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Stereospecific Production of the Herbicide Phosphinothricin (Glufosinate) by Transamination: Isolation and Characterization of a Phosphinothricin-Specific Transaminase from *Escherichia coli*

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An aminotransferase capable of transaminating 2-oxo-4-[(hydroxy)(methyl)phosphinoyl]butyric acid to L-phosphinothricin [L-homoalanine-4-yl-(methyl)phosphinic acid], the active ingredient of the herbicide Basta (Hoechst AG), was purified to apparent homogeneity from *Escherichia coli* K-12. The enzyme catalyzes the transamination of L-phosphinothricin and various analogs with 2-ketoglutarate as the amino group acceptor. The transaminase had a molecular mass of 43 kilodaltons by sodium dodecyl sulfate-gel analysis and an isoelectric point of 4.35. The enzyme was most active in the high-pH region, with a maximum at pH 8.0 to 9.5, and had a temperature optimum of 55°C. Heat stability was observed up to 70°C. Substrate specificity studies suggested that the enzyme is identical with the 4-aminobutyrate:2-ketoglutarate transaminase (EC 2.6.1.19). The first 30 amino acids of the N terminus of the protein were determined by gas phase sequencing. The transaminase was immobilized by coupling to the epoxy-activated carrier VA-Biosynth (Riedel de Haen) and used in a column reactor for the continuous production of L-phosphinothricin. The enzyme reactor was operated for 7 weeks with only a slight loss of catalytic capacity. Production rates of more than 50 g of L-phosphinothricin per liter of column per h were obtained.

L-Phosphinothricin [L-homoalanine-4-yl-(methyl) phosphinic acid] is the active ingredient of the broad-spectrum, nonselective herbicide Basta (Hoechst AG). It is produced as part of the dialanyl tripeptide bialaphos, which was first isolated from *Streptomyces viridochromogenes* by Bayer et al. (3). L-Phosphinothricin is a structural analog of L-glutamic acid, and its herbicidal action is due to the inhibition of the ammonia-fixing enzyme glutamine synthetase (10, 13). The commercially available herbicide is a chemically synthesized racemic mixture and contains 50% of the herbicidally inactive D-enantiomer of phosphinothricin. The aim of our investigations was to develop a method for the stereospecific synthesis of the L-entantiomer.

Studies of the metabolism of phosphinothricin by soil microorganisms and *Escherichia coli* (2, 17) revealed that one major degradation pathway is the conversion of L-phosphinothricin to its corresponding 2-oxoacid, 2-oxo-4-[(hydroxy)(methyl)phosphinoyl]butyric acid (PPO) by transamination. Since enzymatic transamination is a reversible and stereospecific process, we used this type of enzyme reaction for biotechnical production of the herbicidally active isomer of phosphinothricin.

In the present communication we describe the isolation and application of a phosphinothricin-specific transaminase from *E. coli* and provide evidence that this enzyme is identical with 4-aminobutyrate:2-ketoglutarate transaminase (EC 2.6.1.19), the initial enzyme of the 4-aminobutyrate (GABA) degradation pathway (7).

MATERIALS AND METHODS

Chemicals. PPO, DL-homoalanine-4-yl-phosphinic acid, DL-homoalanine-4-yl-phosphonic acid, 3-aminopropyl (methyl)phosphinic acid, D-phosphinothricin, L-phosphinothricin, and DL-phosphinothricin were synthesized at Hoechst AG. Glutamate dehydrogenase (from bovine liver), 6-amino-*n*-caproic acid, 7-aminoheptanoic acid, cadaverine, GABA, gabaculine, aminooxyacetic acid, hydroxylamine, phenylhydrazine, and all protein amino acids were obtained from Sigma, Munich, Federal Republic of Germany. Polymercarrier VA-Epoxy Biosynth was obtained from Riedel de Haen.

