Interaction of L-Aspartate β-Decarboxylase with β-Chloro-L-alanine. β-Elimination Reaction and Active-Site Labeling*

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ABSTRACT: L-Aspartate β-decarboxylase from *Alcaligenes faecalis* catalyzes the conversion of β-chloro-L-alanine into pyruvate, ammonia, and chloride ions. This reaction is accompanied by a slower reaction in which the enzyme becomes inactivated and in which the 3-carbon chain moiety of β-chloroalanine is covalently bound to the enzyme. Close to 1 mole of β-chloroalanine carbon is bound per 60,000 g of enzyme. The data indicate that about 30% of the dialyzed alkylated enzyme is susceptible to decarboxylation by ceric sulfate and that about 20% to decarboxylation by ninhydrin indicating the presence of α-keto and α-amino acid derivatives, *i.e.*, HOO-CCOCH₂-enzyme and HOOCCHNH₂CH₂-enzyme. Similar results were obtained when the enzyme was incubated with *threo*-β-chloro-L-α-aminobutyrate. Incubation of the apoenzyme or of the 4'-deoxypyridoxine 5'-phosphate-enzyme with β-chloroalanine does not lead to inactivation or binding

$\mathbf{I}$n addition to the β decarboxylation of L-aspartate to form L-alanine, L-aspartate β-decarboxylase catalyzes the desulfination of L-cysteinesulfinate (Soda *et al.*, 1964) and transamination between various α-amino and α-keto acids (Novogrodsky *et al.*, 1963; Novogrodsky and Meister, 1964). The enzyme also decarboxylates *threo-* and *erythro*-β-hydroxy-L-aspartate; in the course of this reaction an inactive complex is formed, probably an oxazolidine derivative between the decarboxylation product of β-hydroxyaspartate and the vitamin B₆ cofactor (Miles and Meister, 1967). All of these reactions proceed through an initial step which involves labilization of the proton on the α-carbon atom of the amino acid substrate.

We now report the finding that β-chloro-L-alanine is a substrate for L-aspartate β-decarboxylase; the enzyme catalyzes a β-elimination reaction yielding pyruvate, ammonia, and chloride ion. This relatively rapid catalytic reaction is accompanied by a slower reaction in which the enzyme is irreversibly inactivated. Inactivation is associated with alkylation of the enzyme at a site near or at its active center.

Experimental Section

Materials

L-Aspartate β-decarboxylase was isolated from *Alcaligenes faecalis* (strain N) (ATCC 25094) as described previously (Tate and Meister, 1968).

β-Chloro-L-alanine and β-chloro-D-alanine were obtained from Cyclo Chemical Corp. Uniformly labeled L-serine-¹⁴C (Schwarz BioResearch, Inc.,) and L-serine-3-¹⁴C (ICN Corp.) were converted *via* the methyl ester hydrochloride to the corresponding β-chloro-L-alanine-¹⁴C hydrochlorides by the procedure of Fischer and Raske (1907). *threo*-β-Chloro-L-α-aminobutyric acid was prepared in analogous fashion from L-threonine.

Lactate dehydrogenase (rabbit muscle) and DPNH were obtained from Sigma Chemical Co.

Methods

L-Cysteinesulfinate desulfinase activity was determined as described (Tate and Meister, 1968). Pyruvate was determined by the method of Friedemann and Haugen (1943), and also by use of lactate dehydrogenase and DPNH. In the latter procedure 10 μg of lactate dehydrogenase and 0.15 μmole of DPNH were added per ml of reaction mixture and the absorbance at 340 mμ was recorded with time. Chloride was determined by the Volhard method (Treadwell and Hall, 1942). Ammonia was determined by direct Nesslerization.

The binding of β-chloro-L-alanine-¹⁴C to the enzyme was determined, unless otherwise stated, as follows. Aliquots of mixtures containing the enzyme, β-chloroalanine-¹⁴C, and sodium acetate buffer were added to the top of a column of Sephadex G-25 (1 × 35 cm) and the enzyme was eluted with 0.05 M sodium acetate buffer (pH 5.5). The fractions obtained were analyzed for protein (from the absorbance at 280 mμ), radioactivity, and cysteinesulfinate desulfinase activity.

Thin-layer chromatography was carried out on silica gel coated plastic sheets (Brinkmann Instruments, Inc.) with a solvent consisting of 1-butanol–acetic acid–water (45:5:12.5, v/v); in this solvent β-chloroalanine exhibits an R_F value of 0.40.

Spectrophotometric studies were carried out with a Cary Model 15 recording spectrophotometer equipped with a water-jacketted cuvet compartment.

