# THE CELL-FREE FORMATION OF TRYPTOPHAN SYNTHETASE ENZYME IN ESCHERICHIA COLI*

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Received October 10, 1962

Among the cell-free systems for protein "synthesis" so far reported, special genetic interest is attached to the systems in which DNA-initiated reactions leading to a specific protein formation can be studied (Kameyama and Novelli 1960, Nisman and Fukuhara 1960, Wainwright 1959). We have chosen the tryptophan synthetase (TSase) of E. coli to study the genetic control of a specific enzyme formation at the subcellular level because of the wealth of genetic and enzymatic information available for this system (Yanofsky 1960). The present communication briefly reports some of the results obtained at the initial stage of our investigation along this line.

The wild type E. coli K-12 was grown in three liters of medium E (Vogel and Bonner, 1956) supplemented with 4 mg/ml of Casamino acids and 50 μg/ml of DL-tryptophan. Cells were harvested at the logarithmic phase, centrifuged, resuspended in 500 ml of prewarmed medium E without tryptophan and shaken for 10-15 minutes at 37°C. The partially derepressed cells thus obtained were chilled in crushed ice, centrifuged, washed in water and resuspended in 10 ml of 0.03 M Tris-HCl, pH 7.8 containing 7.5 mM $MgCl_2$ (TMB). The suspension was passed through a French pres-

*This work has been aided in part by grants from The Jane Coffin Childs Memorial Fund for Medical Research, The Rockefeller Foundation and The National Science Foundation.

sure cell at 8000 psi and the resulting extract was centrifuged first at 8000 x g for 15 minutes and then at 30,000 x g for 30 minutes. The final supernatant (crude extract) was pipetted off and stored at -15°C until use.

When the crude extract was incubated with appropriate supplements, an increase in TSase activity was observed. The increase in enzyme activity depends on the presence of ATP and an ATP generating system and is stimulated by an amino acid mixture and ribonucleotide triphosphates (Table 1). Although enzyme assays were usually carried out with 0.2 ml samples of the incubation mixture directly, assays of the $(NH_4)_2SO_4$ precipitates have given quantitatively similar results.

Table 1

Requirements for the cell free formation of TSase

| System | Increase in TSase activity** (unit/ml) |
|---|---|
| complete* | 0.90 |
| - ATP and generator | 0.12 |
| - amino acids | 0.71 |
| - nucleotide triphosphates | 0.60 |

*Complete system (1 ml) contained the following in µmole: Tris-HCl buffer pH 8.4 100; $MgCl_2$ 5; ATP 10; phosphocreatine 5; CTP, GTP, UTP 0.03 each; 18 natural L-amino acids 0.025 each; crude extract that had been dialyzed against TMB for 5 hrs (protein 15 mg).

**Duplicate samples (0.2 ml) were taken before and after incubation at 30°C for 30 min., and were assayed for enzyme activity. This procedure gives automatically the activity of the B protein of TSase, since the A protein is present in 3-4 times excess under these conditions. One unit of enzyme is defined as the amount of enzyme which will convert 0.1 µmole of indole to tryptophan in 20 minutes at 37°C under the standard conditions (Yanofsky and Stadler 1958). However, actual incubation time for enzyme assay was 20-60 min. in different experiments. Initial activity in this experiment was 3.6 ± 0.1 unit/ml.

Additions of DNase, RNase, chloramphenicol, puromycin, streptomycin or L-tryptophan inhibit the activity increase to the varying degrees. Figure 1 shows the result of a typical time course experiment on the action of some of these inhibitors. It is clear that DNase affects the final level of enzyme formation without affecting the initial rate of the activity increase. In contrast, other inhibitors tested affected both the initial rate and the final level attained. The partial inhibition (20-50 %) exhibited by RNase, chloramphenicol or puromycin is in accordance with the partial inhibition of $C^{14}$-leucine incorporation to proteins in this system except that RNase inhibits the incorporation completely. The significance of this RNase effect became clearer when the fractionation studies were carried out and will be considered in detail in a subsequent communication. The inhibitory effect of L-tryptophan appears to be specific since no such effects were found with equimolar amounts of 17 other L-amino acids tested.