Assays. The transaminase was assayed by two different methods. In method 1 (standard assay), it was incubated at 30°C in a volume of 100 μl with 0.1 M Tris, 0.1 mM pyridoxal phosphate, 10 mM 2-ketoglutarate, and 10 mM L-phosphinothricin at pH 7.5. The reaction was stopped by the addition of 200 μl of ethanol, and the glutamate produced was assayed either by reaction with glutamate dehydrogenase as described by Bernt and Bergmeyer (4) or by using a Biotronic LC 5001 amino acid analyzer. In method 2, the transaminase was incubated at 37°C in a volume of 100 μl with 0.1 M Tris, 0.1 mM pyridoxal phosphate, 50 mM PPO, and 150 mM glutamate at pH 8.0. The reaction was stopped by heating the mixture to 100°C for 5 min, and the phosphinothricin produced was determined with a Biotronic LC 5001 amino acid analyzer. Enzyme activities are expressed in katals (1 kat = 1 mol of turnover per s). Protein was determined by the Coomassie brilliant blue binding assay (Bio-Rad Laboratories, Munich, Federal Republic of Germany) of Bradford (5).

Phosphinothricin was determined quantitatively with a Biotronic LC 5001 amino acid analyzer. D- and L-enantiomers of phosphinothricin were determined in the Hoechst analytical laboratory after derivatization with *o*-phthaldialdehyde and *N*-acetyl-L-cysteine. The diastereoisomers were separated on a RP 18 column by using a chiral solvent.

Cultivation of *E. coli* DH 1. *E. coli* DH1 cells were grown at 37°C in Nährbouillon Standard II (Serva, Heidelberg, Federal Republic of Germany) on a rotary shaker or in a medium containing 5 g of peptone from meat per liter, 5 g of yeast extract per liter, and 0.6% NaCl in a 200-liter fermentor under aeration. Under these conditions, the specific transaminase activity was highest in the mid-log growth

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phase. Addition of glucose to the growth medium completely repressed the synthesis of the transaminase. The bacteria were harvested in the mid-log growth phase by centrifugation, washed with 0.9% NaCl, and stored at −20°C until processed.

Enzyme purification. The frozen bacteria were suspended in 20 mM phosphate buffer containing 0.01 mM pyridoxal phosphate, 5 mM 2-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride (pH 7.0) (3 ml of buffer per g of frozen cells) and disrupted by sonication. The resultant slurry was centrifuged, and the supernatant was dialyzed against buffer A [20 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), 10 μM pyridoxal phosphate, 5 mM 2-mercaptoethanol (pH 7.0)]. The dialyzed crude extract was subjected to ammonium sulfate precipitation. The transaminase precipitated between 45 and 70% ammonium sulfate saturation and was dialyzed against buffer A. For heat precipitation, the enzyme solution was heated to 70°C in the presence of 1 mM 2-ketoglutarate for 10 min, and the inactive precipitate was removed by centrifugation. The supernatant was dialyzed against buffer A and placed on a Q-Sepharose (Pharmacia, Freiburg, Federal Republic of Germany) column equilibrated with buffer A. After the column was washed thoroughly with the same buffer, the bound proteins were eluted with a linear gradient from 0 to 1 M KCl in buffer A. The transaminase eluted at ca. 0.3 M KCl. The active fractions were pooled, dialyzed against buffer A, and loaded on a hydroxyapatite column (HA Ultrogel; Serva). The column was eluted with a linear gradient from 0 to 0.5 M phosphate buffer (pH 7.0) in buffer A. The active fractions were pooled, concentrated by ammonium sulfate precipitation (80% saturation), and applied to an Ultrogel AcA 44 (Serva) gel filtration column. The column was operated in buffer A–0.15 M KCl. The transaminase-containing fractions were pooled and subjected to chromatofocusing. Therefore, the enzyme solution was dialyzed against 25 mM imidazole (pH 7.5) and applied to a PBE 94 (Pharmacia) chromatofocusing column equilibrated with the same buffer. The proteins were eluted with 12.5% Polybuffer 74 (Pharmacia), in water, adjusted to pH 3.8. The pH of the eluting fractions was measured and readjusted immediately to pH 7.0. The transaminase eluted at a pH of 4.3. The polybuffer was separated from the enzyme by passing the pooled fractions over a Sephadex G-75 column equilibrated with buffer A.

