Pro Ile Cys Leu Pro Thr Asp Leu Thr Thr Arg Glu Asn
 885 890 895
 Leu Lys Glu Gly Ala Leu Ala Val Val Thr Gly Trp Gly Leu Asn Glu
 900 905 910
 Asn Asn Thr Tyr Ser Glu Met Ile Gln Gln Ala Val Leu Pro Val Val
 915 920 925
 Ala Ala Ser Thr Cys Glu Gln Gly Tyr Gln Asp Ser Gly Leu Pro Leu
 930 935 940
 Thr Val Thr Glu Asn Met Phe Cys Ala Gly Tyr Lys Gln Gly Arg Tyr
 945 950 955 960
 Asp Ala Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Phe Ala Asp Asp
 965 970 975
 Ser Arg Thr Asp Arg Arg Trp Val Leu Glu Gly Ile Val Ser Trp Gly
 980 985 990
 Ser Pro Asn Gly Cys Gly Lys Ser Asn Gln Tyr Gly Gly Phe Thr Lys
 995 1000 1005
 Val Asn Val Phe Leu Ser Trp Ile Arg Gln Phe Ile
 1010 1015 1020

<210> SEQ ID NO 3

<211> LENGTH: 3063

<212> TYPE: DNA

<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 3

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aagtgtggag atccaggata cgtgttcaac gtccccgcga agcaatgtac gtacttctat      180
cgatggaggc cttattgtaa accatgtgac aaactggagg ctaaagatgt gtgtcccaag      240
tacaaacgat gtcaagagtg tagggctggt ctcgacagtt gtgtgagttg tccacctaac      300
aaatatggaa cttggtgtag cggtgagtggt cagtgaaga atgggggtat ttgtgatcag      360
aggacaggag cttgtacatg tcgtgacaga tatgaagggtg tgcattgtga aatccttcaa      420
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ccacaaacta ttgactacag ctgttcacca ggcttcaagc ttaaaggcgt ggcacgtatc      540
acctgtcttc caaatgggca gtggagtagc tttccacca aatgtattcg agaatgttcc      600
atggtttcat ctctagaaca tggcaaagta aactctccta gtgccgatct gatagaagga      660

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tga 3063

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<210> SEQ ID NO 4
<211> LENGTH: 1020
<212> TYPE: PRT
<213> ORGANISM: Limulus polyphemus

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<400> SEQUENCE: 4

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20     25     30
Cys Asp Asp Thr Arg Phe Glu Cys Lys Cys Gly Asp Pro Gly Tyr Val
35     40     45
Phe Asn Val Pro Ala Lys Gln Cys Thr Tyr Phe Tyr Arg Trp Arg Pro
50     55     60
Tyr Cys Lys Pro Cys Asp Lys Leu Glu Ala Lys Asp Val Cys Pro Lys
65     70     75     80
Tyr Lys Arg Cys Gln Glu Cys Arg Ala Gly Leu Asp Ser Cys Val Ser
85     90     95
Cys Pro Pro Asn Lys Tyr Gly Thr Trp Cys Ser Gly Glu Cys Gln Cys
100    105    110
Lys Asn Gly Gly Ile Cys Asp Gln Arg Thr Gly Ala Cys Thr Cys Arg
115    120    125
Asp Arg Tyr Glu Gly Val His Cys Glu Ile Leu Gln Gly Cys Pro Leu
130    135    140
Leu Gln Ser Asp Pro Gln Val Gln Glu Val Lys Asn Pro Pro Asn Asp
145    150    155    160
Pro Gln Thr Ile Asp Tyr Ser Cys Ser Pro Gly Phe Lys Leu Lys Gly
165    170    175
Val Ala Arg Ile Thr Cys Leu Pro Asn Gly Gln Trp Ser Ser Phe Pro
180    185    190
Pro Lys Cys Ile Arg Glu Cys Ser Met Val Ser Ser Leu Glu His Gly
195    200    205
Lys Val Asn Ser Pro Ser Ala Asp Leu Ile Glu Gly Ala Thr Leu Arg
210    215    220
Phe Ser Cys Asp Ser Pro Tyr Tyr Leu Ile Gly Gln Glu Thr Leu Thr
225    230    235    240
Cys Gln Gly Asn Gly Gln Trp Ser Gly Gln Ile Pro Gln Cys Gln Lys
245    250    255
Leu Val Phe Cys Pro Asp Leu Asp Pro Ala Ser His Ala Glu His Gln
260    265    270
Val Lys Ile Gly Leu Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr
275    280    285
Glu Val Thr Tyr Thr Cys Thr Gly Asn Tyr Phe Leu Met Gly Leu Asp
290    295    300
Thr Leu Lys Cys Asn Pro Asp Gly Ser Trp Ser Gly Thr Gln Pro Ser
305    310    315    320
Cys Val Lys Val Ala Asp Arg Glu Val Asn Cys Asp Ser Lys Ala Val
325    330    335

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Asp	Phe	Leu	Asp	Val	Gly	Glu	Pro	Val	Arg	Ile	His	Cys	Pro	Ala
			340				345					350		
Gly	Cys	Ser	Leu	Thr	Ala	Gly	Thr	Val	Trp	Gly	Thr	Ala	Ile	Tyr
		355					360					365		His
Glu	Leu	Ser	Ser	Val	Cys	Arg	Ala	Ala	Ile	His	Ala	Gly	Lys	Val
		370				375					380			Pro
Asn	Ser	Gly	Gly	Ala	Val	His	Val	Val	Asn	Asn	Gly	Pro	Tyr	Ser
385					390					395				Asp
Phe	Leu	Ala	Ser	Asp	Leu	Asn	Gly	Ile	Lys	Ser	Asp	Glu	Leu	Lys
				405					410					415
Leu	Ala	Gln	Ser	Phe	Arg	Phe	Asp	Tyr	Val	Ser	Ser	Ser	Thr	Ala
				420				425					430	Gly
Arg	Lys	Ser	Gly	Cys	Pro	Asp	Gly	Trp	Phe	Glu	Ile	Glu	Glu	Asn
		435					440					445		Cys
Val	Tyr	Val	Thr	Ser	Lys	Gln	Arg	Ala	Trp	Glu	Arg	Ala	Gln	Gly
		450				455					460			Val
Cys	Thr	Asn	Met	Ala	Ala	Arg	Leu	Ala	Val	Leu	Asp	Lys	Asp	Ile
465					470					475				480
Pro	Ser	Ser	Leu	Thr	Glu	Thr	Leu	Arg	Gly	Lys	Gly	Leu	Ala	Thr
				485					490					495
Trp	Ile	Gly	Leu	His	Arg	Leu	Asp	Ala	Asp	Asn	His	Phe	Ile	Trp
			500					505					510	Glu
Leu	Met	Asp	Arg	Ser	Ser	Val	Ala	Leu	Asn	Asp	Ser	Leu	Thr	Phe
		515					520					525		Trp
Ala	Pro	Gly	Glu	Pro	Gly	Asn	Glu	Thr	Asn	Cys	Val	Tyr	Leu	Asp
		530				535					540			Ile
Gln	Asp	Gln	Leu	Gln	Pro	Val	Trp	Lys	Thr	Lys	Ser	Cys	Phe	Gln
545					550					555				Pro
Ser	Ser	Phe	Val	Cys	Met	Met	Asp	Leu	Ser	Asp	Lys	Asn	Lys	Ala
				565					570					575
Cys	Lys	Asp	Pro	Gly	Pro	Leu	Glu	Asn	Gly	His	Ala	Lys	Leu	His
			580					585					590	Gly
Gln	Ser	Ile	Asp	Gly	Phe	Tyr	Ala	Gly	Ser	Ser	Val	Arg	Tyr	Ser
		595					600					605		Cys
Glu	Val	Leu	His	Tyr	Leu	Ser	Gly	Thr	Glu	Thr	Val	Ser	Cys	Thr
		610				615					620			Ser
Asn	Gly	Thr	Trp	Ser	Ala	Pro	Lys	Pro	Arg	Cys	Ile	Lys	Val	Ile
625					630					635				Thr
Cys	Gln	Thr	Pro	Pro	Val	Pro	Ser	Tyr	Gly	Ser	Val	Asp	Ile	Lys
				645					650					655
Pro	Ser	Arg	Thr	Asn	Ser	Ile	Ser	Arg	Val	Gly	Ser	Pro	Phe	Leu
			660					665					670	Arg
Leu	Pro	Arg	Leu	Pro	Leu	Pro	Leu	Ala	Arg	Ala	Ala	Gly	Pro	Pro
			675				680					685		Pro
Lys	Pro	Arg	Ser	Ala	Pro	Pro	Ser	Thr	Val	Asp	Leu	Ser	Ser	Lys
		690				695					700			Val
Lys	Leu	Pro	Glu	Gly	His	Tyr								

