THE INDUCTION OF THYMIDINE PHOSPHORYLASE AND EXCRETION OF DEOXYRIBOSE DURING THYMINE STARVATION

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Received October 8, 1964

It has been reported that xylose isomerase, β-galactosidase, and alkaline phosphatase activities increase when Escherichia coli 15T- are incubated in thymineless media under conditions which induce these enzymes in prototrophs (Cohen and Barner, 1954; Nakada, 1962; McFall and Magasanik, 1962). In contrast, there have been few reports on enzyme changes resulting solely from incubation of thymineless auxotrophs in thymineless media. Gallant (1961) reported no change in dihydroorotic acid dehydrogenase and thymidylate kinase activities during thymine starvation of E. coli B_3 while Biswas et al. (1964) demonstrated an increase in the ribonucleoside diphosphate reductase system of E. coli 15T- under similar conditions.

We have found that E. coli 70V3-462, a thymineless auxotroph of E. coli 15, attained a ten-fold increase in the specific activity of thymidine phosphorylase and excreted deoxyribose when incubated in thymineless media (Fig. 1). Under these conditions uridine phosphorylase activity was constant. This increase in thymidine phosphorylase activity was not observed when cells were incubated in thymineless media deprived of phosphate or in the absence of isoleucine when an isoleucine-requiring strain of E. coli 70V3-462 was employed.

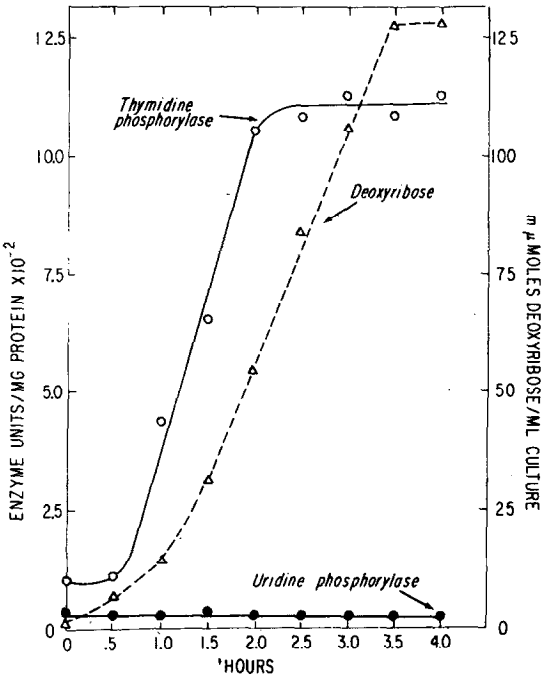

Fig. 1. Increase of thymidine phosphorylase activity and excretion of deoxyribose during thymine starvation of E. coli 70V3-462.

E. coli 70V3-462 was grown in minimal medium (Davis and Mingioli, 1950) containing 0.2% glucose and 20 μM thymine to a concentration of 3×10^8 cells per ml. The cells were washed twice, resuspended at the same concentration in minimal medium containing 0.2% glucose and incubated at 37°. At indicated intervals, cells were removed by centrifugation. The supernatant fraction was analyzed for deoxyribose by Burton's (1956) diphenylamine procedure which measures free, ester, and purine bound deoxyribose. Enzymatic assays were according to Friedkin and Roberts (1954). One unit of enzyme will produce 1 mμmole of thymine or uracil per minute. Further analysis of the supernatant fraction indicated that less than 2% of the diphenylamine-reacting deoxyribose was charcoal adsorbable and that 5% of the non-charcoal adsorbable diphenylamine-reacting deoxyribose precipitated on addition of $Ba(OH)_2$ and ethanol or was adsorbed to Dowex-1. Thus approximately 93% of the deoxyribose was in the free form.

E. coli 70V3-462 is a spontaneous mutant of E. coli 70-462, an X-ray induced thymineless mutant of E. coli 15. Both mutants were isolated by Dr. R. R. Roepke, the latter in 1947 (Roepke and Mercer, 1947). E. coli 15T- (Barner and Cohen, 1954) and strain I (Zamenhof and Griboff, 1954) are derivatives of E. coli 70-462 (Roepke, personal communication) and appear identical to E. coli 70V3-462 on the basis of thymine growth requirements.

Maximal turbidity of growth in minimal medium plus 0.2% glucose was obtained at 100 μM thymine for E. coli 70-462 and 7 μM thymine for E. coli 70V3-462. E. coli 70V3-462 like E. coli 15T- grew equally well on thymine or thymidine while E. coli 70-462 grew better on thymidine.[1]

In contrast to E. coli 70V3-462, incubation of E. coli 70-462 in thymineless media resulted in essentially no increase of thymidine phosphorylase activity or excretion of deoxyribose. Thymidine phosphorylase activity was induced in growing cultures of E. coli 15, 70-462, and 70V3-462 after addition of either thymidine, deoxyuridine, deoxycytidine, or deoxyadenosine.

