The Role of Tris(hydroxymethyl)aminomethane and Cysteine in the Dissociation of Tryptophanase*

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ABSTRACT: The dissociation of tryptophanase into subunits is reversibly effected by tris(hydroxymethyl)-aminomethane (Tris) and other agents, such as cysteine and penicillamine, which react with pyridoxal phosphate. The native enzyme with sedimentation constant 9.4 S is almost completely dissociated into a species with sedimentation constant about 5.6 S. The Tris-induced dissociation exhibits an apparent pK of 7.5 and is dependent upon the relative concentrations of Tris and pyridoxal phosphate. The extent of dissociation is predictable from the dissociation constant (8.28×10^{-3} M) for the Tris–pyridoxal phosphate Schiff base. Anthranilate, a competitive inhibitor of tryptophan degradation, exerts a protective effect against Tris-induced dissociation. Other competitive inhibitors, such as kynurenine and phenylalanine, do not prevent Tris-induced dissociation. Neither sulfhydryl nor disulfide groups appear to be crucial to the association of subunits or to the function of the catalytic site.

The physiological role, purification, and some of the physicochemical properties of the enzyme tryptophanase from *Bacillis alvei* have been reported from this laboratory (Hoch and DeMoss, 1965, 1966; Hoch *et al.*, 1966). The enzyme from *B. alvei* differs in several respects from that of *Escherichia coli*, from which source the enzyme has been crystallized and studied in detail (Newton and Snell, 1964; Newton *et al.*, 1965; Morino and Snell, 1967a–c). The reversible dissociation of tryptophanase from *B. alvei* observed in Tris buffer has been reported by us in an earlier paper (Gopinathan and DeMoss, 1966). In the present paper we present some of the detailed studies on the dissociation of tryptophanase and its mechanism.

Methods

B. alvei tryptophanase was purified by the method of Hoch *et al.* (1966). Sucrose density gradient centrifugation studies (Martin and Ames, 1961) and the enzyme assays were all done as described earlier (Gopinathan and DeMoss, 1966). All enzyme activities are reported as international enzyme units (1 μmole/min).

Column Chromatography on Bio-Gel P-200. A sample (1.5 ml) of the enzyme, dialyzed against 50 mM potassium phosphate (pH 7.5) containing 0.1 mM PLP (500 ml of buffer) for 4 hr, was loaded on a Bio-Gel P-200 (2.5 × 36 cm) column. The loading of the enzyme samples as well as their elution from the column was done from bottom to top of the column using a peristaltic pump (Lab Produkter AB, Stockholm). The individual fractions were analyzed for enzyme activity and the enzymatically active fractions were pooled and concentrated to 1.5 ml, by pressure dialysis in a Diaflo Model 50 cell (Amicon Corp., Cambridge, Mass.). The concentrated preparation was diluted to 5.1 ml with 50 mM Tris (pH 7.5) (not containing PLP[1]) and dialyzed against 1 l. of the same buffer for 18 hr with two changes of buffer. The dialyzed preparation was loaded on the same Bio-Gel P-200 column used before, which had been washed and equilibrated with 50 mM Tris (pH 7.5). The flow rate was maintained at a constant level (20 ml/hr) and 10-min fractions were collected.

The column experiment was also performed in the reverse order. This time the enzyme preparation was started in Tris buffer and then transferred by dialysis into potassium phosphate containing PLP. However, after elution from the column in Tris buffer, the concentrated preparation was frozen for 2 days with an excess of PLP and potassium phosphate prior to dialysis against 50 mM potassium phosphate (pH 7.5) containing 0.1 mM PLP.

Results

The Interconvertibility of the Two Forms of Tryptophanase. Our earlier observations (Gopinathan and DeMoss 1966) concerning the behavior of the enzyme on sucrose density gradients suggested that the two forms of the enzyme (5.6 and 9.4 S) were interconvertible. The reversibility of the reaction has been confirmed

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: PMB, *p*-mercuribenzoate; NEM, *N*-ethylmaleimide; PLP, pyridoxal phosphate; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; Bicine, *N,N*-bis(2-hydroxyethyl)-glycine; Tricine, *N*-tris(hydroxymethyl)methylglycine.

