Effects of D-Serine on Bacterial D-Amino Acid Transaminase: Accumulation of an Intermediate and Inactivation of the Enzyme†

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ABSTRACT: Incubation of pure bacterial D-amino acid transaminase with D-serine or *erythro*-β-hydroxy-DL-aspartic acid, which are relatively poor substrates, leads to generation of a new absorbance band at 493 nm that is probably the quinonoid intermediate. The 420-nm absorbance band (due to the pyridoxal phosphate coenzyme) decreases, and the 338-nm absorbance band (due to the pyridoxamine phosphate or some other form of the coenzyme) increases. A negative Cotton effect at 493 nm in the circular dichroism spectra is also generated. Closely related D amino acids do not lead to generation of this new absorption band, which has a half-life of the order of several hours. Treatment of the enzyme with the good substrate D-alanine leads to a small but detectable amount of the same absorbance band. D-Serine but not *erythro*-β-hydroxyaspartate leads to inactivation of D-amino acid transaminase, and D-alanine affords partial protection. The results indicate that D-serine is a unique type of inhibitor in which the initial steps of the half-reaction of transamination are so slow that a quinonoid intermediate with a 493-nm absorption band accumulates. A derivative formed from this intermediate inactivates the enzyme.

Information on the mechanism of an enzyme can sometimes be obtained by studies on quasi-substrates if significant amounts of intermediates accumulate. For example, L-aspartate transaminase acts on the quasi-substrate *erythro*-β-hydroxy-L-aspartate to generate significant amounts of a quinonoid type of intermediate with a characteristic absorbance around 490 nm (Jenkins, 1961, 1964; Metzler et al., 1988). This intermediate turns over very slowly in either the presence or absence of α-ketoglutarate. The threo isomer of β-hydroxy-L-aspartic acid, on the other hand, does not form detectable amounts of this intermediate. The reason for the different behavior of these two stereoisomers with the enzyme is not known.

It has been assumed that the mechanism of the reaction of D-amino acid transaminase may be related to that of L-aspartate transaminase but of opposite stereospecificity. However, there are few concrete facts to support this idea. Therefore, in an effort to detect spectral bands that could impart information on the mechanism of D-amino acid transaminase, we have examined the effects on the visible spectrum of the enzyme caused by those D amino acids that are relatively poor substrates; D amino acids that are good substrates for the enzyme (Tanizawa et al., 1989a,b) generate a very efficient spectral shift from 420 nm, corresponding to the pyridoxal phosphate internal aldimine structure, to 338 nm, which represents the pyridoxamine form (ketimine) of the enzyme. We have found that with one of the mutants of D-amino acid transaminase in which Trp-139 was changed to a Phe residue this spectral shift was not very efficient presumably because the Trp residue originally at this position was involved in maintaining the geometry of the coenzyme PLP[1] at the active site (Martinez del Pozo et al., 1989a). Using the technique of measuring the efficiency of amino acids in facilitating the spectral shift, we have also found that some L amino acids will undergo the half-reaction with D-amino acid transaminase to form the pyridoxamine form of the enzyme and the corresponding α keto acid. However, during the second half of the reaction, i.e., the formation of a new D amino acid from a keto acid and the PMP form of the enzyme, only D amino acids are formed. Thus, the overall stereochemical fidelity of the enzyme is maintained (Martinez del Pozo et al., 1989b).

There have been few mechanistic studies on D-amino acid transaminase probably because of the sparse quantity of pure enzyme available. However, recently the gene encoding for this enzyme has been cloned and expressed in *Escherichia coli* (Tanizawa et al., 1989a,b), and large amounts of pure enzyme can now be prepared. In the present paper we report further studies on the spectral effects of a good substrate, D-alanine, and of two relatively poor substrates, *erythro*-β-hydroxyaspartate and D-serine.

Materials and Methods

D-Serine, D-alanine, L-aspartate transaminase, and β-hydroxypyruvate (Li salt) were purchased from Sigma. D-Serine and D-alanine were judged pure by elemental analysis (kindly performed by Robert Buzolich of this institution) and by amino acid analysis. The results described were obtained with two different batches of D-serine. Samples of the erythro and threo isomers of β-hydroxy- and β-hydroxy-β-methylaspartic acid were kindly provided by Dr. W. T. Jenkins. Other amino acids tested were from various commercial sources.

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[1] Abbreviations: PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; EDTA, ethylenediaminetetraacetic acid.

