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Kinetic Studies of Tryptophan Synthetase. Interaction of L-Serine, Indole, and Tryptophan with the Native Enzyme*

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ABSTRACT: The synthesis of L-tryptophan from indole and L-serine, as catalyzed by bacterial tryptophan synthetase ($\alpha_2\beta_2$), has been studied with steady-state and rapid reaction techniques. Initial velocity measurements of the reaction have been made utilizing the absorption difference between indole and tryptophan at 289 nm. The results are consistent with both a compulsory sequence of substrate addition, and a random, rapid equilibration between enzyme and substrates. Dissociation constants and/or Michaelis constants for serine and indole were obtained. Temperature-jump, stopped-flow, and combined stopped-flow–temperature-jump measurements were made on solutions containing β_2 protein and an excess of α protein combined with indole, L-serine, L-tryptophan, and indole plus L-serine. The results are compared to data from similar studies in which β_2 protein alone was the catalyst.

The results obtained indicate that (1) L-serine binds much more tightly to $\alpha_2\beta_2$ than to β_2 protein, (2) the binding of L-tryptophan is too fast to measure for the holoenzyme, (3) the enzyme isomerization previously observed for β_2 protein probably does not occur with $\alpha_2\beta_2$, and (4) only the single first-order (rate-limiting) process observed previously can be detected on interaction of the enzyme–serine complex with indole. The mechanism of action of tryptophan synthetase in catalyzing this reaction is discussed in terms of the results obtained.

Tryptophan synthetase isolated from *Escherichia coli* is a multisubunit enzyme consisting of two different subunits (Yanofsky, 1960). The fully associated form, $\alpha_2\beta_2$, where α and β represent different protein chains, is dissociable into two α subunits and a β_2 dimer (Wilson and Crawford, 1964; Hathaway *et al.*, 1969). The overall reaction catalyzed by the intact enzyme is

$$\text{InGP} + \text{Ser} \underset{\text{PLP}}{\overset{\alpha_2\beta_2}{\rightleftharpoons}} \text{Trp} + \text{GP} \qquad (1)$$

which is the sum of reactions catalyzed by the individual subunits

$$\text{InGP} \overset{\alpha}{\rightleftharpoons} \text{In} + \text{GP} \qquad (2)$$

$$\text{In} + \text{Ser} \underset{\text{PLP}}{\overset{\beta_2}{\rightleftharpoons}} \text{Trp} \qquad (3)$$

Here InGP is indole-3-glycerol phosphate, Ser is L-serine, GP is glyceraldehyde 3-phosphate, and PLP is pyridoxal 5′-phosphate. Each subunit has the ability to markedly increase the reaction rate for the process catalyzed by the other protein (Wilson and Crawford, 1965; Hatanaka *et al.*, 1962).

This work represents the second part of a study of the synthesis of tryptophan from L-serine and indole. Steady-state, difference spectral, and rapid reaction techniques were used to examine this reaction in the presence of the fully active tryptophan synthetase molecule ($\alpha_2\beta_2$) and the results obtained are compared to those obtained for catalysis by the β_2 protein alone (Faeder and Hammes, 1970). A model is postulated for the role of α protein in the mechanism of action of the tryptophan synthetase system.

Experimental Section

Materials. The β_2 protein of tryptophan synthetase was purified from the A2/F′A2 mutant of *E. coli* K-12, while α protein was prepared from the B-8 mutant. All preparative procedures were done at 2–5°. Both strains were kindly supplied to us by Dr. C. Yanofsky and Dr. L. Soll. Some of the bacteria were grown by Mr. A. Tannahill of the Department of Chemical Engineering, Cornell University, and some were supplied by the New England Enzyme Center. The procedure used for preparing β_2 protein has been described elsewhere (Wilson and Crawford, 1965; Faeder and Hammes, 1970). The β_2 protein was prepared for experiments by dialyzing against 0.1 M potassium phosphate buffer containing 1×10^{-5} M pyridoxal 5′-phosphate (pH 7.8). The α protein was prepared by a scaled-up version of the method of Henning

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TABLE I: Steady-State Rate Parameters for Tryptophan Synthetase.

