URIDINE-5'-MONOPHOSPHATE PYROPHOSPHORYLASE ACTIVITY FROM *ESCHERICHIA COLI*

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1. Introduction

Uracil is readily incorporated into pyrimidine nucleotides and nucleic acids by many bacteria apparently via its reaction with 5-phosphoribosyl-1-pyrophosphate (PRPP) to give UMP [1–3]. While the enzyme catalysing this reaction has been detected [1, 2] in extracts of *Escherichia coli* it has not been studied in detail. This information has not been available on kinetics or possible effectors of the enzyme. With dialysed crude extracts from *E. coli* we have found that the enzyme is strongly activated by GTP and inhibited by UMP and UTP. Preincubation with GTP is necessary for maximal activity and the specific activity of the extract decreases at extreme dilution. Uracil and PRPP show Michaelis-Menten kinetics as substrates with K_m values of 4×10^{-6} M and 2×10^{-5} M respectively.

2. Materials and methods

Adenine-8-C^{14} (31 μCi/μmole) and uracil-2-C^{14} (50 μCi/μmole) were from the Radiochemical Centre, Amersham, England. Uracil, nucleotides and Tris were obtained from the Sigma Chemical Co., St. Louis, Mo., USA. PRPP was purchased from Sigma Chemical Co. or P:L. Biochemicals, Inc., Milwaukee, Wis., USA as the dimagnesium salt. It was dissolved in 5 mM EDTA at pH 7.5 to give a 4 mM solution by weight. The actual concentration of PRPP in solution was determined by its capacity to convert [^{14}C]-adenine to [^{14}C]-AMP through the reaction catalysed by AMP-pyrophosphorylase.

Cells were harvested by centrifugation from a log phase culture of *Escherichia coli* ABLA in a minimal salts medium containing 0.4% glucose as a carbon source plus supplements of 0.5 μg/ml of thiamin and 20 μg/ml of leucine. They were washed once with 0.9 M NaCl and resuspended in cold 100 mM Tris-HCl, 10 mM KH_2PO_4 buffer at pH 7.8 (Tris-phosphate buffer). Cells were disrupted in an Aminco French pressure cell at a pressure of 10 tons/sq in. and the suspension centrifuged at 10.000 × *g* for 10 min, and then at 200.000 × *g* in a Beckman L2-65 ultracentrifuge for 90 min. The supernatant

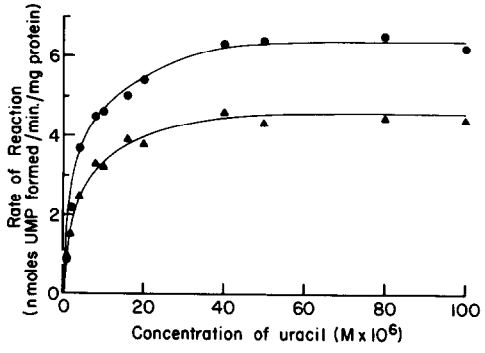

Fig. 1. Effect of uracil concentration on UMP-pyrophosphorylase. 6.2 μg of enzyme extract were preincubated for 5 min with PRPP and GTP in a total volume of 40 μl. The incubation was started by adding varying amounts of uracil in a volume of 10 μl giving final concentrations of 1×10^{-3} M GTP, 2×10^{-4} M or 8×10^{-5} M PRPP and varying uracil as shown. Samples were taken after 5 min incubation and assayed as described. ●——● 2×10^{-4} M PRPP. ▲——▲ 8×10^{-5} M PRPP.

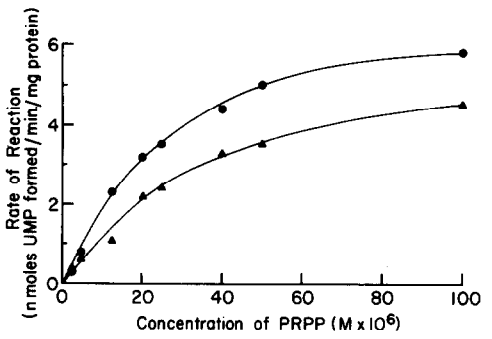

Fig. 2. Effect of PRPP concentration on UMP-pyrophosphorylase. 6.2 μg of enzyme extract were preincubated for 5 min with GTP and varying amounts of PRPP in a total volume of 40 μl. The incubation was started by adding uracil in a volume of 10 μl, giving final concentrations of 1×10^{-3} M GTP, 2×10^{-5} M or 8×10^{-6} M uracil and varying PRPP as shown. Samples were taken after 5-min incubation and assayed as described. ●——● 2×10^{-5} M uracil. ▲——▲ 8×10^{-6} M uracil.

was removed and dialysed against Tris-phosphate buffer for 20 hr at 4°C. It was then used for the study of enzyme activity. Protein content of the extracts was determined by the modified Folin method of Lowry [4].