Approximately 20 compounds involved in nucleic acid metabolism have been isolated from thymineless media in which either thymineless strain had been incubated. Compounds identified are listed in Table I. The excretion of hypoxanthine, uracil, and orotic acid during thymine starvation of E. coli 15T- was reported by Cohen and Barner (1954) who showed that these compounds were synthesized de novo. This finding suggests that all compounds listed in Table I were synthesized de novo and were not degradation products of preformed macromolecules. Preliminary evidence in this laboratory indicates that the excretion of purine and pyrimidine derivatives into the media is accompanied by changes in the intracellular pool. Thus thymine starvation resulted in an intracellular accumulation of both purine and pyrimidine nucleotides (μmoles/g protein) in both thymineless strains with a marked increase in the purine deoxynucleotide fraction only in E. coli 70V3-462. This latter finding is in agreement with the results of Munch-Petersen and Neuhard (1964)

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[1] E. coli 15T- and strain I lack the enzyme thymidylate synthetase (Barner and Cohen, 1959; Mantsavinos and Zamenhof, 1961). Evidence to be presented elsewhere indicates that loss of thymidylate synthetase activity was the first mutation (E. coli 15 → E. coli 70-462) and not the second mutation [E. coli 70-462 → E. coli 70V-462 (E. coli 15T-; strain I)].

Table I

Estimation of Purine and Pyrimidine Compounds and Deoxyribose Excreted by E. coli 70-462 and E. coli 70V3-462 During Two Hours of Thymine Starvation

Compound	Strain 70-462 mμmoles/ml culture	70V3-462 mμmoles/ml culture
Uridine	0.06	0.10
Uracil	2.4	0.63
Hypoxanthine	0.27	0.71
Orotic acid	0.49	0.73
Deoxycytidine-5'-phosphate	0.079	0.040
Deoxyuridine-5'-phosphate	0.74	0.22
Cytidine-5'-phosphate	0.38	0.23
Uridine-5'-phosphate and other uridine-containing nucleotides	0.76	1.6
Deoxyadenosine-5'-phosphate	0.065	
Adenosine-5'-phosphate	0.95	1.8
Other adenine-containing nucleotides	0.73	
Guanine-containing nucleotides	0.35	0.27
Hypoxanthine-containing nucleotides	—	0.030
Deoxyribose	< 2.0	58

Purine and pyrimidine derivatives in the supernatant fraction, obtained as in Fig. 1, were adsorbed and eluted from charcoal and chromatographed on Dowex-1. Individual ultraviolet absorbing peaks were characterized by paper chromatography in four solvent systems and by spectral analysis. The relatively small quantities of some compounds and the many operations required for identification did not permit rigorous quantitation. The mμmoles/ml culture values are therefore estimates. Relative amounts of these compounds varied greatly with time so that comparisons between the two strains must await more thorough analysis at other time periods.

with E. coli 15T-. However, our failure to find a similar enrichment in the purine deoxynucleotide pool with thymine-deprived E. coli 70-462 indicates

that a thymine-containing compound does not exert control on purine deoxynucleotide synthesis. Control by a thymine-containing compound appears to be manifested on purine deoxynucleotide synthesis as a result of the second mutation in E. coli 70V3-462. As yet, the effect of thymine starvation on the pyrimidine deoxynucleotide pool has not been measured.

The nature of the second mutation in E. coli 70V3-462 (in addition to the loss of thymidylate synthetase) is not clear. The inefficient utilization of thymine for DNA synthesis in wild-type E. coli (Crawford, 1958) and E. coli 70-462 is probably due to a deficiency in a source of deoxyribose for thymine to thymidine conversion. Evidence presented in this paper is consistent with the proposition that the second mutation in E. coli 70V3-462 results in greater production of deoxynucleotides. Dephosphorylation would yield deoxynucleosides from which deoxyribose could be transferred to thymine via transdeoxyribosylation and/or coupled deoxynucleoside phosphorylation mechanisms. In absence of thymine, deoxyribose-1-phosphate may accumulate as a result of deoxynucleoside phosphorylase activity and induce thymidine phosphorylase, as suggested by Razzell and Casshyap (1964).

The enzyme system(s) affected by thymine deprivation in E. coli 70-462 and E. coli 70V3-462 and the mechanism of thymine to thymidine conversion are being investigated. Manuscripts in preparation by Dr. R. R. Roepke and us will describe the historical and nutritional aspects of the E. coli 70 strains.

ACKNOWLEDGMENTS

The authors thank Dr. R. R. Roepke for the E. coli strains. One of us (TRB) gratefully thanks Mrs. Grace Campbell for excellent technical assistance on some preliminary experiments.

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