Evidence for a Rate-determining Proton Abstraction in the Serine Deaminase Reaction of the $\beta_2$ Subunit of Tryptophan Synthetase*

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SUMMARY

The $\beta_2$ subunit of tryptophan synthetase of Escherichia coli catalyzes the pyridoxal-P-dependent conversion of L-serine to pyruvate and ammonia. In order to study the effect of substitution of deuterium for the $\alpha$-hydrogen of serine on the rate of this reaction, a new method for the synthesis of $\alpha$-deutero-DL-serine has been developed; analyses of the product by mass spectroscopy and nuclear magnetic resonance spectroscopy are shown. The rate of pyruvate formation from $\alpha$-deuteroserine is one-fourth of that from $\alpha$-proteoserine in the absence of NH$_4^+$ ion. This kinetic isotope effect indicates that the dissociation of the $\alpha$-C--H bond of L-serine is the rate-determining step in the formation of pyruvate under these conditions. The enzyme-substrate intermediate which accumulates before the rate-determining $\alpha$-proton abstraction under these conditions can be observed by its absorption at 420 nm or by its fluorescence. We conclude that this intermediate must be the Schiff base formed between pyridoxal-P and L-serine. The disappearance of this intermediate formed from $\alpha$-deuteroserine and $\alpha$-proteo-DL-serine has been measured by stopped flow experiments in the presence of NH$_4^+$ ion which increases the rate of this step. The results show a kinetic isotope effect of 4.6 on the rate constant for the forward reaction in which this intermediate disappears. These results are direct evidence that a proton is transferred in this step.

Tryptophan synthetase of Escherichia coli is a multienzyme complex with an $\alpha_2\beta_2$ subunit structure (2, 3). The $\beta_2$ subunit alone catalyzes a number of pyridoxal-P-dependent $\beta$ elimination reactions such as the serine deamination reaction (Equation 1) and $\beta$ replacement reactions such as the synthesis of tryptophan (Equation 2) (4).

$\text{L-Serine} \rightarrow \text{pyruvate} + \text{NH}_3$ \hspace{1cm} (1)

$\text{L-Serine} + \text{indole} \rightarrow \text{L-tryptophan} + \text{H}_2\text{O}$ \hspace{1cm} (2)

The $\alpha_2\beta_2$ complex has no activity in the $\beta$ elimination reactions but has a greater activity in the $\beta$ replacement reactions than does the $\beta_2$ subunit alone (4, 5).

The serine deamination reaction is probably carried out through a series of Schiff base intermediates formed between enzyme-bound pyridoxal-P and L-serine (Fig. 1) consistent with the general mechanism of pyridoxal-catalyzed reactions (6). These enzyme-bound intermediates are also thought to be the first three intermediates in $\beta$ addition reactions of the $\beta_2$ subunit and of the $\alpha_2\beta_2$ complex with L-serine (4, 7). Previous studies in this laboratory and by Goldberg et al. have shown that addition of L-serine to the $\beta_2$ subunit results in an enzyme-substrate intermediate which can be observed by its absorbance at 420 nm (7) or by its fluorescence emission at 500 nm (8). We have suggested (7) that this enzyme-substrate intermediate is a Schiff base formed between L-serine and enzyme-bound pyridoxal-P (Intermediate I, Fig. 1). More recently York has found that this fluorescent intermediate, termed the "aqua band," is the first enzyme-substrate intermediate which can be observed by stopped flow methods (9). Furthermore, the conversion of L-serine to pyruvate is rate-limited by the disappearance of the fluorescent complex in the absence of NH$_4^+$ ion (9). These findings suggested that the rate-determining step was the removal of the $\alpha$-proton of L-serine. This report tests this proposal by comparing the rates of pyruvate formation from [α-D]serine and [α-H]serine in the absence of NH$_4^+$ ion.

The rate of disappearance of the fluorescent complex can be determined by stopped flow methods in the presence of NH$_4^+$ ion which accelerates this intermediate step. This report also examines the effect of substituting deuterium for the $\alpha$-hydrogen of serine on the rate of this step in order to determine whether this step involves $\alpha$-proton abstraction.

