Fluorescence Studies of Substrate and Subunit Interactions of the β_2 Protein of *Escherichia coli* Tryptophan Synthetase*

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ABSTRACT: Substrate and subunit interactions of the β_2 protein of *Escherichia coli* tryptophan synthetase were found to have a marked influence on the fluorescence properties of the pyridoxal phosphate prosthetic groups bound to the enzyme. A new, strong fluorescence emission was observed on binding of L-serine, one of the substrates, to the β_2 protein. The emission, termed the aqua band, exhibits an excitation peak at 424 mμ and an emission peak at 500 mμ. Related compounds such as D-serine and L-threonine are ineffective in eliciting the aqua band, showing that a high degree of stereospecificity is involved in the interaction that produces the fluorescence.

The β_2-L-serine species that exhibits the aqua band is probably an intermediate in the deamination of L-serine. This inference is supported by the finding that indole, α subunit, and pH have parallel effects on the rate of deamination of L-serine and on the intensity of the aqua band. Binding of indole, a second substrate of the enzyme, to the β_2-L-serine complex quenches the aqua band and stops the deamination of L-serine. Similarly, addition of 2 moles of α subunit per mole of β_2 protein quenches the aqua band and stops the deamination of L-serine. Finally, both the intensity of the aqua band and the deaminase activity of the β_2 protein are greatest at alkaline pH.

The final step in the synthesis of tryptophan in *Escherichia coli* is catalyzed by tryptophan synthetase, an enzyme composed of two types of readily separable subunits (Crawford and Yanofsky, 1958). The fully associated enzyme complex, $\alpha_2\beta_2$ (Wilson and Crawford, 1965; Goldberg *et al.*, 1966), catalyzes reactions 1–3.

$$\text{indole} + \text{L-serine} \xrightarrow{\beta_2 \text{ or } \alpha_2\beta_2} \text{L-tryptophan} \quad (1)$$

$$\text{indole-3-glycerol phosphate} \xrightleftharpoons{\alpha \text{ or } \alpha_2\beta_2} \text{indole} + \text{D-glyceraldehyde 3-phosphate} \quad (2)$$

$$\text{indole-3-glycerol phosphate} + \text{L-serine} \xrightarrow{\alpha_2\beta_2} \text{L-tryptophan} + \text{D-glyceraldehyde 3-phosphate} \quad (3)$$

The dissociated α subunit catalyzes reaction 2, while the dissociated β_2 protein catalyzes reaction 1 (Crawford and Yanofsky, 1958). In addition, the β_2 protein catalyzes reaction 4 (Crawford and Ito, 1964).

$$\text{L-serine} \xrightarrow{\beta_2} \text{pyruvate} + NH_3 \quad (4)$$

Tryptophan synthetase is a particularly interesting enzyme because the catalytic properties of the α and β_2 subunits are markedly altered on formation of the $\alpha_2\beta_2$ complex. (a) The rates of reactions 1 and 2 are more than tenfold greater for the $\alpha_2\beta_2$ complex than for the isolated subunits. (b) The physiologically significant reaction 3 is a distinct reaction of the $\alpha_2\beta_2$ complex, not merely the sum of reactions 1 and 2 (Crawford and Yanofsky, 1958). (c) The deamination of L-serine (reaction 4) is catalyzed by the β_2 protein, but not by the $\alpha_2\beta_2$ complex (Crawford and Ito, 1964).

The β_2 protein contains two pyridoxal phosphate moieties that serve as prosthetic groups in reactions 1, 3, and 4 (Wilson and Crawford, 1965). The distinctive spectral properties of this coenzyme make it a very useful probe of the active sites of enzymes that contain pyridoxal phosphate (for example, see Morino and Snell, 1967). Goldberg and Baldwin (1967) investigated subunit interactions of tryptophan synthetase by means of a new absorption band they observed from a reaction intermediate bound to the $\alpha_2\beta_2$ complex. We report here our finding of a highly specific fluorescence emission from the pyridoxal phosphate groups in a complex of the β_2 protein and L-serine. Indole, substrate analogs, the α subunit, pH, and NH_4^+ ion were found to influence the fluorescence intensity. The effects of substrate and subunit interactions on the fluorescence intensity suggest that the emitting species is an intermediate in the reaction pathway leading to the deamination of L-serine.

