[2] M. Beljanski and S. Ochoa, these PROCEEDINGS, 44, 494, 1958.
[3] M. B. Hoagland, E. B. Keller, and P. C. Zamecnik, *J. Biol. Chem.*, 218, 345, 1956.
[4] J. E. Snoke and K. Bloch, *J. Biol. Chem.*, 213, 825, 1955.
[5] E. B. Keller and P. C. Zamecnik, *J. Biol. Chem.*, 221, 45, 1956.
[6] H. A. Krebs and R. Hems, *Biochim. et Biophys. Acta*, 12, 172, 1953.
[7] J. A. De Moss and G. D. Novelli, *Biochim. et Biophys. Acta*, 22, 49, 1956.
[8] S. Kaufman, *J. Biol. Chem.*, 216, 153, 1955.

ON THE SEPARATION OF THE TRYPTOPHAN SYNTHETASE OF ESCHERICHIA COLI INTO TWO PROTEIN COMPONENTS*

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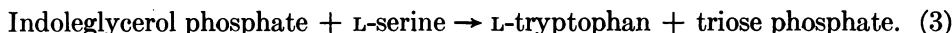
INTRODUCTION

Tryptophan synthetase (TSase), the enzyme catalyzing reaction (1),

$$\text{Indole} + \text{L-serine} \rightarrow \text{L-tryptophan}, \quad (1)$$

has been demonstrated in a variety of micro-organisms.[1] Recent studies with partially purified TSase preparations from *Neurospora crassa* have shown that this enzyme also acts on indoleglycerol phosphate (InGP), a precursor of tryptophan, according to reactions (2) and (3).[2]

$$\text{Indoleglycerol phosphate} \rightleftharpoons \text{indole} + \text{triose phosphate}, \quad (2)$$



$$\text{Indoleglycerol phosphate} + \text{L-serine} \rightarrow \text{L-tryptophan} + \text{triose phosphate}. \quad (3)$$

These studies further indicated that reaction (3) rather than reactions (1) plus (2) probably represents the principal physiological mechanism of tryptophan synthesis in *N. crassa*, since evidence was obtained excluding free indole as an intermediate in this reaction.

Investigations with *Escherichia coli* have shown that two proteins, designated components A and B, are required for the catalysis of reaction (2) and that one of these, component B, is also involved in reaction (1).[3] In view of these findings and the recent observations with preparations from *N. crassa*, a study of reactions (1), (2), and (3) in extracts of *E. coli* was undertaken. It was soon found that the same two separable protein components were required for the catalysis of all three reactions. This paper is concerned with the demonstration of the separability of these two proteins and with certain unusual features of their interaction.

MATERIALS AND METHODS

Organisms.—Two tryptophan auxotrophs of *E. coli* K-12 were used as enzyme sources, strains T-3 and T-8. Mutant T-3 is blocked in the synthesis of anthranilic acid and forms large amounts of TSase when grown on low levels of anthranilic acid.[4] It was considered equivalent to wild-type for the purposes of this study. Mutant T-8 responds to indole or tryptophan but not to anthranilic acid and

accumulates indoleglycerol in its culture filtrates.[4] Extracts of this strain cannot convert InGP to indole or to tryptophan.

Enzyme Assays.—Reaction 1 (indole + serine → tryptophan) was measured as described previously.[3] Indole disappearance was determined from a reaction mixture containing indole, serine, pyridoxal phosphate, sodium chloride, and tris buffer at pH 7.8. Indole disappearance under these conditions was accompanied by the formation of equimolar amounts of tryptophan. One unit of activity corresponds to the conversion of 0.1 μ *M* of indole to tryptophan in 20 minutes at 37° C. under standard assay conditions. Specific activity is expressed as units of activity per milligram protein. Protein was determined by the method of Lowry *et al.*[5]

The conversion of InGP to tryptophan was carried out in a reaction mixture identical with the above except that InGP was substituted for indole. The extent of the reaction was determined by measuring the tryptophan content of a perchloric acid deproteinized sample of the incubation mixture. Tryptophan was determined microbiologically using strain td_4, an auxotroph of *E. coli* K-12 which responds to L-tryptophan specifically.[6] One unit of activity corresponds to the conversion of 0.1 μ *M* of InGP to tryptophan in 20 minutes at 37° C. under standard assay conditions.

