A Thermally Induced Reversible Conformational Transition of the Tryptophan Synthase \( \beta_2 \) Subunit Probed by the Spectroscopic Properties of Pyridoxal Phosphate and by Enzymatic Activity*

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A reversible thermally induced conformational transition of the \( \beta_2 \) subunit of tryptophan synthase from Salmonella typhimurium has been detected by use of the pyridoxal 5'-phosphate coenzyme as a spectroscopic probe. Increasing the temperature converts the major form of pyridoxal 5'-phosphate bound to the \( \beta_2 \) subunit from a ketoenamine species with \( \lambda_{\text{max}} \) at 410 nm to an enolamine species with \( \lambda_{\text{max}} \) at 336 nm \( (T_m = -43^\circ \text{C}) \) and results in loss of the circular dichroism signal at 410 nm and of fluorescence emission at 510 nm. The results indicate that increasing the temperature favors a conformer of the enzyme that binds pyridoxal 5'-phosphate in a more nonpolar environment and leads to loss of asymmetric pyridoxal 5'-phosphate binding. The internal aldimine between pyridoxal 5'-phosphate and the \( \varepsilon \)-amino group of lysine 87 is not disrupted by increased temperature because sodium borohydride treatment of the \( \beta_2 \) subunit below 60°C produces irreversible thermal inactivation \( (T_i = -52^\circ \text{C}) \) and occurs at a much lower temperature than the major reversible unfolding at \( -80^\circ \text{C} \) (Remeta, D. P., Miles, E. W., and Ginsburg, A. (1995) Pure Appl. Chem. 67, 1859-1866). Our new results indicate that the 410 nm absorbing species of pyridoxal 5'-phosphate is the catalytically active form of the cofactor in the \( \beta_2 \) subunit and that the low temperature reversible conformational transition disturbs the active site and causes loss of catalytic activity.

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Pyridoxal 5'-phosphate (PLP)\(^1\) serves as the coenzyme for many enzymes that catalyze a wide variety of reactions involved in the metabolism of amino acids (racemization, transamination, \( \beta \)-elimination, \( \beta \)-replacement, decarboxylation, etc.)\(^1\),\(^2\). In every PLP-dependent enzyme, the PLP coenzyme forms an internal aldimine with the \( \varepsilon \)-amino group of a lysine residue (see structures in Fig. 1A under “Results”). This internal aldimine usually exhibits an absorption maximum at 410–430 nm attributed to the resonance stabilized ketoenamine form II in Fig. 1A (3–5). The dipolar structure II is favored by a more polar environment. Most PLP enzymes also have peaks at 330–340 nm, which may represent the enolamine tautomer (I in Fig. 1A), a neutral species that prefers a nonpolar environment (3–6). Reactions with substrates or inhibitors or changes in \( \varepsilon \) pH often lead to marked alterations in the spectrum of the enzyme-bound PLP\(^2\),\(^5\). Thus PLP serves as a useful chromophoric reporter group for changes in the PLP binding site in enzymes and for reactions with substrates and inhibitors.

The \( \beta_2 \) subunit of tryptophan synthase \( \text{EC} \, 4.2.1.20 \) catalyzes a number of PLP-dependent reactions including a \( \beta \)-elimination reaction with \( L \)-serine (Equation 1) and a \( \beta \)-replacement reaction with \( L \)-serine and indole (Equation 2).

\[
L\text{-serine} \rightarrow \text{pyruvate} + \text{NH}_3
\]  
\[
L\text{-serine} + \text{indole} \rightarrow L\text{-tryptophan} + \text{H}_2\text{O}
\]  

For reviews see Refs. 7–9.) PLP serves as a useful spectrophotometric indicator of alterations in the PLP binding site of the \( \beta_2 \) subunit. For example, the UV-visible spectra \( (10, 11) \) and circular dichroism spectra \( (12, 13) \) of bound PLP are altered by interaction of the \( \beta_2 \) subunit with the \( \alpha \) subunit of tryptophan synthase to form an \( \alpha_2\beta_2 \) complex and by certain \( \beta_2 \) subunit mutations in the \( \alpha_2\beta_2 \) complex \( (11) \).