	Native Enzyme	β_2 Protein[a]
φ_1 (sec)	0.304	6.78
φ_2 (sec M)	5.61×10^{-5}	6.12×10^{-3}
φ_3 (sec M)	4.07×10^{-6}	7.62×10^{-5}
φ_4 (sec M^2)	1.10×10^{-10}	9.42×10^{-8}
K_{Ser} (φ_4/φ_3) (M)	2.70×10^{-5}	1.24×10^{-3}
K_{In} (φ_3/φ_1) (M)	1.34×10^{-5}	1.12×10^{-5}
K_{Ser}' (M)	0.9×10^{-5}[b]	4.0×10^{-3}[c]

[a] Faeder and Hammes (1970). [b] Determined by spectral titration of $\alpha_2\beta_2$ protein with serine. [c] Determined by spectral titration of β_2 protein with serine.

et al. (1962), as modified by Hatanaka *et al.* (1962). Typically, 250 g of bacterial paste was used and protein was eluted from a large column of DEAE-Sephadex A-50, 12.6 $cm^2 \times$ 25 cm. A small amount of Sephadex G-25 (coarse grade) was layered onto the Sephadex A-50 to prevent channeling when the protein was loaded. Two liters of 0.01 M potassium phosphate and 2 l. of 0.4 M potassium phosphate (pH 6.8) were used to establish the linear ionic strength gradient. Fractions containing activity higher than 3500 units/mg were pooled and precipitated with 44 g/100 ml of ammonium sulfate. The suspension was centrifuged at 14,000*g* for 20 min. The precipitate was dissolved in 0.1 M phosphate buffer with a 1×10^{-5} M pyridoxal 5′-phosphate (pH 7.8) at a protein concentration of 8–10 mg/ml, and dialyzed against 1 l. of the same buffer for 8 hr with two buffer changes. After dialysis, the protein was filtered through a Millipore filter (type HA, 0.45-μ mean pore size). Protein prepared in this manner could be stored at 2–5° for at least 1 week with no loss of activity.

Protein concentration was determined by the method of Lowry *et al.* (1951), using human serum albumin as a standard, and multiplying the absorbancy of the β_2 protein by 0.91 to reduce it to an equivalent weight of albumin (Wilson and Crawford, 1965). The activity of α or β_2 protein was measured by the method of Smith and Yanofsky (1962). One unit of activity is defined as the conversion of 0.1 μmole of indole into L-tryptophan in 20 min at 37°. The specific activity of each subunit was measured with a threefold molar excess of the other subunit present. The α protein used in this work had a specific activity between 2500 and 3800 units per mg, and the β_2 protein had a specific activity between 1600 and 2000 units per mg. L-Serine, indole, and pyridoxal 5′-phosphate were purchased from Calbiochem. All other materials used were standard reagent grade.

Methods. Initial velocity measurements of the formation of tryptophan from indole and L-serine were made as previously described (Faeder and Hammes, 1970). The reaction was initiated with 10 μl of enzyme stock solution containing β_2 protein and a concentration of α protein equal to four times that of β_2 protein.

A Durrum-Gibson stopped-flow spectrophotometer was used for stopped-flow measurements. The β-protein concentration ranged from 2.4×10^{-5} to 4.1×10^{-5} M in these experiments. The concentration of α protein was kept at least as high as 2 moles of α to 1 mole of β. A combined stopped-flow–temperature-jump apparatus described elsewhere (Faeder, 1970; Faeder and Hammes, 1970) was also used for kinetic measurements. The concentration of β protein used varied from 2.4×10^{-5} to 6.5×10^{-5} M for these experiments.

