PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Hikaru MIZUMURA, et al.

Docket No: Q270767

Appln. No.: 17/554,128

Group Art Unit: 1656

Confirmation No.: 8200

Examiner: DAVID J STEADMAN

Filed: December 17, 2021

For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

AMENDMENT UNDER 37 C.F.R. § 1.111

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Office Action dated December 20, 2024, please amend the above-identified application as follows on the accompanying pages.

TABLE OF CONTENTS

AMENDMENTS TO THE SPECIFICATION....2

AMENDMENTS TO THE CLAIMS....4

REMARKS....8

CRL Exhibit 1047
CRL v. Seikagaku
IPR2025-00440

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph no. [0035] with the following rewritten paragraph:

[0035]

The Factor C of the present invention may have low susceptibility to inhibition of activity by an ion. The expression "low susceptibility to inhibition of activity by an ion" refers to the state that the Factor C of the present invention exhibits a high residual activity in the presence of an ion, as compared with a control Factor C. Examples of the control Factor C include a recombinant Factor C expressed in insect cell Sf9 and a native Factor C. The meaning of the expression "exhibiting a high residual activity" is not particularly limited, so long as the residual activity of the Factor C of the present invention is higher than that of the control Factor C. The expression "exhibiting a high residual activity" may refer to, for example, the state that the residual activity of the Factor C of the present invention is 1.1 times or higher, 1.2 times or higher, 1.3 times or higher, 1.5 times or higher, 2.0 times or higher, 2.5 times or higher, or 3.0 times or higher than the residual activity of the control Factor C. Specific examples of "low susceptibility to inhibition of activity by an ion" include such a property that the Factor C of the present invention exhibits a higher residual activity in the presence of 21 mM sodium citrate, as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a higher residual activity in the presence of 52 mM sodium hydrogencarbonate, as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a higher residual activity in the presence of 214 mM sodium chloride, as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a higher residual activity in the presence of 16 mM magnesium sulfate, as compared with the control Factor C; and such a property that the Factor C of the present invention exhibits a higher residual

activity in the presence of 2.5 mM calcium chloride, as compared with the control Factor C. Specific examples of "low susceptibility to inhibition of activity by an ion" further include such a property that the Factor C of the present invention exhibits a residual activity of 10% or higher or 20% or higher in the presence of 21 mM sodium citrate~~, as compared with the control Factor C~~; such a property that the Factor C of the present invention exhibits a residual activity of 25% or higher or 35% or higher in the presence of 52 mM sodium hydrogencarbonate~~, as compared with the control Factor C~~; such a property that the Factor C of the present invention exhibits a residual activity of 25% or higher or 35% or higher in the presence of 214 mM sodium chloride~~, as compared with the control Factor C~~; such a property that the Factor C of the present invention exhibits a residual activity of 15% or higher or 25% or higher in the presence of 16 mM magnesium sulfate~~, as compared with the control Factor C~~; and such a property that the Factor C of the present invention exhibits a residual activity of 35% or higher or 45% or higher in the presence of 2.5 mM calcium chloride~~, as compared with the control Factor C~~. The Factor C of the present invention may have any one of the above properties singly or two or more of the above properties in combination. The "residual activity" is defined as a relative reactivity (%) observed for a measurement specimen (endotoxin concentration: 0.05 EU/mL) prepared by adding endotoxin to an ion-containing test specimen, with respect to the reactivity observed for another measurement specimen (endotoxin concentration: 0.05 EU/mL) prepared by adding endotoxin to injection water as 100%, in the assay system shown in Fig. 4.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1. (canceled):

2. (previously presented): The endotoxin assay agent according to claim 3, wherein the Factor C protein has a molecular weight of 115 kDa to 130 kDa, as measured through SDS-PAGE under a non-reducing condition.

3. (currently amended): An endotoxin assay agent comprising a mixture of a horseshoe crab Factor C protein, a horseshoe crab Factor B protein, ~~and~~ a horseshoe crab Pro-clotting enzyme protein, and a detection substrate for the Pro-clotting enzyme protein,

wherein the horseshoe crab is *Limulus polyphemus*,

wherein the Factor C protein is produced through being recombinantly expressed from a Human Embryonic Kidney (HEK) 293 cell,

wherein the Factor B protein is produced through being recombinantly expressed from a Human Embryonic Kidney (HEK) 293 cell,

wherein the Pro-clotting enzyme protein is produced through being recombinantly expressed from a Human Embryonic Kidney (HEK) 293 cell, ~~and~~

wherein the endotoxin assay agent detects an endotoxin through measuring the progress of a cascade reaction of the Factor C protein, the Factor B protein, the Pro-clotting enzyme protein, and the detection substrate, and

wherein the endotoxin assay agent is formulated into a liquid.

