Structure and Function of the Tryptophan Synthase α₂β₂ Complex

ROLES OF β SUBUNIT HISTIDINE 86*

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To probe the structural and functional roles of active-site residues in the tryptophan synthase α₂β₂ complex from Salmonella typhimurium, we have determined the effects of mutation of His86 in the β subunit. His86 is located adjacent to β subunit Lys87, which forms an internal aldimine with the pyridoxal phosphate and catalyzes the abstraction of the α-proton of l-serine. The replacement of His86 by leucine (H86L) weakened pyridoxal phosphate binding ~20-fold and abolished the circular dichroism signals of the bound coenzyme and of a reaction intermediate. Correlation of these results with previous crystal structures indicates that β-His86 plays a structural role in binding pyridoxal phosphate and in stabilizing the correct orientation of pyridoxal phosphate in the active site of the β subunit. The H86L mutation also altered the pH profiles of absorbance and fluorescence signals and shifted the pH optimum for the synthesis of l-tryptophan from pH 7.5 to 8.8. We propose that the interaction of His86 with the phosphate of pyridoxal phosphate and with Lys87 lowers the pKₐ of Lys87 in the wild-type α₂β₂ complex and thereby facilitates catalysis by Lys87 in the physiological pH range.

The tryptophan synthase α₂β₂ complex (EC 4.2.1.20) is a useful system for investigating relationships between protein structure and function, allosteric communication between subunits in a multienzyme complex, and substrate channeling (for reviews, see Refs. 1–5). The β subunit† is the prototypic member of a family of pyridoxal phosphate (PLP)²-dependent enzymes that have distantly related sequences and that catalyze β-replacement and β-elimination reactions (6–8). The crystal structure of the tryptophan synthase α₂β₂ complex from Salmonella typhimurium (9) suggested the probable fold of other members of this family. Recent structures of biosynthetic threonine deaminase from Escherichia coli (10) and of O-acetylserine sulphydrylase from S. typhimurium (11) confirm that these enzymes have the same fold as the tryptophan synthase β subunit.

Multiple alignment of many sequences of several enzymes in this family (Fold type II) showed that the residue on the N-terminal side of the active-site Lys is His in the tryptophan synthase β subunit, Val in O-acetylserine sulphydrylase, Phe or Tyr in threonine deaminase, Phe in threonine synthase, Val or Ile in cystathionine β-synthase, Phe in l-serine dehydratase, Phe or Ile in d-serine dehydratase, and Asn in 1-aminocyclopropane-1-carboxylate deaminase (see Electronic Appendix to Ref. 8). This residue is located at the back of the coenzyme ring in the structures of tryptophan synthase (9), threonine deaminase (10), and O-acetylserine sulphydrylase (11). The authors of the latter work suggested that this residue (Val⁸⁶) may support the coenzyme ring, similar to the functions performed by Ala⁷²⁴ in aspartate aminotransferase (12) and Ala⁷²⁵ in d-alanylglutamic decarboxylase (13), which belong to a different family of PLP-dependent enzymes (Fold type I) (8). Leu²⁰¹ is located in a similar position in the structure of l-α-amino-acid aminotransferase (14), which is classed in Fold type IV (8). Investigations of the effects of mutagenesis of Leu²⁰¹ utilizing biochemical (15) and crystallographic (16) methods support the proposal that Leu²⁰¹ plays a crucial role in the transamination reaction by keeping the pyridoxyl ring in the proper location without disturbing its oscillating motion.

The reaction of l-Ser with the β subunit proceeds through a series of PLP intermediates (Fig. 1A): the gem-diamine intermediate (E-GD), the external aldimine of l-Ser (E-Ser), and the external aldimine of aminoacrylate (E-AA). E-AA reacts with indole to form a quinonoid intermediate (E-Q); protonation of E-Q yields the external aldimine of the l-Trp (E-Trp). In the absence of added indole, E-Ser and E-AA accumulate. The equilibrium distribution of E-Ser and E-AA is affected by pH, temperature, monovalent cations, and allosteric ligands (17).