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<210> SEQ ID NO 5
<211> LENGTH: 1203
<212> TYPE: DNA
<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 5

atggcggtgga tttgtgtgat aacgttgttt gctttggcct ctagtacgtt gagtaataaa      60
gttagtagag tggggatcat ctttcctaag acacagaacg ataataaaca gtgtacagca      120
aaagggtgat taaaagggtc ctgcaagtcc ctcacagact gtcctgctgt cttggetacg      180
ttgaaggata gtttcctctg cgtttgcctc tggaatggtc ggtttcagcc tattgtatgt      240
tgtcctgatg cagcagcacc aagtgtaac acaacagtta caactattgt cctacaaaa      300
gaaacaaaga ttccaagatt acatatacca ggttgtggaa aaagaaaagt aaatgtagat      360
attacaacta ttqqacqtto qqqgtcacca atacttcttc ccatatctac ttctcaaagt      420

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ttgaagggtg ggagaggaat cattgctgga ggtgtagaag ctaaaattgg cgcttggcct 480
tggatggcag ctgtttttgt gaaaaatttt ggcattggca gattccattg tgctggtagc 540
ataatcagta gcaagtacat tttgtctgct gcccacgctt tcctcattgg aggtcgaaaag 600
ctgaccccaa ctcgcttagc tgtcccgcta ggaggccact acgtaaagat gggtcagaaa 660
tatcatgtgg aagatgtgat tatccatcct gactacgtag aaaggagaaa ttacaatgat 720
attgctatca ttgtgttaaa agaggaaactg aattttactg atttggtcgg tccaatctgt 780
ctccctgacc cagaggcagt aacagattca ttaaaaggca gaagggtgac agtagctgga 840
tggggtgatc tggatttcgc cgggccacga agtcaagttc tgcgcgaggt tagtatcccc 900
gttgttccaa tcggtgactg taacaaagcc tatcagaagc tcaacacctt tgctcttaaa 960
aatgggataa cgaaaaagtt tatttgtgct ggattggaag aaggtgggaa agatgcttgt 1020
caaggcgatt ctggtggacc gttgatgcta gtgaacaata gtagttggat agtgacggga 1080
gtggtgtcgt tcggacacaa gtgtgccgag gagggatttc ctggtgtgta cacgcgtgta 1140
gtgagttacc tagagtggat cgcaagggtt acgaactcgt tagaccaggc agtcactaac 1200
tga 1203

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<210> SEQ ID NO 6
<211> LENGTH: 400
<212> TYPE: PRT
<213> ORGANISM: Limulus polyphemus

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<400> SEQUENCE: 6

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Met Ala Trp Ile Cys Val Ile Thr Leu Phe Ala Leu Ala Ser Ser Thr
1           5           10          15
Leu Ser Asn Lys Val Ser Arg Val Gly Ile Ile Phe Pro Lys Thr Gln
20          25          30
Asn Asp Asn Lys Gln Cys Thr Ala Lys Gly Gly Leu Lys Gly Ser Cys
35          40          45
Lys Ser Leu Thr Asp Cys Pro Ala Val Leu Ala Thr Leu Lys Asp Ser
50          55          60
Phe Pro Val Val Cys Ser Trp Asn Gly Arg Phe Gln Pro Ile Val Cys
65          70          75          80
Cys Pro Asp Ala Ala Ala Pro Ser Val Thr Thr Thr Val Thr Thr Ile
85          90          95
Val Pro Thr Lys Glu Thr Lys Ile Pro Arg Leu His Ile Pro Gly Cys
100         105         110
Gly Lys Arg Lys Val Asn Val Asp Ile Thr Thr Ile Gly Arg Ser Gly
115         120         125
Ser Pro Ile Leu Pro Pro Ile Ser Thr Ser Gln Asp Leu Lys Gly Gly
130         135         140
Arg Gly Ile Ile Ala Gly Gly Val Glu Ala Lys Ile Gly Ala Trp Pro
145         150         155         160
Trp Met Ala Ala Val Phe Val Lys Asn Phe Gly Ile Gly Arg Phe His
165         170         175
Cys Ala Gly Ser Ile Ile Ser Ser Lys Tyr Ile Leu Ser Ala Ala His
180         185         190
Ala Phe Leu Ile Gly Gly Arg Lys Leu Thr Pro Thr Arg Leu Ala Val
195         200         205
Arg Val Gly Gly His Tyr Val Lys Met Gly Gln Glu Tyr His Val Glu

