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Thermally induced unfolding of the tryptophan synthase $\alpha_2\beta_2$ multienzyme complex from *Salmonella typhimurium*

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Abstract Tryptophan synthase (TS) from *Salmonella typhimurium* (144,000 M_r) is a bifunctional enzyme composed of two heterodimers arranged in an extended $\alpha\beta\beta\alpha$ geometry [Hyde *et al.*, *J. Biol. Chem. 263*, 17857, 1988]. The thermal unfolding of TS $\alpha_2\beta_2$ and isolated α and β subunits has been studied by differential scanning calorimetry (DSC), circular dichroism, and UV spectroscopy. DSC profiles of the holo-$\alpha_2\beta_2$ complex containing pyridoxal phosphate (PLP) bound to each β chain are characterized by two well-resolved endotherms centered at $\approx$ 52 and $\approx$ 81 °C. Conformational transitions in β chains ($T_m \approx 46$ °C) as well as sequential unfolding reactions in α chains contribute to the low-temperature endotherm whereas major unfolding of the β dimer occurs between 74 and 82 °C. The thermally induced unfolding disrupts $\approx$ 70 % of the secondary structure with a total enthalpy change of 3600 ± 100 kJ mol^{-1} for holo-$\alpha_2\beta_2$. Stabilizing interactions between α and β chains are apparent. The cofactor PLP not only markedly stabilizes β chains in β_2 but also increases the cooperativity of unfolding β chains. Calorimetric and spectral measurements suggest a sequential unfolding pathway for the tryptophan synthase $\alpha_2\beta_2$ complex. Thermodynamic results are correlated with available structural information.

INTRODUCTION

Differential scanning calorimetry (DSC) and temperature-induced spectral changes of oligomeric proteins can provide information on the cooperativity of unfolding/refolding reactions and on thermodynamic domain interactions. Our calorimetric studies on the tryptophan synthase multienzyme complex have been initiated with these goals in mind. This paper summarizes our results and conclusions on the thermal unfolding of the tryptophan synthase complex, as well as those from other laboratories.

Pioneering biochemical and genetic studies in the laboratories of Yanofsky and Crawford established that bacterial tryptophan synthase (EC 4.2.1.20) is an $\alpha_2\beta_2$ complex of dissociable, non-identical subunits (see review 1). More recent reviews (2-4) have emphasized structure-function relationships. The α and β subunits catalyze the final two reactions in the L-tryptophan biosynthetic pathway. The isolated α chains catalyze the reversible cleavage of indole 3-glycerol phosphate in Reaction 1. The β chains exist as a β dimer; each β chain contains one equivalent of the cofactor pyridoxal 5'-phosphate (PLP) bound in Schiff base linkage to Lys-87 and catalyzes the irreversible synthesis of L-tryptophan in Reaction 2:

indole 3-glycerol phosphate $\longleftrightarrow$ indole + D-glyceraldehyde 3-phosphate (1)

indole + L-serine $\longrightarrow$ L-tryptophan + H_2O (2)

The associated $\alpha_2\beta_2$ complex catalyzes the physiologically important Reaction 3, termed the $\alpha\beta$ reaction, which is the sum of the α- and β-reactions (Reactions 1 & 2, respectively):

indole 3-glycerol phosphate + L-serine ⟶ L-tryptophan + D-glyceraldehyde 3-phosphate + H_2O (3)

Affinities for substrates and reaction rates are increased 10- to 100-fold in the $\alpha_2\beta_2$ complex compared to those of the isolated α chains and β dimer. Moreover, the $\alpha_2\beta_2$ complex exhibits greater specificity in catalyzing Reaction 3, since side reactions observed with isolated β_2 are inhibited when α and β chains are associated (2).