EXPERIMENTAL PROCEDURE

Materials

Pyridoxal hydrochloride, pyridoxal-P, DPNH, and D$_2$O were purchased from Sigma Chemical Co. DL-Serine was a product of Aldrich Chemical Co. Lactic dehydrogenase was purchased from...
Nuclear. was from Calatomic. Aquasol was a product of New England trophotometric assay in which pyruvate formation was coupled in Reaction 2. then stored as a frozen paste for use in the assay of the pz subunit monium sulfate step of the procedure of Hatanaka et al. (10) and II). The 01 subunit was partially purified through the first am- (3) or by the new method of Adachi and Miles (Preparation II). Details of the method are available as developed (Adachi and Miles, manuscript in preparation).

Methods

Enzyme Preparations and Assays—The $\beta$ subunit of tryptophan synthetase was purified from the A2/F'A2 strain of E. coli by either the standard method of Wilson and Crawford (Preparation I) (3) or by the new method of Adachi and Miles (Preparation II). The $\alpha$ subunit was partially purified through the first ammonium sulfate step of the procedure of Hatanaka et al. (10) and then stored as a frozen paste for use in the assay of the $\beta$ subunit in Reaction 2.

Serine deaminase activity (Reaction 1) was assayed by a spectrophotometric assay in which pyruvate formation was coupled with lactic dehydrogenase and DPNH (5). A unit of activity in either Reaction 1 or 2 is the appearance of 0.1 amole of product in 20 min at 37°. Assays of Reaction 1 in the absence of NH$_4^+$ ion, contained in 0.9 ml, 0.1 M Tris HCl buffer, pH 7.8; 0.05 mM pyri- doxal-P; 0.1 mM DPNH; $\beta$ subunit (10 to 30 μg), and excess lactic dehydrogenase and were incubated for 10 min at 37° before the addition of mM-serine in 0.1 ml. The decrease in absorbance at 340 nm was determined in a Gilford spectrophotometer for 10 min. Assays in the presence of NH$_4^+$ ion contained a final concentration of 1 mM NH$_4^+$ ion (sulfate counterion).

The tryptophan synthetase activity (Reaction 2) of the $\beta$ subunit was measured in the presence of a high concentration of NH$_4^+$ ion which has been found to greatly increase the rate of this reaction by the $\beta$ subunit alone (19). Each reaction mixture con- tained, in a final volume of 0.25 ml, [2-3H]indole diluted with carrier to 0.125 amole (20,000 cpm); pyridoxal-P (12.5 μmoles); Tris HCl, pH 7.8 (25 μmoles); (NH$_4$)$_2$ citrate (190 μmoles); $\beta$ subunit (1 μg); and mM-serine (10 μmoles or a variable amount in the determination of $K_v$ values). Reactions were terminated after 20 min at 37° by addition of 0.05 ml of 1 N NaOH. Each reaction mixture was extracted three times with 1 ml of toluene to remove indole. An aliquot (0.1 ml) of the aqueous layer was counted in 10 ml of Aquasol to determine the amount of tryptophan formed. The tryptophan synthetase activity (Reaction 2) of the $\alpha$-$\beta$ complex was assayed by a similar procedure. (NH$_4$)$_2$ citrate was replaced by 0.007 ml of a saturated solution of NaCl and a subunit (about 3 units) was added.

Optical Measurements and Chemical Analyses—Absorption spectra were made in a Cary No. 11. Fluorescence measurements were made in an Aminco-Bowman spectrophotofluorimeter on 0.1-ml volumes of solutions in a microcuvet with a path length of 3 mm using 2-mm slits. Stopped flow experiments were performed in an Aminco-Morrow stopped flow apparatus, which was mounted on a Beckman DU monochromator as described by Summers and McPheie (11). Mass spectra were observed by direct insertion probe using an LKB 0000 mass spectrometer at 70 electron volts, 60 ma ionizing current with source temperature 140°. Nuclear magnetic resonance spectra were measured with a Varian A-60 instrument. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