The purified transaminase was stored at −80°C. Prior to use, aliquots of the enzyme were subjected to fast protein liquid chromatography on a Pharmacia Mono Q column. The column was equilibrated with buffer A, and the transaminase was eluted with a linear 0 to 0.5 M KCl gradient in buffer A.

Immobilization of the transaminase. Following the Q-Sepharose step of the purification, the partially purified transaminase was precipitated by ammonium sulfate (70% saturation) and centrifuged. The precipitate was taken up in 1 M phosphate buffer (pH 8.0) and dialyzed against the same buffer. The protein concentration was adjusted to ca. 10 to 15 mg/ml, and 30 ml of the enzyme solution was incubated with 5 g of Polymercarrier VA-Epoxy Biosynth under gentle agitation for 2 days at room temperature. The immobilized transaminase was washed with coupling buffer and 50 mM phosphate buffer (pH 7.0), successively, and unreacted epoxy groups of the carrier were blocked by incubation with 50 mM 2-mercaptoethanol in 50 mM phosphate (pH 7.0) for 1 h at room temperature. The coupled transaminase was stored in 20 mM PIPES–10 μM pyridoxal phosphate–5 mM 2-mercaptoethanol–0.05% sodium azide at 4°C.

TABLE 1. Purification of L-phosphinothricin-specific aminotransferase starting with 10 g of bacteria

Purification step	Total protein (mg)	Total activity (nkat)	Sp act (nkat/mg)	Yield (%)
1. Crude extract	610	195	0.32	100
2. $(NH_4)_2SO_4$ precipitation	288	204	0.71	105
3. Heat precipitation	52	154	2.96	79
4. Q-Sepharose	10.4	138	13.3	71
5. HA-Ultrogel	2.7	121	45.2	62
6. Ultrogel AcA 44	1.5	103	70.3	53
7. Chromatofocusing	0.49	82	167.5	42
8. Mono Q	Aliquots of step 7		245.9	

Amino acid sequencing. The first 30 N-terminal amino acids of the transaminase were determined by gas phase sequencing with a 477a analyzer (Applied Biosystems, Inc., Foster City, Calif.) by using standard procedures.

RESULTS

Purification. A phosphinothricin-specific transaminase activity was measurable in crude extracts of *E. coli* which converted PPO to L-phosphinothricin with L-glutamate as the amino-group donor. Starting with 10 g of bacteria, we purified the transaminase ca. 800-fold following the purification protocol given in Table 1. The chromatofocusing column used in step 7 of the purification procedure revealed an isoelectric point of 4.35 for the transaminase. The purified enzyme was shown to be homogeneous by the criterion of sodium dodecyl sulfate-gel electrophoresis (Fig. 1) and had a molecular mass of ca. 43 kilodaltons. The first 30 amino-terminal amino acids of the purified transaminase were determined by gas phase sequencing. The sequence was H_2N-Met-Asn-Ser-Asn-Lys-Glu-Leu-Met-Gln-Arg-Arg-Ser-Gln-Ala-Ile-Pro-Arg-Gly-Val-Gly-Gln-Ile-His-Pro-Ile-Phe-Ala-Asp-Arg-Ala-.

Substrate specificity. The substrate specificity of the enzyme was studied by using the coupled transaminase-glutamate dehydrogenase assay (method 1; see Materials and Methods), in which the compounds in question were substituted for L-phosphinothricin and 2-ketoglutarate served as the amino-group acceptor. The enzyme showed a high

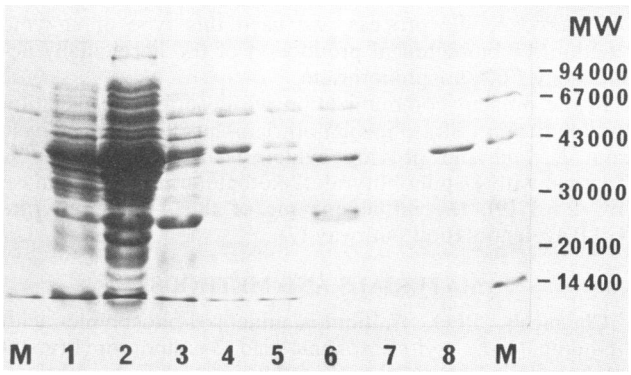

FIG. 1. Sodium dodecyl sulfate-gel analysis of transaminase purification. Lanes: M, molecular weight marker proteins; 1 to 8, protein samples from the purification steps in Table 1. Amounts of protein loaded were 30, 65, 25, 7, 5, 1, 0.5, and 2.3 μg per lane for lanes 1 to 8, respectively. MW, Molecular weight.

