Regulation of Biodegradative Threonine Deaminase

III. EFFECTS OF β SUBSTITUENTS OF SUBSTRATE ON ABSORPTION SPECTRUM AND CIRCULAR DICHROISM OF THE ENZYME-BOUND PYRIDOXAL PHOSPHATE

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SUMMARY

In order to elucidate whether or not the spectral and circular dichroic changes observed during the biodegradative threonine deaminase reaction are caused by the Schiff base formation between the enzyme-bound pyridoxal phosphate and the dehydrated intermediate, the role of substituents and the absolute configuration of the β carbon of substrates was investigated using various analogs of L-threonine. The enzyme was found to catalyze the α,β elimination of L-allothreonine, DL-threo- and DL-erythro-β-hydroxynorvaline, DL-threo- and DL-erythro-β-phenylserine, DL-β-hydroxyvaline, L-β-chlorobutyrylamine, and L-β-chloroalanine in addition to L-threonine and L-serine. When L-threonine or L-β-chlorobutyrylamine was used as substrate, the absorption maximum of the enzyme-bound pyridoxal phosphate shifted from 415 nm to 434 and 442 nm, respectively, but it remained unchanged when L-allothreonine was used as substrate. L-β-Chloroalanine also caused a bathochromic shift of the absorption maximum, but L-serine did not induce such a shift. The positive circular dichroism of the enzyme-bound pyridoxal phosphate at 415 nm disappeared upon the addition of L-threonine or L-β-chloroalanine. On the other hand, L-β-chlorobutyrylamine did induce a negative circular dichroism during the reaction, and L-allothreonine slightly diminished the magnitude of the positive circular dichroism. These results indicate that the substituents as well as the absolute configuration of the β carbon of the substrate participate in the spectral and circular dichroic changes observed during the reaction. Thus, the shift of the absorption maximum and the circular dichroic change at 415 nm appear to occur in the step prior to the β elimination reaction. This conclusion was confirmed by a kinetic analysis of the spectral changes.

The biodegradative threonine deaminase of Escherichia coli (EC 4.2.1.16) catalyzes the α,β elimination of L-threonine and L-serine. The initial reaction is presumed to involve dehydration, which results in the formation of α-aminocrotonate and α-aminocrotylate, respectively (1, 2). These enamino acids are converted to their tautomers, α-iminobutyrate or α-iminocrotonate, which are then hydrolyzed to the corresponding α-keto acids and ammonia.

R-CH(OH)-CH(N walks into the reaction, and L-threonine slightly diminished the magnitude of the positive circular dichroism. These results indicate that the substituents as well as the absolute configuration of the β carbon of the substrate participate in the spectral and circular dichroic changes observed during the reaction. Thus, the shift of the absorption maximum and the circular dichroic change at 415 nm appear to occur in the step prior to the β elimination reaction. This conclusion was confirmed by a kinetic analysis of the spectral changes.

The enzyme exhibits an absorption maximum and a positive peak of circular dichroism at 415 nm due to the enzyme-bound pyridoxal phosphate (3, 4). The absorption maximum shifts to 434 nm with a concomitant loss of the CD when L-threonine is added to the enzyme solution (5). The resulting absorption maximum and CD return to the starting state when the substrate is consumed. Recently, Niedermaier et al. (6) reported that nometabolizable competitive inhibitors such as n-threo-nine and α-aminobutyric acid do not cause any spectral change but do cause a loss in CD. On the basis of this and other lines of evidence, they postulated that the new spectral species observed during the reaction is the Schiff base between α-aminocrotonate and pyridoxal phosphate, and a loss of the CD reflects the accumulation of Schiff bases between pyridoxal phosphate and L-threonine as well as with α-aminocrotonate.

The present study was undertaken to determine whether or not the spectral and circular dichroic changes are attributable to the Schiff base formation between α-aminocrotonate and pyridoxal phosphate. In this paper, evidence is presented that the enzyme shows a broad specificity for the alkyl substituent of β position of the substrate and that the spectral and circular dichroic changes are due to conformational changes of the enzyme protein caused by the binding of substrate rather than by the Schiff base formation between pyridoxal phosphate and the postulated dehydrated intermediate.

EXPERIMENTAL PROCEDURE

Materials

Threonine Deaminase—Crystalline biodegradative threonine deaminase was prepared from extracts of E. coli W (7). Pro-
tein concentration was estimated by the method of Lowry et al. (8).