Experimental Procedures

Materials. The β_2 protein was purified from extracts of the A2/F'A2 strain of *E. coli* by the method of Wilson and Crawford (1965). The β_2 protein was stored in 0.6 M potassium phosphate buffer (pH 7.5) containing 38

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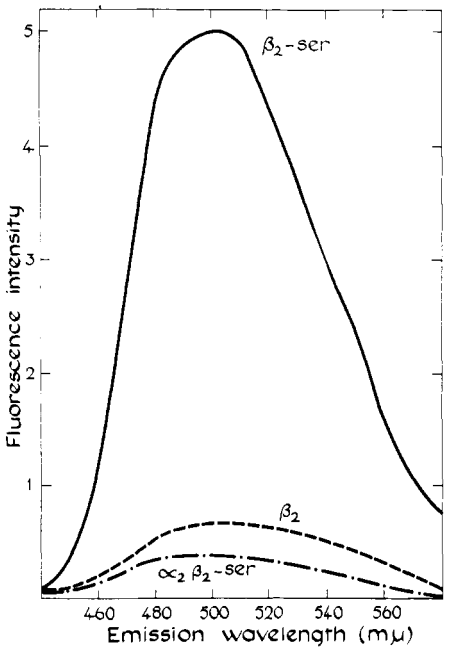

FIGURE 1: Fluorescence emission spectra of 1.76×10^{-6} M β_2 protein (β_2, – – –), of β_2 protein in the presence of 0.018 M L-serine (β_2-Ser, ——) and of β_2 protein in the presence of 0.018 M L-serine and 3.9×10^{-5} M α protein ($\alpha_2\beta_2$-Ser, -·-·-·) at pH 7.4. The excitation wavelength was 405 mμ. The fluorescence of th β_2-L-serine complex will be referred to as the aqua band.

μM pyridoxal phosphate and 10 mM 2-mercaptoethanol, at 5° in the dark. The specific activity in reaction 1 of one of our preparations was 1500 units/mg, which is to be compared with a value of 2700 units/mg reported by Wilson and Crawford (1965). Different preparations of β_2 protein had very similar binding and spectral characteristics, while their ability to complex with the α protein seemed to parallel their activity in reaction 1. The buffer used in studies of the binding of serine, indole, and tryptophan to the β_2 protein was 0.06 M potassium phosphate (pH 7.4). This buffer solution also contained 7.6×10^{-7} M pyridoxal phosphate and 2×10^{-4} M 2-mercaptoethanol as a result of the dilution of the stock solution of β_2 protein.

The α protein was kindly provided by Dr. C. Yanofsky. It was purified from the 5927R38 strain (Somerville and Yanofsky, 1965) of *E. coli* according to the method of Henning *et al.* (1962).

Commercial samples of indole (Eastman), 1-dimethylaminonaphthalene-5-sulfonic acid (Fluka), sodium pyruvate (Schwartz), pyridoxal phosphate (Calbiochem), L-cysteine (Mann), DL-homoserine (Mann), *O*-acetyl-L-serine (Cyclo), and D-serine (Cyclo) were used. Other amino acids and their derivatives were obtained from Calbiochem.

Optical Studies. Absorption spectra were obtained on a Cary Model 14B recording spectrophotometer. Fluorescence emission, excitation, and polarization measurements were performed on a recording spectrofluorimeter described previously (Stryer, 1965). A xenon lamp was used to obtain excitation and polarization spectra, whereas a mercury lamp was used to obtain emission

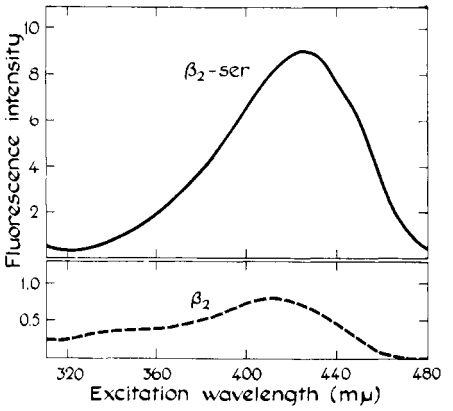

FIGURE 2: Fluorescence excitation spectra of 1.76×10^{-6} M β_2 protein (β_2, – – –), and of β_2 protein in the presence of 0.018 M L-serine (β_2-Ser, ——), at pH 7.4. The emission filter (CS ε-71) transmitted light of wavelengths above 460 mμ.

spectra. Either 365 or 405 mμ was used as the excitation wavelength for emission spectra. A slightly greater emission intensity was observed on excitation at 405 mμ but the scattering was also greater. The corrected fluorescence excitation spectra and polarization measurements were recorded directly using a ratio amplifier. The emission spectra shown here have not been corrected for the variation with wavelength in the sensitivity of the detection system. The relative sensitivity of the detection system was 1.92 at 440 mμ, 1.30 at 480 mμ, 1.03 at 500 mμ, 0.80 at 520 mμ, and 0.62 at 540 mμ. Excited-state lifetimes were obtained using a nanosecond fluorimeter designed by Hundley *et al.* (1967).