The three-dimensional structure of the tryptophan synthase \( \alpha_2\beta_2 \) complex from Salmonella typhimurium revealed that the chains are arranged in a nearly linear \( \alpha\beta\beta\alpha \) order \( (14) \). The larger \( \beta \) chain \( (43,000 \, \text{M}_r) \) in the complex has two domains of about equal size, designated the N- and C-domains, which are folded in similar helix/sheet/helix structures. The active sites of neighboring \( \alpha \) and \( \beta \) subunits are connected by a buried, \( -30 \, \text{Å} \) long hydrophobic tunnel that passes between the N- and C-domains of each \( \beta \) chain. This unique tunnel serves as a likely conduit of indole from the \( \alpha \) site to the \( \beta \) site. The binding site for the PLP coenzyme is located between the N- and C-domains at one end of the tunnel. The single tryptophan in the \( \beta \) chain, Trp-177, is buried in the N-domain.

We report here that the spectral properties of PLP bound to the \( \beta_2 \) subunit of tryptophan synthase from S. typhimurium are altered by temperature. The results provide evidence for a reversible thermal transition in the \( \beta_2 \) subunit at a much lower temperature \( \left(T_m = -41 \text{--} 47 \, ^\circ \text{C}\right) \) than the temperature \( \left(T_i = -80 \, ^\circ \text{C}\right) \) at which the \( \beta_2 \) subunit undergoes a major unfolding transition \( (15--17) \).\(^2\) Irreversible inactivation occurs at the higher temperature \( \left(T_i = -77 \, ^\circ \text{C}\right) \) in the presence of a higher salt concentration that causes protein aggregation \( (18, 19) \). Other studies show that the low temperature transition results in a small, low temperature endotherm in differential scanning calorimetry, perturbation in the environment of Trp-177 but not of tyrosine residues, and loss in the ellipticity of PLP \( (15-17) \).\(^2\)

Our finding that this conformational transition results in loss of activity shows that the conformational transition has biochemical and functional relevance.

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\(^1\) The abbreviation used is: PLP, pyridoxal 5'-phosphate.
RESULTS

Effects of Temperature on the Absorption Spectrum of the $\beta_2$ Subunit—The absorption spectra of the $\beta_2$ subunit display two distinct absorption maxima at 410 and 336 nm at temperatures between 17 and 70 °C (Fig. 1B). These absorption peaks at 24.5 °C have been attributed to the ketoamine form of the internal aldime (II in Fig. 1A) and to the enolimine tautomeric form (I in Fig. 1A), respectively (10, 25). As the temperature is increased, the initial higher absorbance at 410 nm decreases, and the absorbance at 336 nm increases. Between 17 and 50 °C, these temperature-dependent spectral changes are fully reversible, and the spectra exhibit a single isosbestic point at 355 nm (Fig. 1B) consistent with the interconversion of two species (e.g. I and II in Fig. 1A). The loss of activity above 50 °C (see below) is also reversed by cooling. Enzyme heated to 50 °C for 50 min and then cooled had the same activity as an unheated control. Above 50 °C, the isosbestic point is lost, and the change in absorbance at 336 nm is greater than that at 410 nm.

Parallel studies of the temperature dependence of the absorption spectra of a model Schiff base between PLP and l-serine show a smaller decrease in absorbance at 410 nm and an increase in absorbance at 336 nm (see below).

Fig. 2A shows plots of absorbance data versus temperature for the $\beta_2$ subunit and for the model Schiff base. The absorbance changes of the $\beta_2$ subunit occurring below 60 °C were analyzed assuming a simple two-state equilibrium between two forms of the protein, a low temperature form, N, and a high temperature form, I. The equilibrium constant for this reaction, K, can be calculated from the temperature dependence of the absorbance of the solution at any wavelength using Equation 3:

$$K(T) = (1/r)(N) = (A_0(T) - A(T))/[A(T) - A_0(T)]$$

where $A(T)$ is the absorbance of the solution and $A_0(T)$ and $A(T)$ are the absorbances of the low and high temperature forms of the protein at temperature T, where T is absolute temperature in K (26). The temperature dependence of $A(T)$ could not be observed, because it was masked by the more extensive changes occurring above 60 °C (Figs. 1 and 2A). For a two-state unfolding mechanism, the dependence of K on temperature T is given by Equation 4:

