A Mutational Alteration of the Tryptophan Synthetase of *Escherichia coli*

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SUMMARY

A tryptophan auxotroph of *Escherichia coli* produced an altered tryptophan synthetase which could not convert indole to tryptophan but converted indole-3-glycerol phosphate to indole. As distinct from the normal tryptophan synthetase, which also catalysed this reaction, both pyridoxal phosphate and serine stimulated the activity of the mutant enzyme system. Fractionation and chromatography of the mutant tryptophan synthetase separated it into its two protein components, A and B. Examinations of the separated components showed that the A protein was normal, while the B protein was altered. Studies of the effect of serine on the pH-activity response of mutant preparations in the indole-3-glycerol phosphate $\rightarrow$ indole reaction demonstrated that different pH-activity responses were obtained, respectively, in the presence and absence of serine. The curves obtained were characteristic of the serine-requiring and serine-non-requiring reactions, respectively, of normal tryptophan synthetase. The saturation curves of the mutant component B by normal component A, with and without serine added, suggest that one role of serine and pyridoxal phosphate in the stimulation of the indole-3-glycerol phosphate $\rightarrow$ indole reaction is to bind together the A and B proteins in a catalytically effective complex.

INTRODUCTION

Studies performed with washed suspensions of two strains of *Escherichia coli*, a mutant requiring tryptophan for growth (strain 7-4) and a double mutant requiring tryptophan and serine or glycine (strain T2-15), indicated that serine was involved in the synthesis of indole from glucose and ammonium ion (Gibson, Jones & Teltscher, 1956). Although it was also observed that serine was required for anthranilic acid formation, subsequent experiments with bacteria fully adapted to anthranilic acid synthesis showed that serine participated at some point between anthranilic acid and indole, in indole formation by the mutant strains (F. Gibson, unpublished observation). It was not obvious which reaction required serine since in *E. coli* neither the conversion of anthranilic acid to indole-3-glycerol phosphate nor the conversion of indole-3-glycerol phosphate to indole normally involved this amino acid. Recently, DeMoss & Bonner (1959) described a mutationally altered tryptophan synthetase of *Neurospora crassa* (from strain *td₇₁*) which required serine and pyridoxal phosphate for the conversion of indole-3-glycerol phosphate to indole. Serine and pyridoxal phosphate are not required by the wild

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type enzyme in this reaction. In view of this finding it was thought that the unexplained effect of serine on *Escherichia coli* mutant 7-4 might be due to a similar alteration of its tryptophan synthetase. This possibility was examined and an attempt made to localize the alteration to one of the two protein components of the bacterial tryptophan synthetase.

METHODS

Organisms. Three strains of *Escherichia coli* were used in this study. They were the parent strain (518), a mutant strain which required tryptophan for growth (7-4), and a double mutant derived from strain 7-4 which required tryptophan and serine or glycine for growth. These organisms have been described previously (Gibson *et al.* 1956).

Preparation of cell extracts. Organisms were grown on the mineral salts citrate mixture described by Vogel & Bonner (1956) supplemented with 0.16% (w/v) glucose and 0.005% (w/v) acid-hydrolysed casein (neutralized before use). The glucose was added as a sterile solution after sterilization of the medium. For *Escherichia coli* 7-4, L-tryptophan (5 μ g./ml.) was included in the medium. One litre quantities of medium were inoculated with a 6–8 hr. culture in nutrient broth to give an initial population of about 10^6 organisms/ml. The cultures were then shaken vigorously at 37° for 16 hr. After harvesting the bacteria in a Sharples supercentrifuge they were washed once with cold 0.9% (w/v) NaCl solution and resuspended in 0.1 M 2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer (pH 7.8, to give a final concentration of about 4×10^{11} bacteria/ml. The suspension was then disrupted in a 10 Kc Raytheon sonic oscillator for 20 min. Cell debris was removed by centrifugation at 144,000 g in a Spinco Model L centrifuge for 30 min. The supernatant fluid (crude extract) was stored at –15°.

In addition to crude extracts, preparations obtained by precipitating tryptophan synthetase three times with ammonium sulphate were used (ASP enzyme). Solid ammonium sulphate was added to the crude extract to obtain 50% saturation, the addition being made at 0–5° with stirring, and the stirring continued for a further 10 min. after solution. The precipitate was separated by centrifugation at 4600 g for 10 min. and dissolved in an amount of tris buffer (0.1 M; pH 7.8) equal to the original volume of extract. This precipitation was repeated twice and the final precipitate taken up in tris buffer and stored at –15°.

