Stereochemistry and Mechanism of Reactions Catalyzed by Tryptophan Synthetase and Its $\beta_2$ Subunit*

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The synthesis of tryptophan from serine and indole or indoleglycerol phosphate catalyzed by native tryptophan synthetase or $\beta_2$ protein is shown to proceed stereospecifically with retention of configuration at $C_9$. In the $\alpha\beta$ elimination reaction of serine to give pyruvate and ammonia catalyzed by the $\beta_2$ protein, the hydrogen from $C_4$ is transferred intramolecularly and without exchange with solvent protons to $C_9$, where it replaces the OH group with net retention of configuration. In a competing reaction, an abortive transamination in the presence of mercaptoethanol to give pyridoxamine and S-pyruvyl mercaptoethanol, the same proton is transferred to the extent of 70% to $C_4$ of the cofactor when the reaction is carried out in D$_2$O. Together with the finding of Dunathan and Voet (Dunathan, H. G. and Voet, J. G. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 3888) that the cofactor is protonated from the $\beta$ face, these data fully define the geometry of the substrate-enzyme complex and the position of an essential base relative to it. Since no isotope effect was observed in the protonation of $C_4$ of tryptophan synthesized from indole and serine in 50% D$_2$O, the base must be monoprotic. The known inability of the enzyme to degrade tryptophan by $\alpha\beta$ elimination is not due to inability to remove H$_2$ of tryptophan; native enzyme and $\beta_2$ protein catalyze a hydrogen exchange of tryptophan at a rate of 20% and 14%, respectively, of that of tryptophan synthesis.

Tryptophan synthetase (EC 4.1.2.20) (1) is a tetrameric enzyme containing two pairs of identical subunits, which normally catalyzes the reaction:

\[
\text{Indole-3-glycerol phosphate + L-serine} \rightarrow \text{L-tryptophan + d-glyceraldehyde 3-phosphate} \tag{1}
\]

Alternatively, the enzyme can also synthesize tryptophan from L-serine and indole according to Equation 2:

\[
\text{Indole + L-serine} \rightarrow \text{L-tryptophan} \tag{2}
\]

The $\beta_2$ subunit which contains pyridoxal phosphate as the prosthetic group is unable to catalyze reaction 1, but carries out reaction 2 in addition to a number of other reactions, e.g. (3):

\[
\text{L-Serine} \rightarrow \text{pyruvate + ammonia} \tag{3}
\]

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\[
\beta\text{-Mercaptoethanol + L-serine} \rightarrow S\text{-hydroxyethyl-L-cysteine} \tag{4}
\]

\[
\beta\text{-Mercaptoethanol + L-serine + pyridoxal phosphate} \rightarrow S\text{-pyruvyl mercaptoethanol + pyridoxamine phosphate} \tag{5}
\]

As in the case of other pyridoxal phosphate-containing enzymes which carry out $\beta$ replacement and/or $\alpha\beta$ elimination reactions of amino acids, the reaction sequence leads via a series of aldime and ketimine complexes between the substrate amino acid and the cofactor to an enzyme-bound Schiff's base between pyridoxal phosphate and $\alpha$-aminoacrylic acid as a universal intermediate. This intermediate can follow various reaction paths, giving the different observed products.

In this paper we report results which clarify various stereoechemical aspects of these reactions, define the geometry of the coenzyme-substrate complex in the active site and throw some light on several of the protonation steps involved in these reactions. Some of the results have been communicated in preliminary form (2,3).

**EXPERIMENTAL PROCEDURES**

**Materials**—The chemicals used were of reagent grade or of the highest purity commercially available and they were used without further purification. All enzymes obtained commercially were purchased from Sigma. L-[U-14C]Serine (159 mCi/mmol), L-[3-14C]tryptophan (-45 mCi/mmol), and [2-14C]acetate (-50 mCi/mmol) were purchased from Amersham/Searle. Reference indolmycin was a gift from Charles Pfizer and Co. (2S,3R)- and (2S,3S)-[3-'HJserine were purchased analogously using D$_2$O as the solvent in the first step. DL-[2-'H]Serine and N-['H, 3-'H]serine were prepared as described previously (4) whereas (2S,3R)- and (2S,3S)-[3-'H,3-'H]serine were prepared analogously using D$_2$O as the solvent. Double-labeled samples were prepared by mixing the appropriate single-labeled species and constancy of their $^1H/^{13}C$ ratio was usually ascertained by rechromatography of an aliquot in at least one solvent system.

