

METABOLIC REGULATION OF AMINOACYL-tRNA  
SYNTHETASE FORMATION IN BACTERIA

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## SUMMARY

The cellular levels of several aminoacyl-tRNA synthetases vary with the rate of growth of Salmonella typhimurium and of Escherichia coli in different media. Over a seven-fold range in growth rate the levels of arginyl- and of valyl-tRNA synthetase change approximately 2.5-fold; leucyl-tRNA synthetase varies less. The apparent differential rate of formation of arginyl-tRNA synthetase changes to its new, rapid steady state rate immediately after cells are shifted from poor to rich media. This new aspect of synthetase control is provisionally called metabolic regulation.

Discovery of the involvement of aminoacyl-tRNA synthetase in various regulatory processes in bacteria (1) has heightened interest in how the biosynthesis of these indispensable enzymes is controlled. Studies on the control of synthetase formation have been largely at the descriptive level; neither the biochemical nor the genetic elements of synthetase regulation have been identified with certainty. What has been established is that growth conditions which restrict the supply of an amino acid will, in many cases, specifically accelerate the synthesis of the cognate synthetase. This phenomenon has been observed in Escherichia coli with the aminoacyl-tRNA synthetases for phenylalanine (2, 3), isoleucine (2, 4, 5, 6), arginine (4), histidine (4), valine (6), leucine (6), methionine (7), proline (7) and threonine (7), and in Salmonella typhimurium for isoleucine (6), valine (6) and histidine (8). Acceleration of synthetase formation is not always observed, even for the synthetases just listed. The manner in which restriction is imposed, and the nature of the bacterial strain used, seem to have a pronounced effect on the response (cf. 2, 3, 5). This

variability, combined with some aspects of synthetase formation during the recovery of cells from amino acid restriction, leads us to believe that the response of individual synthetases to supply of their cognate amino acids does not by itself define a clear and unambiguous control system for regulating synthetase formation.

Recently we have discovered a facet of synthetase regulation that has hitherto escaped recognition. In this report we show that there is a metabolic regulation which results in the coupling of synthetase formation to growth rate. This discovery has two immediate implications: (a) observations on levels of individual synthetases in cells growing under different conditions must be interpreted in the light of this metabolic control, and (b) the study of synthetase regulation should now be broadened to include possible secondary effects of other cellular control systems.

#### MATERIALS AND METHODS

Organisms. *S. typhimurium* strain NT1 is a prototrophic strain derived from LT2. *E. coli* strain NCl is a prototrophic strain derived from B/r.

Media and methods of cultivation. Bacterial cultures were grown aerobically on a rotary-action New Brunswick shaker at 37°C. All growth studies were carried out in a minimal medium (designated MOPS), consisting of 40 mM potassium morpholinopropane sulfate pH 7.2, 4 mM Tricine (N-tris- [hydroxymethyl]-methyl glycine) pH 7.2, 9.32mM  $\text{NH}_4\text{Cl}$ , 0.218 mM  $\text{K}_2\text{SO}_4$ ,  $5 \times 10^{-4}$  mM  $\text{CaCl}_2$ , 1.0 mM  $\text{MgCl}_2$ , 0.01 mM  $\text{FeSO}_4$ , 1.5 mM NaCl, 1.27 mM  $\text{K}_2\text{HPO}_4$ ,  $3 \times 10^{-7}$  mM  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ ,  $3 \times 10^{-6}$  mM  $\text{CoCl}_2$ ,  $4 \times 10^{-5}$  mM  $\text{H}_3\text{BO}_3$ ,  $8 \times 10^{-6}$  mM  $\text{MnCl}_2$ ,  $1 \times 10^{-6}$  mM  $\text{ZnSO}_4$ , and  $1 \times 10^{-6}$  mM  $\text{CuCl}_2$ . The final pH of the medium was 7.0. Carbon sources used were 0.4% potassium acetate, 0.4% succinic acid disodium salt and 0.4% D-glucose. For richer media the basic medium plus glucose (MOPS-glucose) was supplemented with either 1.5% casamino acids (MOPS-glucose-CA) or 1.0% bacto-tryptone with 0.5% yeast extract (MOPS-TGYE). Shifts in medium composition were accomplished by centrifugation at 4°C and re-

suspension in prewarmed medium. Growth was measured and expressed in the manner previously described (4).

Preparation of extracts. Culture samples were rapidly chilled and cells pelleted by centrifugation at 12,100 x g for 10 minutes at 3°C. The pellet was resuspended in 5 ml of 6 mM potassium phosphate buffer, pH 7.3, containing 6 mM 2-mercaptoethanol. The cells were again centrifuged, resuspended in 2 ml of the same buffer, and then treated with a Branson Sonifier for 0.5-1.0 min at a power setting of 3.5. Control experiments were run to insure that there was no differential loss of enzyme from cells grown in different media, since slowly growing cells are more difficult to disrupt than rapidly growing ones. After sonic disruption, cell debris was removed by centrifugation at 27,000 x g for 15 min. The extract was then dialyzed against 100 volumes of the same buffer at 0-4°C and assayed within 24 hours. Protein content of the extracts was determined by the method of Lowry et. al. (9) using bovine serum albumin as a standard.