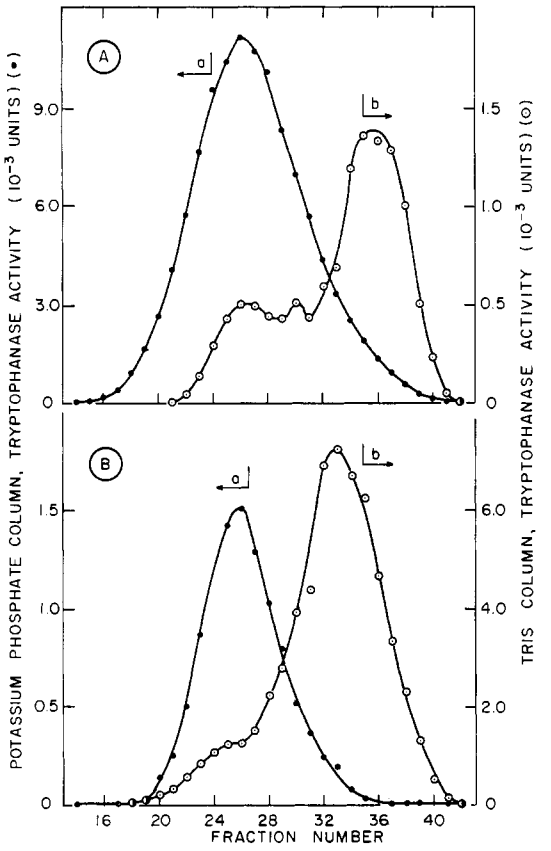

FIGURE 1: Interconvertibility of the two forms of tryptophanase. Details are described in the text under Methods. Curve A-a represents the profile of enzyme in 50 mM potassium phosphate (pH 7.5) containing 0.1 mM PLP, obtained from a column of Bio-Gel P-200 in the same buffer. The active fractions were pooled, concentrated, and dialyzed into 50 mM Tris (pH 7.5) without PLP, filtered through the same column in 50 mM Tris buffer (pH 7.5) (not containing PLP). Curve A-b represents the profile obtained. In B, the enzyme was passed first through the column in Tris buffer (curve B-b), then through the column in potassium phosphate buffer (curve B-a).

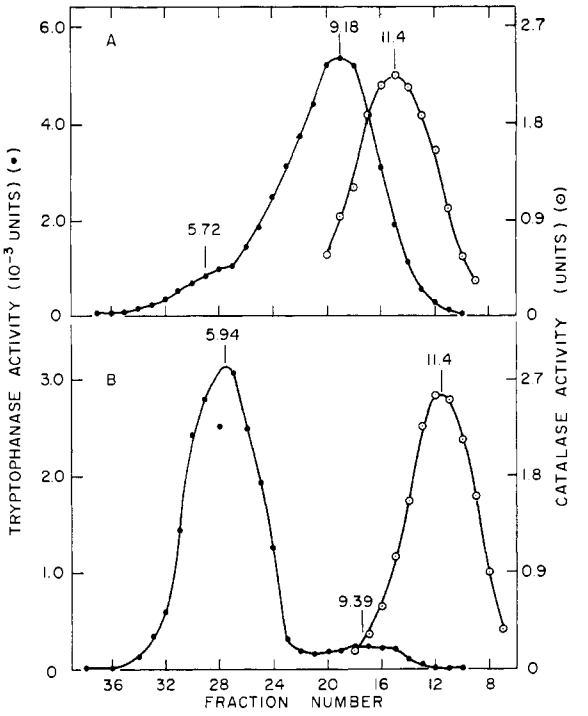

FIGURE 2: Effect of omission or supplementation with excess PLP on the Tris-induced dissociation. Sucrose density gradients were prepared from 5 and 20% sucrose solutions in 50 mM Tris (pH 7.5), containing 1 mM PLP (A) or no PLP (B). The individual sucrose solutions were freshly prepared before making the gradients and 50-μl samples of the purified tryptophanase in 50 mM potassium phosphate buffer (pH 7.0) and containing 0.1 mM PLP were loaded on the gradients. The centrifugations were for 16 hr at 33,000 rpm at 0° in a Spinco Model L ultracentrifuge using a SW 39 rotor. Individual fractions containing 8 drops were collected from each tube. Samples were analyzed for tryptophanase (50 μl) and catalase (10 μl). The tryptophanase assay system contained in a final volume of 0.5 ml, potassium phosphate (pH 7.5) (50 μmoles), tryptophan (4 μmoles), EDTA (0.1 μmole), bovine serum albumin (22 μg), pyridoxal phosphate (25 mμmoles, or more if indicated), and the enzyme. The time of enzymatic incubation and the amount of PLP added depended on the individual experiment. Catalase (Worthington Corp., two-times crystallized) was always included as a marker in the same or parallel gradients. A sedimentation coefficient of 11.4 S for catalase was assumed for calculations. The catalase assay system contained in a final volume of 3.0 ml: 30 μmoles of potassium phosphate (pH 7.5), 18 μmoles of hydrogen peroxide, and the enzyme.

in the present studies using a Bio-Gel P-200 column. The results are presented in Figure 1. The enzyme activity, which had been eluted with maximal activity in fraction 26 in potassium phosphate buffer, was eluted in Tris buffer with three apparent peaks, with the maximal activity in fractions 35–36. On the other hand, the enzyme originally filtered in Tris buffer was eluted with maximal activity in fraction 33 and when transferred to potassium phosphate buffer, gave only one peak of enzyme activity with a maximum in fraction 26. It is evident from these results that the enzyme is dissociated into the smaller particles in Tris and is reaggregated to the native form (9.4 S) in potassium phosphate. However, the loss in activity during dissociation and reaggregation was significant.

