PROTEIN TURNOVER IN ESCHERICHIA COLI

Evidence Against Serine Protease Involvement in Tryptophan Synthetase Degradation

Lee Alexander, C. Schwabe and Shin S. Chang
Department of Biochemistry
Medical University of South Carolina
Charleston, S.C. 29401

Received September 30,1975

SUMMARY: Degradation of E. coli tryptophan synthetase can be observed in a cell-free lysate. This system appears to respond to conditions of growth and nutritional state as expected from living organisms. Evidence is presented that the breakdown of tryptophan synthetase in this cell-free system is due to an enzymatic activity present in proportion to the amount of degrading activity in the bacteria at the time of sonication. Testing specifically for the breakdown of tryptophan synthetase, no evidence could be found for the participation of serine proteases in this process. Instead, our results support the recently advanced proposal that serine protease inhibitors interfere with protein synthesis in the living organism.

Recently serine proteases have been implicated in the catalysis of protein turnover in E. coli (1). Subsequent experiments published by others have shown that serine protease inhibitors might be responsible for the inhibition of protein synthesis rather than degradation (2). Utilizing a cell-free system for the investigation of the degradative mechanisms specific for tryptophan synthetase, we have been unable to demonstrate any effect of serine protease inhibitors. The quasi in vitro system utilized to study protein degradation responds to a variety of conditions analogous to living organisms (3). For example, degradative activity is lowest if the bacteria are harvested during the logarithmic growth phase and is very high if nitrogen or carbon starvation has been imposed prior to sonication. Evidence is presented in this paper that the degradation of tryptophan synthetase as observed in E. coli lysates may well be related to the physiological degradative process and does not seem to depend on serine proteases.

MATERIALS: Several E. coli strains (A2/F'A2, B-8, and a high tryptophan synthetase strain) have been obtained from Dr. C. Yanofsky. Tryptophan and serine

were obtained from Schwarz/Mann and indole, p-dimethylaminobenzaldehyde and pyridoxal phosphate from Eastman Chemical Company. Acid-hydrolyzed casein was prepared by exposing a commercial sample of casein (Schwarz/Mann) to 6 N HCl at 105° for 10 hours. The single-strength basic medium of Vogel and Bonner (4) was used with various supplements.

EXPERIMENTS: Bacteria for these experiments were grown in 2-liter Fernbach-flasks on a platform shaker at 37°. The B-8 mutant of E. coli, which produces only the A protein of tryptophan synthetase, was grown in a minimal media supplemented with 2.5 µg/ml of indole and 500 mg of acid-hydrolyzed casein per liter (5). The A2/F'A2 mutant produces only the B protein of the tetrameric tryptophan synthetase while the wild strain produces both chains. To start a new experiment organisms from slant cultures were spread on agar plates and single colonies isolated for inoculation. After reaching maximum density the cultures were harvested in a Sorvall RC-2B centrifuge using 500 ml polycarbonate bottles. Prior to sonication the bacteria were suspended to a density of 1×10^{11} organisms per ml, usually in a 0.1 M potassium phosphate buffer at pH 7.5. The sonicates were centrifuged at 20,000 rpm in an SS-34 rotor of the Sorvall RC-2B centrifuge. The supernatant thus obtained was incubated with sufficient penicillin and streptomycin (100 units each per ml) to inhibit bacterial growth for at least 48 hours. At the end of each experiment several nutrient agar plates were inoculated to test for survival or contamination. In order to test this system for responsiveness to situations known to stimulate protein degradation in the living organisms, slight modifications of the growth conditions were imposed. To test for the effect of nitrogen and carbon starvation the bacteria were harvested under sterile conditions and resuspended in minimal media devoid of a nitrogen or carbon source. After varying times of starvation the organisms were again harvested, resuspended, and sonicated as before. On other occasions the effect of the serine protease inhibitors administered before sonication was tested by addition of these compounds to the culture prior to the last two divisions.