Spectral measurements were made at 22 ± 1°. 1-Dimethylaminonapthalene-5-sulfonate was used as a standard of quantum yield of 0.37 (Chen, 1966). In view of the reported photosensitivity of pyridoxal compounds (Chen, 1965), care was taken to minimize the exposure to light of solutions containing β_2 protein and pyridoxal phosphate. Most of the fluorescence data were obtained before there was a significant accumulation of products in reactions 1 or 4. Emission spectra were obtained within 3 min after the components were mixed. The time required for a fluorimetric titration was typically 15 min.

Results

Fluorescence Properties of the β_2 Protein and Its Complexes. The pyridoxal phosphate groups bound to the β_2 protein exhibit a weak emission with a peak at 505 mμ (Figure 1). The fluorescence intensity increases markedly on addition of 0.018 M L-serine, and the emission maximum shifts to 500 mμ (Figure 1). This fluorescence of the β_2-L-serine complex[1] will be referred to as the *aqua band.* The effect of L-serine in eliciting the aqua band appears to be highly specific. The aqua band is not formed on addition of 0.05 M D-serine, *O*-acetyl-L-serine, DL-homoserine, DL-α-methylserine, L-threonine,

[1] The β_2 protein in all likelihood binds two molecules of L-serine, since the β_2 protein contains two pyridoxal phosphate residues (Wilson and Crawford, 1965). For simplicity, we denote the complex as β_2-L-serine rather than as β_2-(L-serine)$_2$.

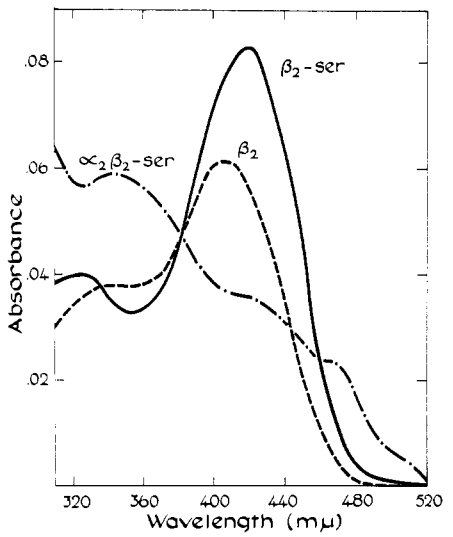

FIGURE 3: Absorption spectra of 4.7×10^{-6} M β_2 protein (β_2, – – –), of β_2 protein in the presence of 0.018 M L-serine (β_2-Ser, ——), and of β_2 protein in the presence of 0.018 M L-serine and 1.08×10^{-5} M α protein ($\alpha_2\beta_2$-Ser, -·-·-·) at pH 7.4.

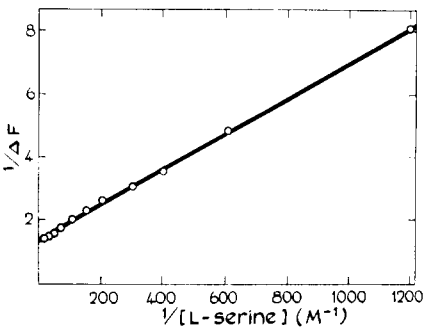

FIGURE 4: Effect of L-serine on the emission intensity at 500 mμ of the β_2 protein. The excitation wavelength was 365 mμ. The reciprocal of the increase in fluorescence intensity ($1/\Delta F$) is shown as a function of the reciprocal of the total concentration of L-serine. The total concentration of L-serine is taken as the unbound concentration since the two are nearly identical. The pH was 7.4. The fluorescence intensities shown in Figures 4–9 are all normalized to a maximal intensity of 1.0 for the β_2-L-serine complex at high pH as defined in Figure 9. At pH 7.4 the β_2-L-serine complex has a relative fluorescence intensity of 0.75.