Reagents. Reagents with the exception of those given below were obtained commercially and not further purified. Indole-3-glycerol phosphate was prepared by the method described by Yanofsky (1956) modified to the extent that it was formed by the indole + triosephosphate reaction (Yanofsky, 1959). The solution used contained 1.8 μ mole indole-3-glycerol phosphate/ml.; 1-(o-carboxyphenylamino)-1-deoxy-ribulose-5-phosphate (anthranilic deoxyribulotide) was prepared as described by Smith & Yanofsky (1960).

Normal A and B proteins from *Escherichia coli* T-8 and *E. coli* T-8, respectively, were purified on diethylaminoethyl (DEAE) cellulose columns (Crawford & Yanofsky, 1958). Proteins A and B from *E. coli* mutant strain 7-4 will be referred to as A₇₋₄ and B₇₋₄.

Methods for assaying enzymic activity. The details of the methods used for estimating the activity of proteins A and B of tryptophan synthetase in the three

reactions which they catalyse were described previously (Crawford & Yanofsky, 1958; Yanofsky & Stadler, 1958; Yanofsky, 1955, 1956). The conversion of indole-3-glycerol phosphate to indole was carried out in a reaction mixture consisting of indole-3-glycerol phosphate and phosphate buffer (pH 7). DL-serine and pyridoxal phosphate were added where desired. Indole was measured by reaction with Ehrlich's reagent as described by Yanofsky (1955). The conversion indole-3-glycerol phosphate $\rightarrow$ tryptophan was carried out in a mixture of indole-3-glycerol phosphate, DL-serine, pyridoxal phosphate, NaCl and tris buffer (pH 7.8). The reaction was followed by measuring the disappearance of indole-3-glycerol phosphate. The remaining indole-3-glycerol phosphate was oxidized to indole-3-aldehyde by meta-periodate at pH 5 and the absorption of indole-3-aldehyde formed was measured at 290 m μ . The conversion indole $\rightarrow$ tryptophan was carried out in the same reaction mixture as that just described except that indole was substituted for indole-3-glycerol phosphate. The reaction was followed by measuring indole disappearance. All tests were carried out in a final volume of 1 ml. at 37°.

One unit of tryptophan synthetase activity is defined as that amount of enzyme which catalyses the disappearance of 0.1 μ mole substrate or the formation of 0.1 μ mole product in 20 min. at 37°.

Chromatography. Column chromatography was carried out on Reagent Grade 'Selectacel' diethylaminoethyl (DEAE) cellulose (Brown Company, Berlin, New Hampshire, U.S.A.). The preparation and use of such columns for the separation and purification of the components of tryptophan synthetase were described by Crawford & Yanofsky (1958). In the present experiments a column 2 $\times$ 90 cm. was used. The gradient elution system consisted of 1 l. of 0.01 M-phosphate buffer (pH 7.0) in the mixing bottle and 1 l. of 0.4 M-phosphate buffer (pH 8.0) in the inlet bottle. Fractions of 7.5 ml. were collected at 18 min. intervals and the buffer was changed to 0.5 M-phosphate (pH 8.0) at fraction 173. All the buffers contained 2×10^{-5} M-pyridoxal phosphate and 10^{-4} M-reduced glutathione.

The crude extract was prepared for the column as follows. Nucleic acids were precipitated by addition of 1.9 ml. M-MnCl₂ to 25 ml. extract. After stirring for 15 min. the mixture was centrifuged and the precipitate removed (15 min., 4600 g). Then 9.67 g. (NH₄)₂SO₄ was added to 26 ml. of the supernatant solution and the mixture stirred for 10 min. After centrifugation (15 min., 4600 g) the precipitate was taken up in dialysis buffer (0.02 M-phosphate) and dialysed for 3 hr. against 1 l. of the same buffer. The final volume was 11.3 ml. of which 11 ml. was chromatographed. All operations were carried out at 0–5°.

