THE DNA-DEPENDENT FORMATION OF TRYPTOPHAN SYNTHETASE IN CELL-FREE EXTRACTS OF ESCHERICHIA COLI

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As reported in our previous communication (Yura et al. 1962), an increase in activity of tryptophan synthetase (TSase) is observed on incubating crude extracts of partially derepressed wild-type cells of E. coli under certain conditions. Fractionation of the crude extract revealed that either a large-particle fraction (P_{30}) or a small-particle fraction (P_{60-180}), each in combination with a supernatant fraction (S_{180}), is capable of developing an increase in TSase activity. Evidence suggested that the P_{30} system was partially dependent on the genetically specific DNA, whereas the P_{60-180} system represented an activation-and-release of preformed protein molecules attached to the ribosomes (Marushige et al. in preparation). The present report will describe the DNA-dependent formation of TSase in the system consisted of DNA, P_{30} and S_{180} fractions obtained from appropriate strains.

Conditions for bacterial culture, preparation of crude extracts and of various fractions were as described before (Yura et al. 1962) with the following modifications. Firstly, in most experiments cells were grown under derepression, i.e., with no tryptophan for

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wild-type strain and with limiting amounts (6 to 10 μg/ml) of DL-tryptophan for mutant strains. Secondly, buffer used for washing cells and P_{30} was 0.03 M Tris-HCl, pH 7.8 containing 7.5 mM $MgCl_2$, 60 mM KCl and 6 mM β-mercaptoethanol. Special care was taken in washing P_{30} to minimize contamination of TSase. For DNA preparation, cells at the log phase were harvested, washed and resuspended in buffer and treated with sodium lauryl sulfate according to Marmur (1961), and DNA extracted by the phenol method.

An increase in activity of TSase-B protein was observed when DNA and P_{30} from derepressed wild-type cells and S_{180} from a tryptophan-requiring deletion mutant (K/1,t-4) were incubated in two steps under appropriate conditions (Table 1). The results show that ATP and its generator, an amino acid mixture, and nucleoside triphosphates are required for the maximum increase in enzyme activity in addition to the DNA, P_{30} and S_{180} fractions. A DNA preparation that had been irradiated with UV light was inactive in this system.

The increase in TSase-B protein activity was markedly inhibited by DNase, RNase and by L-tryptophan. As seen in Table 2a, RNase added either at the step I or at the step II inhibited the activity increase completely. In contrast, DNase was effective only when given at the step I. The inhibition by L-tryptophan was also remarkable when it was added at the step I, but not when added at the step II. These results suggest that L-tryptophan primarily inhibits the reaction or reactions in which DNA participates. Furthermore, the effect of tryptophan in this system is apparently related to the phenomenon of enzyme repression. Thus, when S_{180} from a deletion mutant derived from a non-repressibl strain, R1,try (Cohen and Jacob 1959) was used, L-tryptophan showed no inhibition on the activity increase under otherwise the

Table 1

Requirements for the DNA-dependent formation of TSase-B protein*

System	Omission at step	Increase in TSase-B activity** (unit/ml)
Complete	-	0.12
- ATP and generator	II	< 0.01
- Nucleoside triphosphates	I	0.02
- Amino acids	II	0.03
- DNA	I	< 0.01
- DNA + UVed DNA***	I	< 0.01
- S_{180}	I	< 0.01
- P_{30}	II	< 0.01

* Incubation was carried out in two steps as follows:

Step I ---Complete system (0.9 ml) contained, in μmole unless otherwise noted, Tris-HCl buffer, pH 7.8 33; $MgCl_2$ 3.3; $MnCl_2$ 0.8; spermidine phosphate 0.15; ATP, GTP, CTP, UTP 0.33 each; β-mercaptoethanol 10; DNA from derepressed wild-type cells 20 μg as determined by the diphenylamine reaction; S_{180} (from strain K/1,t-4) that had been dialyzed for 5 hrs. (protein 10 mg). The mixture was incubated at 37°C for 10 min. and the reaction stopped by chilling in ice.

Step II ---Complete system (2.0 ml) contained the first incubation mixture above plus the following; Tris-HCl buffer, pH 7.8 140; ATP 20; phosphocreatine 10; 18 L-amino acids 0.05 each; creatine kinase 80 μg; P_{30} from derepressed wild-type cells (protein 2.5 mg). Duplicate samples (0.9 ml) were taken and one incubated at 30°C for 40 min. while the other kept in ice.

** Assays were performed in the presence of an excess of A protein and 200 μg of chloramphenicol. Definition of enzyme unit and assay conditions are as described by Yanofsky and Stadler (1958), except that the assay volume was doubled, indole concentration adjusted so as to give the maximum resolution, and incubation carried out for 90 min. at 37°C. The actual difference in reading in Klett unit for the complete system was 20 with a No. 54 filter. Initial activity in this experiment was 0.24 unit/ml. This corresponds to about 1/1000 of the total TSase activity in crude extract that contained the amount of P_{30} used.

