PATTERN OF PROTEIN SYNTHESIS IN ESCHERICHIA COLI UNDER LIMITED NITROGEN SUPPLY

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Little is known of the course of protein synthesis in the presence of limited amino acid concentrations. Richmond (1959) studied the effect of different arginine concentrations on protein synthesis and found that in completely synthetic medium without arginine, cultures of Staphylococcus aureus formed no protein, RNA, DNA or various enzymes. The addition of arginine, in a concentration of only 2 µg./ml., resulted in synthesis of all the components investigated. In the absence of amino acids essential for growth, Escherichia coli is incapable of synthesizing nucleic acids (Pardee and Prestidge, 1956; Yčas and Brawerman, 1957). In the absence of nitrogen or amino acids it synthesizes induced beta-galactosidase, i.e. protein synthesis occurs under these conditions (Mandelstam, 1958a). Rickenberg and Lester (1955) found that in diauxic growth no proteins are formed during the diauxic growth lag, although beta-galactosidase is synthesized intensively at this time. They termed synthesis of the enzyme under these conditions "preferential".

It is not yet known whether all proteins are synthesized in the presence of a limited concentration of essential amino acid, or whether one is formed preferentially to the others.

The author therefore attempted to determine the effect of essential amino acid on protein synthesis in general and on the course of the formation of induced beta-galactosidase under these conditions.

Protein synthesis, the incorporation of methionine ^{35}S and beta-galactosidase were studied in a strain of Escherichia coli requiring phenylalanine, in the presence of different concentrations of this amino acid. The bacteria were cultured on synthetic medium (Mandelstam, 1958a) and when the exponential phase was reached the cells were centrifuged, washed twice with phosphate buffer (0.05 M KH_2PO_4, pH = 7) and deprived of the amino acid. They were then centrifuged again, washed twice with phosphate buffer and resuspended in medium containing different concentrations of phenylalanine. This medium contained an inducer (lactose in a concentration of 0.008 M) and 2 µC/ml. methionine ^{35}S (specific activity 500 mC/g.). The bacteria were resuspended in this medium in the same density as that in which they were obtained in the exponential phase. Radioactivity was determined in protein precipitate prepared by the method of Mandelstam (1958b).

Tab.1 gives the results after 60 minutes culture. It shows that proteins were not synthesized in every case during this period. Methionine was incorporated with all the phenylalanine concentrations tested, the amount of radioactive amino acid in the proteins being proportionate to the phenylalanine concentration. The amount of newly synthesized protein was calculated directly by means of the incorporation of methionine, on the basis of the finding that E.coli protein contains 2.6% methionine (Roberts et al., 1957). The results show that protein was synthesized, but in such small quantities that the total protein increase could not be determined by the direct

method. Synthesis of beta-galactosidase was likewise proportionate to the phenylalanine concentration. The increase in enzymatic activity, expressed in units of the enzyme, was also calculated to the amount of enzymatic protein on the basis of the relationship found by Cohen (1957). Enzymatic protein forms only a very small part of the newly synthesized protein, showing that the enzyme is not synthesized preferentially.

Table 1.

Influence of the phenylalanine concentration on protein synthesis, incorporation of methionine ^{35}S and formation of beta-galactosidase in Escherichia coli

Concentr. of phe	++ mg of prot./ml. at 0 min.	++ mg of prot./ml. at 60 min.	+++ cpm per mg of prot.	incr. of prot./hr in µg/mg	+ units of enzyme	µg enzyme per mg. prot. at 60 min.
$2x10^{-4}M$	0,288	0,283	53.490	65,1	6,7	$3,5 \times 10^{-4}$
$1x10^{-5}M$	0,277	0,270	52.750	63,6	5,6	$2,9 \times 10^{-4}$
$5x10^{-6}M$	0,277	0,280	50.400	61,4	5,2	$2,8 \times 10^{-4}$
$1x10^{-6}M$	0,286	0,282	28.000	33,6	1,06	$5,5 \times 10^{-5}$
0	0,292	0,285	22.410	26,8	0,69	$3,6 \times 10^{-5}$