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Protein Purification. D-Amino acid transaminase was purified to homogeneity by the procedures described by Asano (1987), Stoddard et al. (1987), and Tanizawa et al. (1989a,b), with minor modifications (Merola et al., 1989). Before use, each purified protein was dialyzed against a convenient buffer, as indicated below, to remove excess PLP. Amino acid analysis, performed in a Beckman Model 6300 amino acid analyzer, was used to calculate the protein concentration.

Enzyme Assays. Enzyme activity was determined by measuring the rate of pyruvate production from D-alanine and α-ketoglutarate at pH 8.5 and 37 °C. Pyruvate was determined either by an assay employing NADH and lactate dehydrogenase or with salicylaldehyde, as described previously (Martinez-Carrion & Jenkins, 1965; Soper et al., 1977; Jones et al., 1985). One unit of enzyme activity is defined as the amount of protein that catalyzes the formation of 1 μmol of keto acid/min. Specific activity is defined as units per milligram of protein. PLP was not added in these assays since nonspecific and reversible inhibition of enzyme activity was found in the presence of excess PLP as reported previously by Martinez-Carrion and Jenkins (1965) and by us (Martinez del Pozo et al., 1989a). After extensive dialysis of the purified protein to remove excess coenzyme, 1 PLP/subunit was retained on the protein (Merola et al., 1989) as determined by the method of Wada and Snell (1961). This amount of PLP per protein subunit provided maximal enzyme activity.

The activity of L-aspartate transaminase was monitored at 37 °C by recording the production of oxalacetate from L-aspartate and α-ketoglutarate with malate dehydrogenase (Karmen, 1955; Chen et al., 1987).

Spectroscopic Characterization. Absorption spectra were recorded at 25 °C on a Cary 15 UV–vis spectrophotometer with a recording speed of 0.5 nm/s in cells with an optical path of 1.0 cm. Circular dichroism (CD) spectra were obtained on an Aviv-modified Cary 60 dichrograph at a scanning speed of 0.2 nm/s. Cells with an optical path of 1.0 cm were used as described previously (Martinez del Pozo et al., 1989a). The results of the circular dichroism studies are expressed as molar ellipticities, in units of deg cm^2 dmol^{-1}, using 32 000 as the molecular weight per enzyme subunit (Tanizawa et al., 1989a). The protein concentration was 1–2 mg/mL.

Results

Effect of D Amino Acids on the Spectral Properties of D-Amino Acid Transaminase. Using the spectral shift from 420 to 338 nm as a screening procedure, we have studied a variety of D amino acids that had been determined previously to be poor substrates for the transaminase (Tanizawa et al., 1989a). The maximum change in absorbance at 420 nm caused by the good substrate D-alanine (20 mM) was taken as 100% spectral change. The objective was to determine whether any of the poor substrates led to a different spectral band that might be due to accumulated intermediate(s). The results of this survey are summarized in Table I. In most cases the relative efficiency of each amino acid as a substrate did not correlate with the relative degree of spectral shift from 420 to 338 nm. Furthermore, in some cases there was a substantial spectral shift in the presence of L amino acids but absolutely no enzymic activity.

For two of the amino acids tested, D-serine and a racemic mixture of *erythro*-β-hydroxyaspartate, significant amounts of a 493-nm absorption band were observed. Small but detectable amounts of this absorption band were also found with D-alanine when fairly large amounts of enzyme (3–4 mg/mL) were used. Of particular significance was the observation that none of the amino acids that closely resemble D-serine, i.e., D-threonine, D-allothreonine, D-homoserine, *O*-methyl-D-serine, and D-cysteine, were able to generate the 493-nm absorbing species (Table I).

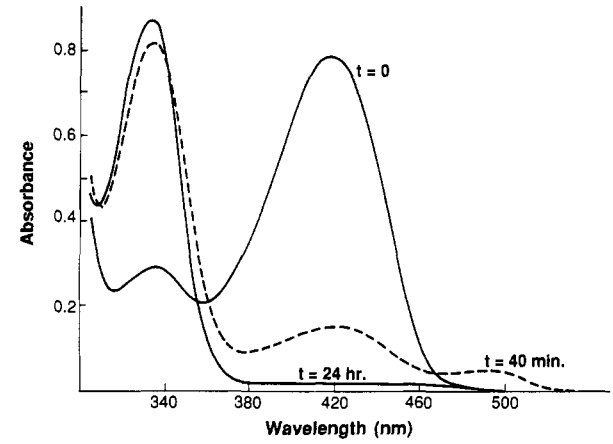

FIGURE 1: Spectral changes of D-amino acid transaminase before and after addition at room temperature of D-alanine. The buffer used was 0.1 M Tris-HCl (pH 7.5) containing 2 mM EDTA. The overnight incubation was for 24 h. The concentration of enzyme was 3.38 mg/mL, and the concentration of D-alanine was 0.1 M.