In 1988, Hyde *et al.* (5) published the three-dimensional structure of the tryptophan synthase $\alpha_2\beta_2$ complex, as determined by x-ray crystallography at 2.5 Å resolution. A ribbon structure of the $\alpha_2\beta_2$ complex is shown in Fig. 1. The view in Fig. 1 is approximately that of looking down the 2-fold axis of symmetry between $\alpha\beta$ pairs. Contact areas between α:β and between β:β chains are indicated by arrows; the corresponding surface areas of β buried are $\approx$ 1110 A^2 in α:β and $\approx$ 1440 A^2 in β:β contacts (5). The quaternary structure of tryptophan synthase has an extended, nearly linear $\alpha\beta\beta\alpha$ geometry 150 A long. Each α chain (29,000 M_r) in the complex has an overall polypeptide chain arrangement of an 8-fold α/β barrel (5,6). The active site of each α chain has been located by difference Fourier analysis of the binding of the competitive inhibitor indole propanol phosphate (IPP). The larger β chain (43,000 M_r) has two domains of about equal size (designated N- and C-domains in Fig. 1), which are folded in similar helix/sheet/helix structures. The site for binding the coenzyme PLP is buried within the interfaces between β structural domains. Active sites of neighboring $\alpha\beta$ pairs are 25 A apart (5). The x-ray structural analysis has revealed that neighboring α and β active sites are connected by a buried, hydrophobic tunnel that has a diameter approximating that of the intermediate indole (5,7). Hyde *et al.* (5) suggested that the tunnel functions by facilitating the diffusion of indole from its site of formation in α to the site of tryptophan synthesis at the β active site. This feature of the tryptophan synthase structure accounts for the inability of investigators to measure free indole during the enzyme catalyzed synthesis of L-tryptophan in Reaction 3 (1). Although channeling of intermediates in multienzyme complexes had been proposed previously to explain their observed greater efficiencies, the identification of an indole tunnel in the tryptophan synthase complex has allowed a direct three-dimensional visualization of how this might occur.

Whereas apo-$\alpha_2\beta_2$ binds the cofactor PLP noncooperatively to noninteracting sites with $K_D' = 10^{-6}$ M at pH 7.5 (20 °C), apo-β_2 binds PLP with marked positive cooperativity (8,9). Calorimetric studies (10,11) also have shown that much more negative changes in heat capacity are associated with the binding of PLP or α chains to apo-β_2 than those measured for PLP binding to apo-$\alpha_2\beta_2$ or for α chains binding to holo-β_2. These observations suggest a concerted mechanism for the binding of PLP to apo-β_2 and a coupling of conformational changes involving substantial decreases in exposed hydrophobic surfaces in β_2 upon binding pyridoxal phosphate or α chains. Studies of Goldberg and associates (12,13) on a nicked form of apo-β_2 have indicated that there is an energetic coupling between the N- and C-terminal domains of each β chain and between β chains in the dimer. Unfortunately, atomic structures for the isolated α chain, apo-β_2, and holo-β_2 are not yet available, but it is anticipated that some of these structures will differ substantially from those found in the holo-$\alpha_2\beta_2$ complex shown in Fig. 1 (5).

The β:β contacts in apo- or holo-β_2 are remarkably stable. High pressure studies (to 1600 bar) by Silva *et al.* (14) have provided dissociation constants at 25 °C and 1 bar (pH 7.5) of $\approx 4\times10^{-9}$ M for apo-β_2, 4×10^{-10} M for holo-β_2, and $\approx 6 \times 10^{-11}$ M for borohydride reduced holo-β_2. Recent pressure studies (15) on the dissociation of β_2 in the $\alpha_2\beta_2$ complex (in the presence of 5-fold excess α) at 10 °C and 1 bar (pH 7.8) have yielded K_D values and reaction volumes of dissociation (ΔV) for the apo- and holo-β_2 components of 4×10^{-9} M with $\Delta V = -196$ ml mol^{-1} and $\approx 10^{-18}$ M with $\Delta V = -632$ ml mol^{-1}, respectively (P. Bartholmes, personal communication). Thus, β:β contacts in holo-β_2 are further stabilized by the binding of α chains.

Each $\alpha\beta$ pair of the tryptophan synthase $\alpha_2\beta_2$ complex contains only one tryptophanyl residue. The residue Trp-177 is buried in the hydrophobic core of the N-terminal domain of each β chain and this region is responsive to substrate binding at the pyridoxal phosphate site (C. C. Hyde, personal communication). The location of Trp-177 is shown in Fig. 1 for one of the two β chains in the complex. Trp-177 has been a useful intrinsic probe for monitoring thermally induced unfolding reactions in the $\alpha_2\beta_2$ complex and the isolated β_2 subunit.