$$\Delta G(T) = \Delta H - T \Delta S = \Delta H(1 - T/T_m)$$

where $\Delta G(T)$, $\Delta H$, and $\Delta S$ are the changes in free energy, enthalpy, and entropy of the reaction and $T_m$ is the midpoint and where $\Delta G(T) = 0$, assuming $\Delta C_p = 0$. A control experiment with the model Schiff base of PLP with l-serine showed a linear temperature dependence (Fig. 2A). The temperature-dependent decreases in absorbance at 410 nm and increases at 336 nm.

EXPERIMENTAL PROCEDURES

Chemicals and Buffer—Buffer P (50 mM sodium phosphate, pH 7.8) and Buffer B (50 mM sodium N,N-bis(2-hydroxyethyl)glycine containing 1 mM EDTA at pH 7.8) were used as indicated. [4'-3H]PLP (specific activity, ~8 × 10^6 cpm/nmol) was prepared previously (20). The model Schiff base of PLP with l-serine was prepared by mixing 0.05 mM PLP (Sigma) with 50 mM l-serine in Buffer P, 1,4-Dioxane (99%; Janssen Chimica) was shaken with heated alumina to remove peroxide prior to use.

Enzymes—The $\beta_2$ subunit from S. typhimurium was purified to homogeneity as described (21) or by heat precipitation of the $\alpha$ subunit from the $\alpha_{\beta_2}$ complex (22). The apo $\beta_2$ subunit prepared as described in Ref. 12 (21 nmol in Buffer P) was treated with [4'-3H]PLP (~23 nmol) at room temperature; unbound cofactor was removed by gel filtration in Buffer P. Protein concentrations were determined from the specific absorbance at 278 nm (22).

Spectroscopic and Analytical Methods—Absorption spectra were made using a Hewlett-Packard 8452 diode array spectrophotometer thermostated by a circulating water bath (Method 1) or using a Hewlett-Packard 8450 diode array spectrophotometer thermostated by a Peltier junction temperature controlled cuvette holder (Method 2). An external water bath was interfaced with the Peltier unit for temperature control below 20 °C in Method 2. Absorption spectra of the $\beta_2$ subunit (1.17 mg/ml in Buffer P) were recorded after equilibration for at least 10 min at each temperature (Method 1) or for 11 min at each temperature between 0–20 °C and for 3 min at each temperature between 22–70 °C (Method 2). Because different preparations of $\beta_2$ subunits had different contents of PLP, the absorption values were normalized to millimolar extinction values from the absorbance of the solution saturated with PLP at pH 7.6 and 25 °C (E_2ε2 nm = 5.4 m M^-1 cm^-1 (23)). Fluorescence emission at 510 nm with excitation at 420 nm was determined using a Perkin-Elmer model MPF-44B fluorimeter thermostated by a circulating water bath. Circular dichroism measurements (mean residue ellipticity in degrees cm^2/dmol) of the $\beta_2$ subunit in Buffer P were made in a Jasco J-500C spectropolarimeter, equipped with a DP-500N data processor (Japan Spectroscopic Co., Easton, MD) and thermostated by a circulating water bath. Single wavelength melting curves were determined at 360 and 410 nm. Spectrophotometric assays at single wavelengths were made in a Cary 110 spectrophotometer. Aliquots of aqueous solutions containing [4'-3H]PLP or its reaction products were counted in 1 ml of OptiPhase "HiSafe II" (LKB) using a Beckman LS3800 scintillation counter.

