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PURIFICATION AND CHARACTERISATION OF D-AMINO ACID AMINOTRANSFERASE FROM *RHIZOBIUM JAPONICUM*

J.P. GOSLING and P.F. FOTTRELL

Department of Biochemistry, University College, Galway (Republic of Ireland)

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Summary

Rhizobium japonicum has D-amino acid aminotransferase and alanine racemase activities. The D-amino-acid aminotransferase has been partially purified and characterized. This enzyme has a broad specificity and is very active with D-α-aminobutyrate and D-aspartate as well as D-alanine and D-glutamate. The stereospecificity of the enzyme for D-amino acids was apparently absolute with respect to product inhibition, pyridoxamine formation as well as catalytic activity. The apparent molecular weight was 58 000 and the pH optimum was 7.8—7.9. The equilibrium constant in the direction of D-glutamate formation was 1.9. Initial-velocity kinetic studies indicate the enzyme acts by a ping-pong mechanism. The dissociation constant for pyridoxal phosphate and the Michaelis constants (± standard errors) for D-alanine and 2-oxoglutarate were determined to be 0.51 ± 0.06 μM, and 2.13 ± 0.18 and 0.058 ± 0.005 mM respectively. The enzyme is moderately inhibited (30%) by 4 mM *p*-chloromercuribenzoate.

Introduction

Following the biological fixation and assimilation of nitrogen, aminotransferases are actively involved in the metabolism of amino groups (see Nagatani et al., [1]). Previous studies from this laboratory have described the characterization of L-aspartate aminotransferase from soybean (Glycine max.) root nodules and *Rhizobium japonicum* [2,3]. In a previous report [4] we described the presence of a highly active D-amino acid aminotransferase in *R. japonicum* and soybean root nodules. This latter enzyme was called D-alanine aminotransferase in accordance with Martinez-Carrion and Jenkins [5,6]. However, until the precise function of the enzyme, and of a similar one from pea seedlings [7], are established, it is now proposed to employ the less specific name, D-amino acid aminotransferase.

The present paper describes more detailed studies on the kinetics and other characteristics of D-amino acid aminotransferase from *R. japonicum*.

Materials and Methods

Plants

Soybeans (Glycine max. var. vent; seeds obtained from the U.S. Department of Agriculture, Beltsville, Md.) were grown in nitrogen-free medium and inoculated as described previously [8] with *R. japonicum* strain J392 (obtained from the Agricultural Institute, Johnstown Castle, Wexford, Ireland). *R. japonicum* was grown in defined medium [9] and cell-free extracts were prepared by shaking 10 g of bacteria, 50 g glass beads and 10 ml of 0.1 M KH_2PO_4/KOH buffer, pH 7.0 in a Braun Disintegrator, cooled with CO_2, for 40 s. The resulting suspension was then diluted with another 10 ml of the same buffer and centrifuged at 30 000 × *g* for 1 h. The sediment was then re-extracted by the same procedure and the supernatants combined.

Enzyme assays

L-aspartate aminotransferase (EC 2.6.1.1) activity was measured at 30°C as described previously [3]. To assay L-alanine aminotransferase (EC 2.6.1.2) the following mixture was used; 80 mM L-alanine, 135 μM pyridoxal-5-phosphate, 667 μM 2-oxoglutarate, 20 μM NADH, excess lactate dehydrogenase (EC 1.1.2.7), (Sigma Chemical Co.) and 100 mM Tris · HCl buffer, pH 7.9. The assay mixture for D-amino acid aminotransferase contained 33 mM D-alanine and 1.67 mM 2-oxoglutarate. The other constituents were the same as for L-alanine aminotransferase except that, where indicated, different buffers were used.

Alanine racemase (EC 5.1.1.1) was estimated by measurement of D-alanine formation on incubating the enzyme with 120 mM L-alanine, 0.2 mM pyridoxal-*P*, 50 mM HEPES pH 7.9 for 10 min. The enzyme was then inactivated by boiling and an aliquot was transferred to 200 μM NADH, 50 mM HEPES, pH 7.9 containing 50 μg D-amino acid oxidase (Sigma Chemical Co.) and excess catalase. After recording the absorbance excess lactate dehydrogenase was added and the new stable absorbance recorded.

