Importance of Conserved and Variable C-terminal Residues for the Activity and Thermal Stability of the β Subunit of Tryptophan Synthase*

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To assess the functional roles of helix 13 and of the conserved and variable residues in the C-terminal region (residues 378–397) of the tryptophan synthase β subunit, we have constructed four C-terminal truncations and 12 point mutations. The effects of these mutations on kinetic and spectroscopic properties and thermal stability are reported here. The mutant β subunits all form stable αβ2 complexes that have been purified to homogeneity. The mutant αβ2 complexes are divided into two classes on the basis of activity in the reaction of L-serine with indole to form tryptophan. Class I enzymes, which have mutations at Arg-379 or Asp-383 or truncations (382–397), exhibit very low activity (<1% of wild type). Class II enzymes, which have mutations at Lys-382 or Asp-383 or truncations (383–397 or 383–397), exhibit significant activity (1–38% of wild type). Although Class II enzymes have drastically reduced activity in the reaction of L-serine with indole and an altered distribution of enzyme-substrate intermediates in the reaction of L-serine with β-mercaptoethanol, they retain activity in the reaction of β-chloro-L-alanine with indole. Correlation of the results with the three-dimensional structural model of the αβ2 complex suggests that Lys-382 and Asp-383 serve important roles in a proposed "open" to "closed" conformational change that occurs in the reaction of L-serine. Because mutant β subunits having C-terminal truncation (383–397 or 384–397) undergo much more rapid thermal inactivation at 60 °C than the wild type β subunit, the C-terminal helix 13 stabilizes the β subunit.

The bacterial tryptophan synthase αβ2 complex (EC 4.2.1.20) is a useful model system for probing the relationships between enzyme structure and function and the mechanisms of intersubunit communication (for reviews see Refs. 1–4). The three-dimensional structure of the αβ2 complex from Salmonella typhimurium revealed that the four polypeptide chains are arranged in a nearly linear αββα order (5). The larger pyridoxal phosphate-dependent β subunit contains two domains of nearly equal size, termed the N-domain (residues 1–52 and 85–204 shown in yellow in Fig. 1A) and the C-domain (residues 53–84 and 205–397 shown in cyan and red in Fig. 1A). The pyridoxal phosphate coenzyme is located in the interface between these two domains and interacts with residues from both domains. Portions of the two domains possess a high level of structural homology and are nearly superimposable, suggesting that they may have evolved by gene duplication and fusion. The core region of the C-domain terminates at residue 377 and thus excludes the C-terminal residues 378–397. Residues 383–393 form a helix (helix 13) that protrudes from the center of the β subunit into solvent. Three residues in helix 13 (Ile-384, His-388, and Leu-391) are involved in β/β interaction (Fig. 1B). The side chains of Lys-382 and Glu-350 form a salt bridge located below the plane of the tryptophan phosphate ring in the active center of the β subunit (Fig. 1B and Fig. 5 in Ref. 6).

Alignment of the sequences of the tryptophan synthase β subunit or β domain from 24 species (see supplementary material in the electronic appendix to Ref. 7) reveals that residues corresponding to 384–397 of the β subunit from S. typhimurium are variable, whereas the preceding residues 378–383 are identical (Fig. 2). Several other pyridoxal phosphate-dependent enzymes, including dehydratases and synthases, have also been aligned with the tryptophan synthase β subunit and assigned to the β family (8) or to Fold type II (7). The sequence similarity of these other enzymes with the β subunit is very low in the C-terminal region (beyond Glu-350 in the β subunit from S. typhimurium).

To investigate the roles of helix 13 and of the conserved and variable residues in the C-terminal region, we have constructed four C-terminal truncations and 12 point mutations (Fig. 2) using methods based on PCR1 (6, 9). The effects of these mutations on kinetic and spectroscopic properties and thermal stability are reported here. Our results show that residues in the C-terminal region are important for the thermal stability and activity of the αβ2 complex but are not essential for catalysis. Alteration or deletion of Lys-382 or Asp-383 drastically reduces activity in the reaction of L-serine with indole and alters the substrate specificity and spectroscopic properties. We correlate these results with data on the three-dimensional structure of the αβ2 complex (5, 10)2 and with models depicting ligand-mediated conformational changes of the tryptophan synthase αβ2 complex (11–16). Our results suggest that Lys-382 and Asp-383 are involved in an open to closed conformational transition that activates the αβ2 complex. Initial aspects of portions of this work have been reported (6, 9).