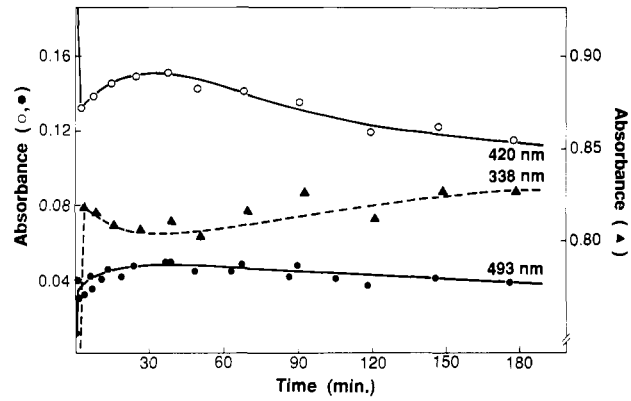

FIGURE 2: Kinetics of spectral changes of D-amino acid transaminase induced by D-alanine. The conditions are the same as those described in the legend to Figure 1. The wavelengths plotted are 493 (●), 420 (○), and 338 nm (▲).

Effects of D-Alanine on the Spectrum of D-Amino Acid Transaminase. The effect of D-alanine on the spectrum of native, wild-type D-amino acid transaminase is shown in Figure 1. The efficiency of D-alanine in promotion of the spectral shift from 420 nm (due to the PLP form of the enzyme) to 338 nm (due to the PMP form of the enzyme) has been observed previously to be rapid and efficient (Martinez del Pozo et al., 1989a). In the experiment shown in Figure 1, fairly large amounts of enzyme and an expanded scale were employed to detect a small amount of a spectral band with an absorbance near 493 nm. We have tentatively assigned this band to the quinonoid intermediate of the coenzyme–substrate complex by analogy with L-aspartate transaminase (Jenkins, 1961, 1964: Chen et al., 1987: Metzler et al., 1988).

Kinetics of Spectral Changes Produced by D-Alanine on D-Amino Acid Transaminase. The kinetics of formation of A_{493} band and the disappearance of the other absorbance bands are shown in Figure 2. A small, steady-state concentration of this intermediate was reached within 40 min and persisted over a fairly relatively long period of time (Figure 2). Most of the enzyme was rapidly converted to the PMP form absorbing at 338 nm. In contrast to the results with D-serine (see below), the amount of this species was relatively low, and after overnight exposure to D-alanine, none of either the 493- or the 420-nm absorbance bands remains (Figure 1).

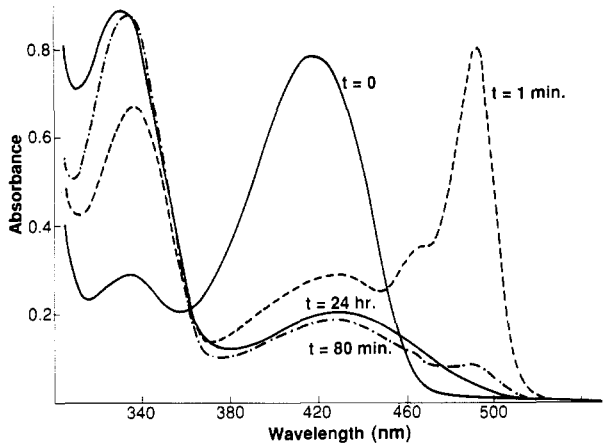

FIGURE 3: Spectra of D-amino acid transaminase before and after addition of D-serine. The enzyme concentration was 3.38 mg/mL in 0.1 M Tris (pH 7.5) containing 2 mM EDTA. The final concentration of D-serine was 0.1 M. The times indicated were representative of those when the most significant changes were noted. All the other conditions are the same as those described in Figure 1.