Enzyme Assays—One unit of activity in any reaction is the formation of 0.1 μmol of product in 20 min at the indicated temperature. Assays were performed in Buffer P containing 0.05 mM PLP. Activity in the β-elimination reaction (Equation 1) was measured by a spectrophotometric assay coupled with lactate dehydrogenase at temperatures up to 50 °C (24). Because lactate dehydrogenase was inactive above 50 °C, the assay between 50 and 70 °C was allowed to proceed in a water bath for 5 min, stopped by addition of 1 N HCl, and neutralized with NaOH. Pyruvate concentration was then determined by the addition of lactate dehydrogenase and NADH at 37 °C. Activity in the β-replacement reaction (Equation 2) was measured by a direct spectrophotometric assay (22). The concentration of l-serine (100 mM) used for assays was saturating because either activity gave the same rate with 50 mM, 100 mM, and 200 mM l-serine at 14 and 50 °C.
were much smaller than those observed with the enzyme-bound PLP. Absorbance changes at 410 nm were fitted to Equations 3 and 4 using the PC-MLAB program (Civilized Software, Bethesda, MD) to derive values of $\Delta H$ and $T_m$ using the assumption that the absorbance of the native protein $A_n(T)$ showed the same linear temperature dependence as that of the model Schiff base and that the high temperature form of the protein $A_h(T)$ showed a parallel linear dependence. The fitted curve ($T_m = 43.2 \pm 0.5 ^\circ C; \Delta H = -180 \pm 30 \text{kJ/mol}$) is shown in Fig. 2A.

Effect of Temperature on the Circular Dichroism Spectrum of the $\beta_2$ Subunit—The binding of PLP to the $\beta_2$ subunit induces a positive ellipticity band at 415 nm due to the asymmetric orientation on the enzyme of the otherwise optically inactive chromophore (12, 13). Remeta et al. (15) have recently found that the ellipticity band at 415 nm of the $\beta_2$ subunit in the $\alpha_2\beta_2$ complex disappears with heating at a low temperature transition ($T_m = 46.4 ^\circ C$) and that the $\beta_2$ subunit alone exhibits a similar transition. Measurements of the ellipticity of the $\beta_2$ subunit as a function of temperature detected a decrease in ellipticity at 410 nm (Fig. 2B) but very little ellipticity at 336 nm at any temperature. The ellipticity changes at 410 nm were analyzed assuming a simple two-state equilibrium between two forms of the protein as above and fit to Equations 3 and 4. Adequate fits were obtained assuming that the ellipticities of the two forms of the protein were independent of temperature (Fig. 2B). The complete loss of ellipticity at 410 nm indicates that the observed temperature-induced transition ($T_m = -41 ^\circ C; \Delta H = -150 \pm 40 \text{kJ/mol}$) results in loss of asymmetric binding of PLP. However, PLP must remain bound to the enzyme at 60 °C, because the absorbance maximum is at 336 nm, whereas free PLP in solution has an absorbance maximum at 388 nm (see below).

Effects of Temperature on the Activities of the $\beta_2$ Subunit—Fig. 3A shows the effect of temperature on the activities of the $\beta_2$ subunit in the $\beta$-elimination (Equation 1) and $\beta$-replacement (Equation 2) reactions (Fig. 3A) to the predicted rates (dashed lines in Fig. 3B) are plotted versus temperature in °C. D. The logarithm of the fluorescence emission at 510 nm upon excitation at 420 nm is plotted versus temperature in °C for the $\beta_2$ subunit alone (€), $\beta_2$ subunit + L-serine (€), and model Schiff base (€). Intensities are plotted on a semi-logarithmic scale versus temperature in °C in order to show all of the data on the same chart and to simplify the temperature dependence of the fluorescence of the native protein. Curves were fitted to the untransformed data as explained in the text. Intensities were measured on solutions in Buffer P containing 61 µM $\beta_2$ subunit alone, 3.7 µM $\beta_2$ subunit in the presence of 0.1 mM L-serine, or 0.05 mM PLP in the presence of 50 mM L-serine and were normalized to 3.7 µM PLP. Measurements on the $\beta_2$ subunit alone and of the model Schiff base of PLP with L-serine were made on a single solution of each after equilibration for 10–15 min at each temperature. Each measurement on the $\beta_2$ subunit in the presence of L-serine was made on a separate solution after equilibration at the indicated temperature for 10 min. Two sets of data were collected in the presence of L-serine. One set was collected in the presence 50 µM PLP; the other was collected in the absence of added excess PLP. Each data set gave the same $T_m$. The temperature of the $\beta_2$ subunit alone was gradually increased up to 73 °C and then cooled to 4.5 °C. The finding that the fluorescence emission of the previously heated $\beta_2$ subunit was 86% of that of the unheated enzyme at 4.5 °C demonstrates that the thermal transition is largely reversible. The derived values of $T_m$ are given and are marked on the curves by small arrows.