The binding constant for the interaction between α and β_2 protein was measured by following the spectral changes occurring when aliquots of α protein were added to 1.00 ml of β_2 protein solution. Equal aliquots of buffer were added to 1.00 ml of β_2 protein solution in the reference cell. The apparent binding constant for L-serine to the $\alpha_2\beta_2$ protein was determined by addition of aliquots of 1.00×10^{-3} M L-serine to 1.00 ml of β_2 protein (with four times the concentration of α protein) solution. Again equal aliquots of buffer were added to 1.00 ml of the same protein solution in the reference cell. A Cary 14 recording spectrophotometer equipped with a 0.0–0.1 absorbancy slide-wire and thermostatted at 24.5° (±0.5) was used. The slit width was maintained constant at 0.05 mm.

Results and Treatment of Data

Steady-State Experiments. The α and β_2 proteins form a tight complex in the presence of pyridoxal 5′-phosphate and L-serine (Creighton and Yanofsky, 1966). The overall enzymatic reaction catalyzed by the $\alpha_2\beta_2$ enzyme can be considered as a two-substrate reaction producing a single product since pyridoxal 5′-phosphate binds quite tightly to the enzyme. As for the β_2 protein alone (Faeder and Hammes, 1970), the initial steady-state velocity, v, obeys the rate law

$$\frac{(E_0)}{v} = \varphi_1 + \frac{\varphi_2}{(Ser)} + \frac{\varphi_3}{(In)} + \frac{\varphi_4}{(In)(Ser)} \quad (4)$$

where (E_0) is the total concentration of β and the φ's are constants. Initial velocities were measured for permutations of eight indole concentrations (7.36×10^{-6}–1.86×10^{-4} M) and five serine concentrations (3.82×10^{-5}–2.29×10^{-4} M), and the values of the φ's obtained from a weighted least-squares analysis are summarized in Table I. (See Faeder, 1970, for a compilation of the data.) The estimated uncertainty in these parameters is ±15%. The simplest mechanisms consistent with the data are a compulsory sequence of addition of substrates to the enzyme with L-serine binding first, and a random, rapid equilibration of enzyme and substrate. In the former case the dissociation constant for the first substrate, K_{Ser}, is φ_4/φ_3 and the Michaelis constant for indole, K_{In}, is φ_3/φ_1. In the latter case the ratio φ_4/φ_3 is still the enzyme–serine dissociation constant, but φ_3/φ_1 is now the equilibration dissociation constant for indole with the enzyme–serine complex rather than a Michaelis constant. Furthermore, φ_2/φ_1 is the dissociation constant for the reaction of serine with the enzyme–indole complex. The value of φ_2/φ_1 (1.84×10^{-4} M) is markedly different from φ_4/φ_3 (2.7×10^{-5} M), which implies an increase in the dissociation constant for indole from the enzyme–serine complex (1.3×10^{-5} M) relative to that for dissociation from free enzyme (2.0×10^{-6} M).

Binding Experiments. Creighton and Yanofsky (1966) have studied the binding of α and β_2 protein to form the $\alpha_2\beta_2$ complex utilizing activity measurements. This interaction can also be studied by following the spectral changes accompanying the binding process. The absorbance increases with the maximum change occurring at about 405 nm. If the

assumption is made that the overall reaction can be written as (Hathaway *et al.*, 1969)

$$2\alpha + \beta_2 \overset{K_{\alpha_2\beta_2}}{\rightleftharpoons} \alpha_2\beta_2 \qquad (5)$$

the dissociation constant, $K_{\alpha_2\beta_2}$, can be written in terms of the quantities measurable from the difference spectrum

$$K_{\alpha_2\beta_2} = \frac{[(A_0) - 2\Delta a/\Delta\epsilon]^2[(B_0) - \Delta a/\Delta\epsilon]}{\Delta a/\Delta\epsilon} \qquad (6)$$