Recent crystal structures of enzyme-substrate intermediates formed by the tryptophan α₂β₂ complex provide further insight into the roles of His⁸⁶ and of other active-site residues. The structure of E-Ser was obtained by cryocystallography of a mutant (αD60N) tryptophan synthase α₂β₂ complex in the presence of l-Ser (18) (Fig. 1B). The structure of E-AA was obtained with the wild-type α₂β₂ complex in the presence of l-Ser and fluoroindole-3-propanol phosphate using a flow cell (19) (Fig. 1C). Both structures show that one nitrogen of His⁸⁶ is within hydrogen-bonding distance of the phosphate of PLP and the hydroxyl group of Ser⁴⁵⁵. The phosphate of PLP also interacts with the hydroxyl group of Ser⁴⁵⁵ and with the carboxyl oxygens of Ser⁴⁵⁵ and of three glycine residues in the glycine-rich loop (residues 232–234). The primary difference between the two structures is that His⁸⁶ can form a hydrogen bond with the ε-amino group of Lys⁸⁷ (3.09 Å) in the structure of E-AA, but is too far (3.8 Å) to form a stable hydrogen bond with Lys⁸⁷ in the structure of E-Ser. However, because the temperature factor of the ε-amino group of Lys⁸⁷ is high in both structures, this group undergoes fluctuations in its position.

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| The term β₂ subunit is used for the isolated enzyme in solution; β subunit is used for the enzyme in the α₂β₂ complex or to describe a specific residue in the β subunit.
| The abbreviations used are: PLP, pyridoxal phosphate; MOPS, 3-morpholino propane sulfonic acid; Bicine, N,N-bis(2-hydroxyethyl) glycine.

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The presence of the hydrogen bond between His\textsuperscript{86} and Lys\textsuperscript{87} in E-AA (19) is consistent with evidence from spectroscopic experiments that the abstracted \( \alpha \)-proton of L-Ser is sequestered in a solvent-excluded site and possibly stored in a low barrier hydrogen bond (20). The interactions of His\textsuperscript{86} with PLP and with other residues in the active site would be expected to alter the \( pK_a \) values of His\textsuperscript{86}, Lys\textsuperscript{87}, and the phosphate of PLP. Indeed, the \( ^{31}P \) nuclear magnetic resonance chemical shift of the phosphate of the PLP, which is bound to the tryptophan synthase apo form, is pH-dependent, with \( pK_a \) values of 6.5 to 8.6, whereas the PLP \( ^{31}P \) chemical shift of a model PLP derivative exhibits a \( pK_a \) value of 6.23 (21). The results suggest that the phosphate group of PLP is excluded from interaction with water and is fixed in its dianionic form by means of a salt bridge to a positively charged region of the enzyme, leading to a considerable lowering of the \( pK_a \) value for the mononion-dianion (21). This proposal is supported by the crystallographic results (Fig. 1, A and C) that show that His\textsuperscript{86} is within hydrogen-bonding distance of the phosphate of PLP. Interestingly, the \( ^{31}P \) shift of PLP bound to d-serine dehydratase from E. coli is pH-dependent, with \( pK_a \) = 6.4 (22). D-Serine dehydratase is related in sequence to the tryptophan synthase \( \beta \) subunit, but has His\textsuperscript{117} at the position equivalent to His\textsuperscript{86} in the \( \beta \) subunit (see Electronic Appendix to Ref. 8). These observations provide additional evidence that His\textsuperscript{86} in the \( \beta \) subunit stabilizes the phosphate of PLP as the dianion.

Early studies of the effects of substitution of \( \beta \) subunit His\textsuperscript{86} by leucine (H86L) in the \( \alpha_2\beta_2 \) complex concluded that His\textsuperscript{86} did not play an important role in catalysis or in substrate binding (23). Here we report more detailed investigations of the effects of pH on kinetic and spectroscopic properties of the H86L \( \alpha_2\beta_2 \) complex. The results provide evidence that His\textsuperscript{86} facilitates catalysis by lowering the \( pK_a \) of the catalytic residue Lys\textsuperscript{87} and also plays a structural role by binding and supporting the PLP coenzyme.