L-alanine, L-cysteine, sodium pyruvate, indole, L-tryptophan, or 0.024 M D-tryptophan to the β_2 protein.

There is a striking decrease in the fluorescence intensity of the β_2-L-serine complex on addition of the α protein (Figure 1). In fact, the fluorescence intensity of the $\alpha_2\beta_2$-L-serine complex is distinctly less than that of the β_2 protein alone. The quantum yields of the pyridoxal phosphate fluorescence are 0.016 for β_2 protein, 0.14 for the β_2-L-serine complex, and less than 0.01 for the $\alpha_2\beta_2$-L-serine complex, at pH 7.4.

The excitation spectra of the pyridoxal phosphate emission of the β_2 protein and of the aqua band of the β_2-L-serine complex are shown in Figure 2. The β_2 protein exhibits an excitation peak at 415 mμ. There is a pronounced minimum in the excitation spectrum of the aqua band near 320 mμ.

The emission of the β_2 protein and of the β_2-L-serine complex is highly polarized, as expected for an emitting species that is tightly bound to a protein (for example, see Haugland and Stryer, 1967). On excitation at 405 mμ, the emission anisotropy of the β_2 protein is 0.321, whereas that of the aqua band of the β_2-L-serine complex is 0.297. The corresponding values of the fluorescence polarization are 0.415 and 0.388, respectively. The excited-state lifetime of the aqua band is 4.0 nsec.

Absorption Spectra of the β_2 Protein and Its Complexes. The absorption spectrum, as well as the emission spectrum, of the β_2 protein is altered on addition of L-serine and α protein. The absorption spectra of the β_2 protein, the β_2-L-serine complex, and the $\alpha_2\beta_2$-L-serine complex, between 320 and 520 mμ, are shown in Figure 3. Addition of 0.018 M L-serine to the β_2 protein results in a shift of the absorption maximum from 417 to 421 mμ and a substantial increase in extinction coefficient at those wavelengths. Subsequent addition of α protein leads to a marked decrease of the absorbance between 400 and 440 mμ. At wavelengths beyond about 450 mμ, $\alpha_2\beta_2$-L-serine complex has a higher absorbance than either the β_2 protein or the β_2-L-serine complex. Addition of α protein to the β_2 protein in the absence of L-serine did not lead to a spectral change.

The absorption spectrum of a mixture of β_2 protein and L-serine changes with time. Several lines of evidence indicate that the time-dependent increase in absorbance at wavelengths shorter than 360 mμ is primarily the absorbance contribution of the pyruvate formed by the enzymatic deamination of L-serine. (a) The magnitude of the increase of absorbance parallels the amount of L-serine available for deamination; (b) the rate of increase of absorbance is similar to the known rate of deamination; (c) both the time-dependent increase in absorbance and the formation of pyruvate are stopped by the addition of α protein to form $\alpha_2\beta_2$-L-serine; and (d) gel filtration of a reaction mixture of β_2 protein and L-serine yields a high molecular weight fraction which has the absorption spectrum of β_2 protein, and a low molecular weight fraction which has a spectrum like that of pyruvate.

Dependence of the Aqua Band upon L-Serine Concentration. These results show that either fluorescence or absorption spectroscopy can be used to study substrate and subunit interactions of the β_2 protein. We chose fluorescence for several reasons. (a) Less protein is needed than for absorption studies; (b) the relative changes of the emission intensity on formation of complexes are much larger than corresponding changes of the optical density; and (c) the aqua band is a highly specific expression of the environment of the bound pyridoxal phosphate moiety.

The intensity of the aqua band was used as a means of following the binding of L-serine to the β_2 protein. The increase in fluorescence intensity at 500 mμ (ΔF) is related to the concentration of L-serine by an expression analogous to the Lineweaver–Burk equation in which ΔF is proportional to the fraction of β_2 protein complexed with L-serine, $1/\Delta F = 1/\Delta F_{max} + K_s'/\Delta F_{max}$[L-serine], where ΔF_{max} is the fluorescence increment when