**Amino Acids—**L-ß-Chloroalanine was prepared from L-serine by the procedure of Fisher and Raske (9). The authentic sample of L-ß-chloroalanine was kindly donated by Dr. Y. Morino, Department of Biochemistry, Osaka University Medical School, Osaka, Japan. L-ß-Chlorobutyric acid was synthesized from L-threonine by the same procedure. DL-ß-Phenylserine was prepared by the condensation of glycine with benzaldehyde in the presence of sodium hydroxide, and the -ß- and -erythro-diestereoisomers were resolved according to Shaw and Fox (10). Diastereoisomeric purities were checked by paper chromatography as described under "Methods." DL-ß-Hydroxy-norvaline was synthesized by the mercuration of 2-pentenic acid in methanol followed by bromination and amination and was resolved to the diastereoisomers by the procedure of Izumiya (11). The diastereoisomeric purity of the resolved amino acids was determined as its N-trifluoroacetyl ester as described by Schrauth and Seiler (13). The values of elemental analyses of the synthesized amino acids, which gave one spot with silica gel thin layer chromatography, were in good agreement with the theoretical values. The melting points were identical with those described in the literature (Table I). L-Threonine and L-serine were products of Kyowa-Hakko Kogyo Ltd. (Tokyo). L-Allothreonine was a gift of Dr. Y. Izumi, Division of Organic Chemistry, Institute for Protein Research, Osaka University. L-Threonine and L-allothreonine were found to be diastereisomERICALLY pure by gas liquid chromatography.

**Other Materials—**Sodium pyruvate, sodium a-ß-ketobutyrate, sodium a-ß-ketoisovalerate and sodium phenylpyruvate were purchased from Sigma, and a-ß-ketonorvaleric acid and 3-methylcrotonic acid from Nakarai Chemicals Ltd. (Kyoto). Neopentyglycol succinate on Chromosorb W was a product of Nishio Kogyo Ltd. (Tokyo). Thin layer plates, Silica Gel 60, were purchased from E. Merck (Darmstadt). Adenosine 5'-monophosphate was kindly donated by Kohjin Company Ltd. (Tokyo), Yamasa Shoyu Co., Ltd. (Choshi), and Ajinomoto Company Ltd. (Kawasaki). All other chemicals were obtained commercially and were of analytical grade.

**Methods**

**Gas Chromatography** Gas liquid chromatography of amino acids was carried out using a Packard model 7300 under the following conditions: glass column (4 mm x 3 m), 3% neopentyglycol succinate on Chromosorb W, temperature, 180°C; carrier gas, nitrogen (25 ml per min); detector, flame ionization. The N-trifluoroacetylated esters of amino acids were prepared according to Stalling et al. (12). Under the above conditions, the retention times of threonine, allothreonine, threo-ß-hydroxy-norvaline, and erythro-ß-hydroxy-norvaline were 3.8, 5.4, 3.8, and 5.4 min, respectively.

**Paper Chromatography—**As described by Drell (14). The keto acids formed during the deamination reaction were identified as their 2,4-dinitrophenylhydrazones by ascending paper chromatography with Whatman No. 1 filter paper with a system of isopropyl alcohol-glacial acetic acid-water (70:5:25) as described by Drell (14). A-ß-keto acids formed during the deamination reaction were identified as their 2,4-dinitrophenylhydrazones by ascending paper chromatography with Whatman No. 1 filter paper using 1-butanol-ethanol-water (5:1:4) as solvent (15). \( R_f \) values for 2,4-dinitrophenylhydrazones of a-ß-keto acids under the above conditions were as follows: pyruvic acid, 0.58 (trans) and 0.68 (cis); a-ketobutyric acid, 0.75; a-ß-ketorvaleric acid, 0.80; a-ß-ketoisovaleric acid, 0.81; phenylpyruvic acid, 0.83.

**Thin Layer Chromatography—**Thin layer chromatography of amino acids was performed on silica gel thin layer plates in a solvent system of phenol-water (75:25).

**Assay of Threonine Deaminase—**The activity of the threonine deaminase was determined spectrophotometrically by measurement of the formation of the a-ß-keto acid as its 2,4-dinitrophenylhydrazone with a Hitachi model 124 spectrophotometer (16). The details of the assay conditions are described in the legends to Fig. 1 or Table II. The enzyme activity was also determined by measuring ammonia formation with the microdiffusion method of Conway and O'Malley (17).