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210	215	220
Asp Val Ile Ile His Pro Asp Tyr Val Glu Arg Glu Asn Tyr Asn Asp		
225	230	235 240
Ile Ala Ile Ile Val Leu Lys Glu Glu Leu Asn Phe Thr Asp Leu Val		
	245	250 255
Arg Pro Ile Cys Leu Pro Asp Pro Glu Ala Val Thr Asp Ser Leu Lys		
	260	265 270
Gly Arg Arg Val Thr Val Ala Gly Trp Gly Asp Leu Asp Phe Ala Gly		
	275	280 285
Pro Arg Ser Gln Val Leu Arg Glu Val Ser Ile Pro Val Val Pro Ile		
	290	295 300
Gly Asp Cys Asn Lys Ala Tyr Gln Lys Leu Asn Thr Leu Ala Leu Lys		
305	310	315 320
Asn Gly Ile Thr Lys Lys Phe Ile Cys Ala Gly Leu Glu Glu Gly Gly		
	325	330 335
Lys Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu Met Leu Val Asn		
	340	345 350
Asn Ser Ser Trp Ile Val Thr Gly Val Val Ser Phe Gly His Lys Cys		
	355	360 365
Ala Glu Glu Gly Phe Pro Gly Val Tyr Thr Arg Val Val Ser Tyr Leu		
	370	375 380
Glu Trp Ile Ala Lys Val Thr Asn Ser Leu Asp Gln Ala Val Thr Asn		
385	390	395 400

<210> SEQ ID NO 7

<211> LENGTH: 1203

<212> TYPE: DNA

<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 7

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atggcgtgga tttgtgtgat aacgttggtt gctttggctt ctagtacgtt gagtaataaa    60
gttagtagag tggggatcat ctttcctaag acacagaacg ataataaaca gtgtacagca    120
aaagtggtat taaaagggtc ctgcaagtcc ctcacagact gtcttctgtt cttggctacg    180
ttgaaggata gtttccttgt cgtttgctct tggaatggtc ggtttcagcc tattgtatgt    240
tgtcctgatg cagcagcacc aagtgttaacc acaacagtta caactattgt ccctacaaaa    300
gaaacaaaga ttccaagatt acatatacca ggttgtggaa aaagaaaagt aaatgtagat    360
attacaacta ttggacgttc ggggtcacca atacttcttc ccatatctac ttctcaagat    420
ttgaagggtg ggagaggaat cattgctgga ggtgtagaag ctaaaattgg cgccctggcct    480
tggatggcag ctgtttttgt gaaaaatttt ggcattggca gattocattg tgctggtagc    540
ataatcagta gcaagtacat tttgtctgct gccacgctt tcctcattgg aggtcgaaa    600
ctgaccccaa ctcgcttagc tgtccgcgta ggagccact acgtaaagat ggggtcaagaa    660
tatcatgtgg aagatgtgat tatccatcct gactacgtag aaaggagaaa ttacaatgat    720
attgctatca ttgtgttaaa agaggaaactg aattttactg atttggcccg tccaatctgt    780
ctccctgacc cagaggcagt aacagattca ttaaaaggca gaagggtgac agtagctgga    840
tggggtgatc tggatttcgc cgttcacaga agtcaagttc tgcgcgaggt tagtatcccc    900
gttgttccaa tcagtgattg taacaaagcc tatcagaatc tcaacaccct tgctcttaaa    960
aatgggataa cgaaaaagtt tattttgtgt ggattggaag aaggtgggaa agatgcttgt   1020

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caaggcgatt ctggtggacc gttgatgcta gtgaacaata gtagttggat agtgacggga 1080
gtggtgtcgt tcggacacaa gtgtgccgag gagggatttc ctggtgtgta cacgcgtgta 1140
gtgagttacc tagagtggat cgcgaagggt acgaactcgt tagaccaggc agtcactaac 1200
tga 1203

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<210> SEQ ID NO 8
<211> LENGTH: 400
<212> TYPE: PRT
<213> ORGANISM: Limulus polyphemus

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<400> SEQUENCE: 8

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Met Ala Trp Ile Cys Val Ile Thr Leu Phe Ala Leu Ala Ser Ser Thr
1      5      10      15
Leu Ser Asn Lys Val Ser Arg Val Gly Ile Ile Phe Pro Lys Thr Gln
20     25     30
Asn Asp Asn Lys Gln Cys Thr Ala Lys Gly Gly Leu Lys Gly Ser Cys
35     40     45
Lys Ser Leu Thr Asp Cys Pro Ala Val Leu Ala Thr Leu Lys Asp Ser
50     55     60
Phe Pro Val Val Cys Ser Trp Asn Gly Arg Phe Gln Pro Ile Val Cys
65     70     75     80
Cys Pro Asp Ala Ala Ala Pro Ser Val Thr Thr Val Thr Thr Ile
85     90     95
Val Pro Thr Lys Glu Thr Lys Ile Pro Arg Leu His Ile Pro Gly Cys
100    105    110
Gly Lys Arg Lys Val Asn Val Asp Ile Thr Thr Ile Gly Arg Ser Gly
115    120    125
Ser Pro Ile Leu Pro Pro Ile Ser Thr Ser Gln Asp Leu Lys Gly Gly
130    135    140
Arg Gly Ile Ile Ala Gly Gly Val Glu Ala Lys Ile Gly Ala Trp Pro
145    150    155    160
Trp Met Ala Ala Val Phe Val Lys Asn Phe Gly Ile Gly Arg Phe His
165    170    175
Cys Ala Gly Ser Ile Ile Ser Ser Lys Tyr Ile Leu Ser Ala Ala His
180    185    190
Ala Phe Leu Ile Gly Gly Arg Lys Leu Thr Pro Thr Arg Leu Ala Val
195    200    205
Arg Val Gly Gly His Tyr Val Lys Met Gly Gln Glu Tyr His Val Glu
210    215    220
Asp Val Ile Ile His Pro Asp Tyr Val Glu Arg Glu Asn Tyr Asn Asp
225    230    235    240
Ile Ala Ile Ile Val Leu Lys Glu Glu Leu Asn Phe Thr Asp Leu Val
245    250    255
Arg Pro Ile Cys Leu Pro Asp Pro Glu Ala Val Thr Asp Ser Leu Lys
260    265    270
Gly Arg Arg Val Thr Val Ala Gly Trp Gly Asp Leu Asp Phe Ala Gly
275    280    285
Pro Arg Ser Gln Val Leu Arg Glu Val Ser Ile Pro Val Val Pro Ile
290    295    300
Ser Asp Cys Asn Lys Ala Tyr Gln Asn Leu Asn Thr Leu Ala Leu Lys
305    310    315    320