Since, as will be shown presently, two protein components, A and B, are involved in the reactions described above, procedures were devised for quantitatively measuring each component. Component A assays were performed as described above with the addition of a three-fold or greater excess of component B, and component B assays were performed in the presence of a three-fold or greater excess of component A. The activity detected in either case was expressed as units of component A (assayed with excess B) or units of component B (assayed with excess A), where the unit again refers to the conversion of 0.1 μ *M* of substrate to product in 20 minutes at 37° C. Partially purified component A, free of component B activity, was used for the assay of component B, while T-8 preparations served as a source of component B for the assay of component A. A small correction was necessary in component A assays when indole → tryptophan activity was measured, since T-8 preparations exhibit a slight amount of indole → tryptophan activity in the absence of component A. This activity is increased twenty to thirty fold, however, in the presence of component A.

Partial Purification of TSase.—All operations were performed at 0–6° C. Extracts of *E. coli* T-3 were prepared by disrupting lyophilized cells suspended in 0.1 *M* potassium phosphate buffer, pH 7.8, with No. 130 Superbrite glass beads in a Nossal vibrator. After centrifugation for 30 minutes at 20,000 × *g*. to remove debris, these extracts were heated at 58° C. for 3 minutes and the denatured protein removed by centrifugation. Nucleic acids were precipitated by the addition of one-half volume of 20 per cent streptomycin sulfate. The supernatant solution was then brought to pH 6.2–6.3 by the addition of 1 *M* sodium acetate buffer, pH 4.5. Proteins precipitating from this solution between 33 and 43 per cent saturation with ammonium sulfate were collected by centrifugation and redissolved in column buffer. The column buffer was 0.01 *M* potassium phosphate at pH 7.0, generally supplemented with pyridoxal phosphate and reduced glutathione at final concentrations of 2×10^{-5} *M* and 10^{-4}*M*, respectively. The

