ENHANCEMENT OF URIDINE KINASE ACTIVITY IN *Escherichia coli* B CULTIVATED IN THE PRESENCE OF METABOLIC INHIBITORS

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The cultivation of *Escherichia coli* B in the presence of sub-bacteriostatic concentrations of 5-azauracil or 5-azacytidine resulted in the increase of uridine kinase activity assayed in a cell-free bacterial extract. The activities of thymidine, thymidylate, and uridylate kinases were not changed under similar conditions. The increase of uridine kinase following both 5-azapyrimidines is dose-dependent and occurs also in the inhibited culture of *E. coli*. Uridine and uracil competing to different degree with the uptake of 5-azauracil and 5-azacytidine into nucleic acids reverse completely the stimulating effect of both drugs. Mild inhibition of the growth of *E. coli* by chloramphenicol had no effect on the observed increase of uridine kinase activity.

Uridine kinase, a key enzyme in the pyrimidine salvage pathway[1-3], seems to be under different and unique regulatory control. This conclusion is based on our earlier observations on eukaryotic systems[4-6], mainly that various drugs and metabolic inhibitors result in the enhancement of hepatic uridine kinase under conditions of a blocked RNA, DNA, and protein synthesis. Compounds increasing the activity of uridine kinase belong to various classes of inhibitors affecting different cellular processes[5,6]. In this paper an attempt to demonstrate the increase of uridine kinase in bacterial system was made. *Escherichia coli* B cultivated in a mineral medium supplemented with glucose was chosen as a model. The results obtained are in agreement with our earlier findings and show that 5-azacytidine and similarly 5-azauracil increase the activity of uridine kinase also in a simple prokaryotic system.

EXPERIMENTAL

Chemicals. Analogues of nucleic acid components were prepared in the Department of Organic Synthesis in this Institute. Adenosine 5'-triphosphate and natural pyrimidine precursors of nucleic acids were delivered by Calbiochem, Luzern. 5-Azacytidine-[4-^{14}C] (3·37 μCi/μmol), 5-azauracil-[2,4-^{14}C] (2·47 μCi/μmol) and 6-azauridine-[4,5-^{14}C] (44 μCi/μmol) were prepared in the Institute for Research, Production and Uses of Radioisotopes, Prague.

Cultivation of E. coli B was carried out stationarily at 37°C for 16 h in 200 ml of a synthetic medium containing glucose[7]. Compounds tested and/or pyrimidine precursors were added

to the medium immediately before inoculation. Inoculation was carried out with 4 ml of a freshly prepared *E. coli* culture. The cell-free extracts were prepared after centrifugation and washing of bacteria with cold 0·9% NaCl. The harvested bacteria were suspended in 8 ml of ice-cold 0·05M-Tris-HCl buffer (pH 7·5) and disrupted by sonication (70 s, 2 °C, 1·5 kc). The sonicates were centrifuged (10000 *g*, 3°C, 20 min) and supernatant fractions used immediately for the measurement of uridine kinase.

Uridine kinase was assayed with $5 . 10^{-5}$M 6-azauridine-[2,4-^{14}C] as a substrate[8] during a 10-min incubation period at 37°C with $3 . 10^{-3}$M adenosine 5′-triphosphate and $1·5 . 10^{-3}$M $MgCl_2$ in 0·05M-Tris-HCl buffer (pH 7·5). The reaction was linear over a 20—30 min period of incubation and 0·5—0·6 mg of crude proteins in 0·1 ml of cell-free bacterial extract added to the total volume of 0·3 ml of the incubation mixture. Analysis of the mixture was carried out by chromatography on Whatman No 1 paper in the solvent system composed of n-butanol–acetic acid–water (10 : 1 : 3) or isobutyric acid–ammonium hydroxide–water (66 : 1·5 : 33). The radioactive zones of 6-azauridine-[2,4-^{14}C] and of the corresponding 5′-monophosphate were cut out and their radioactivity was measured using Packard liquid scintillation spectrometer. The activity of uridine kinase is expressed in μmol of 6-azauridine 5′-phosphate formed during the 10 min incubation period in the presence of 1 mg of crude bacterial proteins in the incubation mixture. Activities of thymidine, thymidylate and uridylate kinases were measured as described earlier[5].