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1 The abbreviations used are: PCR, polymerase chain reaction; E-Ser, external aldimine of L-serine; E-AA, external aldimine of aminocrotylate; E-Q, quinonoid intermediate formed by addition of β-mercaptoethanol to E-AA.

2 S. Rhee, K. D. Parris, C. C. Hyde, S. A. Ahmed, E. W. Miles, and D. R. Davies, submitted for publication.
Enzymes and Chemicals—Enzymes and chemicals used for recombinant DNA methods and DNA sequencing were as before (6). Indole-3-glycerol phosphate was prepared as described (17). b-Chloro-L-alanine, D-glyceraldehyde-3-phosphate dehydrogenase, and other common chemicals were from Sigma. Buffer B was 50 mM sodium N,N-bis(2-hydroxyethyl)glycine containing 1 mM EDTA at pH 7.8. A synthetic peptide (NH₂-DIFTVHDILKA-COOH), corresponding to b-subunit residues 383–393, was custom synthesized by Bio-Synthesis Corp., Lewisville, TX.

Bacterial Strains and Plasmids, Growth of Cells, and Purification of Enzymes—The Escherichia coli host strain CB149 lacks the trp operon (17). Plasmids pEBA-10, pEBA-6, and pEBA-4A8 (6) express the S. typhimurium tryptophan synthase α₂β₂ complex, β subunit, and α subunit, respectively. Cultures of the host harboring wild type or mutant plasmids (see below) were grown, and enzymes were induced with IPTG as described (6). Enzymes were purified from the disrupted cells from 100-ml cultures. Purification of the wild type and mutant α₂β₂ complexes (18) utilized crystallization from crude extracts followed by recrystallization. The combined yields of α and β subunits were greater than 70% of the total soluble proteins, as reported recently for the five complexes with mutations in β subunit residue 382 expressed under the same conditions (6). The amounts of purified α₂β₂ complex obtained from 100-ml cultures ranged from 23 to 90 mg. Each purified α₂β₂ complex gave only two bands on SDS-polyacrylamide gel electrophore-

**Experimental Procedures**

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**FIG. 2. Sequence of C-terminal residues 378–397 of the tryptophan synthase β subunit from S. typhimurium and structural elements found in the three-dimensional structure (5) (r, random coil; h, helix; s, cannot be seen). Multiple alignment of 24 β subunit or domain sequences (7) shows that residues corresponding to 378–383 (underlined) of the β subunit from S. typhimurium are identical, whereas 384–397 are variable. We have constructed and characterized the four indicated C-terminal truncation mutant proteins and 12 mutant proteins having single amino acid replacements at positions 379, 381, 382, and 383 marked by *; the construction and initial characterization of five mutant β subunits altered at position 382 has been reported recently (6).**
C Terminus of Tryptophan Synthase β Subunit

TABLE I

| Enzyme | α Rxn (IGP → G3P) | Ratio of αβ Rxn / α Rxn | β Rxn (Ser + Ind = Trp) | Mutant
|--------|-----------------|--------------------------|------------------------|--------
| WT     | 41 (100)        | 100%                     | 100%                   |        |
| R379Q  | 23 (57)         | 43%                      | 15%                    | I      |
| R379K  | 28 (68)         | 44%                      | 15%                    | I      |
| R379P  | 6 (15)          | 14%                      | 14%                    | I      |
| D381N  | 26 (64)         | 19%                      | 20%                    | I      |
| D381S  | 37 (91)         | 20%                      | 14%                    | I      |
| D381Y  | 31 (78)         | 21%                      | 13%                    | I      |
| D382G  | 29 (73)         | 12%                      | 6%                     | I      |
| D382R  | 27 (68)         | 13%                      | 8%                     | I      |
| D382E  | 25 (54)         | 14%                      | 8%                     | I      |
| D383A  | 30 (69)         | 17%                      | 10%                    | I      |
| D383G  | 14 (35)         | 19%                      | 4%                     | I      |
| D383K  | 27 (68)         | 13%                      | 4%                     | I      |
| D384R  | 13 (33)         | 12%                      | 5%                     | I      |
| D385R  | 15 (38)         | 13%                      | 11%                    | I      |