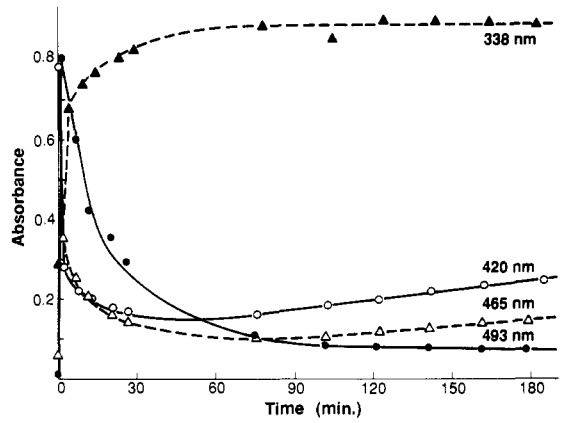

FIGURE 4: Kinetics of spectral changes induced by D-serine. The changes at three different absorbance bands observed after the addition of D-serine (0.1 M) to a solution of enzyme (3.38 mg/mL). The conditions employed were the same as those described in Figure 3. The wavelengths chosen had the most significant changes and are 493 (●), 420 (○), 338 (▲), and 465 nm (△).

Effect of D-*Serine on the Spectrum of* D-*Amino Acid Transaminase.* The spectrum of D-amino acid transaminase taken 1 min after mixing with D-serine is different from that found with D-alanine (Figure 3). In addition to the usual PLP (420 nm) and PMP (338 nm) absorbance bands, there is a large new absorbance band present at 493 nm with a shoulder at 465 nm. Equivalent amounts of pure enzyme were used in the spectral studies with D-alanine and D-serine (Figures 1 and 3, respectively) so that the relative amounts of 493-nm bands formed with each substrate could be readily compared. However, with D-serine the 493-nm band can also be observed with as little as 0.1 mg/mL of enzyme. This 493-nm band, which closely resembles that found with L-aspartate transaminase and *erythro*-β-hydroxy-L-aspartate (Jenkins, 1961, 1964), has tentatively been assigned to the presence of a quinonoid-type intermediate. However, final proof of structure for the intermediate formed with D-amino acid transaminase must await its isolation and characterization. *erythro*-β-Hydroxy-DL-aspartate also results in formation of the same spectral changes as D-serine.

Kinetics of Spectral Changes of D-*Amino Acid Transaminase upon Addition of* D-*Serine.* As shown in Figure 4 the putative quinonoid intermediate that absorbs at 493 nm has a half-life that can be readily measured. Thus, the

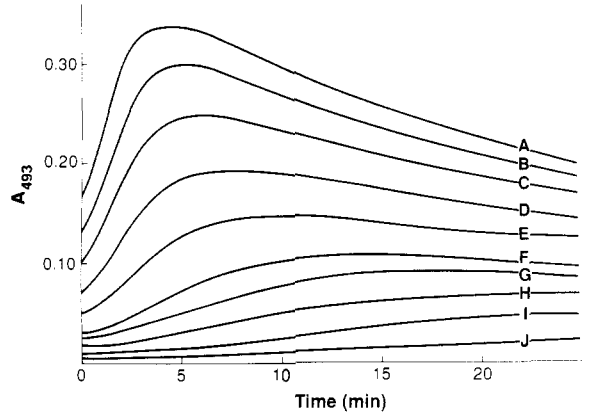

FIGURE 5: Effect of varying concentration of enzyme on the absorbance band at 493 nm. The absorbance at 493 nm is plotted versus time for a fixed concentration of D-serine (0.1 M) and different amounts of D-amino acid transaminase. Protein concentrations: (A) 1.24, (B) 1.09, (C) 0.93, (D) 0.78, (E) 0.62, (F) 0.47, (G) 0.39, (H) 0.31, (I) 0.23, and (J) 0.16 mg/mL.

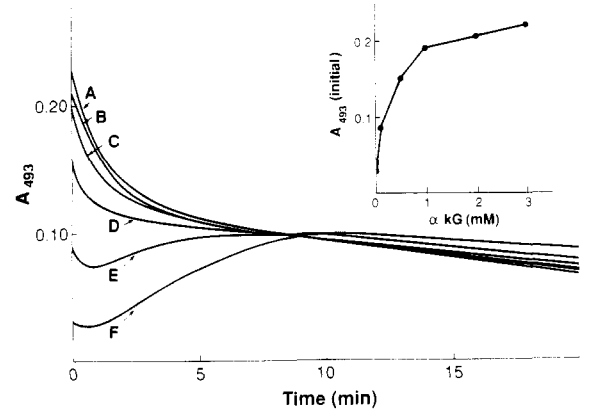

FIGURE 6: Effect of α-ketoglutarate on the rate of formation and amount of the 493-nm absorbing species. The absorbance at 493 nm is plotted versus time for fixed concentrations of D-amino acid transaminase (0.47 mg/mL) and D-serine (0.1 M) and variable amounts of α-ketoglutarate: (A) 3.0, (B) 2.0, (C) 1.0, (D) 0.5, and (E) 0.1 mM; (F) no α-ketoglutarate. (Inset) Initial absorbance at 493 nm just after the addition of D-serine plotted versus α-ketoglutarate concentration.

