INHIBITION OF BACTERIAL URACIL PHOSPHORIBOSYLTRANSFERASE BY URIDINE AND 6-AZAURIDINE 5'-MONOPHOSPHATES

A. ČIHÁK

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received June 21st, 1973

Uracil phosphoribosyltransferase in a cell-free extract of *Escherichia coli* is inhibited by uridine 5'-monophosphate and its synthetic analogue, 6-azauridine 5'-phosphate. 50 per cent inhibition of the enzyme is achieved at $6 . 10^{-4}$ and $3 . 10^{-3}$M concentrations, respectively. Orotidine 5'-phosphate is less effective and its inhibitory action is not due to the decarboxylation to uridine 5'-monophosphate. Contrary to uracil phosphoribosyltransferase orotate phosphoribosyltransferase is almost insensitive to uridine 5'-phosphate but is strongly inhibited by orotidine 5'-phosphate. The interference of uridine phosphorylase with the assay of uracil phosphoribosyltransferase is discussed.

Uracil phosphoribosyltransferase catalyzes the reaction between uracil and 5-phosphoribosyl-1-pyrophosphate leading to the formation of uridine 5'-monophosphate. The enzyme has no sharp specificity and a similar transfer of the phosphoribosyl residue was observed in 6-azauracil[1] 5-fluorouracil[2-4], 5-azauracil[5,6] and several other base analogues as substrates[7]. The enzyme is widely spread in bacteria[8-10] and differs from orotate phosphoribosyltransferase[11-13].

In view of the remarkable inhibitory effect of orotidine 5'-phosphate on hepatic orotate phosphoribosyltransferase[14,15] and inhibition of bacterial adenine phosphoribosyltransferase by adenosine 5'-monophosphate[16,17] we were interested if a similar regulatory mechanism was operating in case of uracil phosphoribosyltransferase. The present report deals with the effect of different pyrimidine 5'-monophosphates on uracil phosphoribosyltransferase present in a cell-free extract of *E.coli*.

EXPERIMENTAL

Material. Uridine and adenosine 5'-triphosphates, orotidine and uridine 5'-monophosphates and 5-phosphoribosyl-1-pyrophosphate were obtained from Calbiochem, Luzern. 6-Azauridine and 6-azacytidine 5'-monophosphates were prepared in this Institute. Orotic-[2-^{14}C] acid (44 mCi/mmol) and uracil-[2-^{14}C] (48 mCi/mmol) were purchased from the Institute for Research, Production and Uses of Radioisotopes, Prague.

Abbreviations used: UTP, uridine 5'-triphosphate; OMP, orotidine 5'-phosphate; UMP, uridine 5'-monophosphate; 6-AzUMP, 6-azauridine 5'-phosphate; UR, uridine and PRPP, 5-phosphoribosyl-1-pyrophosphate.

Bacterial extract. The cultivation of *E. coli* B was carried out at 37°C for 16 h in 2 litres of a synthetic medium containing glucose[5]. The harvested bacteria were washed with 0·9% NaCl and suspended in 15 ml of cold 0·05M-Tris-HCl buffer (pH 7·5); the sample was disrupted by sonication (70 s, 2°C, 1·5 kc) and the sonicate was centrifuged (10 000 *g*, 20 min, 2°C). The supernatant fraction was stored at −20°C for 5 days without detectable decrease of the enzyme activity.

Uracil and orotate phosphoribosyltransferases were measured[6,10] during a 10 min incubation period at 37°C in 0·06M-Tris-HCl buffer (pH 7·5) with 0·1 mM uracil-[2-^{14}C] or orotate-[2-^{14}C], generally 0·4 mM 5-phosphoribosyl-1-pyrophosphate with equimolar Mg^{2+}-ions and 0·1 ml of cell-free extract (corresponding to 2·2 mg protein) in a total volume of 0·5 ml of incubation mixture. Aliquots were analyzed without deproteinization by chromatography using Whatman No 1 paper in a solvent system composed of isobutyric acid–water–ammonium hydroxide (66 : 33 : 1·5). Radioactive spots of the newly formed products were located on chromatograms according to standards, and their radioactivity was measured in a liquid scintillation spectrometer (Packard).

Orotidine 5'-phosphate decarboxylase and uridine 5'-monophosphatase were assayed as described above using 2 mM orotidine-[2-^{14}C] 5'-phosphate or uridine-[2-^{14}C] 5'-monophosphate as substrates for the respective enzyme reactions.