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TABLE 2. Substrate specificity of phosphinothricin transaminase with PPO or 2-ketoglutarate as the amino-group acceptor

Amino-group donor	Relative activity[a]	
	PPO	2-Ketoglutarate
L-Glutamic acid	100	
L-Phosphinothricin		33.3
D-Phosphinothricin		0
GABA	81.3	100
3-Aminopropyl(methyl)phosphinic acid	73.6	54.2
DL-Homoalanine-4-yl-phosphinic acid	11.2	33.3
DL-Homoalanine-4-yl-phosphonic acid	6.5	25.0
L-Glutamine	2.6	1.3
7-Aminoheptanoic acid	0	0
n-Amylamine	0	0
Protein amino acids[b]	0	0

[a] PPO and 2-ketoglutarate are used as amino-group acceptors.
[b] These are L-Ala, L-Arg, L-Asp, L-Cys, Gly, L-His, L-Ile, L-Lys, L-Met, L-Phe, L-Pro, L-Ser, L-Thr, L-Try, and L-Val.

specificity for L-phosphinothricin and some structurally related compounds. The highest enzyme activity, however, was observed with GABA (Table 2). With PPO as the amino-group acceptor (method 2; see Materials and Methods), the enzyme showed high substrate affinity toward glutamate, GABA, and 3-aminopropyl (methyl) phosphinic acid (Table 2). With both amino-group acceptors, however, no enzyme activity was detectable with other protein amino acids than L-glutamate and L-glutamine. The molecular structures of the transaminase substrates are given in Fig. 2.

Inhibitors. The effects of various compounds on the enzymatic activity of the transaminase were tested by addition of the substance in question at 1 mM to the reaction mix. The enzyme activity was determined as described above, and the results are listed in Table 3. Typical inhibitors of pyridoxal phosphate-dependent enzymes such as phenylhydrazine, hydroxylamine, and aminooxyacetic acid, as well as gabaculine, known as an irreversible GABA-transaminase inhibitor (6, 15, 19), strongly inactivated the enzyme. Similar results were obtained by incubating the enzyme with the sulfhydryl reagents *N*-ethylmaleimide and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). In contrast to the results of Yonaha and Toyama (19), who reported strong inhibition of

TABLE 3. Effects of inhibitors on phosphinothricin transaminase

Inhibitor	Relative activity[a]
None	100
Aminooxyacetate	0
Hydroxylamine	11
Phenylhydrazine	9
L-Cysteine	95
D-Cysteine	100
DTNB	0
N-Ethylmaleimide	15
$HgCl_2$	5
Gabaculine	12
EDTA	100
Divalent metal ions[b]	100

[a] Remaining transaminase activity in the presence of 1 mM compound in question in the test mix.
[b] Metal ions from $CaCl_2$, $CdCl_2$, $CoCl_2$, $MnCl_2$, $FeSO_4$, $BaCl_2$, and $MgCl_2$.

GABA-transaminase from *Pseudomonas* sp. by D- and L-cysteine, these compounds had no effect in our experiments. With the exception of mercury, none of the divalent metal ions tested affected the transaminase.

Effect of pH and temperature. The effect of pH and temperature on the transamination between L-phosphinothricin and 2-ketoglutarate was examined by using assay method 1. The pH dependence of the enzyme reaction was determined by substituting the following buffers for Tris: citrate-NaOH buffer (pH 4 to 4.5), 2-(*N*-morpholino)ethanesulfonic acid (MES) (pH 5 to 6), PIPES (pH 6.5 to 7.5), and 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) (pH 9.5 to 11.5). The enzyme was most active in the high-pH region, with a maximum at pH 8 to 9.5 (Fig. 3). Almost no transaminase activity was detectable below pH 6.

