BBA 66418

THE ROLE OF URIDINE–CYTIDINE KINASE IN THE REGULATION OF PYRIMIDINE RIBONUCLEOTIDE SYNTHESIS IN *TETRAHYMENA PYRIFORMIS* GL

WILLIAM PLUNKETT* AND J. G. MONER
Department of Zoology, University of Massachusetts, Amherst, Mass. 01002 (U.S.A.)

(Received May 17th, 1971)

SUMMARY

1. Uridine–cytidine kinase (ATP:5'-uridine phosphotransferase, EC 2.7.1.48) has been purified 500-fold from *Tetrahymena pyriformis* GL, and some of its properties have been investigated.

2. The molecular weight of the enzyme has been estimated as 195 000 by gel filtration.

3. A bivalent cation is necessary for activity. Mg^{2+} is the most effective but may be partially replaced by Mn^{2+} or Fe^{2+}.

4. Uridine and cytidine are readily phosphorylated to UMP and CMP, respectively, however 2'-deoxyuridine, 2'-deoxycytidine, 5-methyluridine and 5-fluoro-2'-deoxyuridine do not serve as phosphate acceptors.

5. The kinase utilizes ATP, dATP, GTP, dCTP and dUTP as phosphate donors at selected concentrations, while CTP, UTP and dGTP are not effective in this capacity.

6. Substrate phosphorylation is inhibited by the addition of either UTP or CTP to the assay mixture. Inhibition by CTP is competitive with the phosphate donor and noncompetitive with the phosphate acceptor.

7. It is suggested that UTP and CTP act as feedback inhibitors of uridine–cytidine kinase. Such control of pyrimidine ribonucleotide synthesis may be of importance in *Tetrahymena*, an organism which is unable to synthesize the pyrimidine ring by the *de novo* pathway.

INTRODUCTION

Nutritional studies have shown that *Tetrahymena pyriformis*, like other ciliate protozoans, is unable to synthesize the pyrimidine ring[1]. Cell growth is dependent on the presence of uracil, uridine or cytidine[2]. Since control of *de novo* synthesis of the type found in bacterial[3] and mammalian[4,5] cells is not possible, regulation of salvage

* Present address: Department of Microbiology, University of Colorado Medical Center, Denver, Colorada, 80220, U.S.A.

enzyme[6-8] activity may control pyrimidine ribonucleotide synthesis in *Tetrahymena*.

Salvage enzymes, which convert preformed bases and nucleosides to nucleotides, catalyze two routes by which *Tetrahymena* synthesizes UMP, the first of which consists of two reactions, 1 and 2. Reaction 1 is reversibly catalyzed by uridine phosphorylase (uridine:orthophosphate ribosyltransferase, EC 2.4.2.3) (refs. 9–11), while uridine–cytidine kinase (ATP:uridine 5'-phosphotransferase, EC 2.7.1.48) (ref. 10) irreversibly phosphorylates the nucleoside (Reaction 2).

$$\text{Uracil} + \text{ribose 1-phosphate} \leftrightharpoons \text{uridine} + P_i \quad (1)$$

$$\text{Uridine} + \text{ATP} \rightarrow \text{UMP} + \text{ADP} \quad (2)$$

$$\text{Uracil} + \text{PRPP} \rightarrow \text{UMP} + PP_i \quad (3)$$

A second route (3) involves the direct synthesis of UMP from uracil and 5-phosphoribosyl-1-pyrophosphate (PRPP) by UMP pyrophosphorylase (UMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.9)[10,12,13].

A study of uridine–cytidine kinase has been undertaken for several reasons. First, since *Tetrahymena* is unable to synthesize the pyrimidine ring, this enzyme may play a major role in the salvage of preformed pyrimidine nucleosides and their conversion to nucleotides. Second, in fulfilling a salvage function, the kinase may be sensitive to a form of end product regulation similar to that operative in other cells[14-17]. In *Tetrahymena*, this enzyme irreversibly catalyzes the initial reaction of a metabolic pathway (pyrimidine ribonucleotide synthesis), a general property of regulatory enzymes[18,19]. Finally, uncertainty exists over the presence of uridine–cytidine kinase in *Tetrahymena*. A report[10] of weak kinase activity in extracts is in disagreement with work by other investigators[11,20] who were unable to detect such kinase activity. Consequently, several schemes which omit uridine–cytidine kinase have been constructed for the metabolism[11,21] and regulation[20] of pyrimidines by *Tetrahymena*.