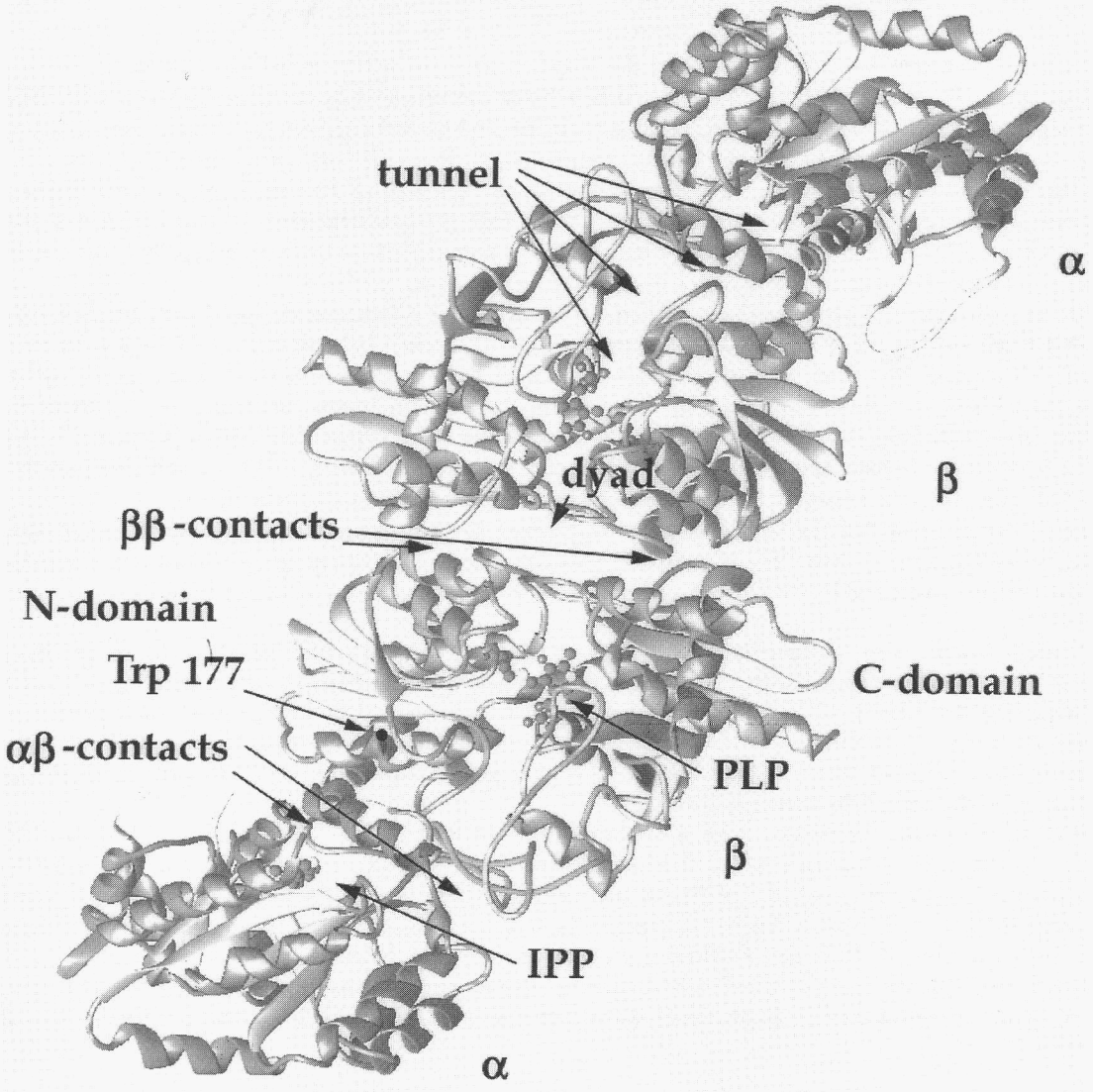

Fig. 1. View of the tryptophan synthase holo-$\alpha_2\beta_2$ complex from *S. typhimurium* looking down the two-fold axis of symmetry between αβ pairs. The figure is based on the x-ray structure of Hyde *et al.* (5) and constructed using the RIBBONS Program written by Mike Carson, University of Alabama, through the courtesy of C. Craig Hyde (NIAMS, NIH). In one αβ pair, the location of indole propanol phosphate (IPP) at the active site of an α chain, the binding site of pyridoxal phosphate (PLP) buried between N- and C-domains of a β chain, Trp-177 in the N-domain of β, and the α:β contacts are indicated by arrows. The β:β contact surface, the dyad axis, and the location of the hydrophobic tunnel connecting α and β active sites in the upper αβ pair also are identified.