Protein estimations. Protein was estimated by using the Folin phenol reagent as described by Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

The site of the serine effect

Indole is now considered to be a breakdown product of indole-3-glycerol phosphate rather than, as previously thought, a normal intermediate in tryptophan synthesis (Yanofsky, 1960). A number of bacterial mutants have been isolated which accumulate indole but, except in the case of *Escherichia coli* strain 7-4, there has been no report of serine being required for indole production. If the effect of serine on the

production of indole by *E. coli* strain 7-4 were due to the fact that, as with *Neurospora crassa* mutant *td₇₁* (De Moss & Bonner, 1959), it stimulated the conversion indole-3-glycerol phosphate $\rightarrow$ indole, restriction of the available serine should cause the accumulation of indole-3-glycerol phosphate. Restriction of available serine could be achieved in two ways, either by using the double mutant which also required serine or glycine for growth (*E. coli* strain T2-15) or, alternatively, by using cell-free extracts which could not form serine from the available substrates.

Experiments with whole organisms. From a nutrient agar slope (24 hr., 37°) of the double mutant *Escherichia coli* strain T2-15, three flasks of mineral salts + citrate + glucose medium (10 ml. in 50 ml. flasks), each containing a different concentration of serine, were inoculated to give an initial population of about 2×10^8 bacteria/ml. The flasks were incubated for 18 hr. at 37° with vigorous shaking and then sampled for indole and indole-3-glycerol phosphate determinations. Table 1 shows that as the concentration of serine was decreased 20-fold the ratio of indole-3-glycerol phosphate to indole increased some 100-fold, indicating that serine probably did play some part in the conversion of indole-3-glycerol phosphate to indole by this strain.

Table 1. *The accumulation products of Escherichia coli strain T2-15 (double mutant requiring tryptophan and serine or glycine) when grown with limiting amounts of serine*

Organisms grown on basal medium* for 18 hr. with 5 μ g. L-tryptophan/ml. and DL-serine as indicated.

Final concentration of DL-serine in basal medium (M)	Product formed (μ mole/ml.)		Ratio: InGP/indole
	Indole	InGP†	
2×10^{-2}	9.1	0.45	0.049
10^{-2}	9.6	1.0	0.104
10^{-3}	0.026	0.15	5.8

* Medium as described in methods for preparation of cell extracts with the casein hydrolysate omitted.

† The accumulated material was probably predominantly indole-3-glycerol but was considered equivalent to InGP for the purposes of the experiment.

Experiments with cell extracts. The results given above were confirmed with cell extracts of *Escherichia coli* mutant strain 7-4 which were allowed to metabolize anthranilic deoxyribulotide, the intermediate immediately preceding indole-3-glycerol phosphate (Smith & Yanofsky, 1960). Table 2 shows that indole formation from anthranilic deoxyribulotide was stimulated 25-fold by the addition of serine. Furthermore, the conversion indole-3-glycerol phosphate $\rightarrow$ indole was increased fivefold when serine was added.

Examination of the effect of serine on indole formation

Indole formation in the presence and absence of serine. Table 2 shows that some indole was formed in the absence of serine when the ASP enzyme was incubated with indole-3-glycerol phosphate. Since the enzyme had been precipitated 3 times with ammonium sulphate it seemed unlikely that this indole formation was due to traces of serine remaining from the crude extract. It appeared more likely that

Table 2. *Effect of serine on the formation of indole-3-glycerol phosphate and indole by a cell-free extract of Escherichia coli strain 7-4*

Reaction mixture A consisted of anthranilic deoxyribulotide (0.44 μ mole) and phosphate buffer (pH 7.9; 50 μ moles); mixture B contained indole-3-glycerol phosphate (0.18 μ mole), phosphate buffer (pH 7.0; 50 μ mole) and *Escherichia coli* strain 7-4 ASP extract (0.02 ml.) in a final volume of 1.0 ml. with the additions shown. Incubation was at 37° for 20 min. The contents of the tubes were then assayed for indole and indole-3-glycerol phosphate (InGP; see Methods).