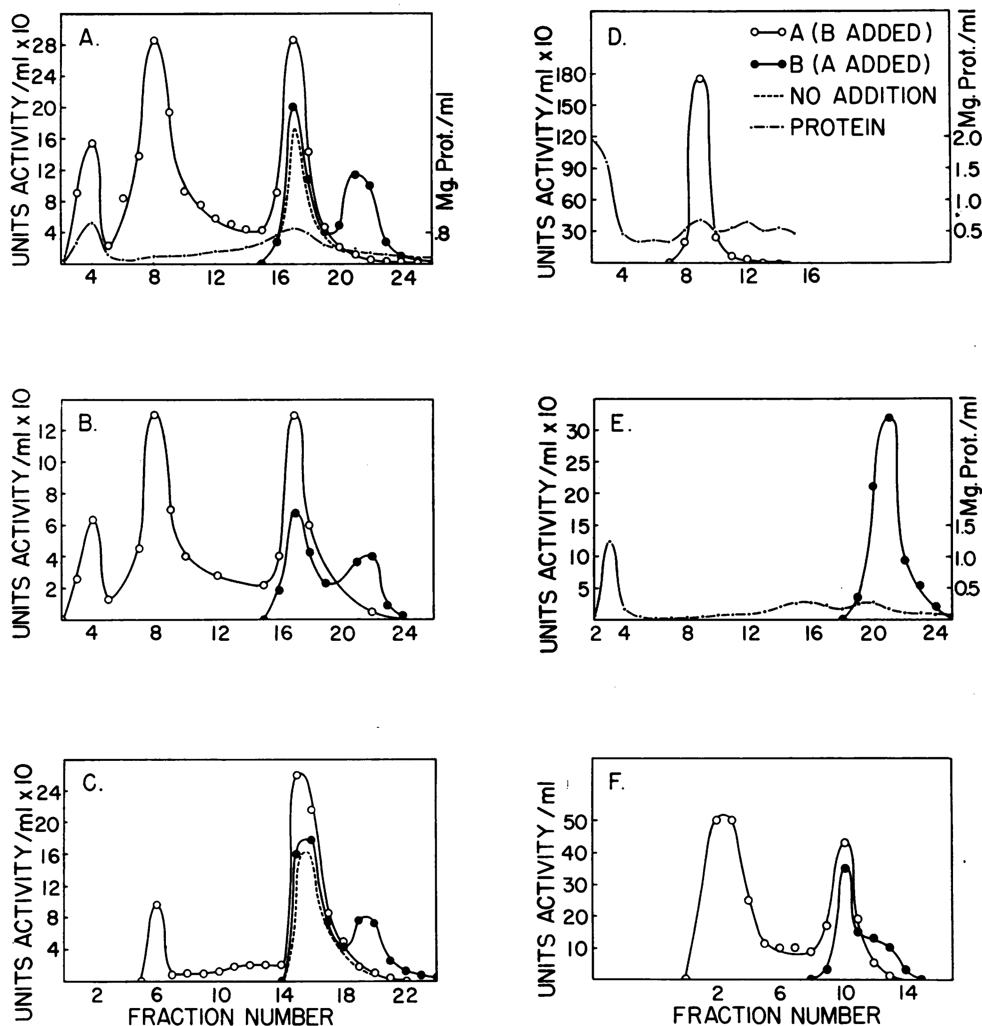


FIG. 1.—Chromatography of partially purified *E. coli* preparations on diethylaminoethyl cellulose. (*A*) T-3 TSase assayed by indole → tryptophan. (*B*) T-3 TSase assayed by InGP → tryptophan. (*C*) Rechromatography of center (combined) peak fractions. (*D*) Partially purified T-3 component A. (*E*) Partially purified T-8 component B. (*F*) Mixture of chromatographically purified T-3 component A and T-8 component B. *C–F* assayed by indole → tryptophan. For additional explanation see text.

enzyme preparation was dialyzed for 1–2 hours against 50 volumes of column buffer. The next step in the purification procedure involved chromatography on diethylaminoethyl cellulose.[7, 8] The diethylaminoethyl cellulose employed was washed and suspended in 0.01 *M* potassium phosphate buffer at pH 7. When formed into a column of the desired diameter and at least 20 cm. in height, it was washed with about 2 volumes of column buffer. The dialyzed enzyme sample was then added to the column and washed on with one-half column volume of buffer. The amount of protein placed on a column never exceeded 2 mg./cc. of cellulose. Elution was accomplished by a gradient system employing approximately five times the column volume of supplemented buffer in the mixing bottle

and the same buffer made 0.5 M with respect to sodium chloride in the inlet bottle. The rate of elution was 1 column volume every 2–10 hours.

RESULTS

Chromatography of TSase.—The distribution of protein and enzymatic activities obtained in a typical chromatogram is shown in Figure 1 *A*. Assays of indole → tryptophan activity were performed in the presence of added component A or component B or without the addition of either. It can be seen from the curves obtained that there is one peak of activity (tubes 16–20) in the absence of added component A or B. When components A and B were assayed separately, however, component B exhibited a bimodal distribution, while three peaks of component A activity were observed. The recovery of indole → tryptophan activity in the fractions from this experiment was only 52 per cent. The major reason for this apparent loss of activity was obviously the separation of components A and B. The recoveries of these two components were 96 per cent (A) and 80 per cent (B). When these values were taken into consideration, most of the indole → tryptophan activity was actually recovered.

The distribution of component A activity in Figure 1, *A*, is clearly triphasic. In a number of similar experiments the appearance of the first small peak of A activity was quite variable. More constant features of the chromatography were the appearance of the bulk of component A at the relative position of the second or largest "A" peak (around fraction 8) and the appearance of less, but appreciable, amounts of A activity in the fractions separating this peak from the fractions exhibiting both A and B activity. The early minor peak of component A activity parallels a peak of protein-reacting material, some of which may be streptomycin. The behavior of component A under certain other conditions suggests that both early A fractions may be identical. However, the possibility that two different proteins may exhibit component A activity has not been excluded.

Tryptophan Formation from InGP.—Figure 1, *B*, shows the distribution of component A and B activities in the same chromatogram as Figure 1, *A*, when tryptophan formation from InGP is measured (reaction [3]). Clearly, the same protein pair is responsible for both reactions. Assays performed on fractions from a similar chromatogram indicate that the separation of the components required for the conversion of InGP to indole also follows the same pattern as that observed when reactions (1) and (3) are measured. It appears from these findings that all three reactions are catalyzed by the same pair of proteins.