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**Fig. 2.** Effect of temperature on spectral properties and activities of the $\beta_2$ subunit. A, plot of normalized extinction coefficients versus temperature in °C for the absorbance changes at 410 nm (€) using Method 1 (see Fig. 1B), and additional data were collected (€) using Method 2. The deviation of the data obtained by the two methods at higher temperatures may result from the fact that the enzyme used for Method 1 was expressed by a plasmid encoding the trpB gene (21), whereas the enzyme used in Method 2 was prepared by heat precipitation of the $\alpha$ subunit from the $\alpha_2\beta_2$ complex (22). The data were fitted to Equations 3 and 4 as described under "Results." The derived values of $T_m = 43.2 \pm 0.8$ is given. The changes in the m extinction at 410 nm (€) for the model Schiff base of L-serine and PLP (0.05 mM PLP with 50 mM...
low temperature form (N) and an inactive, high temperature form (I), then the fraction of the protein (α) that is in the active form at a given temperature is given by Equation 6:

$$\alpha(T) = \frac{\text{measured rate at } T}{\text{extrapolated rate at } T}$$  \hspace{1cm} (Eq. 6)

and the equilibrium constant for the reaction between N and I is given by Equation 7:

$$K(T) = \frac{(I)/(N)}{(1 - \alpha(T))/\alpha(T)}$$  \hspace{1cm} (Eq. 7)

The temperature dependence of (K)T is again given by Equation 4, where T is absolute temperature in K. The low temperature data in Fig. 3A were fitted to the Arrhenius equation using PC-MLAB, and the derived constants were used to predict rates at higher temperatures (dashed lines in Fig. 3B). These values together with the measured rates were used to calculate the values of α(T) for both reactions, shown in Fig. 3B, which were then fitted to Equation 4 to derive the values of T_m, shown in Fig. 2C. Equations 4 and 5 were combined to predict the temperature dependence of the measured activities (Fig. 3B, solid lines) and the rate ratios (Fig. 2C, solid and dashed lines).

Effects of Temperature on the Fluorescence Emission of the $\beta_2$ Subunit in the Absence and Presence of L-Serine—PLP bound to the $\beta_2$ subunit exhibits a weak fluorescence emission with a peak at 505 nm (27). The fluorescence emission intensity increases markedly upon addition of L-serine due to formation of E-Ser (27). Fig. 2D shows a semi-logarithmic plot of the effects of temperature on the fluorescence emission intensity at 510 nm (uncorrected for inner filter effect) of the $\beta_2$ subunit in the presence and the absence of 0.1 M L-serine and of the model Schiff base of PLP with L-serine. The fluorescence intensities of the native (pretransition) forms of the protein and of the model Schiff base exhibit marked temperature dependence as described previously for base-line trends (28). The base-line data for the enzyme show linear dependence on temperature below 40 °C in a semi-logarithmic plot (Fig. 2D), whereas the behavior of the model Schiff base of PLP with L-serine is linear throughout the temperature range, as would be expected from Equation 21 in the work of Eftink (28). This linear dependence was used to extrapolate the behavior of the native proteins in the presence and the absence of L-serine into their transition zones. The fluorescence of the high temperature forms of the proteins were assumed to parallel that of the model Schiff base, and two state transition curves were fitted to Equations 3 and 4 (26) using the PC-MLAB program. The fitted curves and the derived values of T_m are shown in Fig. 2D.

Effect of Temperature on the Integrity of the Internal Aldimine Bond in the $\beta_2$ Subunit—It is important to determine whether the enzyme-bound PLP at 60 °C exists as the enolimine species in a nonpolar environment (I in Fig. 1A) or as PLP in a nonpolar environment with its carbonyl group free. Studies of the effects of polarity on the absorption spectra of PLP analogs using dioxane/water mixtures have shown that reducing the polarity results in a blue shift of about 30–50 nm in the absorbance maximum (29–31). The absorption spectra of PLP in dioxane/water mixtures (Fig. 4) show that PLP has an absorption maximum of 390 nm in water and 350 nm in 98% dioxane. Our finding that free PLP in a nonpolar environment absorbs at a significantly higher wavelength (350 nm) than the species observed at 60 °C (336 nm) indicates that the enzyme-bound species at 60 °C is not free PLP but is an internal aldime, linkage in a nonpolar environment.