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<210> SEQ ID NO 9
<211> LENGTH: 1203
<212> TYPE: DNA
<213> ORGANISM: Limulus polyphemus
```

<400> SEQUENCE: 9

atggcgctgga	tttgtgtgat	aacgttggtt	gctttggctt	ctagtacgtt	gagtaataaa	60
gttagtagag	tggggatcat	ctttcctaag	acacagaacg	ataataaaca	gtgtacagca	120
aaagggtgat	taaaagggtc	ctgcaagtcc	ctcacagact	gtcctgctgt	cttggctacg	180
ttgaaggata	gtttccctgt	cgtttgtctc	tggaatggtc	ggtttcagcc	tattgtatgt	240
tgtctctgatg	cagcagcacc	aagtgtaac	acaacagtta	caactattgt	cctacaaaa	300
gaaacaaaga	ttccaagatt	acatatacca	ggttgtggaa	aaagaaaagt	aaatgtagat	360
attacaacta	ttggacgttc	ggggtcacca	atacttctc	ccatatctac	ttctcaagat	420
ttgaagggtg	ggagaggaat	cattgctgga	ggtgtagaag	ctaaaattgg	cgcctggcct	480
tggatggcag	ctgtttttgt	gaaaaatttt	ggcattggca	gattccattg	tgtctgtagc	540
ataatcagta	gcaagtacat	tttgtctgct	gcccacgctt	tcctcattgg	aggtcgaaag	600
ctgaccccaa	ctcgcttagc	tgtccgcgta	ggaggccact	acgtaaagat	gggtcaagaa	660
tatcatgtgg	aagatgtgat	tatccatcct	gactacgtag	aaaggggagaa	ttacaatgat	720
attgctatca	tttgtgtaaa	agaggaactg	aattttactg	atttggctcg	tccaatctgt	780
ctccctgacc	cagaggcagt	aacagattca	ttaaaaggca	gaagggtgac	agtagctgga	840
tggggtgatc	tggatttcgc	cggtcacaga	agtcaagttc	tgcgcgaggt	tagtatcccc	900
gttgttccaa	tcagtgattg	taacaaagcc	tatcagaatc	tcaacaccc	tgtctttaa	960
aatgggataa	cgaacaagtt	tattttgtct	ggattggaag	aaggcgggaa	agatgcttgt	1020
caaggcgatt	ctggtggacc	gttgatgcta	gtgaacaata	gtagtggat	agtgacggga	1080
gtggtgtcgt	tcggacacaa	gtgtgccgag	gagggtatcc	ctggtgtgta	cacgcgtgta	1140
gtgagttacc	tagagtggat	cgcaagagtt	acgaactcgt	tagaccaggc	agtcactaac	1200
tga						1230

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<210> SEQ ID NO 10
<211> LENGTH: 400
<212> TYPE: PRT
<213> ORGANISM: Limulus polyphemus
```

<400> SEQUENCE: 10

Met Ala Trp Ile Cys Val Ile Thr Leu Phe Ala Leu Ala Ser Ser Thr
1 5 10 15

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<210> SEQ ID NO 11
<211> LENGTH: 1203
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<212> TYPE: DNA

<213> ORGANISM: *Limulus polyphemus*

<400> SEQUENCE: 11

```

atggcgtgga tttgtgtgat aacgttggtt gctttggctt ctagtacgtt gagtaataaa    60
gttagtagag tggggatcat ctttctaag acacagaacg ataataaaca gtgtacagca    120
aaaggtggat taaaagggtc ctgcaagtcc ctcacagact gtcctgctgt cttggctacg    180
ttgaaggata gtttccctgt cgtttgctct tggaatggtc ggtttcagcc tattgtatgt    240
tgtctgatg cagcagcacc aagtgttaacc acaacagtta caactattgt cctacaaaa    300
gaaacaaaga ttccaagatt acatatacca ggttgtggaa aaagaaaagt aaatgtagat    360
attacaacta ttggacgttc ggggtcacca atacttcctc ccatatctac ttctcaagat    420
ttgaaggggtg ggagaggaat cattgtgga ggtgtagaag ctaaaattgg cgctggcct    480
tggatggcag ctgtttttgt gaaaaatttt ggcattggca gatttcattg tgctggtagc    540
ataatcagta gcaagtacat tttgtctgct gccacgctt tcctcattgg aggtcgaaa    600
ctgaccccaa ctgccttagc tgtccgcgta ggaggccact acgtaaagat gggtaagaa    660
tatcatgtgg aagatgtgat tatccatcct gactacgtag aaaggagaa ttacaatgat    720
attgctatca ttgtgttaaa agaggaaactg aattttactg atttggctcg tccaatctgt    780
ctccctgacc cagaggcagt aacagattca ttaaaaggca gaagggtgac agtagctgga    840
tggggtgatc tggatttcgc cgtccacga agtcaagttc tgcgcgaggt tagtatcccc    900
gttgttccaa tcagtgtattg taacaaagcc tatcagaatc tcaacaccct tgctcttaaa    960
aatgggataa cgaacaagtt tattttgtgt ggattggaag aaggcgggaa agatgcttgt   1020
caaggcgatt ctggtggacc gttgatgcta gtgaacaata gtagttggat agtgacggga   1080
gtggtgtcgt tcggacacaa gtgtgcccag gagggatttc ctggtgtgta cagcgtgta   1140
gtgagttacc tagagtggat cgcgaagggt acgaactcgt tagaccaggc agtcactaag   1200
tga                                                    1203

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<210> SEQ ID NO 12

<211> LENGTH: 400

<212> TYPE: PRT

<213> ORGANISM: *Limulus polyphemus*

<400> SEQUENCE: 12

```

Met Ala Trp Ile Cys Val Ile Thr Leu Phe Ala Leu Ala Ser Ser Thr
 1             5             10             15
Leu Ser Asn Lys Val Ser Arg Val Gly Ile Ile Phe Pro Lys Thr Gln
          20             25             30
Asn Asp Asn Lys Gln Cys Thr Ala Lys Gly Gly Leu Lys Gly Ser Cys
          35             40             45
Lys Ser Leu Thr Asp Cys Pro Ala Val Leu Ala Thr Leu Lys Asp Ser
          50             55             60
Phe Pro Val Val Cys Ser Trp Asn Gly Arg Phe Gln Pro Ile Val Cys
65             70             75             80
Cys Pro Asp Ala Ala Ala Pro Ser Val Thr Thr Thr Val Thr Thr Ile
          85             90             95
Val Pro Thr Lys Glu Thr Lys Ile Pro Arg Leu His Ile Pro Gly Cys
100             105             110