where (A_0) and (B_0) are the total concentrations of α and β_2, respectively, Δa is the difference absorbancy, and $\Delta\epsilon$ is the difference molar extinction coefficient at 405 nm. Since stock solutions of α and β_2 are similar in concentration, $K_{\alpha_2\beta_2}$ must be determined by first obtaining a good estimate for $\Delta\epsilon$, using a large excess of α. Values of (A_0), (B_0), and Δa are then used to obtain $K_{\alpha_2\beta_2}$ for a series of solutions. The value of $\Delta\epsilon$ is then varied to obtain a set of $K_{\alpha_2\beta_2}$ values with the minimum standard deviation from the average (*cf.* Hammes and Schimmel, 1966). The measured value for $K_{\alpha_2\beta_2}$ from four difference spectral measurements is $1.8 \pm 0.7 \times 10^{-10}$ M^{-2}, with $\Delta\epsilon = 3.4 \pm 0.9 \times 10^3$ M^{-1} cm^{-1}. From this value it can be estimated that under the conditions of the rapid reaction studies, at least 80% of the β_2 protein present is in the $\alpha_2\beta_2$ complex.

The equilibrium dissociation constant characterizing the binding of L-serine to the $\alpha_2\beta_2$ enzyme can be measured by a difference spectral titration (Faeder and Hammes, 1970). In this study, the concentrations of L-serine used were comparable to the concentration of complex (since $K_{Ser} \sim (E_0)$), and an iterative procedure similar to that used above to obtain $K_{\alpha_2\beta_2}$ permitted calculation of K_{Ser}

$$K_{Ser} = \frac{((B_0) - \Delta a/\Delta\epsilon_{Ser})((Ser_0) - \Delta a/\Delta\epsilon_{Ser})}{\Delta a/\Delta\epsilon_{Ser}} \qquad (7)$$

where now (B_0) is the total concentration of β protein, (Ser_0) is the initial concentration of L-serine, Δa is the difference absorbancy, and $\Delta\epsilon_{Ser}$ is the difference molar extinction coefficient at 420 nm. The value obtained for K_{Ser} ($0.9 \pm 0.3 \times 10^{-5}$ M) is similar to the value obtained by steady-state methods (2.7×10^{-5} M).

Fast Reaction Experiments. A single relaxation process is observed in the temperature-jump apparatus with α and β_2 protein mixed together. The effect is observed at 420 nm, but not at 330 nm (absorption maxima) and has an extremely small amplitude. The value of the reciprocal relaxation time is $2.85 \pm 0.38 \times 10^3$ sec^{-1} for a β concentration of 5.6×10^{-5} M. This value is essentially identical with the reciprocal relaxation time of $2.95 \pm 0.46 \times 10^3$ sec^{-1} observed for β_2 protein alone, but the relaxation amplitude is much smaller.

The interaction of L-tryptophan with the $\alpha_2\beta_2$ enzyme was studied over a concentration range of tryptophan from 5×10^{-4} to 4×10^{-3} M in the temperature-jump apparatus, and from 1×10^{-4} to 3.5×10^{-3} M in the stopped-flow apparatus. Two processes were observed. The faster relaxation time, observed at 420 nm, is independent of the L-tryptophan concentration ($1/\tau = 6.8 (\pm 0.4) \times 10^3$ sec^{-1}), which suggests that the conformational change occurring in the free enzyme also occurs when tryptophan is bound, but the associated rate constants are slightly altered. Alternatively a different conformational change of the enzyme–tryptophan complex may be responsible for the observed process. The second relaxation process characterized by τ_2 was observed at longer times, both with temperature-jump and stopped-flow methods, at 420 and 330 nm, and was also associated with a concentration independent relaxation time ($1/\tau = 24.9 (\pm 2.5)$ sec^{-1}). These results suggest a relatively slow isomerization of the $\alpha_2\beta_2$-tryptophan complex occurs. The estimated binding constant for L-tryptophan ($2\text{–}3 \times 10^{-4}$ M; Creighton, 1970) is in accord with the essentially concentration independent value of the relaxation time over the accessible concentration range.