4. (withdrawn): A method for measuring endotoxin in a test specimen using an endotoxin assay agent according to claim 3.

5. (previously presented): The endotoxin assay agent according to claim 3, wherein the Factor C protein contains (α-2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding Factor C protein recombinantly expressed using Sf9 as a host cell.

6. - 14. (canceled)

15. (previously presented): The endotoxin assay agent according to claim 3, wherein the Factor C protein is secreted into a culture supernatant and recovered from the culture supernatant.

16. (previously presented): The endotoxin assay agent according to claim 3, wherein the Factor B protein is secreted into a culture supernatant and recovered from the culture supernatant.

17. (previously presented): The endotoxin assay agent according to claim 3, wherein the Pro-clotting enzyme protein is secreted into a culture supernatant and recovered from the culture supernatant.

18. (previously presented): The endotoxin assay agent according to claim 3, wherein the Factor C protein has no peptide added to the C-terminus.

19. (canceled).

20. (previously presented): The endotoxin assay agent according to claim 3, wherein the detection substrate is a synthetic substrate represented by formula X-Y-Z, wherein X represents a protection group, Y represents a peptide, and Z represents a dye linked to Y via amide bond.

21. (previously presented): The endotoxin assay agent according to claim 20, wherein Z is selected from the group consisting of a p-nitroaniline, a 7-methoxycoumarin-4-acetic acid, a 2,4-dinitroaniline, and a Dansyl dye.

22. (currently amended): The endotoxin assay agent according to claim 20, wherein Y comprises at least one selected from the group consisting of Leu-Gly-Arg (LGR), Ile-Glu-Gly-Arg （IEGR) (SEQ ID NO: 10), Val-Pro-Arg (VPR), and Asp-Pro-Arg (DPR).

23. (new): A liquid formulation comprising an endotoxin assay agent, wherein the endotoxin assay reagent comprises a mixture of a horseshoe crab Factor C protein, a horseshoe

crab Factor B protein, a horseshoe crab Pro-clotting enzyme protein, and a detection substrate for the Pro-clotting enzyme protein,

wherein the horseshoe crab is *Limulus polyphemus*,

wherein the Factor C protein is produced through being recombinantly expressed from a Human Embryonic Kidney (HEK) 293 cell,

wherein the Factor B protein is produced through being recombinantly expressed from a Human Embryonic Kidney (HEK) 293 cell,

wherein the Pro-clotting enzyme protein is produced through being recombinantly expressed from a Human Embryonic Kidney (HEK) 293 cell,

wherein the endotoxin assay agent detects an endotoxin through measuring the progress of a cascade reaction of the Factor C protein, the Factor B protein, the Pro-clotting enzyme protein, and the detection substrate.

REMARKS

In the present Amendment, paragraph [0035] of the specification has been amended to delete some of the phrases "as compared with the control Factor C", which did not exist in original Japanese PCT specification, but were inadvertently included in the English specification.

Claim 3 has been amended to delete an extra “and?, to recite “An endotoxin assay agent comprising a mixture of a horseshoe crab Factor C protein, a horseshoe crab Factor B protein, and a horseshoe crab Pro-clotting enzyme protein, …” and that “the endotoxin assay agent is formulated into a liquid”. Support for the amendment is found, for example, in paragraphs [0074] and [0075] of the specification.

Claim 22 has been amended to recite “Ile-Glu-Gly-Arg （IEGR) (SEQ ID NO: 10)”. Support for the amendment is found, for example, in paragraph [0072] of the specification.

New claim 23 has been added. Support for claim 23 is found, for example, in claim 3 and paragraphs [0074] and [0075] of the specification.

No new matter has been added, and entry of the Amendment is respectfully requested.

Upon entry of the Amendment, claims 2-5, 15-18 and 20-23 will be pending, of which claim 4 is withdrawn from consideration.

Request for Rejoinder

If claim 3 is found to be allowable, Applicant respectfully requests rejoinder of withdrawn method claim 4 pursuant to MPEP §821.04(b).

Response to Claim Objection

At page 3 of the Office Action, claims 3 and 22 are objected to because, per the Examiner,

> Claim 3 is objected to in the recitation of "a horseshoe crab Factor B protein, and a horseshoe crab Pro-clotting enzyme protein, and a detection substrate for the Pro-clotting enzyme protein" and in the interest of improving claim form and grammar, it is suggested that the noted phrase be amended to recite (with markings to show changes made) "a horseshoe crab Factor B protein, ~~and~~ a horseshoe crab Pro-clotting enzyme protein, and a detection substrate for the Pro-clotting enzyme protein."
>
> Claim 22 is objected to for reciting the sequence Ile-Glu-Gly-Arg (IEGR) that is encompassed by the definitions for sequences set forth in 37 CFR 1.821(a)(1) and (a)(2) yet is not identified with a sequence identifier, i.e., "SEQ ID NO:" (see MPEP 2422.01).