Enzyme activity is expressed as nmol substrate transformed per min per mg protein or ml enzyme solution, except for the initial velocity studies where it is given as the rate of decrease in absorbance per min. Protein was determined by a micro-biuret procedure with bovine serum albumin as standard.

Purification of D-amino acid aminotransferase

Crude extract (90 ml), prepared from 40 g (wet weight) of *R. japonicum* with pyridoxal-*P* (2 mM) added, was heated to 46°C for 20 min and then centrifuged at 34 000 × *g* for 30 min. The supernatant was passed through a column (35 × 3.4 cm) of Sephadex G-25 previously equilibrated with 10 mM 2-mercaptoethanol/20 mM Tris · HCl, pH 8.4. Fractions containing protein were pooled and applied to a column (20 × 3.4 cm) of DEAE-cellulose (Whatman DE52) equilibrated with the aforementioned buffer. The D-aminotransferase was eluted with a 600-ml linear gradient of 20—285 mM Tris · HCl, pH 8.4 containing 10 mM 2-mercaptoethanol. Fractions (10 ml) were collected in tubes containing 1 ml of 1.0 M KH_2PO_4/KOH buffer, pH 5.8 and 0.1 ml of 4 mM pyridoxal-*P*. To separate the enzyme from any remaining L-aspartate

aminotransferase activity, the fractions were pooled, re-equilibrated with 10 mM mercaptoethanol, 20 mM Tris · HCl, pH 8.4 and rechromatographed on another DEAE-cellulose column (30 × 1.4 cm). The D-amino acid aminotransferase was then eluted with a 150 ml linear gradient of 100—300 mM Tris · HCl, 10 mM 2-mercaptoethanol, pH 8.4. The fractions containing the latter enzyme were combined and concentrated by vacuum dialysis after which the enzyme solution was applied to a Sephadex G-200 column (50 × 2.6 cm) equilibrated with 50 mM KH_2PO_4/KOH, pH 7.0. The D-amino acid aminotransferase was eluted from the column as a single peak.

Molecular weight

A Sephadex G-200 column (52 × 2.6 cm) equilibrated with 50 mM KH_2PO_4/KOH, pH 7.0 was calibrated with the following proteins, α-chymotrypsinogen A (mol. wt. 27 000), ovalbumin (45 000), bovine serum albumin (67 000), pig heart L-alanine aminotransferase (115 000) and phycoerythrin (291 000). With the log molecular weight as dependent variable and the elution volume/void volume ratio as independent variable, a calibration line was fitted to the standard points by unweighted least squares analysis. The correlation coefficient was —0.98.

pH optimum

KH_2PO_4/KOH (20 mM) buffers were used in the assay mixture over the entire pH range and the pH of the assay media was also determined when the assays were completed. Detectable alteration in the pH values during the assay occured only at the highest pH levels and was never greater than 0.3 units. The reaction was measured at the following pH values: 6.8, 7.0, 7.4, 7.6, 7.8, 8.0, 8.1, 8.4, 8.7.

Equilibrium constant

The equilibrium constant was determined with the enzyme operating in both forward and reverse directions. The reaction in the forward direction was measured at a volume of 1 ml in the presence of 50 μmol HEPES/HCl (pH 7.9), 50 μmol pyruvate, 50 μmol of D-glutamate and 1.5 units of purified enzyme. The reaction was started by the addition of enzyme and samples of 50 μl each were removed periodically and added to 50-μl volumes of 10% (v/v) $HClO_4$. These samples were later assayed for pyruvate with NADH and lactate dehydrogenase. The same method was used to measure the reverse reaction but with 50 μmol of 2-oxoglutarate and 50 μmol of D-alanine instead of pyruvate and D-glutamate.

Substrate specificity

Two procedures were used to study the substrate specificity of the enzyme:

(a) Dehydrogenase-linked spectrophotometric assay. The formation of 2-oxo-acids when the enzyme was incubated with 667 μM 2-oxoglutarate, 167 μM pyridoxal-*P* and 40 mM concentrations of different amino acids, was measured with 200 μM NADH and excess lactate dehydrogenase or malate dehydrogenase; the rate of NADH oxidation being followed spectrophotometrically. Sufficient lactate dehydrogenase was used to reduce 2-oxobutyrate at a

very high rate. In these experiments, the reactions were initiated by the addition of the 2-oxoglutarate.