When L-ß-chloroalanine or L-ß-chlorobutyric acid was used as substrate, the chloride formed was determined as silver chloride (1) by the following procedure. The reaction mixture (1.0 ml) containing 100 /imole of potassium phosphate (pH 7.4), 10 /imoles of AMP, 50 /imoles of substrate, and 2.1 /g of the enzyme was incubated for 4 min at 37°C. The reaction was stopped with 0.5 ml of n nitric acid, after which was added 0.1 ml of freshly prepared 10 mm silver nitrate solution. The resulting solution was allowed to stand for 20 min in the dark at room temperature. The absorption at 500 nm was then measured in a cuvette of 1-cm light path with a Hitachi model 124 spectrophotometer.

**Measurement of Absorption Spectra and CD—**Absorption spectra were measured with a Cary model 15 recording spectrophotometer. CD was measured with a Jasco ORD/UV-5 recording spectropolarimeter with a CD attachment.

**RESULTS**

**Substrate Specificity of Enzyme—**Five a-ß-hydroxyamino acids and two a-ß-chloroamino acids were chemically synthesized as shown in Table I. The deamination of these compounds, in addition to naturally occurring amino acids, was then investigated in order to study the interactions between the ligands of threonine deaminase and the a-ß substituents of the substrate. Table II summarizes \( V_{max} \) and \( K_m \) values for each substrate calculated from double reciprocal plots. As shown in Table I, the enzyme was found to catalyze the deamination of L-allothreonine, L-ß-chloroalanine, L-ß-chlorobutyric acid, DL-ß-phenylserine, and DL-ß-hydroxy-norvaline in addition to L-threonine and L-serine. The keto acids formed were identified as their 2,4-dinitrophenylhydrazones by paper chromatography as described above. Since L-threonine, L-serine, and DL-ß-chloroalanine did not serve as substrates, and only one-half of the added DL-threonine and DL-ß-phenylserine was deaminated after prolonged incubations, the enzyme appears to be specific for L- or D-enantiomers.

The \( V_{max} \) and \( K_m \) values for each substrate in Table II indicate that the absolute configuration of the a-ß carbon in...
moiety at the $\beta$ carbon play important roles in determining the catalytic activity of the enzyme. The configuration of the $\beta$ carbon as well as the bulk of the alkyl $\alpha$-alkyl substituents of the substrate, and that the absolute stereospecific interactions between the enzyme protein and the $\alpha$-aminobutyric acid ($\alpha$-threonine) to that for the corresponding $\beta$ carbon. Namely, the ratio of the $V_{\text{max}}$, for L-threo-$\beta$-hydroxy-phenylserine is 195-196° (10). These results suggest that there do exist influences the kinetic parameters of the enzyme significantly; the $V_{\text{max}}$ values for three-$\alpha$-amino acids are greater than those for the erythro-diastereoisomers, whereas $K_m$ values for the corresponding three-diastereoisomers. Furthermore, the ratio of the $V_{\text{max}}$ value for the three-$\alpha$-amino acid to that for the erythro-diastereoisomer increases with increasing bulk of the alkyl substituent at the $\beta$ carbon. Namely, the ratio of the $V_{\text{max}}$ for L-threo-$\beta$-hydroxy-$\alpha$-amino butyric acid (L-threonine) to that for the corresponding L-erythro-diastereoisomer (L-allothreonine) is 4.4, whereas those for L-threo-$\beta$-hydroxynorvaline and L-$\beta$-phenylserine are 3.8 and 190, respectively. These results suggest that there do exist stereospecific interactions between the enzyme protein and the $\beta$-alkyl substituents of the substrate, and that the absolute configuration of the $\beta$ carbon as well as the bulk of the alkyl moiety at the $\beta$ carbon play important roles in determining the catalytic activity of the enzyme.

The enzyme is known to be inactivated during the deamination of L-serine (19), and it is also inactivated during the deamination of L-$\beta$-chloroalanine by threonine deaminase. A reaction mixture (1 ml) containing 100 $\mu$moles of potassium phosphate (pH 7.4), 10 $\mu$moles of AMP, 0.42 $\mu$g of threonine deaminase, and 100 $\mu$moles of L-serine (10) or 2.0 $\mu$g of the enzyme and 10 $\mu$moles of L-$\beta$-chloroalanine (O), was incubated at 37°. Pyruvate formed was determined spectrophotometrically as its 2,4-dinitrophenylhydrazone (16). $V_{\text{max}}$ and $K_m$ values for each substrate were calculated from double reciprocal plots.