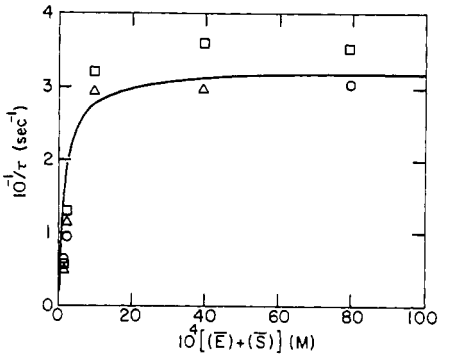

FIGURE 1: Variation of $1/\tau_3$ with L-serine concentration. The data points were obtained with the stopped flow technique at 470 (△), 420 (○), and 330 (□) nm. The solid line has been calculated with eq 9 and the parameters given in the text. The β protein concentration ranged from 2.6×10^{-5} to 2.8×10^{-5} M; $(\alpha)/(\beta)$ ranged from 2.0 to 2.4.

The interaction of the $\alpha_2\beta_2$ enzyme with L-serine was studied over a concentration range of L-serine of 2.5×10^{-4} to 5.0×10^{-3} M in the stopped-flow–temperature-jump apparatus, and from 1.25×10^{-4} to 8.00×10^{-3} M in the stopped flow apparatus. Only one relaxation process was observed. The very fast process previously observed for β_2 protein and L-serine with the stopped flow–temperature jump was no longer present. A relaxation process corresponding to that seen with β_2 protein alone (Faeder and Hammes, 1970) could be seen with both temperature-jump and stopped-flow techniques, although it was characterized primarily by the stopped-flow method. Figure 1 shows the reciprocal relaxation time, $1/\tau_3$, as a function of L-serine concentration and wavelength at 470, 420, and 330 nm. The values of τ_3 are approximately independent of wavelength. The experimental error is estimated to be about ±10% for relaxation times obtained at 470 and 420 nm and about ±15% for those obtained at 330 nm. The shape of the curve (sharply rising and leveling off when (Ser) > K_{Ser}) is consistent with the formation of an enzyme–serine intermediate. The small value of K_{Ser} makes it experimentally difficult to quantitatively determine the shape of the curve at low values of $[(\bar{E}) + (\bar{S})]$, since the amplitude is markedly decreased at low concentrations. Assuming that τ_3 characterizes a simple isomerization of the $\alpha_2\beta_2$-serine complex

$$E + S \overset{K}{\rightleftharpoons} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES' \qquad (8)$$

then (*cf.* Amdur and Hammes, 1966)

$$1/\tau_3 = k_{-2} + \frac{k_2}{1 + K/[(\bar{E}) + (\bar{S})]} \qquad (9)$$

where K is an apparent dissociation constant for the first

step, E represents the $\alpha_2\beta_2$ enzyme, S is serine, and the second step has been assumed to equilibrate slowly relative to the first step. The dissociation constant, K, is related to the overall dissociation constant, K_{Ser}, by the relationship $K_{Ser} = K/(1 + k_2/k_{-2})$. The curve in Figure 1 was calculated according to eq 9 using $K = 1.76 \times 10^{-4}$ M, $k_2 = 30$ sec^{-1}, and $k_{-2} = 2.5$ sec^{-1}. The calculated value of K_{Ser} is 1.4×10^{-5} M, which is in reasonable agreement with the values obtained by steady-state (2.7×10^{-5} M) and difference spectral (0.9×10^{-5} M) methods.

The relaxation spectrum of the mixture of α and β proteins was unchanged by the addition of indole over the concentration range 5.02×10^{-5}–9.06×10^{-4} M. The one relaxation process previously observed with the enzyme alone remained unchanged.