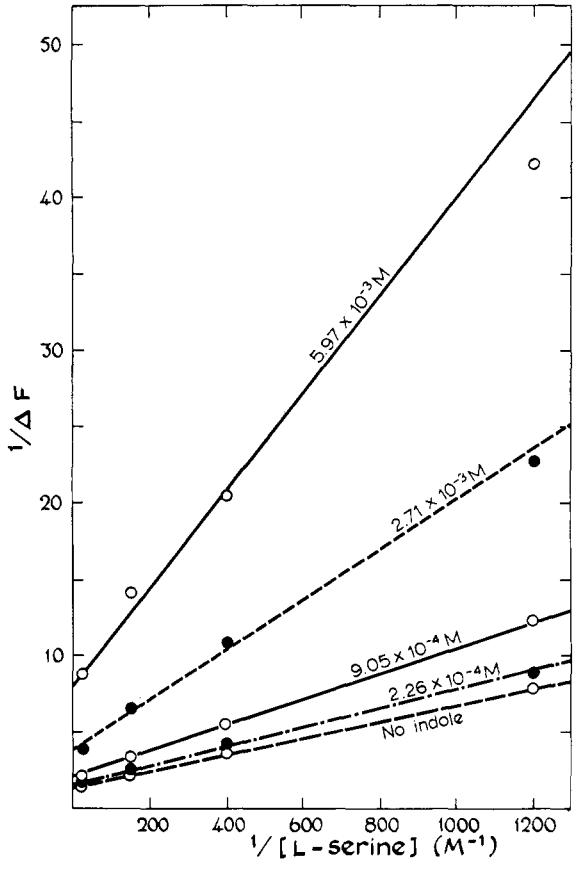

FIGURE 5: Effect of indole on the fluorescence increment obtained at 500 mμ on addition of L-serine to 1.47 × 10^{-6} M β_2 protein. The excitation wavelength was 365 mμ. The reciprocal of the increase in fluorescence intensity ($1/\Delta F$) is shown as a function of the reciprocal of the concentration of L-serine, in the presence of 0, 2.26, 9.05, 27.1, and 59.7 × 10^{-4} M indole. The pH was 7.4.

the β_2 protein is completely complexed with L-serine, [L-serine] is the concentration of unbound L-serine, and K_s' is the apparent dissociation constant for L-serine. K_s' is not a true equilibrium constant since the system is at a steady state rather than at equilibrium because of the deamination of L-serine. A plot of $1/\Delta F$ *vs.* 1/[L-serine] shows that K_s' is 4.2 × 10^{-3} M at pH 7.4, in the absence of other substrates or inhibitors (Figure 4).

Effect of Indole on the Aqua Band. Addition of 6 × 10^{-3} M indole to the β_2 protein has no effect on its fluorescence intensity at 500 mμ. In contrast, indole has a large effect on the fluorescence intensity of a mixture of β_2 protein and L-serine. In Figure 5, $1/\Delta F$ *vs.* 1/[L-serine] is shown for several concentrations of indole. These data are consistent with the interpretation that the K' for L-serine is hardly altered by the binding of indole to the β_2 protein. The K' for L-serine is 4.1 × 10^{-3} M in the absence of indole and 4.4 × 10^{-3} M in the presence of the indole. The K' for indole is 1.4 × 10^{-3} M. Rather, indole has a marked effect on the aqua band intensity of the β_2-L-serine complex. The fluorescence intensity at 500 mμ of a mixture of 3.52 × 10^{-6} M β_2 protein and 0.02 M L-serine was measured at several concentrations of indole. The decrease in fluorescence intensity is related to the concentration of indole by the expression

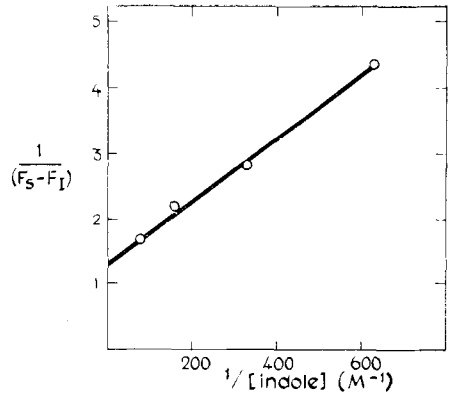

FIGURE 6: Effect of indole on the fluorescence intensity of 3.52 × 10^{-6} M β_2 protein in the presence of 0.02 M L-serine at pH 7.4. The excitation wavelength was 405 mμ. The reciprocal of the difference in fluorescence intensity in the absence and presence of indole is plotted as a function of the reciprocal of indole concentration.