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Gly	Lys	Arg	Lys	Val	Asn	Val	Asp	Ile	Thr	Thr	Ile	Gly	Arg	Ser	Gly
		115					120					125			
Ser	Pro	Ile	Leu	Pro	Pro	Ile	Ser	Thr	Ser	Gln	Asp	Leu	Lys	Gly	Gly
	130					135					140				
Arg	Gly	Ile	Ile	Ala	Gly	Gly	Val	Glu	Ala	Lys	Ile	Gly	Ala	Trp	Pro
145					150					155					160
Trp	Met	Ala	Ala	Val	Phe	Val	Lys	Asn	Phe	Gly	Ile	Gly	Arg	Phe	His
				165					170					175	
Cys	Ala	Gly	Ser	Ile	Ile	Ser	Ser	Lys	Tyr	Ile	Leu	Ser	Ala	Ala	His
			180					185					190		
Ala	Phe	Leu	Ile	Gly	Gly	Arg	Lys	Leu	Thr	Pro	Thr	Arg	Leu	Ala	Val
		195					200					205			
Arg	Val	Gly	Gly	His	Tyr	Val	Lys	Met	Gly	Gln	Glu	Tyr	His	Val	Glu
	210					215					220				
Asp	Val	Ile	Ile	His	Pro	Asp	Tyr	Val	Glu	Arg	Glu	Asn	Tyr	Asn	Asp
225					230					235					240
Ile	Ala	Ile	Ile	Val	Leu	Lys	Glu	Glu	Leu	Asn	Phe	Thr	Asp	Leu	Val
				245					250					255	
Arg	Pro	Ile	Cys	Leu	Pro	Asp	Pro	Glu	Ala	Val	Thr	Asp	Ser	Leu	Lys
			260					265					270		
Gly	Arg	Arg	Val	Thr	Val	Ala	Gly	Trp	Gly	Asp	Leu	Asp	Phe	Ala	Gly
		275					280					285			
Pro	Arg	Ser	Gln	Val	Leu	Arg	Glu	Val	Ser	Ile	Pro	Val	Val	Pro	Ile
	290					295					300				
Ser	Asp	Cys	Asn	Lys	Ala	Tyr	Gln	Asn	Leu	Asn	Thr	Leu	Ala	Leu	Lys
305				310						315					320
Asn	Gly	Ile	Thr	Asn	Lys	Phe	Ile	Cys	Ala	Gly	Leu	Glu	Glu	Gly	Gly
				325					330					335	
Lys	Asp	Ala	Cys	Gln	Gly	Asp	Ser	Gly	Gly	Pro	Leu	Met	Leu	Val	Asn
		340						345					350		
Asn	Ser	Ser	Trp	Ile	Val	Thr	Gly	Val	Val	Ser	Phe	Gly	His	Lys	Cys
		355					360					365			
Ala	Glu	Glu	Gly	Phe	Pro	Gly	Val	Tyr	Thr	Arg	Val	Val	Ser	Tyr	Leu
	370					375					380				
Glu	Trp	Ile	Ala	Lys	Val	Thr	Asn	Ser	Leu	Asp	Gln	Ala	Val	Thr	Lys
385				390						395					400

<210> SEQ ID NO 13

<211> LENGTH: 1128

<212> TYPE: DNA

<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 13

atgttggtga ataacatggt ttcattgctg tgtttccac tcctgatgct tatgttttagc	60
tgcagtagtc tcggcagaca gcgtagacag tttgttttcc ccgatgatga agaatacatgc	120
tcaaaccgat ttactaacga tggaatatgt aaagatgttt tgaattgtag agatctttta	180
caaaaaaatg attataatct actgaaagaa tcaatatgctg gttttgaagg cataacaccc	240
aaagtttgtt gtccgaaaca aagtattgta aatccaataa cagaagcacc tccaaaaacc	300
actacaactg aacgaccgcc aatacggata ccatccaatc ttcctaaaca gtgtggaaat	360
cgtaatatca caactaccag gattattgga gggcaggaag caacacctgg agcctggccc	420

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tggatggctg ctgtctatat caaacaagga ggaatcagaa gtgttcagtg tggaggtgcg	480
cttgtcacca acaggcacgt gattacagca tcgcactgtg ttgtaaacag tttaggaaca	540
gatgtgatgc gagctgacgt attctcggtt cgcctaggtg aacacaattt atatagcacc	600
aatgacagtt cagatccaat tgattttgca gttacgtcag tgaaacatca tgaaaacttt	660
gtgctcgcga cgtatttgaa tgatatcgca attctgaagt taaacgacac tgttacgttt	720
acgcacaaaa ttaaaccaat ttgtctacct tatgaaagct taaggatatga ggatctagca	780
atgagaaaacc catttgctgc cggatgggga acaacagcat ttaatggccc atctagtga	840
gtattacgag aagtgcagtt accaatatgg ggacacgagc cctgcaggca ggctacgag	900
aaggatttaa atattacaaa cgtgtatatg tgtgctgggt atgcagatgg cggtaaagat	960
gcttgccagg gtgattctgg aggtccaatg atgttgctg ataaaagcgg gaacttttat	1020
ctcgttgcaa ttgtgtcttt cggaaagaaa tgcgcgttgc ctggatttcc tggggtttac	1080
acaaaagtga ccgaattttt agattggatt gcagtaaata tgggtgtag	1128

<210> SEQ ID NO 14

<211> LENGTH: 375

<212> TYPE: PRT

<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 14

Met Leu Val Asn Asn Met Phe Ser Leu Leu Cys Phe Pro Leu Leu Met	
1 5 10 15	
Ser Met Phe Ser Cys Ser Ser Leu Gly Arg Gln Arg Arg Gln Phe Val	
20 25 30	
Phe Pro Asp Asp Glu Glu Ser Cys Ser Asn Arg Phe Thr Asn Asp Gly	
35 40 45	
Ile Cys Lys Asp Val Leu Asn Cys Arg Asp Leu Leu Gln Lys Asn Asp	
50 55 60	
Tyr Asn Leu Leu Lys Glu Ser Ile Cys Gly Phe Glu Gly Ile Thr Pro	
65 70 75 80	
Lys Val Cys Cys Pro Lys Gln Ser Ile Val Asn Pro Ile Thr Glu Ala	
85 90 95	
Pro Pro Lys Thr Thr Thr Thr Glu Arg Pro Pro Ile Arg Ile Pro Ser	
100 105 110	
Asn Leu Pro Lys Gln Cys Gly Asn Arg Asn Ile Thr Thr Thr Arg Ile	
115 120 125	
Ile Gly Gly Gln Glu Ala Thr Pro Gly Ala Trp Pro Trp Met Ala Ala	
130 135 140	
Val Tyr Ile Lys Gln Gly Gly Ile Arg Ser Val Gln Cys Gly Gly Ala	
145 150 155 160	
Leu Val Thr Asn Arg His Val Ile Thr Ala Ser His Cys Val Val Asn	
165 170 175	
Ser Leu Gly Thr Asp Val Met Arg Ala Asp Val Phe Ser Val Arg Leu	
180 185 190	
Gly Glu His Asn Leu Tyr Ser Thr Asn Asp Ser Ser Asp Pro Ile Asp	
195 200 205	
Phe Ala Val Thr Ser Val Lys His His Glu Asn Phe Val Leu Ala Thr	
210 215 220	
Tyr Leu Asn Asp Ile Ala Ile Leu Lys Leu Asn Asp Thr Val Thr Phe	
225 230 235 240	