To establish definitively that PLP is bound to the $\beta_2$ subunit at 60 °C in the form of an internal aldime, we have reconstituted the apo $\beta_2$ subunit with [4'-H]PLP as described under "Experimental Procedures" and have treated the enzyme with...
sodium borohydride at 15 or 60 °C as described (20). The absorption spectra of both treated enzymes exhibited peaks at 318 nm, showing that reduction of PLP had occurred. However, this result could not distinguish whether free PLP was reduced to pyridoxine 5'-phosphate or whether the internal aldimine was reduced to 5'-phosphopyridoxyl lysine because these products have similar absorption spectra. Acid precipitation of the proteins followed by analysis of the radioactivity in the supernatant solutions (Table I) showed that more than 80% of the [4-3H]PLP became covalently attached by reduction of the β2 subunit at either 15 or 60 °C. Thus the PLP must be bound as an internal aldimine at 60 °C and must be reduced by sodium borohydride. The low but significant amount of radioactivity in the supernatants of both sodium borohydride treated samples (Table I) may be due to dissociation of some of the cofactor before reduction or to binding of a radioactive impurity that does not form an internal aldimine.

**DISCUSSION**

The PLP coenzyme has been used previously as a sensitive chromophoric probe to investigate the structure and function of many PLP-dependent enzymes. However, there have been very few studies to our knowledge of the effects of temperature on the spectroscopic properties of PLP-dependent enzymes, of PLP itself, or of PLP derivatives.4 Recent studies from Ginsburg's group (15–17) and the studies reported here show that PLP serves as a useful probe of the effects of temperature on the structure and function of the tryptophan synthase β2 subunit and α/β2 complex.

Evidence for a Thermally Induced Reversible Conformational Transition—Our results provide evidence for a reversible thermal transition of the β2 subunit that occurs at a much lower temperature than the major unfolding transition at −80 °C (15–18). This low temperature transition alters the absorption spectrum of enzyme-bound PLP (Figs. 1 and 2A) and leads to loss of fluorescence emission of the internal and external aldime at 510 nm (Fig. 2D) and of ellipticity at 410 nm (Fig. 2B) and to loss of activity (Figs. 2C and 3, A and B). Temperature affects the activities of the β2 subunit in β-elimination and

β-replacement reactions (Equations 1 and 2) in two ways; the activities first increase in rate with temperature until maximal rates are achieved at −55 °C and then decrease due to thermal inactivation (Fig. 3A). In the region prior to inactivation (below 45 °C), the data are described by the Arrhenius equation (Equation 5) and can be fit to the straight lines shown in Fig. 3B. Analysis of the data (Fig. 2C) shows that the β2 subunit reversibly loses one-half of its activity under assay conditions at a much lower temperature (T_m = −52 °C) than the temperature (T_c = −77 °C) at which the β2 subunit undergoes irreversible inactivation (18).

The values of T_m for the absorption and circular dichroism changes for the internal aldime forms (in the absence of L-serine) shown in Fig. 2 (A and B) vary from −40 to −43 °C, whereas the values of T_c for reversible inactivation (Fig. 2C) and of T_m for fluorescence changes for the external aldime forms (in the present of L-serine) (Fig. 2D) are significantly higher (−49 to −53 °C). Although formation of the external aldime forms in the presence of L-serine appears to stabilize the enzyme,5 the spectral changes and inactivation observed in the

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4 Metzler's group (32) has measured absorption spectra of 5-deoxy-pyridoxal over the temperature range 2.5–68 °C in acidic, neutral, and basic conditions. Band shape analysis of spectra at pH 5.8 shows that the proportion of the dipolar ion is relatively constant, whereas the ratio of hydrate to uncharged species decreases with increasing temperature. Parallel studies with Schiff base derivatives of PLP have not been carried out previously. Schiff base derivatives, which do not form hydrates, might be expected to be less affected by temperature as shown by our results in Fig. 2.