The dissociated particle does not possess any enzyme activity, as shown by the absence of activity in these fractions in the presence of tryptophan alone. Although there was some activity in these fractions in the presence of PLP and tryptophan, the activity was much lower than that obtained with tryptophan, PLP, and potassium phosphate. Thus potassium phosphate also showed a beneficial effect in the reaggregation of the enzyme, possibly owing to the presence of potassium.

The Effect of Tris Concentration on Activity and Tris-Induced Dissociation of Tryptophanase. Since the treatment of tryptophanase with Tris resulted in the inhibition of the enzyme activity, the effect of varying the substrate concentration at different concentrations of Tris (pH 7.5) was tested. The results were analyzed by the Lineweaver–Burk graphical method and the Tris inhibition was found to be noncompetitive with respect to tryptophan.

The effect of varying the concentration of Tris in sucrose density gradients was also studied in a series of experiments in which the final concentrations of

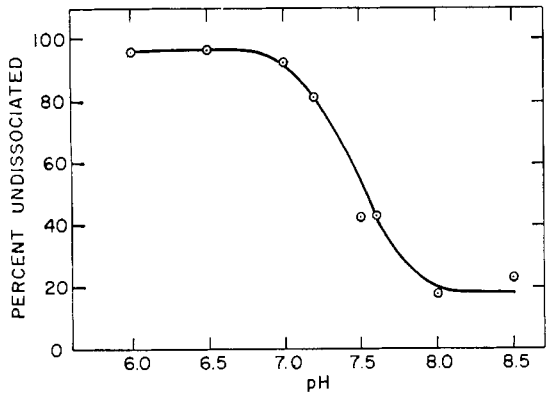

FIGURE 3: Effect of varying the pH of sucrose gradients prepared in 50 mM Tris. Each gradient was prepared in 50 mM Tris at the indicated pH. Larger amounts (50 μl) of the tryptophanase were loaded on the gradients at pH 8.0 and 8.5 than at the other pH values (25 μl).

Tris employed were 5, 10, 50, and 100 mM, respectively. The pH of the gradients were adjusted to 7.5 and all contained 0.1 mM PLP. These studies showed that the dissociation of the enzyme was dependent upon the concentration of Tris. At the lowest concentration of Tris (5 mM), the majority of the enzyme remained as the 9.4S material whereas at 50 mM Tris, there was 60 and 40%, respectively, of 5.6S and 9.4S particles. The Tris effect was reversed by excess concentrations of PLP, even at higher pH values (Figure 2A), whereas the omission of PLP from the gradients, prepared in Tris, lead to almost complete dissociation into the 5.6S subunit (Figure 2B). Raising the concentration of PLP from 0.1 to 1.0 mM in sucrose gradients prepared in 50 mM Tris (pH 7.5) resulted in an increase in the proportion of 9.4S material (Table I).

Effect of pH on Activity and Tris-Induced Dissociation of Tryptophanase. In 50 mM Tris, the effect of varying the pH of the gradient from 6.0 to 8.5 was also examined and the results are summarized in Figure 3. It is evident that the Tris effect is dependent upon pH. At pH 6.0, the majority of the enzyme remained as the 9.4S material, whereas at pH 7.5 almost half of the enzyme was dissociated. At pH 8.5, the enzyme dissociation was even greater and there appeared to be at least four particles with s values estimated to be 9.23, 7.21, 5.90, and 4.80. The estimated peaks contained, respectively, 23, 26, 40, and 11% of the enzyme activity. A similar set of experiments was done using 50 mM potassium phosphate buffer, from pH 6.0 to 8.0 and in all these gradients, the enzyme remained almost completely in the 9.4S form; even at pH 8.5 more than 95% was the 9.4S species. The estimated fraction of undissociated enzyme in the Tris gradient experiment series is plotted as a function of pH (Figure 3). The enzyme is 50% dissociated at about pH 7.5.