$$\frac{1}{F_S - F_I} = \frac{1}{F_S - F_{ESI}} + \frac{K_I'}{(F_S - F_{ESI})[\text{indole}]}$$

where F_S is the fluorescence intensity of the β_2-L-serine complex in the absence of indole, F_I is the intensity in the presence of indole, F_{ESI} is the fluorescence intensity of the β_2-L-serine–indole complex, and K_I' is the apparent dissociation constant for indole. A plot of $1/(F_S - F_I)$ *vs.* 1/[indole] is shown in Figure 6, which gives a K' for indole of 3.7 × 10^{-3} M and an F_{ESI} of 0.01. This latter fluorescence intensity is subject to considerable error because of the nature of the extrapolation in Figure 6. The fluorescence intensities of the β_2 protein alone and of the β_2-L-serine complex are 0.10 and 0.75, respectively. Thus, binding of indole to the β_2-L-serine complex decreases the fluorescence intensity to a very low value, probably below that of the β_2 protein alone.

Effect of Tryptophan and Other Compounds on the Aqua Band. The intensity of the aqua band is decreased

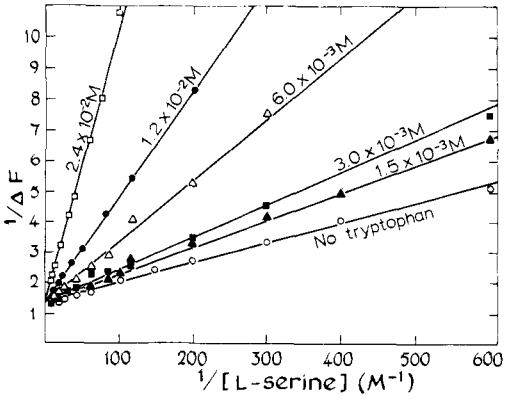

FIGURE 7: Effect of L-tryptophan on the fluorescence increment obtained at 500 mμ on addition of L-serine to 1.93 × 10^{-6} M β_2 protein. The excitation wavelength was 365 mμ. The reciprocal of the increase in fluorescence intensity ($1/\Delta F$) is shown as a function of the reciprocal of the concentration of L-serine, in the presence of 0, 1.5, 3.0, 6.0, 12.0, and 24.0 × 10^{-3} M L-tryptophan. The pH was 7.4.

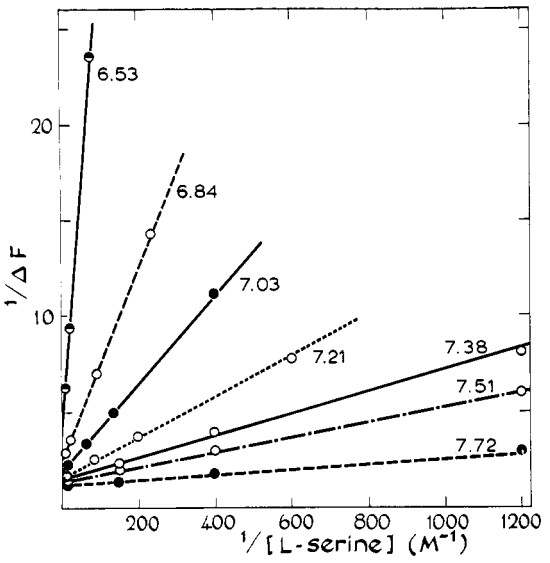

FIGURE 8: Effect of pH on the fluorescence increment obtained at 500 mμ on addition of L-serine to 1.47×10^{-6} M β_2 protein. The excitation wavelength was 365 mμ. The reciprocal of the increase in fluorescence intensity ($1/\Delta F$) is shown as a function of the reciprocal of the concentration of L-serine at pH 6.53, 6.84, 7.03, 7.21, 7.38, 7.51, and 7.72.

on addition of L-tryptophan to a mixture of β_2 protein and L-serine. In Figure 7, $1/\Delta F$ is plotted *vs.* 1/[L-serine], at several concentrations of L-tryptophan. These data show that L-tryptophan competes with L-serine for binding to the β_2 protein. In the presence of tryptophan, a competitive inhibitor, the slopes of plots of $1/\Delta F$ *vs.* 1/[L-serine] are increased by the factor $[1 + ([\text{tryptophan}]/K_T)]$, where K_T is the equilibrium dissociation constant of tryptophan. From Figure 7, K_T is 2.7×10^{-3} M.

