PSEUDOURIDINE KINASE OF ESCHERICHIA COLI: A NEW ENZYME

L. R. Solomon and T. R. Breitman

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

Received May 18, 1971

Summary: Pseudouridine kinase activity has been demonstrated in extracts of some pyrimidine auxotrophs of Escherichia coli. This enzyme has a pH optimum of 7, and requires ATP, Mg^{++}, and either K^+ or NH_4^+. Pseudouridine kinase is distinct from uridine kinase and deoxythymidine kinase. The presence of pseudouridine kinase and pseudouridylate synthetase activities in strains capable of growth on pseudouridine (Ψrd) as the sole pyrimidine source supports the following pathway for Ψrd utilization:

$$\Psi\text{rd} + \text{ATP} \xrightarrow{\text{pseudouridine kinase}} \text{pseudouridylate (}\Psi\text{MP)} + \text{ADP} \qquad (1)$$

$$\Psi\text{MP} \xrightleftharpoons{\text{pseudouridylate synthetase}} \text{uracil} + \text{ribose-5-P} \qquad (2)$$

Introduction

A previous report from this laboratory (1) described pseudouridylate synthetase activity in some pyrimidine auxotrophs of Escherichia coli and related this activity to their ability to utilize pseudouridine (Ψrd) as a pyrimidine source. The following two-step pathway for Ψrd utilization was proposed:

$$\Psi\text{rd} + \text{ATP} \xrightarrow{\text{pseudouridine kinase}} \text{pseudouridylate (}\Psi\text{MP)} \qquad (1)$$

$$\Psi\text{MP} \xrightleftharpoons{\text{pseudouridylate synthetase}} \text{uracil} + \text{ribose-5-P} \qquad (2)$$

Reaction (1) requires the participation of an enzyme which has not been heretofore described. The present study provides the first direct evidence for the existence of pseudouridine kinase.

Experimental Procedures

Exponential-phase cells of E. coli strains Bu⁻ and B5RU (1), were grown as described previously (1), harvested by centrifugation, and washed with cold 0.85% NaCl. The cells were suspended to a density of 1.4×10^{10} cells/ml in 10 ml of 55 mM potassium phosphate buffer, pH 7, containing 10 mM dithiothreitol. Cell extracts were prepared by sonification as described (1) and were clarified by centrifugation at 31,000 x g for 30 min.

The pseudouridine kinase assay mixture contained (per ml): $MgCl_2$, 20 μmoles; ATP, 6 μmoles; β-Ψrd-2-^{14}C (CalAtomic), 1 μmole; potassium phosphate buffer (pH 7.65), 55 μmoles; and 0.25 ml of cell extract. The pH of the reaction mixture was 7. Incubation was at 37°. At timed intervals 15 μl samples were added to 10 μl of a solution containing 10 mM uracil, 10 mM Ψrd, and 10 mM UMP, and the mixture was applied to Whatman 3 MM paper (20 x 40 cm). Electrophoresis was performed on a Camag HVE system using 40 mM sodium borate buffer, pH 8.65 (2) at 5000 volts (125 V/cm) for 30 min. The relative mobilities in the direction of the anode were: UMP and ΨMP, 1; Ψrd, 0.23; and uracil, -0.05. The paper was dried and the ultraviolet absorbing areas were cut out and placed in counting vials. Samples were counted with 5 ml toluene phosphor in a liquid scintillation spectrometer. One unit of pseudouridine kinase is defined as the amount of enzyme that will produce one nmole of pseudouridylate per minute at 37°.

