*REVERSIBLE DISSOCIATION OF TRYPTOPHANASE INTO PROTEIN SUBUNITS**

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Communicated by S. Spiegelman, August 3, 1966

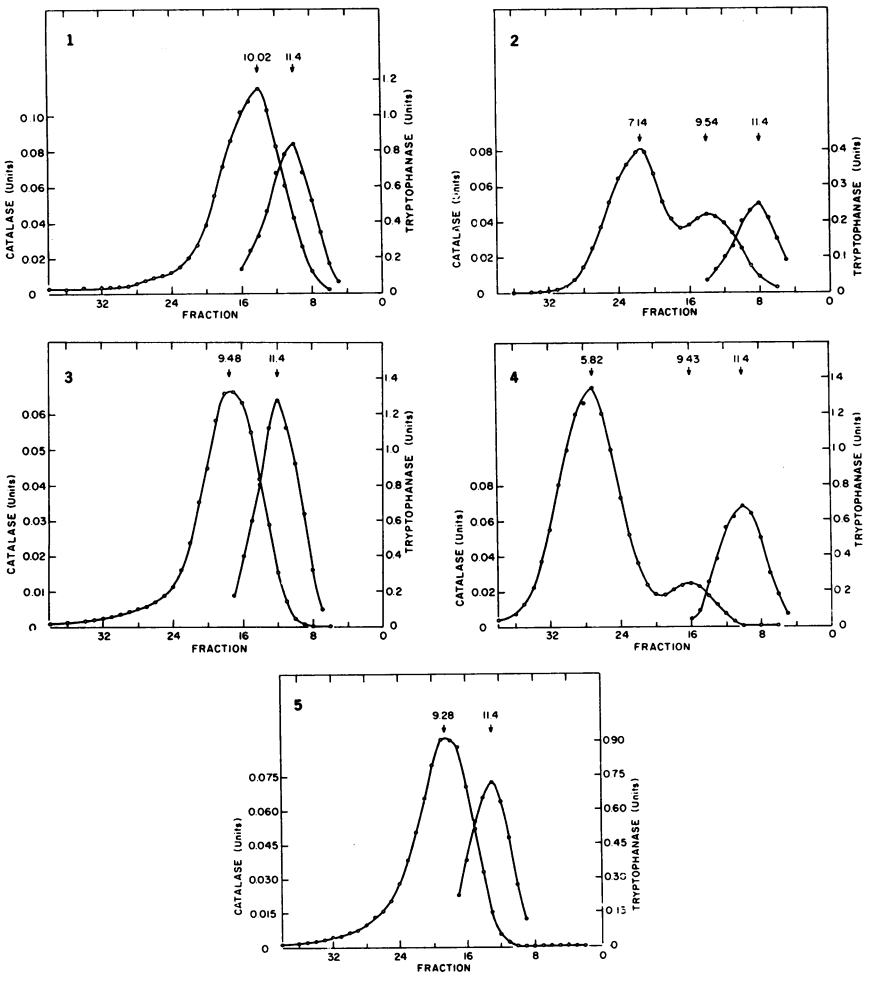
Crystalline tryptophanase from *Escherichia coli* strain B/1t7-A has been shown to behave as a single entity in the analytical ultracentrifuge with an $S_{20,\,w}$ value of 9.65, whereas sucrose density gradient centrifugation of crude extracts of the organism showed that tryptophanase sedimented predominantly as a particle with $S_{20,\,w}$ value of 6.85.[1] Since the crystalline enzyme was isolated in 60 per cent yield, it was suggested that two species of the enzyme coexist in crude extracts and are interconvertible.[1] The sedimentation constant of a purified tryptophanase preparation from *E. coli* T3, a tryptophan auxotroph of *E. coli* K12, was determined[2] to be 9.0*S*. Crude extracts of *E. coli* T3 also gave two peaks with sedimentation values 9.4*S* (minor peak) and 6.9*S* (major peak) by the sucrose density gradient method.[3] A purified tryptophanase from *Bacillus alvei* has been shown[4] to behave as a single peak in the analytical ultracentrifuge; the sedimentation coefficient was calculated to be 10.3*S* by extrapolation to infinite dilution.

All of the sedimentation results cited above can be explained consistently by the observations reported herein. We describe some of our findings on the behavior of tryptophanases from *E. coli* T3 and *B. alvei*.

Methods.—The cell-free extracts, prepared from *E. coli* by sonic disintegration, and the purified enzyme from *B. alvei*[4] were used as enzyme sources. The sucrose density gradient centrifugation studies were carried out essentially as described by Martin and Ames,[5] except that 50 mM potassium phosphate buffer was used in place of 50 mM Tris-Cl wherever indicated. Pyridoxal phosphate was also included in all the gradients to a final concentration of 40 μM. Magnesium acetate was included (0.1 mM) wherever mentioned. Catalase (Worthington Biochemical Corp., N. J.) was used as the reference standard. It was established in separate experiments in the analytical ultracentrifuge that the sedimentation constant of catalase (11.4*S*) was the same in either 50 mM potassium phosphate buffer or 50 mM Tris-Cl buffer. The tryptophanase and catalase activities were determined as described by Feiss and DeMoss,[3] and Martin and Ames,[5] respectively.

