THE EFFECT OF ETHIONINE ON THE SYNTHESIS OF β-GALACTOSIDASE: FORMATION OF AN IMMUNOLOGICALLY CROSS-REACTING PROTEIN

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Received November 11, 1968

Ethionine, an analogue of methionine, inhibits growth of some microorganisms (Maw, 1961; Smith and Salmon, 1965a,b), is incorporated into proteins (Smith and Salmon, 1965 a,b; Gross and Tarver, 1955; Parks, 1958; Maw, 1961, 1966) and inhibits the synthesis of certain enzymes (Wainwright, 1959; Bernheim, 1959).

Yoshida (1958) and Yoshida and Yamasaki (1959) described the incorporation of ethionine into the α-amylase of Bacillus subtilis. When studying the purified enzyme, they found that one third of methionine was substituted by ethionine. However, the physico-chemical and biological properties of the isolated enzyme were not altered.

We studied the effect of ethionine on the synthesis of β-galactosidase in Escherichia coli. Escherichia coli M1-30 (i^+, z^+, y^+) producing inducible β-galactosidase was employed throughout. Medium "56" (Cohn and Monod, 1951) containing 0.2% glycerol as a carbon source was used for cultivation.

The growth of the bacterial culture was followed by measuring optical density in a spectrophotometer CF 4(Optica Milano) at 600 nm. Proteins were determined according to Lowry et al.(1951).

Thiomethyl -ß-D-galactopyranoside (TMG) at a concentration of 0.001 M served as inducer of ß-galactosidase. Activity of ß-galactosidase was assyed according to Janeček and Rickenberg (1964). Specific ß-galactosidase activity was defined as the number of millimicromoles of o-nitrophenyl-ß-D-galactopyranoside hydrolysed per min per mg of protein. Antiserum against pure ß-galactosidase was prepared according to the method of Janeček and Rickenberg (1964). Rabbits were injected into their footpads at 2-week intervals over a 6-week period, with 1 mg per injection of the ß-galactosidase preparation (specific activity 500.000) diluted in 1 ml of Freund's adjuvant. Two weeks after the final injection the animals were bled from heart and the serum collected. The serum was further purified by adsorption with an extract prepared from non-induced ML-30 cells. The precipitate was discarded and the supernate used in further experiments. The method of Masters and Pardee (1962) was used to detect immunologically cross-reacting material (CRM).

It was found that ethionine inhibits the synthesis of ß-galactosidase in the strain used. The differential rate of ß-galactosidase synthesis decreases with increasing ethionine concentrations, 0.01 M ethionine bringing about almost complete suppression of the synthesis of enzymically active ß-galactosidase.

Several possibilities should be considered concerning the mechanism of inhibition of the synthesis of ß-galactosidase by ethionine. First of all, the transport of the inducer and, hence, the enzyme induction might be inhibited. However, this mechanism of inhibition is invalidated by the fact that in the strain ML-308 of Escherichia coli (i^-, z^+, y^+)

with constitutive β-galactosidase production ethionine also inhibits β-galactosidase synthesis (Spížek and Janeček, 1967). Another possibility that might be considered was a differential inhibition of the transcription of the lac operon, However, this possibility was ruled out in experiments (Spížek and Janeček, 1968), in which it was shown that the mRNA specifying the lac operon enzymes was still synthesized in the presence of ethionine. A third possibility was, therefore, considered, viz. that the replacement of methionine by ethionine alters the enzymic activity of β-galactosidase. Such a protein should be comparable with β-galactosidase in many respects. Immunological tests were performed to find out if the bacteria in the presence of ethionine produced a cross-reacting material instead of normal enzyme. The test used (Masters and Pardee, 1962) detects cross-reacting material (CRM) by its ability to prevent combination and precipitation of antibody and the active enzyme. Various amounts of the enzyme were added to the same concentration of the antiserum against normal β-galactosidase and the mixture left to precipitate overnight at 4°C. If the enzyme activity remaining in the supernatant is plotted against the enzyme activity added, the point where activity is first detected in the supernatant is shifted toward the origin when CRM is present in the mixture. Enzyme preparations obtained by homogenizing cells grown in the presence of 0.001 M, 0.005 M and 0.01 M ethionine were treated as above. The homogenate of bacteria grown without ethionine served as control. It can be seen in Fig. 1 that the incorporation of ethionine into β-galactosidase resulted in the formation of CRM. The concentration of CRM increased proportionally with increasing concentration of ethionine in the medium. The synthesis of