Incorporation of ^{14}C-labelled 5-azauracil and 5-azacytidine into the fraction of total nucleic acids in *E. coli* was followed using the technique of membrane filters. Bacteria from 1 ml medium were washed with 5 ml of cold 5% trichloroacetic acid, twice 10 ml distilled water and the filters were dried for 10 min at 100°C.

RESULTS

We were interested in the effect of various pyrimidine analogues and metabolic inhibitors, affecting hepatic uridine kinase[5,6], on the activity of this enzyme in *E. coli*. Table I summarizes the effect of the compounds tested. As in the liver, the addition of 5-azacytidine into the medium results in the enhancement of uridine kinase in bacterial cells. 5-Azauracil, not effective in the liver, had a similar stimulatory effect. This compound is first phosphoribosylated and its action in *E. coli* is then similar to 5-azacytidine. No stimulatory (or inhibitory) effect on the activity of *E. coli* uridine kinase was observed during the cultivation of bacteria with thioacetamide, cycloheximide or related glutarimide antibiotics resulting in the enhancement of liver uridine kinase[6,9].

The increase of the enzyme activity depends on the concentration of both 5-azapyrimidines in the culture medium (Fig. 1). The data shown in the figure reflect the enhancement of uridine kinase in the presence of low doses of the drugs, permitting the growth of bacteria. The optical density of control and analog-treated cultures was the same. At higher concentrations of 5-azacytidine ($2·5—3·5 . 10^{-5}$M) the cells display pronounced morphological changes manifested by the formation of filamentous forms[10] and also their number decreases. However, even under these conditions the activity of uridine kinase, calculated per 1 mg of crude proteins in cell-free bacterial extracts, was many times increased.

TABLE I

Effect of Various Metabolic Inhibitors on Uridine Kinase Activity in Cell-Free Extracts of *E. coli*

The experiments were carried out in triplicates.

Compound	Concentration M	Uridine kinase μmol/mg protein ± S.E.	Compound	Concentration M	Uridine kinase μmol/mg protein ± S.E.
Control	—	8·2 ± 1·1	5-azacytidine	$1 . 10^{-5}$	24·7 ± 1·8
5-Fluorouracil	$2 . 10^{-6}$	8·8 ± 0·9	6-azacytidine	$5 . 10^{-4}$	8·4 ± 0·5
5-Azauracil	$1 . 10^{-5}$	20·4 ± 2·3	5-fluorodeoxyuridine	$2 . 10^{-7}$	9·1 ± 1·2
6-Azauracil	$2 . 10^{-5}$	7·8 ± 0·6	5-azadeoxycytidine	$1 . 10^{-5}$	8·7 ± 1·0
5-Fluorouridine	$2 . 10^{-7}$	9·2 ± 1·1	5-fluoroorotate	$1 . 10^{-4}$	8·6 ± 0·5
6-Azauridine	$3 . 10^{-4}$	8·0 ± 0·3	5-azaorotate	$1 . 10^{-4}$	7·6 ± 0·6

TABLE II

Activity of Pyrimidine Kinases in a Cell-Free Extract of *E. coli* Cultivated in the Presence of 5-Azauracil and 5-Azacytidine

The activity of enzymes is expressed in μmol of reacted substrates ($5 . 10^{-5}$ M) during a 10 min incubation period at 37°C in a reaction mixture containing 1 mg of proteins from cell-free bacterial extracts. Experiments were carried out in triplicates. Controls = 100 per cent.