In response, claim 3 has been amended as suggested by the Examiner. Claim 22 has been amended to add "SEQ ID NO: 10" for the sequence Ile-Glu-Gly-Arg (IEGR).

In view of the above, reconsideration and withdrawal of the claim objection are respectfully requested.

Response to § 103 Rejection

Claims 2, 3, 5, 15-18, and 20-22 are rejected under 35 U.S.C. § 103 as being unpatentable over Tamura et al. (US 2009/0208995 A1; cited on the IDS filed on February 2, 2022; hereafter "Tamura") in view of Ding et al. (US 5,858,706; cited on the IDS filed on February 2, 2022; hereafter "Ding") and Koshiba et al. (*J. Biol. Chem.* 282:3962-3967, 2007; cited on the IDS filed on February 2, 2022; hereafter "Koshiba").

This rejection should be withdrawn because Tamura, Ding, and Koshiba do not disclose or render obvious the claimed invention, either alone or in combination.

In the Amendment Under 37 C.F.R. § 1.114(c) filed September 10, 2024, Applicant argued that it is common knowledge that in the cascade reaction of the Factor C protein, the Factor B protein, the Pro-clotting enzyme protein, and a detection substrate, the three proteases

(*i.e.,* Factor C, Factor B, and Pro-clotting enzyme) sequentially hydrolyze their respective target in this order in an aqueous medium; therefore, it is clearly recited in amended claim 3 that the assay is conducted in an aqueous medium.

In response, the Examiner states that the claims are drawn to an endotoxin assay agent, *not an assay* and according to the instant specification, "[t]he endotoxin assay agent of the present invention may be formulated into any preparation such as solid, liquid, or gel" (paragraph [0074]) and "[i]n the endotoxin assay agent of the present invention, the factors and other components may be present as a mixture or present separately" (paragraph [0075]). Therefore, the Examiner states that the claims are *not* directed to an assay and given a broadest reasonable interpretation in light of the specification, the claimed endotoxin assay agent is not required to be in the form of an aqueous medium.

To address the Examiner's concern, claim 3 has been amended to recite that the endotoxin assay agent is formulated into a liquid.

Also, new claim 23 recites a liquid formulation comprising an endotoxin assay agent which has the same composition as that recited in claim 3.

In the Amendment filed September 10, 2024, Applicant also argued that the Third Mizumura Declaration provides data showing that an endotoxin assay agent comprising *Limulus polyphemus* Factor C protein, Factor B protein, and Pro-clotting enzyme protein are unexpectedly less susceptive to endotoxin-independent activation and have significantly superior storage stability in solution.

Applicant further argued that the reagents for endotoxin measurement using the cascade reaction are used in an aqueous solution as described above, even if they are in solid form at the

time of distribution. Thus, Applicant argued that the invention recited in amended claim 3 is commensurate in scope with the results in the Third Mizumura Declaration.

In response, the Examiner states that Applicant's result is based on an endotoxin assay agent comprising a mixture of *Limulus polyphemus* Factor C protein, Factor B protein, Pro-clotting enzyme protein, and detection substrate in an aqueous solution. Per the Examiner, given a broadest reasonable interpretation in light of the specification, the claimed endotoxin assay agent is not required to be a mixture of *Limulus polyphemus* Factor C protein, Factor B protein, Pro-clotting enzyme protein, and detection substrate in an aqueous solution, and is interpreted as encompassing *Limulus polyphemus* Factor C protein, Factor B protein, Pro-clotting enzyme protein, and detection substrate as separate components and/or each component in the form of a solid or a gel.

To address the Examiner's concern, claim 3 has been amended to recite an endotoxin assay agent comprising **a mixture of** a horseshoe crab Factor C protein, a horseshoe crab Factor B protein, a horseshoe crab Pro-clotting enzyme protein, and a detection substrate for the Pro-clotting enzyme protein, and that the endotoxin assay agent is formulated into a liquid. Accordingly, the data is commensurate in scope with the claims.

In view of the above, the present claims, including new claim 23, are not obvious and are patentable over Tamura, Ding and Koshiba, either alone or in combination. Reconsideration and withdrawal of the § 103 rejection based on Tamura, Ding and Koshiba are respectfully requested.

Response to Double Patenting Rejection

Claims 2, 3, 5, 15-18, and 20-22 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 10, 11, and 14 of co-pending application 17/272,805 (reference application) in view of Tamura, Ding, and Koshiba.

This rejection should be withdrawn for at least the same reasons that the § 103 rejection based on Tamura, Ding, and Koshiba should be withdrawn as discussed above.

In view of the above, reconsideration and withdrawal of the double patenting rejections are respectfully requested.

Allowance is respectfully requested. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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