Gradients in HEPES and TES. To determine if the

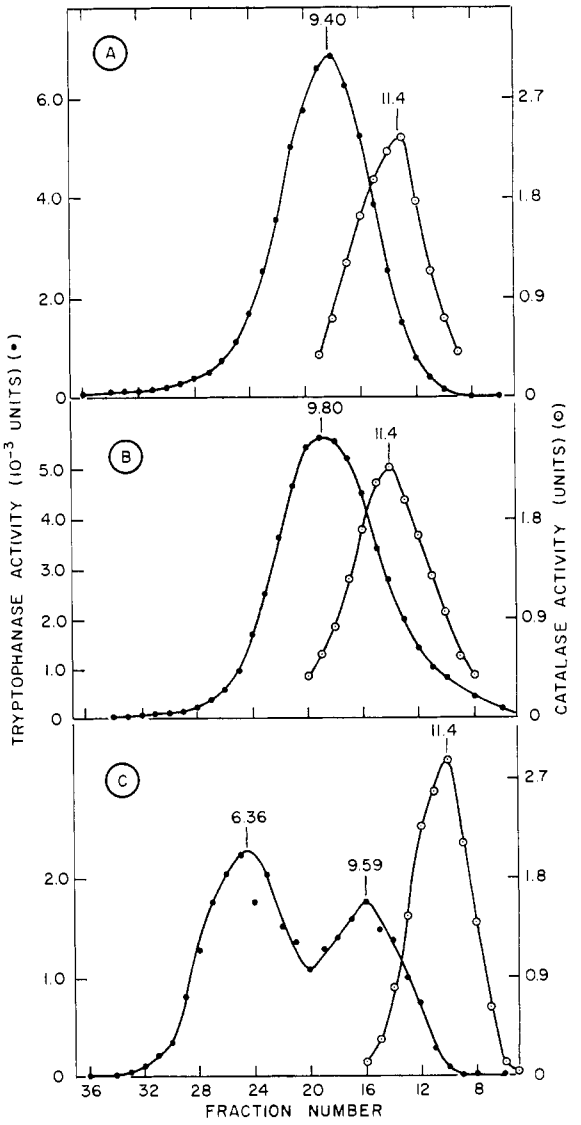

FIGURE 4: Gradient centrifugation in HEPES and TES. Conditions as in Figure 2 except that the individual sucrose solutions were prepared in 50 mM HEPES (A), TES (B), or Tris (C) buffers, all at pH 7.5, and all contained 0.1 mM PLP.

TABLE I: Effect of PLP Concentration on the Tris-Induced Dissociation of Tryptophanase.[a]

PLP (M)	Relative Activity		
	Total	9.4 S	% 9.4 S
1×10^{-4}	0.487	0.249	51.1
2×10^{-4}	0.753	0.491	65.2
5×10^{-4}	1.175	1.108	94.2
1×10^{-3}	1.103	0.952	86.3

[a] Details as described under Figure 5 except that the individual sucrose solutions were prepared in 50 mM Tris (pH 7.5). The PLP concentration was varied as indicated, and cysteine was omitted. The distribution of enzyme activity was plotted and the percentage of 9.4S material was computed from the graphs. The area under each curve was measured using a Model 39231 planimeter (Gelman Instrument Co., Ann Arbor, Mich.).

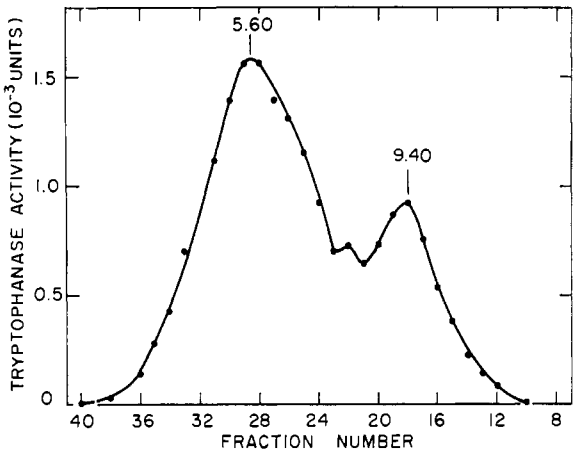

FIGURE 5: The effect of cysteine on tryptophanase dissociation. The sucrose density gradient was prepared from 5 and 20% sucrose solutions in 50 mM potassium phosphate (pH 7.5) containing 0.1 mM PLP and 1 mM cysteine.

dissociation of tryptophanase observed in Tris gradients at pH 7.5 is a specific Tris effect, the sucrose density gradients prepared in other buffers were also studied. The results are presented in Figure 4. It is evident that in HEPES or TES buffers, the enzyme sedimented as the 9.4S material with little dissociation.