Studies on the decarboxylation of the alkylated enzyme were carried out as follows. The holoenzyme (1 mg/ml) was incubated at 37° for 40 min in 0.2 M sodium acetate buffer (pH 5.5) containing 10 mM β-chloro-L-alanine-¹⁴C, after which the solution was exhaustively dialyzed against several changes of 0.05 M sodium acetate buffer (pH 6.0). Aliquots (0.1 ml) of the dialyzed solution were mixed with 0.1 ml of a saturated solution of ceric sulfate in 2 M sulfuric acid in a tightly stoppered bottle. Prior to stoppering the two solutions were kept separate by a ridge in the bottle. After mixing, the carbon dioxide evolved was absorbed into 0.05 ml of ethanol-
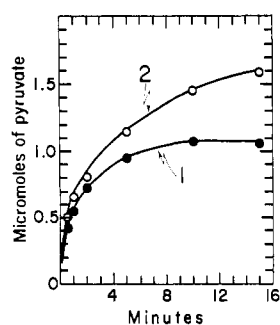
FIGURE 1: Enzymatic formation of pyruvate from β-chloro-L-alanine. The reaction mixtures (1 ml) contained β-chloro-L-alanine (1 mM, curve 1; 10 mM, curve 2), enzyme (100 μg), and 0.1 M sodium acetate buffer (pH 5.5); incubated at 37°. Pyruvate was determined by the method of Friedemann and Haugen (1943).

FIGURE 2: Effect of β-chloro-L-alanine concentration on the initial rate of pyruvate formation. The reaction mixtures contained β-chloro-L-alanine, aspartate β-decarboxylase (25 μg), 0.05 M potassium phosphate buffer (pH 7.0), DPNH (0.15 μmole), and lactate dehydrogenase (10 μg); final volume, 1 ml; 37°. The inset shows a double-reciprocal plot of the data. (Abscissa, $10^{-3}/S$).

amine placed on a glass rod suspended in the center of the bottle. The bottle was slowly rotated at 25° for 4 hr and the ethanolamine was then transferred into 10 ml of liquid scintillation medium and counted.

Treatment with ninhydrin was carried out in the following manner. Aliquots (0.1 ml) of the enzyme solution were mixed with 0.4 ml of 0.2 M sodium citrate buffer (pH 2.2) containing 50 mg of ninhydrin. The solution was placed in a boiling-water bath for 15 min during which time the CO_2 evolved was flushed out with nitrogen and bubbled into two traps connected in series; each trap contained 10 ml of liquid scintillation medium and 0.1 ml of ethanolamine. The decarboxylation reactions with ceric sulfate and ninhydrin were standardized and controlled by carrying out determinations with authentic samples of sodium pyruvate-1-^{14}C and alanine-1-^{14}C, respectively.

Results

Conversion of β-Chloro-L-Alanine into Pyruvate, Cl^-, and Ammonia. When aspartate β-decarboxylase was incubated with 0.001 M β-chloro-L-alanine, this amino acid was completely converted into pyruvate (Figure 1, curve 1). Under the same conditions but with 0.01 M β-chloro-L-alanine, the formation of pyruvate decreased rapidly and came to a stop when only a small fraction of the added substrate was utilized (Figure 1, curve 2); this suggested that the enzyme is inactivated under these conditions. The formation of pyruvate was confirmed in these studies by preparation and paper chromatography of the corresponding 2,4-dinitrophenylhydrazone by the procedure of El Hawary and Thompson (1953). Thin-layer chromatography of the reaction mixture used in the experiment described in Figure 1 (curve 2) after incubation for 15 min revealed only a single ninhydrin-positive compound which exhibited an R_F value characteristic of β-chloroalanine.

As indicated by the data given in Table I the conversion of β-chloro-L-alanine into pyruvate is accompanied by equimolar formation of Cl^- and ammonia.

The pH dependence of the reaction was determined over the range 5.5–8.0. The reaction proceeded most rapidly at pH 7.5; the velocity at pH 5.5 was about 75% of that at pH 7.5.

The effect of β-chloro-L-alanine concentration on the initial rate of pyruvate formation was determined at pH 7.0 in a system containing lactate dehydrogenase and DPNH; the rate of
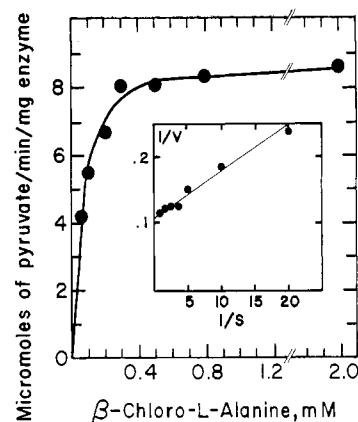
disappearance of reduced pyridine nucleotide was measured as described under Methods (Figure 2). When the data of Figure 2 were plotted in the double-reciprocal manner, a straight line was obtained; $V_{max} = 9.0$ μmoles of pyruvate/min per mg of enzyme; $K_m = 6.3 \times 10^{-5}$ M.