The reaction velocity increased dramatically with temperature and exhibited a maximum at 55°C (Fig. 4). The thermal stability of the protein was examined at pH 8.0. After the enzyme was incubated at various temperatures for 10 min, the remaining activity was determined by the standard assay. The enzyme was found to be stable up to 70°C but inactivated at higher temperatures (Fig. 5). The enzyme preparation showed no significant loss of activity during 3 weeks of storage at room temperature.

Enzyme immobilization and L-phosphinothricin production. For immobilization of the transaminase, a partially purified

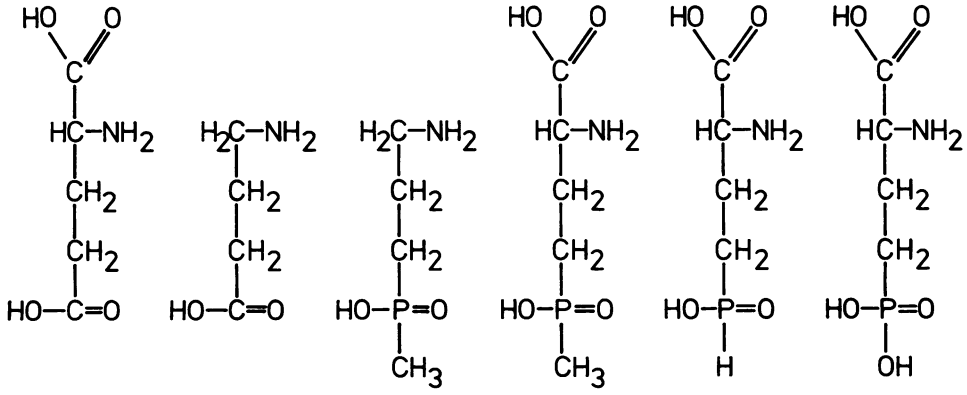

FIG. 2. Molecular structures of amino-group donor substrates of the phosphinothricin transaminase (see Table 2). Structures: 1, L-glutamic acid; 2, GABA; 3, 3-aminopropyl (methyl) phosphinic acid; 4, L-phosphinothricin; 5, DL-homoalanine-4-yl-phosphinic acid; 6, DL-homoalanine-4-yl-phosphonic acid.

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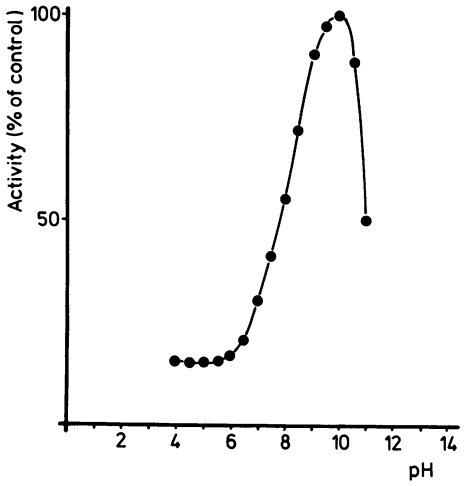

FIG. 3. pH optimum of the transamination between L-phosphinothricin and 2-ketoglutarate. For details, see the text.

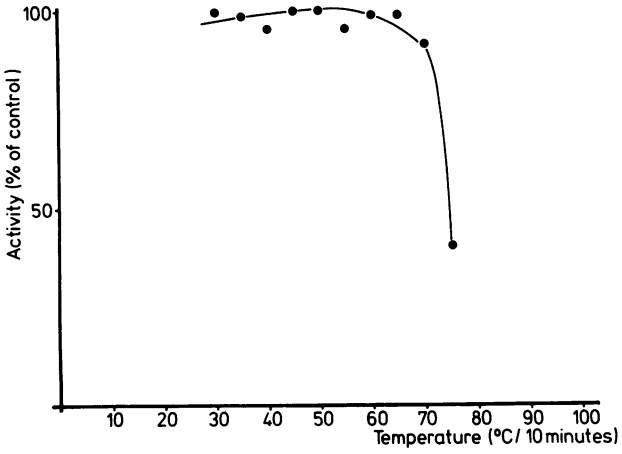

FIG. 5. Thermal stability of the L-phosphinothricin transaminase. The enzyme was incubated at various temperatures for 10 min at pH 8.0. The remaining enzymatic activity was determined by the standard assay.

enzyme preparation (20% transaminase) was incubated with Polymercarrier VA-Epoxy Biosynth. Approximately 95% of the protein bound to the matrix, exhibiting ca. 50% of the initial transaminase activity.