(b) Amino acid analysis. The ability of the purified enzyme to catalyse the transamination of different D- or L-amino acids with 2-oxo-acids was measured in 500 μl containing 25 μmol of HEPES buffer (pH 7.9), 33 μmol of the amino acid and 33 μmol of the 2-oxo-acids and 5 μmol pyridoxal-*P*. After the addition of 0.06 units of enzyme the reaction was allowed to proceed for 60 min at 30°C. The enzyme was inactivated by placing the reaction tubes in a boiling water bath for 2 min. The formation of new amino acids was determined with a Jeol-5AH amino acid analyser.

Absorbance spectra

Excess pyridoxal-*P* was removed from the enzyme preparation by dialysis with 50 mM KH_2PO_4/KOH, pH 6.8, 5 mM mercaptoethanol, 0.01% NaN_3 for 16 h at 4°C and then concentrated by vacuum dialysis. The final preparation could be activated a little (15%) by addition of excess pyridoxal-*P*. Spectra were recorded with the enzyme at a concentration of 57 units/ml in quartz microcuvettes with a 2-cm lightpath.

Preparation of apoenzyme

Apoenzyme was prepared by incubating the enzyme, for 30 min, at 30°C with 50 mM D-alanine, 0.9 M KPO_4/KOH, pH 5.5 and then dialysing against 1 l of 10 mM mercaptoethanol, 10 mM KH_2PO_4/KOH, pH 6.8 for 16 h. Recovery of enzyme activity was 40% and the enzyme exhibited 14% of its final activity when assayed in the absence of pyridoxal-*P*. Dialysis or gel filtration using buffers other than phosphate resulted in much lower recoveries of enzyme activity. Following resolution of apoenzyme and pyridoxal-*P* the enzyme steadily lost activity but this was prevented by high phosphate concentration (50—900 mM).

Michaelis constants

The dissociation constant for pyridoxal-*P* was determined with apoenzyme prepared as described above and with 50 mM HEPES/HCl, pH 7.9 as the assay buffer; the final concentration of phosphate in this mixture was 500 μM. Preliminary determination of the inhibition constant (5—10 mM) of phosphate for reassociation of the enzyme indicated that 500 μM phosphate could increase the apparent dissociation constant for the enzyme and pyridoxal-*P* by less than 10%. Enzyme activity due to the small proportion of unresolved enzyme was subtracted out.

Data from each initial velocity experiment, was fitted to the rate equation which best explained the observed kinetic behaviour. Computer programmes developed by Cleland [10] and supplied to us by Dr. B. Orsi (Dublin) were used.

Inhibition by p-chloromercuribenzoate

Solutions of enzyme and 4 mM *p*-chloromercuribenzoate with and without 30 mM D-alanine were incubated at 30°C and samples taken at intervals for enzyme assay. Care was taken that sufficient lactate dehydrogenase was added to ensure an excess of active enzyme in the assay cuvette.

Results

Enzymic activities in crude extracts of bacteria and soybean root nodules

D-amino acid aminotransferase was much more active than L-alanine aminotransferase in crude extracts of *R. japonicum* while the activity of L-aspartate aminotransferase was on order of magnitude higher again (Table I). Alanine racemase was also comparatively active and probably contributed to the measured L-alanine aminotransferase activity. All the latter enzymes were also active in extracts of soybean nodules infected with *R. japonicum* and the relative activities of the enzymes were similar to those in Table I. Both alanine racemase and D-amino acid aminotransferase were detected in the bacterial and non bacterial fractions of extracts of soybean nodules. The activities of the L-aspartate, L-alanine and D-amino acid aminotransferase in crude extracts of *R. japonicum* without pyridoxal-*P* added, were only 10% of the activities obtained when excess pyidoxal-*P* was added. In contrast, the activities of these enzymes in crude nodule extracts were not markedly influenced when additional pyridoxal-*P* was added [11]. The activity of alanine racemase was not stimulated by the addition of pyridoxal-*P* to either of the two aforementioned sources.