5 Some of this variation may be due to experimental errors. The precise determination of the thermodynamic parameters of any conformational transition requires the accurate measurement of the temperature-dependent behaviors of both the high and low temperature forms of the protein, so that they can be extrapolated into the transition zone. This allows a good estimate of the extent of reaction (26). In many of the experiments presented herein, it was not possible to follow the behavior of the inactive β2 subunit at high temperatures, because the data were obscured by the overlapping unfolding transition, which occurs around 77 °C. In the absence of any better information, high temperature changes were assumed to parallel those of the low temperature form of the protein or of the model PLP Schiff base with L-serine. This uncertainty increases the experimental error in the fitted values of T_m.

6 One discrepant observation is that the T_m for fluorescence changes in the absence of added L-serine (−47 °C) is only 2 degrees lower than
presence and the absence of L-serine probably reflect the same transition. Our results also indicate that the species of the cofactor that absorbs at 410 nm exhibits optical activity at 410 nm and emits fluorescence at 510 nm upon excitation at 420 nm is the catalytically competent species.

The observed low temperature transition does not result in dissociation of the internal aldimine between PLP and the ε- amino group of Lys-87, because treatment with sodium borohydride results in covalent attachment of PLP to the protein at both 15 and 60 °C (Table I). Our results indicate that PLP is bound to the inactive conformer of the β subunit as the enolimine tautomer of the internal aldimine (I in Fig. 1A), a neutral species that prefers a nonpolar environment (3, 4).

Structure of the Inactive Conformer That Results from the Low Temperature Transition—Information on the structure of the inactive conformer of the β subunit is available from studies using differential scanning calorimetry, circular dichroism, and UV spectroscopy (15–17) and from the results presented here. The differential scanning calorimetry profiles of the holo β subunit exhibit a reproducible broad, low temperature endotherm (Tm = 47 °C; ΔHcal = –180 kJ/mol), whereas major unfolding, which disrupts ~70% of the secondary structure, occurs between 74 and 82 °C (15). The low temperature endotherm is consistent with a small conformational transition. The single tryptophan in each β subunit (Trp-177), which is buried in the hydrophobic core of the N-terminal domain of each β subunit, is a useful intrinsic probe for monitoring thermally induced unfolding in the αβ2 complex (15) and the isolated β2 subunit. Thermally induced perturbations in Trp and Tyr environments were independently monitored using second derivative UV absorption spectroscopy of tryptophan synthase β2 subunit and αβ2 complex (15). The results revealed perturbations of Trp-177 but not of tyrosyl residues in β chains in the low temperature transition (15). Perturbation of Trp-177 may result from solvent exposure or change in the polarity of the environment. The absence of perturbation of tyrosyl residues suggests that partial unfolding and solvent exposure does not occur and supports a change in polarity of the environment of Trp-177 resulting from a small conformational transition.

Analysis of the spectral properties of the bound PLP show that both a change in the tautomeric form of the internal aldimine (Figs. 1 and 2A) and loss of the ellipticity at 410 nm (Fig. 2B) occur in the low temperature transition. Thus, the low temperature transition involves several reversible changes in the structure of the β subunit: 1) perturbation in the environment of Trp-177, 2) change in the environment of the PLP, and 3) a conformational transition that contributes to the low temperature endotherm. This transition must reflect a small change in structure or a conformational change because it does not break the internal aldimine of PLP (Table I) or result in exposure of the buried tyrosine residues in the β subunit. The low temperature transition is clearly biologically relevant because it results in loss of catalytic activity. An additional important conclusion is that the species of PLP that absorbs at 410 nm, exhibits optical activity at 410 nm, and emits fluorescence at 510 nm upon excitation at 420 nm is the catalytically active form of the cofactor in the β2 subunit.