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<210> SEQ ID NO 15
<211> LENGTH: 1128
<212> TYPE: DNA
<213> ORGANISM: Limulus polyphemus
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atggttggtga	ataacatggt	ttcattgctg	tgtttccac	tctgatgtc	tatgtttagc	60
tgcagtagtc	tccgcagaca	gcgtagacag	tttgttttcc	ccgatgatga	agaatcatgc	120
tcaaacccgat	ttactaacga	tggaaatagt	aaagatgttt	tgaattgtag	agatctttta	180
caaaaaaatg	attataattt	actgaaagaa	tcaatatgcg	gttttgaagg	cataacaccc	240
aaagtttggt	gtccgaaaca	aagtattgta	aatccaataa	cagaagcacc	tccaaaaacc	300
actacaactg	aacgaccgcc	aatacggata	ccatccaatc	ttcctaaaca	gtgtggaaat	360
cgtaatat	caactaccag	gattattgga	gggcaggaag	caacacctgg	agcctggccc	420
tggatggctg	ctgtctatat	caacaagga	ggaatcagaa	gtgctcagt	tggaggtgcg	480
cttgtcacca	acaggcacgt	gattacagca	tgcactgtg	ttgtaaacag	tttaggaaca	540
gatgtgatgc	gagctgatgt	attctcggtt	cgcctaggtg	aacacaattt	atatagcacc	600
aatgacagtt	cagatccaat	tgattttgca	gttacgtcag	tgaaacatca	tgaaaacttt	660
gtgctcgoga	cgtatttgaa	tgatatcgca	attctgaagt	taaacgacac	tgttacgttt	720
acgcacaaaa	ttaaaccaat	tgtgtacct	tatgaaagct	taaggtagta	ggatctagca	780
atgagaaacc	catttgtcgc	cggatgggga	acaacagcat	ttaatggccc	atctagtgc	840
gtattacgag	aagtgcagtt	accaatatgg	ggacacgagc	cctgcaggca	ggcctacgag	900
aaggatttaa	atattacaaa	cgtgtatatg	tgtgctgggt	atgcagatgg	cggtaaagat	960
gcttgccagg	gtgattctgg	agggtccaat	atgttgccctg	ataaaagcgg	gaacttttat	1020
ctcgttggaa	ttgtgtcttt	cggaaagaaa	tgcgcgttgc	ctggatttcc	tggggtttac	1080
acaaaagtga	ccgaattttt	agattggaat	gcagtaataa	tgggtgaag		1128

<210> SEO ID NO 16

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<211> LENGTH: 375
<212> TYPE: PRT
<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 16
Met Leu Val Asn Asn Met Phe Ser Leu Leu Cys Phe Pro Leu Leu Met
1          5          10          15
Ser Met Phe Ser Cys Ser Ser Leu Gly Arg Gln Arg Arg Gln Phe Val
20          25          30
Phe Pro Asp Asp Glu Glu Ser Cys Ser Asn Arg Phe Thr Asn Asp Gly
35          40          45
Ile Cys Lys Asp Val Leu Asn Cys Arg Asp Leu Leu Gln Lys Asn Asp
50          55          60
Tyr Asn Leu Leu Lys Glu Ser Ile Cys Gly Phe Glu Gly Ile Thr Pro
65          70          75          80
Lys Val Cys Cys Pro Lys Gln Ser Ile Val Asn Pro Ile Thr Glu Ala
85          90          95
Pro Pro Lys Thr Thr Thr Thr Glu Arg Pro Pro Ile Arg Ile Pro Ser
100         105         110
Asn Leu Pro Lys Gln Cys Gly Asn Arg Asn Ile Thr Thr Thr Arg Ile
115         120         125
Ile Gly Gly Gln Glu Ala Thr Pro Gly Ala Trp Pro Trp Met Ala Ala
130         135         140
Val Tyr Ile Lys Gln Gly Gly Ile Arg Ser Ala Gln Cys Gly Gly Ala
145         150         155         160
Leu Val Thr Asn Arg His Val Ile Thr Ala Ser His Cys Val Val Asn
165         170         175
Ser Leu Gly Thr Asp Val Met Arg Ala Asp Val Phe Ser Val Arg Leu
180         185         190
Gly Glu His Asn Leu Tyr Ser Thr Asn Asp Ser Ser Asp Pro Ile Asp
195         200         205
Phe Ala Val Thr Ser Val Lys His His Glu Asn Phe Val Leu Ala Thr
210         215         220
Tyr Leu Asn Asp Ile Ala Ile Leu Lys Leu Asn Asp Thr Val Thr Phe
225         230         235         240
Thr His Lys Ile Lys Pro Ile Cys Leu Pro Tyr Glu Ser Leu Arg Tyr
245         250         255
Glu Asp Leu Ala Met Arg Asn Pro Phe Val Ala Gly Trp Gly Thr Thr
260         265         270
Ala Phe Asn Gly Pro Ser Ser Ala Val Leu Arg Glu Val Gln Leu Pro
275         280         285
Ile Trp Gly His Glu Pro Cys Arg Gln Ala Tyr Glu Lys Asp Leu Asn
290         295         300
Ile Thr Asn Val Tyr Met Cys Ala Gly Tyr Ala Asp Gly Gly Lys Asp
305         310         315         320
Ala Cys Gln Gly Asp Ser Gly Gly Pro Met Met Leu Pro Asp Lys Ser
325         330         335
Gly Asn Phe Tyr Leu Val Gly Ile Val Ser Phe Gly Lys Lys Cys Ala
340         345         350
Leu Pro Gly Phe Pro Gly Val Tyr Thr Lys Val Thr Glu Phe Leu Asp
355         360         365
Trp Ile Ala Val Asn Met Val

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370                               375

<210> SEQ ID NO 17
<211> LENGTH: 1128
<212> TYPE: DNA
<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 17

atgttggtga ataacatggt ttcattgctg tgtttccac tcctgatgtc tatgttttagc   60
tgcagtagtc tcggcagaca gcgtagacaa tttgttttcc ccgatgatga agaatacatgc   120
tcaaacccgat ttactaacga aggaatatgt aaagatgttt tgaattgtag agaactttta   180
caaaaaaatg attataatgt actgaaagaa tcaatatgag gttttgaagg cataacaccc   240
aaagtttggt gtccgaaaca aagtattgta aatccaataa cagaagcacc tccaaaaact   300
actacaactg aacgaccgcc aatccgtata ccatccaatc ttcctaaaca gtgtggaat   360
cgtaatatta caactaccag gattattgga gggcaggaag caacacctgg agcctggccc   420
tggatggctg ctgtctatat caaacaagga ggaatcagaa gtgttcagtg tggaggtgag   480
cttgtcacca acaggcacgt gattacagca tcgcactgtg ttgtaaacag tttaggaaca   540
gatgtgatgc gagctgacgt attctcggtt cgcctaggtg aacacaatgt atatagcacc   600
aatgacagtt cagatccaat tgattttgca gttacgtcag tgaaacatca tgaaaacttt   660
gtgctcgcga cgtatttgaa tgatatcgca attctgaagt taaacgacac tgttacgttt   720
acgcacaaaa ttaaaccaat ttgtctacct tatgaaagct taagggtatga ggatctagca   780
atgagaaacc catttgctgc cggatgggga acaacagcat ttaatggccc atctagtga   840
gtattacgag aagtgcagtt accaatatgg ggacacgagc cctgcaggca ggcctacgag   900
aaggatttaa atattacaaa cgtgtatatg tgtgctgggt atgcagatgg cggtaaagat   960
gcttgccagg gtgattctgg aggtccaatg atgttgctg ataaaagcgg gaacttttat   1020
ctcgttgaa ttgtgtcttt cggaaagaaa tgcgcgttgc ctggatttcc tggggtttac   1080
acaaaagtga ccgaattttt agattggatt gcagtaaata tgggtgtag   1128