Purification of D-amino acid aminotransferase

The first DEAE-cellulose step separated the two alanine aminotransferase but although the recovery of D-alanine aminotransferase was good (60—70%) the recovery of L-alanine aminotransferase was only about 3% (Fig. 1). The overall recovery of D-alanine aminotransferase activity was 30% with a 160-fold purification (Table II). The purified enzyme was free of L-alanine aminotransferase, L-aspartate aminotransferase and alanine racemase activities.

Molecular weight

The elution volume ratio (V_e/V_0) of D-amino acid aminotransferase was determined for a G-200 column, calibrated with standards of known molecular weight. Unweighted least squares analysis of these data gave a log molecular weight of 4.762 ± 0.225 (95% confidence limits of $\hat{Y}$) for D-amino acid aminotransferase. The apparent molecular weight of 58 000 is, therefore, significantly different from the estimated molecular weight of 115 000 for pig heart L-alanine aminotransferase [12]. It is similar to the values of 53 000, 60 000 and 65 000 for D-amino acid aminotransferase from *Bacillus subtilis* [5], *Bacillus sphaericus* [13] and pea seedlings [7] respectively.

TABLE I

SPECIFIC ACTIVITIES OF SOME ENZYMES IN CRUDE EXTRACTS OF *RHIZOBIUM JAPONICUM*

All enzymes were assayed in the presence of excess pyridoxal-*P*.

Enzyme	Specific activity nmol/min per mg
D-alanine aminotransferase	21
L-alanine aminotransferase	6
Alanine racemase	150
L-aspartate aminotransferase	352

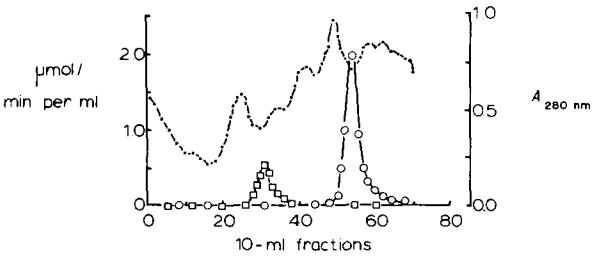

Fig. 1. Chromatography on the first (20 × 3.4 cm) DEAE-cellulose column. Conditions are given in the text. Absorbance at 280 nM (●———●); D-amino acid aminotransferase activity (D-AAT) (○———○) and L-alanine aminotransferase activity, ×10 (L-ALAT) (□———□) are shown.

pH optimum

The activity of the enzyme at both extremes of the pH range employed was 50% of the activity at the optimum. The pH optimum of the enzyme was 7.8—7.9 with 20 mM KH_2PO_4/KOH (see Materials and Methods for details) and was not significantly different when 100 mM Tris · HCl was used.

Equilibrium constant

Both substrate pairs (D-alanine and 2-oxoglutarate, and D-glutamate and pyruvate) were employed to measure the approach to equilibrium (Fig. 2). From these results the equilibrium constant was calculated to be 1.9.

Specificity

The ability of D-amino acid aminotransferase to transaminate to 2-oxoglutarate using various amino acid donors was measured by adapting the direct spectrophotometric assay. Negligible activity was observed with L-aspartate or L-alanine, while D-aspartate and α-D-aminobutyrate were good donors (Table III). The specificity of the enzyme was also examined with a greater range of substrate pairs using an amino acid analyser. As before, negligible activity was obtained with L-amino acids, and with D-serine or D-leucine whereas D-alanine, D-aminobutyrate, D-glutamate and D-aspartate were good substrates. Optimum activity was obtained with D-alanine and 2-oxybutyrate. Glycine, D-serine and D-norleucine gave negligible activity with 2-oxoglutarate. This specificity is similar to D-alanine aminotransferase from *Bacillus subtilis* [5] and D-amino

TABLE II

PURIFICATION OF D-ALANINE AMINOTRANSFERASE FROM *RHIZOBIUM JAPONICUM*

Step	Volume (ml)	Total activity (μmol/min)	Protein (mg)	Specific activity (units/mg)	Yield (%)	Purification (-fold)
Crude extract	89	177	26	0.05	100	1
Heat	90	135	18	0.08	115	1.6
1st DE52	88	97	4	0.28	83	5.5
2nd DE52	43	51	0.6	2.0	44	40
G-200	36	37	0.12	8.3	32	166