Indole and L-serine were simultaneously mixed with enzyme and observed by stopped-flow–temperature-jump and stopped-flow methods. The L-serine concentration was fixed at 1×10^{-3} M, saturating the enzyme, and the indole concentration varied from 1.27×10^{-5} to 4.80×10^{-4} M. There were no rapid effects observed in the time range accessible to the temperature jump, and only the single stopped-flow effect observed with the enzyme–serine system was seen. The relaxation time is essentially unchanged by the addition of indole, and varies very little with wavelength: $\tau = 32.3$ (± 3.2) msec at 470 nm, 30.3 (± 3.1) msec at 420 nm, and 26.4 (± 2.5) at 330 msec nm. The overall reaction can be followed by the stopped-flow method, and the results are consistent with the turnover number expected for the $\alpha_2\beta_2$–enzyme complex.

Discussion

A comparison of the steady-state parameters for the β_2 and $\alpha_2\beta_2$ proteins is presented in Table I. The Michaelis constant for the dissociation of indole remains essentially unchanged, while the dissociation constant for serine decreases by about a factor of 100 when α is added to β_2 protein. These results differ from others in the literature (*cf.* Hatanaka *et al.*, 1962); however, a recent kinetic investigation of the indole-3-glycerol phosphate plus serine reaction (eq 1) with $\alpha_2\beta_2$ protein (Creighton, 1970), gives a value for the serine dissociation constant, at 37° (6×10^{-5} M), in good agreement with this work. Furthermore, the value obtained by spectrophotometric titration is consistent with the steady-state constant.

When the steady-state data are analyzed in terms of a random, rapid equilibration mechanism a marked difference between the β_2- and $\alpha_2\beta_2$-catalyzed reactions is seen. For the $\alpha_2\beta_2$ enzyme, the binding of one substrate decreases the affinity of $\alpha_2\beta_2$ for the second substrate, while the binding of substrate to β_2 does not affect the affinity for the second. Therefore, if the mechanism is random, rapid equilibration, the α protein causes a marked change in the binding properties of the β_2–substrate complexes.

On the basis of the steady-state results presented here, a distinction cannot be made between a compulsory sequence of substrate addition and a random, rapid equilibration between enzyme and substrate (*cf.* Faeder and Hammes, 1970). In principle, tryptophan inhibition experiments can differentiate between these two mechanisms, but the indole–tryptophan difference spectral assay cannot be used for such experiments because of the high concentrations of tryptophan required, which gave a prohibitively large background absorbancy. Creighton (1970) has done such experiments for the overall reaction where the substrate is indole-3-glycerol phosphate instead of indole. His results support an ordered mechanism or a random, rapid equilibrium mechanism with a dead-end complex of enzyme, tryptophan, and indole-3-glycerol phosphate.

A relaxation process was observed for the $\alpha_2\beta_2$ complex, with a similar relaxation time to that found for an analogous process with free β_2 although the amplitude was greatly diminished. Some unbound β_2 is present in the α–β_2 mixture, and this is probably sufficient to account for the observed process, which has an extremely small amplitude. The failure to observe a very fast relaxation process with the $\alpha_2\beta_2$-serine complex supports this hypothesis since serine effectively removes uncomplexed β_2 protein.

The large difference in the dissociation constants measured here for the formation of $\alpha_2\beta_2$ and that reported elsewhere ($\sim 1 \times 10^{-15}$ M^2, Creighton and Yanofsky, 1966) is due to the absence of serine in this work, and is in agreement with qualitative ultracentrifuge studies reported by Creighton and Yanofsky (1966). It has been proposed that the enzyme–pyridoxal 5′-phosphate Schiff base exists in two different protonated forms; a neutral species which prefers a hydrophobic environment, and gives rise to the 330-nm absorption peak; and a dipolar, resonance-stabilized species which prefers a polar environment and gives rise to the 420-nm absorption peak (Faeder and Hammes, 1970). The titration of β_2 protein with α protein results in an increased absorbancy at 420 nm as more $\alpha_2\beta_2$ is formed. This implies that in the complex, pyridoxal 5′-phosphate is forced into a more polar environment, making it more accessible to the serine. This may account for the tighter binding of serine to the $\alpha_2\beta_2$ complex. Furthermore, the disappearance of the isomerization process in the $\alpha_2\beta_2$ complex, as previously discussed, probably means that α protein locks the β_2 protein and the pyridoxal phosphate into a fixed conformation.