The effect of several other compounds on the intensity of the aqua band was investigated. The intensity of the aqua band was appreciably diminished in the presence of 2.4×10^{-2} M D-tryptophan, 5×10^{-2} M L-threonine, or 5×10^{-3} M pyruvate. In these experiments, a single concentration of inhibitor was used. Assuming competitive inhibition, as seems likely from the fact that much of the intensity of the aqua band was restored on increasing the L-serine concentration to 0.05 M, the dissociation constants are 0.006 M for D-tryptophan, 0.1 M for L-threonine, and 0.02 M for pyruvate. The maximal intensity of the aqua band and the apparent dissociation constant for L-serine were not affected by 0.05 M D-serine, L-alanine, DL-α-methylserine, or DL-homoserine.

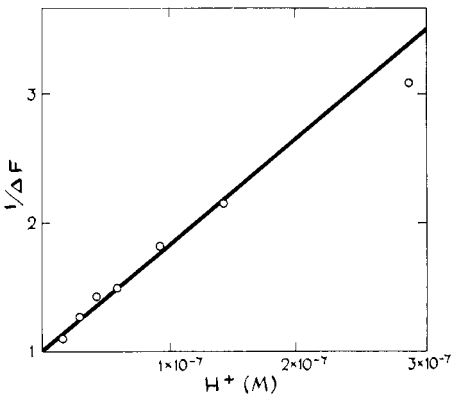

FIGURE 9: Effect of pH on the fluorescence increment at 500 mμ of the β_2-L-serine complex. The increase in fluorescence intensity of 1.47×10^{-6} M β_2 protein when completely complexed with L-serine was estimated from the data of Figure 8 by extrapolation to an infinite concentration of L-serine. The reciprocal of this maximal fluorescence increment at each pH is shown here as a function of H^+ ion concentration.

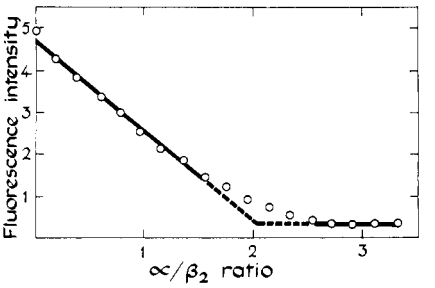

FIGURE 10: Effect of α protein on the fluorescence intensity at 500 mμ of the β_2-L-serine complex. Aliquots (30 μl) of 3.42×10^{-5} M protein were added to 3 ml of pH 7.4 buffer containing 1.76×10^{-6} M β_2 protein, 0.05 M L-serine, 1.5×10^{-5} M pyridoxal phosphate, and 0.01 M β-mercaptoethanol. The excitation wavelength was 405 mμ.

Effect of pH on the Aqua Band. Both the binding affinity for L-serine and the intensity of the aqua band when the β_2 protein is completely complexed with L-serine depend upon pH (Figure 8) whereas the fluorescence intensity of the β_2 protein alone is unaffected by pH in this range. K' for L-serine decreases from 8×10^{-2} to 1.5×10^{-3} M as the pH is raised from 6.53 to 7.72. This 53-fold decrease in the apparent dissociation constant of L-serine concomitant with a 15.5-fold decrease in $[H^+]$ indicates that more than one ionizing group is involved in the binding of L-serine. The pK's of these groups cannot readily be ascertained from these data. On the other hand, the effect of pH on the maximal fluorescence increment ΔF at a given pH can be interpreted more definitively, as shown by Figure 9, in which $1/\Delta F$ is plotted as a function of $[H^+]$. These data are consistent with the interpretation that a single ionizing group determines the fluorescence intensity of the β_2-L-serine complex and that the protonated form of the complex is virtually nonfluorescent. The dissociation constant, K_H, of the group involved in the equilibrium $H^+ - \beta_2$-L-serine $\rightleftarrows H^+ + \beta_2$-L-serine, and ΔF_u, the fluorescence intensity of the unprotonated form of the β_2-L-serine complex, minus that of the β_2 protein alone, are related to the observed fluorescence increment ΔF and $[H^+]$ by the expression $1/\Delta F = 1/\Delta F_u + [H^+]/K_H\Delta F_u$. From Figure 9, ΔF_u is 1.0 and K_H is 1.2×10^{-7} M. Thus, the unprotonated form of the β_2-L-serine complex has a fluorescence quantum yield of 0.19, and the ionizing group which controls the fluorescence of this complex has a pK of 6.9.