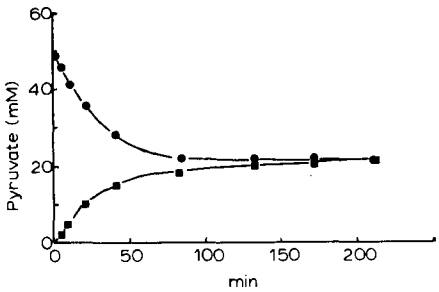

Fig. 2. Determination of the equilibrium of the reaction of D-amino acid aminotransferase. The reaction in the forward direction was started at 50 mM pyruvate and 50 mM D-glutamate (●———●), and in the reverse direction at 50 mM 2-oxoglutarate and 50 mM D-alanine (■———■). For further details see Materials and Methods.

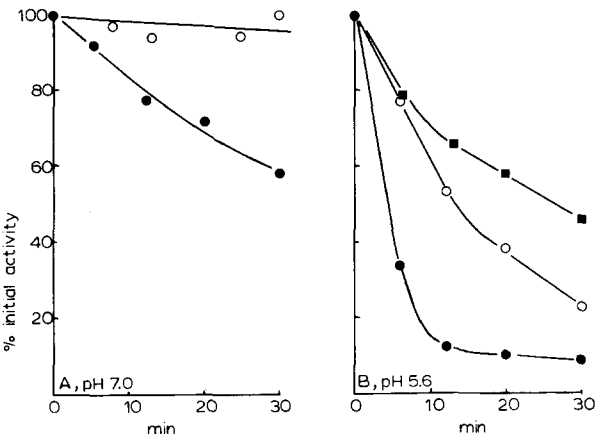

Fig. 3. The dissociation of D-amino acid aminotransferase. Purified enzyme was incubated at 30°C under the conditions described below, samples were taken at intervals and assayed in the absence of added pyridoxal-*P*. Addition of pyridoxal-*P* restored the original activity. A. At pH 7.0 enzyme was incubated in 0.8 M KH_2PO_4/KOH, pH 7.0 (○———○), or in the same buffer with 50 mM D-alanine added (●———●). B. At pH 5.6 enzyme was incubated in 0.8 M KH_2PO_4/KOH, pH 5.6 (○———○), or in the same buffer with 50 mM D-alanine added (●———●). The enzyme was also incubated in 0.04 M KH_2PO_4/KOH, 50 mM D-alanine, pH 5.6 (■———■).

TABLE III

TRANSAMINATION FROM SOME AMINO ACIDS TO 2-OXOGLUTARATE

The rates of pyruvate, 2-oxobutyrate or oxaloacetate formation were directly determined spectrophotometrically.

Amino acid donor	Relative activity
D-alanine	100
L-alanine	5
α-D-aminobutyrate	62
D-aspartate	82
L-aspartate	5

TABLE IV

PROMOTION OF D-ALANINE AMINOTRANSFERASE DISSOCIATION BY AMINO COMPOUNDS

Aliquots of enzyme were incubated in 40 mM KH_2PO_4/KOH, pH 5.6 at 30°C for 40 min with the additions indicated, then assayed (± pyridoxal-*P*) and % dissociation calculated.

Compound (40 mM)	% dissociation
None	7
Glycine	1
D-alanine	92
L-alanine	6
D-glutamate	90
L-glutamate	8
D-aspartate	88
α-D-aminobutyrate	89
D-norleucine	79
1,6-diaminohexane	35

acid aminotransferase from *B. sphaericus* [13]. D-amino acid aminotransferase from pea seedlings [7], however, showed highest activity with D-aspartate and 2-oxoglutarate.

Dissociation of the apoenzyme and coenzyme

Dissociation of the holoenzyme into apoenzyme and coenzyme was promoted by the amino acid substrate, D-alanine at pH 7.0 and pH 5.6 (Fig. 3, A & B). At pH 5.6 a high phosphate concentration alone was sufficient to promote considerable dissociation but the most efficient dissociation was induced by the combination of D-alanine, low pH, and high phosphate concentration. The efficiencies with which a range of amino and amino acid compounds promoted dissociation at low pH (5.6) and low phosphate concentration (40 mM) were determined (Table IV). Efficient substrates were efficient promoters of dissociation but norleucine, though a poor substrate, was an efficient promoter. Diaminohexane also caused significant dissociation but glycine had no effect. The inability of L-amino acids to promote dissociation indicates the stereo-specificity of pyridoxamine formation at the active site [14].