Results.—The purified enzyme from *B. alvei* sedimented as two peaks, a major peak with sedimentation constant 5.5*S* and a minor peak at 9.4*S* in a sucrose density gradient, prepared in 50 mM Tris-Cl. However, when potassium phosphate was used in place of Tris-Cl, the enzyme tended to aggregate predominantly to a larger particle with the sedimentation value of 9.4. The sedimentation behavior of the enzyme from *E. coli* also was examined in Tris-Cl and in potassium phosphate buffers, and the results with both enzymes are shown in Figures 1–4. The sedimentation constants obtained for the enzymes from *E. coli* and *B. alvei* in a series of experiments are presented in Table 1. The presence of Mg^{++} had no significant influence on the sedimentation constants observed.

A sample of the enzyme used for the sedimentation experiment shown in Figure 3 was dialyzed against Tris-Cl buffer. When the Tris-Cl enzyme was sedimented



FIGS. 1 and 2.—The enzyme from *E. coli* in 50 mM potassium phosphate and 50 mM Tris-Cl buffers, respectively.

FIGS. 3–5.—The enzyme from *B. alvei* in 50 mM potassium phosphate, 50 mM Tris-Cl, and 50 mM potassium phosphate buffers, respectively.

Distribution of tryptophanase and catalase activities in sucrose density gradients. The sucrose density gradients were prepared as described by Martin and Ames.[5] The centrifugations were carried out for 16–17 hr at 33,000 rpm in a Spinco model L ultracentrifuge using an SW39 AN rotor, at 0°. Eight drops each were collected in individual fractions, 50-μl samples were analyzed for tryptophanase, and 10-μl samples for catalase activities.

The tryptophanase assay system contained, in a final volume of 0.5 ml, the following substances: potassium phosphate (pH 7.5), 10 μmoles; tryptophan (4 μmoles); pyridoxal phosphate (22 mμmoles); EDTA (0.1 μmole); bovine serum albumin (22 μg); and the enzyme. The enzymatic reactions were incubated for 30 min at 37°. The reactions were terminated by addition of 3.0 ml of the p-dimethylaminobenzaldehyde reagent. The tryptophanase unit is defined as the amount needed to cause a change in optical density of 1.00 per 30 min at 568 mμ and corresponds to 2.85×10^{-3} international enzyme units.

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. The catalase unit is defined as the amount needed to cause a change in optical density of 1.00 per 2 min at 240 mμ and corresponds to 25 international enzyme units. O—O Tryptophanase; ●—● catalase.

TABLE 1

SEDIMENTATION CONSTANTS OF TRYPTOPHANASE IN POTASSIUM PHOSPHATE AND TRIS BUFFERS

Source of enzyme	Potassium Phosphate		Tris			
			Without Mg^{++}		With Mg^{++}	
	Without Mg^{++}	With Mg^{++}	Major peak	Minor peak	Major peak	Minor peak
E. coli	10.09	9.98	7.15	9.70	6.56	9.67
	10.02	9.82	7.15	9.70	7.14	9.54
	...	...	7.32	9.87	...	...
Mean	10.06	9.90	7.20	9.76	6.85	9.61
B. alvei	9.06	9.33	5.77	9.35	5.12	9.42
	9.11	9.62	5.41	9.49	5.82	9.43
	...	9.28	5.58	9.06	5.21	9.45
Mean	9.09	9.41	5.59	9.30	5.38	9.43

The sucrose density gradients were prepared as described by Martin and Ames,[5] in 50 mM Tris-Cl or 50 mM potassium phosphate buffer, pH 7.5, as indicated. All of the gradients contained pyridoxal phosphate (40 μM); magnesium acetate (0.1 mM) was present wherever mentioned. Catalase was included as the standard (11.4*S*). Other experimental conditions were as described under Figs. 1–5.

through a Tris-Cl sucrose gradient, the results shown in Figure 4 were obtained. The enzyme was largely disaggregated upon replacing potassium phosphate by Tris-Cl. When the Tris-Cl enzyme was sedimented through a potassium phosphate sucrose gradient, the results were essentially the same as in Figure 4, and only a slight tendency to reaggregate was noted. Dialysis of the Tris-Cl enzyme against potassium phosphate buffer resulted in nearly complete reaggregation. Sedimentation of the latter enzyme yielded the results shown in Figure 5.

It is evident from these data that the enzyme exists primarily as the smaller particle in Tris-Cl buffer, whereas the larger component is the predominant form in potassium phosphate buffer. Moreover, these findings give direct evidence for the interconvertibility of the two forms. All of the previously published sedimentation constants cited above were derived from experiments with potassium phosphate buffer where the analytical ultracentrifuge was used, and with Tris-Cl buffer where sucrose density gradients were used.

It is known that Tris inhibits the enzymatic activity of tryptophanase.[6] We conclude that the smaller particle (about 6*S*), observed in the experiments with both *E. coli* and *B. alvei*, is enzymatically inactive. The small particle can be located in the sucrose gradient, however, because the enzyme assay contains potassium phosphate buffer which encourages the formation of the larger (about 9.5*S*) enzymatically active particle.

Summary.—Tryptophanase is dissociable into enzymatically inactive protein subunits. The subunits are half the size of the active enzyme, as deduced from their behavior in sucrose density gradient sedimentation experiments.

Dissociation of tryptophanase is facilitated by Tris; aggregation to the active enzyme is encouraged by potassium phosphate.

* This work was supported in part by a research grant (AI-2971) from the National Institute of Allergy and Infectious Diseases.

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