EXPERIMENTAL

The tryptophan synthase $\alpha_2\beta_2$ complex and the α and β_2 subunits were purified and assayed for activity according to Ruvinov and Miles (16). The apo-β_2 subunit was prepared as described by Miles *et al.* (17). Reduction of the Schiff base linking PLP to β subunits to produce reduced holo-$\alpha_2\beta_2$ was accomplished by slowly mixing a 10-fold excess of dry $NaBH_4$ with holo-$\alpha_2\beta_2$ in a well buffered solution at pH 7.8 while monitoring the complete shift in the cofactor absorbance from 410 to 320 nm. Proteins were dialyzed against three 1000-fold volumes of 10 mM K-PO_4 buffer (pH 8) which contained 1.0 mM EDTA, 0.1 mM dithiothreitol (DTT), and 0.2 mM PLP for holo-$\alpha_2\beta_2$ and holo-β_2, all components except PLP for apo-β_2 and borohydride reduced $\alpha_2\beta_2$, and only 1.0 mM EDTA for α chain.

DSC experiments were performed with a MicroCal Model MC-2 calorimeter equipped with tantalum cells (sample cell volume: 1.220 mL) as described previously (18). Protein concentrations of 1.5 - 3.8 mg mL^{-1} and 60 K h^{-1} scan rates from 10 to 95 °C under ~ 10 bar nitrogen pressure were used. After subtracting the instrument base line (obtained by scanning the dialysate buffer in both sample and reference cells under the same run conditions), DSC data were normalized for scan rate and protein concentration (expressed in mol of $\alpha_2\beta_2$ complex, α chain, apo-β_2, or holo-β_2). DSC data were analyzed using the Origin software provided by MicroCal, Inc. The area over the base line corresponds to the enthalpy of unfolding (ΔH_{cal}).

The cooperative ratio (CR) is defined as $\Delta H_{cal}/\Delta H_{vH}$, where ΔH_{vH} is the van't Hoff enthalpy calculated from $\Delta H_{vH} = 4RT_m^2C_{pm}/\Delta H_{cal}$ (19). Deviation from a two-state mechanism of unfolding toward intermolecular cooperativity is indicated by CR < 1, whereas the presence of multiple intramolecular domains is suggested by CR > 1.

Spectral studies were conducted on a Hewlett Packard Model 8450 rapid scan, diode array spectrophotometer equipped with a Peltier junction temperature-controlled cuvette holder operated at a heating rate of ~ 30 K h^{-1} (20,21). Thermally induced changes in tryptophanyl and tyrosyl residue exposure were monitored by second derivative peak-trough absorbance differences calculated from 120 averaged spectra using [A″(295 nm) – A″(292 nm)] for Trp (21) and [A″(290 nm) – A″(285 nm)] for Tyr (22), where A″ is the second derivative absorbance.

Mean molar residue ellipticities were measured with a JASCO Model J-710 circular dichroism (CD) spectropolarimeter. Water-jacketed, cylindrical cells (pathlengths of 0.01 and 1.0 cm for far and near UV, respectively) were connected to a NESLAB programmable bath (± 0.1 °C) for temperature control and heating at 50 K h^{-1}. Thermally induced changes in Trp and Tyr exposures and molar ellipticities at 290 nm and 415 nm (bound PLP perturbation) were analyzed by the EXAM program of Kirchhoff (23). Temperature induced changes in 90° light scattering were monitored using a Perkin Elmer Model 650/40 spectrofluorometer (excitation and emission wavelengths set at 360 nm with 2 nm slits) at a heating rate of ~ 60 K h^{-1}.

RESULTS AND DISCUSSION

Solvent conditions for studying the thermal unfolding of the tryptophan synthase $\alpha_2\beta_2$ complex have been investigated before conducting DSC. Phosphate stabilizes the complex and the enzyme remains soluble when heated in the presence of 10 mM K-PO_4 at pH 8. Light scattering measurements indicate that $\alpha_2\beta_2$ does not dissociate under these conditions (24), although some higher order aggregates are formed above 82 °C. When lower pH and higher ionic strengths are used, the enzyme precipitates during heating.