For comparison of the catalytic properties, 0.2 nkat of the immobilized and the free enzyme was incubated in 5 ml of standard reaction mix containing substrate concentrations of 30 g of PPO per liter and 90 g of L-glutamate per liter. The time courses of L-phosphinothricin production for both experiments are shown in Fig. 6. The L-phosphinothricin production by the immobilized enzyme remained approximately linear for more than 30 days, whereas the free enzyme was unstable under these conditions and L-phosphinothricin synthesis stopped after 2 days.

Owing to its high stability, the carrier-fixed transaminase seemed to be suitable for continuous L-phosphinothricin production in an enzyme reactor. Therefore, a 20-ml column was packed with the immobilized enzyme. Various concentrations of the substrate PPO and the amino-group donor L-glutamate were used in the enzyme reactor for the transamination process (Table 4). Earlier investigations had demonstrated that at least a fourfold molar excess of glutamate with respect to PPO was necessary to drive the transaminase reaction to near completion. Under the given reaction conditions, 76 to 90% of the applied PPO was converted to L-phosphinothricin. The conversion rate dropped with increasing substrate concentrations, indicating an insufficient contact time of enzyme and substrate. Higher conversion rates at high substrate concentrations were obtained by lowering the flow rate of the enzyme reactor (data not shown). Experiments to determine the long-term operational stability of the immobilized enzyme were carried out in which the catalytic activity of the enzyme reactor was measured over a total period of 53 days. During the experiment, the enzyme column was operated intermittently for 7 weeks for periods of 1 day at a time. When not in use, the enzyme column was stored in substrate solution at 42°C. Only a slight loss in the activity of the immobilized protein was detectable; at the end of the test period, the enzyme still exhibited ca. 75% of its initial catalytic capacity. The specific L-phosphinothricin production rate of the enzyme reactor was compared with the production rate obtained by biotrans-

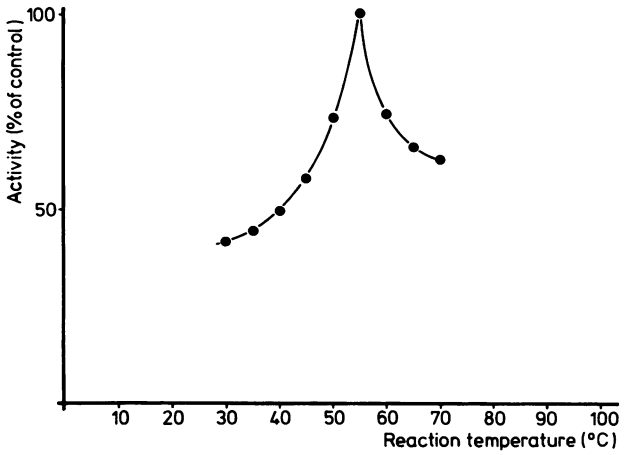

FIG. 4. Temperature optimum of the transamination between L-phosphinothricin and 2-ketoglutarate. For details, see the text.