Aminoacyl-tRNA synthetase assays. The aminoacylation of tRNA was measured in reaction mixtures identical to those previously described (10). Mixtures were incubated at 37°C and terminated at 10 min (5 min for valyl-tRNA synthetase) by the addition of 5% trichloroacetic acid (TCA) containing 1 mg/ml of the appropriate L-amino acid. The precipitates were collected on glass fiber filters (Reeve Angel 934 AH) and washed with 5% TCA and 67% ethanol. Samples were dried and counted in a Beckman LS-230 scintillation counter. Specific activities are given in units/mg protein, 1 unit being the amount of enzyme attaching 1  $\mu$ mole of amino acid to tRNA/hr under the conditions employed. Relative specific activities are normalized to that of extracts from cells grown in MOPS-glucose.

#### RESULTS AND DISCUSSION

Cultures of S. typhimurium NT1 were established in balanced growth in various media, and samples were taken for enzyme assay. The results obtained for arginyl-tRNA synthetase are presented in Fig. 1. Over a 7-fold

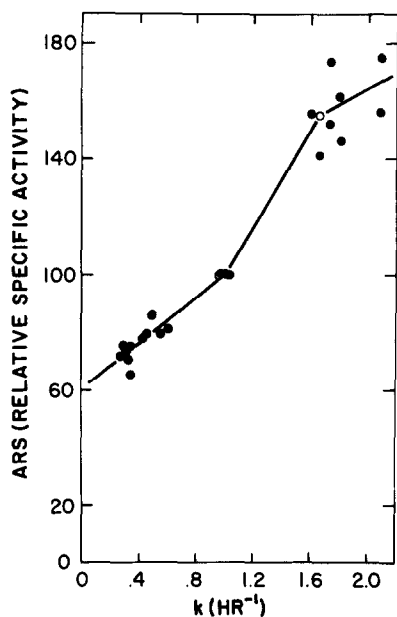


Fig. 1.

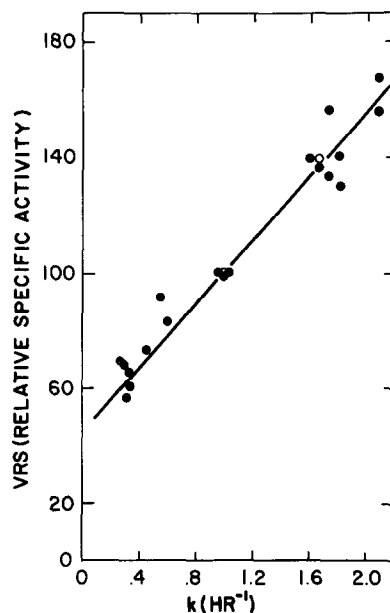


Fig. 2.

Fig. 1. Level of arginyl-tRNA synthetase at different growth rates in *S. typhimurium* NT1. The relative specific activity (ARS) is plotted as a function of the first order constant for growth ( $k$ ). Each solid circle represents an independent measurement of growth rate and synthetase level in a different culture; the open circles indicate the coincidence of two or more values. The values clustered about  $k = 0.3$  are for cells growing in MOPS-acetate;  $k = 0.5$ , MOPS-succinate;  $k = 1.0$ , MOPS-glucose;  $k = 1.7$ , MOPS-glucose-CA;  $k = 2.1$ , MOPS-TGYE. The absolute level of synthetase in cells growing in MOPS-glucose was 0.18 units/mg protein.

Fig. 2. Level of valyl-tRNA synthetase (VRS) at different growth rates in *S. typhimurium* NT1. For explanation see legend to Fig. 1. The absolute level of synthetase in cells growing in MOPS-glucose was 0.50 units/ml protein.

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range of growth rate there is a variation of approximately 2.5-fold in enzyme activity. Fig. 2. contains data for valyl-tRNA synthetase. The correlation of specific activity with growth rate is virtually the same as for the arginine enzyme. Results not shown indicate a definite but much less marked variation in the specific activity of leucyl-tRNA synthetase with growth rate.

Similar measurements, but fewer in number, have been made on cultures of E. coli NC1. As shown in Fig. 3, the results for the arginine, leucine and valine enzymes resemble qualitatively the results in S. typhimurium. Variations consistent with these, but over a lesser growth rate range may be seen in the data of Hirshfield and Zamecnik (11) for the glutamate, arginine and lysine enzymes of E. coli K12.