Preparation of DL-[α-D]Serine—A method for the preparation of DL-[α-D]serine has been reported by Walsh et al. (12). The preparation reported here is based on the method of Posner and Flavin for the preparation of DL-[α-D]homoserine (13) and has some advantages of simplicity. When DL-serine is incubated in D$_2$O at alkaline pH with pyridoxal and aluminum ions, pyridoxal catalyzes the exchange of the α-hydrogen with deuterium. The exchange of the α-hydrogen, which leads to racemization of L-serine, predominates over other pyridoxal-catalyzed reactions of serine under these conditions (14). Pyridoxal hydrochloride (204 mg, 1 mmole) was first converted to the free base on a column (0.9 X 30 cm) of Dowex 50-H$^+$. Pyridoxal was eluted with 2 N NH$_3$OH, dried in vacuo, and suspended in 30 ml of D$_2$O (99.7%). mM-Serine (1.05 g, 10 mmoles) was added; the pD was adjusted to 9.6 with 5 N KOH in D$_2$O. The solution was again lyophilized, dissolved in 20 ml of D$_2$O, and combined with A$_2$(SO$_4$)$_3$·18 H$_2$O (204 mg, 0.4 mmole) which had been previously lyophilized from 1 ml of D$_2$O. The pD was adjusted to 9.6 with 5 N KOH in D$_2$O. This solution was incubated in the dark for 48 hours at 23°. The reaction was followed by periodic measurement of the NMR spectrum of an aliquot as shown in Fig. 2. The α- and β-protons of serine are superimposed at δ = 3.5 to 4.0, and this region showed a complex splitting pattern at zero time which simplified to a single peak after 24 hours. A small side band remained after an additional 24 hours and was probably due to an impurity. During the first 24-hour period the integrated signal area decreased by about one-third, equivalent to the disappearance of one proton (Fig. 2).

4 After 48 hours the reaction mixture was treated with 20 ml of ethanol alcohol and the precipitate (about 0.8 g) was collected by filtration, redissolved in water, treated with activated charcoal, and recrystallized several times from 50% EtOH.

$\text{C}_6\text{H}_4\text{D}_2\text{NO}_4$

Calculated: C 34.0, H 6.7, N 13.2

Found: C 34.1, H 6.65, N 13.2

5 These spectra were generously determined and interpreted by Dr. Henry M. Fales, Laboratory of Chemistry, National Heart and Lung Institute.

6 These spectra were kindly determined by Mr. Noel Whittaker, Laboratory of Chemistry, National Institute of Arthritis, Metab- olism, and Digestive Diseases.

7 The calculated content of H was corrected for $1/7$ D.

Fig. 1. Scheme for the conversion of L-serine to pyruvate and enzyme-bound pyridoxal-P(E-PLP) forms a Schiff base intermediate with the α-proton to form Intermediate II and of the β-hydroxyl to form Intermediate III. This is converted by one or more steps to pyruvate and ammonia.

Worthington Biochemical Corp. [2-3H]Indole (1 mCi per mmole) was from Calatomic. Aquasol was a product of New England Nuclear.
The product and DL-[a-H]-serine were also analyzed by electron impact mass spectroscopy (Fig. 3). The spectrum of DL-[a-H]-serine (Fig. 3A) showed no molecular ion peak at m/e 105, but this is to be expected for amino acids examined by this technique (15). The first two fragmentation peaks at m/e 74 and 75 can be assigned to the loss of CH₂OH and CH₂O from the C-3 position. The major fragmentation peak at m/e 69 corresponds to the loss of COOH from the C-1 position; this is the most common fragmentation product of amino acids in electron impact mass spectroscopy. The fragmentation peak at m/e 42 results from the loss of H₂O from the (M-COOH)⁻, while that at m/e 28 results from the loss of CH₂OH from the (M-COOH)⁻. Each of the fragmentation peaks described above for Fig. 3A is seen in Fig. 3B at one higher mass number. The relative proportions of the major peaks in Fig. 3A are closely similar to those in Fig. 3B. Since fragments ascribed to the loss of CH₃OH and CH₂O from the C-3 position. The major fragmentation peak at m/e 60 corresponds to the loss of COOH to the loss of the C-1 at m/e 61 and 43, to the loss of C-3 at m/e 75 and 62, and to the loss of C-1 and C-2 at m/e 79 in Fig. 3B, all have one higher mass number than the corresponding fragments in Fig. 3A, this proves that 1 deuterium has been specifically incorporated in the C-2 position. It also confirms the assignment of the fragmentation products of serine. A comparison of the relative intensities of the fragmentation peaks at m/e 61 and 60 in Fig. 3B shows that incorporation of deuterium into the C-2 position has been at least 95%.