Incubation of the enzyme with 0.01 M β-chloro-D-alanine did not lead to formation of pyruvate nor was inactivation (see below) observed.

Inactivation of Aspartate β-Decarboxylase by β-Chloro-L-alanine. When the enzyme was incubated with 10 mM β-chloro-L-alanine, there was a very rapid loss of cysteinesulfinate desulfinase activity (Figure 3, curve 1). Attempts to restore activity by (a) gel filtration through a column of Sephadex G-25, (b) extensive dialysis against 0.05 M sodium acetate buffer (pH 5.5), or (c) treatment with pyridoxal 5′-phosphate, were not successful. Similar treatment of the apoenzyme with β-chloro-L-alanine followed by gel filtration and reconstitution with pyridoxal 5′-phosphate did not lead to loss of activity (Figure 3, curve 3). It is thus evident that pyridoxal 5′-phosphate is required for inactivation.

TABLE I: Conversion of β-Chloro-L-Alanine into Pyruvate, Cl^-, and Ammonia by L-Aspartate β-Decarboxylase.[a]

Incubn Period (min)	Product (μmoles)		
	Pyruvate	Cl^-	NH_3
1	0.60	0.62	0.70
5	1.1	1.2	1.2
10	1.3	1.4	1.5

[a] The reaction mixtures contained β-chloro-L-alanine (4 mM), enzyme (200 μg), and 0.1 M sodium acetate buffer (pH 5.5) in a final volume of 1 ml; 37°. Pyruvate was determined by the method of Friedemann and Haugen (1943); Cl^- and NH_3 were determined as described under Methods.

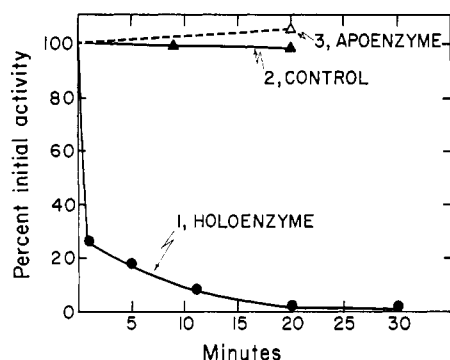FIGURE 3: Inactivation of aspartate β-decarboxylase by β-chloro-L-alanine. Curve 1: the holoenzyme (100 μg) was incubated at 37° in 0.2 ml of 0.25 M sodium acetate buffer (pH 5.5) containing 10 mM β-chloro-L-alanine; aliquots (10 μl) were removed for determination of cysteinesulfinate desulfinase activity. Curve 2: the experiment was the same as for curve 1, except that β-chloro-L-alanine was omitted; 37°. Curve 3: the apoenzyme (810 μg) was incubated in 1 ml of 0.25 M sodium acetate buffer (pH 6.0) containing 10 mM β-chloro-L-ananine for 20 min at 37°; the mixture was then added to the top of a Sephadex G-25 column (1 × 35 cm) and the enzyme was eluted with 0.05 M sodium acetate buffer (pH 6.0). The cysteinesulfinate desulfinase activity was determined after reconstitution with pyridoxal 5'-phosphate (Tate and Meister, 1968).

TABLE II: Effect of Dialysis and Other Procedures on the ¹⁴C Bound by the β-Chloro-L-alanine-¹⁴C-Treated Enzyme.

Experiment	¹⁴C/mg of Enzyme (cpm)
A. ¹⁴C-Labeled enzyme obtained by gel filtration (from expt in Figure 5)	1200
B. An aliquot of A was incubated with 0.01 M β-chloroalanine-¹²C for 1 hr at 37° and then dialyzed extensively against acetate buffer	1100
C. An aliquot of A (0.5 ml) was brought to 100° for 2 min. The denatured protein was separated by centrifugation, dissolved in 50 μl of hyamine, and then mixed with 10 ml of liquid scintillation medium.	900
D. An aliquot of A (0.5 ml) was mixed with 1 ml of 20% trichloroacetic acid. After standing at 0° for 10 min, the denatured protein was separated by centrifugation and its radioactivity was determined.	1300