Specific activities of wild type and mutant αββ complexes

Specific activities in the α, β, and αβ reactions were determined as described under “Experimental Procedures” in the presence of a 3-fold excess of wild type α subunit. The activity of the α subunit alone in the α reaction was 0.6 units/mg or 1–2% that of the wild type αββ complex. IGP, indole-3-glycerol phosphate; G3P, β-glyceraldehyde 3-phosphate; WT, wild type; Ind, indole; Rxn, reaction.

One unit of activity is the amount of enzyme that gives rise to formation of 0.1 μmol of product per 20 min at 37 °C. Activities of the αββ complex in the conversion of indole (0.2 mM) and L-serine or β-chloro-l-alanine (40 mM) to tryptophan were determined by a spectrophotometric assay in the presence of an approximately 3-fold excess of α subunit (16, 20). CsCl (0.5 M) was added in some assays where indicated. The activities of the αββ complex in the α reaction (conversion of indole-3-glycerol phosphate to indole and β-glyceraldehyde 3-phosphate) and in the αβ reaction (conversion of indole-3-glycerol phosphate and L-serine to tryptophan and β-glyceraldehyde 3-phosphate) were measured by a spectrophotometric assay coupled with β-glyceraldehyde-3-phosphate dehydrogenase in the presence of excess α subunit (21).

Spectroscopic Methods—Absorption spectra utilized a Hewlett-Packard 8452 diode array spectrophotometer. Fluorescence measurements were made in a Δ-Scan 1 (Photon Technology International) dual excitation spectrophotofluorimeter. Fluorescence titrations of enzymes with L-serine measured the increase in fluorescence emission at 510 nm (excitation at 420 nm) upon incubation of the αββ complex (0.26–0.52 μM αβ pair) with 0.01–10 mM L-serine at 25 °C (22–24). K<sub>f</sub> values for L-serine were obtained by hyperbolic curve fitting of the change in fluorescence in ∆fluence using Equation 1:

\[
\Delta F = \Delta F_{max} \times [\text{L-serine}]/K_f + [\text{L-serine}]
\]

(K<sub>f</sub> values for β-chloro-L-alanine were obtained from the competitive inhibition by β-chloro-L-alanine of the fluorescence of the enzyme complexes with L-serine. In Method 1, several concentrations of β-chloro-L-alanine were used (0, 0.2, 0.5, 1, and 3 mM for the wild type and 0, 10, 20, 40, and 60 mM for the K382N αββ complex, and K<sub>f</sub> values were obtained by linear fit of R<sub>flu</sub>/K<sub>f</sub> versus [β-chloro-L-alanine] using Equation 2:

\[
K_f = 1 + [\text{β-chloro-L-alanine}]/K_f
\]

In Method 2, a single concentration of β-chloro-L-alanine was used (0.5 mM) and K<sub>f</sub> values were obtained from double-reciprocal plots of ∆F<sub>versus</sub> [L-serine] using Equation 3:

\[
\text{intercept on } 1/[\text{L-serine}] = -1/K_f (1 + [\text{β-chloro-L-alanine}]/K_f)
\]
work because these residues do not interact with other residues and have not been implicated previously as important residues. Initial studies on five mutant proteins altered at position 382 have been reported (6). The engineered mutant subunits were all expressed in high yield and purified as stable αβ2 complexes (see “Experimental Procedures”).

Evidence for Structural Integrity of the Mutant αβ2 Complexes: Activation of the α Subunit—Interaction of the wild type α and β subunits stimulates the catalytic activity of the α subunit in the α reaction (Equation 4) about 50-fold (see Ref. 1 and footnote to Table I).