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REFERENCES

1. Snell, E. E. (1986) in Coenzymes and Cofactors (Dolphin, D., Paulson, R., and Av Arramovic, O., eds) Vol. I, part A, pp. 1–12, J ohn Wiley & Sons, Inc., New York
2. Davis, L., and Metzler, D. E. (1972) in The Enzymes (Boyer, P. D., ed) Vol. 7, pp. 33–74, Academic Press, New York
3. Heinert, D., and Martinez, A. E. (1962) J. Am. Chem. Soc. 84, 3257–3263
4. Metzler, C. M., Cahilli, A., and Metzler, D. E. (1980) J. Am. Chem. Soc. 102, 6075–6082
5. Kalien, R. G., Kopolica, R., Martelli, A. E., Matsushima, Y., Metzler, C. M., Metzler, D. E., Morozov, Y. V., Ralston, I. M., Savin, F. A., Torchinsky, Y. M., and Ueno, H. (1985) in Transaminases (Christen, P., and Metzler, D. E., eds) pp. 37–106, John Wiley & Sons, Inc., New York
6. Shaltiel, S., and Cortijo, M. (1970) Biochem. Biophys. Res. Commun. 41, 594–600
7. Miles, E. W. (1991) Adv. Enzymol. Relat. Areas Mol. Biol. 64, 93–172
8. Miles, E. W. (1979) Adv. Enzymol. 49, 127–186
9. Miles, E. W. (1995) in Proteins: Structure, Function, and Protein Engineering (Biswas, B. B., and Roy, S., eds) Vol. 24, pp. 207–254, Plenum Publishing Corp., New York
10. Faeder, E. J., and Hammes, G. G. (1971) Biochemistry 10, 1041–1045
11. Ahmed, S. A., Ruvinov, S. B., Kayastha, A. M., and Miles, E. W. (1991) J. Biol. Chem. 266, 21548–21557
12. Miles, E. W., and Morishita, M. (1977) J. Biol. Chem. 252, 6594–6599
13. Balk, H., Merki, I., and Bartholmes, P. (1981) Biochemistry 20, 6391–6395
14. Hyde, C. C., Ahmed, S. A., Padan, E. A., Miles, E. W., and Davies, R. F. (1988) J. Biol. Chem. 263, 17857–17871
15. Remeta, D. P., Miles, E. W., and Ginsburg, A. (1995) Pure Appl. Chem. 67, 1859–1866
16. Remeta, D. P., Miles, E. W., and Ginsburg, A. (1992) Biophys. J. 61, 213 (abstr.)
17. Remeta, D. P., Miles, E. W., and Ginsburg, A. (1993) Biophys. J. 64, 175 (abstr.)
18. Ruvinov, S. B., and Miles, E. W. (1994) J. Biol. Chem. 269, 11703–11706
19. Chaffotte, A. F., and Goldberg, M. E. (1983) Biochemistry 22, 2708–2714
20. Miles, E. W., Hock, D. R., and Floss, H. G. (1982) J. Biol. Chem. 257, 14203–14210
21. Yang, X.-J., Ruvinov, S. B., and Miles, E. M. (1992) Protein Expression Purif. 3, 347–354
22. Miles, E. W., Bauerle, R., and Ahmed, S. A. (1987) Methods Enzymol. 142, 398–414
23. Lane, A. N., and Kirschner, K. (1983) Eur. J. Biochem. 129, 571–582
24. Crawford, I. P., and Itou, J. (1964) Proc. Natl. Acad. Sci. U. S. A. 51, 390–397
25. Faeder, E. J., and Metzler, G. G. (1970) Biochemistry 9, 4043–4049
26. Pace, N. C. (1990) Trends Biotechnol. 8, 93–97
27. Goldberg, M. E., York, S., and Stryer, L. (1968) Biochemistry 7, 3662–3667
28. Eftink, M. R. (1994) Biophys. J. 66, 482–480
29. Metzler, D. E., and Snell, E. E. (1955) J. Am. Chem. Soc. 77, 2431–2437
30. Nakamoto, K., and Martelli, A. E. (1959) J. Am. Chem. Soc. 81, 5857–5863
31. Nakamoto, K., and Martelli, A. E. (1959) J. Am. Chem. Soc. 81, 5863–5869
32. Harris, C. M., Johnson, R. J., and Metzler, D. E. (1976) Biochemistry 15, 181–194