maximal amount of the intermediate occurs in about 1 min under these particular conditions, and then it slowly disappears over the next few hours. Concurrently, after the initial rapid changes, steady-state levels of the PLP (420 nm) and the PMP (338 nm) forms of the enzyme persist. After an overnight incubation (approximately 24 h) at room temperature, the band in the 420-nm wavelength range formed with D-serine does not completely disappear (Figure 3) as it does with D-alanine (Figure 1). This band is now centered at 430 nm, which indicates that it represents a species different from the original PLP form of the enzyme. The amount of quinonoid-like species formed is dependent on the initial concentration of D-serine up to a concentration of 50 mM at a constant enzyme concentration (0.5 mg/mL) (data not shown). When the concentration of D-serine is maintained constant at 0.1 M and the amount of enzyme is increased over a 10-fold range, the initial amount of quinonoid species and the rate of its formation increase slowly (Figure 5).

Effect of Keto Acid Cosubstrates on the Formation of the 493-nm Species. As shown in Figure 6, the presence of α-ketoglutarate has a significant effect on the initial rate of formation of the 493-nm absorbing material but not on the steady-state amount. At initial times in the presence of α-ketoglutarate, there is an increased amount of 493-nm species,

Scheme I

(1) D-Ala + E-PLP ⇌ E-PLP-Ala ⇌ Quinonoid Intermediate[1] ⇌ E-PMP-Pyr ⇌ E-PMP + Pyr
(internal aldimine) (external aldimine) (A_{493}) (ketimine)

(2) D-Ser + E-PLP ⇌ E-PLP-Ser ⇌ Quinonoid Intermediate[2] ⇌ E-PMP-βOH-Pyr ⇌ E-PMP + β-OH-Pyr
(internal aldimine) (external aldimine) (A_{493}) (ketimine)

↓

(E-D-Ser-Quinonoid Derivative)

↓

E (inactive)

Table I: Comparison of Relative Activity, Spectral Shift, and Presence of 493-nm Absorbance Band with Various Amino Acids and D-Amino Acid Transaminase

amino acid	relative activity[a] (%)	spectral shift[b] (%)	493-nm absorbance band
D-Ser	1.2	80	+++
DL-*erythro*-β-OH-Asp[c]	nd[h]	92	+++
D-α-aminobutyric acid	98.0	100	–
D-α-*O*-Me-Ser	nd	79	–
D-Ala	100.0	100	+[g]
D-Asp	60.0	93	–
D-cyclo-Ser	nd	100	–
D-Cys	0.2	95	–
D-Glu	58.0[d]	100	–
D-His	1.9	90	–
DL-Hse[c]	nd	100	–
D-penicillamine	nd	100	–
D-Thr	<0.1	89	–
D-allo-Thr	nd	90	–
DL-α-Me-Ser[c]	nd	7	–
DL-Hcy	nd	97	–
DL-Ser *O*-phosphate[c]	nd	5	–
DL-*erythro*-β-OH-β-Me-Asp[c]	nd	23	–
DL-*threo*-β-OH-β-Me-Asp[c]	nd	34	–
DL-*threo*-β-OH-Asp[c]	nd	67	–
L-α-aminobutyric acid	nd	0	–
L-Ala	*f*	62	–
L-Asp	0	21	–
L-cyclo-Ser	nd	*e*	–
L-Glu	0	25	–
L-Hse	nd	51	–
L-Ser	0	18	–
L-Thr	0	50	–
L-allo-Thr	nd	2	–
α-aminoisobutyrate	nd	2	–
α,β-diaminobutyrate	nd	100	–

[a] From Tanizawa et al. (1989a) with 25 mM α-ketoglutarate as the acceptor. [b] With 20 mM of amino acid, the spectral shift is that from 420 to 338 nm with the 100% value taken as the amount of change found with 20 mM D-Ala. The protein concentration was 0.5–1.0 mg/mL. [c] 40–45 mM concentration of amino acid was used. [d] Determined with 25 mM α-ketobutyrate as acceptor (Tanizawa et al., 1989a). [e] L-cyclo-Ser caused a complete spectral shift from 420 to 385 nm. [f] Estimated to undergo the half-reaction of transamination about 3000 times slower than D-Ala (Martinez del Pozo et al., 1989b). [g] Detectable only with relatively high concentrations of enzyme (4 mg/mL). [h] nd = not detectable.