α Reaction: indole 3-glycerol phosphate ↔ indole

\[ + \text{d-glyceraldehyde 3-phosphate} \quad \text{(Eq. 4)} \]

Activities in the α reaction of αβ2 complexes containing mutant β subunits and wild type α subunit range from 15 to 91% that of the wild type αβ2 complex (Table I). Thus, the mutant β subunits all stimulate the catalytic activity of the α subunit. This result provides partial evidence for the structural integrity of the mutant αβ2 complexes.

Activity Measurements Distinguish Two Classes of Mutant β Subunits—Table I gives the specific activities of the wild type and mutant αβ2 complexes in the synthesis of l-tryptophan from l-serine and indole (β reaction, Equation 5) and from l-serine and indole-3-glycerol phosphate (αβ reaction, Equation 6).

β Reaction: l-serine + indole → l-tryptophan + H₂O (Eq. 5)

αβ Reaction: l-serine + indole-3-glycerol phosphate → l-tryptophan

\[ + \text{d-glyceraldehyde 3-phosphate} + \text{H}_2\text{O} \quad \text{(Eq. 6)} \]

The αβ reaction is essentially the sum of the α and β reactions and requires coupled catalysis by the α and β subunits. Reaction of l-serine at the β site in the αβ2 complex stimulates the rate of indole-3-glycerol phosphate cleavage approximately 20-fold as shown by the ratio of αβ reaction/α reaction in Table I.

We have arbitrarily classified the mutant αβ2 complexes on the basis of the effects of the mutations on the activity in the β reaction, the reaction of l-serine with indole. “Class I” enzymes, which have mutations at positions 379 and 381 or truncations after residue 383 (384–397 or 385–397), exhibit significant activity (1–38% of wild type). “Class II” enzymes, which have mutations at positions 382 or 383 or truncations including residues 382 or 383 (382–397 or 383–397), exhibit very low activity (<1% of wild type). The absence of l-serine stimulation of indole-3-glycerol phosphate cleavage by Class II enzymes (Ratio αβ reaction/α reaction = ~1 in Table I) can be attributed to the lack reaction of l-serine at the β site.

Some mutant forms of tryptophan synthase have considerably higher activity in the overall αβ reaction than in the individual β reaction (25, 26). These results were partly due to a reduced association of the mutant α subunit with the β subunit; addition of indole-3-glycerol phosphate during catalysis of the β reaction or αβ reaction increased association and activity. The finding that the activity of each mutant protein in Class I is quite similar in the αβ and β reactions (Table I) shows that Class I mutations have no effects on association between the α and β subunits that can be detected by these assays of enzymatic activity.

Class II Mutant αβ2 Complexes Have Altered Substrate Specificity—Table II compares the activities of the wild type and mutant enzymes in the reaction of β-chloro-L-alanine with indole to activities in the reaction of l-serine with indole. It is striking that all of the mutant enzymes, except the Class I enzymes with mutations at position 379, have rather high activities with β-chloro-L-alanine (42–140% of the wild type enzyme). Thus, the Class II mutations, which lead to loss of
activity with L-serine and indole, do not prevent the analogous reaction with β-chloro-L-alanine. Consequently, the substrate specificity ratio, which is defined as the ratio of activity with β-chloro-L-alanine and indole to activity with L-serine and indole, is much higher for the Class II enzymes (25–500) than for the wild type αβ2 complex (0.24) or the Class I mutant proteins (0.11 to 8.7).

Effects of Mutations on Binding Constants of L-serine and β-Chloro-L-alanine—Table III compares the binding constants of L-serine and β-chloro-L-alanine for the wild type and several mutant αβ2 complexes. The results were obtained by determining the effect of L-serine concentration on the change in fluorescence emission at 510 nm in the presence or absence of fixed concentrations of β-chloro-L-alanine, a competitive inhibitor that does not form a fluorescent external aldimine (22–24).