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<210> SEQ ID NO 18
<211> LENGTH: 375
<212> TYPE: PRT
<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 18

Met Leu Val Asn Asn Met Phe Ser Leu Leu Cys Phe Pro Leu Leu Met
1      5      10      15
Ser Met Phe Ser Cys Ser Ser Leu Gly Arg Gln Arg Arg Gln Phe Val
20     25     30
Phe Pro Asp Asp Glu Glu Ser Cys Ser Asn Arg Phe Thr Asn Glu Gly
35     40     45
Ile Cys Lys Asp Val Leu Asn Cys Arg Glu Leu Leu Gln Lys Asn Asp
50     55     60
Tyr Asn Leu Leu Lys Glu Ser Ile Cys Gly Phe Glu Gly Ile Thr Pro
65     70     75     80
Lys Val Cys Cys Pro Lys Gln Ser Ile Val Asn Pro Ile Thr Glu Ala
85     90     95
Pro Pro Lys Thr Thr Thr Thr Glu Arg Pro Pro Ile Arg Ile Pro Ser
100    105    110

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Asn	Leu	Pro	Lys	Gln	Cys	Gly	Asn	Arg	Asn	Ile	Thr	Thr	Thr	Arg	Ile
	115						120					125			
Ile	Gly	Gly	Gln	Glu	Ala	Thr	Pro	Gly	Ala	Trp	Pro	Trp	Met	Ala	Ala
	130					135					140				
Val	Tyr	Ile	Lys	Gln	Gly	Gly	Ile	Arg	Ser	Val	Gln	Cys	Gly	Gly	Ala
	145				150				155						160
Leu	Val	Thr	Asn	Arg	His	Val	Ile	Thr	Ala	Ser	His	Cys	Val	Val	Asn
			165						170						175
Ser	Leu	Gly	Thr	Asp	Val	Met	Arg	Ala	Asp	Val	Phe	Ser	Val	Arg	Leu
			180					185					190		
Gly	Glu	His	Asn	Leu	Tyr	Ser	Thr	Asn	Asp	Ser	Ser	Asp	Pro	Ile	Asp
	195						200					205			
Phe	Ala	Val	Thr	Ser	Val	Lys	His	His	Glu	Asn	Phe	Val	Leu	Ala	Thr
	210					215					220				
Tyr	Leu	Asn	Asp	Ile	Ala	Ile	Leu	Lys	Leu	Asn	Asp	Thr	Val	Thr	Phe
	225				230					235					240
Thr	His	Lys	Ile	Lys	Pro	Ile	Cys	Leu	Pro	Tyr	Glu	Ser	Leu	Arg	Tyr
			245						250					255	
Glu	Asp	Leu	Ala	Met	Arg	Asn	Pro	Phe	Val	Ala	Gly	Trp	Gly	Thr	Thr
		260						265					270		
Ala	Phe	Asn	Gly	Pro	Ser	Ser	Ala	Val	Leu	Arg	Glu	Val	Gln	Leu	Pro
	275						280					285			
Ile	Trp	Gly	His	Glu	Pro	Cys	Arg	Gln	Ala	Tyr	Glu	Lys	Asp	Leu	Asn
	290					295					300				
Ile	Thr	Asn	Val	Tyr	Met	Cys	Ala	Gly	Tyr	Ala	Asp	Gly	Gly	Lys	Asp
	305				310					315				320	
Ala	Cys	Gln	Gly	Asp	Ser	Gly	Gly	Pro	Met	Met	Leu	Pro	Asp	Lys	Ser
			325					330						335	
Gly	Asn	Phe	Tyr	Leu	Val	Gly	Ile	Val	Ser	Phe	Gly	Lys	Lys	Cys	Ala
		340					345						350		
Leu	Pro	Gly	Phe	Pro	Gly	Val	Tyr	Thr	Lys	Val	Thr	Glu	Phe	Leu	Asp
		355				360						365			
Trp	Ile	Ala	Val	Asn	Met	Val									
	370					375									

<210> SEQ ID NO 19

<211> LENGTH: 1128

<212> TYPE: DNA

<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 19

atgttggtga ataacatggt ttcattgctg tgtttccac tcctgatgtc tatgtttagc	60
tgcagtagtc tcggcagaca gcgtagacaa tttgttttcc ccgatgatga agaatacatgc	120
tcaaaccgat ttactaacga tggaatatgt aaagatgttt tgaattgtag agatctttta	180
caaaaaaatg attataatgt actgaaagaa tcaatatgag gttttgaagg cataaacacc	240
aaagtgtgtt gtccgaaaca aagtattgta aatccaataa cagaagcacc tccaaaaaat	300
actacaactg aacgaccgcc aatccgtata ccatccaatc ttcctaaaca gtgtggaaat	360
cgtaatatga caactaccag gattattgga gggcaggaag caacacctgg agcctggccc	420
tggatggctg ctgtctatat caaacaagga ggaatcagaa gtgttcagtg tggaggtgag	480

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cttgtcacca acaggcacgt gattacagca tcgcactgtg ttgtaaacag ttaggaaca 540
gatgtgatgc gagctgacgt attctcgggt cgcctagggt aacacaattt atatagcacc 600
aatgacagtt cagatccaat tgattttgca gttacgtcag tgaaacatca tgaaaacttt 660
gtgctcgcga cgtatttgaa tgatategca attctgaagt taaacgacac tgttacgttt 720
acgcacaaaa ttaaccaat ttgtctacct tatgaaagct taaggatga ggatctagca 780
atgagaaaacc catttgtgcg cggatgggga acaacagcat ttaatggccc atctagtga 840
gtattacgag aagtgcagtt accaatatgg ggacacgagc cctgcaggca ggcctacgag 900
aaggatttaa atattacaaa cgtgtatatg tgtgctgggt atgcagatgg cggtaaagat 960
gcttgccagg gtgattctgg aggtccaatg atgttgcttg ataaaagcgg gaacttttat 1020
ctcggttgaa ttgtgtcttt cggaagaaa tgcgcgttgc ctggatttcc tggggtttac 1080
acaaaagtga ccgaattttt agattggatt gcagtaaata tgggtgtag 1128