FIG. 1. Intermediates in the reaction of the tryptophan synthase \( \alpha_2\beta_2 \) complex with L-Ser (A) and three-dimensional structures of two intermediates: E-Ser (B) and E-AA (C). A, shown are the intermediates in the reaction with L-Ser: internal aldimine (E), gem-diamine (E-GD), the external aldimine of L-Ser (E-Ser), and the external aldimine of aminoacrylate (E-AA). E-AA reacts with an added indole to form a quinonoid intermediate (E-Q); protonation of E-Q yields the bound product (E-Trp). In the absence of an added nucleophile, E-Ser and E-AA accumulate. B, the structure of E-Ser was obtained by cryocrystallography of a mutant (D60N) tryptophan synthase \( \alpha_2\beta_2 \) complex in the presence of L-Ser and indole-3-propanol phosphate (18). C, the structure of E-AA was obtained with the wild-type \( \alpha_2\beta_2 \) complex in the presence of L-Ser and fluoroindole-3-propanol phosphate using a flow cell (19). B and C were prepared with selected residues from the Protein Data Bank (codes 1BEU and 1A5S, respectively) using Rasmol. Enz, enzyme.

EXPERIMENTAL PROCEDURES

Chemicals and Buffers—PLP and L-serine were purchased from Sigma. MOPS and proline were from Fluka. Bicine was from U. S. Biochemical Corp. MBP buffer (17), containing 50 mM MOPS, 50 mM Bicine, 50 mM proline, and 1 mM EDTA, was used for kinetic and spectroscopic studies with additional 100 mM NaCl. The pH was raised with NaOH to pH 11.2; the solution was then back-titrated with HCl to the desired pH value.

Bacterial Strains, Plasmids, Enzyme Preparations, and Enzyme Assays—The plasmid pEBA-10 (24) was used to express the wild-type tryptophan synthase \( \alpha_2\beta_2 \) complex from S. typhimurium in E. coli CB149 (25), which lacks the trp operon. The plasmid pSTB7 (25) containing the H86L mutant trpB gene from the S. typhimurium \( \alpha_2\beta_2 \) complex was used to express the H86L \( \alpha_2\beta_2 \) complex in E. coli CB149 (23). The mutant and wild-type \( \alpha_2\beta_2 \) complexes were purified by a method using crystallization from crude extracts (23). The \( \alpha \) subunit was expressed and purified as described previously (26). Protein concentrations were determined from the specific absorbance at 278 nm using \( A_{1 \text{nm}}^{1\text{cm}} = 6.0 \) for the \( \alpha_2\beta_2 \) complex and \( A_{1 \text{nm}}^{1\text{cm}} = 4.4 \) for the \( \alpha \) subunit (27). The apo forms of the wild-type and mutant \( \alpha_2\beta_2 \) complexes were
prepared by a method utilizing treatment with hydroxylamine and KSCN (28).

The activity of tryptophan synthase in the β-replacement reaction (L-Ser + indole → L-Trp + H₂O) was determined by fitting the initial rate data to Equation 1,

\[ v = V \cdot S \left( \frac{K_m + S}{K_m + S} \right) \]  

(Eq. 1)

where \( v \) is the initial rate and \( S \) is the concentration of L-serine in the presence of 0.2 mM indole.

Spectroscopic Methods—Absorption spectra were measured using a Hewlett-Packard 8452 diode array spectrophotometer. Fluorescence spectra were made using a Photon Technology International spectrofluorometer. The pH dependence of the absorption and fluorescence spectra was measured in the presence of 100 mM L-Ser. Circular dichroism spectra were recorded on a Jasco J-715 spectropolarimeter in the absence and presence of L-Ser (100 mM). The final concentration of the protein used for the spectroscopic measurements was 10 μM. All spectra were obtained in the presence of excess α subunit at 25 °C.