Figure 2 shows normalized DSC profiles obtained for the tryptophan synthase $\alpha_2\beta_2$ complex when the dialysis buffer of the holo-enzyme contains no added PLP (A), 0.2 mM free PLP (B), and 1.0 mM free PLP (**C**). Two resolved endotherms centered at 51.2 - 54.9 °C and 78.8 - 81.1 °C are observed. The high-temperature endotherm in Fig. 2 (A) is smaller than those in (B) and (C), and also has a pronounced shoulder from ~ 58 - 70 °C. This is due to the presence of some apo-$\alpha_2\beta_2$ that is formed during dialysis in the absence of added PLP (*see* Fig. 3 *below*). Although there are only minor differences in the high-temperature endotherms of Fig. 2 (B) and (C), the low-temperature endotherm appears to be stabilized by increasing concentrations of free PLP [T_{max} = 52.2 °C (B) versus 54.9 °C (C)]. After constructing a cubic base line for the best fit of the pre- and post-transition data in Fig. 2 (B) and (C), areas above the base line for the low- and high-temperature endotherms are 1590 ± 60 and 2060 ± 30 kJ mol^{-1}, respectively; total ΔH_{cal} = 3600 ± 100 kJ mol^{-1}.

The fact that the unfolding enthalpy is not changed from 0.2 to 1.0 mM PLP is evidence that the cofactor remains saturating throughout the temperature range in Fig 2. DSC profiles (T_m values and endotherm shapes) for the enzyme complex are essentially the same at scan rates of 30, 45, 60, and 90 K h^{-1}, which suggests that unfolding reactions can be treated as reversible processes (25). After slow cooling from 95 ° C in the calorimeter, however, subsequent rescans exhibit no endotherms and the enzyme is inactive following the second scan. Possibly, the high-temperature T_m approaches the T^* in the Lumry-Eyring Model II (25). In such a model, a reversible unfolding reaction between native and unfolded states is followed by an irreversible denaturation (with a large activation energy) to a state D at T^*.

Normalized DSC profiles for the isolated α, apo-β_2, and holo-β_2 subunits of tryptophan synthase are shown in Fig. 3. The temperature at the maximum excess heat capacity (C_{pm}) is 49.4 °C for α chain, 57.5 °C for apo-β_2, and 80.0 °C for holo-β_2. By comparing Figs. 2 and 3, it is apparent that the low- and high-temperature endotherms of the holo-$\alpha_2\beta_2$ complex occur at approximately the same temperatures as the unfolding of α chains and holo-β_2, respectively. The binding of the cofactor PLP markedly stabilizes β_2 as evidenced by the ≈ 23 °C difference in T_m values observed for holo- and apo-β_2 (26). Furthermore, the thermal unfolding of holo-β_2 is significantly more cooperative than that of apo-β_2 (26). In fact, CR ≈ 4 for apo-β_2 (ΔH_{cal} ≈ 1370 kJ mol^{-1}) whereas CR ≈ 2.4 for holo-β_2 (ΔH_{cal} ≈ 1610 kJ mol^{-1}). The DSC profiles of holo-β_2 exhibit a reproducible broad, low-temperature endotherm (T_m ≈ 47 °C; ΔH_{cal} ≈ 180 kJ mol^{-1}) which

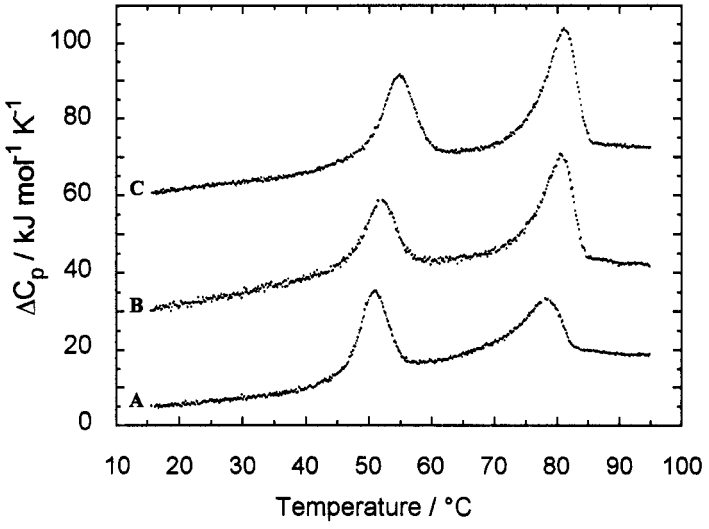

Fig. 2. Normalized DSC data for the tryptophan synthase $\alpha_2\beta_2$ complex dialyzed against the standard 10 mM K-PO4 buffer (pH 8.0) without added PLP (A), and in the presence of 0.2 mM free PLP (B), and 1.0 mM free PLP (C). The DSC profiles are from experiments with 2.75, 2.42, and 3.75 mg mL^{-1} of protein in (A), (B), and (C), respectively.

