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Inability of Low Thymine-requiring Mutants of *Escherichia coli* Lacking Phosphodeoxyribomutase to Be Induced for Deoxythymidine Phosphorylase and Deoxyriboaldolase

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In a previous communication (T.R. Breitman and R. M. Bradford, Biochim. Biophys. Acta **138**:217, 1967), we reported that *Escherichia coli* 70V3, a low thymine-requiring mutant of *Escherichia coli* 15, lacked deoxyriboaldolase activity, establishing a biochemical basis for the low requirement. Also presented were data indicating that both deoxythymidine phosphorylase and deoxyriboaldolase were induced coordinately when *E. coli* was grown on deoxythymidine. We suggested that deoxyribose 5-phosphate (dR-5-P) was more directly involved with these inductions than was deoxyribose 1-phosphate (dR-1-P), the compound implicated by W. E. Razzell and P. Casshyap (J. Biol. Chem. **239**:1789, 1964) as the inducer of deoxythymidine phosphorylase. We also proposed that the failure of the latter workers to observe induction of deoxythymidine phosphorylase with two thymineless mutants of *E. coli* K-12, grown previously on deoxythymidine (dThd), occurred because these mutants lacked phosphodeoxyribomutase activity and, therefore, could not synthesize dR-5-P from dThd by the following enzymatic reactions.

$$\text{dThd} + P_i \xleftrightarrow[\text{phosphorylase}]{\text{deoxythymidine}} \text{thymine} + \text{dR-1-P} \quad (1)$$

$$\text{dR-1-P} \xleftrightarrow[\text{mutase}]{\text{phosphodeoxyribo-}} \text{dR-5-P} \quad (2)$$

Subsequently, we have investigated other low thymine-requiring mutants derived from *E. coli* 70 (a high thymine-requiring mutant derived from strain 15). As shown in this report, strains lacking phosphodeoxyribomutase (and therefore unable to synthesize dR-5-P from dThd) are not induced for either deoxythymidine phosphorylase or deoxyriboaldolase by growth on dThd.

Growing cultures of strains 15T$^-$ (H. D. Barner and S. S. Cohen, J. Bacteriol. **68**:80, 1954), I, and II, a reversion of strain I to thymine independence (S. Zamenhof and G. Griboff, Nature **174**:306, 1954), are similar to strain 70V3 in that they degrade dThd to thymine more slowly than strains 15 and 70 and that they are unable to cleave deoxyribosephosphates (Table 1).

The data in Table 2 show that both strain 15T$^-$ and strain I have lost phosphodeoxyribomutase activity and that this deficiency is maintained in the back-mutation of strain I to strain II. These strains exhibit no increase in either deoxyriboaldolase or deoxythymidine phosphorylase activities when grown on dThd, establishing that the production of dR-5-P is required for induction. When grown on thymine, strains lacking phosphodeoxyribomutase have higher aldolase and phosphorylase activities than strains 15 and 70. Phosphodeoxyribomutase is induced by growth on dThd.

These results on the deoxythymidine phosphorylase levels of strain 15T$^-$ confirm those of A. P. Harrison (J. Gen. Microbiol. **41**:321, 1965).

TABLE 1. *Metabolism of deoxythymidine (dThd) by growing cultures of Escherichia coli*[a]

Strain	Thymine (μmole/ml of culture)	Deoxyribose (μmole/ml of culture)
15	0.89	0.04
70	0.85	0.04
70V3	0.29	0.29
15T$^-$	0.27	0.28
I	0.27	0.27
II	0.29	0.31

[a] Cells (4×10^7/ml) were grown on 1 mM dThd to a concentration of 4×10^8/ml, and the cell-free supernatant fluid was analyzed for thymine and deoxyribose as described previously (T. R. Breitman and R. M. Bradford, Biochim. Biophys. Acta **138**:217, 1967). Growth of strains 15T$^-$, I, and II was inhibited by dThd, as has been described for strain 70V3 (T. R. Breitman and R. M. Bradford, J. Bacteriol. **93**:845, 1967).

TABLE 2. *Phosphodeoxyribomutase, deoxyriboaldolase, and deoxythymidine phosphorylase activities of cells grown on thymine and deoxythymidine (dThd)*[a]

Strain	Phosphodeoxyribomutase	Deoxyriboaldolase	Deoxythymidine phosphorylase
Grown on thymine			
15	12	16	48
70	13	18	52
70V3	3	<2	22
15T⁻	<1	48	170
I	<1	46	188
II	<1	40	204
Grown on dThd			
15	42	296	525
70	52	348	609
70V3	51	<2	415
15T⁻	<1	48	187
I	<1	51	197
II	<1	46	245

[a] Cells were grown on either 1 mM thymine or 1 mM dThd and were assayed for deoxythymidine phosphorylase and deoxyriboaldolase as described previously (T. R. Breitman and R. M. Bradford, Biochim. Biophys. Acta **138**:217, 1967). For assay of phosphodeoxyribomutase, 7.5×10^{10} spheroplasts, prepared according to M. H. Malamy and B. L. Horecker (Biochemistry **3**:1893, 1964), were lysed in 7.2 ml of cold 25 mM $MnCl_2$. After 30 min in an ice bath, 0.4 ml of 1 M tris(hydroxymethyl)aminomethane-chloride, *p*H 7.3, and 0.8 ml of 1 M NaF were added to the suspension, which was then centrifuged at 31,000 × *g* for 10 min. The supernatant fraction was used as the enzyme source. Reaction mixtures contained, in a final volume of 0.1 ml: deoxyribose 1-phosphate, 0.2 μmole; NaF, 80 mmoles; and 0.2 to 1.0 mg of enzyme protein. Reactions were incubated at 37 C for various time periods up to 180 min and were stopped by the addition of 0.9 ml of cold 0.5 N perchloric acid and assayed for inorganic phosphate (P. S. Chen et al., Anal. Chem. **28**:1756, 1956). The decrease of inorganic phosphate is a measure of the conversion of the acid-labile deoxyribose 1-phosphate to an acid-stable form (deoxyribose 5-phosphate and glyceraldehyde 3-phosphate). All activities are expressed as mμmoles of substrate converted per min per mg of protein.

A. Munch-Peterson (*personal communication*) has found that several independently derived low thymine-requiring mutants of *E. coli* K-12 lack phosphodeoxyribomutase. A similar observation has been made by R. C. Bockrath (*personal communication*) for a mutant derived from *E. coli* 15T⁻. P. A. Hoffee (J. Bacteriol. **95**:449, 1968) has reported that some low thymine-requiring mutants of *Salmonella typhimurium* are deoxyriboaldolase deficient, and, in agreement with our previous results and the data in Table 2, presents data indicating that deoxyriboaldolase and deoxythymidine phosphorylase are both induced by dR-5-P.