**Enzymes and Assay Procedures**—The native tryptophan synthetase was purified from Neurospora crassa according to the procedures of Meyer et al. (6) and Yanofsky (7). Tryptophan synthetase a protein was partially purified from Escherichia coli B$_1$ (8), omitting the final DEAE-cellulose column step, to give preparations of 4.7 IU/mg of protein. Tryptophan synthetase $\beta_2$ protein was purified from E. coli A$_v$/A$_S$ according to the procedure of Adachi and Miles (9) and had a specific activity of 14.6 IU/mg of protein. Both E. coli mutants were kindly provided by Dr. R. Somerville. Protein determinations were carried out by Lowry's method (10) using serum albumin as standard.

Tryptophan synthetase activity was measured by following indole disappearance colorimetrically in the reaction between indole and serine to form tryptophan (11).
glacial acetic acid/water, 2:1:1 (descending, serine 0.38, S-hydroxyethyl cysteine 0.51). Amino acids were visualized with ninhydrin spray

**Radioactivity Determinations**—Radioactivity on chromatograms was located using a Packard model 7201 radiochromatogram scanner. The radioactivity of compounds in solution was determined in a Packard model 340 liquid scintillation counter. The radioactivity of mixtures of fumarate plus malate after equilibration with fumarase. Parallel incubations from which fumarase was omitted were also carried out to give malate.

**Deamination of Serine**—The incubation mixture contained in 1 ml of 0.1 M potassium phosphate buffer, pH 7.8: 40 μmol of L-serine samples (0.5 μCi), 10 mM glutathione, 0.2 mM pyridoxal phosphate, 80 μmol of NADH, 5 IU of the β2 protein of tryptophan synthetase from *E. coli*, and excess lactate dehydrogenase from pig heart. For the experiments in columns 1 to 4 of Table II, the 125I-labeled serum (0.09 μCi/μl) was diluted with unlabeled L-serine. For the experiment of column 5, Table II, the 125I-labeled serum was diluted with L-serine before labeling at the α position with deuterium and the resulting mixture was used directly, whereas in the experiment of column 6 the carrier serine consisted of a 64-fold excess of DL-serine. The incubation was carried out at 37°C for 4 h and stopped by boiling for 3 min. The decrease in the absorbance at 340 nm was followed during the incubation. The proteins were separated from the small molecules by dialysis. The lactate samples were isolated by paper chromatography in System C and further purified by acidification with HCl and extraction with ether, with the yields varying from 20% to 50%. The resulting lactate samples were then oxidized to acetate by heating with 12 ml of oxidation mixture (153 mg of K2Cr2O7; 24 ml of concentrated H2SO4; made up to 100 ml with water) on a steam bath for 20 min under an argon atmosphere (14). The acetate samples were then isolated by steam distillation and analyzed for deuterium by the method of Cornforth et al. (15) and Anderson and co-workers (16).

The stable isotope experiments were carried out under the same conditions and the lactate samples were analyzed for deuterium distribution by proton NMR.

**Abiotic Transamination of Serine**—300 μl of the β2 protein of tryptophan synthetase from *E. coli* were first dialyzed against 0.1 M potassium phosphate buffer (pH 7.8) containing 50 mM β-mercaptoethanol to remove the pyridoxal phosphate. The apoenzyme was then dialyzed against 0.1 M potassium phosphate buffer in D2O (pD 7.8) containing 50 mM serine and 50 mM β-mercaptoethanol. NMR analysis of the enzyme solution (in a volume of 50 ml) indicated >98.5% of D2O content. The reaction was started by addition of 10 μmol of pyridoxal phosphate followed by incubation at 37°C for 2 h, and was stopped by boiling for 3 min. Transamination was monitored spectrophotometrically by the decrease in the absorption at 410 nm and the increase at 325 nm during incubation. The substrates and products were separated from the enzyme by dialysis and then passed onto the top of an anion exchange column (Dowex AG-1-X8, formate form, 200 to 400 mesh, 1.8 × 34 cm). Elution with a formic acid gradient (500 ml of 1 N formic acid and 500 ml of D2O) (17) gave a fraction containing 1.5 μmol of pyridoxamine phosphate which was found to be pure by UV spectroscopy. After the solvent had been removed by evaporation under reduced pressure, the product was dissolved in MeOH containing 1.5 pmol of pyridoxamine phosphate which was found to be pure by UV spectroscopy. After the solvent had been removed by evaporation under reduced pressure, the product was dissolved in MeOH containing 1.5 13C/molecule (18). The reaction mixture was run through an anion exchange column (Dowex 50 X-8, formate form, 200 to 400 mesh, 1.8 × 34 cm) and analyzed for deuterium content. The substrate and product in each case were assayed for the ratio of the parent peaks M + 2/M + 1 by comparing the ratio of the peak areas of M + 2 and M + 1 (m/e 170/169) with that of authentic unlabeled pyridoxamine phosphate as an internal standard. The deuterium content was determined as 26% by comparing the ratio of the peak areas of M + 2 and M + 1 (m/e 172/171) with that of authentic unlabeled pyridoxamine phosphate. From these data it was estimated that each of the fragment ions showed patterns consistent with this isotope ratio.