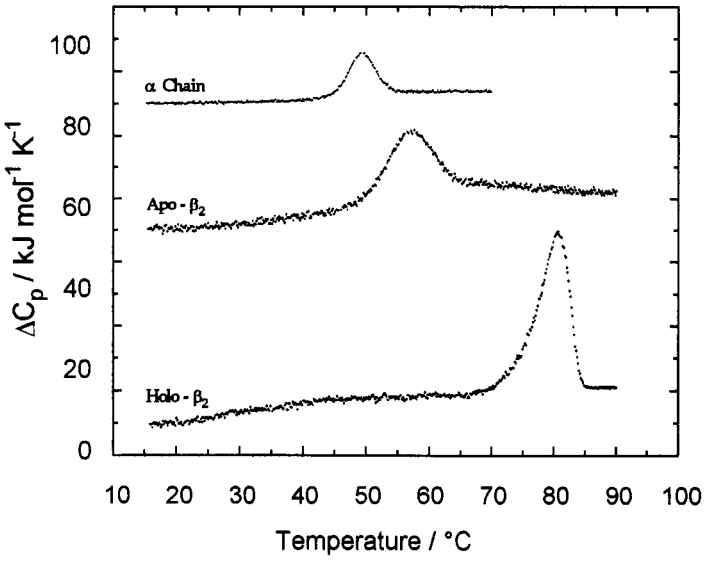

Fig. 3. Normalized DSC data for the TS α chain (1.56 mg mL^{-1}), apo-β_2 (1.56 mg mL^{-1}), and holo-β_2 (1.80 mg mL^{-1}) subunits dialyzed against 10 mM K-PO4 buffer (pH 8.0). Transition temperatures of 49.4, 57.5, and 80.0 °C are observed for the α chain, apo-β_2, and holo-β_2, respectively.

may be correlated to perturbations in β chains involving partial exposure of Trp-177 and changes in the environment of bound PLP (27). This observation corroborates results from deconvolutions of DSC data obtained for the $\alpha_2\beta_2$ complex, since the low-temperature endotherm of holo-β_2 in $\alpha_2\beta_2$ is masked by larger contributions from α chain unfolding.

For isolated α chains in Fig. 3, CR ≅ 0.6 which indicates that intermolecular interactions occur during unfolding reactions. Indeed, light scattering measurements recorded as a function of increasing temperature indicate that α chains self-associate after unfolding. When the ionic strength is decreased from 10 to 1 mM K-PO_4 at pH 8, T_m = 51.7 °C, ΔH_{cal} ≅ 410 kJ mol^{-1}, and CR ~ 0.7 for α chains. Yutani and associates (28) have conducted DSC studies on the thermal unfolding of α chains of tryptophan synthases from *Escherichia coli and Salmonella typhimurium* in 1 mM Na-tetraborate and 1 mM EDTA buffer. Over the pH range of 8 to 9.2, the *E. coli* α chain is more stable than that from *S. typhimurium*. However, denaturation enthalpies of these proteins are similar at the same T_m and a value of ΔC_p = 18.2 kJ mol^{-1} K^{-1} has been determined for α chain unfolding by Sugisaki *et al.* (28). The area of the endotherm for α chain in Fig. 3 is ~ 90 % of that predicted, although the ΔC_p value in K-PO_4 buffer may well be different (29,30).

The magnitude of the areas (ΔH_{cal}) obtained for thermally unfolding the isolated α and β_2 subunits (Fig. 3) are expected to be greater than their contributions in the partial unfolding of the nondissociated holo-$\alpha_2\beta_2$ complex (24). CD measurements at 222 nm in the absence and presence of 6 M guanidine.HCl indicate that approximately 70 % of the secondary structure of $\alpha_2\beta_2$ is disrupted at 90 °C (27).