Addition to reaction mixture	Product (m μ mole)	
	InGP	Indole
Addition to reaction mixture A		
None	84	2
DL-serine (30 μ mole)	32	50
Addition to reaction mixture B		
None	—	19
DL-serine (30 μ mole)	—	100

some serine-independent indole formation was taking place. The components and additions involved in this serine-independent reaction will be called the basal system. Those mutant preparations and additions required to form indole in the presence of serine will be called the serine system. Hydroxylamine, a potent inhibitor of those reactions of tryptophan synthetase which require pyridoxal phosphate, slightly stimulates as a rule the conversion indole-3-glycerol phosphate $\rightarrow$ indole (Yanofsky & Rachmeler, 1958). Table 3 shows that the basal system was not affected by hydroxylamine, whereas in the serine system, hydroxylamine acted as an inhibitor, apparently competing with serine for the available pyridoxal phosphate. Inhibition by low concentrations of hydroxylamine provided indirect evidence that pyridoxal phosphate might also be involved with serine in the stimulation of the conversion of indole-3-glycerol phosphate to indole by this altered tryptophan synthetase.

Table 3. *Effect of hydroxylamine on indole formation by the basal and serine systems*

The reaction mixture contained: indole-3-glycerol phosphate, 0.18 μ mole; phosphate buffer (pH 7.0), 50 μ mole and in Expt. 1, 0.01 ml. crude *Escherichia coli* strain 7-4 extract, or in Expt. 2, 0.02 ml. of ASP enzyme together with various amounts of DL-serine and hydroxylamine as shown. Incubation time, 20 min. Final volume 1 ml.

Experiment	Substance added (μ mole)		Indole formed (m μ mole)
	DL-Serine	Hydroxylamine	
1	None	None	5
	None	1	4
	60	None	88
	60	1	5
2	None	None	4
	100	None	80
	100	0.01	71
	100	0.1	19
	100	1	5
	10	None	62
	10	0.01	23
	10	0.1	5
	10	1	5

Effect of pH value on basal and serine reactions. The pH value of the incubation mixture over the range pH 7—9 (Fig. 1) affected the basal and serine systems differently. The curves obtained may be compared with those in Fig. 2 obtained with normal tryptophan synthetase. The curve for indole formation by the basal system (Fig. 1) resembles that for the conversion indole-3-glycerol phosphate $\rightarrow$ indole by normal tryptophan synthetase (Fig. 2). The curve for indole formation by the serine system (Fig. 1) more closely resembles that for the serine and pyridoxal

Fig. 1

Fig. 2

Fig. 3

Fig. 1. The effect of pH on the conversion indole-3-glycerol phosphate $\rightarrow$ indole by the serine and basal systems with *Escherichia coli* strain 7-4 extract. The reaction mixtures were: for serine system, InGP (0.18 μ mole), DL-serine (60 μ mole), pyridoxal phosphate (0.03 μ mole), normal A (86 units*) crude *E. coli* 7-4 extract (0.01 ml.); for basal system, InGP (0.18 μ mole), hydroxylamine (1 μ mole), normal A (86 units*) crude *E. coli* strain 7-4 extract (0.04 ml.) together with 0.5 M-tris buffer (50 μ mole) in final volume 1 ml. Incubation times: serine system 20 min.; basal system 80 min. Serine system, ●—●; basal system, x—x.

* Units measured in indole $\rightarrow$ tryptophan reaction.

Fig. 2. The effect of pH value on the conversion indole-3-glycerol phosphate (InGP) $\rightarrow$ tryptophan and InGP $\rightarrow$ indole by normal tryptophan synthetase. The reaction mixtures were: for InGP $\rightarrow$ tryptophan reaction; InGP (0.18 μ mole), DL-serine (60 μ mole), pyridoxal phosphate (0.03 μ mole), saturated aqueous NaCl solution (0.03 ml.), normal component A (10 units*), normal protein B (2 units*); for InGP $\rightarrow$ indole reaction; InGP (0.18 μ mole), hydroxylamine (1 μ mole), normal component A (150 units*), normal component B (40 units*), with tris buffer (50 μ mole). Incubation time 20 min. Final volume 1 ml. InGP $\rightarrow$ tryptophan, ●—●; InGP $\rightarrow$ indole, x—x.

* Units measured in indole $\rightarrow$ tryptophan reaction.

Fig. 3. The effect of serine concentration on the rate of indole formation. The reaction mixture contained indole-3-glycerol phosphate (0.18 μ mole), phosphate buffer, pH 7.0 (50 μ mole), ASP enzyme from *Escherichia coli* strain 7-4 (0.02 ml.) with serine as indicated. Final volume 1 ml.; incubation time 20 min.

phosphate-dependent formation of tryptophan by normal tryptophan synthetase (Fig. 2).