**a-Hydrogen Exchange of Tryptophan in D2O**—The reaction mixture in a volume of 0.75 ml of D2O contained: 0.1 M potassium phosphate buffer, pH 7.8: 1-tryptophan, 1.5 mg; pyridoxal phosphate, 0.2 mM; β-mercaptoethanol, 10 mM; phenylmethylsulfonylfluoride, 0.5 mM; and the native tryptophan synthetase (mixture of α2; β; 0.85/0.35 IU of β; 1.31/0.45 IU of α; and 1.91/0.625 IU of β. Parallel incubations from which fumarase was omitted were also carried out to give malate.

**Degradation of Stereospecifically Trinitiated Tryptophan**—The tryptophan samples (5 μCi) obtained from (2S,3R)- and (2S,3S)-[3-13]H]serine with the native tryptophan synthetase from *N. crassa* were diluted with L-[1-13C]tryptophan and diluted with 5 mg of carrier tryptophan. A small aliquot was assayed for its 13C/12C ratio at 37°C. The reaction mixtures were boiled for 2 min and the tryptophan was isolated by successive chromatography in Systems A and B.

**a-Hydrogen Exchange of Tryptophan in D2O**—The reaction mixture in a volume of 0.75 ml of D2O contained: 0.1 M potassium phosphate buffer, pH 7.8: 1-tryptophan, 1.5 mg; pyridoxal phosphate, 0.2 mM; β-mercaptoethanol, 10 mM; phenylmethylsulfonylfluoride, 0.5 mM; and the native tryptophan synthetase (mixture of β and excess α protein) or its β2 protein. The incubation was carried out at 37°C for 20 min and stopped by boiling for 3 min. The enzyme activity was determined by the ratio M + 2/M + 1 in the mass spectra at unknown institution, on September 29, 2009

**Radioactivity Determinations**—Radioactivity on chromatograms was located using a Packard model 7201 radiochromatogram scanner. The radioactivity of compounds in solution was determined in a Packard model 340 liquid scintillation counter. The radioactivity of mixtures of fumarate plus malate after equilibration with fumarase. Parallel incubations from which fumarase was omitted were also carried out to give malate.
Stereochemistry of Tryptophan Synthetase

Table 1

Stereochemistry of tryptophan synthesis with different preparations and substrates of tryptophan synthetase.

<table>
<thead>
<tr>
<th>T/°C of</th>
<th>Native enzyme from N. crassa 1-[3-³H]-</th>
<th>Native enzyme from N. crassa 1-[3-³H]-</th>
<th>Native enzyme from E. coli 1-[3-³H]-</th>
<th>Native enzyme from E. coli 1-[3-³H]-</th>
<th>β₃ protein from E. coli 1-[3-³H]-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>serine + indole</td>
<td>serine + indole</td>
<td>indole + phosphate</td>
<td>indole + phosphate</td>
<td>indole + phosphate</td>
</tr>
<tr>
<td></td>
<td>3R 3S</td>
<td>3R 3S</td>
<td>3R 3S</td>
<td>3R 3S</td>
<td>3R 3S</td>
</tr>
<tr>
<td>Serine</td>
<td>2.10 2.06</td>
<td>2.52 3.85</td>
<td>3.29 3.22</td>
<td>3.29 3.22</td>
<td>3.29 3.22</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>2.10 2.06</td>
<td>2.52 3.85</td>
<td>3.29 3.22</td>
<td>3.29 3.22</td>
<td>3.29 3.22</td>
</tr>
<tr>
<td>Aspartate</td>
<td>1.86 1.63</td>
<td>2.28 2.17</td>
<td>3.33 3.16</td>
<td>3.21 3.13</td>
<td>3.31 3.14</td>
</tr>
<tr>
<td>Malate</td>
<td>0.33 1.68</td>
<td>0.33 1.68</td>
<td>(3.21)* (3.16)*</td>
<td>(2.94)* (2.90)*</td>
<td>(2.94)* (2.90)*</td>
</tr>
<tr>
<td>Fumarate + Malate</td>
<td>2.64 0.086</td>
<td></td>
<td>3.00 0.14</td>
<td>2.82 0.14</td>
<td>3.23 0.28</td>
</tr>
<tr>
<td>Indolmycin</td>
<td></td>
<td></td>
<td>(2.99)* (0.07)*</td>
<td>(3.11)* (0.09)*</td>
<td>(2.21)* (0.09)*</td>
</tr>
</tbody>
</table>