The partial purification and some properties of uridine–cytidine kinase from *Tetrahymena* are presented in this report. End product inhibition of enzymatic activity suggests that the kinase may be a regulatory site in the synthesis of pyrimidine ribonucleotides. A preliminary report of this work has been published elsewhere[22].

EXPERIMENTAL

Materials

[2-^{14}C]Uridine (50.5 mC/mmole), [5-^{3}H]cytidine (26.4 C/mmole) and [5-^{3}H]uridine (28.3 C/mmole) were purchased from New England Nuclear Corp. One lot of [2-^{14}C]uridine contained impurities which gave high blank levels in enzyme assays. After purification by paper chromatography the specific activity was determined to be 44.0 mC/mmole. Streptomycin sulfate and the sodium salt of all nucleotides were obtained from Sigma Chemical Co. Diethylaminoethylcellulose paper (Whatman DE-81) was purchased from H. Reeve Angel Corp. (Clifton, N.J.). Triton X-100 was a gift from Rohm and Haas Co. Medical X-ray film (NS-54T) was a product of Eastman Kodak Co. Proteins used were from the following sources: calf intestinal mucosa alkaline phosphatase (EC 3.1.3.1), *Aspergillus niger* catalase (EC 1.11.1.6) and bovine

pancreas chymotrypsinogen-A, P-L Biochemicals; horse heart cytochrome *c* (EC 1.9.3.1), and yeast hexokinase (EC 2.7.1.1), Nutritional Biochemicals Corp.; bovine hemoglobin, Pentex, Inc (Kankakee, Ill). Sephadex G-200 and Blue Dextran 2000 were obtained from Pharmacia Fine Chemical, and DEAE-cellulose (Cellex-D) was purchased from BioRad Laboratories.

Organisms and growth medium

Cultures of *T. pyriformis* strain GL and *T. pyriformis* strain W (kindly provided by Dr. Virginia Dewey, Amherst College) were grown at 28° in organic medium containing 2.0% (w/v) proteose-peptone and 0.4% (w/v) liver fraction "L" (Nutritional Biochemicals Corp.). Salts prescribed for medium A of KIDDER AND DEWEY[23] were added omitting the phosphates. Cells were aerated by shaking on either a reciprocal or a gyratory shaker at 75–90 cycles/min. Cell number was determined with an electronic cell counter (Coulter Electronic, Inc., Model A).

Enzyme assay

The standard assay mixture contained 8.56 μmoles Tris–HCl (pH 8.5), 0.64 μmole $MgCl_2$, 0.54 μmole ATP, 37.0 nmoles labelled nucleoside and 2.0 munits of enzyme in a final volume of 107 μl. All substrate concentrations were saturating and gave maximal enzyme activity. The reaction was started by the addition of enzyme to the pre-equilibrated (30°) mixture. After 2–4 min, the conversion of substrates to nucleoside monophosphate and ADP was measured either chromatographically or by the filter disk method of BREITMAN[24]. Chromatography was used primarily to identify products of the reaction when crude extracts were the enzyme source, while the filter disk method was employed with the purified enzyme.

In the chromatographic method, the reaction was stopped in a boiling water bath for one min before removal of protein by centrifugation. A 10-μl portion was chromatographed on No. 1 Whatman paper using isobutyric acid–water–NH_4OH (66:33:1, v/v/v) as a solvent. Tritiated products were detected by the ultraviolet absorption of known carrier compounds while ^{14}C-labelled products were subjected to radioautography as well. Radioactive spots were cut out, placed in 10 ml of Omnifluor (New England Nuclear Corp.) and counted in a Nuclear Chicago scintillation counter.

When using the filter disk assay, a 50-μl portion of the reaction mixture was pipetted onto a 17-mm DE-81 disk which was dropped into a beaker containing 20 ml of 10 mM ammonium formate per disk. Three washes in formate and two rinses in water were followed by dehydration in ethanol and then ether prior to scintillation counting. Preliminary experiments showed that radioactivity on control disks (heat-inactivated enzyme added to the reaction mixture) was due entirely to impurities in labelled nucleoside substrate, not to substrate retention or cross contamination with product released from other disks in the washing procedures. After washing and dehydration, control levels were always below 250 counts/min when $1.0 \cdot 10^5$ counts/min had been placed on the disk. Repurification of labelled substrate was necessary only once (see *Materials*). Retention of the phosphorylated product during preparation of the disks and scintillation counting was 100%.