RESULTS

Rates of Reactions of Tryptophan Synthetase with [a-D]-Serine and [a-H]-Serine—The kinetics of the reactions of the βₐ subunit and the αβₐ complex with [a-D]-serine and [a-H]-serine was determined under several conditions (Table I and Fig. 4). The largest kinetic isotope effect observed was in the reaction of the βₐ subunit with serine in the absence of NΗ₄⁺ ion (Table I, Experiment A, and Fig. 4). Substitution of deuterium for the α-hydrogen of serine had no effect on the Kₐ but decreased the Vₐ max 4-fold. The kinetic isotope effect was reduced from 2.5 to 1.8 in the presence of NΗ₄⁺ ion and to 1.4 in the presence of the α subunit (Table I).

Stopped Flow Study of the Reaction of the βₐ Subunit with αH-[a-H]-Serine and αH-[a-D]-Serine—Fig. 5 is a semilogarithmic plot showing the observed decrease in absorbance at 420 nm after the βₐ subunit was mixed in the presence of NΗ₄⁺ ion with [α-H]-serine or [α-D]-serine. An initial increase in absorbance at 420 nm occurred during the dead time of the instrument which was 25 ms under the experimental conditions used. The rate constant for the disappearance of the enzyme-[α-H]-serine intermediate which absorbs at 420 nm (kₐdis = 6.34 s⁻¹) was 2.3 times faster than the rate constant for the disappearance of the enzyme-[α-D]-serine intermediate (kₐdis = 2.74 s⁻¹).

Fig. 6 shows fluorescence spectra (A) and absorption spectra...
FIG. 4. Effect of the concentration of \(\alpha\)-D-serine (\(\Delta\)) and \(\alpha\)-H-serine (\(\bigcirc\)) on the serine deaminase activity of the \(\beta_1\) subunit in the absence of \(\text{NH}_3^+\) ion. Serine deaminase activity was measured as described under "Experimental Procedure" using 0.01 to 0.2 M \(\text{DL-}[\alpha\text{-D}]\text{serine}\) and 0.01 to 0.2 M \(\text{DL-}[\alpha\text{-H}]\text{serine}\) and 10 to 25 \(\mu\)g of \(\beta_1\) subunit per ml. The concentration of serine plotted on the abscissa is that of the L isomer present in the \(\text{DL}\) mixture. A control experiment (not shown) with \(\text{L-}[\alpha\text{-H}]\text{serine}\) gave identical results showing that the presence of \(\text{L-}\) serine in the \(\text{DL}\) mixture has no effect on the kinetics of the reaction.

FIG. 5. Disappearance of an enzyme-substrate intermediate formed by the reaction of \(\beta_2\) subunit with \(\text{L-}[\alpha\text{-H}]\text{serine}\) (\(\bullet\)) or \(\text{L-}[\alpha\text{-D}]\text{serine}\) (\(\Delta\)) in the presence of \(\text{NH}_3^+\) ion. The decrease in the absorbance at 420 nm is plotted as a first order reaction for \(\beta_2\) subunit (2.6 mg per ml) reacting with 0.1 M \(\text{DL-}[\alpha\text{-H}]\text{serine}\) or \(\text{DL-}[\alpha\text{-D}]\text{serine}\) in 1.5 M \(\text{NH}_4^+\) ion (phosphate counterion) at pH 7.8 containing \(2.5 \times 10^{-3} \text{ M pyridoxal-P}\) and 1 mM EDTA. The final steady state absorbance values \(A = 0.25 \text{ for } [\alpha\text{-D}]\text{serine and 0.16 for } [\alpha\text{-H}]\text{serine}\) (see Fig. 6A and "Discussion"). Rate constants calculated from straight lines drawn through the data gave \(k = 6.34 \text{ s}^{-1} \text{ for } [\alpha\text{-H}]\text{serine and } k = 2.74 \text{ s}^{-1} \text{ for } [\alpha\text{-D}]\text{serine, } k_{\text{H}}/k_{\text{D}} = 2.3\). Preparation II of the \(\beta_2\) subunit was used.