Results and Discussion

General Characteristics of the Reaction

Figure 1 shows that the decrease of Ψrd could be accounted for by the appearance of ΨMP and uracil. The identities of the products were confirmed by subjecting the reaction mixture to descending paper chromatography using isobutyric acid: NH_4OH: H_2O(66:1:33) which separates uracil, uridine, Ψrd, ΨMP and UMP from one

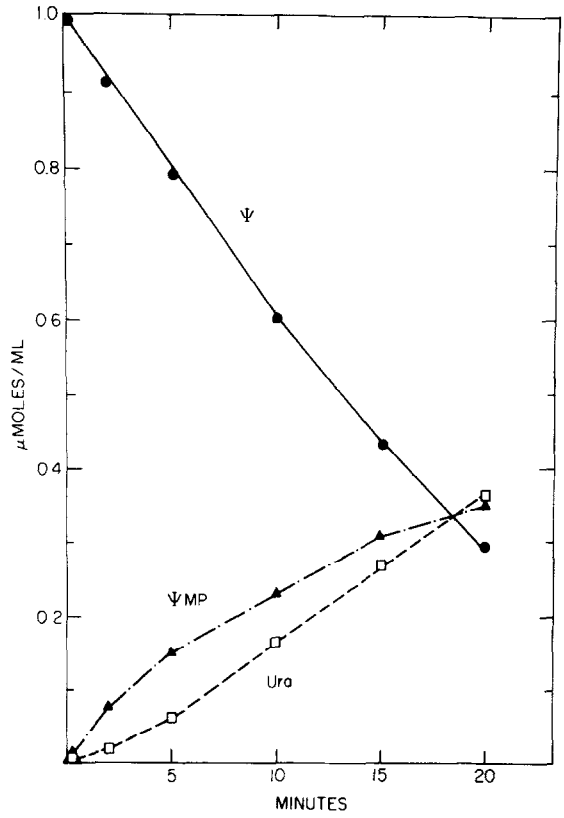

Fig. 1. The kinetics of formation of pseudouridylate (ΨMP) and uracil from pseudouridine (Ψrd). Cells of strain Bu⁻, growing exponentially on 200 μM uracil and 27 mM glycerol, were harvested at a density of 6.3×10^8 cells/ml. Cell extracts were prepared and the enzyme assayed as described in Experimental Procedures.

---

another (3). ΨMP was eluted off the paper and further characterized by treatment with E. coli alkaline phosphatase. Subsequent chromatography showed radioactivity only in Ψrd.

The kinetics of the reaction indicate that ΨMP is the first product formed (Fig. 1). The subsequent formation of uracil would be expected from the action of pseudouridylate synthetase on ΨMP. The following evidence supports this reaction sequence and eliminates the possibility that uracil is an intermediate in the formation of ΨMP: a) the addition of unlabeled uracil to the reaction mixture at a concentration of 2 mM did not alter the rate of synthesis of radioactive ΨMP from Ψrd-^{14}C; b) no

synthesis of ΨMP or UMP occurred when uracil-2-^{14}C was substituted for Ψrd-2-^{14}C; and c) NH_4SO_4 precipitation at neutral pH separated pseudouridine kinase activity (precipitated between 0 and 50% of saturation) from pseudouridylate synthetase activity (precipitated between 50% and 67% of saturation). In the standard reaction mixture, this purified pseudouridine kinase fraction catalyzed the formation of ΨMP with no detectable formation of uracil.

With the partially purified kinase, a Ψrd-dependent formation of ADP was demonstrated by coupling it to the oxidation of NADH (4). The same pseudouridine kinase activities were obtained by measuring either the rate of NADH oxidation or the rate of ΨMP-^{14}C synthesis. These results indicate that ADP is a product of the pseudouridine kinase reaction.