A unit of enzyme activity is defined as the amount of enzyme necessary to catalyze the phosphorylation of one μmole of nucleoside to nucleoside monophosphate

per min under the conditions of the standard assay. Specific activity is defined as units of enzyme activity per mg protein. The protein was estimated by the method of LOWRY *et al.*[25] using bovine serum albumin for a standard.

RESULTS

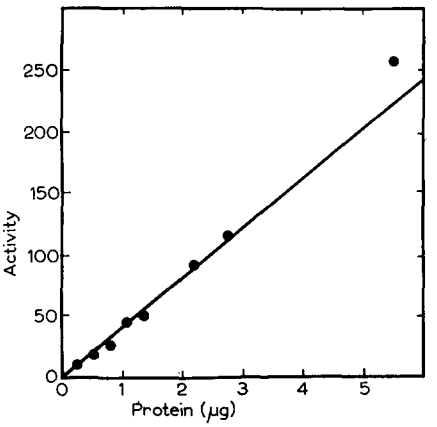
Purification of uridine–cytidine kinase

Cells in the late exponential phase of growth ($5 \cdot 10^5$ cells/ml) were harvested by centrifugation at 450 × *g* for one min from 6 l of culture medium. All subsequent steps were carried out at 0–4°. After two washes in phosphate buffer (50 mM NaCl, 2 mM KCl, 12.5 mM Na_2HPO_4, pH 7.4) and one wash in the same buffer containing 0.25 M sucrose, cells were suspended in three times the packed cell volume of the latter solution. Complete lysis was effected by the addition of 0.2 vol. of phosphate buffer–0.25 M sucrose containing 0.5% (w/v) Triton X-100. After centrifugation at 30 000 × *g* for 20 min, the sediment was discarded and 0.3 vol. of 5% (w/v) streptomycin sulfate, made up in phosphate buffer–0.25 M sucrose, was added to the supernatant fluid (Fraction I). Following 90 min of stirring, the precipitate was removed by centrifugation at 30 000 × *g* for 15 min. The supernatant fluid (Fraction II) was then brought to 40% $(NH_4)_2SO_4$ saturation according to the nomograph of DI JESO[26]. The precipitate was removed by centrifugation and the supernatant fluid was brought to 50% $(NH_4)_2SO_4$ saturation. After 4 h of stirring, the precipitate was recovered by centrifugation and dissolved in a minimal volume of phosphate buffer (Fraction III). The enzyme preparation was pipetted on a column of Sephadex G-200 (6.2 cm^2 × 48.0 cm) which had been equilibrated with 10 mM Tris–HCl (pH 8.5). The enzyme was eluted with the same buffer at a flow rate of 15 ml/h. 3-ml fractions were collected and assayed for kinase activity by the filter disk method. Eluted protein was determined spectrophotometrically at 280 nm. Fractions containing the highest specific activity were pooled (Fraction IV) and applied to a DEAE-cellulose column (4.5 cm^2 × 15 cm) which had been equilibrated with 10 mM sodium phosphate (pH 7.4). The kinase was eluted with a 200-ml linear gradient of 10–100 mM sodium phosphate (pH 7.4). 3-ml fractions were collected and those containing the highest specific activity were pooled to form Fraction V. All experiments to be presented utilized this preparation unless otherwise indicated. A summary of the purification is given in Table I.

TABLE I

PURIFICATION OF URIDINE KINASE

Fraction	*Vol. (ml)*	*Total protein (mg)*	*Total activity (units)*	*Specific activity (units/mg)*	*Purification*
I. Extract	365	2956	10.6	0.0036	1
II. Streptomycin sulfate	455	3094	12.7	0.0041	1
III. $(NH_4)_2SO_4$	3	46	2.27	0.0493	13
IV. Sephadex G-200	18	19	1.64	0.0863	24
V. DEAE--cellulose	15	0.83	1.55	1.867	518



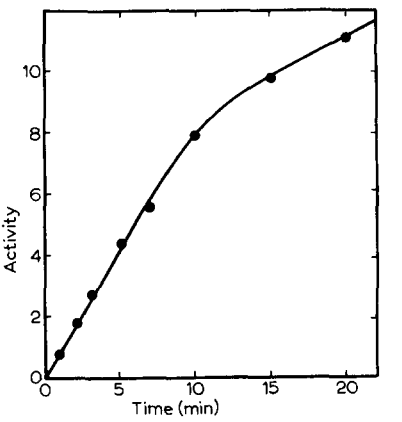


Fig. 1. Uridine kinase activity as a function of protein concentration. The indicated amount of Fraction V protein was added to the standard assay mixture. Enzyme activity was determined by the filter disk method and expressed as pmoles [2-^{14}C]uridine phosphorylated per min.

