EFFECT OF THE ANTIBIOTIC BORRELIDIN ON THE REGULATION OF THREONINE BIOSYNTHETIC ENZYMES IN E. COLI

G. Nass, K. Poralla, and H. Zähner
Max-Planck-Institut für Molekulare Genetik, Berlin, Germany,
and Institut für Biologie der Universität, Tübingen, Germany

Received November 26, 1968

The biosynthesis of threonine in E. coli is regulated by multivalent repression: both amino acids, threonine and isoleucine, must be present in excess for the repression of the threonine biosynthetic enzymes to occur (Freundlich 1963). Recently it has been observed, that thiaisoleucine resistant mutants of E. coli K12, having an altered isoleucyl-tRNA-synthetase, exhibit derepressed levels of the homoserinedehydrogenase (Dwyer and Umbarger 1968). This indicates that isoleucyl-tRNA-synthetase takes part in forming the repressor for the threonine biosynthetic enzymes and therefore that the free amino acid itself is not the repressor.

In this report evidence will be presented that also the threonyl-tRNA-synthetase is involved in the repression of the threonine-biosynthetic enzymes. This will be shown by means of the antibiotic Borrelidin.

Borrelidin is known to prevent the growth of E. coli cells and to inhibit the enzymatic activity of the threonyl-tRNA-synthetase in vitro (Hütter et al. 1966). It therefore seemed suitable for investigating the question of whether the threonyl-tRNA-synthetase takes part in the repression of the threonine biosynthetic enzymes.

Methods

1. Growth of Bacteria

E. coli K12 and B wild strains were used.

Cultures were grown aerobically at 37° in minimal medium (Fraenkel and Neidhardt 1961) supplemented with 0.2% $(NH_4)_2SO_4$ and 0.4% glucose. In all of the experiments to be described this medium was supplemented with 2.5×10^{-3} molar L-lysine in order to repress the lysine-sensitive aspartokinase in these cells (Stadtman et al. 1961). When indicated Borrelidin (7 μg/ml final concentration) was added to exponentially growing cultures and the cells were allowed to grow for at least three generations in the presence of the antibiotic.

2. Preparation of cell-free extracts

Cell-free extracts from 300 ml samples of the cultures were prepared as described previously (Nass 1967); the buffers used for washing and resuspending the cells are listed with every enzyme assay.

3. Enzyme assays

All enzyme activities reported are averages of double determinations at two different protein concentrations.

a. Aminoacyl-tRNA-synthetases

were measured by determining the rate of attachment of the C^{14}-amino-acid to tRNA (Berg et al. 1961; Nass 1967). Enzyme activities are expressed as μMoles amino acid attached to tRNA per hour per mg protein

b. Threonine deaminase was measured by the method of Umbarger and Brown 1958. Enzyme activities are expressed as O. D. 540 mμ per mg protein per 10 min.

c. Aspartokinase-measurements

were performed according to the methods described by Black and Wright 1954 and Stadtman et al. 1961. Enzyme extracts were prepared in 0.03 molar potassium phosphate-buffer pH 6.8, containing 0.02 molar ethylmercaptan. The 1 ml incubation mixture contained: 20 μMoles ATP, 20 μMoles $MgCl_2$, 100 μMoles Tris-HCl pH 10.0, 200 μMoles KOH, 200 μMoles $NH_2OH.HCl$, and 28 μMoles Aspartate. The KOH and $NH_2OH.HCl$-solutions were mixed immediately before pipetting into the assay tube. The final pH of the incubation mixture was pH 8.0. The enzyme reaction took place for 30 minutes at 30°; it was stopped by the addition of 2 ml $FeCl_3$-reagent. After centrifugation of the assay tube for 30 minutes at 9000 rpm (Sorvall RC2B rotor SS34), the amount of asparto-hydroxamate-iron complex formed was determined by reading the OD of the supernatant in a Zeiss Spektrophotometer (CPMQ II) at 540 mμ. For each assay tube a control tube was run as the blank throughout the assay; it contained all the components except the substrate aspartate. Enzyme activities are expressed as OD 540 mμ per mg protein per 30 min.

d. Homoserinedehydrogenase assay

was performed according to the method described by Gibson et al. 1962. The extracts were prepared in 0.03 molar Potassiumphosphate-buffer pH 6.8 containing 0.02 molar Ethylmercaptan. The OD change of the reaction mixture at 340 mμ at 37° was followed for 5 minutes and read in intervals of 0.5 minutes after adding the cell-free extract. Enzyme activities are expressed as OD 340 mμ change per 0.5 minutes per mg protein multiplied by 1000.

4. The protein concentration of the cell-free extracts was determined by the standard colorimetric method (Lowry et al. 1951).

Results and Discussion

Table 1 shows that the threonyl-tRNA-synthetase activity in cell-free extracts of E. coli K12 grown in the presence of Borrelidin is inhibited, whereas the activity of all other aminoacyl-tRNA-synthetases tested stayed unchanged. The same pattern of aminoacyl-tRNA-synthetases activities is observed when Borrelidin is added to cell-free extracts in vitro 0.7 μg/ml final concentration (not shown in Table 1), i. e. the threonyl-tRNA-synthetase is inhibited almost completely, whereas the activities of the other amino-acyl-tRNA-synthetases remain unaffected. Similar results were obtained with extracts of E. coli B. The crucial experiment was to determine the activity of the threonine biosynthetic enzymes in extracts of cells grown in the absence and in the presence of growth limiting concentrations of Borrelidin.