Specificity of L-serine. Some amino acids were tested as possible substitutes for DL-serine in the serine system (Table 4). L-Serine was twice as active as DL-serine on a molar basis, while D-serine had a slight stimulatory effect; this may have been due to contamination of the D-serine with small amounts of L-serine. The other amino acids tested were inactive.

Table 4. *Indole synthesis with various amino acids related to serine*

The reaction mixture was as in Table 3. Enzyme from *Escherichia coli* strain 7-4 (ASP preparation, 0.02 ml.) with the amino acids as shown.

Addition	Final concentration (M)	Indole formed (μmole)
None	—	10
DL-Serine	0.06	93
L-Serine	0.03	100
D-Serine	0.03	25
DL-Homoserine	0.06	10
DL-Threonine	0.06	8
L-Cysteine	0.03	14
Glycine	0.03	14

Serine saturation curve. Figure 3 shows the effect of increasing concentrations of serine on indole formation from indole-3-glycerol phosphate. The K_A for DL-serine estimated from the curve is about 1.3×10^{-3} M which is of the same order as the K_M for serine with normal tryptophan synthetase (K_M for L-serine = 1.7×10^{-3} M; C. Yanofsky, unpublished).

Pyridoxal phosphate and the serine reaction

The inhibition of the serine stimulation by hydroxylamine was strong presumptive evidence that pyridoxal phosphate was concerned in the serine system. However, indole formation by the ASP enzyme was only stimulated to a variable small degree or not at all by added pyridoxal phosphate, despite the fact that ammonium sulphate precipitations usually removed much of the bound pyridoxal phosphate (C. Yanofsky, personal communication). However, after dialysis of 0.5 ml. of ASP preparation for 2 hr. against 1 l. of 0.02 M tris buffer (pH 7.8) it was possible by adding pyridoxal phosphate in the serine system to show stimulation of indole formation (1.5-fold) by mutant preparations.

Separation of the A and B proteins of the tryptophan synthetase of Escherichia coli mutant strains 7-4

The two protein components A and B of *Escherichia coli* tryptophan synthetase can be separated by chromatography on cellulose ion exchangers (Crawford & Yanofsky, 1958). To learn more about the peculiarities of the tryptophan synthetase of *E. coli* strain mutant 7-4 the two component proteins were separated and examined individually. After chromatography of a cell-free extract of *Escherichia coli* strain 7-4 on DEAE cellulose (see Methods) the fractions obtained were assayed

for component A activity in the indole $\rightarrow$ tryptophan reaction in the presence of an excess (10 units) of normal B protein (Fig. 4*a*). After the first peak of component A (A_{7-4}) was located the fractions were examined for component B by measuring the conversion indole-3-glycerol phosphate $\rightarrow$ indole (in the presence of serine and an excess of A_{7-4}). Figure 4*b* shows that B_{7-4} activity was detected in two peaks. Examination of fraction 154 indicated that the addition of A_{7-4} was not necessary for this fraction to catalyse the conversion indole-3-glycerol phosphate $\rightarrow$ indole. Therefore, the first B_{7-4} peak probably represented an AB complex (Crawford & Yanofsky, 1958). This was confirmed by assaying fractions 140 to 190 for component A activity using excess normal B protein and measuring the conversion

Fig. 4. Chromatography of tryptophan synthetase from *Escherichia coli* strain 7-4 on DEAE cellulose. Details of assay methods in text. (a) Component A activity, $\times-\times$; (b) component B activity, $\bullet-\bullet$; protein concentration, $\times-\times$.

indole $\rightarrow$ tryptophan (Fig. 4*a*). The second peak of B_{7-4} activity contained only low amounts of A_{7-4} . Both A_{7-4} and B_{7-4} were concentrated by pooling the appropriate fractions, precipitating the proteins with ammonium sulphate at 60% of saturation, and redissolving the precipitates in 0.1M-tris buffer (pH 7.8). Purified B_{7-4} protein was stored in buffer containing pyridoxal phosphate and reduced glutathione (see Methods).