and this amount declines to the same steady-state value at each keto acid concentration. In this study lower concentrations of enzyme were used than employed in Figure 3 to make the spectral changes easier to follow. The concentration dependence of α-ketoglutarate in the formation of the 493-nm absorbing species indicates a saturation function for α-ketoglutarate (Figure 6, inset). The effect of α-ketoglutarate is most likely due to accelerated turnover of the PMP form of the enzyme to regenerate more of the PLP form, which reacts with D-serine to form more of the quinonoid intermediate (see Scheme I). Eventually, after 24 h, the 493-nm spectral band completely disappears, indicating a very slow turnover.

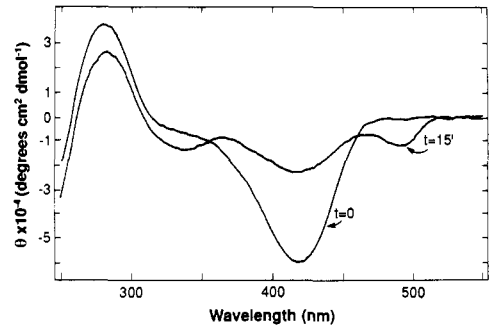

FIGURE 7: Circular dichroism spectra of D-amino acid transaminase in the presence of 20 mM D-serine. Two different times (0 and 15 min) are shown. The buffer was 0.1 M Tris-HCl (pH 7.5) containing 2 mM EDTA.

Effect of pH on the Rate of Formation of the Quinonoid-like Species. The amount of quinonoid species that accumulates and the rate of its formation are also a function of the pH (data not shown). Thus, at low pH values its rate of formation is slow, and the total amount is low. However, as pH values approach 8, both the rate of formation and the amount formed are increased until, at pH values above 8, these amounts are constant (data not shown). When these data are plotted, the midpoint of the line corresponds to an apparent pK_a of about 6.8. The relevance of this particular value is unknown at the present time, but it could constitute the pK_a value of an important amino acid side chain at the active site of the enzyme.

Effect of D-Serine on the Circular Dichroism Spectrum of D-Amino Acid Transaminase. As shown in Figure 7, treatment of the transaminase with D-serine generates a significant negative Cotton effect at 493 nm in the visible circular dichroism spectrum. At the same time there is a decreased negative Cotton effect at 420 nm. These changes correspond to the absorption changes described above upon the addition of D-serine to native D-amino acid transaminase (Figure 3).

Effect of D-Serine on Activity of D-Amino Acid Transaminase. D-Serine not only promotes spectral changes in D-amino acid transaminase but also leads to inactivation of this enzyme. As shown in Figure 8, enzyme activity is slowly

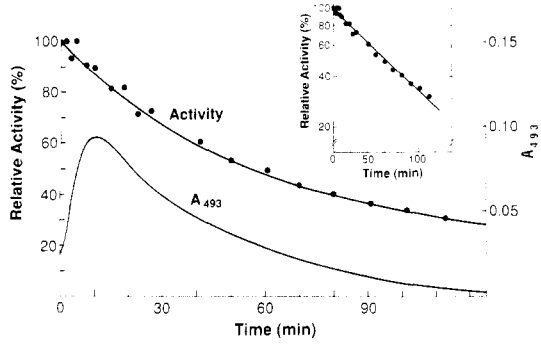

FIGURE 8: Relationship between inactivation of the enzyme and amount of 493-nm absorbing species. The generation of absorbance at 493 nm, its disappearance, and inactivation kinetics of the enzyme are plotted. This last measurement is shown as the percentage of activity remaining as a function of time. The concentration of D-serine was 0.1 M, and the enzyme concentration was 0.47 mg/mL in 0.1 M Tris-HCl (pH 7.5) containing 2 mM EDTA. (Inset) Semilogarithmic representation of the activity of the enzyme in the presence of D-serine versus time.

lost in the presence of a relatively high concentration of D-serine. The rate of loss in enzyme activity is the same whether the lactate dehydrogenase or the salicylaldehyde assay is used. The rate of disappearance of the 493-nm absorption band parallels inactivation of the enzyme (Figure 8). The inactivation by D-serine follows pseudo-first-order kinetics (Figure 8, inset) with a half-life of about 1 h under these conditions. Addition of 3.3 μM PLP to the assay mixture did not result in reactivation of the enzyme, suggesting that the inactivation was not due to removal of the coenzyme. The inactivated enzyme does not regain any activity after overnight dialysis against 0.1 M Tris-HCl (pH 7.5) buffer containing 2 mM EDTA, and the spectral bands at 338 and 430 nm are still present (also see studies below on spectrum of the inactive enzyme).