normal enzyme was considerably inhibited by 0.005 M and 0.01 M ethionine and practically only CRM was formed.

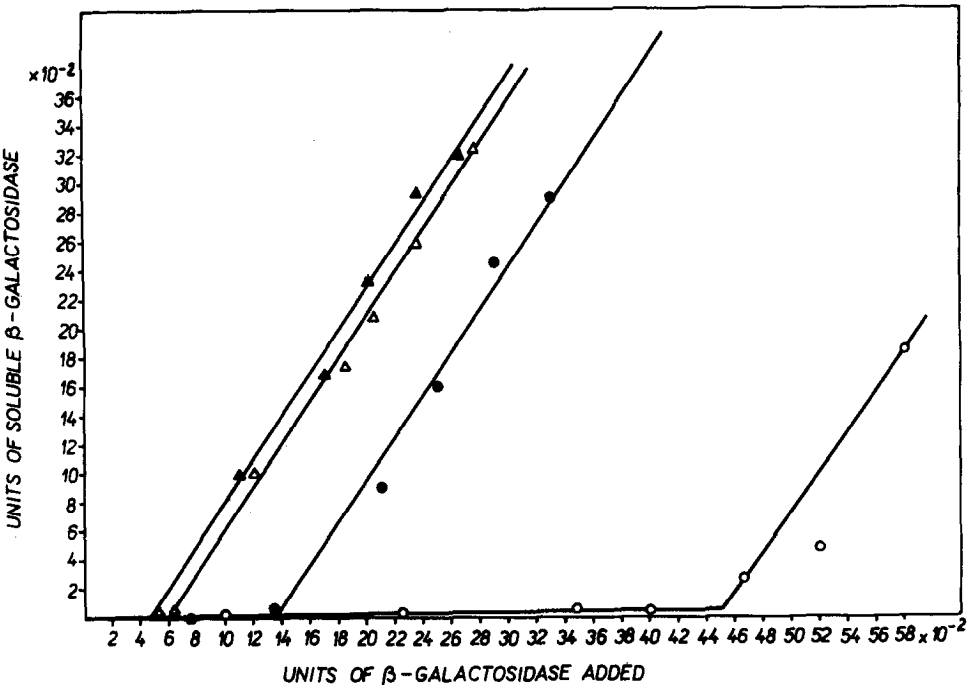

Figure 1. The immunological demonstration of cross-reacting material formed as a result of the inhibition of β-galactosidase synthesis by various concentrations of ethionine.
o—o control, ●—● ethionine 0.001 M, Δ—Δ ethionine 0.005 M, ▲—▲ ethionine 0.01 M
β-galactosidase activity is expressed as millimicromoles of ONPG hydrolyzed per min at 37°C.

It follows from the comparison of the enzyme concentration in the preparation with the concentration of CRM that the former decreased proportionally with increasing concentration of the latter and that the sum of both equaled 100% of the total β-galactosidase synthesized in the culture not inhibited by ethionine. This held for all used concentrations of ethionine.

It can be concluded that the replacement of methionine

with ethionine in the molecule of ß-galactosidase resulted in the formation of a protein which did not exhibit the enzyme activity but immunologically cross-reacted with the antiserum against normal ß-galactosidase. The results suggest that methionine might be involved in the active site of ß-galactosidase. Experiments are under way to isolate the CRM and study its physico-chemical and biological properties.

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