Assays of the degradation of either the A or B protein in the B-8 or A2/F'A2 mutant respectively were performed with excess of the complimentary chain added at the time of tryptophan synthetase assay. The degradation of the single A or B chains in the extracts was always allowed to proceed without addition of the complimentary chain until the tryptophan synthetase assay was performed. The disappearance of tryptophan synthetase from various lysates of starved E. coli cultures grown in the presence of serine protease inhibitors or from lysates, supplemented with serine protease inhibitors after sonication, was tested by incubating the extracts at 37°. Aliquots were withdrawn at approximately hourly intervals and tested for tryptophan synthetase activity remaining, using the assay conditions of Wilson and Crawford (6).

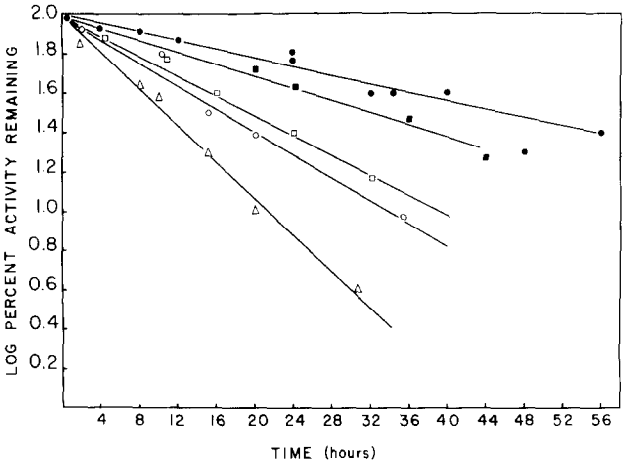

Figure 1. Disappearance of tryptophan synthetase activity from the lysates of various E. coli strains.

●—● B-8 (A protein),

■—■ W 3110 plus exogenous B protein, □—□ W3110,

○—○ A2/F'A2 (B protein),

△—△ A2/F'A2 lysed after nitrogen starvation. The B protein was added to the wild-type extract after each determination of tryptophan synthetase activity (open squares). A second determination was then performed with the additional B protein present (closed squares). Addition of A instead of B protein did not lead to any increase of activity.

RESULTS: The results obtained with extracts from B-8, A2/F'A2, and wild-type 3110 are depicted in Figure 1. Tryptophan synthetase does disappear from these extracts as a function of time and A protein seems to be much more stable than the B protein of the same enzyme. The quasi half-lives for A protein and B protein are 26 and 12 hours respectively and the disappearance of the B protein is approximately parallel to the disappearance of total tryptophan synthetase activity in wild-type 3110. These results suggest that the wild-type E. coli might have a limiting content of B chain and that the addition of B chain to the extract after several hours incubation should restore tryptophan synthetase activity to the level commensurate with the level of the remaining A chain. The result depicted in Figure 2 appears to support this assumption although other less likely interpretations are possible. The effect of nitrogen and carbon starvation seems as expected from studies of the living microorganism (7). The degradative activity in these cultures is much greater than in cultures harvested during the logarithmic growth phase. Further evidence for the enzymatic nature of the degradation is derived from an experiment where the extract of a starved culture was heated to 56° for three minutes prior to the determination of the tryptophan synthetase half-life. Heating under these conditions appears to denature the degradative activity and, as a consequence, stabilizes the tryptophan synthetase such that its apparent half-life becomes twice that of the unheated extract

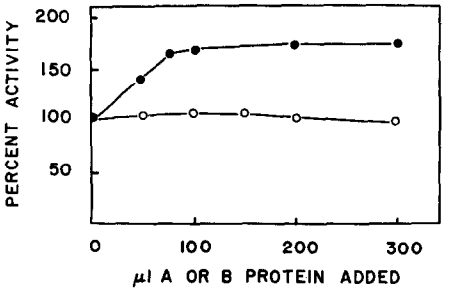

Figure 2. To a lysate of W3110 either nothing or A protein was added prior to tryptophan synthetase determination O—O (both lines identical). If B protein was added a significant increase in total tryptophan synthetase was observed ●—●. Assays were performed immediately after sonication.