Effect of Cysteine, Mercaptoethanol, and Penicillamine on Activity and Dissociation of Tryptophanase. Cysteine was found to effect dissociation of the enzyme when incorporated into the gradients. The enzyme, which

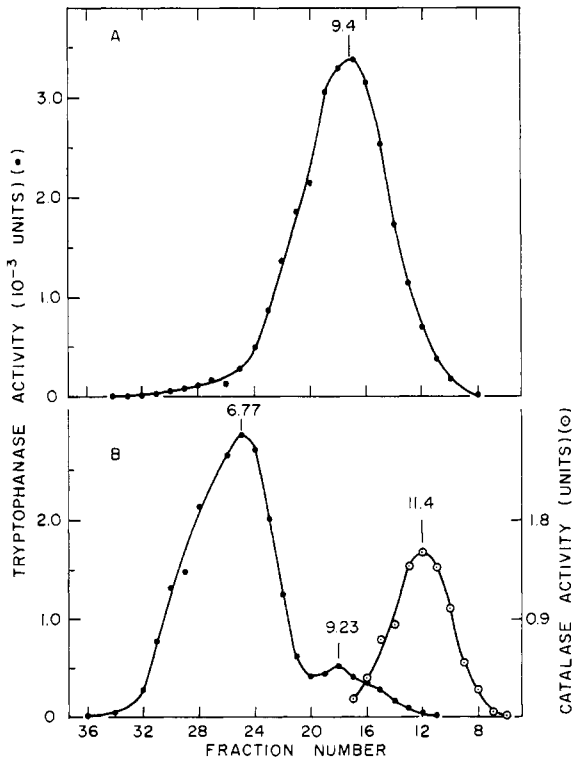

FIGURE 6: The effect of PLP on tryptophanase dissociation. Conditions as in Figure 5 except in A, the gradient contained 5 mM PLP and 1 mM cysteine, and in B, both cysteine and PLP were omitted.

TABLE II: Effect of PLP and Iodoacetate on Cysteine Inhibition of Tryptophanase.[a]

Cysteine Final Concn (mM)	Percentage Activity		
	No PLP + No Iodoacetate	PLP before Iodoacetate	PLP after Iodoacetate
1.0	7.77	38.8	41.6
0.5	18.88	65.2	61.1
0.1	76.3	100	92.0
0.05	92.0	100	100
0.01	92.0	100	100

[a] Samples (50 μl) of purified enzyme (1.25 μg of protein) were incubated with the noted amounts of cysteine for 5 min at room temperature (about 22°), 0.05 ml of 10 mM PLP was added, and the incubation was continued for another 5 min, then followed by the addition of 0.1 ml of 100 mM iodoacetate (pH adjusted to 6.5 with potassium hydroxide). After incubation for an additional 5 min at room temperature, 2 drops of toluene and 0.4 ml of tryptophanase assay mixture (containing a final concentration of 10 mM potassium phosphate (pH 7.5) and 10 mM tryptophan) were added. The reaction mixtures were incubated at 37° for 15 min. The reactions were terminated by addition of 3.0 ml of *p*-dimethylaminobenzaldehyde reagent and the absorbancy of the samples were read at 568 mμ after 20 min. To the samples in column 4, PLP was added after iodoacetate.

normally sediments as the 9.4S material in sucrose gradients prepared in 50 mM potassium phosphate, was dissociated into at least two subunits (Figure 5). The cysteine-induced dissociation suggests two possible mechanisms, *viz.*, the removal of PLP by cysteine, or the reduction of some disulfide bonds by cysteine leading to the dissociation of the enzyme, or both. The incorporation of cysteine into the gradients resulted in the disappearance of the yellow color of PLP suggesting formation of a thiazolidine with PLP (Heyl *et al.*, 1948). If that is the case, the cysteine-induced dissociation should be overcome by an excess of PLP or, conversely, omission of PLP from the gradients prepared in potassium phosphate, even in the absence of cysteine should lead to dissociation of the enzyme. This was actually found to be the case, as evidenced from Figure 6.