RESULTS

For the assay of uracil phosphoribosyltransferase a highly sensitive radioactive method was used[10]. The enzyme activity is expressed as the amount of newly synthesized uridine 5'-monophosphate. During the incubation uridine 5'-monophosphate undergoes simultaneously metabolic transformation and uridine 5'-triphosphate is formed. The level of uridine 5'-mono- and triphosphate as well as the inhibitory effect of different pyrimidine 5'-monophosphates on the activity of uracil phosphoribosyltransferase are shown in Table I. Time course of the reaction and unexpected enhancement of the level of radioactive uridine in the mixture containing uracil-[2-^{14}C] with 10 mM uridine 5'-monophosphate are presented in Fig. 1. In the presence of 10 mM orotidine 5'-phosphate the activity of uracil phosphoribosyltransferase is also inhibited but there is no change in the synthesis of uridine.

From the experiments shown in Table II it is evident that the enhanced synthesis of uridine taking place in the presence of uridine 5'-monophosphate does not require 5-phosphoribosyl-1-pyrophosphate. While the synthesis of uridine 5'-monophosphate from uracil and 5-phosphoribosyl-1-pyrophosphate decreases, the level of newly formed uridine is increased. It is evident (Table III) that uridine 5'-monophosphate is at first split to uridine which is utilized in a reaction catalyzed by uridine phosphorylase[18]. If the splitting is inhibited, no uridine is formed and the reaction catalyzed by uridine phosphorylase does not take place.

Both orotidine and uridine 5'-phosphates depress the phosphoribosyl transfer; however, no significant kinetic data with respect to reacting molecules could be obtained in unpurified bacterial extract. The extent of the inhibitory effect of both

5'-phosphates on uracil phosphoribosyltransferase is seen from Fig. 2. On molar concentration basis uridine 5'-monophosphate exhibits significantly higher depression of the enzyme activity than orotidine 5'-phosphate. The data presented in Fig. 3 show that the inhibition of uracil phosphoribosyltransferase observed in the presence

TABLE I

Inhibition of Bacterial Uracil Phosphoribosyltransferase

Incubation was carried out for 10 min at 37°C in 0·06M Tris-HCl buffer (pH 7·5) in a total volume of 0·5 ml with 0·1 ml cell-free extract, 0·1 mM uracil-[2-^{14}C], 0·4 mM 5-phosphoribosyl-1--pyrophosphate and equimolar Mg^{2+}-ions.

Added 10 mM	Newly formed, nmol			Reacted %
	UTP	UMP	Uridine	
0	2·26	20·83	1·51	24·60
OMP	0·77	11·32	1·36	13·45
UMP	0·49	1·24	1·20[a]	2·93
6-AzUMP	0·70	4·23	0·92	5·85

[a] Corrected for transribosylation.

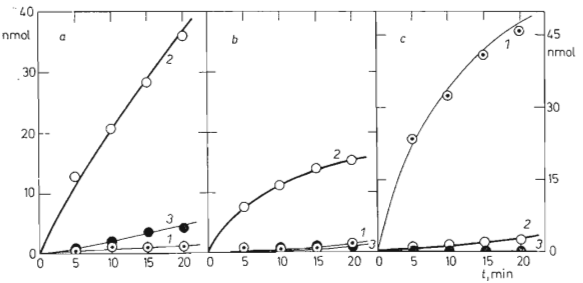

FIG. 1

Time Course of the Reaction Catalyzed by Uracil Phosphoribosyltransferase in the Presence of Orotidine and Uridine 5'-Monophosphates

Incubation was carried out at 37°C in 0·06M-Tris-HCl buffer (pH 7·5) in a total volume of 0·5 ml with 0·1 ml of cell-free extract, 0·1 mM uracil-[2-^{14}C], 0·4 mM 5-phosphoribosyl-1-pyrophosphate and equimolar Mg^{2+}-ions. The amount of newly formed uridine (*1*), uridine 5'-monophosphate (*2*) and 5'-triphosphate (*3*) is expressed in nmol. *t*, Duration of the incubation in min. *a*, Control; *b*, 10 mM OMP added; *c*, 10 mM UMP added.

TABLE II

Synthesis of Uridine and Uridine 5′-Monophosphate from Uracil in *E.Coli* Cell-Free Extract

Incubation was carried out for 10 min at 37°C in 0·06M Tris-HCl buffer (pH 7·5) in a total volume of 0·5 ml with 0·1 ml cell-free extract and 0·1 mM uracil-[2-^{14}C].

UMP mM	With 0·4 mM PRPP		Without PRPP	
	UMP formed nmol	UR formed nmol	UMP formed nmol	UR formed nmol
0	23·0	1·5	0·7[a]	1·7
1	5·8	5·1	0·8[a]	6·3
10	1·8	32·5	0·6[a]	36·4

[a] Within the range of standard error of the method.