Table 2 demonstrates that both strains K12 and B show more than a fourfold derepression of the two threonine biosynthetic enzymes when grown in the presence of the antibiotic. The specificity of the derepression process is demonstrated by the fact that the activity of threonine deaminase is the same in cells grown with or without Borrelidin (Table 2). Threonine deaminase was chosen as a control enzyme because the intracellular level of this enzyme reflects the isoleucine- and indirectly the threonine-pool in E. coli (Dwyer and Umbarger 1968). When E. coli K12 and B are grown in the presence of threonine (100 μg/ml) and Borrelidin (21 μg/ml) the growth limitation is almost abolished, but there is still a threefold derepression of the threonine specific aspartokinase. The activity of acetylornithase, another less related amino acid biosynthetic enzyme was also not influenced in cells

Table 1: Activity of various aminoacyl-tRNA-synthetases in E. coli K12 grown in the presence of Borrelidin

Aminoacyl-tRNA synthetase	cell-free extracts of cells grown without Borrelidin	in the presence of Borrelidin
threonyl-tRNA synthetase	0.024	0.002
methionyl-tRNA synthetase	0.030	0.031
arginyl-tRNA synthetase	0.119	0.117
phenylalanyl-tRNA synthetase	0.080	0.072
isoleucyl-tRNA synthetase	0.052	0.053
leucyl-tRNA synthetase	0.063	0.075
tyrosyl-tRNA synthetase	0.071	0.080
lysyl-tRNA synthetase	0.030	0.034
valyl-tRNA synthetase	0.168	0.192

E. coli K12 was grown for more than three generations in the absence and in the presence of growth limiting amounts Borrelidin (7 μg Borrelidin/ml final concentration). Cell-free extracts were prepared and the activity of various aminoacyl-tRNA-synthetases determined as described in the Methods-section.

grown in the presence of Borrelidin. Further control experiment showed that adding Borrelidin in vitro (0.7 μg/ml final concentration) had no effect on the measured activity of the aspartokinase and homoserine-dehydrogenase in cell-free extracts of E. coli K12 and B.

The reported results are compatible with the assumption that the threonyl-tRNA-synthetase is involved in the formation of the repressor for the threonine-biosynthetic enzymes out of threonine. This

Table 2: Effect of Borrelidin on growth rate and enzyme synthesis in E. coli K12 and B

Strain	Lysine containing Minimal-Medium supplemented with	growth rate (gen/hour)	Specific Activity of Aspartokinase	Homoserine-dehydrogenase	Threonine-deaminase
K12B	-	0.64	0.052	3.0	0.054
K12B	Borrelidin	0.33	0.305	11.0	0.048
B	-	1.05	0.050	2.0	0.306
B	Borrelidin	0.52	0.250	11.0	0.350

E. coli K12 and B were grown for more than three generations in minimal medium supplemented with the substance indicated in the table. Growth rates were calculated, cell-free extracts prepared and enzyme assays performed as described in the Methods-section.

assumption is based on two observations: Borrelidin inhibits specifically the enzymatic activity of the threonyl-tRNA-synthetase, and the threonine biosynthetic enzymes are derepressed in cells grown in the presence of Borrelidin. These results can be explained by assuming that Borrelidin inhibits the threonyl-tRNA-synthetase in vivo, to an extent that not enough threonine-charged tRNA can be produced, leading to growth limitation, in addition, the inhibition of the threonyl-tRNA-synthetase by Borrelidin causes a derepression of the threonine biosynthetic enzymes, since the formation of repressor from threonine by the threonyl-tRNA synthetase is also impaired. However, the alternative hypothesis that Borrelidin, beside inhibiting the threonyl-tRNA-synthetase also interferes with another unknown reaction leading from threonine to the actual repressor for the threonine biosynthetic enzymes has not yet been experimentally excluded. Analysing the molecular changes in Borrelidin resistant E. coli mutants might eludicate further the role of the threonyl-tRNA-synthetase in the regulation of threonine biosynthetic enzymes.

Acknowledgements: G. N. wishes to thank the Deutsche Forschungsgemeinschaft for generous support. The skillful technical assistance of Renate Hasenbank is gratefully acknowledged.

References

Berg, P., Bergmann, F. H., Ofengand, E. J., and Dieckmann, M.: J. Biol. Chem. 236, 1726 (1961)
Black, S., and Wright, N. G.: J. Biol. Chem. 213,27 (1955)
Dwyer, S. B., and Umbarger, H. E.: J. Bacteriol. 95,1680 (1968)
Fraenkel, D., and Neidhardt, F. C.: Biochim. Biophys. Acta 53, 96 (1961)
Freundlich, M.: Biochem. Biophys. Res. Commun. 10, 277 (1963)
Gibson, K.D., Neuberger, A., and Tait, G. H.: Biochem. J. 84, 483 (1962)

Hütter, R., Poralla, K. Zachau, H. G., and Zähner, H.: Biochem. Ztschr. 344, 190 (1966)
Lowry, O. H. Rosebrough, N. J., Farr, A. L., and Randall, R. J.: J. Biol. Chem. 193 265 (1951)
Nass, G.: Molec. Gen. Genetics 100, 216 (1967)
Stadtman, E. R., Cohen, G. N., Lebras, G., and H. D. Robichon-Szulmajster: J. Biol. Chem. 236, 2033 (1961)
Umbarger, H. E., and Brown, B.: J. Biol. Chem. 233, 415 (1958)