* Figures in parentheses are the results from the parallel experiments in the presence of 0.2 M NaCl.

RESULTS

Stereochemistry of the Replacement Reaction at the β-Carbon Atom—The replacement of the hydroxyl group at Cβ of serine by the indolyl group may proceed either with retention or with inversion of configuration at Cβ. To distinguish between these alternatives, we converted (2S,3R) and (2S,3S)-[U-14C, 3-³H]serine, prepared as described earlier (4), into L-tryptophan using tryptophan synthetase purified from Neurospora crassa by the method of Meyer et al. (6) or from Escherichia coli (8,9). As shown in Table I, the T/°C ratios of the substrates do not change during this transformation, indicating complete retention of the tritium. Samples of tryptophan prepared in this way with the Neurospora enzyme were then degraded according to the scheme in Fig. 1 to determine their configuration at the β-carbon atom of the side chain. Initial attempts to carry out direct ozonolysis of N-acetyltryptophan led to extensive tritium exchange and racemization at Cβ, presumably due to formation of a readily enolizable carbonyl function adjacent to the benzene ring and the methylene group. This problem was largely circumvented by Birch reduction of tryptophan to give the 4,7-dihydro derivative (10), which was then subjected to oxidation. The results of the degradation show that the tryptophan sample obtained from (3R)-[3-³H]serine gave malate which lost most of its tritium in the fumarase reaction. Since fumarase removes the pro-3R hydrogen of L-malate (18,19), it follows that this tryptophan sample had 3s configuration. Conversely, the tryptophan sample from (3S)-[3-³H]serine was found to have 3R configuration. The replacement reaction therefore proceeds with retention of configuration at the β-carbon atom.

We next used these tryptophan samples to calibrate a simple system with which the configuration at Cβ of tritiated tryptophan could be analyzed more readily. For this we chose intact cells of Streptomyces griseus, which proceeds with loss of one of the two p hydrogens (12):

\[
\begin{align*}
\text{NHCH}_3 & \quad \text{C}_2 \quad \text{H}_3 \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\end{align*}
\]

Conversion of the two tryptophan samples of established configuration into indolmycin (Table I) showed that these were, within the limits of error, completely stereospecifically labeled and that the pro-3R hydrogen is eliminated in indolmycin formation (20). Using this analytical system we then determined the stereochemical course of tryptophan formation with the native tryptophan synthetase isolated from E. coli, using either indole or indole-3-glycerol-P as the second substrate, with tryptophan synthetase β₃ protein from E. coli in the presence of excess indole (21). As shown in Table I, the reaction in each case is completely stereospecific and occurs with retention of configuration.