CsCl Partially Repairs Activities of the Mutant αβ2 Complexes—Because CsCl activates certain mutant αβ2 complexes (11), including those having single amino acid replacements of β subunit Lys-382 (6), we have also determined activity in the β reaction in the presence of 0.5 mM CsCl (Table II). CsCl activates all of the mutant αβ2 complexes that have low activity but has little effect on the R379Q and R379K αβ2 complexes that have relatively high activities. CsCl results in a striking 16-fold activation of the R379Q αβ2 complex, which has a much lower activity than R379Q and R379K under standard conditions. CsCl also activates the wild type β subunit (11).

Spectroscopic Properties of the Mutant αβ2 Complexes—The wild type αβ2 complex and β subunit catalyze a β replacement reaction of L-serine with β-mercaptoethanol that proceeds through a series of pyridoxal phosphate-substrate intermediates that have characteristic absorption spectra (see Fig. 3 for structures and abbreviations) (27, 28). Whereas an equilibrium mixture of these intermediates accumulates with the wild type αβ2 complex, the E-Ser intermediate predominates with the wild type β subunit and most of the mutant αβ2 complexes (data not shown). Conversion of E-Ser to E-AA is rate-limiting with the wild type β subunit (29). Because CsCl and NH4Cl alter the rate of this conversion by the β subunit (29) and alter the rates and spectroscopic properties of some mutant enzymes (11), we have determined the absorption spectra obtained upon reaction of L-serine and of L-serine and β-mercaptoethanol in the presence of 0.5 mM CsCl (Fig. 3 and Table II).

Table III

Dissociation Constants of L-serine and β-chloro-L-alanine for the Wild Type (WT) and Representative Mutant αβ2 Complexes

| Enzyme   | Mutant class | L-Serine, Kd (mM) | β-Chloro-L-alanine, Kd (mM) |
|----------|--------------|------------------|----------------------------|
| WT       |              | 0.36 ± 0.05      | 0.95 ± 0.06                |
| R379Q    | I            | 0.32 ± 0.02      | 0.47                       |
| K382N    | II           | 2.9 ± 0.1        | 78 ± 6                     |
| D383A    | II           | 0.21 ± 0.01      | 0.73                       |
| ∆383–397 | II           | 0.29 ± 0.04      | 1.2                        |

Table III shows the spectra obtained in the presence of 0.5 mM CsCl with the R379Q αβ2 complex (a representative of Class I) and with the ∆383–397 αβ2 complex (a representative of Class II), respectively. The three spectra obtained with the R379Q αβ2 complex are essentially identical to those of the wild type αβ2 complex under the same conditions. The enzyme alone exhibits a major peak centered at 410 nm due to the external aldimine (E). Reaction with L-serine yields a complex spectrum with a major peak centered at 340–350 nm, which is ascribed to the external aldimine between pyridoxal phosphate and aminoacylalylase (E-AA). Reaction of L-serine and β-mercaptoethanol yields a major peak at 468 nm, which is ascribed to a quinonoid (E-Q) intermediate formed upon addition of β-mercaptoethanol to the aminoacylalylase intermediate. The spectrum is unchanged by the further addition of β-mercaptoethanol.

Table II presents the wavelength of maximum absorbance in the presence of L-serine or of L-serine plus β-mercaptoethanol for the wild type αβ2 complex and β subunit and for each mutant αβ2 complex in the presence of CsCl. In general, the three spectra for each mutant αβ2 complex in Class I were similar to those of the wild type αβ2 complex, whereas the spectra for each mutant αβ2 complex in Class II were similar.
The four β subunit C-terminal truncations and 12 point mutations were all expressed in high yield as stable αβ complex ("Experimental Procedures" and Ref. 6), contained bound pyridoxal phosphate that forms enzyme-substrate intermediates (Fig. 3 and Table II), and activated indole-3-glycerol phosphate cleavage by the β subunit (Table I). Thus, the residues deleted (Δ382–397) or altered (379, 381, 382 and 383) are not required for overall folding of the β subunit, for association of the β subunit with the α subunit to form an αβ complex, or for activation of the α subunit. These results document the structural integrity of these mutant β subunits. The β subunit C-terminal helix 13 may serve a structural role because the mutant proteins having residues 383–397 or 384–397 deleted were much more thermolabile (Fig. 4).