Absorption spectra

The absorption spectra of L-alanine aminotransferases from rat liver [12] and pig heart [15] are characterized by prominent absorption peaks at 335 and 425 nm. The peak at 425 nm decreases in intensity with increasing pH, while the peak at 335 nm increases, especially in the enzyme from rat liver. On the other hand the absorption spectra of D-alanine aminotransferase from *B. subtilis* [5] and *B. sphaericus* [13] were experimentally indistinguishable when recorded over the pH range 5.2—9.2. These spectra had peaks at 325—330 nm and 415 nm. Spectra at pH 6.90 of the partially purified D-amino acid aminotransferase from *R. japonicum* exhibited an absorption peak between 410 and 420 nm, and at 325 nm there was a slight shoulder adjacent to the protein peak. When the pH was increased to 8.9 the absorbance near 315 nm was reduced, whereas absorbance at 325 nm was not significantly altered (Fig. 4A). As shown in Fig. 4B, addition of D-alanine to the enzyme solution at pH 6.90 had a more pro-

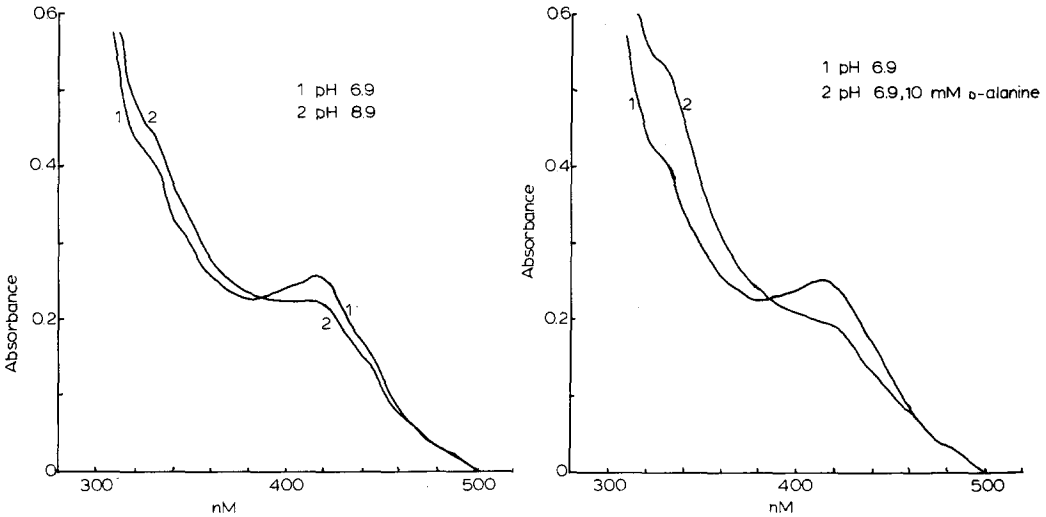

Fig. 4. Absorption spectra of D-amino acid aminotransferase. A. Curve 1. Enzyme was analysed in 0.5 ml of 50 mM KH_2PO_4/KOH, 5 mM mercaptoethanol, pH 6.90. Curve 2. A spectrum of the same solution after the pH had been increased to pH 8.90 by the addition of 10 μl of 1 M Na_2CO_3. B. Curve 1. Enzyme in 0.5 ml of 100 mM KH_2PO_4/KOH, 5 mM mercaptoethanol, pH 6.95. Curve 2. The same solution after addition of 5 μl of 1 M D-alanine (final concentration 10 mM D-alanine).

nounced effect than increasing pH; the absorbance at 415 nm was markedly decreased and the shoulder at 320—330 nm became more pronounced (Fig. 4B). These changes accord with the supposition that the enzyme-pyridoxamine phosphate complex absorbs at 325 nm [6].

Dissociation constant for pyridoxal phosphate

The resolved enzyme (i.e. apoenzyme), was preincubated with a range of pyridoxal-*P* concentrations and the reaction velocity determined at high D-alanine and 2-oxoglutarate concentrations. Analysis of these data by fitting to a hyperbola gave K_m = 0.51 ± 0.06 μM (± S.E.). The K_m for pyridoxal-*P* with D-amino acid aminotransferase from *B. sphaericus* is 2.3 μM [13].