Experiments with the separated A_{7-4} and B_{7-4} components

Component A_{7-4} appeared to be a normal A protein; in the presence of normal B protein it catalysed all reactions characteristic of normal A protein. Component B_{7-4} was obviously an altered component; in the presence of A_{7-4} or other normal component A it failed to convert indole to tryptophan. Furthermore, serine stimulated the conversion indole-3-glycerol phosphate $\rightarrow$ indole by a mixture of B_{7-4} and normal A protein.

The addition of serine to a mixture of normal A and B proteins in the presence of

indole-3-glycerol phosphate and pyridoxal phosphate eliminated indole formation from indole-3-glycerol phosphate because of the much faster conversion of the latter to tryptophan (Table 5).

On the other hand, a mixture of B₇₋₄ and normal A protein formed indole from indole-3-glycerol phosphate both in the presence and absence of serine (Table 5) as expected on the basis of the experiments with the ASP extract. Purified B₇₋₄ protein had some activity when measured in the serine system without added A protein (Table 5); this can be explained by the small amount of A₇₋₄ that was present (see Fig. 4).

Table 5. *The effect of serine on the conversion of indole-3-glycerol phosphate to indole with B₇₋₄ protein and the components of normal tryptophan synthetase*

The reaction mixture consisted of the reagents listed in the Table together with indole-3-glycerol phosphate (0.18 μ mole) and phosphate buffer (pH 7.0; 50 μ mole). In Expts. 1 and 3 (a), hydroxylamine (1 μ mole) was added; in Expts. 2 and 3 (b), pyridoxal phosphate (0.03 μ mole) was added. Final volume 1 ml. Incubation times: Expt. 1, 80 min.; Expt. 2, 30 min.; Expt. 3, 30 min.

Experiment	Normal A protein (units)*	A ₇₋₄ protein (units)*	Normal B protein (units)*	B ₇₋₄ protein (ml.)	Serine added (μ mole)	Indole formed (m μ mole/ 20 min.)
1 (a)	560	—	—	0.02	—	22
(b)	—	—	—	0.02	—	< 1
2 (a)	56	—	—	0.02	60	87
(b)	—	—	—	0.02	60	5
3 (a)	—	26	80	—	—	31
(b)	—	13	40	—	60	< 1

* Units measured in the indole $\rightarrow$ tryptophan reaction. In Expts. 1 (a) and 2 (a) the normal A protein added was sufficient to saturate the B₇₋₄ protein used.

It was impossible to obtain reaction rates for the three reactions catalysed by tryptophan synthetase with either of the two components in excess, since the B₇₋₄ protein of course failed to catalyse the indole $\rightarrow$ tryptophan reaction. Further, this B protein could not be used in excess in the basal system, since at the concentration of enzyme required the preparation became inhibitory to the reaction.

The ratio of reaction rates for the indole $\rightarrow$ tryptophan and indole-3-glycerol phosphate $\rightarrow$ indole reactions with normal A protein and excess normal B protein in the first case, and normal A and excess B₇₋₄ plus serine and pyridoxal phosphate in the second case, was 1:0.05. The ratio of reaction rates for the same two reactions using normal A protein with normal B component was 1:0.08 (Yanofsky, 1960). It is clear then, that the addition of serine to the mutant system restored the ratio to nearly normal but did not accelerate the indole-3-glycerol phosphate $\rightarrow$ indole reaction to a point where it would be comparable with the serine requiring reactions carried out by normal A and B components.

Saturation of B₇₋₄ by normal A. With normal tryptophan synthetase components about a three-fold excess of one component was necessary to saturate fully the other component for maximal activity in the conversion indole $\rightarrow$ tryptophan (Crawford & Yanofsky, 1958). In Fig. 5 it can be seen that the curve for the saturation of normal protein B by normal A in the conversion indole-3-glycerol phosphate $\rightarrow$

indole was almost identical with that obtained for the conversion indole $\rightarrow$ tryptophan (Fig. 5).

It was of interest to examine the saturation curves for B_{7-4} protein in both the serine and basal systems (Fig. 6). A striking difference was detected; in the absence of serine 25 times more protein A was required for half saturation of B_{7-4} than in its presence.