TABLE III

Uridine Phosphorylase Activity in *E.coli* Cell-Free Extract

Incubation was carried out for 10 min at 37°C in 0·06M Tris-HCl buffer (pH 7·5) (and 50 mM Na_2HPO_4, pH 7·5, eventually) in a total volume of 0·5 ml with 0·1 ml cell-free extract and 0·1 mM uracil-[2-^{14}C].

Added 10 mM	Uridine-[2-^{14}C] formed, nmol	
	Tris	Tris + 50 mM phosphate
0	4·87	4·12
Uridine	61·20	51·63
UMP	48·35	4·82

TABLE IV

Inhibition of Bacterial Orotate Phosphoribosyltransferase

Incubation was carried out for 10 min as in Table I with 0·1 mM orotic-[2-^{14}C] acid, 0·4 mM 5-phosphoribosyl-1-pyrophosphate and equimolar Mg^{2+}-ions.

Added 10 mM	Newly formed, nmol			Reacted %
	OMP	UMP	UR + Uracil	
0	5·90	91·42	1·69	99·01
OMP	7·63	1·93	0·94	10·50
UMP	1·83	89·48	1·55	92·86

of orotidine 5'-phosphate cannot be explained by its decarboxylation to uridine 5'-monophosphate. Orotidine 5'-phosphate is probably bound to enzyme molecules and this prevents further attachment of uridine 5'-monophosphate.

6-Azauridine 5'-phosphate[19] inhibits also uracil phosphoribosyltransferase, while 6-azacytidine 5'-phosphate is without any effect (Fig. 4). 50% inhibition of uracil phosphoribosyltransferase was achieved at $6 . 10^{-4}$ and $3 . 10^{-3}$ M concentrations of uridine and 6-azauridine 5'-monophosphates, respectively. Synthesis of 6-azauridine 5'-monophosphate from 6-azauracil catalyzed by bacterial pyrimidine phosphoribosyltransferase is also inhibited by uridine 5'-monophosphate (50% inhibition at about $1 . 10^{-3}$M concentration).

Cell-free extract isolated from *E.coli* possesses highly active orotate phosphoribo-

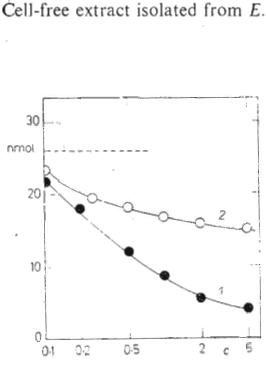

FIG. 2

Inhibitory Effect of Uridine 5'-monophosphate (1) and Orotidine 5'-phosphate (2) on Uracil Phosphoribosyltransferase in *E. coli* Cell-free Extract

Incubation was carried out as in Fig. 1 for 10 min at 37°C. The enzyme activity is expressed as newly formed uridine 5'-monophosphate in nmol. *c*, Concentration of 5'-mononucleotides added to the incubation mixture in mol . 10^3. Dotted, activity of the control.

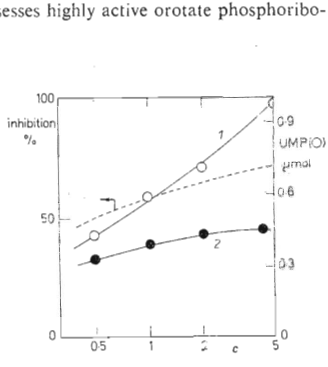

FIG. 3

Actually Observed and Expected Inhibition of Uracil Phosphoribosyltransferase by Orotidine 5'-phosphate and by Newly Formed Uridine 5'-monophosphate

Orotidine [2-^{14}C] 5'-phosphate (c) was incubated for 10 min at 37°C in 0·06M Tris-HCl buffer (pH 7·5) in a total volume of 0·5 ml with 0·1 ml enzyme extract. Levels of the newly formed uridine 5'-monophosphate (1) are expressed as μmol. Uracil phosphoribosyltransferase was assayed as described in Fig. 1. Actual inhibition of the enzyme by the added orotidine 5'-phosphate (2) and expected inhibition of the enzyme by the newly formed uridine 5'-monophosphate (dotted) are given in per cents.

syltransferase. In this case (Table IV) uridine 5'-monophosphate added to the incubation mixture is almost without inhibitory effect while orotidine 5'-phosphate strongly blocks the phosphoribosyltransferase reaction.

DISCUSSION

Phosphoribosyltransferase isolated from ascitic cells of P 388/38 280 murine leukemia catalyzes the phosphoribosyl transfer to various pyrimidine bases and base analogues including uracil and orotic acid[3,4]. Our results as well as findings of other workers[10–13] speak in favour of two different enzymes utilizing uracil and orotic acid as the substrates. Inhibitory effect of uridine 5'-monophosphate on bacterial uracil phosphoribosyltransferase reminds of the end-product inhibition of adenine phosphoribosyltransferase by adenosine 5'-monophosphate studied in *E.coli* K_{12} and in *Mycoplasma mycoides*[17] and the inhibition of adenine and hypoxanthine phosphoribosyltransferases present in Ehrlich ascites tumour cells[20].