Michaelis constants for D-alanine and 2-oxoglutarate

The initial velocity of the reaction was measured with excess pyridoxal-*P* and different concentrations of D-alanine and 2-oxoglutarate. As shown in Fig. 5A and B the results are consistent with a bi-reactant ping-pong mechanism. The Michaelis constants for D-alanine and 2-oxoglutarate are 2.13 ± 0.18 (S.E.) and 0.058 ± 0.005 mM respectively.

Inhibition by substrates

D-glutamate inhibited the transfer by the enzyme of amino groups from D-alanine to 2-oxoglutarate. When the 2-oxoglutarate concentration was varied at high D-alanine concentration (Fig. 6A), D-glutamate inhibition was non-competitive, K_{ii} and K_{is} values being 29.6 mM ± 2.3. (± S.E.) and 33.40 ± 9.0 mM respectively [16]. In contrast, at high 2-oxoglutarate concentration and with varying concentrations of D-alanine the inhibition by D-glutamate was compe-

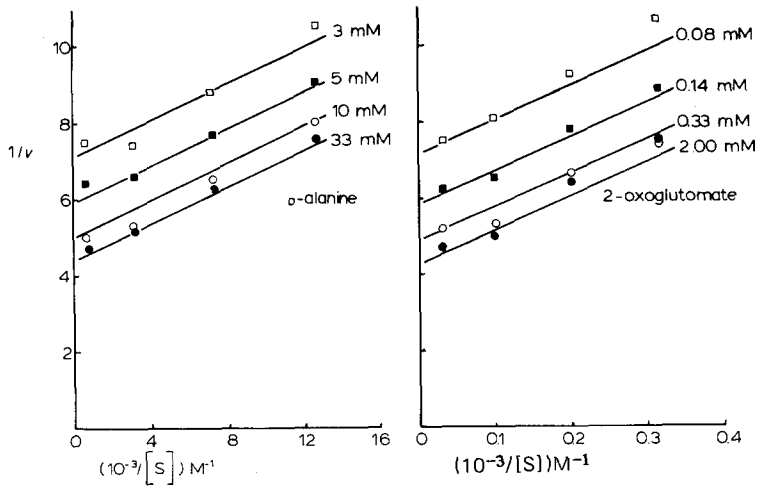

Fig. 5. Double reciprocal plots of initial velocity measurements for D-amino acid aminotransferase at various concentrations of 2-oxoglutarate and D-alanine. The same data is used in both graphs, A and B. A. The concentration of 2-oxoglutarate was varied at 3 (□———□), 5 (■———■), 10 (○———○) or 33 mM (●———●) D-alanine. B. The concentration of D-alanine was varied at 0.08 (□———□), 0.14 (■———■), 0.33 (○———○) and 2.00 (●———●).

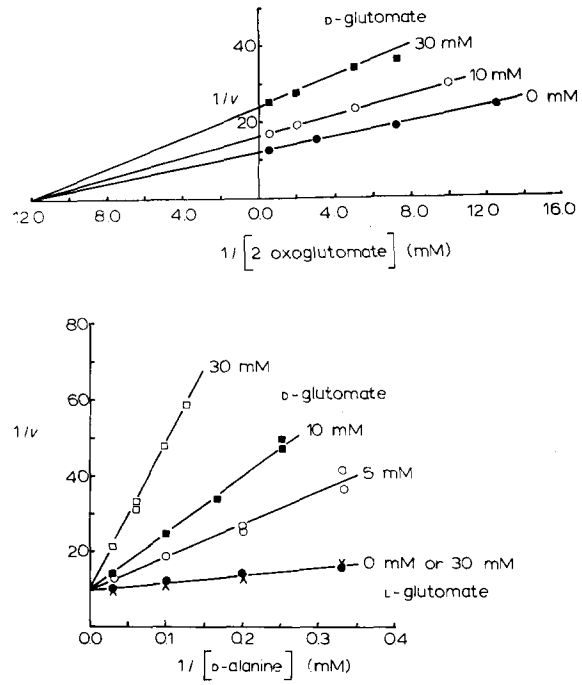

Fig. 6. Double reciprocal plots of initial velocity measurements for D-amino acid aminotransferase in the presence of various concentrations of the product D-glutamate, A. At 33 mM D-alanine the concentration of 2-oxoglutamate was varied at 0, (●———●) 10, (○———○) or 30 mM D-glutamate (■———■). B. At 2.0 mM 2-oxoglutarate the concentration of D-alanine was varied at 0, (●———●) 5, (○———○) 10, (■———■) or 30 mM D-glutamate (□———□) and at 30 mM L-glutamate (X———X).