Fig. 5

Fig. 6

Fig. 5. The saturation of normal B protein by normal A protein as measured in the conversion indole $\rightarrow$ tryptophan and the conversion indole-3-glycerol phosphate (InGP) $\rightarrow$ indole. The reaction mixtures were: (a) indole $\rightarrow$ tryptophan: phosphate buffer pH 7.0 (50 μ mole), indole (0.4 μ mole), DL-serine (60 μ mole), pyridoxal phosphate (0.03 μ mole), saturated aqueous NaCl solution (0.03 ml.), normal B (3.2 units), various amounts of normal A protein; (b) InGP $\rightarrow$ indole: InGP (0.18 μ mole), phosphate buffer pH 7.0 (50 μ mole), hydroxylamine (1 μ mole), normal B (26 units), various concentrations of normal A protein. Incubation time 20 min. for both tests. Units are measured in the indole $\rightarrow$ tryptophan reaction. Indole $\rightarrow$ tryptophan, ●—●; InGP $\rightarrow$ indole, x---x.

Fig. 6. The saturation of B_{7-4} protein by normal A protein in the serine and basal systems. The reaction mixtures were: for serine system; indole-3-glycerol phosphate (0.18 μ mole), DL-serine (60 μ mole), pyridoxal phosphate (0.03 μ mole), phosphate buffer pH 7.0 (50 μ mole), purified B_{7-4} protein preparation (0.03 ml.), and normal B protein as indicated; for basal system; as above with serine and pyridoxal phosphate omitted and hydroxylamine (1 μ mole) added. Final volume 1 ml.; incubation times: serine system 30 min.; basal system 80 min. Serine system, ●—●; basal system, x---x.

DISCUSSION

It seems probable from the findings reported here that the mutation which affects the tryptophan synthetase in *Escherichia coli* 7-4 leads to the production of an altered enzyme system with characteristics similar to those of the tryptophan synthetase of the mutant of *Neurospora crassa* described by DeMoss & Bonner (1959). Experiments with whole organisms and cell extracts of *E. coli* strain 7-4 have shown that serine and probably pyridoxal phosphate have a marked effect on the conversion indole-3-glycerol phosphate $\rightarrow$ indole although neither of these compounds has any effect on this reaction with normal tryptophan synthetase. The present observations thus extend the similarities of mutational effects on this enzyme system in the two organisms. A major difference between the two systems, however, is the ready dissociation of the tryptophan synthetase from *E. coli* into

two protein components A and B (Crawford & Yanofsky, 1958). This property of the *E. coli* system has permitted the localization of the mutational defect in *E. coli* strain 7-4 to the B protein. The participation of serine and pyridoxal phosphate in a reaction involving an altered B protein lends further support to the suggestion of Crawford & Yanofsky (1958) that protein B provides the surface on which the serine + pyridoxal phosphate complex is combined during those reactions of tryptophan synthetase in which this complex is involved.

Three types of experiments differentiate the conversion indole-3-glycerol phosphate $\rightarrow$ indole in the presence and in the absence of serine: (i) the serine system is strongly inhibited by a low concentration of hydroxylamine; (ii) the pH-activity curves for the two systems are different; (iii) curves for the saturation of B₇₋₄ protein by normal A protein have marked differences in the serine and basal systems.

The function of serine (presumably as a Schiff base with pyridoxal phosphate) in the serine system is of interest. It does not act as a substrate in the conversion indole-3-glycerol phosphate $\rightarrow$ indole but it is possible that it is changed to some other compound by the A B protein complex during the course of the reaction. It was thought that if serine were attacked during indole formation, pyruvate would be a likely end product. Experiments designed to detect pyruvate production by the specific method of Friedmann & Haugen (1943) were clearly negative. The affinity for serine is fairly low (K_s about $1.3 \times 10^{-3} M$) and with the concentrations of serine necessary to obtain a significant reaction it was not practicable to look for serine removal,

It has been suggested (DeMoss & Bonner, 1959) that in mutants of *Neurospora crassa* similar to *Escherichia coli* strain 7-4, serine and pyridoxal phosphate might be required to maintain the active site of the altered enzyme in its 'proper' configuration for cleavage of indole-3-glycerol phosphate. This suggestion is supported and extended by the experiment shown in Fig. 4. The amount of normal A protein required for half saturation of B₇₋₄ protein in the basal system is about 25 times that required for the serine system. This suggests that one function of serine and pyridoxal phosphate in the conversion indole-3-glycerol phosphate $\rightarrow$ indole by the tryptophan synthetase of *E. coli* strain 7-4 is to assist in binding the A and B components into an enzymically effective complex.

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