**TABLE II**

$V_{\text{max}}$ and $K_m$ values for substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$V_{\text{max}}$</th>
<th>$K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$moles/min/mg</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>L-Serine*</td>
<td>1000</td>
<td>30</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>880</td>
<td>15</td>
</tr>
<tr>
<td>L-Allothreonine</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>L-$\beta$-Chloroalanine*</td>
<td>87</td>
<td>0.5</td>
</tr>
<tr>
<td>L-$\beta$-Chlorobutyric</td>
<td>36</td>
<td>0.6</td>
</tr>
<tr>
<td>L-threo-$\beta$-Hydroxynorvaline</td>
<td>220</td>
<td>30*</td>
</tr>
<tr>
<td>L-erythro-$\beta$-Hydroxynorvaline</td>
<td>25</td>
<td>15*</td>
</tr>
<tr>
<td>L-$\beta$-Hydroxyvaline</td>
<td>0.34</td>
<td>50*</td>
</tr>
<tr>
<td>L-thero-$\beta$-Phenylserine</td>
<td>0.77</td>
<td>50*</td>
</tr>
<tr>
<td>L-erythro-$\beta$-Phenylserine</td>
<td>0.004</td>
<td>15*</td>
</tr>
</tbody>
</table>

* Incubation time was 30 s to minimize the inactivation of enzyme.

* The numbers are one-half of the experimental values on the assumption that only the $\alpha$-enantiomers are active.

Fig. 1. Time course of the deamination of L-serine and L-$\beta$-chloroalanine by threonine deaminase. A reaction mixture (1 ml) containing 100 $\mu$moles of potassium phosphate (pH 7.4), 10 $\mu$moles of AMP, 0.42 $\mu$g of threonine deaminase, and 100 $\mu$moles of L-serine (10) or 2.0 $\mu$g of the enzyme and 10 $\mu$moles of L-$\beta$-chloroalanine (O), was incubated at 37°. Pyruvate formed was determined spectrophotometrically as its 2,4-dinitrophenylhydrazone at 415 nm.

**TABLE I**

Melting points and analytical data of $\beta$-hydroxy- and $\beta$-chloroamino acids

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Melting point (with decomposition)</th>
<th>Carbon</th>
<th>Hydrogen</th>
<th>Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Literature value</td>
<td>Calculated</td>
<td>Found</td>
<td>Calculated</td>
</tr>
<tr>
<td>L-$\beta$-Chloroalanine</td>
<td>190-191°</td>
<td>29.16</td>
<td>5.95</td>
<td>4.90</td>
</tr>
<tr>
<td>L-$\beta$-Chlorobutyric</td>
<td>190-191°</td>
<td>34.92</td>
<td>6.12</td>
<td>6.12</td>
</tr>
<tr>
<td>L-thero-$\beta$-Phenylserine</td>
<td>190-191°</td>
<td>59.58</td>
<td>6.12</td>
<td>6.12</td>
</tr>
<tr>
<td>L-erythro-$\beta$-Phenylserine-$\frac{1}{2}$H$_2$O</td>
<td>189-190° (10)</td>
<td>56.83</td>
<td>6.12</td>
<td>6.12</td>
</tr>
<tr>
<td>L-thero-$\beta$-Hydroxynorvaline</td>
<td>217-226°</td>
<td>45.10</td>
<td>8.33</td>
<td>8.33</td>
</tr>
<tr>
<td>L-erythro-$\beta$-Hydroxynorvaline</td>
<td>250-257° (11)</td>
<td>45.10</td>
<td>8.33</td>
<td>8.33</td>
</tr>
<tr>
<td>L-$\beta$-Hydroxyvaline</td>
<td>217-221°</td>
<td>45.10</td>
<td>8.33</td>
<td>8.33</td>
</tr>
</tbody>
</table>