Effect of α Protein and NH_4^+ on the Aqua Band. The aqua band disappears on binding of the α protein to the β_2-L-serine complex (Figures 1 and 10). Two moles of α protein per mole of the β_2-L-serine complex is required

to quench the aqua band (Figure 10). It is also evident that the dissociation constant of the α protein in its complex with β_2-L-serine is less than 10^{-6} M, since the fluorescence intensity of 1.8×10^{-6} M β_2-L-serine complex follows the stoichiometric line over most of the titration (Figure 10).

The effect of NH_4^+ ion on the aqua band was investigated since this ion is known to alter the enzymatic properties of the β_2 protein. We find that the NH_4^+ ion diminishes the intensity of the aqua band. In 1.0 M ammonium citrate–0.06 M potassium phosphate (pH 7.4) the β_2-L-serine complex exhibits the same fluorescence as the β_2 protein alone. This effect of NH_4^+ ion cannot be attributed solely to an increase in ionic strength, since the intensity of the aqua band in 1.0 M sodium citrate–0.06 M potassium phosphate (pH 7.4) is 1.38-fold greater than in 0.06 M potassium phosphate at the same pH.

Discussion

The fluorescence properties of the pyridoxal phosphate groups of the β_2 protein are sensitive to substrate and subunit interactions of the enzyme. L-Serine, one of the substrates of the β_2 protein, is required for formation of the aqua band. Related compounds such as D-serine, *O*-acetyl-L-serine, DL-homoserine, L-threonine, L-alanine, and L-cysteine are ineffective in eliciting the aqua band, showing that a high degree of stereospecificity is involved in the interaction that gives rise to the fluorescence. The displacement of L-serine by L- or D-tryptophan, but not by indole, suggests that the fit of L-serine and indole at the active site is rather precise. Furthermore, the highly polarized emission of the pyridoxal phosphate groups of the β_2 protein and of the β_2-L-serine complex also indicates that the prosthetic group has little rotational flexibility when bound at the active site.

The β_2-L-serine species that exhibits the aqua band is probably an intermediate in the deamination of L-serine. This inference is supported by the following observations. Indole, α subunit, and pH have parallel effects on the rate of deamination of L-serine (Hatanaka *et al.*, 1962; Crawford and Ito, 1964), and on the intensity of the aqua band. The deamination of L-serine is stopped and the aqua band disappears on binding of indole to the β_2-L-serine complex. Similarly, the $\alpha_2\beta_2$-L-serine complex is virtually nonfluorescent and L-serine is not deaminated in that complex. The intensity of the aqua band, greatest at alkaline pH, appears to be influenced by an ionizing group with a pK of 6.9. The deaminase activity of the β_2 protein has a pH optimum in the range 7.8–8.2.

Another enzyme-bound pyridoxal phosphate species, the "amber complex," was identified by Goldberg and Baldwin (1967). The amber complex is characterized by an absorption band centered at 468 mμ. It is formed when the $\alpha_2\beta_2$ protein, L-serine, and a second substrate such as indole or 2-mercaptoethanol are present. The amber band has been attributed to an intermediate in the formation of tryptophan or *S*-(2-hydroxyethyl)-cysteine.

Indole, α subunit, and NH_4^+ ion have opposite effects on the aqua band and on the amber band. Indole diminishes the intensity of the aqua band and increases that of the amber band. The α subunit has the same effect. The effect of NH_4^+ ion on the β_2 protein is more complex. High concentrations of NH_4^+ ion lead to a small increase (typically threefold) in the rate of deamination of L-serine (Crawford and Ito, 1964). The effect of high concentrations of NH_4^+ ion on the rate of formation of L-tryptophan by the β_2 protein is more pronounced; the 20-fold increase approaches the increase obtained on formation of the $\alpha_2\beta_2$ complex (Hatanaka *et al.*, 1962). In this regard, it is interesting to note that NH_4^+ ion effectively substitutes for the α subunit in eliciting the amber band (Goldberg and Baldwin, 1967), whereas it decreases the intensity of the aqua band.

These data clearly demonstrate that the aqua and amber bands come from quite different species. The amber band is associated with an intermediate in the formation of L-tryptophan (Goldberg and Baldwin, 1967). Our studies suggest that the aqua band is associated with a species capable of being deaminated. This species may also be an intermediate in the formation of L-tryptophan. Stopped-flow studies of the aqua band probably will give an answer to this question, thus providing a better understanding of the catalytic mechanism and of the way in which the α subunit modifies the reactivity of the β_2 protein.

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