Residues 379 and 381–397 are not essential for catalysis because all of the mutant enzymes exhibit some activity in the reaction of L-serine with indole (0.01–38% of wild type) and quite significant activity in the reaction of β-chloro-L-alanine with indole (10–140% of wild type) (Table II). We have divided the mutant proteins into two classes, I and II, based on activity in the reaction of L-serine with indole. Class I mutant proteins are more similar to the wild type enzyme than Class II mutant proteins in activity with L-serine and indole, in substrate specificity, and in spectroscopic properties (Tables I and II and Fig. 3). The results with Class I mutant proteins show that Arg-379, Asp-381, and residues 384–397 are not very important for catalytic activity. Although substitution of Arg-379 by Lys or Glu has minor effects on the catalytic properties, substitution by Pro (R379P) has more drastic effects. This result is consistent with the finding that E. coli cells having this mutation in the trpB gene are unable to convert indole to tryptophan (32). Our results do not explain why the R379P mutant cells were found to produce more indole than cells with a trpB mutation at position 382 (32). The activity in the α reaction is in fact lower than that of any of the other mutant proteins. The proline replacement may alter the backbone geometry and have a more deleterious effect on the conformation of the β subunit than the more conservative Lys and Glu replacements.

Class II mutant proteins have extremely low activities in the reaction of L-serine and indole but have activities close to that of wild type in the reaction of β-chloro-L-alanine with indole (Tables I and II). These mutant proteins all have a substitution or deletion of Lys-382 or of Asp-383. These results support and extend observations that the mutation of Lys-382 results in inactivation (1, 32, 33). The D383A and Δ383–397 mutant proteins lack the conserved residue Asp-383. The finding that these mutant proteins have extremely low activity in the reaction of L-serine and indole provides the first evidence that Asp-383 is very important for the catalysis of this reaction. The effects of the Class II mutations on activity in the reaction of L-serine with indole are not attributable to changes in the binding of L-serine because the Kd values of L-serine for the mutant enzymes (Table III) are all lower than the concentration of L-serine (40 mM) used in the assay. The importance of Lys-382 for tight binding of L-serine and β-chloro-L-alanine may be attributable to formation of a salt bridge between Lys-382 and Glu-350 (see Fig. 1B and Ref. 6). This salt bridge could stabilize the substrate binding site. An additional role for Lys-382 in a substrate-induced conformational change is discussed below.

Relation of Class II Mutant Proteins to a Model for Ligand-mediated Conformational Changes—Class II mutant proteins exhibit altered substrate specificity and an altered distribution of enzyme-substrate intermediates in the reaction of L-serine and β-mercaptoethanol (Table II and Fig. 3). These results are now discussed in relation to previous studies of the conformational changes of the Class II mutant proteins and of the wild type enzyme, to elucidate a model for ligand-mediated conformational changes.
tional states of tryptophan synthase. Dunn and co-workers (12-15) have drawn upon data from a number of investigations of tryptophan synthase by different groups of investigators to formulate a model depicting ligand-mediated conformational changes that occur during the course of the αβ reaction. The model postulates that the α and β subunits undergo transitions between open and closed conformations which function to coordinate the catalytic activities and promote diffusion of the indole intermediate. Recent investigations using 8-anilino-1-naphthalenesulfonate binding as a probe have identified three distinct conformations of the αβ complex associated with different liganded states of the α and β subunits (13). The conformation of the β subunit stabilized by E-AA and E-Q has been designated as closed, whereas the conformation stabilized by E-Ser and E-S-hydroxyethyllysylene has been designated as open (see diagram at the top of Fig. 3) (13).