\+ beta-galactosidase determined by the method of Rickenberg and Lester (1955)
++ protein determined by the method of Lowry et al. (1951)
+++ radioactivity measured by a 2π counter

The above results were verified by electrophoresis of the proteins of a culture of Escherichia coli phe$^-$ grown for 60 minutes in the presence of different phenylalanine concentrations, under the same conditions as in the previous experiment. After 60 minutes the bacteria were homogenized in a Hughes homogenizer (Hughes, 1951). Intact cells and non-protein particles were removed by centrifugation (15 minutes at 15,000 r.p.m.), the clear supernatant fluid being used for electro-

phoretic separation. The specimen contained 4% protein and 10 µg. protein was applied at the start. Electrophoresis was carried out in a starch block measuring 0.4 x 1 x 45 cm. in veronal-citrate buffer, pH = 8.6, μ = 0.1, with 220 V in the electrodes during the first 24 hours and 300 V during the next 24 hours at 10°C. After completing electrophoresis the starch block was cut into 1 cm. sections and the proteins were shaken out into distilled water. The protein content, radioactivity and enzymatic activity were determined in the supernatant fluid.

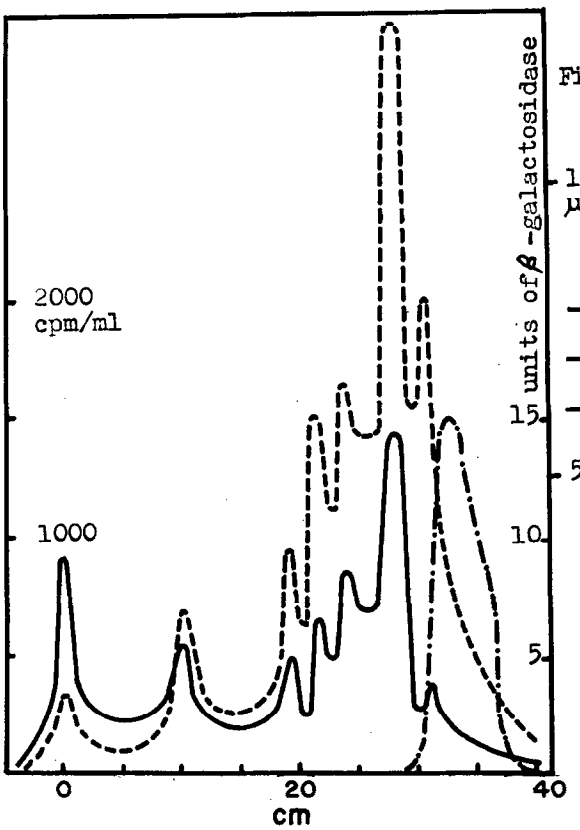

Fig.1. Separation of proteins, radioactivity and enzyme on starch-block electrophoresis.

---- proteins µg/ml

——— radioactivity cpm/ml

—·—·— micro moles of ONPG hydrolyzed/mg proteins /hour.

Fig.1 shows the separation of proteins, radioactivity and the enzyme obtained from a culture grown in the presence of 2.5 x 10^{-6}M phenylalanine. The picture in cultures grown in the presence of an adequate phenylalanine concentration

(2×10^{-4}M) and in the absence of phenylalanine was similar. These results show that on limiting protein synthesis by the essential amino acid, all proteins are probably synthesized; in no case was preferential synthesis of one protein fraction or of beta-galactosidase observed. Limitation was manifested in a decrease in protein synthesis only.

It is probable that in synthesis of beta-galactosidase during diauxic growth (in which, however, there is no nitrogen limitation), all proteins are also synthesized, though so slowly that the increase cannot be determined by the total protein content.

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