Inhibitor $1 . 10^{-5}$ M	Enzyme activity, %			
	thymidine kinase	thymidylate kinase	uridine kinase	uridylate kinase
Control	21·3 ± 2·1 (100)	10·1 ± 0·6 (100)	8·4 ± 1·1 (100)	21·8 ± 2·7 (100)
5-Azauracil	20·9 ± 2·0 (98)	10·7 ± 0·8 (106)	21·0 ± 1·6 (248)	23·2 ± 3·4 (106)
5-Azacytidine	22·5 ± 1·6 (105)	6·7 ± 0·3 (65)	23·6 ± 1·9 (274)	20·6 ± 1·7 (95)

Uridine kinase seems to be unique in its stimulatory response to both 5-aza analogs. No similar effect was observed with thymine, thymidylate, and uridylate kinases (Table II). Pyrimidine precursors of nucleic acid components added at $2 . 10^{-4}$M concentration simultaneously with both analogs ($1 . 10^{-5}$M) completely reverse the enhancement of uridine kinase. There was no difference between the action of free bases and nucleosides (unpublished). Also thymine and thymidine at higher concentrations counteract the stimulatory action of 5-azacytidine (Fig. 2) and similarly

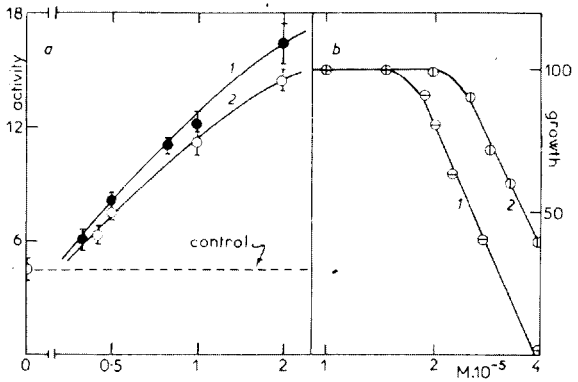

FIG. 1

Increase of Uridine Kinase Activity (*a*) in *E. coli* Cultivated in the Presence of Increasing Concentrations of 5-Azauracil (*2*) and 5-Azacytidine (*1*) (in M . 10^{-5}); Growth of Bacteria (*b*) Under Similar Conditions (in %)

The activity of uridine kinase is expressed in μmol of the newly formed 6-azauridine 5′-monophosphate per 1 mg of proteins; the growth is expressed in % of the control (= 100 per cent).

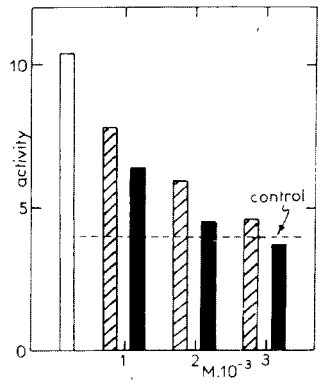

FIG. 2

Reversion of the Stimulatory Effect of $1 . 10^{-5}$M 5-Azacytidine (□) on Uridine Kinase Activity by the Cultivation of *E. coli* with Increasing Concentrations of Thymine (⊡) and Thymidine (■)

The activity of uridine kinase is expressed as in Fig. 1.

that of 5-azauracil. This finding is somewhat unexpected, since uracil, cytosine and thymine do not reverse the growth inhibition of *E. coli* caused by 5-azacytidine[10] (but do reverse it in case of 5-azauracil-mediated inhibition).

To get more insight into this phenomenon we measured further the uptake of labelled 5-azacytidine and 5-azauracil into the fraction of nucleic acids in *E. coli*. The data shown in Table III are in accordance with the reversion of the growth inhibition caused by 5-azacytidine and 5-azauracil, but do not give the explanation of the complete reversion of the enhanced uridine kinase. Again, both uracil and uridine

TABLE III

Different Effects of Uracil and Uridine on the Uptake of 5-Azauracil and 5-Azacytidine by Nucleic Acids in *E. coli*

$1 . 10^{-5}$M 5-Azauracil-[2,4-^{14}C] (2·47 μCi/μmol) and/or $1 . 10^{-5}$M 5-azacytidine-[4-^{14}C] (3·37 μCi/μmol) were present in the medium of *E. coli* simultaneously with $2 . 10^{-4}$M uracil and/or uridine. Cultivation of *E. coli* was carried out 12 h at 37°C in duplicate. 1 ml samples of the cultures were used for the measurement of the labelling of total nucleic acids. Controls = = 100 per cent.