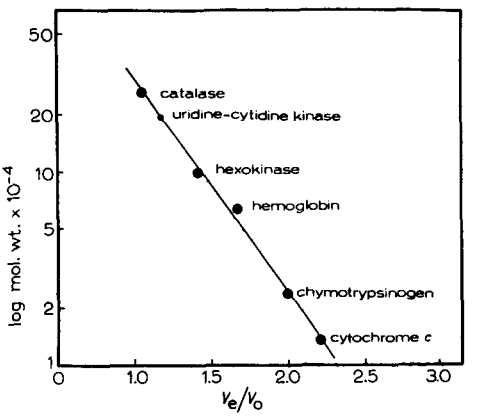
Fig. 2. Uridine kinase activity as a function of time. The reaction mixture contained 34.4 μmoles Tris–HCl (pH 8.5), 2.56 μmoles $MgCl_2$, 2.16 μmoles ATP, 148 nmoles [2-^{14}C]uridine and 1.1 μg of Fraction V protein in a final volume of 425 μl. Aliquots were withdrawn at the indicated times and assayed for kinase activity by the filter disk method. Enzyme activity is expressed as moles [2-^{14}C]uridine phosphorylated per mg protein.

Fractions IV and V showed no decrease in activity when kept at 4° for 60 days. After this time, lost activity could be partially restored by the addition of 2.5 mM dithiothreitol or mercaptoethanol to the reaction mixture. Addition of reducing agents to the fresh preparation had no effect on enzyme activity.

The final preparation, Fraction V, appeared to be free of contaminating enzyme activities. Thus, neither uridine phosphorylase activity[9–11], which could catalyze the phosphorolysis of uridine, nor phosphatases[27], capable of hydrolyzing the product, either UMP or CMP, were detected. Mononucleotide kinase activity[20] was absent from Fractions III–V. While cytidine was readily converted to uridine by crude extracts, no deaminase activity[11,27,28] was found in the final preparation. ATPase activity, measured by the detection of ADP with a coupled spectrophotometric assay[29], was not present in Fraction V.

UMP formation was proportional to the amount of Fraction V protein added to the standard assay mixture, as shown in Fig. 1. Uridine kinase activity was linear for 8 min under standard conditions (Fig. 2). This relationship was checked for each experiment to ensure linear monophosphate production at the time of sampling. All assays to be reported were carried out for 2–4 min.

The radioactive product of the reaction had an R_F value identical with those of UMP and CMP by paper chromatography in the following solvent systems: (1) isobutyric acid–NH_4OH–water (66:1:33, v/v/v), (2) *n*-butanol–formic acid–water (84:11:5, v/v/v) and (3) 4.0 M formic acid in 0.1 M ammonium formate. Systems (1) and (2) were run on Whatman No. I paper while system (3) utilized Whatman DE-81 paper[30]. Spectral ratios of 280/260 nm and 250/260 nm of the uridine reaction product compared favorably with those published by Bock *et al.*[31] for UMP. With uridine as the substrate, the reaction product was further characterized by treatment with



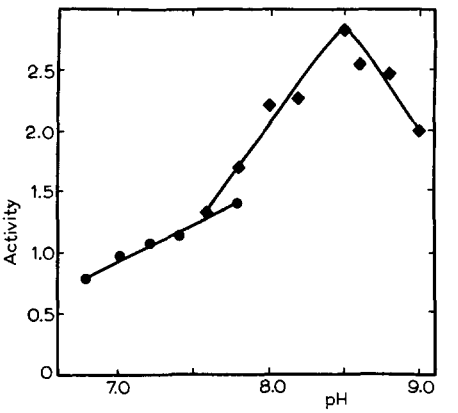


Fig. 3. Estimation of the molecular weight of uridine–cytidine kinase by gel filtration. Fraction III protein (20 mg) was pipetted on top of a Sephadex G-200 (6.15 cm^2 × 48.2 cm) which had been equilibrated with 10 mM Tris–HCl (pH 8.5) at 5°. The protein was eluted with the equilibrating buffer at a flow rate of 12 ml/h and collected in 3-ml fractions, each of which was assayed for activity by the filter disk method. The column was calibrated with the following proteins of known molecular weight: catalase (225 000 mol wt)., hexokinase (96 000 mol. wt.), hemoglobin (64 000 mol. wt.) chymotrypsinogen-A (23 300 mol. wt.) and cytochrome *c* (13 000 mol. wt.). Void volume was determined using Blue Dextran 2000 ($2 \cdot 10^6$ mol. wt.). Abscissa, ratio of elution volume, V_e, divided by void volume, V_0. Ordinate logarithm of the molecular weight of the protein.