FIG. 6. Fluorescence emission spectra (A) and absorption spectra (B) of the \(\beta_2\) subunit and its enzyme-substrate complexes with \(\text{L-}[\alpha\text{-H}]\text{serine and } [\alpha\text{-D}]\text{serine}\) in the presence and absence of \(\text{NH}_3^+\) ion. Curve 1, \(\beta_2\) subunit in the presence or absence of \(\text{NH}_3^+\) ion. Curve 2, \(\beta_2\) subunit + \(\text{L-}[\alpha\text{-H}]\text{serine or } [\alpha\text{-D}]\text{serine}\) in the absence of \(\text{NH}_3^+\) ion. Curve 3, \(\beta_2\) subunit + \(\text{L-}[\alpha\text{-D}]\text{serine}\) in the presence of \(\text{NH}_3^+\) ion. Curve 4, \(\beta_2\) subunit + \(\text{L-}[\alpha\text{-H}]\text{serine in the presence of } \text{NH}_3^+\) ion. Buffer plus \(\text{NH}_3^+\) ion was as in Fig. 5. Buffer minus \(\text{NH}_3^+\) ion contained 0.1 M potassium phosphate, pH 7.8, 1 mM EDTA, and \(2.5 \times 10^{-3} \text{ M pyridoxal-P}\). The final concentration of \(\text{DL-}[\alpha\text{-D}]\text{serine or } \text{DL-}[\alpha\text{-H}]\text{serine}\) was 0.1 M in A and B. The final concentration of \(\beta_2\) subunit was 0.77 mg per ml in A and 2.6 mg per ml in B. Fluorescence emission spectra (A) were measured with excitation at 420 nm. Preparation II of the \(\beta_2\) subunit was used.

DISCUSSION

The conversion of L-serine to pyruvate and \(\text{NH}_3^+\) ion by the \(\beta_2\) subunit occurs through several enzyme-substrate intermediates involving enzyme-bound pyridoxal-P as shown in Fig. 1 (7). Although several enzyme-substrate intermediates have been observed in studies of the \(\beta_2\) subunit under steady state conditions (7, 8) and under pre-equilibrium conditions (9), no definitive assignment of structures to the observed intermediates have been made and no individual intermediate step has been clearly identified. We have attempted to identify one such step, the step in which the \(\alpha\)-proton of L-serine is removed, and the inter-
mediate which precedes this step, by investigating the effects of substituting deuterium for the α-hydrogen of serine under various reaction conditions. Our finding that this substitution decreases the overall rate of pyruvate formation 4-fold in the absence of NH₄⁺ ion indicates that the rate-limiting step under these conditions is the abstraction of the proton on the α-C of L-serine, the conversion of Intermediate I to Intermediate II in Fig. 1. The enzyme-substrate intermediate which accumulates before this rate-limiting step and which has been observed by its absorption at 420 nm (7) and by its fluorescence (8, 9) must have the structure of Intermediate 1 or that of a closely related intermediate which still retains the α-proton.