Cell-free extracts isolated from *E.coli* utilize uridine 5'-monophosphate for the synthesis of labelled uridine from uracil-[2-^{14}C]. The enhanced synthesis of uridine (Table II) under conditions of blocked uracil phosphoribosyltransferase by the added uridine 5'-monophosphate can be explained assuming the degradation of the mononucleotide and the utilization of the newly formed uridine for the reaction catalyzed by uridine phosphorylase[18].

Uridine 5'-monophosphate inhibits thus two enzyme reactions leading to its synthesis: uracil phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase[21,22]. It seems that decarboxylase activity is more sensitive to the inhibitory action of uridine 5'-monophosphate in the liver[22] than in bacteria (Table IV). Uridine kinase, catalyzing the synthesis of uridine 5'-monophosphate by the salvage pathway

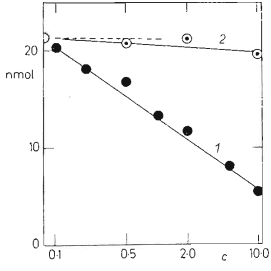

FIG. 4

Inhibitory Effect of 6-Azauridine 5'-Phosphate on Uracil Phosphoribosyltransferase in *E.coli*

Incubation was carried out as in Fig. 1 for 10 min in the presence of 6-azauridine 5'-monophosphate (*1*) or 6-azacytidine 5'-monophosphate (2). The activity of enzyme is expressed as newly formed uridine 5'-monophosphate in nmol. *c*, Concentration of 6-azaanalogues added to the mixture in mol . 10^3. Dotted, activity of the control.

is inhibited by several nucleoside 5'-triphosphates[23] but not by uridine 5'-monophosphate (unpublished observation). For the further work concerning the inhibitory effect of uridine and 6-azauridine 5'-monophosphates the purification of uracil phosphoribosyltransferase from crude bacterial extract is necessary.

I am greatly indebted to Mr H. Jahn Calbiochem, Luzern, for the generous gift of 5-phosphoribosyl-1-pyrophosphate.

REFERENCES

1. Rubin R. J., Jaffe J. J., Handschumacher R. E.: Biochem. Pharmacol. *11*, 563 (1962).
2. Dahl J. L., Way J. L., Parks R. E.: J. Biol. Chem. *234*, 1050 (1959).
3. Reyes P.: Biochemistry *8*, 2057 (1969).
4. Kessel D., Deakon J., Coffey B., Bakamjian A.: Mol. Pharmacol. *8*, 731 (1972).
5. Čihák A., Škoda J., Šorm F.: This Journal *29*, 814 (1964).
6. Čihák A., Šorm F.: Biochem. Pharmacol. *21*, 607 (1972).
7. Hatfield D., Wyngaarden J. B.: J. Biol. Chem. *239*, 2580 (1964).
8. Crawford I., Kornberg A., Simms E. S.: J. Biol. Chem. *226*, 1093 (1957).
9. Canellakis E. S.: J. Biol. Chem. *227*, 329 (1957).
10. Čihák A., Šorm F.: This Journal *30*, 3513 (1965).
11. Brockman R. W., Davis J. M., Stutts P.: Biochim. Biophys. Acta *40*, 22 (1960).
12. Markoff L. J., Handschumacher R. E.: Biochem. Pharmacol. *15*, 761 (1966).
13. O'Donovan G. A., Neuhard J.: Bacteriol. Rev. *34*, 278 (1970).
14. Umezu K., Amaya T., Yoshimoto A., Tomita K.: J. Biochem. *70*, 249 (1971).
15. Čihák A.: Arch. Biochem. Biophys., in press.
16. Hochstadt-Ozer J., Stadtman E. R.: J. Biol. Chem. *246*, 5294 (1971).
17. Sin I. L., Finch L. R.: J. Bacteriol. *112*, 439 (1972).
18. Kraut A., Yamada E. W.,: J. Biol. Chem. *246*, 2021 (1971).
19. Handschumacher R. E.: J. Biol. Chem. *235*, 2917 (1960).
20. Murray A. W.: Biochem. J. *103*, 271 (1967).
21. Creasey W. A., Handschumacher R. E.: J. Biol. Chem. *236*, 2058 (1961).
22. Blair D. G. R., Potter V. R.: J. Biol. Chem. *236*, 2503 (1961).
23. Anderson E. P., Brockman R. W.: Biochim. Biophys. Acta *91*, 380 (1964).

Translated by J. Veselý.