JOURNAL OF BACTERIOLOGY, July 1970, p. 264-265

Vol. 103, No. 1
Printed in U.S.A.

Pseudouridylate Synthetase of *Escherichia coli*: Correlation of Its Activity with Utilization of Pseudouridine for Growth

T. R. BREITMAN

Laboratory of Physiology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

Received for publication 19 January 1970

A direct correlation exists between the pseudouridylate synthetase activity and the ability of pyrimidine auxotrophs of *Escherichia coli* to grow on pseudouridine.

A previous report from this laboratory (2) described pyrimidine auxotrophs of *Escherichia coli* which can use pseudouridine (5-ribosyluracil) as their sole pyrimidine source. Pyrimidine auxotrophs either did not grow, grew slowly, or grew rapidly on pseudouridine (Table 1). Those that grew rapidly had the same growth rates on either pseudouridine or uracil.

In seeking a biochemical basis for these variations, the following two-step enzymatic pathway for conversion of pseudouridine to uracil was considered:

$$\text{pseudouridine} + \text{ATP} \xrightarrow{\text{pseudouridine kinase}} \text{pseudouridine 5'-phosphate} \quad (1)$$

$$\text{pseudouridine 5'-phosphate} \xrightarrow[\text{synthetase}]{\text{pseudouridylate}} \text{uracil} + \text{ribose 5-phosphate} \quad (2)$$

Although there have been no reports on the existence of pseudouridine kinase, the enzyme catalyzing the first reaction, evidence for its presence in *E. coli* may be inferred from the work of Ginsberg and Davis (5), who isolated labeled pseudouridine mono-, di-, and triphosphates from the metabolic pool of a pyrimidine-requiring mutant of *E. coli* 15 incubated in the presence of pseudouridine-2-^{14}C and uracil. Ginsberg and Davis concluded that little dilution of exogenous pseudouridine occurred during its conversion to the pseudouridine phosphate derivatives, a finding consistent with the presence of pseudouridine kinase.

Pseudouridylate synthetase, the enzyme catalyzing reaction 2, has been purified from *Tetrahymena pyriformis* (6) and *Agrobacterium tumefaciens* (8). No other source of this enzyme has been reported.

If the two-step reaction sequence described above was the pathway for utilization of pseudouridine, it would be expected that the specific activity of at least one of the enzymes would be related to the growth response of a strain on pseudouridine. In addition, if the activity of one of the enzymes constituted a growth-limiting step, an estimation of the rate of nucleic acid-pyrimidine synthesis in the growing culture would be a measure of the activity of this enzyme in vivo.

The data in Table 2 indicate a close correlation between the pseudouridylate synthetase activity and the ability of pyrimidine auxotrophs to grow on pseudouridine. The close agreement between the values for the rates of nucleic acid-pyrimidine synthesis and the pseudouridylate synthetase activity in vitro for strains B_u- and W 63-86 makes it likely that the activity of this enzyme is probably the growth-limiting step for these strains growing on pseudouridine. Associated with the mutations in strains B 5RU and W 5RU is a pseudouridylate synthetase activity

TABLE 1. *Growth characteristics and source of pyrimidine auxotrophs*

Strain	Growth rate[a] on		Parental strain and origin
	Uracil	Pseudouridine	
	min	*min*	
B-148	50	No growth	Isolated from *E. coli* B by M. Lubin
B_u-	50	360	*E. coli* B (3)
B 5RU	50	50	*E. coli* B_u- (2)
W 63-86	65	360	Isolated from *E. coli* W by B. D. Davis (ATCC 12632)
W 5RU	65	65	*E. coli* W 63-86 (2)

[a] Mass-doubling time for cultures grown in minimal medium (4) under conditions described previously (2).

TABLE 2. *Pseudouridylate synthetase activity and rates of nucleic acid-pyrimidine synthesis in different strains of Escherichia coli*

Strain	Pseudouridylate synthetase activity[a]	Rate of nucleic acid-pyrimidine synthesis on pseudouridine[b]
B	<0.017	
B-148	<0.017	0
B_u-	0.40	0.28
B 5RU	11.35	2.05
W 63-86	0.40	0.28
W 5RU	3.50	1.55