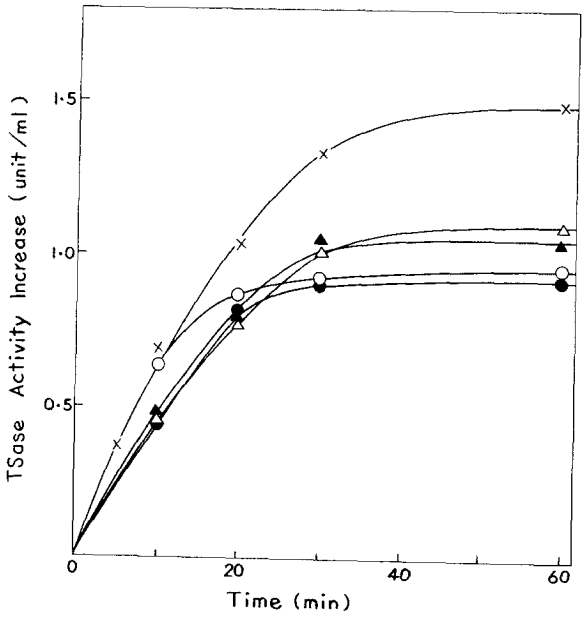


Fig. 1 Time course of the inhibition

—×— complete
—○— + DNase (10 μg/ml)
—●— + RNase (20 μg/ml)
—△— + Chloramphenicol (200 μg/ml)
—▲— + L-tryptophan (1 mg/ml)

Non-dialyzed crude extract was used in this experiment. The initial activity was 6.2 units/ml. For other conditions and procedures, see Table 1.

Newly formed TSase activity has been located entirely in the supernatant fraction obtained by centrifuging the incubation mixtures at 105,000 x g for 180 minutes. The immunological assays for TSase were also performed and the results are shown in Table 2. Since the extract used does not contain an appreciable amount of immunologically active but enzymatically inactive proteins, these results indicate that the increase in enzyme activity is accompanied by the similar increase in protein immunologically reactive with anti-TSase serum.

Table 2

Immunological assays for TSase*

| Sample | Additions | TSase activity found (units) | TSase activity neutralized (units) |
|---|---|---|---|
| 0 min. control | buffer | 0.90 | -- |
| " | Antiserum | 0.51 | 0.39 |
| 30 min. incubated | buffer | 1.18 | -- |
| " | Antiserum | 0.78 | 0.40 |

*0.2 ml of each sample was incubated with 0.3 ml of either buffer or antiserum (made against extracts of strain T3) at 2°C for 15 minutes and was assayed for TSase activity.

In addition to the B protein of TSase so far described, activities of the A protein of TSase and of indoleglycerol phosphate synthetase also increased to the similar extent in the present system. Thus a series of enzymes in the tryptophan pathway seem to be "formed" under the conditions used.

A preliminary experiment (Haruna and Yura, unpublished) suggests that some $C^{14}$-labelled amino acids can actually be incorporated to form peptide bonds in the crystals of the A protein. Further experiments are being carried out to evaluate how much, if

any, of the enzyme activity increase represents the de novo synthesis of the A protein.

Meanwhile, the crude extract has been fractionated by centrifugations at 105,000 x g and the three fractions obtained ($P_{30}$, $P_{60-180}$ and $S_{180}$) were tested either alone or in combination for TSase formation. As can be seen in Table 3, either $P_{30}$ or $P_{60-180}$ in combination with $S_{180}$ gave rise to the increase in TSase activity. However, these two systems are quite distinct in that the activity increase in the $P_{30}$ + $S_{180}$ system, but not in the $P_{60-180}$ + $S_{180}$ system, is dependent on the genetically specific DNA. The characterization of these systems will be a subject of subsequent communications.

Table 3

Activities of various fractions in TSase formation

| Fraction used* | Increase in TSase activity** (unit/ml) |
|---|---|
| $P_{30}$ | 0.18 |
| $P_{60-180}$ | 0.24 |
| $S_{180}$ | 0.12 |
| $P_{30}$ + $S_{180}$ | 1.15 |
| $P_{60-180}$ + $S_{180}$ | 1.25 |

*All the centrifugations were performed at 105,000 x g at 4°C and the pellets obtained were washed and resuspended in TMB.
$P_{30}$: Pellet after 30 min. run; pellet from 4 ml crude extract was used (protein 5.4 mg).
$P_{60-180}$: Pellet after 180 min. centrifugation of the supernatant obtained by 60 min. centrifugation. Pellet from 4 ml crude extract was used (protein 16.8 mg).
$S_{180}$: Supernatant after 180 min. run. Supernatant from 0.5 ml crude extract was used (protein 6.4 mg).
**For procedures and conditions, see Table 1.

We are grateful to Drs. T. Kameyama of Kanazawa University and K. Okamoto of Osaka University for their kind help in initiating the present study.

## References

Kameyama, T. and Novelli, G. D., Biochem. Biophys. Res. Communs., 2, 393 (1960)
Nisman, B. and Fukuhara, H., Compt. rend., 250, 410 (1960)
Vogel, H. J. and Bonner, D. M., Microbial Genetics Bull., 13, 43 (1956)
Wainwright, S. D., Can. J. Biochem., 37, 1417 (1959)
Yanofsky, C., Bacteriol. Rev., 24, 221 (1960)
Yanofsky, C. and Stadler, J., Proc. Nat. Acad. Sci., 44, 245 (1958)