Table 1 summarizes apparent T_m values obtained from different measurements monitoring the thermal unfolding of tryptophan synthase. The loss of secondary structure observed in the far UV CD at 222 nm occurs in the regions of the low- and high-temperature DSC endotherms. The cofactor PLP bound to holo-$\alpha_2\beta_2$ exhibits a large positive, extrinsic Cotton effect at 415 nm (31) which decreases during heating; temperature progress curves have been fitted with a two-state model of unfolding (23) to derive T_m for the loss in ellipticity at 415 nm. Thermally induced changes in the near UV CD show both a low- and high-temperature decrease at 290 nm due to losses in tertiary structure and aromatic residue ellipticity. Exposures of Trp-177 in β chains of $\alpha_2\beta_2$ occur in the temperature ranges of CD decreases at 415 and 290 nm. Moreover, Tyr exposure occurs only at high temperature in the complex even though Tyr is exposed (T_m ≅ 47 °C) during thermal unfolding of isolated α chains (27). Finally, irreversible inactivation temperatures for α- and β-activities measured by Ruvinov and Miles (16) correspond approximately to T_{m1} and T_{m2}, respectively. Thus, perturbations in the environment of Trp-177 and a loss in the ellipticity of bound PLP are early events in the partial unfolding of the $\alpha_2\beta_2$ complex.

Figure 4 illustrates temperature-induced CD changes at 290 nm (Panel A) and DSC results (Panel B) obtained with the reduced pyridoxal-$\alpha_2\beta_2$ complex. The DSC profile has two distinct endotherms centered at 53.4 and 79.7 °C. The low-temperature endotherm is shifted approximately 1.6 °C above that observed

TABLE 1. Apparent T_m values for thermally induced partial unfolding of the tryptophan synthase holo-$\alpha_2\beta_2$ complex at pH 8.0 in 10 mM K-PO_4, 0.2 mM PLP, 1.0 mM EDTA, and 0.1 mM DTT.

Measurement	T_{m1} / °C	T_{m2} / °C	Ref.
DSC	51.8	80.6	Fig. 2 (B)
CD ($\Theta_{222\,nm}$)	49.5	74.5	(27)
CD ($\Theta_{415\,nm}$)	46.4	-	(27)
CD ($\Theta_{290\,nm}$)	48.2	75.1	(27)
Trp Exposure	45.6	77.3	(27)
Tyr Exposure	-	75.3	(27)
α Activity Loss	54	-	(16)*
β Activity Loss	-	78	(16)*

* Irreversible inactivation in 50 mM Bicine/NaOH and 1.0 mM EDTA (pH 7.8).