Rechromatography of TSase-containing Fractions.—The appearance of both separate and combined peaks of components A and B on diethylaminoethyl cellulose chromatograms suggested that there was an association of the two proteins under the elution conditions employed. To examine this association further, fractions comprising the center or combined peak of the TSase chromatogram were concentrated by ammonium sulfate precipitation and rechromatographed under standard conditions. The distribution of activities obtained is shown in Figure 1, *C*. It is clear that appreciable amounts of free A and B separated from the combined peak fractions; thus the essential characteristics of the first chromatogram were duplicated, suggesting that the center peak represents an AB association.

Chromatography of a Mixture of Purified Components A and B.—As a further

test of AB association, a chromatogram was performed with a mixture of purified components A and B. For this purpose, modifications of the purification procedure described previously were utilized which yielded workable amounts of chromatographically homogeneous A and B. With component A the modification consisted of substituting a pH precipitation step for the ammonium sulfate precipitation in the purification procedure detailed above. Lowering the pH to 3.9 by the addition of 1 *M* sodium acetate buffer, pH 3.0, resulted in the precipitation of component B along with other contaminating proteins. After thorough dialysis, chromatography of the supernatant solution yielded the distribution of proteins and component A activity shown in Figure 1, *D*. Extracts of the mutant T-8 were used as a source of component B, since, as mentioned previously, T-8 preparations exhibit little or no A activity under the assay conditions employed. The purification procedure paralleled that used for T-3 TSase except for the ammonium sulfate step. With T-8 preparations those proteins precipitating between 0 and 33 per cent of saturation were carried on to constitute the column charge. A chromatogram of this partially purified component B is illustrated in Figure 1, *E*. When fractions containing chromatographically homogeneous A and B so obtained were combined and used as a column charge, the distribution of activities shown in Figure 1, *F*, was obtained. Even though an excess of component A was present, the similarity to Figure 1, *A* and *C*, is readily apparent. It is possible to obtain greater separation of components A and B from combined peak fractions and from mixtures if the protein solution is diluted before addition to the column and if the elution rate is decreased. However, conditions have not yet been found which give complete separation of A and B on diethylaminoethyl cellulose columns.

Recovery of A from Fractions Containing Excess B.—Although all the early chromatographic fractions rich in component A are incapable of converting indole to tryptophan, all the purified component B fractions obtained to date exhibit low levels of this activity. The level of activity observed with the best chromatographically purified T-3 and T-8 component B fractions is generally 3–5 per cent of that obtainable on the addition of component A. Experiments involving pH 4.0 precipitation were performed with such preparations in an attempt to determine whether or not the low level of activity detected was due to small amounts of contaminating component A. At least half the "inherent" indole to tryptophan activity of a chromatographically purified T-3 component B fraction could be accounted for in this manner, while no acid-stable material exhibiting component A activity could be recovered from T-8 component B fractions. Further studies are required before it can be established whether or not contaminating component A (probably an altered A in the case of T-8, since T-8 fractions show no InGP → tryptophan activity) is responsible for the low level of indole → tryptophan activity observed.

Properties of Purified Components A and B.—In its response to physical agents,[3] its susceptibility to destruction by trypsin, and its ability to elicit the production of neutralizing and precipitating antibodies,[9] component B exhibits the properties of a typical protein. Chromatographically purified component A is also protein-like in behavior, undergoing denaturation at temperatures above 55° C. and at extremely high and low pH values. In addition, incubation with either trypsin or pepsin results in the rapid inactivation of component A.

Further purification of T-3 component A has yielded a preparation with a specific activity of about 4,000, which migrates as a single component in the ultracentrifuge.[10] This specific activity represents a value approximately 1,000 times that obtained with crude extracts of strain K-12. The most highly purified component B prepared to date has a specific activity of 1,000, or 400 times greater than the value obtained with crude extracts of strain K-12.