Precursor added	Uptake of 5-azauracil-[2,4-^{14}C]		Uptake of 5-azacytidine-[4-^{14}C]	
	dpm per ml	%	dpm per ml	%
—	470—530	100	860—970	100
Uracil	128—165	29	680—740	78
Uridine	86— 92	18	117—123	13

TABLE IV

5-Azacytidine-Stimulated Uridine Kinase in *E. coli* Cultivated in the Presence of Low Doses of Chloramphenicol

5-Azacytidine ($1 . 10^{-5}$M) and/or chloramphenicol were added to the medium before inoculation. Growth of *E. coli* without chloramphenicol = 100 per cent.

Chloramphenicol μg/ml	Without 5-azacytidine		5-Azacytidine-treated	
	uridine kinase μmol/mg proteins ±S.E.	growth %	uridine kinase μmol/mg proteins ±S.E.	growth %
0	6·9 ± 0·7	100	22·8 ± 2·3	100
2	7·4 ± 1·0	95	30·2 ± 4·1	90
4	6·7 ± 0·6	88	24·2 ± 2·7	81

compete with the uptake of 5-azauracil whereas uridine only depresses the incorporation of 5-azacytidine. Addition of uracil simultaneously with labelled 5-azacytidine results in a partial depression of its uptake only.

In the liver the increase of uridine kinase occurs when the synthesis of total cellular proteins is blocked by cycloheximide. Moreover, the drug alone results in the liver in the enhancement of the activity of this enzyme[9]. In the present study we used chloramphenicol to test the effect of inhibitors of protein synthesis on the enhacement of bacterial uridine kinase. From the data shown in the Table IV it can be seen that also in *E. coli* the increase of uridine kinase mediated by 5-azacytidine occurs in the presence of an inhibitor of protein synthesis. The level of chloramphenicol did not change substantially the growth of *E. coli* and the drug itself had no effect on the activity of uridine kinase.

DISCUSSION

The finding of enhanced uridine kinase activity in *E. coli* cultivated in the presence of 5-azacytidine or 5-azauracil offers a new system for the study of the molecular mechanism of the observed phenomenon. Both hepatocytes and prokaryotic cells respond thus similarly to the action of 5-azacytidine and enhance uridine kinase activity. The stimulatory effect of 5-azauracil observed in *E. coli* but not in the liver reflects the different metabolic transformation of the drug in both systems. Whereas in bacteria 5-azauracil undergoes phosphoribosylation resulting in the synthesis of the corresponding 5'-phosphate, this reaction was not observed in the liver[11].

The ability of natural pyrimidine bases completely to counteract the enhancement of uridine kinase activity mediated by 5-azacytidine is difficult to explain with certainity at present. One explanation is, that the inducing effect of 5-azacytidine is not necessarily related to its incorporation (Table III). However, this presumption should be verified further, *e.g.* by using various strains of *E. coli* sensitive and resistant to 5-azacytidine and/or 5-azauracil, and by measurement of the stimulatory response of uridine kinase in these bacterial strains.

We do not know whether the enhanced activity of uridine kinase is paralleled by the increased rate of the *de novo* enzyme synthesis. In fact, the enhanced rate of the synthesis of uridine kinase is hardly probable in view of the known stimulatory effect of cycloheximide on the activity of this enzyme in the liver[9]. Similarly, the growth of *E. coli* in the presence of chloramphenicol (Table IV) had no effect on the 5-azacytidine mediated increase of uridine kinase. The elucidation of the mechanism of uridine kinase enhancement is the aim of our present study.

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