D-Serine undergoes turnover to yield a compound (presumably β-hydroxypyruvate) that is a substrate for lactate dehydrogenase. Under these conditions D-serine is only about 5% as effective a substrate as D-alanine with D-amino acid transaminase. The presence of D-alanine (0.1 M) provides significant but not complete protection against inactivation by D-serine. The protection afforded by D-alanine at early times of incubation is slowly overcome so that by 24 h the inactivation is complete. The profile of inactivation and protection is consistent with the suggestion that D-serine acts as an enzyme-activated inhibitor of D-amino acid transaminase.

β-Hydroxypyruvate is also capable of reacting with the PMP form of the enzyme to generate the same spectral changes and inactivation of the enzyme. When D-alanine is added to the PLP form of the enzyme, the PMP form of the enzyme (338 nm) is formed. Addition of β-hydroxypyruvate to the *PMP form* leads to generation of the 493-nm band, consistent with the formation of D-serine through transamination of β-hydroxypyruvate and D-alanine. At the initial time of formation of this band, the enzyme is fully active. The slow disappearance of the band at 493 nm parallels the subsequent inactivation of the enzyme as described above with D-serine.

erythro-β-Hydroxy-DL-aspartate does *not* inactivate the enzyme, even though the spectral changes are practically identical with those generated by D-serine. We have also found that *erythro*-β-hydroxy-DL-aspartic acid does not inhibit L-aspartate transaminase under conditions where a very intense absorption band centered at 490 nm is observed, as described by Jenkins (1961, 1964). In addition, neither D- nor L-serine induces any spectral change or inactivation of L-aspartate transaminase, which has a more restricted substrate specificity than D-amino acid transaminase; i.e., L-serine is apparently not a substrate for L-aspartate transaminase, not even a poor one.

Spectral Properties of the Inactive Enzyme. D-Amino acid transaminase (3.5 mg/mL) inactivated by D-serine as described above was applied to a Sephadex G-25 (fine) column (2 × 38 cm) equilibrated in 50 mM potassium phosphate (pH 7.4). Gel filtration in this system has been shown to successfully resolve the apoenzyme from the phenylhydrazine-modified coenzyme (Wada & Snell, 1961). The protein, which eluted in the void volume of the column, retained spectroscopic features identical with those shown in Figure 3 for the enzyme incubated with D-serine for 24 h and it was still completely inactive. The A_{338}/A_{280} ratio, which was 0.21 for the protein after inactivation, was between 0.17 and 0.20 for the fractions containing the protein peak. Measurement of the absorbance at 280 nm indicated that 91% of the protein loaded was recovered and 79% of the coenzyme eluted in the same position as measured by the absorbance at 338 nm. No coenzyme was eluted in any other area of the column. These results indicate that the inactivation of the enzyme by D-serine is not simply due to the loss of some form of the coenzyme or the coenzyme–substrate complex.

DISCUSSION

The results in the present paper are consistent with the suggestion that the mechanism of the transamination of D amino acids catalyzed by bacterial D-amino acid transaminase is likely to be similar to that of L-aspartate transaminase but of opposite stereospecificity. However, the negative Cotton effect at 490 nm in the circular dichroism spectrum of L-aspartate transaminase induced by the quasi-substrate *erythro*-β-hydroxyaspartate (Torchinsky & Braunstein, 1979; Metzler et al., 1978) is similar both in sign and wavelength to that generated in D-amino acid transaminase by D-serine. The significance of these findings in terms of the relative optical specifities of these enzymes is not known at present and must await the results of the structural studies currently in progress (Stoddard et al., 1987).