NH₄⁺ ion has been shown to increase the rate of disappearance of this intermediate measured under pre-equilibrium conditions (9). Our results show that the rate of disappearance of this intermediate in the presence of NH₄⁺ ion is decreased 2.3-fold by the substitution of deuterium for the α-hydrogen of serine. This is further evidence that this intermediate is Intermediate I and that its conversion to Intermediate II involves abstraction of the α-proton. The rate constant for the conversion of Intermediate I to Intermediate II with [α-H]serine as substrate which we have obtained from stopped flow absorption measurements \(k_{\text{obs}} = 6.3 \text{ s}^{-1}\) is about 2 times higher than the rate constant obtained by York \(k_{\text{obs}} = 3.6 \text{ s}^{-1}\) using stopped flow fluorescence measurements at a lower protein concentration (9). These numbers are in reasonably close agreement and provide further evidence that the fluorescence and absorbance observed under these conditions are due to the same intermediate.

Two enzyme-substrate intermediates accumulate in the presence of NH₄⁺ ion under steady state conditions: Intermediate I and an intermediate which absorbs at 330 nm. York has shown that the latter intermediate, which he terms the “pale” species, is formed as one-half of Intermediate I disappears before the steady state condition is reached (9). Therefore, the reaction of the β subunit with L-serine in the presence of NH₄⁺ ion appears to contain two rate-limiting steps which occur after each of the two enzyme-substrate intermediates which accumulate under steady state conditions (9). These observations can be represented by a minimal kinetic scheme (Equation 1) in which ES-I represents Intermediate 1 which absorbs at 420 nm and ES-II represents the “pale” species which absorbs at 330 nm. One or more other enzyme-substrate intermediates connected by fast steps could occur between ES-I and ES-II without affecting this kinetic scheme.

\[
E + S \xrightleftharpoons{k_{-1}} ES-I \xrightarrow{k_2} \text{II}^+ \xrightarrow{k_3} \text{H}^+ \xrightarrow{k_4} E + P
\]

(420 nm) fast (330 nm) slow slow

Our finding (Fig. 6) that a larger amount of Intermediate I accumulates under steady state conditions when [α-D]serine is the substrate than when [α-H]serine is the substrate is a result of the decrease in the rate of proton removal when deuterium is substituted for the α-hydrogen of serine; the rates of subsequent steps should be the same with the two substrates. Thus a different steady state mixture of the Intermediate I (ES-I) and the “pale” intermediate (ES-II) would be expected with [α-D]serine. The observation in the stopped flow experiment (Fig. 5) that the decrease in absorbance at 420 nm observed with [α-D]serine was less than that observed with [α-H]serine is a consequence of the higher steady state level of Intermediate I formed from [α-D]serine.

If one assumes that the conversion of ES-I to ES-II is a rate-limiting step and that ES-I and ES-II are the only forms of the enzyme present under the steady state conditions in the presence of a high concentration of substrate, then:

\[
k_{\text{obs}} = k_1 + k_2
\]

and

\[
K_{\text{eq}} = \frac{k_{-1}}{k_1} = \frac{ES-I}{ES-II} = \frac{k_z}{k_{-z}} = 1
\]

where \(k_z\) and \(k_{-z}\) are the rate constants for the forward and back reactions, respectively.

\(K_{\text{eq}}\) can be calculated from the data of Fig. 6. A value of approximately 1 when [α-H]serine is the substrate and approximately 3 when [α-D]serine is the substrate. These equilibrium constants and the values of \(k_{\text{obs}}\) from Fig. 5 can be used to calculate \(k_z\) and \(k_{-z}\) for each substrate from Equations 2 and 3.

For [α-H]serine,

\[
k_{\text{obs}} = 6.34 \text{ s}^{-1} = k_1 + k_2
\]

Therefore, \(k_z = k_2 = 3.17 \text{ s}^{-1}\).

For [α-D]serine,

\[
k_{\text{obs}} = 2.74 \text{ s}^{-1} = k_1 + k_{-z}; k_z/k_{-z} = 3.
\]

Therefore, \(k_z = 3 k_{-z}; k_z = 2.05 \text{ s}^{-1}\) and \(k_{-z} = 0.685 \text{ s}^{-1}\).