Further Experiments on the Reaction between Proteins A and B.—In assaying combined peak chromatogram fractions (e. g., *17* and *18*, Fig. 1, *A*) containing equivalent amounts (activity) of the two components, it became apparent that neither component was fully active. Only when an excess of one component was added, was an accurate estimate of the other obtained. This relationship was examined further in a saturation experiment employing varying amounts of component A (Fig. 2). The results obtained show that approximately a three-fold

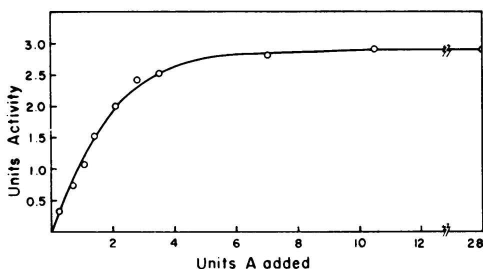


FIG. 2.—Saturation of component B with component A. Reaction mixtures (1-ml. volume) contained 0.4 μM indole, 4 $\times$ 10^{-5} M pyridoxal phosphate, 6 $\times$ 10^{-2} M DL-serine, 0.5 mg. bovine albumin, 10 mg. NaCl, 2.9 units of chromatographically purified T-8 component B, and varying amounts of chromatographically purified T-3 component A.

excess of component A is required for B to be maximally active. In other studies of AB interaction the rate at which these two proteins combine and interact was found to be quite rapid. On mixing the two components at 37° C., the lag observed prior to attaining the maximal rate of indole to tryptophan conversion was, at most, about 2 minutes. Dilution of the enzyme components in the presence of constant substrate concentrations did not bring about a detectable diminution in the reaction rate, though experimental difficulties limited this experiment to a tenfold dilution, and the reliability of the assay procedures at these levels probably was not better than 10 per cent.

Experiments Excluding a Small Molecular-Weight Intermediate.—With two protein components required for the catalysis of several rather complex reactions, even though a physical combination of the proteins appears to occur, it becomes necessary to search assiduously for a free intermediate compound. Two types of experiments were performed in the search for such a compound. The first approach, similar to that employed in studies with *N. crassa*,[2] tested the possible participation of free indole as an intermediate in reaction (3). In this experiment an extract of a mutant (td_2) of *E. coli* was used which was unable to convert indole or InGP to tryptophan but could hydrolyze InGP to indole. When mixed with an extract of a *N. crassa* mutant (A 78) possessing the ability to form tryptophan from indole but unable to act on InGP at an appreciable rate (Rachmeler, unpublished), tryptophan formation from InGP at a rate equal to that of the T-3 preparation would be expected if free indole were an intermediate in reaction (3). As can be seen from the data in Table 1, however, the amount of tryptophan formed by the A 78-td_2 mixture was insignificant compared with the amount formed by the T-3 extract.

Thus in *E. coli* as in *N. crassa*, free indole is not an intermediate in reaction (3).

The second experimental approach employed in the search for an intermediate involved measuring the various reactions when the two protein components were separated by a dialysis membrane (Table 2). Experiments 1 and 2 show that InGP is not converted to indole or to tryptophan when components A and B are so separated. Similarly, in experiment 3, indole disappearance was not significantly greater when the two components were separated than when each was present alone, while appreciable amounts disappeared when the two components were on the same side of the membrane.

Pyridoxal Phosphate Dependence.—Pyridoxal phosphate is required for the conversion of indole to tryptophan in both *Neurospora*[11] and *E. coli*. The pyridoxal phosphate dependence of reactions (2) and (3) was also investigated with *E. coli* preparations, and it was found that, of the three reactions catalyzed by this enzyme system, only those involving serine (reactions [1] and [3]) require pyridoxal phosphate. Pyridoxal phosphate also serves to stabilize both *Neurospora* TSase[12]

TABLE 1

EXCLUSION OF FREE INDOLE AS INTERMEDIATE IN CONVERSION OF InGP TO TRYPTOPHAN IN *E. coli*

	Activity Detected		
Extracts Tested	InGP → indole (μM/30 min)	Indole → tryptophan (μM/30 min)	InGP → tryptophan (μM/30 min)
T-3 0.1 ml.	0.03	0.57	0.3
td_2* 0.02 ml.	0.045	0	0
A78† 0.02 ml.	0	0.6	0.01
A78 0.02 ml. + td_2 0.02 ml.	....	...	0.04
T-3 0.1 ml. + td_2 0.02 ml.	....	...	0.29

* td_2 is a mutant of *E. coli* with an altered component B. Extracts of this strain will convert InGP → indole but will not convert InGP → tryptophan or indole → tryptophan.
† A78 is a tryptophan auxotroph of *N. crassa*. Partially purified TSase preparations from this organism exhibit indole → tryptophan activity but convert InGP → indole and InGP → tryptophan very poorly as compared with TSase preparations from wild-type *N. crassa* (Rachmeler, unpublished).