The hypothesis was also tested by the following experiment. The enzyme preparation, after treatment with cysteine, was incubated with iodoacetate, at a final concentration of 50 mM, to carboxymethylate any newly formed sulfhydryl groups. Subsequently, PLP was added to achieve reaggregation. As shown in Table II, the dissociation induced by cysteine resulted in inhibition of enzyme activity, and was completely reversed by excess PLP whether iodoacetate had been added before or after the treatment with PLP. The

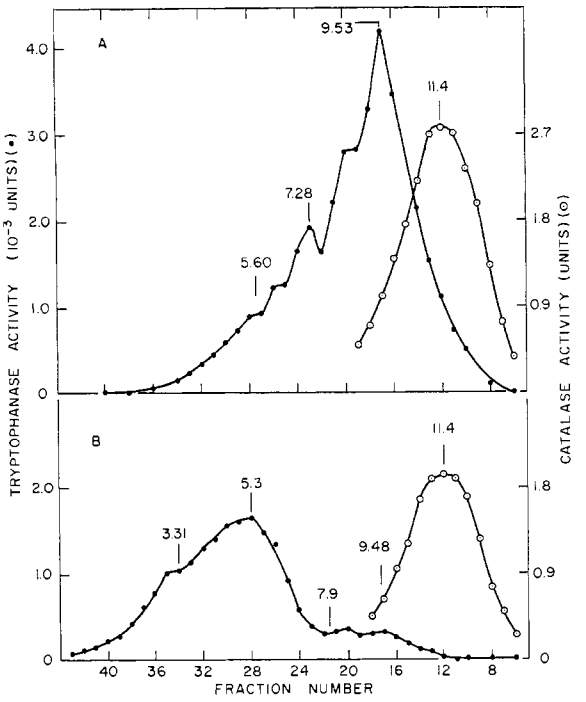

FIGURE 7: Effect of L-penicillamine on tryptophanase dissociation. Conditions as in Figure 5 except that the gradients contained 0.1 mM L-penicillamine (A) and 10 mM L-penicillamine (B). The values given on the curves are calculated sedimentation constants for the positions indicated.

results suggest that the cysteine treatment does not involve the reduction of any disulfide groups which may be required for enzyme activity.

Mercaptoethanol, when incorporated into the gradients up to a final concentration of 1 mM (tenfold greater than the concentration of PLP) did not lead to the dissociation of the enzyme.

On the other hand, L-penicillamine, which forms a thiazolidine with PLP, also leads to the dissociation of the enzyme, when included in the gradients. At a final concentration of 0.1 mM (equal to that of PLP), the enzyme was partially dissociated, whereas at 1 mM there was almost complete dissociation (Figure 7).

The treatment of the enzyme with sulfhydryl-reactive agents such as PMB, NEM, or iodoacetate, at a final concentration of 1 mM, showed absolutely no inhibition of the enzyme activity, indicating the absence of involvement of free SH groups in enzyme activity.

Protection against the Tris-Induced Dissociation of Tryptophanase. The effect of certain amino acids and other related compounds on the Tris-induced dissociation of tryptophanase was tested. The compounds were incorporated into a final concentration of 1 mM in each of the 5 and 20% sucrose solutions in Tris or potassium phosphate (50 mM) used for the preparation of gradients. Only anthranilate showed an effect whereas L-serine, L-threonine, L-phenylalanine, D-tryptophan, tryptamine, L-histidine, and DL-kynurenine exerted no effect.

The effect of incorporating anthranilate into sucrose gradients is presented in Figure 8. It is clear that anthranilate, a competitive inhibitor of tryptophan degradation, at a final concentration of 1 mM, affords complete protection of the enzyme from dissociation in Tris.

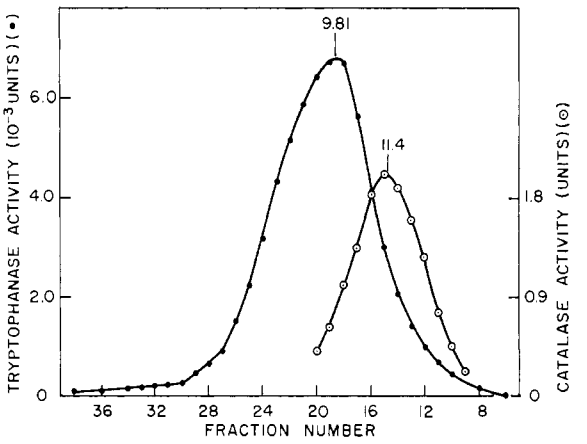

FIGURE 8: The protective effect of anthranilate against the Tris-induced dissociation of tryptophanase. Conditions as in Figure 2 except that the sucrose solutions contained 1 mM anthranilate and 0.1 mM PLP.

Incorporation of urea, at concentrations greater than 1.0 M, into the sucrose gradients, resulted in the complete and irrecoverable loss of enzyme activity. At a final concentration of 0.1 mM sodium dodecyl sulfate, the enzyme sedimented as the native 9.4S material.