titive (Fig. 6B) with an inhibition constant of 1.56 ± 0.09 mM. These results also are suggestive of a ping-pong reaction mechanism for the present D-amino acid aminotransferase. No inhibition by 30 mM L-glutamate was detectable (Fig. 6B), which demonstrates the sterospecificity that exists in initial binding to the active site of the enzyme. In common with aspartate aminotransferase [17] chloride inhibited the D-amino acid aminotransferase competitively with 2-oxoglutarate (K_i for KCl = 13.9 ± 2.5 (±S.E.) mM. In all the aforementioned kinetic studies the assay mixtures were buffered with 20 mM KH_2PO_4/KOH because when 100 mM Tris · HCl was employed anomolous plots were obtained.

Inhibition by p-chloromercuribenzoate

Incubation of D-amino acid aminotransferase with *p*-chloromercuribenzoate (4 mM) caused about 30% inhibition and this was not significantly increased by extending the time of incubation to 30 min. D-alanine (33 mM) protected the enzyme against inhibition when the incubation period was about 1 min but it did not protect if incubation was continued for 30 min. L-alanine aminotransferase [12,18] and D-amino acid aminotransferase from *B. sphaericus* [13] were strongly inhibited by sulphydryl reagents whereas Martinez-Carrion and Jenkins [6] were unable to detect inhibition of D-alanine aminotransferase from *B. subtilis* by *p*-chloromercuribenzoate or other sylphydryl reagents even after incubation for 30 min in the absence of substrates.

Discussion

Among the notable properties of the enzyme purified and characterized in this paper is that, in common with other D-amino acid aminotransferases [3,5,7], it has a relatively broad substrate specificity when compared with mammalian L-alanine aminotransferase [15]. For instance, the bacterial D-amino acid aminotransferases will actively transfer amino groups from α-D-aminobutyrate, D-alanine and D-glutamate to their 2-oxoacid equivalents [5,13]. The enzyme from pea seedlings is also very active with the aforementioned substrates (except the pair α-D-aminobutyrate and 2-oxoglutarate) but has optimal activity with D-aspartate and 2-oxoglutarate [7].

Like other D-amino acid aminotransferases [5—7,13] the present enzyme also displays considerable sterospecificity. For instance, the enzyme was not inhibited by L-glutamate or dissociated to apoenzyme and cofactor by L-alanine or L-glutamate, in contrast with the results obtained with the D-isomers of these amino acids. An interesting finding was that compounds which were inefficient substrates for the enzyme such as D-norleucine promoted dissociation of the holoenzyme probably by condensing with pyridoxal-*P* bound to the enzyme. The dissociation of the holoenzyme by 1,6-diaminohexane is similar to results previously obtained [19] with L-aspartate aminotransferase from the same organism used in the present studies.

As regards the function of the present enzyme, the roles of alanine racemase and D-amino acid aminotransferase in bacterial cell wall synthesis are recognised [20] but there is at present no apparent role for such enzymes in higher plants. D-alanine has been found in pea seedling tissues [7] and the catalysis of

D-amino acid metabolism by maize plants [21] and by enzyme preparations from plants [4,7,22,23] has been reported. An aminotransferase specific for D-methionine has been partially purified from germinating peanut seeds [23]. Ogawa et al. [7] were unable to detect alanine racemase in pea seedlings although D-amino acid aminotransferase was present. It is difficult therefore to ascertain the function of D-amino acid aminotransferase in plants.

The roles of both alanine racemase and D-amino acid aminotransferase activities in *R. japonicum* and in the soluble and bacteroid fractions of soybean root nodules infected with *R. japonicum* is also of interest in the context of the present studies. One possible role is in the biosynthesis of bacteroid cell walls. Another possibility is in the metabolism of D-amino acids during bacterium-plant symbiosis.

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