Inactivation of the enzyme by β-chloro-L-alanine is associated with a marked change in absorbance spectrum (Figure 4). Thus, the maximum at 355 mμ exhibited by the holoenzyme (curve 1) is shifted to about 320 mμ (curve 2) after incubation with β-chloro-L-alanine. When the β-chloro-L-alanine-treated enzyme (Figure 4, curve 2) was incubated with 1 mM sodium α-ketoglutarate there was no change in the spectrum. When the β-chloro-L-alanine-treated enzyme was passed through a column of Sephadex G-25 and then dialyzed against 1000 volumes of 0.05 M sodium acetate buffer (pH 5.5) at 5°
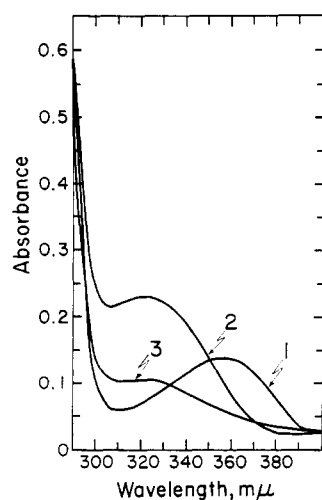

FIGURE 4: Spectral changes associated with inactivation of L-aspartate β-decarboxylase by β-chloroalanine. Curve 1: spectrum of the holoenzyme (1 mg/ml in 0.25 M sodium acetate buffer; pH 5.5). Curve 2: spectrum of the holoenzyme (as in curve 1) 10 min after addition of 10 mM β-chloro-L-alanine. Curve 3: spectrum of the β-chloro-L-alanine-treated enzyme (curve 2) after passage through a Sephadex G-25 column followed by extensive dialysis as described in the text.

for 18 hr, an inactive form of the enzyme was obtained which exhibited a spectrum (Figure 4, curve 3) intermediate between that of the treated holoenzyme and the apoenzyme (Novogrodsky and Meister, 1964; Wilson and Meister, 1966). This finding indicates that gel filtration and dialysis of the β-chloroalanine-treated enzyme resulted in loss of some of the cofactor; similar treatment of the holoenzyme does not lead to loss of the enzyme-bound pyridoxal 5'-phosphate. After the β-chloro-L-alanine-treated apoenzyme (Figure 4, curve 3) was incubated with an excess of pyridoxal 5'-phosphate (followed by dialysis) the spectrum was somewhat similar to that of the holoenzyme (curve 1) and exhibited a broad maximum between 330 and 350 mμ; an estimate based on fluorimetric titration (Tate and Meister, 1969) indicated that about 0.7 mole of pyridoxal 5'-phosphate was bound per 60,000 g of enzyme.[1] The inactivated enzyme (after dialysis) is capable of binding α-keto acid. Thus, experiments carried out as previously described (Tate and Meister, 1969) with 4 × 10⁻⁴ M α-ketoglutarate-¹⁴C and 1.1 mg of enzyme indicated binding of 0.67 mole of keto acid/60,000 g of enzyme.

Electrophoresis of the β-chloro-L-alanine-treated enzyme (after gel filtration and dialysis) in polyacrylamide gel at pH 8 revealed that about 70% of the material migrated as the 19S species; the remainder exhibited the mobility of the 6S species. Previous studies have shown that, under these conditions (pH 8, 25°), the apoenzyme exists entirely in the 6S form (Tate and Meister, 1968, 1969).

Binding of ¹⁴C to the Enzyme after Incubation with β-Chloroalanine-¹⁴C. The data given in Figure 5 were obtained in an experiment in which the enzyme was incubated with uniformly labeled β-chloro-L-alanine-¹⁴C; the enzyme was then precipitated by adding ammonium sulfate to 60% of

[1] Recent studies (Bowers et al., 1968) indicate that the molecular weight of the enzyme is close to 720,000 and that it is composed of 12 subunits of molecular weight of about 60,000.

TABLE III: Decarboxylation of the β-Chloro-L-alanine-^{14}C-Treated Enyzme with Ceric Sulfate and with Ninhydrin.[a]

Enzyme Treated	Total ^{14}C in Enzyme (cpm)	$^{14}CO_2$ Released (cpm)		
		With Ce(SO$_4$)$_2$ (A)	With Ninhydrin (B)	Sum
β-Chloro-L-alanine-1-^{14}C	1195	360[b]	224	584
β-Chloro-L-alanine-3-^{14}C	4260	60	0	60

[a] The enzyme was incubated with either β-chloro-L-alanine-1-^{14}C or β-chloro-L-alanine-3-^{14}C; after dialysis, samples of the alkylated enzyme were decarboxylated by treatment with ceric sulfate (4 hr) or ninhydrin (15 min) (see Methods).
[b] No further release of $^{14}CO_2$ occurred after 19 hr.