Fig. 4. Effect of pH on uridine kinase activity. The standard assay mixture was employed using either Tris–maleate (●) or Tris–HCl (◆) buffer at a final concentration of 80 mM. The pH of each assay mixture was determined at 30°, the temperature at which the assay was conducted. Enzyme activity was assayed by the filter disk method and is expressed as μmoles [2-^{14}C]uridine phosphorylated per min per mg protein Fraction V.

alkaline phosphatase, an enzyme known to hydrolyze the phosphate from 5'-mononucleotides[32]. Subsequent paper chromatography demonstrated the R_F of the phosphatase reaction product to be identical with that of uridine.

Properties of uridine–cytidine kinase

The molecular weight of the enzyme, estimated by the gel filtration procedure of LEACH AND O'SHEA[33], was 195 000 (Fig. 3). Identical results were obtained when

TABLE II

EFFECT OF BIVALENT CATIONS ON URIDINE KINASE ACTIVITY

The standard assay mixture was used except that the dichloride salt of the indicated cation was added to a final concentration of 6 mM. The uridine kinase activity of Fraction V protein was determined by the filter disk method.

Cation	*pmoles [^{14}C]uridine phosphorylated per 3 min*	*% of Mg^{2+} activity*
Mg^{2+}	232	100
Mn^{2+}	132	56
Fe^{2+}	79	34
Co^{2+}	57	24
Ca^{2+}	25	10
Cu^{2+}	0	0
Zn^{2+}	0	0
None	10	4

the column was pre-equilibrated at pH 7.5 with the same buffer. A similar value may be estimated for NOVIKOFF ascites tumor uridine–cytidine kinase from the data presented by ORENGO (ref. 16, Fig. 1).

Uridine kinase exhibits activity over a broad pH range with a maximum at pH 8.5 (Fig. 4). Similar broad activity optima, but at somewhat lower pH scales have been reported for uridine kinase from both Ehrlich[34] and Novikoff[16] ascites tumor cells and from *Chlorella*[35].

Uridine kinase activity depends upon the presence of a bivalent cation in the reaction mixture (Table II). Maximum activity is achieved with 6.0 mM Mg^{2+}, and increasing the concentration to 20 mM gave no inhibitory effect. Similar partial replacement of the Mg^{2+} requirement by Mn^{2+} and Fe^{2+} has been demonstrated for the uridine kinase of Ehrlich ascites tumor cells[34].

Several lines of evidence indicate that the phosphorylation of both uridine and cytidine is catalyzed by a single enzyme in *Tetrahymena*. Uridine and cytidine are equally suitable substrates for the kinase, the ratio of uridine kinase activity to cytidine kinase activity remaining at 1.2 throughout the purification. Fraction III protein applied to Sephadex G-200 and Fraction IV protein eluted from DEAE-cellulose both show parallel uridine and cytidine kinase activity profiles[36]. In addition, cytidine competes with uridine for the enzyme (Fig. 5). The strict stereospecific demands of the enzyme for the phosphate acceptor were demonstrated by the inability of either 2′-deoxyuridine or 2′-deoxycytidine to compete with uridine for the enzyme (Fig. 5). Similarly, neither 5-methyluridine nor 5-fluoro-2′-deoxyuridine competed with uridine for the enzyme (not shown).

The ability of physiological phosphate donors to support uridine kinase activity is shown in Table III. ATP and dATP gave maximum activity at all concentrations employed while dUTP, dCTP and GTP supported maximal phosphorylation at 1.0 mM and 0.5 mM. This lack of specificity of the kinase for the phosphate donor is