Measurement of PLP Dissociation Constants—The apparent dissociation constants of PLP for the wild-type and H86L αβ₂ complexes were determined by measuring the activity of the apoenzymes in the β-replacement reaction (L-Ser + indole → L-Trp + H₂O) in a reaction mixture containing 100 mM L-Ser, 0.2 mM indole, 100 mM NaCl, 50 mM MBP buffer, and a 10-fold molar excess of α subunit at pH 7.3, 8.0, or 9.2 and at 37 °C. PLP concentrations were 0–1 μM for the wild-type enzyme and 0–30 μM for the H86L enzyme.

RESULTS

The H86L Mutation Alters the pH Dependence of the Absorption and Fluorescence Spectra of the Tryptophan Synthase αβ₂ Complex and of the Reaction Intermediates with L-Ser—The absorption spectrum of the H86L αβ₂ complex in the absence of L-Ser (Fig. 2) was similar to that of the wild-type αβ₂ complex (spectrum not shown). However, the absorbance at 420 nm of the H86L αβ₂ complex exhibited a small decrease at alkaline pH (Fig. 2, inset), which is not observed with the wild-type enzyme (17). The fluorescence spectra of the wild-type and H86L αβ₂ complexes (Fig. 3A) and plots of fluorescence at 510 nm versus pH (Fig. 3B) also revealed differences between the two enzymes. The fluorescence intensity of the wild-type enzyme was essentially pH-independent in the absence of L-Ser, whereas that of the H86L enzyme decreased at alkaline pH.

The reaction of L-Ser with the wild-type αβ₂ complex from S. typhimurium in the absence of indole yielded several intermediates: E-GD, E-Ser, and E-AA (Fig. 1A). The equilibrium distribution of these intermediates under steady-state conditions is strongly pH-dependent (17). E-Ser (λ_max = 420 nm) predom-
The absorbance at 420 nm and the fluorescence intensity of the fluorescence emission at 510 nm (excitation at 420 nm) (30). The absorbance at 420 nm and the fluorescence intensity of the intermediates formed in the reaction of the wild-type αβ2 complex with l-Ser exhibit very similar pH dependences (17).

The effects of pH on the absorption spectra (Fig. 2) and fluorescence spectra (Fig. 3C) of the H86L αβ2 complex in the presence of l-Ser were significantly different from the effects of pH on the spectra of the wild-type enzyme reported previously (17). The absorbance at 420 nm and the fluorescence emission at 510 nm (excitation at 420 nm), which are attributed to E-Ser, of the H86L αβ2 complex increased significantly only above pH 8 (Figs. 2 and 3, C and D), whereas the analogous signals of the wild-type enzyme increased significantly between pH 7 and 8 (Fig. 3, C and D) (17). The major absorbance peak of the H86L enzyme in the presence of l-Ser (λmax = 320–340 nm) (Fig. 2) differed from that of the wild-type enzyme (λmax = 350 nm) (17). The low wavelength peak of the H86L enzyme shifted from 340 to 320 nm with increasing pH.

To further characterize the low wavelength peak of the H86L enzyme, fluorescence emission spectra (excitation at 355 nm) were recorded for the wild-type and H86L αβ2 complexes in the presence of l-Ser at three pH values (Fig. 4). The H86L enzyme, unlike the wild-type enzyme, exhibited a strong fluorescence emission at 400 nm at pH 7.6 in the presence of l-Ser. The intensity of this peak decreased with increasing pH. These results provide evidence for the presence of a PLP derivative with saturated C-4' in the reaction of H86L with l-Ser (31, 32), most likely the gem-diamine (E-GD in Fig. 1A).