FIGURE 5: Binding of ^{14}C to the enzyme after incubation with β-chloro-L-alanine-^{14}C. The enzyme (15 mg) was incubated in 10 ml of 0.25 M sodium acetate buffer (pH 5.5) containing 10 mM β-chloro-L-alanine-^{14}C (14 $\times$ 10^6 cpm) for 2 hr at 37°. After cooling to 0°, solid ammonium sulfate was added to obtain 60% of ammonium sulfate saturation, and the precipitated protein was collected by centrifugation. It was then dissolved in 1.5 ml of 0.05 M sodium acetate buffer (pH 5.5) and this solution was added to the top of a column (1.5 $\times$ 60 cm) of Sephadex G-25; elution was carried out with 0.05 M sodium acetate buffer (pH 5.5). Fractions of 1.8 ml were collected. The absorbance of these fractions at 280 mμ and their radioactivity were measured.

saturation and it was then dissolved in acetate buffer and subjected to gel filtration. The recovered enzyme contained an amount of ^{14}C equivalent to about 0.96 mole of β-chloro-L-alanine-^{14}C/60,000 g of inhibited enzyme. The ^{14}C-labeled enzyme (Table IIA) was incubated with an excess of β-chloro-L-alanine-^{12}C and then dialyzed for 48 hr against two changes of 1000 volumes of 0.05 M sodium acetate buffer (pH 5.5). As indicated in Table IIB there was no significant loss of radioactivity from the enzyme after dialysis; the spectrum of the dialyzed enzyme was identical with that shown in Figure 4 (curve 3). Heating the enzyme preparation (A) at 100° for 2 min did not release a very large fraction of the bound radioactivity (Table IIC). Precipitation of the ^{14}C-labeled enzyme (A) with trichloroacetic acid was not accompanied by loss of bound ^{14}C (Table IID).

Incubation of the enzyme with 10 mM uniformly labeled β-chloro-L-alanine-^{14}C led to greater than 90% inhibition within 10 min and, under these conditions, the binding of ^{14}C to the enzyme approached a value of 1 mole/60,000 g of enzyme (Figure 6). In this experiment and also in one in which the concentration of β-chloro-L-alanine was varied (Figure 7), both the binding of ^{14}C to the enzyme and inactivation were parallel within experimental error. The agreement of the data obtained with uniformly labeled β-chloro-L-alanine-^{14}C (Figure 6) with those obtained with β-chloro-L-alanine-3-^{14}C (Figure 7) indicates that the 3-carbon chain of β-chloro-L-alanine is bound to the enzyme.

When the enzyme was incubated with β-chloro-L-alanine-1-^{14}C followed by dialysis (cf. Figure 4; curve 3), and then treated with a saturated solution of ceric sulfate in 2 M sulfuric acid, approximately one-third of the radioactivity was liberated as ^{14}C-labeled carbon dioxide (Table III). Treatment of the inactivated enzyme with ninhydrin led to the release of about 20% of the radioactivity as ^{14}C-labeled carbon dioxide. Thus, close to 50% of the radioactivity of the inactivated enzyme was released by treatment with ceric sulfate and ninhydrin. Although the data indicate the presence of both α-amino and α-keto acid derivatives of the enzyme,
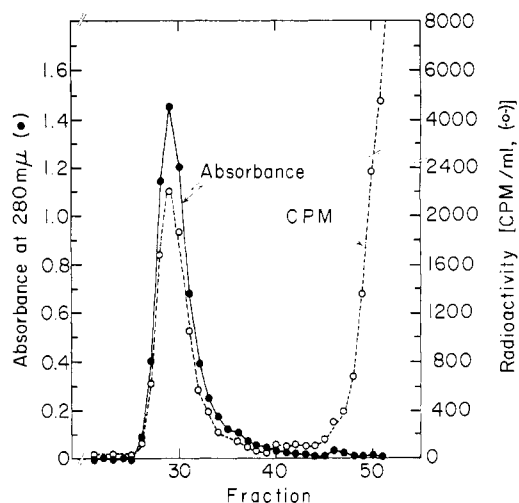
the presence of an additional form (or forms) of the enzyme that is not susceptible to decarboxylation under these conditions also seems indicated. When enzyme inactivated in the same way by incubation with β-chloro-L-alanine-3-^{14}C was treated with ceric sulfate less than 2% of the radioactivity was released as $^{14}CO_2$ and no radioactivity was released on treatment with ninhydrin.