The equations shown in Scheme I are a working hypothesis to explain some of the findings in the present paper. D-Serine is a relatively poor substrate for bacterial D-amino acid transaminase compared with D-alanine, a good substrate (Tanizawa et al., 1989a). Whether or not this is due to a difference in binding or in some catalytic step is not known. Nevertheless, it is clear from the data in the present paper that with both substrates an enzyme-bound component with a spectral band at 493 nm can be detected, but with D-serine and *erythro*-β-hydroxyaspartate there is significantly more of this species than with D-alanine. By analogy with the spectral changes found with L-aspartate transaminase and *erythro*-β-hydroxyaspartate (Jenkins, 1961, 1964) this component is tentatively assigned the structure of a quinonoid intermediate. It is likely that this quinonoid intermediate has a much higher extinction coefficient than either the PLP or PMP form of the enzyme, but the exact value is not presently known. With D-alanine (Scheme I, eq 1) a low steady-state concentration of the putative quinonoid intermediate is attained rather quickly (Figure 1) and most of the enzyme is in the PMP form, consistent with the known efficiency of D-alanine as a substrate. After overnight exposure to D-alanine, there is no detectable 493-nm band. In contrast, with the same amount of enzyme in the presence of D-serine, there is significantly more of the component(s) absorbing in the 490–500-nm region. This component is formed rapidly and slowly disappears concom-

itant with inactivation of the enzyme. It is conceivable that with D-serine the presence of a β-hydroxyl group stabilizes the intermediate so that more of it accumulates and less of the ketimine is formed. Hence, turnover of D-serine would be less efficient. After accumulation of significant amounts of the quinonoid intermediate, the data in Figure 8 show that it slowly disappears at a rate comparable to inactivation of the enzyme. This result could be explained by hydrolysis of this quinonoid intermediate formed with D-serine to generate a released quinonoid derivative that would subsequently react with and inactivate the enzyme. Although the structure of this putative highly reactive intermediate is not known, one possibility is that it is formed by β-elimination of the β-hydroxyl group of the quinonoid derivative formed with D-serine. Such an event would serve to distinguish between the reactivity of the different types of quinonoid formed with D-serine and D-alanine; with the latter substrate no such β-elimination would occur. These possibilities are currently being evaluated through studies with a radiolabeled substrate.

We also noted that there is accumulation of a compound with a similar spectral band at 493 nm when the substrate is *erythro*-β-hydroxyaspartic acid, but there is no inactivation of the enzyme. This finding is analogous to that found earlier with the same substrate and L-aspartate transaminase (Jenkins, 1961, 1964). It is conceivable that with *erythro*-β-hydroxyaspartic acid there is no release of the quinonoid intermediate from the active site, nor would β-elimination be favored because the pseudo-substrate may be stabilized at the active site by its β-carboxyl group, which may have a binding pocket on the enzyme. These possibilities are reminiscent of the effects of two related compounds on L-aspartate transaminase. Thus, this enzyme catalyzes only β-elimination of *threo*-β-chloro-L-glutamate without inactivation (Manning et al., 1968), but with L-serine *O*-sulfate both β-elimination and inactivation take place (John & Fasella, 1969; Ueno et al., 1982). Evidence for a quinonoid type of intermediate and no inactivation has also been obtained in another pyridoxal phosphate dependent enzyme system, serine transhydroxymethylase (Schirch & Jenkins, 1964). This enzyme, for the most part, acts on L amino acids but it also reacts with D-alanine (Hopkins & Schirch, 1986; Shostak & Schirch, 1988).

In the reaction with D-serine there would be *slow* formation of both the quinonoid and ketimine (PMP) form of the coenzyme. Treatment with α-ketoglutarate in the presence of D-serine leads to more rapid initial formation of the quinonoid intermediate (Figure 6) because the PMP form of the enzyme is converted to the PLP form, which in turn reacts with D-serine to produce more of the 493-nm band. The result supports the suggestion in Scheme I. α-Ketoglutarate also accelerates the rate at which the equilibrium concentration of quinonoid is attained (Figure 6). However, all of the quinonoid disappears after 24 h.

The concept that certain amino acids may be toxic to a particular organism has been appreciated for some time. The type of inhibition reported here may be similar to that found earlier on the effect of L-serine on L-serine deaminase (Wood & Gunsalus, 1949). It has been known for many years that D-serine inhibits the growth of many different types of bacteria (Maas & Davis, 1951). It has been suggested that it does so by interfering with amino acid transport. The results in the present paper indicate that it is also possible that D-serine inhibits bacterial growth by acting as an inhibitor of bacterial D-amino acid transaminase, an enzyme that is a target for the development of novel antimicrobial agents (Soper & Manning, 1985).

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Registry No. D-Ser, 312-84-5; DL-*erythro*-β-OH-Asp, 6532-76-9; D-Ala, 338-69-2; D-amino acid transaminase, 50864-42-1.

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