Stereochemistry of the Replacement of —OH by —H at Cβ in the aβ Elimination Reaction—In the aβ elimination reaction catalyzed by tryptophan synthetase β₃ protein, a hydrogen is added at the β carbon atom of serine, replacing the OH—group, and one can ask whether this protonation step is stereospecific. To examine this question, (2S,3R)- and (2S,3S)-[U-14C, 3-³H]serine were incubated with tryptophan synthetase β₃ protein in D₂O and the resulting pyruvate was trapped in situ as lactate by reduction with excess lactate dehydrogenase and NADH. The lactate samples were isolated and oxidized to acetate and the chirality of the methyl group in the acetate samples was determined by the method of Cornforth et al. (15) and Arigoni and co-workers (16). In this analysis procedure, which involves conversion to malate with malate synthetase followed by reaction with fumarase, (R)-[2-³H, 2-³H]lactate gives rise to malate which retains more than half of its tritium in the fumarase reaction, whereas the S isomer produces malate which retains less than half of its
tritium in the fumarase reaction. The results which are shown in Table II (Columns 1 and 2) indicated that the methyl group in both samples was achiral or racemic. This is in contrast to the same reaction catalyzed by tryptophanase (3) and by tyrosine phenol-lyase (21) and to the deamination of D-serine by D-serine dehydratase (22), all of which are stereospecific and occur with retention of configuration. A conclusion that by tryptophan synthetase α is nonstereospecific (3) is predicated on the assumption that one, and only one, atom of D is incorporated into the methyl group from the D₂O medium. This assumption was subsequently shown to be incorrect. NMR analysis of a sample of lactate prepared from nonlabeled serine in D₂O by the same reaction showed the presence of no deuterium within the limits of detection. Thus, the third hydrogen of the methyl group must originate from within the enzyme-substrate complex. We therefore prepared (2S,3S)-[3-'H, 3-'H]serine and subjected it to the α,β elimination reaction with tryptophan synthetase β₃ protein in H₂O. Chirality analysis (Table II, columns 3 and 4) showed that in both cases the methyl group was chiral. 3S-Serine had produced R-acetate and 3R-serine had given S-acetate, indicating that the protonation at C₆ does occur stereospecifically from the same side at which OH has been removed.

**Origin of the Third Hydrogen of the Methyl Group of Pyruvate Formed by α,β Elimination**—The data presented show that the hydrogen which is added at C-3 of serine to give the pyruvate methyl group must originate from within the enzyme-substrate complex and must be transferred without appreciable exchange with solvent protons. The only other nonexchangeable hydrogen in the substrate is H₆, which must therefore be the source of the third methyl hydrogen. This was demonstrated to be true by repeating the experiment in column 1, Table II, with substrate carrying deuterium in position 2, in addition to the stereospecific tritium label at C-3. dl-(3R)-(2-'H, 3-'H]serine, in contrast to the unlabelled sample, gave pyruvate containing a chiral methyl group (Table II, Column 5). In a second experiment, it was shown that the transfer of the α-hydrogen to C₆ is intramolecular. For this experiment, (2S,3S)-[3-'H, 3-'H]serine was mixed with a large (64-fold) excess of [2-'H]serine and converted into lactate. Chirality analysis (Table II, Column 6) showed that the methyl group was chiral and had R configuration. Had intermolecular transfer of the α-hydrogen occurred to even a small extent, the majority of tritiated methyl groups would have been deuterated. The difference in apparent chiral purity of the acetate samples reported in Columns 4, 5, and 6 of Table II, although appreciable, is not statistically significant since the error of the chiral analysis is approximately ±5% absolute.

### Table II

**Stereochemistry of the α,β elimination/deamination of serine catalyzed by tryptophan synthetase β₃ protein**

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3R</td>
<td>3S</td>
<td>3R</td>
<td>3S</td>
<td>3R</td>
</tr>
<tr>
<td>Substrate</td>
<td>2.00</td>
<td>2.00</td>
<td>1.94</td>
<td>4.22</td>
<td>2.57</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.09</td>
<td>2.04</td>
<td>2.90</td>
<td>8.50</td>
<td>5.20</td>
</tr>
<tr>
<td>Acetate</td>
<td>3.02</td>
<td>2.90</td>
<td>3.56</td>
<td>6.30</td>
<td>1.52</td>
</tr>
<tr>
<td>Malate</td>
<td>2.56</td>
<td>2.47</td>
<td>2.08</td>
<td>2.35</td>
<td>1.27</td>
</tr>
<tr>
<td>Fumarate</td>
<td>1.31</td>
<td>1.27</td>
<td>2.04</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>% H retention in fumarase reaction</td>
<td>51.1</td>
<td>51.4</td>
<td>37.3</td>
<td>71.1</td>
<td>64.8</td>
</tr>
</tbody>
</table>

* [2-'C]Acetate was added as reference.

NMR analysis of a [2-'C]Acetate was added as reference. These samples were labeled in such a way that essentially every tritiated molecule also contained deuterium.