Deuterium isotope effects can be calculated for each rate constant from these data:

\[
\frac{k_H}{k_D} = 4.6 \text{ for } k_1 \text{ and } \frac{k_H}{k_D} = 1.5 \text{ for } k_2.
\]

Thus, the kinetic deuterium isotope effect for the forward reaction \(k_1\) is twice as large as that for the \(k_{-1}\), which is the sum of the forward and back reactions if the assumptions made are correct. The isotope effect of 4.6 for this step is close to the observed isotope effect for pyruvate formation in the absence of NH₄⁺ ion (Fig. 4) and supports the proposal that this is the step in which the α-proton is removed. Our calculated isotope effect for the back reaction \(k_1/k_D = 1.5\) for \(k_{-1}\) is slightly lower than the theoretical value of 1.0. We have observed isotope effects for the over-all rates of synthesis of pyruvate or tryptophan from L-serine in the presence of NH₄⁺ ion of 2.5 and 1.8, respectively (Table I). These findings are consistent with the proposal that α-proton removal is one of two rate-limiting steps in the presence of NH₄⁺ ion.

When L-serine is added to the αβ₂ complex, there is a transient increase in fluorescence due to Intermediate I which rapidly disappears (9). York concludes that the disappearance of the fluorescent intermediate is no longer rate-determining in reactions of the αβ₂ complex. Our finding (Table I) of a very small kinetic isotope effect (1.4) in Reaction 2 catalyzed by the αβ₂ complex also indicates that abstraction of the α-proton of L-serine is not rate-determining in the conversion of L-serine and indole to tryptophan by the αβ₂ complex.

Most of pyridoxal-P-dependent β elimination and β replacement reactions catalyzed by the β₂ subunit of tryptophan synthetase can also be catalyzed by tryptophanase of E. coli (2, 16). The conversion of serine to pyruvate by the two enzymes can both be visualized as proceeding through the same intermediates shown in Fig. 1 (2, 16). However, Morino and Snell (16) have demonstrated that in reactions catalyzed by tryptophanase the removal of the β substituent (conversion of Intermediate II to Intermediate III in Fig. 1) is the rate-determining step. With certain substrates an enzyme-substrate intermediate which absorbs at 500 nm accumulates (16). This intermediate has been identified as a quinonoid intermediate which lacks the
\(\alpha\)-proton of the bound amino acid and which is a tautomeric form of Intermediate II or analogous \(\beta\) substituted compounds where the \(\beta\)--OH = R. Under these conditions exchange experiments in the presence of D\(_2\)O or T\(_2\)O showed that the \(\alpha\)-H of the amino acid was labilized at rates faster than the overall rate of the reaction (16). Thus the rate-determining steps of the \(\beta\) subunit and of tryptophanase are different, occurring after Intermediates I and II, respectively.

Antonini et al. (17) have concluded that the rate-determining step in the \(\beta\) elimination reaction of \(\alpha\)-\(\beta\)-chloroglutamate catalyzed by aspartate aminotransferase is the labilization of the \(\alpha\)-proton; this slow step had a rate constant of 7.5 s\(^{-1}\). Banks et al. (18) have reported that the rate-determining step in the transamination reaction catalyzed by aspartate aminotransferase is the labilization of the \(\alpha\)-hydrogen of L-glutamate and L-aspartate; their conclusions are based on observed deuterium kinetic isotope effects of 1.7 and 1.9. They observed a larger isotope effect (3.5) in a model transamination reaction. Large deuterium kinetic isotope effects \((k_\text{H}/k_\text{D} = 3 \text{ to } 7)\) have also been observed in other model transamination systems (19--21), and the authors have concluded that the labilization of the \(\alpha\)-hydrogen is rate-determining and base-catalyzed in model systems.

The rate-determining \(\alpha\)-proton abstraction step in transamination reactions has been shown to be catalyzed by imidazole in model systems (22). Histidyl residues in aspartate aminotransferase have been implicated in this role (23). We have recently shown that histidyl residues are essential for the activity of the \(\beta\) subunit (1, 24) and have proposed that they have a catalytic role in the rate-determining \(\alpha\)-proton abstraction step which has been established in this paper.

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Evidence for a Rate-determining Proton Abstraction in the Serine Deaminase Reaction of the β2 Subunit of Tryptophan Synthetase

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