# MULTIVALENT REPRESSION IN THE BIOSYNTHESIS OF THREONINE IN SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI*

Martin Freundlich**
Long Island Biological Association, Cold Spring Harbor, N. Y.

Received January 15, 1963

The repression of the formation of biosynthetic enzymes by the ultimate endproduct of their action is a widespread regulatory pattern in bacteria (Vogel, 1961). Recently this type of control mechanism has been examined in pathways where more than one endproduct is formed from common intermediates and two distinct phenomena, which appear to permit the efficient control of these endproducts, have been reported. In the first of these studies, it has been shown that more than one enzyme catalyzes the formation of the common intermediate. Each of these enzymes is specifically controlled by its respective endproduct (Stadtman et al., 1961; Smith et al., 1962). A second method for the regulation of the formation of enzymes of branched pathways has been reported in the synthesis of isoleucine, valine, and leucine (Freundlich et al., 1962). In this system isoleucine, valine, and leucine are required for the repression of the enzymes common to the formation of the three amino acids. This phenomenon has been termed multivalent repression.

In view of these results, it appeared interesting to examine other pathways for the occurrence of multivalent repression. This report presents evidence that the enzymes leading to threonine biosynthesis in Salmonella typhimurium and Escherichia coli are also subject to multivalent repression in that both threonine and isoleucine must be in excess for these enzymes to be repressed.

---

* This work was supported by grant AI-03501-03 from the National Institute of Allergy and Infectious Diseases, USPHS.

** Postdoctoral fellow of the National Institutes of Health, USPHS.

Aspartate $\xrightarrow{1}$ Aspartyl-P $\xrightarrow{2}$ Aspartic semialdehyde $\xrightarrow{3}$ Homoserine $\xrightarrow{4}$ $\xrightarrow{5}$

Threonine $\xrightarrow{6}$ → → → Isoleucine

Fig. 1. Pathway of threonine biosynthesis.

## METHODS

The following derivatives of S. typhimurium LT-2 were used: strain ile 217 which lacks threonine deaminase (enzyme 6, Fig. 1) and which therefore requires isoleucine, and strain thr 201, a threonine auxotroph blocked after the formation of homoserine. Two mutants of E. coli K-12 were also employed, namely: strain JHM544, a threonine deaminase negative mutant and strain 12B14, which is blocked after homoserine formation. The bacteria were grown in a chemostat (Novick and Szilard, 1950) in minimal medium (Davis and Mingioli, 1950), supplemented as indicated. Extracts were prepared by ultrasonic oscillation and aspartokinase activity (enzyme 1, Fig. 1) tested according to the methods of Stadtman et al. (1961). Homoserine dehydrogenase activity (enzyme 3, Fig. 1) was measured by following the reduction of $NADP^+$ in the presence of 100 μmoles of DL-homoserine. The procedure of Gibson et al. (1962) was used except that the reaction mixture was incubated at room temperature instead of 37°C. The assay for threonine synthetase (enzymes 4, 5 Fig. 1) was that of Watanabe and Shimura (1955). The threonine formed was measured microbiologically using S. typhimurium strain thr 201.

## RESULTS AND DISCUSSION

By utilizing threonine and isoleucine auxotrophs of S. typhimurium, it was found that the enzymes leading to threonine formation could be repressed only in the presence of excess threonine and isoleucine (Table 1). When either of these amino acids were added in limiting amounts, (i.e. 10 μg per ml), in the presence of an excess of the other compound, the enzymes were derepressed. Other investigators, using E. coli (Cohen and Hirsch, 1954;

Wormser and Pardee, 1958; Stadtman et al., 1961) and other bacteria (Gibson et al., 1962; Nara et al., 1961), have found that the levels of these enzymes were not affected by the presence of exogenous threonine. Therefore, to determine whether the present results were specific for Salmonella, the experiments were repeated with E. coli. As may be seen from the data in Table 2, the results obtained were the same as with S. typhimurium. The failure of other workers to find repression in the threonine pathway can probably be attributed, at least in the case of E. coli, to the use of strains prototrophic for threonine.

TABLE 1

Effect of Threonine and Isoleucine on Repression of the Threonine Enzymes in S. typhimurium.

| Strain | Growth Supplement | Enzyme Activity | | |
|---|---|---|---|---|
| | | "threonine" aspartokinase* | homoserine dehydrogenase | threonine synthetase |
| thr 201 | Threonine + Isoleucine (50 µg/ml) (50 µg/ml) | 11 | 0.081 | enzyme |
| | Threonine + Isoleucine (10 µg/ml) (50 µg/ml) | 84 | 1.391 | absent |
| ile 217 | Isoleucine + Threonine (50 µg/ml) (50 µg/ml) | 10 | 0.052 | 0.13 |
| | Isoleucine + Threonine (10 µg/ml) (50 µg/ml) | 81 | 0.928 | 1.44 |

* "Threonine" aspartokinase activity is considered as total aspartokinase activity measured in the presence of 10 µmoles of L-lysine.