Studies in which the p-mercuribenzoate derivative of the enzyme (Tate and Meister, 1968) was incubated with β-chloro-L-alanine gave results which were the same as those
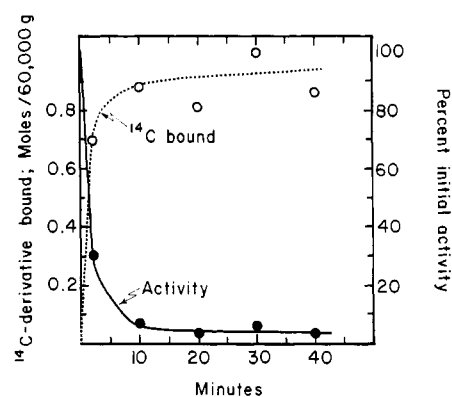

FIGURE 6: Effect of incubation of the enzyme with 10 mM β-chloro-L-alanine-^{14}C on binding of ^{14}C to the enzyme and on activity. The enzyme (1 mg) was incubated at 37° in 1 ml of 0.25 M sodium acetate buffer (pH 5.5) containing 10 mM uniformly labeled β-chloro-L-alanine-^{14}C. At the intervals indicted, the reaction was terminated by applying the reaction mixture to the top of a Sephadex G-25 column and elution was carried out as described in Figure 5.
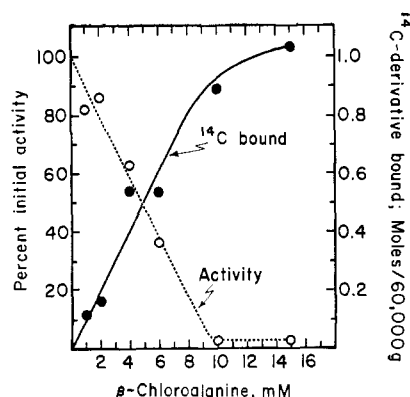
FIGURE 7: Effect of β-chloro-L-alanine-^{14}C concentration on the binding of ^{14}C to the enzyme and on activity. The enzyme (1 mg) was incubated for 20 min at 37° with β-chloro-L-alanine-3-[14]C (in the concentrations indicated) in 0.2 M sodium acetate buffer (pH 5.5) in a final volume of 1 ml. Aliquots of the reaction mixtures were assayed for cysteinesulfinate desulfinase activity. The binding of ^{14}C to the enzyme was determined after extensive dialysis against 0.05 M acetate buffer (pH 5.5).

FIGURE 9: Proposed pathways for the conversion of β-chloro-L-alanine to pyruvate, ammonia, H+, and Cl-, and for the alkylation of the enzyme (see the text).

observed with the holoenzyme (Figure 3; curve 1). When the apoenzyme (1 mg) was incubated in a reaction mixture (1 ml) containing 0.05 M sodium acetate buffer (pH 6) and 10 mM β-chloro-L-alanine-^{14}C for 20 min at 37°, there was no loss in enzyme activity (tested in the presence of added pyridoxal 5'-phosphate), nor was ^{14}C bound to the enzyme. Treatment of the 4'-deoxypyridoxine 5'-phosphate–enzyme (Tate and Meister, 1969) with 10 mM β-chloro-L-alanine-^{14}C under the same conditions (pH 6, 37°, 20 min) did not result in binding of ^{14}C to the enzyme.

Interaction of the Enzyme with threo-β-Chloro-L-α-aminobutyrate. Incubation of the enzyme with *threo*-β-chloro-L-α-aminobutyrate led to results qualitatively similar to those observed with β-chloro-L-alanine. Thus, α-keto acid (identified as α-ketobutyrate by chromatography of the 2,4-dinitrophenylhydrazone derivative (El Hawary and Thompson, 1953)) was formed from *threo*-β-chloro-L-α-aminobutyrate (Figure 8). α-Keto acid formation took place at about 10%

of the rate observed with β-chloro-L-alanine. The enzyme was also inactivated during the course of incubation, but at a rate substantially less than that observed with β-chloro-L-alanine.

Discussion

The experiments reported here show that β-chloro-L-alanine is a substrate for aspartate β-decarboxylase; as indicated in Table IV, the enzyme exhibits a somewhat higher affinity for β-chloro-L-alanine than for L-aspartate and L-cysteinesulfinate. The conversion of β-chloro-L-alanine into pyruvate, ammonia, and chloride and hydrogen ions (eq 1)

$$\text{ClCH}_2\text{CHCOO}^- + \text{H}_2\text{O} \longrightarrow \text{CH}_3\text{CCOO}^- + \text{NH}_4^+ +$$
$$\underset{+}{\overset{|}{\text{NH}_3}} \qquad \qquad \overset{\|}{\text{O}}$$

$$\text{H}^+ + \text{Cl}^- \quad (1)$$

is evidently an α,β-elimination reaction of the type observed by Metzler *et al.* (1954) in nonenzymatic systems. Thus, loss
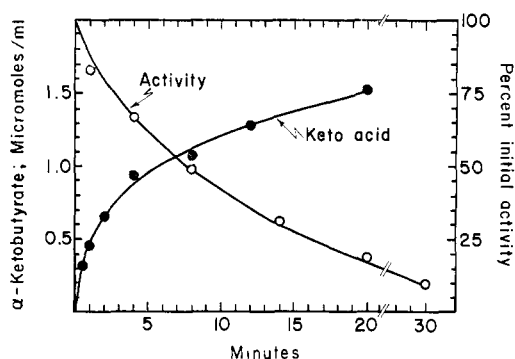