TABLE 2

EXCLUSION OF DIALYZABLE INTERMEDIATES

	Inside Dialysis Bag		Outside Dialysis Bag		Activity	
Experiment*	Enzyme	Substrate	Enzyme	Substrate	μM Indole	μM Tryptophan
1	A	InGP	B	InGP	...	0
	...	InGP	A + B	InGP	...	+1.59
2	B	InGP	A	InGP	0	...
	...	InGP	A + B	InGP	+0.15	...
3	A	Indole	B	Indole	−0.35	...
	A	Indole	...	Indole	−0.03	...
	...	Indole	B	Indole	−0.29	...
	...	Indole	A + B	Indole	−1.46	...

* Experiment 1: Each 4-ml. reaction mixture (1-ml. inside 1/4-inch dialysis bag, 3 ml. outside) contained 1.6 μM InGP, 2.4 × 10^{-2} *M* serine, 4 × 10^{-5} *M* pyridoxal phosphate, 9 × 10^{-2} *M* tris buffer pH 7.8, 1.7 × 10^{-1} *M* NaCl, partially purified T-3 component A and T-8 component B distributed as indicated.
Experiment 2: Each 2-ml. reaction mixture (0.5 ml. inside 1/4-inch dialysis bag, 1.5 ml. outside) contained 0.3 μM InGP, 0.1 *M* KPO_4 buffer pH 7.0, 7.5 × 10^{-3} *M* NH_4OH, partially purified T-3 component A and T-8 component B distributed as indicated.
Experiment 3: Reaction mixtures identical to experiment 1 except for the substitution of 1.6 μM of indole for InGP. Partially purified T-3 components A and B distributed as indicated.

and *E. coli* TSase. It was readily determined that this stabilizing effect was exerted on protein B specifically.

Experiments with P^{32}-Triose Phosphate.—If, as proposed on the basis of studies with *N. crassa*,[2] enzyme-bound indole serves as an intermediate in reaction (3), the glycerol phosphate moiety of InGP might be expected to exchange with triose phosphate. In a series of experiments employing P^{32}-triose phosphate and C^{14}-

indole, this possibility was examined (Table 3). The results obtained indicate that no such exchange occurs (experiment 1, tube No. 4). Furthermore, although indole and triose phosphate were readily converted to InGP, the presence of even small amounts of InGP in the reaction mixture suppressed the appearance of P^{32} and C^{14} in the isolated InGP. Since the incorporation of both indole and triose phosphate was inhibited to approximately the same extent (experiments 1 and 3), it appears that incorporation of either of these compounds into InGP is associated with the synthesis of InGP.

TABLE 3*

INCORPORATION OF P^{32}-TRIOSE PHOSPHATE AND C^{14}-INDOLE INTO InGP

Experiment	Enzymes Added	Units Added	Supplements InGP (μM)	Supplements Indole (μM)	Observed CPM in InGP	Per Cent Inhibition
1	A + B	28A + 11B	....	1	11,700	
1	A + B	28A + 11B	0.3	1	3,250	74
1	A + B	28A + 11B	0.6	1	1,140	91
1	A + B	28A + 11B	0.6	....	155	
1 control	A + B	28A + 11B	....	....	170	
2	A + B	30B + 1.8A	....	1	2,027	
2	A	200A	....	1	894	
2	A	200A	0.3	1	248	79
2	B	60B	....	1	58	
2 control	...	...	....	1	72	
3	A + B	28A + 11B	....	1	80	
3	A + B	28A + 11B	0.3	1	36	69
3	A + B	28A + 11B	0.6	1	28	81
3 control	...	...	....	1	16	