**The H86L Mutation Alters the pH Dependence of the Kinetic Parameters**—The pH dependences of activity (Vmax) for the wild-type and H86L αβ2 complexes were determined for the β-elimination reaction (Fig. 6A) and for the β-replacement reaction (Fig. 6B). The pH profiles of the wild-type enzyme in the two reactions were very different. Although the activity was maximal at pH ~7.5 in the β-elimination reaction as reported recently (38), the activity in the β-elimination reaction increased between pH 6.6 and 9.2. In contrast, the activity of the H86L enzyme was very low at pH 7 and increased markedly above pH 7.5 in both reactions. The H86L mutation shifted the maximum of the bell-shaped curve of Vmax versus pH in the β-replacement reaction from pH 7.5 to 8.8.

**DISCUSSION**

Our studies of the effects of a mutation (H86L) in the active site of the β subunit of tryptophan synthase demonstrate that
His<sup>86</sup> interacts with the phosphate of PLP and with Ser 235 in the glycine-rich loop and is located behind the PLP ring (Fig. 1, inset). The interaction of His<sup>86</sup> with the phosphate of PLP with residues 232–235 in the glycine-rich loop and is located behind the PLP ring (46). The loss in the His<sup>86L</sup> α<sub>b</sub>β<sub>2</sub> complex of the circular dichroism signals that are observed in the wild-type α<sub>b</sub>β<sub>2</sub> complex and in its reaction intermediate with l-Ser (Fig. 5) suggests that the double bond and the PLP ring have relaxed in the mutant enzyme. Several other mutant forms of the tryptophan synthase α<sub>b</sub>β<sub>2</sub> complex exhibit optically active PLP coenzymes (35). The only exception observed previously is the K87T α<sub>b</sub>β<sub>2</sub> complex, which has very weak circular dichroism at 400 nm (35). The results demonstrated the importance of the internal aldime linkage (E in Fig. 1A) for rigid or asymmetric binding of PLP (35). Our new finding that His<sup>86</sup> is also needed for the rigid or asymmetric binding of PLP leads to the conclusion that multiple interactions are required for the proper orientation of PLP in the active site of the β subunit: the formation of an aldime, interactions of the phosphate of PLP with residues 232–235 in the glycine-rich loop and with His<sup>86</sup>, and the location of His<sup>86</sup> behind the PLP ring (Fig. 1).

His<sup>86</sup> Stabilizes Cofactor Binding and Orientation—Our findings that the His<sup>86L</sup> mutation increased the apparent dissociation constant for PLP (K<sub>d</sub><sub>PLP</sub>) by 18-fold (Table I) and increased the rate of removal of PLP by dialysis provide evidence that His<sup>86</sup> plays a role in cofactor binding. This functional role is consistent with the crystallographic evidence that His<sup>86</sup> interacts with the phosphate of PLP and with Ser<sup>235</sup> in the Gly-rich loop and is located behind the PLP ring (Fig. 1, B and C). Dialysis has been used previously to compare the rate of PLP dissociation from the wild-type and K87T α<sub>b</sub>β<sub>2</sub> complexes and to demonstrate that PLP is bound more weakly to the mutant enzyme (36). The results established the importance of the internal aldime (E in Fig. 1A) for tight binding of PLP.

The interaction of the phosphate of PLP with a Gly-rich loop is a common feature of PLP enzymes in Fold types I, II, and IV (38). Mutations (Gly to Asp) at either of two positions in the Gly-rich loop of β-serine dehydratase, an enzyme related to tryptophan synthase and in Fold type II, led to loss of activity and to a great increase in K<sub>d</sub><sub>PLP</sub> (39, 40). The results indicated that the mutant β-serine dehydratases bind PLP weakly, but do not hold the cofactor in a catalytically competent orientation. The tryptophan synthase β subunit is the only member of Fold type II that has His adjacent to the active-site Lys. Consequently, the interactions of His<sup>86</sup> with the phosphate of PLP and Ser<sup>235</sup>, as shown in Fig. 1 (B and C), are likely to be features specific to tryptophan synthase that are important for binding PLP and for holding PLP in the catalytically competent orientation. Thus, mutation of His<sup>86</sup> in tryptophan synthase has effects similar to those of mutation of Gly in the Gly-rich loop of β-serine dehydratase.