* The mechanism of inactivation will be published elsewhere.
Effects of various $\beta$-alkyl-substituted substrates on the absorption spectrum of threonine deaminase. The absorption spectrum of the enzyme solution (1.0 ml) containing 100 mM potassium phosphate (pH 7.4), 10 mM AMP, and 1.0 mg of threonine deaminase was recorded at 24° in a cuvette of 1-cm light path in the absence (-----) and presence of 100 mM L-threonine (----), L-allothreonine (-----), L-\(\beta\)-chlorobutyrate (-----) or DL-three-$\beta$-hydroxynorvaline (-----). The addition of L-p-chlorobutyrate to the enzyme solution, it returned to the starting maximum (Fig. 2). When L-threonine was added to the enzyme solution, the absorption maximum immediately shifted to 434 nm and, after the depletion of L-threonine, it returned to the starting maximum (Fig. 2) as reported previously (5). The addition of L-\(\beta\)-chlorobutyrate to the enzyme solution caused a shift of absorption maximum to 442 nm. On the other hand, L-allothreonine, the diastereoisomer of L-threonine, did not induce such a shift, although it has the same $K_m$ value as that of L-threonine. DL-three-$\beta$-Hydroxynorvaline also did not cause a shift of the absorption maximum. Furthermore, L-serine did not cause the shift of the absorption maximum but L-\(\beta\)-chloroalanine induced a shift similar to that caused by L-\(\beta\)-chlorobutyrate (Fig. 3). Absorption maxima of the absolute and difference spectra in the presence of these analogs are presented in Table III.

**Table III**

Effects of $\beta$-substituents of substrates on the absorption spectrum of the enzyme

The data on the absolute absorption maxima of the enzyme in the absence and presence of substrates were taken from Figs. 2 and 3. Difference spectra were measured at 24° under the following conditions. The experimental cuvettes contained 100 mM potassium phosphate (pH 7.4), 10 mM AMP, 100 mM substrates, and 1.9 mg of the enzyme in a final volume of 1 ml. The reference cuvettes contained the same components except for the omission of substrates.

<table>
<thead>
<tr>
<th>Substrate (R; X; CHX; CH(NH)$\text{CO}_2$H)</th>
<th>$\mu$m</th>
<th>Product formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-serine</td>
<td>H OH</td>
<td>415</td>
</tr>
<tr>
<td>L-(\beta)-Chloroalanine</td>
<td>H Cl</td>
<td>442</td>
</tr>
<tr>
<td>L-Threonine (threo)</td>
<td>CH$_2$ OH</td>
<td>434 450</td>
</tr>
<tr>
<td>L-Allothreonine (erythro)</td>
<td>CH$_2$ OH</td>
<td>415 434</td>
</tr>
<tr>
<td>L-(\beta)-Chlorobutyrate (erythro)</td>
<td>CH$_2$ Cl</td>
<td>442 456</td>
</tr>
<tr>
<td>DL-three-$\beta$-Hydroxynorvaline</td>
<td>C$_2$H$_5$ OH</td>
<td>415 434</td>
</tr>
</tbody>
</table>

Analogues are metabolised through the corresponding enamino acid to form $\alpha$-keto acids. In order to determine whether or not the spectral intermediate is due to the enamino acid, effects of the substituents as well as the absolute configuration of the $\beta$ carbon of the substrate on the absorption spectrum were investigated using various analogs of L-threonine. The results of typical experiments are presented in Figs. 2 and 3. When L-threonine was added to the enzyme solution, the absorption maximum immediately shifted to 434 nm and, after the depletion of L-threonine, it returned to the starting maximum (Fig. 2) as reported previously (5). The addition of L-\(\beta\)-chlorobutyrate to the enzyme solution caused a shift of absorption maximum to 442 nm. On the other hand, L-allothreonine, the diastereoisomer of L-threonine, did not induce such a shift, although it has the same $K_m$ value as that of L-threonine. DL-three-$\beta$-Hydroxynorvaline also did not cause a shift of the absorption maximum. Furthermore, L-serine did not cause the shift of the absorption maximum but L-\(\beta\)-chloroalanine induced a shift similar to that caused by L-\(\beta\)-chlorobutyrate (Fig. 3). Absorption maxima of the absolute and difference spectra in the presence of these analogs are presented in Table III.

Effects of Substrates on Circular Dichroism of Enzyme—As the bathochromic shift of the absorption maximum caused by L-threonine is accompanied by the disappearance of the positive CD band at 415 nm (3), the role of the $\beta$-substituents and the absolute configuration of the $\beta$ carbon in the circular dichroic changes at 415 nm were also studied using L-threonine, L-allothreonine, L-\(\beta\)-chlorobutyrate, and L-\(\beta\)-chloroalanine. Fig. 4 represents time courses of the circular dichroic changes at 415 nm after addition of these compounds. As shown in this figure a transient loss of CD was observed when L-threonine or L-\(\beta\)-chloroalanine was added to the enzyme solution. The CD was restored to the starting state with depletion of the substrate. L-Allothreonine, however, slightly decreased the magnitude of the positive CD of the enzyme, and L-\(\beta\)-chlorobutyrate did induce a transient negative CD during the reaction.