*** Irradiated with a UV germicidal lamp (Toshiba 15W x 2) for 10 min. at a distance of 15 cm. at 5°C.

same condition (Table 2a, Exp. 2). The present system was also found to be sensitive to actinomycin, streptomycin and to chloramphenicol (Table 2b).

Table 2

Inhibition of the increase in TSase-B protein activity*

(a)

System	Concentration (μg/ml)	Addition at step	Increase in TSase activity** (unit/ml)	
			Exp. 1	Exp. 2***
Complete	-	-	0.10	0.09
+ DNase	10	I	< 0.01	-
+ DNase	10	II	0.09	-
+ RNase	20	I	< 0.01	-
+ RNase	20	II	< 0.01	-
+ L-tryptophan	500	I	< 0.01	0.09
+ L-tryptophan	500	II	0.10	0.09

(b)

			Exp. 3	Exp. 4
Complete	-	-	0.06	0.07
+ Actinomycin	5	I	-	0.02
+ Streptomycin	250	I	0.03	-
+ Streptomycin	1000	I	-	0.01
+ Puromycin	100	II	0.01	< 0.01
+ Chloramphenicol	100	II	0.02	0.01

* For procedures and conditions, see Table 1. Non-dialyzed S_{180} was used in these experiments.

** Initial activities (unit/ml) were 0.23 (Exp. 1), 0.25 (Exp. 2), 0.24 (Exp. 3) and 0.06 (Exp. 4).

*** S_{180} used in this experiment was obtained from strain R1,try/1,t-1, a tryptophan-requiring deletion mutant derived from a non-repressible mutant, R1,try. The medium used for this strain was supplemented with 0.1 % Difco yeast extract.

As shown in Table 3, a DNA preparation from the wild-type strain is effective in developing an increased activity of both A and B proteins of TSase, whereas that from a deletion mutant (K/1,t-4) is inactive with respect to either protein. Furthermore, DNA from A or B mutant strain was shown to be active only with respect to B or A protein of the enzyme, respectively. These results show the genetic specificity of the DNA required in the present system. It should be noted, however, that the increase in activity of A protein was barely detectable under these conditions.

Table 3

Activity of various DNA preparations in the formation of TSase*

DNA preparation**		Increase in activity (unit/ml)***	
Strain	Grown under	A protein	B protein
Wild	derepression§	0.02	0.06
Wild	repression	-	< 0.01
K/1,t-4	derepression	< 0.01	< 0.01
B_4	derepression	0.01	< 0.01
A_2	derepression	< 0.01	0.06
R1,try	repression	-	0.05

* Procedures and conditions are as in Table 1, except non-dialyzed S_{180} was used and the activity of A protein was assayed with an excess of B protein. DNA was prepared from the strains indicated.

** These preparations had not been treated with RNase, since they proved equally active compared with RNase-treated preparations at least for derepressed wild-type strain tested.

*** Initial activity (unit/ml) in this experiment was 0.24 for A protein and 0.26 for B protein.

§ Derepression means growth without tryptophan for wild type and with a limiting amount of DL-tryptophan for tryptophan-requiring strains. Repression means growth with high concentration (50 µg/ml) of DL-tryptophan.

Table 3 further shows that a DNA preparation obtained from repressed wild-type cells was almost inactive. It thus appears that growth under repression has in some way altered the activity of the DNA as manifested in the present system. This alteration in the DNA may be brought about by a cytoplasmic repressor, since DNA preparation from the R1,try mutant grown with high concentration of tryptophan was found to be active. The R1,try mutant had been shown to be defective in the formation of a hypothetical repressor substance (Cohen and Jacob 1959).

In view of the results so far presented, DNA in the present system seems to be playing certain genetic roles in bringing about an ultimate increase in the enzyme activity whatever the precise mechanisms may be involved. Preliminary experiments have shown that when P_{30} obtained from strain K/1,t-4 was substituted for that from derepressed wild-type cells so far used, a significant activity (0.02-0.04 unit/ml) of TSase-B protein appeared against the "zero" (< 0.01 unit/ml) activity for the control in which the second incubation was omitted. A DNA preparation from strain K/1,t-4 was inactive in this system. Although further characterization of the system as well as of the strain K/1,t-4 is required to draw a definite conclusion, these results suggest that at least in such a system, we may be dealing with de novo synthesis of protein molecules.

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References

Cohen, G. and Jacob, F., Compt. rend., 248, 3490 (1959).
Marmur, J., J. Mol. Biol., 3, 219 (1961).
Yanofsky, C. and Stadler, J., Proc. Nat. Acad. Sci., 44, 245 (1958).
Yura, T., Marushige, K., Imai, M. and Watanabe, I., Biochem. Biophys. Res. Communs., 9, 545 (1962).