The cells were grown in a chemostat for 3 generations. The glucose concentration of the repressed cells was limited in order to maintain equivalent turbidity. The L-enantiomorph of the amino acid was used in all cases. Enzyme activity is expressed as follows: aspartokinase - OD units x 1000 per mg protein per 30 min; homoserine dehydrogenase - µmoles NADPH formed per mg protein per hr; threonine synthetase - µmoles threonine formed per mg protein per hr. For other conditions see text.

TABLE 2

Effect of Threonine and Isoleucine on

Repression of the Threonine Enzymes in E. coli.

| Strain | Growth Supplement | Enzyme Activity | | |
|---|---|---|---|---|
| | | "threonine" aspartokinase* | homoserine dehydrogenase | threonine synthetase |
| JHM544 | Threonine + Isoleucine (50 µg/ml) (50 µg/ml) | 36 | 0.043 | enzyme |
| | Threonine + Isoleucine (10 µg/ml) (50 µg/ml) | 180 | 0.870 | absent |
| 12B14 | Isoleucine + Threonine (50 µg/ml) (50 µg/ml) | 24 | 0.037 | 0.19 |
| | Isoleucine + Threonine (10 µg/ml) (50 µg/ml) | 156 | 0.696 | 3.82 |

For conditions see Table 1.

Since the intermediates for isoleucine biosynthesis are formed directly from threonine, the physiological role for the additional requirement of excess isoleucine for repression of the threonine enzymes is obscure. However, if the cell contains separate, non-interchangeable threonine pools for protein and for isoleucine, it is conceivable that an excess of threonine in one pool could, by repression, stop the flow of threonine into the other pool. This would be prevented if isoleucine, in addition to threonine, was necessary for the repression of the threonine synthesizing enzymes.

That separate pools for threonine and isoleucine may exist is indicated by the fact that the enzymes leading to isoleucine and valine biosynthesis are derepressed when a threonine auxotroph is grown with limiting threonine (Table 3). Derepression of these enzymes under these conditions does not occur if excess isoleucine is present. Thus, the increase in the isoleucine-valine enzymes is not caused directly by limiting amounts of threonine but by

a decrease in isoleucine formation. It would seem unlikely that threonine and isoleucine could simultaneously be growth limiting. However, if the threonine pool leading to isoleucine formation became depleted before that of the threonine-protein pool, isoleucine would be the only amino acid being limited. This limitation would account for the simultaneous derepression of the threonine and isoleucine-valine enzymes. This explanation is, of course, highly speculative.

TABLE 3

Effect of Threonine and Isoleucine on the Repression of the Isoleucine-Valine Enzymes in *typhimurium thr* 201

| Growth Supplement | Enzyme Activity | |
|---|---|---|
| | threonine deaminase | dihydroxy acid dehydrase |
| Threonine (50 μg/ml) | 4.5 | 2.1 |
| Threonine (10 μg/ml) | 130.3 | 53.0 |
| Threonine (10 μg/ml) + Isoleucine (50 μg/ml) | 10.0 | 3.4 |

Enzyme activity is expressed as μmoles keto acid formed per mg protein per hr. The methods for measuring both enzymes have been described (Freundlich *et al.*, 1962). For other conditions see Table 1.

The finding that threonine and isoleucine are both required for the repression of the enzymes leading to threonine, as well as the previous report of multivalent repression in the biosynthesis of isoleucine and valine (Freundlich *et al.*, 1962), point to a significant role for this type of control mechanism in the regulation of pathways that serve more than one physiological function.

## REFERENCES

Cohen, G. N., and Hirsch, M-L., J. Bacteriol., 67, 182 (1954).
Davis, B. D., and Mingioli, E. S., J. Bacteriol., 60, 17 (1950).
Freundlich, M., Burns, R. O., and Umbarger, H. E., Proc. Natl. Acad. Sci., 48, 1804 (1962).
Gibson, K. D., Neuberger, A., and Tait, G. H., Biochem. J., 84, 483 (1962).
Nara, T., Samejima, H., Fujita, C., Ito, M., Nakayama, K. and Kinoshita, S., Agric. Biol. Chem. (Japan), 25, 532 (1961).
Novick, A., and Szilard, L., Science, 112, 715 (1950).
Smith, L. C., Ravel, J. M., Lax, S. R., and Shive, W., J. Biol. Chem. 237, 3566 (1962).
Stadtman, E. R., Cohen, G. N., LeBras, G., and deRobichon-Szulmajster, H., J. Biol. Chem., 236, 2033 (1961).
Vogel, H. J., in Control Mechanisms in Cellular Processes, ed. D. M. Bonner, Ronald Press Co., New York (1961).
Watanabe, Y., and Shimura, K., J. Biochem. (Japan), 42, 181 (1955).
Wormser, E. H. and Pardee, A. B., Arch. Biochem. Biophys., 78, 416 (1958).