FIGURE 8: Interaction of *threo*-β-chloro-L-α-aminobutyrate with aspartate β-decarboxylase. The reaction mixtures (1 ml) contained 0.2 M sodium acetate buffer (pH 5.5), 0.0033 M *threo*-β-chloro-L-α-aminobutyrate, and 0.9 mg of enzyme; incubated at 37°. Aliquots were withdrawn for the determination of α-ketobutyrate (method of Friedemann and Haugen, 1943) and of cysteinesulfinate desulfinase activity.
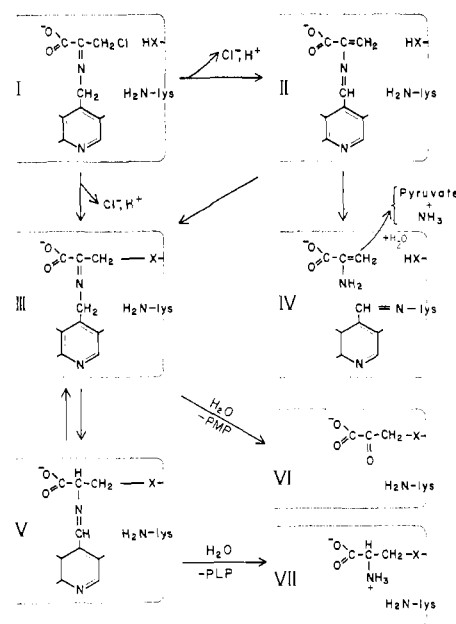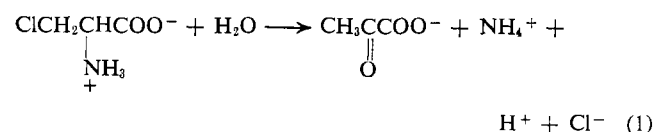
TABLE IV: Activities of L-Aspartate β-Decarboxylase.

Reaction Catalyzed	K_m (M)	$V_{max}{}^a$
L-Aspartate $\rightarrow$ L-alanine + CO_2	6.8×10^{-4}	122
L-Cysteinesulfinate $\rightarrow$ L-alanine + SO_2	4.8×10^{-3}	99.0
β-Chloro-L-alanine $\rightarrow$ pyruvate + Cl- + H+ + NH4+	6.3×10^{-5}	9.0

^{a} In micromoles per minute per milligram of enzyme.

of chloride from the ketimine Schiff base (Figure 9, I) would yield the aldimine of α-aminoacrylate (II), hydrolysis of which would give pyruvate, H$^+$, ammonia, and the pyridoxal–enzyme (IV). This reaction is therefore analogous to those catalyzed by such enzymes as tryptophanase (Newton and Snell, 1964; Newton et al., 1965; Morino and Snell, 1967), serine and threonine dehydrases, cystathionase, allinase, and β-tyrosinase (for reviews, see Snell, 1958, Braunstein, 1960, and Meister, 1965). It is of interest that the L-serine dehydrase of Streptococcus rimosus catalyzes the deamination of β-chloroalanine to form pyruvate (Szentirmai and Horvath, 1962), and that this reaction is also catalyzed by rat liver preparations (Gregerman and Christensen, 1956), which do not appear to exhibit aspartate β-decarboxylase activity. Evidence that glutamate–aspartate transaminase from pig heart catalyzes dehydrofluorination and deamination of β-fluoroaspartate has been reported by Kun et al. (1960), and Manning et al. (1968) have found that this enzyme catalyzes β elimination of both the threo and erythro isomers of β-chloroglutamate to yield chloride, ammonia, and α-keto-glutarate. It thus appears that a number of vitamin B$_6$ enzymes can catalyze β-elimination reactions of the type observed here with substrates that possess electronegative substituents on the β-carbon atom.

L-Aspartate β-decarboxylase does not interact with cysteine, serine, or S-methylcysteine, but does combine with cysteinesulfinate (as a substrate) and with β-cyanoalanine (an inhibitor; Tate and Meister, 1969) and asparagine (an inhibitor; S. S. Tate and A. Meister, unpublished data). It is notable that all of the active compounds have an electronegative or negatively charged β substituent. The molecular models of these compounds exhibit interesting similarities; further study and analysis of these along lines employed in work on glutamine synthetase (Meister, 1968) may be useful.