(Fig. 3). The effect of a variety of serine protease inhibitors on the degradation of tryptophan synthetase is depicted in Table I. The half-life of tryptophan synthetase in the presence of these inhibitors is unchanged or even shorter than the control values. This is true for the typical degradative rate observed in our system as well as for the accelerated degradative rate induced

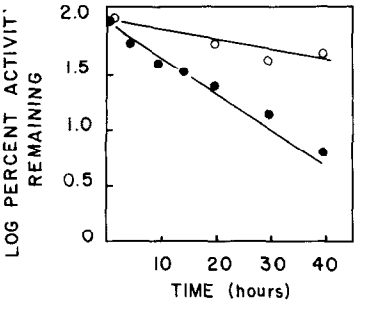

Figure 3. The effect of heating on the stability of the B protein in an A2/F'A2 lysate. ●—● Untreated material, ○—○ heated to 56° for 3 min prior to incubation at 37° for the degradation rate determination.

TABLE I

THE EFFECT OF VARIOUS SERINE PROTEASE INHIBITORS ON THE RATE OF DISAPPEARANCE OF TRYPTOPHAN SYNTHETASE ACTIVITY IN LYSATES FROM CONTROL AND STARVED CULTURES OF THE A2/F'A2 MUTANT OF *E. COLI*

Inhibitor	In Vitro Half-Lives (hrs)	
	Control	C-starved
None	16 ± 4	8 ± 4
TLCK	12	3
TPCK	10	4
Benzamidine	14	8
Phenylarsonate	16	7
PMSF	13	8
ε-Amino Caproate	16	9

by carbon or nitrogen starvation. If the serine protease inhibitors are added to the culture of E. coli prior to the last two divisions, the only effect observed after sonication is a lower initial activity of tryptophan synthetase but an unchanged slope of disappearance as a function of time. This result corroborates the finding that macromolecular synthesis rather than degradation might be inhibited.

DISCUSSION: From the experiments presented it appears that E. coli can either produce or activate a proteolytic enzyme in response to unfavorable nutritional states. These enzymes, once activated, should be detectable even after lysis and thus be amendable to investigations in cell-free systems. Advantages of such systems are great, but the uncertainty of appropriate correlation to the living organism is ever present. The fact that the response of the living organism can be duplicated in our experiments speaks strongly for their validity in terms of adaptive protein turnover. In addition, β-galactosidase, known to be a very stable enzyme, did not decay measurably in our system (not shown).

The inhibitors, tested extensively by two different persons several months apart, did not inhibit tryptophan synthetase disappearance but appeared to stimulate it in some cases. Addition of serine protease inhibitors to the cultured medium prior to the last two division cycles led to a 50% reduction of trytophan synthetase present in the extract. These findings corroborate those of Rossman et al. (2), who reported a reduction of macromolecular synthesis under the influence of serine protease inhibitors.

It is of great interest to note that, in the tetrameric protein tryptophan synthetase, one kind of subunit is so much more susceptible to degradation than its partner protomer. The experiment depicted in Figure 2 suggests that this situation prevails in the living organisms as shown by the excess of A protein. We have not been able to alter significantly the half-life of A protein by any of the measures that would greatly influence the stability of the B protein. This finding suggests that short-term regulation of tryptophan synthesis may be due to modulation of B protein levels.

REFERENCES

1. Prouty, W. F., and Goldberg, A. L., (1972) J. Biol. Chem 247, 3341-3352
2. Rossman, T., Morris, C., and Troll, W., (1974) J. Biol. Chem. 249, 3412-3417.
3. Schwabe, C. (1975) in Intracellular Protein Catabolism, (H. Hanson and P. Boley, Eds.), Ambrosius Barth Verlag, Leipzig.
4. Vogel, H. J., and Bonner, D. M (1956) J. Biol. Chem. 218, 97-101.
5. Yanofsky, C. (1971) J. Am. Med. Assoc. 218, 1026-1035.
6. Wilson, D. A., and Crawford, I. P., (1965) J. Biol. Chem. 240, 4801-4808.
7. Pine, M. J. (1975) in Intracellular Protein Turnover, (R. T. Schimke and N. Katunuma, Eds.) Academic Press, N.Y., pp 65-76.