The reaction of L-tryptophan with the enzyme also changes when α is present. The bimolecular step previously observed is not found and instead two isomerization processes are detected, although the faster one is probably due to the isomerization of the β_2 protein (rather than of $\alpha_2\beta_2$). The failure to observe the bimolecular step probably means that tryptophan binds more rapidly to β_2 in the presence of α. This may be due to the exposed pyridoxal group, as mentioned above, and a more rigid enzyme conformation. The specific nature of the isomerization processes cannot be ascertained. The initial association of either β_2 or $\alpha_2\beta_2$ with serine is too fast for study.

The single relaxation process observed for serine and $\alpha_2\beta_2$ protein was relatively slow and might be due to actual Schiff base formation or to an isomerization after the Schiff base is formed. The formation of this intermediate corresponds to the formation of a similar intermediate for β_2 protein alone (Faeder and Hammes, 1970), and probably to the formation of "aqua" complex observed by fluorescence (Goldberg *et al.*, 1968). The maximum value of the reciprocal relaxation time is approximately the same for β_2 and $\alpha_2\beta_2$. The associated spectral change for the $\alpha_2\beta_2$-serine complex is a decrease at 420 nm, and an increase at 470 and 330 nm. This may mean that the Schiff base formed between serine and pyridoxal 5′-phosphate is in a hydrophobic environment. However, the shift for β_2 protein alone showed an increase in absorbancy at 420 nm with a corresponding decrease at 330 nm, implying that the serine–pyridoxal Schiff base is in a more polar environment. This model is consistent with the observation that serine can be readily deaminated by β_2

protein alone, a reaction which is markedly inhibited by α protein (Crawford and Ito, 1964), since the Schiff base formed would be much more exposed to water in the absence of α protein. Furthermore, indole might be expected to react better with $\alpha_2\beta_2$-serine, which is in a more hydrophobic sterically blocked environment, than with the more polar, bulkier indole-3′-glycerol phosphate, which has a similar dissociation constant (0.7×10^{-5} M; Creighton, 1970). This is consistent with previous observations (De Moss, 1962; Creighton, 1970).

The spectrum of the $\alpha_2\beta_2$-serine–indole complex has a definite maximum at 470 nm, which is not observed in the absence of α protein. This can be attributed to an aminoacrylic intermediate (Goldberg and Baldwin, 1967). However, formation of this intermediate cannot be rate limiting since the associated relaxation time is the same at 330, 420, and 470 nm, and no other kinetic processes are observed at 470 nm. The formation of this absorption peak must therefore be rapid compared to the observed rate. Thus the rate-limiting process in the overall reaction is probably addition of indole to the aminoacrylic intermediate.

The results of this investigation are consistent with the following mechanistic model for the tryptophan synthetase system. The separate subunits, α and β_2, interact to form a complex in which each protein exerts a conformational change on the other, and a particular conformation is stabilized where the active sites of the β_2 subunit are more exposed to solvent than in the absence of α protein. This results in a tighter binding of serine; on the other hand, the $\alpha_2\beta_2$ enzyme forms a hydrophobic pocket for the pyridoxal 5′-phosphate–serine Schiff base intermediate, which increases the specificity of the reaction (relative to that in the absence of α protein). The most important aspect of the interaction between α and β_2 for this model is the stabilization of particular conformations of subunits, rather than a change in the basic mechanism of catalysis.

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