A notable feature of the interaction between aspartate β-decarboxylase and β-chloro-L-alanine is the associated inactivation of the enzyme.[2] Such inactivation appears to require Schiff base formation; thus, incubation of the apoenzyme or of the 4'-deoxypyridoxine 5'-phosphate enzyme with β-chloro-L-alanine does not lead to either inactivation or binding of the analog. Inactivation is associated with binding to the enzyme of the 3-carbon chain moiety of β-chloro-L-alanine, and the evidence indicates that a covalent linkage is formed at a site very close to or at the active site of the enzyme. The findings also show that some of the vitamin B$_6$ cofactor can be readily removed from the inactivated enzyme. The inactivation process may be viewed as resulting from a nucleophilic attack by a group on the enzyme on the β-carbon atom of the substrate analog or the analogous α-aminoacrylate–Schiff base. In either case, structure III (Figure 9) would be formed. Hydrolysis of III followed by removal of pyridoxamine 5'-phosphate would lead to an α-keto derivative of the enzyme containing the 3-carbon chain of β-chloroalanine (VI). Tautomerization of III to the corresponding aldimine (V) followed by hydrolysis and loss

of pyridoxal 5'-phosphate would give the analogous α-amino derivative of the enzyme (VII). The data taken as a whole indicate that treatment of the holoenzyme with β-chloro-L-alanine results in formation of alkylated forms of the enzyme which retain some affinity for α-keto acid and vitamin B$_6$ cofactor but which are not able to bind aspartate. Further studies on the properties of the alkylated enzyme and in particular directed to determining the site of attachment to the enzyme are subjects of continued study in this laboratory. Such investigations may offer information about the amino acid structure of the enzyme at the active site.

References

Bowers, W. F., Czubaroff, V., and Haschemeyer, R. H. (1968), 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968, Abstract No. 177.

Braunstein, A. E. (1960), Enzymes 2, 113.

El Hawary, M. F. S., and Thompson, R. H. S. (1953), Biochem. J. 53, 340.

Fischer, E., and Raske, E. (1907), Ber. 40, 3717.

Friedemann, T. E., and Haugen, G. E. (1943), J. Biol. Chem. 147, 415.

Gregerman, R. I., and Christensen, H. N. (1956), J. Biol. Chem. 220, 765.

John, R. A., Fasella, P., Thomas, J. G., and Tudball, N. (1968), Proc. 5th Meeting Fed. European Biochem. Soc., 96.

Kun, E., Fanshier, D. W., and Grassetti, D. R. (1960), J. Biol. Chem. 235, 416.

Manning, J. M., Khomutov, R. M., and Fasella, P. (1968), European J. Biochem. 5, 199.

Meister, A. (1965), Biochemistry of the Amino Acids, Vol. 1, New York, N. Y., Academic, p 402.

Meister, A. (1968), Advan. Enzymol. 31, 183.

Metzler, D. E., Ikawa, M., and Snell, E. E. (1954), J. Am. Chem. Soc. 76, 648.

Miles, E. W., and Meister, A. (1967), Biochemistry 6, 1734.

Morino, Y., and Snell, E. E. (1967), J. Biol. Chem. 242, 2793.

Newton, W. A., Morino, Y., and Snell, E. E. (1965), J. Biol. Chem. 240, 1211.

Newton, W. A., and Snell, E. E. (1964), Proc. Natl. Acad. Sci. U. S. 51, 382.

Novogrodsky, A., and Meister, A. (1964), J. Biol. Chem. 239, 879.

Novogrodsky, A., Nishimura, J. S., and Meister, A. (1963), J. Biol. Chem. 238, PC 1903.

Phillips, A. T., Whanger, P. D., Rabinowitz, K. W., Shada, J. D., and Wood, W. A. (1968), in Pyridoxal Catalysis: Enzymes and Model Systems, Snell, E. E., et al., Ed., New York, N. Y., Interscience, p 549.

Snell, E. E. (1958), Vitamins Hormones 16, 77.

Soda, K., Novogrodsky, A., and Meister, A. (1964), Biochemistry 3, 1450.

Szentirmai, A., and Horvath, I. (1962), Acta Microbiol. Acad. Sci. Hung. 9, 23.

Tate, S. S., and Meister, A. (1968), Biochemistry 7, 3240.

Tate, S. S., and Meister, A. (1969), Biochemistry 8, 1056, 1660.

Treadwell, F. P., and Hall, W. T. (1942), Analytical Chemistry, Vol. II, New York, N. Y., Wiley, p 650.

Wilson, E. M., and Meister, A. (1966), Biochemistry 5, 1166.

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[2] Phillips et al. (1968) have described an apparently analogous inactivation of threonine dehydrase by L-serine, and John et al. (1968) have reported that glutamate–aspartate transaminase, which catalyzes conversion of L-serine O-sulfate into NH$_4^+$, SO$_4^{2-}$, H$^+$, and pyruvate, is inactivated when incubated with this substrate.