This behavior is interesting from several view points. (a) Synthetases now join other parts (tRNA and ribosomes) of the cell's translation apparatus that are expanded in number during rapid growth. (b) It is difficult to imagine that this response is mediated by changes in the levels of aminoacylated tRNA (as has been suggested for the behavior of histidyl-tRNA synthetase (8)), unless one postulates a decrease in tRNA charging during rapid growth in rich media. (c) Either an active or a passive control system could be responsible for this aspect of synthetase regulation. One can envision, for example, the structural cistrons for these enzymes being responsive to varying degrees to the positive (or negative) control elements that regulate ribosome synthesis. Or one can employ the cell model of Maaløe (12) and imagine that this metabolic regulation of synthetase passively reflects the increased availability of transcription-translation machinery resulting from widespread repression of most catabolic and biosynthetic operons during growth in rich media.

Whichever is the case, the experiment presented in Figure 4 emphasizes the reality of this mode of synthetase regulation. Shift of a culture from MOPS-acetate medium to MOPS-glucose-CA medium causes a nearly immediate acceleration of the differential rate of arginyl-tRNA synthetase formation, as for ribosome synthesis.

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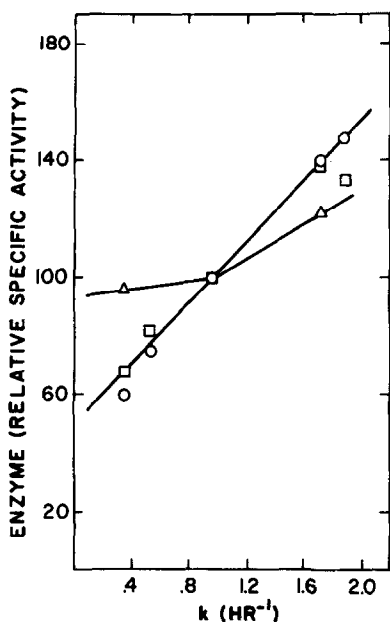


Fig. 3.

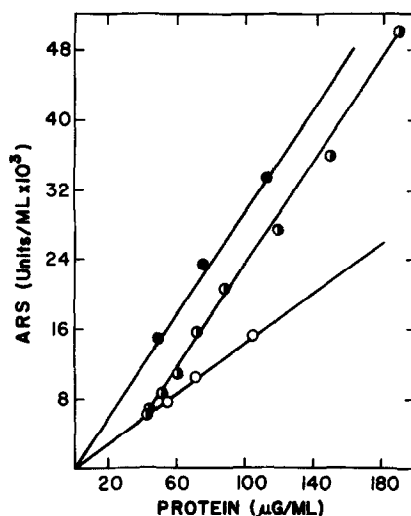


Fig. 4.

Fig. 3. Synthetase levels at different growth rates in *E. coli* NCl. The manner of plotting the results and the nature of the growth media are as described for Fig. 1. Arginyl-tRNA synthetase, ○; valyl-tRNA synthetase, ◻; leucyl-tRNA synthetase, △. The absolute values of the synthetase levels in cells growing in MOPS-glucose were for arginine, 0.20, for valine, 0.60, and for leucine, 0.12 units/mg protein.

Fig. 4. Differential rate of accumulation of arginyl-tRNA synthetase in *S. typhimurium* NT1 at slow growth rate, fast growth rate, and after a shift up. Enzyme activity (ARS) per ml of culture is plotted as a function of total protein per ml of culture. One culture was in steady state growth in MOPS-acetate, ○; one culture was in steady state growth in MOPS-glucose-CA, ●; one culture growing in MOPS-acetate was transferred at the time indicated by the arrow to MOPS-glucose-CA, ◐.

#### REFERENCES

1. Neidhardt, F. C., *Bacteriol. Rev.* **30**, 701 (1966).
2. Nass, G. and Neidhardt, F. C., *Biochim. Biophys. Acta* **134**, 347 (1967).
3. Nass, G., in *Molecular Genetics*, edited by Wittmann, H. G. and Schuster, H., Springer-Verlag, Berlin (1968) p. 147.
4. Williams, L. S. and Neidhardt, F. C., *J. Mol. Biol.* **43**, 529 (1969).
5. Gahr, M. and Nass, G., *Molec. Gen. Genetics*, in press.
6. Archibold, E. R. and Williams, L. S., *J. Bacteriol.* **109**, 1020 (1972).
7. McGinnis, E. and Williams, L. S., *J. Bacteriol.* **108**, 254 (1971).
8. McGinnis, E. and Williams, L. S., *J. Bacteriol.* **109**, 505 (1972).
9. Lowry, D. H., Rosebrough, N. V., Farr, A. L. and Randall, R. J., *J. Biol. Chem.* **193**, 265 (1951).

10. Anderson, J. J. and Neidhardt, F. C., *J. Bacteriol.* 109, 307 (1972).
11. Hirshfield, I. N. and Zamecnik, P. C., *Biochim. Biophys. Acta* 259, 330 (1972).
12. Maaløe, O., *Developmental Biology, Suppl.* 3, 33 (1969).