Effect of pH and Buffer Composition on Activity of Tryptophanase. Because of the dissociation of tryptophanase observed in Tris buffer, the activity of the enzyme as

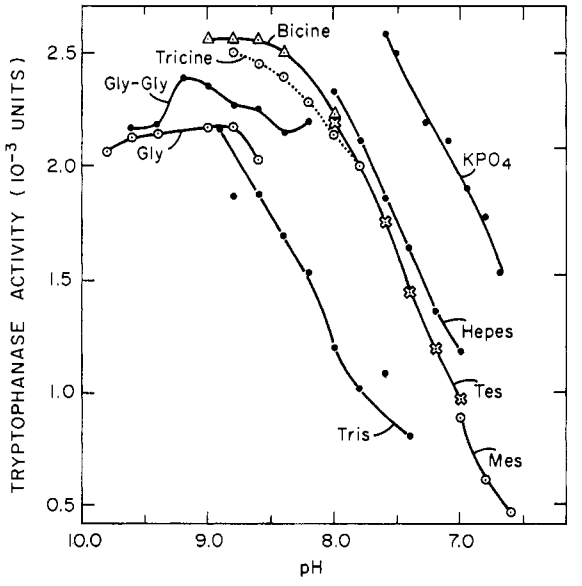

FIGURE 9: Effect of pH on tryptophanase activity. The assay system in a final volume of 0.5 ml contained 25 μl (2.15 μg of protein) of purified tryptophanase, 20 μmoles of the appropriate buffer, 4 μmoles of tryptophan, 0.8 μmole of PLP, and 3 drops of toluene. The enzyme incubations were for 15 min at 37° and the reactions were terminated with 3.0 ml of *p*-dimethylaminobenzaldehyde reagent. The absorbancies of the samples were read at 568 mμ after 20 min.

a function of pH was tested in a variety of buffers. The activity patterns observed in nine of the buffers tested are shown in Figure 9. The relatively high activity observed in Tris was due to the presence of a large excess of PLP in the reaction mixture. The relatively low activity in HEPES, TES, and MES buffers in comparison with potassium phosphate may be a consequence of potassium deficiency. The enzyme is much more stable in buffers around pH 7.0 than at higher pH values as demonstrated by storage or gradient centrifugation studies.

Discussion

The rapid and essentially quantitative removal of PLP from serine transhydroxymethylase by thiazolidine formation with cysteine has been used previously for the resolution as well as determination of PLP bound to the enzyme (Schirch and Mason, 1962, 1963). L-penicillamine, which also combines with PLP, brings about the same effect on tryptophanase as does cysteine and this effect also was reversed by excess concentrations of PLP, as evidenced by the sedimentation behavior of the enzyme in sucrose density gradients. Incorporation of mercaptoethanol into these gradients had no effect on the sedimentation behavior of the enzyme, either as an inducer or as an inhibitor of dissociation. The involvement of mercaptoethanol in the formation of a new absorption band when the α and β_2 subunits of tryptophan synthetase are associated in presence of PLP and serine has been reported by Goldberg and Baldwin (1967). The behavior of tryptophanase with respect to cysteine and mercaptoethanol suggests that no accessible sulfhydryl or disulfide bonds are important in enzyme activity.

Since the present results indicated that the dissociation of tryptophanase could be achieved by removal of PLP, the mechanism of dissociation observed in Tris buffer was examined in greater detail. The interaction of Tris with PLP to form a Schiff base was reported by Matsuo (1957) and Tris was shown to inhibit the activation of homoserine dehydrogenase by PLP (Matsuo, 1957). In the present studies it was shown that Tris noncompetitively inhibits tryptophanase. The Tris-induced dissociation was found to be dependent on the concentrations of both Tris and PLP in the gradients and upon pH.

The data of Table I, which represent the dissociation of tryptophanase as a function of PLP concentration in Tris buffer, may be used to support the values published earlier (Hoch and DeMoss, 1966) for the dissociation constant of the enzyme–PLP reaction. For the kinetic arguments used (Hoch and DeMoss, 1966), K_1^a was the dissociation constant for the reaction, E(PLP) $\rightleftarrows$ E + (PLP), where E is enzyme, and was estimated to be 14.4 μM. K_2^a, the dissociation for the reaction, $E(PLP)_2 \rightleftarrows E(PLP) + (PLP)$, was estimated to be 1.14 μM. At PLP concentrations from 10^{-4} to 10^{-3} M, the amount of E(PLP) is 1.0–0.1% of the amount of $E(PLP)_2$ and all enzyme which contains PLP can be considered to be in the form of $E(PLP)_2$. The over-all dissociation constant, K_0^a, for the reaction $E(PLP)_2 \rightleftarrows E + 2(PLP)$, is $K_1^a K_2^a = K_0^a$, *i.e.*, $K_0^a = 16.4\ (\mu M)^2$. Thus, the enzyme is 50% in the dissociated form at 4.05×10^{-6} M free PLP. The dissociation constant for the reaction of Tris with PLP, Tris–PLP $\rightleftarrows$ Tris + PLP, K_t = (Tris)(PLP)/(Tris–PLP), may be calculated from these data (Table I). The enzyme is half in the dissociated form at 8.28×10^{-5} M PLP total concentration or 4.05×10^{-6} M unbound PLP. Since the amount of enzyme-bound PLP is negligible in comparison with the total PLP

$$K_t = \frac{(5 \times 10^{-2})(4.05 \times 10^{-6})}{(8.28 \times 10^{-5} - 0.405 \times 10^{-5})} = 2.57 \times 10^{-3}\ \text{M}$$