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**Stereochemistry of Tryptophan Synthetase**

- **Transfer of H₆ to C-4' of Pyridoxal Phosphate in the Abortive Transamination Reaction**—In order to obtain further information on the geometry of the enzyme substrate complex we determined the origin of the hydrogen which is added at C-4' of the cofactor in the abortive transamination reaction to give pyridoxamine phosphate (Reaction 5). Unlabeled serine and mercaptoethanol were incubated with tryptophan synthetase β₃ protein in D₂O and the pyridoxamine phosphate formed was isolated from the reaction mixture. After purification it was analyzed for its deuterium content by chemical ionization and electron impact mass spectrometry. The data showed the presence of only 0.25 and 0.26 atom of deuterium per mol, respectively, in the two analyses, implying that about 75% of the hydrogen added at C-4' must have originated from within the enzyme-substrate complex, most likely from the α position of serine. Such a 1 → 3 hydrogen shift from C₆ to C-4' has been observed in pyridoxamine-pyruvate transaminase (23). Since the hydrogen being transferred undergoes little exchange with the medium, the transfer can be assumed to be intramolecular. However, since the experiment requires a very large amount of enzyme, no attempt was made to further examine this point.

- **Nature of the Base Group Catalyzing Protonation/Deprotonation at C₆**—An attempt was made to establish the origin of the hydrogen which is added at C₆ of the tryptophan formed in the tryptophan synthetase reaction. Incubation of the native enzyme with nonlabeled indole and serine in D₂O gave tryptophan which was analyzed for deuterium content by mass spectrometry and by proton NMR. Mass spectrometry indicated the presence of 0.86 atom of deuterium, whereas integration of the signal for H₆ in the proton NMR spectrum showed the presence of 0.09 atom of H. The α-hydrogen of tryptophan thus originates predominantly from the solvent, but the presence of a small amount of H is consistent with some internal transfer of a proton within the enzyme substrate complex, presumably from C₆ of serine. Repetition of the above experiment in a medium of 50% D₂O in H₂O gave tryptophan which by mass spectral analysis was shown to contain 49.8% deuterium in the α position. Thus, the protonation of this site proceeds without an appreciable isotope effect. Based on the arguments put forth by O'Leary (24), these data indicate that the proton is transferred to C₆ by a monoprotic base group in the enzyme, consistent with the

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1 These samples were labeled in such a way that essentially every tritiated molecule also contained deuterium.
results of Miles and Kumagai (25) implicating a histidyl residue in the abstraction of the \( \alpha \)-proton of serine.

**Rate of \( \alpha \)-Hydrogen Exchange of Tryptophan**—One of the perplexing features of tryptophan synthetase \( \beta_2 \) protein is the fact that it can deaminate serine, but not tryptophan. As one conceivable explanation, we considered the possibility that tryptophan cannot undergo \( \alpha \)-hydrogen cleavage, thus preventing an essential step of the reaction sequence. To examine this question we measured the exchange of the \( \alpha \)-hydrogen of tryptophan against deuterium catalyzed by \( \beta_2 \) protein and native tryptophan synthetase in D\(_2\)O. Since the enzyme used was not homogeneous, the rates were related to the overall rate of tryptophan synthesis by the native enzyme. It was found that the native enzyme catalyzes \( \alpha \)-hydrogen exchange of tryptophan at 20% of the rate of tryptophan synthesis. The \( \beta_2 \) protein catalyzes \( \alpha \)-hydrogen exchange of tryptophan at 70% of the rate observed for the native enzyme. Thus, inability to catalyze C\(_6\)-H bond cleavage in tryptophan does not account for the inability of tryptophan synthetase \( \beta_2 \) protein to carry out the \( \alpha \beta \) elimination reaction with tryptophan as substrate.

**DISCUSSION**

The stereochemistry observed for the \( \beta \) replacement reaction catalyzed by tryptophan synthetase, which was independently confirmed by Fuganti’s group (26), conforms to that seen in all the pyridoxal phosphate-catalyzed \( \beta \) replacement reactions studied, i.e. in the replacement reactions catalyzed by tryptophanase (3, 27), tyrosine phenol-lyase (28, 29), O-acetylserine sulfhydrolase (4), and \( \beta \)-cyanoalanine synthetase.\(^5\) This may have an implication for the reaction mechanism. As was discussed in a previous paper (4), formation and cleavage of a bond at C\(_6\) is expected to require an orthogonal orientation of this bond relative to the \( \pi \) plane and a corresponding alignment of the incoming substituent and the leaving group. Retention of configuration requires orthogonal alignment of these two groups on the same side of the \( \pi \) plane implying, since two objects cannot be in the same place at the same time, that the reaction either proceeds by a ping-pong mechanism or involves a conformational change of the enzyme during the reaction which reorients the incoming and the leaving group relative to the \( \pi \) plane. For O-acetylserine sulfhydrolase a ping-pong mechanism has recently been demonstrated (30).