* All experimental tubes contained phosphate buffer at pH 7.0, 3 μM of hexosediphosphate and crystalline aldolase. In experiments 1 and 2 the hexosediphosphate was labeled (P^{32}), while in experiment 3 the indole was labeled (C^{14}). After a 30-minute incubation period the InGP formed was adsorbed on charcoal, the charcoal washed repeatedly, plated, and counted. In the experiment with C^{14}-indole, the residual indole was extracted with toluene, and the incubation mixture was treated with periodate to convert InGP to indole aldehyde. The indole aldehyde was extracted with ethyl acetate, and an aliquot of the extract was evaporated and counted. Labeled hexosediphosphate was prepared using P^{32} inorganic phosphate, and was purified by chromatography on Dowex-1 ion-exchange resin. Triose phosphate was generated from hexosediphosphate during the course of the experiment with aldolase. C^{14} indole was isolated from the culture filtrates of an indole-accumulating mutant of *E. coli* grown on C^{14}-acetate.

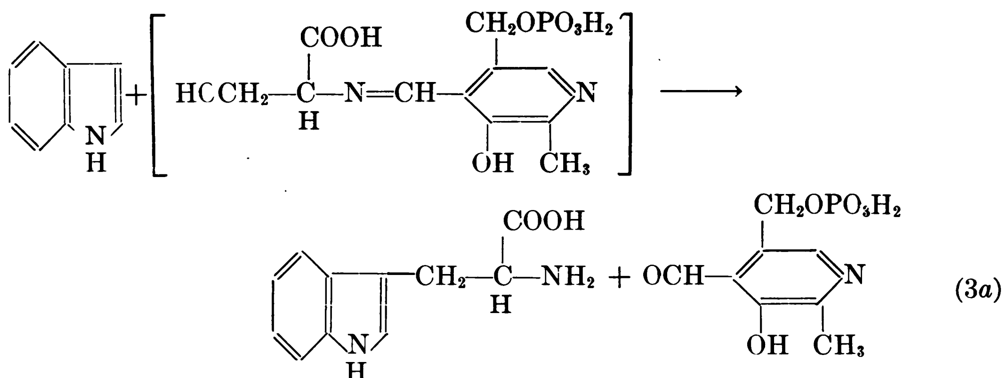
Examination of our most highly purified component A preparations revealed that they do incorporate P^{32}-triose phosphate into a charcoal-adsorbable compound in the presence of indole (experiment 2). As can also be seen in experiment 2, per unit of component A activity this conversion is only 1 per cent of that obtained in the presence of component B. This incorporation appears to represent InGP synthesis, since the reaction was indole-dependent and was strongly inhibited by InGP. T-8 component B exhibited no activity in the absence of added component A.

DISCUSSION

The chromatography of proteins is becoming an increasingly powerful tool in the study of enzymes. To our knowledge, however, it has not previously uncovered an enzymatic relationship analogous to that shown by the two proteins catalyzing the final step in tryptophan biosynthesis in *E. coli.* The evidence presented in this paper clearly indicates that these two proteins must undergo physical contact prior to attaining full activity, forming thereby a complex with new catalytic, as well as chromatographic, properties.[13] It seems unlikely that free intermediates are involved in any of the three reactions catalyzed by this

protein pair, and it appears probable that all three reactions are catalyzed on a single surface made active by the joining of components A and B. The name "tryptophan synthetase" will be retained for the A-B association catalyzing reactions (1), (2), and (3). It is of interest that the genes controlling proteins A and B are very closely linked on the bacterial chromosome and may in fact be adjacent to one another (C. Yanofsky and E. Lennox, unpublished).

Mechanistically viewed, the three reactions are not dissimilar. Reaction (3) may be considered analogous to reaction (2), the hydrolysis of InGP, except that a pyridoxal phosphate–serine compound of the type formulated by Braunstein and others [14–17] substitutes for water (reaction [3*a*]):



In the same manner reaction (1) may be considered analogous to the reverse of reaction (2), with the pyridoxylidine azomethine compound (in brackets) substituting for triose phosphate. It should be noted that reaction (2), in either direction, is much slower than the reactions involving pyridoxal phosphate and serine; it proceeds at less than 5 per cent the rate of reactions (1) and (3).