In the reaction of L-serine with β-mercaptoethanol, the E-AA (closed) intermediate predominates in the wild type and Class I mutant enzymes, whereas the E-Ser (open) intermediate predominates in the Class II mutant enzymes (Table II and Fig. 3). This result indicates that the mutations hinder the ligand-mediated transition from an open to a closed structure either by stabilizing the open structure or by destabilizing the closed structure. The E-Ser intermediate also predominates in several other αβ complexes having mutations in the β subunit (6, 11, 16). We have postulated that the wild type β subunit and these mutant αβ complexes have a conformation (termed “open”) that results in a poor alignment of the weak hydroxyxyl leaving group of L-serine for protonation and β-elimination. These enzymes may have much higher activity with β-chloro-L-alanine because this substrate has a strong leaving group that does not require protonation. The finding that addition of CsCl (0.5 M) activates a number of open mutant proteins and the wild type β subunit suggests that Cs+ may stabilize a more active alternative conformation of these enzymes (6, 11). CsCl also increases the very low activities of the D383A, Δ382–397, and Δ383–397 mutant proteins 10–80-fold (Table II). CsCl may stabilize the more active, closed conformation of the β subunit and partially shift the equilibrium from the open to the closed form (11).

Relation of the Mutation Studies to Crystallographic Results—Fig. 1, A and B, shows the locations of residues that have been altered or deleted in the present investigation in the three-dimensional structure of the wild type αβ complex. The region containing altered residues (residues 378–393) is shown in red. The C-terminal residues 394–397 are not clearly visible in the structure and are not shown. The C-terminal helix 13 from each β subunit is located near the β/β interaction site and protrudes from the center of the β subunit into solvent. Three residues in helix 13 are involved in β/β interactions (Fig. 1B). These interactions are Ile-384 to Pro-144, His-388 to Phe-147, and Leu-391 to Phe-147. Two of the conserved residues mutated (Arg-379 and Asp-381) also make β/β interactions: the two Arg-379 residues are stacked with each other; Asp-381 interacts with Arg-148. Lys-382 forms an internal salt bridge with Glu-350 of the same β subunit. Although the four C-terminal truncations remove the three β/β interactions made by Ile-384, His-388, and Leu-391, the mutant β subunits all form stable αβ complexes. Thus, disruption of interactions between six pairs of residues in the αβ complex is not sufficient to cause disruption of the β/β interface. Similarly, single amino acid substitution of Arg-379 or Asp-381 does not cause disruption of the β/β interface.

How are ligand-mediated conformational changes in tryptophan synthase related to structural changes? Crystallographic investigations are only beginning to reveal structural effects of ligands. Recent studies reveal that exchange of K+ or Cs+ for Na+ induces local and long range changes in the three-dimensional structure of the tryptophan synthase αβ complex (10). Studies of a mutant form of the enzyme (βK87T) having an external aldimine of L-serine at the β site and indole-3-propanol phosphate or DL-α-glycerol 3-phosphate at the α site show conformational changes that may be related to formation of the closed conformation in solution. Conformational changes observed in these structures of the residues investigated here include movements of Arg-141 toward Thr-386 and of Glu-142 toward Lys-382 and Asp-383. The possible involvement of these interactions in the closed conformation may explain why the mutation or deletion of Asp-383 or of Lys-382 in Class II mutant enzymes appears to destabilize the closed form of the enzyme and prevent the open to closed transition.

Because proteins are stabilized by a large network of interactions, mutations are quite likely to interfere with this complex network and destabilize the protein or alter the conformation of the enzyme needed for optimal activity (34). Some proteins are stabilized by better attachment of the N and C termini to the rest of the molecule to prevent “fraying” (34). Deletion of one of these termini may remove these stabilizing interactions and promote fraying. Although the C-terminal helix 13 of the β subunit is partially exposed to solvent, it does make several interactions in the β/β interface. The removal of helix 13 may destabilize the β subunit to heat and loosen β/β interaction by removing these interactions as discussed above. Addition of a synthetic peptide corresponding to residues 383–393 did not restore the catalytic activity or the spectroscopic properties of the mutant β subunit having residues 383–397 deleted.

Conclusions—Our results show that helix 13 is important for thermal stability and that residues 379, 381, 382, and 383 in the C terminus of the β subunit are important, but not essential, for catalytic activity. Lys-382 and Asp-383 appear especially important for stabilization of the closed conformation of the enzyme that has optimal activity with L-serine.

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