**DISCUSSION**

Although some pyridoxal enzymes such as tryptophanase (20) and homoserine deaminase (21) show fairly broad specificity with respect to the nature and the position of leaving group, the biodegradative threonine deaminase exhibits a rigid specificity for the leaving group. Namely the elimination reaction is specific for the hydroxyl and chlorine groups at the $\beta$ carbon of the L-$\alpha$-amino acid. The specificity for the $\beta$-alkyl substituent is relatively broad; however, a correlation between the absolute configuration of the $\beta$ carbon and the $V_{\text{max}}$ value was observed. This finding indicates that the $\beta$ substituents of the substrate interact stereospecifically with the ligands of the enzyme protein and influence the elimination reaction.

In recent studies, the spectral change during the deamination
mental cuvettes contained 100 mM potassium phosphate (pH 7.4), 10 mM AMP, 10 mM substrate, and 1.0 mg of the enzyme in a final volume of 1 ml. The reference cuvettes contained the same components except for the omission of the substrate. Experiments were performed at 24°C. α-Ketobutyrate formed was determined as described under “Methods” by change in difference absorption at 450 nm; ○, formation of α-ketobutyrate. A, L-threonine; B, L-allothreonine; and C, L-β-chlorobutyryl.

![Fig. 5. Time course of the accumulation of α-ketobutyrate and changes in the difference absorption at 450 nm.](image)

![Fig. 6. Relationships between the reaction velocities and ΔA_{450} obtained from the data in Fig. 5. The reaction velocity was estimated by the procedure of Shizuta et al. (22). Substrates: L-threonine (○), L-allothreonine (●), and L-β-chlorobutyryl (Δ).](image)

The reaction has been presumed to reflect the formation of pyridoxal phosphate enamino acid azomethine (6). Although α-aminoacrylate has been postulated to be the common intermediate of the deamination of L-threonine, L-allothreonine, and L-β-chlorobutyryl, and α-aminoacrylate is presumed to be the common intermediate of L-serine and L-β-chloroalanine, spectral changes caused by these substrates are grossly different, indicating that the β-leaving group and the absolute configuration of the β carbon contribute to the spectral changes during the deamination reaction of the substrate. This fact appears to indicate that the shift of the absorption maximum does occur in the step prior to the β elimination reaction. There remains, however, a possibility that some of the different absorption spectra observed with the various substrates could be due to species formed after β elimination if the kinetics of various substrates differ, and consequently the steady state concentration of the intermediate is also different, and one sees different steady state spectra. To examine this possibility, the relationships between the reaction velocities and the changes in the difference absorption at 450 nm (ΔA_{450}) with various substrates were studied as reported previously (22). In Fig. 5 the changes in the difference absorption at 455 nm during the entire course of the reaction (lower curves, open circles) are shown, as well as the accumulation of the reaction product, α-ketobutyrate (upper curves, closed circles). When the relative velocities of overall reaction at different time intervals are calculated from the data shown in Fig. 5 by the procedure of Shizuta et al. (22) against the relative amounts of ΔA_{450}, none of the reaction velocities were directly proportional to ΔA_{450}, and every plot displayed downward curvature (Fig. 6). These results suggest that the spectral changes caused by L-threonine, L-allothreonine, and L-β-chlorobutyryl are not derived from the obligatory intermediate such as aminocrotonate, because in such a case, the reaction velocity should be directly proportional to ΔA_{450}.

It has been assumed from a recent study (6) that the loss of the CD at 415 nm reflects the accumulation of Schiff bases between pyridoxal phosphate and L-threonine as well as with α-aminoacrylate. The fact that L-allothreonine, the diastereoisomer of L-threonine, hardly affects the CD and that L-β-chlorobutyryl, the β-chlorine-substituted derivative of L-allothreonine, induces a negative CD at 415 nm indicates that the substituents and the absolute configuration of the β carbon of the substrate do participate in the circular dichroic change.

Available evidence is therefore inconsistent with the interpretation that the changes of the visible absorption spectrum and circular dichroism observed after addition of L-threonine to the enzyme are ascribable to the azomethine linkage between pyridoxal phosphate and α-aminoacrylate as has been postulated by previous investigators. Rather the spectral shifts and changes in CD appear to be the results of the conformational changes of the enzyme due to the binding of L-threonine.

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REFERENCES

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