The measured K<sub>d</sub><sub>PLP</sub> is an apparent dissociation constant because it is determined from activity in the presence of l-Ser and indole. l-Ser may alter PLP binding in two ways: first by converting the internal aldime to one or more external aldimes (Fig. 1A) and second by inducing a conformational change that alters catalysis and increases subunit association (41). The occurrence of a conformational change triggered by reaction with l-Ser is supported by kinetic results (42, 43) and by crystallographic results (19, 44). A true K<sub>d</sub> (−0.2 μM in Table I) for the wild-type α<sub>b</sub>β<sub>2</sub> complex obtained from activity in the presence of l-Ser and indole is lower than the true K<sub>d</sub> (1 μM) demonstrates that l-Ser does increase the affinity for PLP.

The induced circular dichroism signal from enzyme-bound PLP has been proposed to largely originate from out-of-plane twisting of the internal aldime double bond with respect to the pyridine ring of PLP (46). The loss in the His<sup>86L</sup> α<sub>b</sub>β<sub>2</sub> complex of the circular dichroism signals that are observed in the wild-type α<sub>b</sub>β<sub>2</sub> complex and in its reaction intermediate with l-Ser (Fig. 5) suggests that the double bond and the PLP ring have relaxed in the mutant enzyme. Several other mutant forms of the tryptophan synthase α<sub>b</sub>β<sub>2</sub> complex exhibit optically active PLP coenzymes (35). The only exception observed previously is the K87T α<sub>b</sub>β<sub>2</sub> complex, which has very weak circular dichroism at 400 nm (35). The results demonstrated the importance of the internal aldime linkage (E in Fig. 1A) for rigid or asymmetric binding of PLP (35). Our new finding that His<sup>86</sup> is also needed for the rigid or asymmetric binding of PLP leads to the conclusion that multiple interactions are required for the proper orientation of PLP in the active site of the β subunit: the formation of an aldime, interactions of the phosphate of PLP with residues 232–235 in the glycine-rich loop and with His<sup>86</sup>, and the location of His<sup>86</sup> behind the PLP ring (Fig. 1).

His<sup>86</sup> Alters the pH Dependence of Catalytic Properties—Our results show that mutation of β subunit His<sup>86</sup> (His<sup>86</sup>L) altered the pH dependence of the absorption and fluorescence spectra of the tryptophan synthase α<sub>b</sub>β<sub>2</sub> complex in the absence of l-Ser (Figs. 2 (inset) and 3 (A and B)) and in the presence of l-Ser (Figs. 2, 3 (C and D), and 4) and altered the pH dependence of V<sub>max</sub> in the β-elimination and β-replacement reactions (Fig. 6, A and B, respectively). The His<sup>86L</sup> α<sub>b</sub>β<sub>2</sub> complex formed very little E-Ser between pH 7 and 8 (Fig. 3D) and had very low activity in either the β-elimination reaction (Fig. 6A) or the β-replacement reaction (Fig. 6B) between pH 7 and 8. Under the same conditions, the wild-type enzyme formed significant E-Ser (Fig. 3D) and had significant activity (Fig. 6, A and B). Furthermore, the His<sup>86L</sup> α<sub>b</sub>β<sub>2</sub> complex exhibited an intense fluorescence emission peak at 405 nm (excitation at 335 nm) at pH 7.6 and 8.0 that decreased in intensity at pH 9.3 (Fig. 4) and that was not seen with the wild-type enzyme. This emission peak is characteristic of PLP–gem-diamines (47) (E-GD in Fig. 1A) and other PLP derivatives that have saturated C-4 and sp<sup>3</sup> character at C-4′ (31, 32). A gem-diamine intermediate has been detected previously in the reaction of the wild-type α<sub>b</sub>β<sub>2</sub> synthase complex with the intermediate analogues oxindolyl-L-alanine and 2,3-dihydro-L-tryptophan (48). A novel derivative that has an emission maximum at 384 nm upon excitation at 340 nm is formed during the inactivation of a mutant (S377D) tryptophan synthase α<sub>b</sub>β<sub>2</sub> complex (49).
contrast, enolimine tautomers of Schiff bases exhibit maximal fluorescence near 500 nm upon excitation at the wavelength of maximal absorbance (320–350 nm) as reported for native phosphorylase (32).