From the data of Matuso (1957), the comparable value of 8.28×10^{-3} M may be calculated for K_t. The similar value of 6.64×10^{-3} M for the Tris–PLP dissociation constant was computed using the data from sucrose gradient analyses in which the Tris concentration was varied from 5 to 100 mM in the presence of 10^{-4} M PLP. In view of the errors inherent in the sucrose gradient analyses, the differences are probably not significant.

The Tris-induced dissociation of enzyme was shown to be dependent upon pH (Figure 3). An apparent pK, corresponding to 50% dissociation of the enzyme, occurs at about pH 7.5. This pH does not correspond to any of the pK's reported for PLP (Williams and Neilands, 1954), *i.e.*, $pK_1 = <2.5$, primary ionization of phosphate ester group; $pK_2 = 4.14$, ionization of phenolic hydroxy group; $pK_3 = 6.20$, secondary ionization of phosphate ester group; $pK_4 = 8.69$, ionization of pyridinium nitrogen, and appears too far removed to correspond to the Tris pK of 8.10. In unpublished experiments, involving spectral studies, we find no indication of a pK near pH 7.5 for the Tris–PLP Schiff base. Thus, the apparent pK of 7.5 would seem to apply to an ionizable group on the enzyme which affects the binding of PLP.

An important finding was that the anthranilate afforded complete protection from dissociation in Tris gradients, so that the enzyme sedimented as the undissociated 9.4S species. Anthranilate has been shown to be a competitive inhibitor of the tryptophanase reaction and has a lower K_i value than the K_m for tryptophan (Hoch *et al.*, 1966). The binding of anthranilate to the enzyme may thus be leading to a structural modification such that at least one of the PLP molecules on the enzyme is no longer available to react with Tris. The protective effect of anthranilate is under further study. In contrast, kynurenine, another competitive inhibitor of tryptophanase reaction, did not show any protective effect on Tris-induced dissociation. None of the other compounds tested afforded any protection from Tris-induced dissociation, including L-serine, which is another substrate for tryptophanase, or D-tryptophan.

Tryptophanase from *B. alvei* differs from that of *Escherichia coli* in some of its biochemical properties (Hoch *et al.*, 1966; Hoch and DeMoss, 1966). One

important difference between the enzymes from the two sources is that the *B. alvei* tryptophanase contains only 2 moles of PLP/mole of enzyme whereas the *E. coli* enzyme contains 4 moles of PLP/mole, although both have a molecular weight of approximately 220,000. The *E. coli* enzyme is a tetramer and dissociation of the enzyme was achieved by guanidine hydrochloride, urea, sodium dodecyl sulfate, or even K^+ or Na^+ ions (Morino and Snell, 1967a). The removal of PLP apparently does not necessarily lead to the dissociation of the enzyme and in fact tryptophanase from *E. coli* is crystallized much more easily as the apoenzyme after the removal of PLP (Newton *et al.*, 1965). In contrast, the *B. alvei* enzyme dissociates into subunits, when made free of PLP, particularly when the enzyme preparations are dilute. Based on the evidence obtained from the sucrose gradients, it appears that the *B. alvei* tryptophanase also may be composed of four subunits, the active holoenzyme being the particle sedimenting as the 9.4S material. PLP may be involved in the binding of subunits. Thus PLP may have a function other than in the catalytic activity of the enzyme. Since the *B. alvei* enzyme has only 2 moles of PLP/mole of enzyme, if one PLP has a strictly structural function, then the enzyme would possess only one catalytic site for tryptophanase activity. This speculation is consistent with the earlier observation that although no cross-precipitating antibodies are elicited by the *B. alvei* and *E. coli* tryptophanases (Hoch *et al.*, 1966), common antigenic sites, possibly the enzyme active sites, do exist on the two enzymes as judged by enzyme activity neutralization experiments (unpublished experiments).

After this report was prepared, additional data were published by Morino and Snell (1967d,e), derived from experiments with tryptophanase from *E. coli*.

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