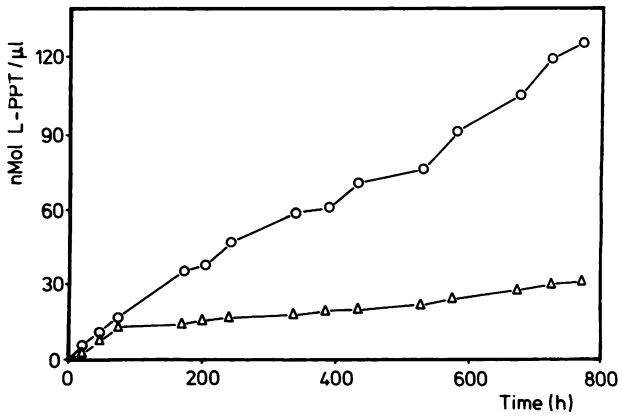

FIG. 6. Time course of L-phosphinothricin (L-PPT) production from PPO by transamination. Carrier-fixed (○) and free (△) transaminase (0.2 nkat each) were incubated with 5 ml of PPO (30 g/liter) and L-glutamate (90 g/liter) adjusted to pH 8.0 in the presence of 10 µM pyridoxal phosphate and 0.05% sodium azide at 37°C under gentle agitation. Samples were taken over a total period of 800 h and analyzed for L-phosphinothricin.

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TABLE 4. L-Phosphinothricin production with immobilized transaminase in a column reactor[a]

Substrate concn (g/liter)		Product concn (g/liter)	Conversion rate (%)
PPO	L-Glutamic acid		
20	60	18.6	90
30	90	25.5	85
60	180	49.2	82
100	300	76.1	76

[a] The enzyme column had a volume of 20 ml and was operated at 42°C at pH 8.0 with a flow rate of 0.5 ml/min in the presence of 10 μM pyridoxal phosphate, 5 mM 2-mercaptoethanol, and 0.05% sodium azide.

formations with resting cells (2) of *E. coli* DH1 at identical substrate concentrations. Whereas the highest production rates by biotransformation did not exceed 0.2 g of L-phosphinothricin per liter per h, production rates of more than 50 g of L-phosphinothricin per liter per h were obtained with the enzyme reactor.

Purification of L-phosphinothricin. After enzymatic conversion, the reaction broth contained L-phosphinothricin, glutamate and 2-ketoglutarate in a molar ratio of 1:3:1, plus trace amounts of unreacted PPO. To isolate the L-phosphinothricin, we subjected the reaction mixture to a two-step ion-exchange column chromatography. First, the two amino acids were purified from the ketoacids by binding the amino acids to a cation exchanger (Amberlite 200). The amino acids were eluted with NH_4OH, applied to an anion exchanger (Dowex-1 acetate), and separated from each other by eluting the column with acetic acid. The phosphinothricin-containing fractions were pooled and concentrated to dryness under reduced pressure. Analysis of the L-phosphinothricin produced in the Hoechst analytical laboratory revealed an optical purity of >99.9%.

DISCUSSION

We have isolated a transaminase from *E. coli* K-12 that is capable of catalyzing the synthesis of the herbicidal compound L-phosphinothricin from the corresponding keto acid (PPO) with L-glutamic acid, GABA, or 3-aminopropyl (methyl) phosphinic acid as an efficient amino-group donor. When 2-ketoglutarate was used as the amino-group acceptor, the enzyme exhibited the highest activity with GABA as the amino-group donor and the next-highest activity with L-phosphinothricin as the amino-group donor.

From these studies we conclude that the enzyme is a GABA:2-ketoglutarate transaminase (EC 2.6.1.19), an enzyme that has previously been described as being involved in the GABA utilization pathway of *E. coli* K-12 (7). Our substrate specificity studies demonstrate that the enzyme activity depends on the spacing between the amino group and a negatively charged group in the substrate. The charged group can be either a carbonyl, a phosphino, or a phosphono group. The enzyme was most active on substrates with three carbon atoms interposed between these functions (Fig. 2), whereas molecules with longer or shorter carbon chains inserted were not transaminated. As the only exception, ornithine could serve as an amino-group donor when the reaction was carried out at pH 9.5 (data not shown).

Microbial GABA transaminases have previously been isolated and partially characterized by Voellmy and Leisinger (18) and Yonaha and Toyama (19), who isolated the enzymes from *Pseudomonas aeruginosa* and a *Pseudomonas* sp., respectively. The authors also report a strict dependence of enzyme activity on the distance between the amino and carboxyl groups of the substrates. From these studies, however, it was not known whether phosphono or phosphino analogs of GABA or glutamate can be transaminated by the *Pseudomonas* enzymes.