The combined spectroscopic results (Figs. 2–4) provide evidence that the H86L αβ complex reacts with 1-Ser between pH 7 and 8 to form the gem-diamine intermediate (E-GD in Fig. 1A) and that the conversion of E-GD to E-Ser is low under these conditions. The formation of a low level of E-Ser between pH 7 and 8 probably results from the low catalytic activity of the H86L αβ complex in β-elimination and β-replacement reactions in this pH range (Fig. 6).

We now ask how the H86L mutation alters the catalytic properties. The presence of His86 behind the PLP ring in the wild-type enzyme may facilitate expulsion of Lys87 from the gem-diamine. In addition, His86 in the wild-type enzyme very likely alters the pK_a of Lys87. His86, Lys87, and the phosphate of PLP are involved in a complex system of interactions with each other and with other residues in the β site (positions 232–235) in E-Ser (Fig. 1B) and E-AA (Fig. 1C). X-ray crystallography cannot assign the location of protons shared between these groups. However, 31P nuclear magnetic resonance studies of tryptophan synthase demonstrate that the PLP phosphate is a diion and suggest that the diion is stabilized by means of a salt bridge to a positively charged region of the active center, leading to a considerable lowering of the pK_a value for the monoanion-diamion (21). The crystallographic results (Fig. 1) suggest that His86 is the positively charged residue that forms an ion pair with the phosphate of PLP. This ion pair may stabilize the protonated His86, raise the pK_a of His86, and reduce the pK_a of the phosphate of PLP. The proximity of the protonated His86 to Lys87 may decrease the pK_a of Lys87 and increase the ability of Lys87 to accept the α-proton of l-serine at neutral pH. This suggestion is supported by the observation that replacement of His86 by a neutral leucine residue (H86L) increased the pK_a of Lys87 and shifted the pH activity profile (Fig. 6).

Our recent investigations of the pH dependence of the kinetic parameters of the wild-type αβ complex in the β-replacement reaction provided evidence that Lys87 has a pK_a value of ~7.2, which is much lower than the pK_a of the ε-amino group of lysine in solution (~10.5) (38). Our new results and interpretation of the crystallographic data provide a plausible explanation for the low pK_a of Lys87 in the wild-type enzyme.

The kinetic studies of the wild-type enzyme (38) also provide evidence that the decrease in activity in the β-replacement reaction at high pH (pK_a = 8.3) (Fig. 6B) is associated with a conformational change that yields an open form of the αβ complex. Interestingly, the pH profile of V_max for the β-elimination reaction by the wild-type enzyme (Fig. 6A), that has not been reported previously, was very different from that for the β-replacement reaction (Fig. 6B). The β-elimination reaction increased nearly linearly between pH 6.7 and 9.3. A plausible explanation is that the open form of the αβ complex formed at high pH has increased activity in the β-elimination reaction. We have proposed previously that the open form of the αβ complex, which is induced by mutation (50–53), by solvents (54, 55), or by guanidine HCl or urea (56), has increased activity in the β-elimination reaction relative to activity in the β-replacement reaction because the E-AA intermediate is more accessible to hydrolysis by solvent water in the open form and because catalysis of the β-replacement reaction is less efficient in the open form. Our finding of increasing β-elimination activity with increasing pH is consistent with the previous conclusion that the open conformation is formed at high pH (38). The H86L αβ complex also showed an almost linear increase in β-elimination activity between pH 7.5 and 9.3, but showed much lower activity at low pH than the wild-type enzyme. The decreased activity at low pH likely results from the increase in the pK_a of the catalytic Lys87 caused by the mutation. In conclusion, our results provide evidence that His86 facilitates catalysis by altering the pK_a of Lys87 and plays a structural role in binding and orienting the PLP coenzyme.

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REFERENCES

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