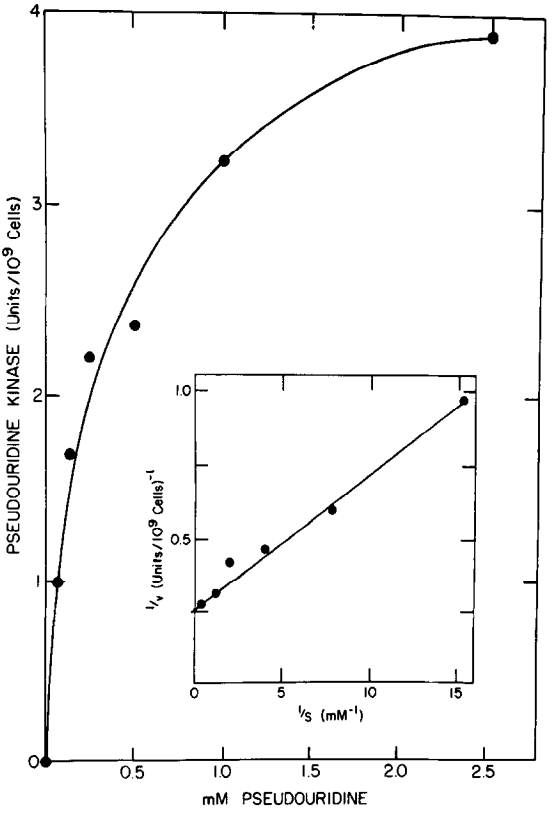

Fig. 2. Pseudouridine kinase activity as a function of pseudouridine concentration. The experimental procedure was as described in Fig. 1, using 3.5 x 10^9 cells/ml of assay mixture.

Pseudouridine kinase activity was completely dependent on ATP (apparent Km of 2.9×10^{-3} M), Mg^{++} (apparent Km of 3.3×10^{-3} M) and K^{+} (apparent Km of 4×10^{-2} M). NH_4^{+} but neither Li^{+} nor Na^{+} could substitute for K^{+}. The apparent Km for Ψrd was 1.8×10^{-4} M (Fig. 2). The pH optimum for the reaction was 7.0 with either phosphate or morpholinopropane sulfonic acid buffers.

In crude extracts, pseudouridine kinase activity was more than 30-fold greater than either uridine kinase or deoxythymidine kinase activities. In addition, uridine and deoxythymidine had no effect on the formation of ΨMP from Ψrd (Table I). Thus, pseudouridine kinase appears to be distinct from uridine kinase and deoxythymidine kinase.

Table I

Effect of Uridine and Deoxythymidine on Pseudouridine Kinase Activity

	Pseudouridine Kinase (Units/10^8 cells)	Relative Activity
Complete system	9.81	1
+ 1 mM Uridine	9.81	1
+ 10 mM Uridine	8.97	0.91
+ 1 mM Deoxythymidine	11.23	1.14
+ 10 mM Deoxythymidine	9.42	0.96

Cells of strain B5RU growing exponentially on 200 μM uracil and 27 mM glycerol, were harvested at a density of 5×10^8 cells/ml, washed with cold 0.85% NaCl and suspended to a density of 9.4×10^8 cells/ml in 11 mM morpholinopropane sulfonic acid buffer (MOPS), pH 7, containing 10 mM dithiothreitol. The cell extract was prepared and assayed as described in Experimental Procedures except that potassium phosphate buffer was replaced with an equimolar amount of MOPS buffer, pH 7, and 300 μmoles of KCl were added per ml of reaction mixture. The concentration of pseudouridine was 1 mM.

Distribution

In this report, Ψrd kinase activity was demonstrated in strains Bu^{-} and B5RU.

We have also observed this activity in W63-86 and W5RU, strains which, like Bu^- and B5RU, grow on Ψrd as a sole pyrimidine source. In contrast, the pyrimidine auxotroph B148, which does not grow on Ψrd (5), had no detectable pseudouridine kinase activity. This distribution of kinase activity parallels that of pseudouridylate synthetase activity (1) and indicates that both activities are necessary for strains to be capable of growth on Ψrd.

References

1. Breitman, T. R., J. Bacteriol. 103, 264 (1970).
2. Simmonds, H. A., Clin. Chim. Acta 23, 319 (1969).
3. Suzuki, J., and Hochster, R. M., Can. J. Biochem. 44, 259 (1966).
4. Reichard, P. and Sköld, O., in Methods of Enzymology, Vol. 6, p. 194. Colowick, S. P. and Kaplan, N. O. (Eds.), Acad. Press (1963).
5. Breitman, T. R. and Scher, C. D., J. Bacteriol. 96, 1873 (1968).