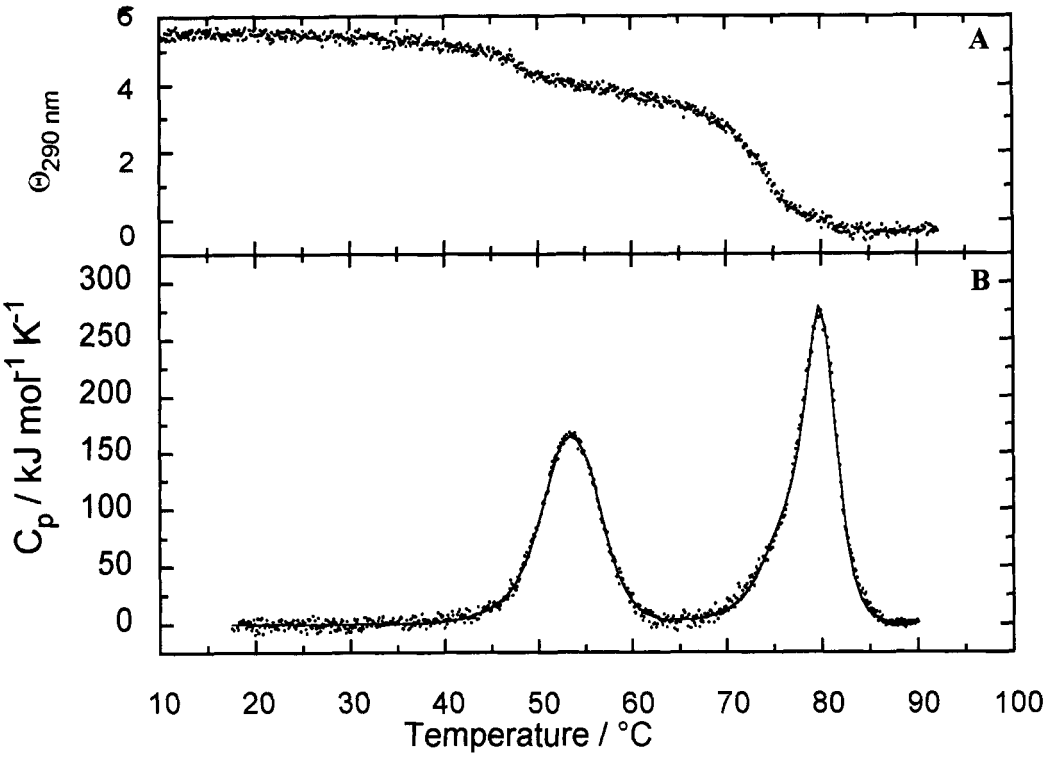

Fig. 4. Thermal unfolding transitions of borohydride-reduced holo-$\alpha_2\beta_2$ complex. Panel A shows CD measurements at 290 nm (units 10^3 deg cm^2 dmol)$^{-1}$ as a function of temperature. Panel B shows normalized DSC data (•) from an experiment with 2.44 mg/ml protein (after subtraction of a cubic base line constructed from pre- and post-transition DSC data). The solid curve in Panel B is calculated for a model of six sequential, two-state transitions (see text).

in the presence of 0.2 mM PLP (Table 1), which suggests that covalently bound reduced PLP stabilizes some structures that unfold in this temperature range. Thermally induced changes in tertiary structures of the reduced holo-$\alpha_2\beta_2$ complex occur at 48.4 and 74.2 °C as evidenced by the ellipticity decreases at 290 nm (Fig. 4A). The CD changes at 290 nm occur in the temperature ranges of Trp and Tyr exposures (Table 1) and are at the onsets of the two endotherms observed by DSC. The overall enthalpy change for the partial unfolding of the reduced holo-enzyme ($\Delta H_{cal} \approx 3000$ kJ mol^{-1}) is ≈ 83% of that measured in the presence of 0.2 mM PLP (see above). Most of this difference is in the area of the high-temperature endotherm. A model of six sequential, two-state transitions has been fitted to the DSC data in Fig. 4B (solid line), yielding T_m values of 50, 52, 55.5, 76, 79, and 80.6 °C with ≈ 11, 16, 18, 17, 26, and 12 % of the total heat absorbed in Steps 1 - 6, respectively. Although the fit of the sequential model to the data in Fig. 4B is good, the parameters of the fit should be considered as estimates since ΔH values in the model are assumed to be temperature-independent and the high-temperature endotherm may be influenced by irreversible denaturation (25).

CONCLUSIONS

Spectral and DSC data are consistent with a model in which seven states are populated during a sequential, partial unfolding of the tryptophan synthase holo-$\alpha_2\beta_2$ multienzyme complex (32,33):

$$\alpha^F\beta^F\beta^F\alpha^F \rightarrow \alpha^F\beta^f\beta^f\alpha^F \rightarrow \alpha^U\beta^f\beta^f\alpha^F \rightarrow \alpha^U\beta^f\beta^f\alpha^U \rightarrow \alpha^U\beta^u\beta^u\alpha^U \rightarrow \alpha^U\beta^U\beta^u\alpha^U \rightarrow \alpha^U\beta^U\beta^U\alpha^U$$

where each transition in the nondissociated enzyme complex is two-state and the superscripts are defined: $F \equiv$ folded, native chains; $f \equiv$ conformation after a marked decrease in the ellipticity of bound PLP and partial exposure of Trp-177 in β chains; $u \equiv$ intermediate after further unfolding the f conformation with both Trp and Tyr residues exposed and tertiary structure disrupted; and, $U \equiv$ final state after thermal unfolding. The area of the low-temperature endotherm (steps 1-3) is more than twice that for unfolding an isolated α chain and contains contributions from the conformational change of β chains in step 1, as observed in DSC profiles of holo-β_2 (Fig. 3). The different stabilities of α chains in the holo-enzyme complex is consistent with a previous report of negative cooperativity for binding α to $\alpha\beta_2$ in the presence of phosphate (34). The high-temperature endotherm includes the main transitions for unfolding β chains in steps 4-6. The bound cofactor PLP links the unfolding of the two structural domains of each β chain (Fig. 1). Thus, calorimetric and spectral measurements allow characterization of the thermal unfolding pathway for holo-$\alpha_2\beta_2$ which permits correlation of thermodynamic and structural information.

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