Assay mixtures contained PRPP, uracil-2-C^{14} and $MgCl_2$ with an appropriate amount of protein in 50 μl Tris-phosphate buffer pH 7.8. The mixtures were incubated at 37°C and sampled by the removal of 5 μl volumes for spotting on to polyethyleneimine thin layers [5] for chromatography. After development of the chromatograms twice with water, they were dried and the origins (containing the product UMP) and the fronts (containing unchanged uracil) were cut out and placed in scintillation vials with 2.5 ml of scintillation mixture (5 g of 2,5-diphenyl oxazole (PPO) and 0.3 g of dimethyl POPOP/litre of toluene) for counting in a liquid scintillation spectrometer.

The chromatograms were developed in one direction only with several samples per sheet. To prevent interference from adjacent samples or distortion of spots by uneven flow rates the sheet was divided into a number of strips, each 1.2 cm wide, by scouring out 0.5 mm divisions between them. At the top of the sheet (4.5 cm above the origin) 0.5 cm of the layer was scraped off. Samples were spotted 1.2 cm from the bottom of the strips.

Table 1
Effect of preincubation on UMP-pyrophosphorylase.

Compound(s) preincubated with enzyme	2 min preincubation	10 min preincubation
	Rate of reaction (nmoles UMP formed/ min/mg protein)	
NIL	6.1	3.4
GTP	9.8	10.0
Uracil	5.9	2.7
PRPP	5.4	4.3
GTP + uracil	5.1	6.1
GTP + PRPP	7.4	10.0

The assays were carried out as described in the text, with 9.8 μg protein/50 μl, 1×10^{-4} M uracil, 1×10^{-3} M GTP and 2×10^{-4} M PRPP in the incubation. Components of the incubation mixture were preincubated as shown, then the additional components were added for an incubation period of 5 min before sampling.

3. Results and discussion

Preliminary experiments indicated a barely detectable UMP-pyrophosphorylase activity in the 200,000 × *g* supernatant extract. This activity was greatly increased by the inclusion of GTP in the incubation mixture with the stimulation being half maximal at a GTP concentration of 1×10^{-4} M and maximal at 1×10^{-3} M. Thereafter, GTP was routinely included in the incubation mixtures at the latter concentration. Study of the time-course of the reaction showed a lag of approximately 2 min before a constant rate was reached. This lag could be overcome by preincubation of the extract with GTP (table 1). Preincubation of the extract with PRPP and GTP for 5 min, then adding uracil to start the reaction, resulted in the rate remaining constant for at least 20 min, providing sufficient substrates were available.

Under these conditions the rate of reaction was proportional to the amount of extract in the incubation provided that the extract was not too dilute. With greater dilution the specific activity was decreased. A variety of treatments using combinations

Table 2
Effect of nucleotides on UMP-pyrophosphorylase.

Nucleotide(s) added for preincubation	Rate of reaction as percentage of control
10^{-3} M GTP (Control)	100
NIL	3
10^{-3}M ATP	2
10^{-3}M GTP + 10^{-3}M ATP	110
10^{-3}M GTP + 10^{-3}M ATP + 10^{-3}M UTP	47
10^{-3}M GTP + 10^{-3}M UTP	29
10^{-3}M GTP + 10^{-4}M UTP	66
10^{-3}M GTP + 10^{-3}M UMP	28
10^{-3}M GTP + 10^{-4}M UMP	77
10^{-3}M GTP + 10^{-3}M CTP	100
10^{-3}M GTP + 10^{-3}M CMP	100

Enzyme protein (6.2 μg) was preincubated in a volume of 40 μl for 10 min with PRPP and nucleotides as shown above, then 10 μl of labelled uracil was added to give a total volume of 50 μl containing 2×10^{-5} M uracil, 2×10^{-4} M PRPP and nucelotides at the stated concentrations. Samples were taken after 5 min incubation for assay as described in text.

of bovine serum albumin, β-mercaptoethanol or dithiothreitol, increased $[Mg^{2+}]$, glass distilled water and increased [EDTA] were tested for their effect on the enzymic activity. While bovine serum albumin, β-mercaptoethanol and increased $[Mg^{2+}]$ were effective in increasing activity they did not abolish the effect of dilution in reducing the specific activity of the enzyme.

The results described above served to establish suitable assay conditions for kinetic studies. We have shown that all the product (that is radioactive material remaining at the origin) formed under these conditions co-chromatographs with carrier UMP. The dependence of rate of reaction on uracil and PRPP concentrations is shown in fig. 1 and fig. 2 respectively. Double reciprocal plots [6] of these results indicate K_m values of 4×10^{-6} M for uracil and 2×10^{-5} M for PRPP.

The effect of some other nucleotides on enzymic activity is shown in table 2. The results demonstrate the requirement for GTP and the inhibitory effect of the uridine nucleotides. Such a relationship could be instrumental in maintaining a balance between the concentrations of purine and pyrimidine nucleotides within the bacterial cell during growth in a medium containing uracil.

Acknowledgement

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