The stereochemical data reported define the geometry of the substrate cofactor complex and of the reaction intermediates in the active site and the position of an essential base relative to these. These relationships are summarized in Fig. 2. A monoprotic base, presumably a histidine residue, catalyzes the abstraction of the \( \alpha \)-hydrogen (H\(_1\)) and a completely intramolecular transfer of this hydrogen to C\(_6\). Since this \( \alpha \)-hydrogen shift is intramolecular it must be suprafacial, and since the replacement of the OH group at C\(_6\) by H\(_1\) occurs with retention of configuration, the configuration around the bond \( \alpha \)-hydrogen cleavage bond \( \alpha \)-hydrogen cleavage at C\(_6\) must be as shown in Fig. 2, i.e. H\(_1\) is displayed orthogonal to the \( \pi \) plane on the side which corresponds to the si face at C-4'. This conclusion is predicated on the widely accepted assumption (32) that the configuration of the Schiff's base around the C\(_6\)-C\(_4\) bond is syn, allowing hydrogen bonding between the nitrogen and the phenolic hydroxyl group. The atoms C\(_6\)-N-C\(_4\)-C\(_5\) thus lie in a plane, underneath which the essential base is situated which functions in the abstraction of the \( \alpha \)-hydrogen of the substrate and the protonation of C\(_6\) to give the product. Under certain conditions this base can also donate the proton to sites other than C\(_6\).

The finding that H\(_1\) is transferred completely to C\(_6\) in the \( \alpha \beta \) elimination reaction, but only to the extent of \( \frac{75}{100} \) in the abortive transamination reaction, requires some comment. Reaction 5 is much slower than Reaction 3, and Reaction 5 is at least partially reversible (17) whereas Reaction 3 is not. After formation of the aminoaacrylate Schiff's base, protonation at C\(_6\) by transfer of H\(_1\) from the protonated base and addition of RS\(^{-} \) at C\(_6\) are competing reactions. Following addition of RS\(^{-} \), protonation at C-4' may be a slower process, allowing for some exchange of the hydrogen on the base with solvent protons in this species.
It has been shown (33, 34) that the tryptophan synthetase \( \beta_2 \) protein upon binding of the \( \alpha \) subunit undergoes a conformational change, which increases its catalytic efficiency. The small difference in the rates of \( \alpha \)-hydrogen abstraction from tryptophanase with which it shares the tryptophan:enzyme complex.

It is interesting to compare tryptophan synthetase \( \beta_2 \) protein with the enzyme tryptophanase, with which it shares many catalytic capabilities. As has been pointed out (1), there are many similarities between these two enzymes. The reactions seems to involve essentially the same intermediates and, as shown in this and the following paper (27), the overall geometry of the coenzyme:substrate complexes in the two enzymes as well as the steric course of the reactions catalyzed are very similar or identical. A major difference is the fate of the hydrogen abstracted from the \( \alpha \) position. In tryptophanase it is used to protonate the leaving group, whereas in tryptophan synthetase \( \beta_2 \) protein it can be transferred quantitatively to \( \mathrm{Co} \), implying that it is not used to protonate the leaving group. Another difference is the inability of tryptophan synthetase \( \beta_2 \) protein to catalyze \( \alpha,\beta \) elimination of tryptophan, and, as the data show, this cannot be explained by inability to cleave the \( \mathrm{C}_\alpha-H \) bond in this substrate. Perhaps related to this may be the inability of tryptophan synthetase to catalyze synthesis of tryptophan from indole, pyruvate, and ammonia, a reaction readily catalyzed by tryptophanase (35, 36). Since this presumably reflects the ability or inability of these enzymes to abstract a hydrogen from the methyl group of pyruvate it is particularly perplexing, because both enzymes can stereospecifically protonate \( \mathrm{C}_m \) to generate the methyl group and both must thus have a base group proximal to this site. It is possible that the difference lies in the nature of the base groups in the two enzymes and/or in the exact position of the bases relative to the pyrdaldehyde amino acid complex.

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