[a] Bacteria were grown overnight at 37 C in minimal medium (4) containing 400 μM uracil. The cultures were diluted 10-fold in fresh medium to give a concentration of approximately 10^8 cells/ml and grown to a concentration of approximately 8×10^8 cells/ml. The cells from these exponentially growing cultures were harvested, washed twice with 0.9% NaCl, and suspended at a concentration of 8×10^9 cells/ml in 10 ml of 10 mM tris(hydroxymethyl)aminomethane (Tris) containing 10 mM dithiothreitol and 2.17 M glycerol, and adjusted to *p*H 7.5 with HCl at 25 C. The cells were disrupted by treating for 1 min with a Branson S-75 Sonifier as described previously (1). Pseudouridylate synthetase was assayed in a reaction mixture containing in a final volume of 0.5 ml: uracil-5-^3H, 1.06 μmoles (7.5×10^6 dpm); ribose 5-phosphate, 4.03 μmoles; $MgCl_2$, 10 μmoles; 0.2 ml of a buffer containing 0.1 M Tris-hydrochloride and 10 mM dithiothreitol (*p*H 8.6); and 0.1 ml of disrupted cell suspension. Incubations were at 37 C for various time periods up to 120 min and were stopped by cooling in an ice-bath followed by the addition of 1.5 ml of a 4% activated charcoal (Norit A) suspension. The mixture was filtered through a membrane filter; a portion of the filtrate (1 ml) was added to a scintillation vial, and 10 ml of scintillation fluid (containing 2 volumes of toluene phosphor and 1 volume of Triton X-100) were added. Samples were counted in a liquid scintillation spectrometer at a counting efficiency of approximately 20%. This new assay for pseudouridylate synthetase activity takes advantage of the fact that tritium is released, presumably as tritiated water, during the formation of the carbon-carbon bond between the C-5 of uracil-5-^3H and the C-1 position of ribose 5-phosphate. Under the conditions described above, the release of tritium from uracil-5-^3H was linear for the 120-min incubation period. No tritium was released in the absence of ribose 5-phosphate. Comparable activities were obtained when uracil-2-^{14}C was substituted for uracil-5-^3H, and the formation of ^{14}C-labeled pseudouridine and pseudouridylate were analyzed by paper chromatography according to Suzuki and Hochster (8). The absence of pseudouridylate synthetase activity in strain B-148 probably was not due to the presence of an inhibitor, since addition of an extact of strain B-148 had no effect on the pseudouridylate synthetase activity in an extract of strain B 5RU. Enzyme activity is expressed as nanomoles of pseudouridylate formed per minute per 10^9 cells.

[b] Expressed as nanomoles synthesized per minute per 10^9 cells. The rates of nucleic acid-pyrimidine synthesis in cultures growing on pseudouridine were calculated by the method of Monod et al. (7) using a value of 150 nmoles of nucleic acid-pyrimidine per 10^9 cells and the growth rates listed in Table 1.

with a capacity, as measured in vitro, to produce uracil from pseudouridylate at a rate much greater than the rate of nucleic acid-pyrimidine synthesis in vivo. This, therefore, appears to be the biochemical basis for the ability of these strains to grow as well on pseudouridine as on uracil.

I thank A. George Klein for valuable technical assistance.

LITERATURE CITED

1. Breitman, T. R., and R. M. Bradford. 1967. The absence of deoxyriboaldolase activity in a thymineless mutant of *Escherichia coli* strain 15: a possible explanation for the low thymine requirement of some thymineless strains. Biochim. Biophys. Acta 138:217–220.
2. Breitman, T. R., and C. S. Scher. 1968. Growth of pyrimidineless strains of *Escherichia coli* on 5-ribosyluracil (pseudouridine). J. Bacteriol. 96:1873–1874.
3. Cohen, S. S., J. Lichtenstein, H. D. Barner, and M. Green. 1957. Studies on the biosynthesis of bacterial and viral pyrimidines. IV. Utilization of pyrimidine bases and nucleosides by bacterial mutants. J. Biol. Chem. 228:611–619.
4. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. 60: 17–28.
5. Ginsberg, T., and F. F. Davis. 1968. The biosynthesis of pseudouridine in ribonucleic acids of *Escherichia coli*. J. Biol. Chem. 243:6300–6305.
6. Heinrikson, R. L., and E. Goldwasser. 1964. Studies of the biosynthesis of 5-ribosyluracil 5'-monophosphate in *Tetrahymena pyriformis*. J. Biol. Chem. 239:1177–1187.
7. Monod, J., A. M. Pappenheimer, Jr., and G. Cohen-Brazire. 1952. La cinétique de la biosynthèse de la β-galactosidase chez *E. coli* considérée comme fonction de la croissance. Biochim. Biophys. Acta 9:648–660.
8. Suzuki, T., and R. M. Hochster. 1966. On the biosynthesis of pseudouridine and of pseudouridylic acid in *Agrobacterium tumefaciens*. Can. J. Biochem. 44:259–272.