The hypothetical pyridoxylidine azomethine compound shown has been assumed to be stabilized in an active form by metal chelation[16] or by attachment to a protein molecule.[18] The involvement of this hypothetical compound with its many tautomeric forms might explain the finding of Tatum and Shemin[19] that the α-hydrogen of serine is probably removed during the reaction between indole and serine.

The available data do not permit any definite conclusions on the function of each of the protein components of TSase. The finding that trace amounts of InGP → indole activity are associated with purified component A and that component B is stabilized by pyridoxal phosphate, however, suggests that component A may contribute the InGP combining site and component B the pyridoxylidine azomethine combining site to the surface on which reactions (1), (2), and (3) are catalyzed. Observations consistent with this hypothesis are the following: (1) the *td* series of mutants of *E. coli* represents a group in which alteration of component B[3] leaves the organism incapable of performing the reactions involving serine; (2) a component B-antibody complex can still participate in the conversion of InGP to indole while being essentially inactive in the pyridoxal phosphate–serine requiring conversion of indole to tryptophan;[3] and (3) mutational

alterations of component A seem to affect adversely the ability to act on InGP while permitting a limited conversion of indole to tryptophan.

Finally, it is of interest that similar studies performed in an attempt to detect an AB association in *N. crassa* have as yet been unsuccessful (Rachmeler, unpublished). Futhermore, neither component A nor component B from *E. coli* stimulates the activity of *N. crassa* TSase. Whether *N. crassa* TSase consists of two subunits, comparable to *E. coli* components A and B, which are linked by a peptide bond, or are associated more firmly, remains to be determined.

SUMMARY

E. coli tryptophan synthetase, the enzyme catalyzing the terminal step in the biosynthesis of tryptophan, was found to consist of two separable protein components. These two components combine with each other, and this combination seems to be responsible for the enzymatic activities detected.

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† Postdoctoral fellow, National Foundation for Infantile Paralysis.

[1] C. Yanofsky in W. D. McElroy and B. Glass, *Amino Acid Metabolism* (Baltimore: Johns Hopkins Press, 1955); see also n. 17.

[2] C. Yanofsky and M. Rachmeler, *Biochim. et Biophys. Acta*, **28**, 640, 1958.

[3] C. Yanofsky and J. Stadler, these PROCEEDINGS, **44**, 245, 1958; C. Yanofsky, *Biochim. et Biophys. Acta* (in press).

[4] C. Yanofsky, *J. Biol. Chem.*, **224**, 783, 1957.

[5] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265, 1951.

[6] C. Yanofsky, *J. Bact.*, **68**, 577, 1954.

[7] E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*, **78**, 751, 1956.

[8] H. A. Sober, F. J. Gutter, M. M. Wyckoff, and E. A. Peterson, *J. Am. Chem. Soc.*, **78**, 756, 1956.

[9] P. Lerner and C. Yanofsky, *J. Bact.*, **74**, 494, 1957.

[10] Ultracentrifugal experiments were kindly performed by Dr. Fu-Chuan Chao.

[11] W. Umbreit, W. A. Wood, and I. C. Gunsalus, *J. Biol. Chem.*, **165**, 731, 1956.

[12] C. Yanofsky, in *Methods in Enzymology*, Vol. 2 (New York: Academic Press, 1955).

[13] Recently Richards (these PROCEEDINGS, **44**, 162, 1958) has presented evidence indicating that the enzyme subtilisin hydrolyzes a single peptide bond in ribonuclease, liberating a polypeptide 20 amino acid residues in length. Since both the liberated polypeptide and the residual protein are required for full enzymatic activity, this artificial two-component system resembles the AB interaction reported here.

[14] A. E. Braunstein and M. M. Shemyakin, *Doklady Akad. Nauk. SSSR*, **85**, 1115, 1952.

[15] A. E. Braunstein and M. M. Shemyakin, *Biokhimiya*, **18**, 393, 1953.

[16] D. Metzler, M. Ikawa, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648, 1954.

[17] J. Harley-Mason, *Experientia*, **10**, 134, 1954.

[18] A. Braunstein, *Proc. Intern. Symposium Enzyme Chemistry* (Tokyo, 1957).

[19] E. L. Tatum and D. Shemin, *J. Biol. Chem.*, **209**, 671, 1954.