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<210> SEQ ID NO 20

<211> LENGTH: 375

<212> TYPE: PRT

<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 20

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Met Leu Val Asn Asn Met Phe Ser Leu Leu Cys Phe Pro Leu Leu Met
1           5           10          15
Ser Met Phe Ser Cys Ser Ser Leu Gly Arg Gln Arg Arg Gln Phe Val
20        25        30
Phe Pro Asp Asp Glu Glu Ser Cys Ser Asn Arg Phe Thr Asn Asp Gly
35        40        45
Ile Cys Lys Asp Val Leu Asn Cys Arg Asp Leu Leu Gln Lys Asn Asp
50        55        60
Tyr Asn Leu Leu Lys Glu Ser Ile Cys Gly Phe Glu Gly Ile Thr Pro
65        70        75        80
Lys Val Cys Cys Pro Lys Gln Ser Ile Val Asn Pro Ile Thr Glu Ala
85        90        95
Pro Pro Lys Asn Thr Thr Thr Glu Arg Pro Pro Ile Arg Ile Pro Ser
100       105       110
Asn Leu Pro Lys Gln Cys Gly Asn Arg Asn Ile Thr Thr Thr Arg Ile
115       120       125
Ile Gly Gly Gln Glu Ala Thr Pro Gly Ala Trp Pro Trp Met Ala Ala
130       135       140
Val Tyr Ile Lys Gln Gly Gly Ile Arg Ser Val Gln Cys Gly Gly Ala
145       150       155       160
Leu Val Thr Asn Arg His Val Ile Thr Ala Ser His Cys Val Val Asn
165       170       175
Ser Leu Gly Thr Asp Val Met Arg Ala Asp Val Phe Ser Val Arg Leu
180       185       190
Gly Glu His Asn Leu Tyr Ser Thr Asn Asp Ser Ser Asp Pro Ile Asp
195       200       205
Phe Ala Val Thr Ser Val Lys His His Glu Asn Phe Val Leu Ala Thr
210       215       220
Tyr Leu Asn Asp Ile Ala Ile Leu Lys Leu Asn Asp Thr Val Thr Phe
225       230       235       240
Thr His Lys Ile Lys Pro Ile Cys Leu Pro Tyr Glu Ser Leu Arg Tyr

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<210> SEQ ID NO 22
<211> LENGTH: 375
<212> TYPE: PRT
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-continued

<213> ORGANISM: *Limulus polyphemus*

<400> SEQUENCE: 22

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Met Leu Val Asn Asn Met Phe Ser Leu Leu Cys Phe Ser Leu Leu Met
 1           5           10          15

Ser Met Phe Ser Cys Ser Ser Leu Gly Arg Gln Arg Arg Gln Phe Val
 20          25          30

Phe Pro Asp Asp Glu Glu Ser Cys Ser Asn Arg Phe Thr Asn Asp Gly
 35          40          45

Ile Cys Lys Asp Val Leu Asn Cys Arg Asp Leu Leu Gln Lys Asn Asp
 50          55          60

Tyr Asn Leu Leu Lys Glu Ser Ile Cys Gly Phe Glu Gly Ile Thr Pro
 65          70          75          80

Lys Val Cys Cys Pro Lys Gln Ser Ile Val Asn Pro Ile Thr Glu Ala
 85          90          95

Pro Pro Lys Thr Thr Thr Thr Glu Arg Pro Pro Ile Arg Ile Pro Ser
100          105          110

Asn Leu Pro Lys Gln Cys Gly Asn Arg Asn Ile Thr Thr Thr Arg Ile
115          120          125

Ile Gly Gly Gln Glu Ala Thr Pro Gly Ala Trp Pro Trp Met Ala Ala
130          135          140

Val Tyr Ile Lys Gln Gly Gly Ile Arg Ser Val Gln Cys Gly Gly Ala
145          150          155          160

Leu Val Thr Asn Arg His Val Ile Thr Ala Ser His Cys Val Val Asn
165          170          175

Ser Leu Gly Thr Asp Val Met Arg Ala Asp Val Phe Ser Val Arg Leu
180          185          190

Gly Glu His Asn Leu Tyr Ser Thr Asn Asp Ser Ser Asp Pro Ile Asp
195          200          205

Phe Ala Val Thr Ser Val Lys His His Glu Asn Phe Val Leu Ala Thr
210          215          220

Tyr Leu Asn Asp Ile Ala Ile Leu Lys Leu Asn Asp Thr Val Thr Phe
225          230          235          240

Thr His Lys Ile Lys Pro Ile Cys Leu Pro Tyr Glu Ser Leu Arg Tyr
245          250          255

Glu Asp Leu Ala Met Arg Asn Pro Phe Val Ala Gly Trp Gly Thr Thr
260          265          270

Ala Phe Asn Gly Pro Ser Ser Ala Val Leu Arg Glu Val Gln Leu Pro
275          280          285

Ile Trp Gly His Glu Pro Cys Arg Gln Ala Tyr Glu Lys Asp Leu Asn
290          295          300

Ile Thr Asn Val Cys Met Cys Ala Gly Tyr Ala Asp Gly Gly Lys Asp
305          310          315          320

Ala Cys Gln Gly Asp Ser Gly Gly Pro Met Met Leu Pro Asp Lys Ser
325          330          335

Gly Asn Phe Tyr Leu Val Gly Ile Val Ser Phe Gly Lys Lys Cys Ala
340          345          350

Leu Pro Gly Phe Pro Gly Val Tyr Thr Lys Val Thr Glu Phe Leu Asp
355          360          365

Trp Ile Ala Val Asn Met Val
370          375

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<210> SEQ ID NO 23
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: peptide

<400> SEQUENCE: 23

Ile Glu Gly Arg
1

<210> SEQ ID NO 24
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 24

ctgcaggaat tcgatttttt tttttttttt ttttt 35

<210> SEQ ID NO 25
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 25

atcgataagc ttgatgatct gggcttgtgt gatga 35

<210> SEQ ID NO 26
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 26

ctgcaggaat tcgat 15

<210> SEQ ID NO 27
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 27

ctacaccaag ttcca 15

<210> SEQ ID NO 28
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 28

gtaaaccatg tgacaaactg gaggc 25

<210> SEQ ID NO 29
<211> LENGTH: 25

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 29

aataaggcct ccatcgatag aagta 25

<210> SEQ ID NO 30
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 30

ggggaattca agcttgccac catggtacta gcgtcggtc 39

<210> SEQ ID NO 31
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 31

gggctcgagt caaatgaact gccgaatcca cgata 35

<210> SEQ ID NO 32
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 32

atcgataagc ttgatcacat gcaaggaaaa gttct 35

<210> SEQ ID NO 33
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 33

ctgcaggaat tcgatcactg tttaaacaaa ctgaa 35

<210> SEQ ID NO 34
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 34

atcgataagc ttgatagacc agagtgggtct ttctg 35

1. A recombinant protein which is any of the following:
 - (A) a recombinant protein that contains the amino acid sequence represented by SEQ ID NO: 2 or 4;
 - (B) a recombinant protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 2 or 4, and has factor C activity;
 - (C) a recombinant protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 1 or 3, and has factor C activity; and
 - (D) a recombinant protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 1 or 3 under stringent conditions, and has factor C activity.
 2. Use of the recombinant protein according to claim 1 for activating the following protein:
 - (A) a protein that contains the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12;
 - (B) a protein that contains an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 6, 8, 10, or 12, and has factor B activity;
 - (C) a protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 5, 7, 9, or 11, and has factor B activity; or
 - (D) a protein that is encoded by a DNA hybridizing to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 5, 7, 9, or 11 under stringent conditions, and has factor B activity.
 3. A method for detecting an endotoxin in a specimen, comprising a step of bringing the recombinant protein according to claim 1 into contact with the specimen.
 4. An endotoxin detecting agent, comprising the recombinant protein according to claim 1.
- * * * * *