The use of transaminases for the production of protein amino acids has become an important technique in biotechnology and was reviewed recently (16). The major advantages of these enzymes are their stereoselectivity, their high catalytic rates, and their ability to react with a variety of structurally related substrate analogs, thus allowing even nonprotein amino acids to be produced by this group of enzymes. Moreover, the application of an immobilized-transaminase process leads to significantly higher production rates than those of a fermentation process.

For these reasons, we decided to investigate the utility of the *E. coli* phosphinothricin-transaminase for continuous L-phosphinothricin production in an enzyme reactor. Purification of the amounts of transaminase needed for a large-scale technical process is economically not justifiable. Therefore, we used a partially purified enzyme preparation (with transaminase as 10 to 20% of the total protein) for the immobilization experiments, since this grade of purity can be obtained in crude protein extracts of genetically engineered bacteria overproducing the transaminase (1). When used for the continuous production of L-phosphinothricin, this enzyme preparation showed a significant reduction of activity within 2 days (Fig. 6). After immobilization by coupling to the carrier, however, the transaminase remained active for more than 1 month. This long-term activity may be due to a stabilization of the active configuration of the transaminase by covalent binding to the matrix. Furthermore, immobilization of the proteins may prevent proteases present in the relatively crude enzyme preparation from digesting the transaminase.

In a column reactor the immobilized enzyme was stable for at least 53 days and produced L-phosphinothricin at a rate of ca. 50 g/liter per h. These results are of the same order of magnitude as those described for L-alanine synthesis with an immobilized commercially available glutamic-pyruvic aminotransferase from porcine heart tissue (16).

An inherent disadvantage of the use of transamination reactions for the production of amino acids is their equilibrium constant, which is near unity, resulting in an incomplete conversion of the keto acid to the desired amino acid. One possible way to overcome this problem is by decomposition of the keto acid by-product of the transamination, thus eliminating the equilibrium constraint of the reaction. The feasibility of this approach in large-scale amino acid production with aspartic acid as the amino donor has been described by Walter and Sherwin (J. F. Walter and M. B. Sherwin, United Kingdom patent application GB 2161 159 A, 1986), who demonstrated that the reaction by-product, oxalacetic acid, could be decarboxylated to pyruvic acid in the presence of various metal ions, thus allowing an amino acid yield of more than 90%. Alternatively, oxalacetic acid can be degraded enzymatically by oxaloacetate decarboxylase (EC 4.1.1.3) (16). However, for application of this technique to a transamination process with glutamic acid as the obligatory amino group donor, the by-product 2-ketoglutarate has to be recycled to glutamate with aspartate: ketoglutarate transaminase (EC 2.6.1.1) in a coupled reaction. Since the coordinated use of two immobilized enzymes in a bioreactor is difficult to control, this approach seems to be limited to biotransformations in which both enzymes are present in the same microorganism.

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For the technical application of phosphinothricin transaminase, we therefore used an excess of the amino substrate glutamate to shift the reaction equilibrium to the desired product. A fourfold molar excess of glutamate over the 2-keto acid precursor resulted in production yields of more than 90% L-phosphinothricin (Table 4). During the purification of L-phosphinothricin from the reaction broth by ion-exchange chromatography, the unreacted glutamate could be separated from the other reaction products and be recycled into the transamination process.

E. coli is one of the most powerful sources of transaminases for biotechnological processes. The nucleotide sequences of *E. coli* genes coding for the branched-chain amino acid transaminase (*ilvE*) (12) imidazolylacetolphosphate:glutamate transaminase (*hisC*) (11), aspartate aminotransferase (*aspC*) (9), aromatic aminotransferase (*tyrB*) (9), alanine:valine transaminase (*avtA*) (14), and 3-phosphoserine aminotransferase (*serC*) (8) are already known. A comparison of the deduced amino acid sequences of these genes shows no homology to the 30 N-terminal amino acids of the transaminase described in this paper. The cloning, characterization, and overexpression of the corresponding transaminase gene are reported in the accompanying article (1).

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