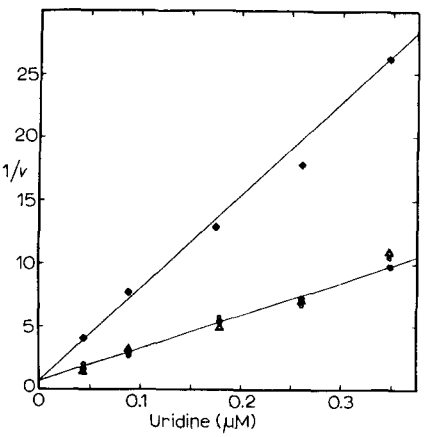


Fig. 5. The effect of added pyrimidine nucleosides on uridine kinase activity. The standard assay mixture was employed except the uridine concentration was varied and the following pyrimidine nucleosides were added at a final concentration of 92 μM: cytidine (◆), 2′-deoxycytidine (△), 2′-deoxyuridine (○), and no addition (●). Uridine kinase activity was assayed by the filter disk method and expressed as nmoles [2-^{14}C] uridine phosphorylated per min per mg protein Fraction V.

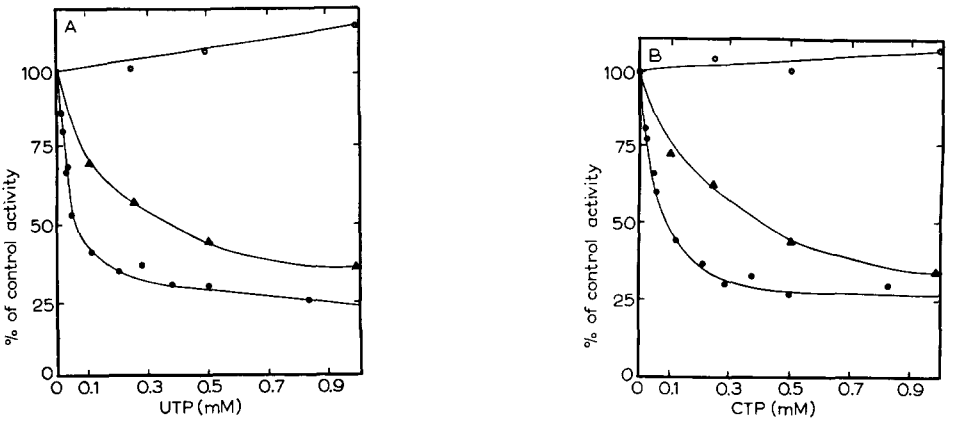


Fig. 6. Effect of pyrimidine nucleotides on uridine kinase activity. Each assay mixture contained 8.56 μmoles Tris–HCl (pH 8.5), 0.64 μmoles $MgCl_2$, 0.11 μmole ATP, and 37 nmoles [2-^{14}C]uridine and 0.28 μg Fraction V protein in a final volume of 107 μl. A. UTP (●), dUTP (○) or UDP (▲) was added to the assay mixture at the indicated concentration. B. CTP (●), dCTP (○) or CDP (▲) was added to the assay mixture at the indicated concentrations. Ordinate values are expressed as uridine kinase activity in the presence of the added pyrimidine nucleotide times 100, divided by uridine kinase activity with no addition. The latter value (control) was 0.5 μmole [2-^{14}C]uridine phosphorylated per min per mg protein Fraction V (average of four determinations).

contrasted by the inability of the enzyme to utilize UTP, CTP, or dGTP in this capacity.

Kinase activity was inhibited by the addition of UTP or CTP to the assay mixture as shown in Fig. 6. The presence of either UTP (Fig. 6a) or CTP (Fig. 6b) in the assay mixture resulted in inhibition of UMP formation. The apparent K_i values calculated by the method of DIXON[37] were 57 μM for UTP and 63 μM for CTP. Higher concentrations of the corresponding nucleoside diphosphates (Figs. 6a and 6b, respectively) were needed to give inhibition equal to that of UTP and CTP. In contrast to the inhibition caused by the ribonucleoside triphosphates, enzyme activity was

TABLE III

EFFECT OF PHYSIOLOGICAL PHOSPHATE DONORS ON URIDINE KINASE ACTIVITY

The standard assay was used except that the sodium salt of the phosphate donor was added at the indicated concentration. Uridine kinase activity is expressed as μmoles of [^{14}C]uridine phosphorylated per min per mg protein Fraction V.

Donor	*Phosphate donor conc. (mM)*		
	5.0	*1.0*	*0.5*
ATP	2.65	1.25	0.91
dATP	2.54	1.27	0.85
GTP	1.71	1.11	0.80
dGDP	0.25	0.49	0.09
CTP	0.31	0.14	0.09
dCTP	0.31	1.24	0.93
UTP	0.07	0.07	0.07
dUTP	0.29	1.09	0.95
None	0.02		

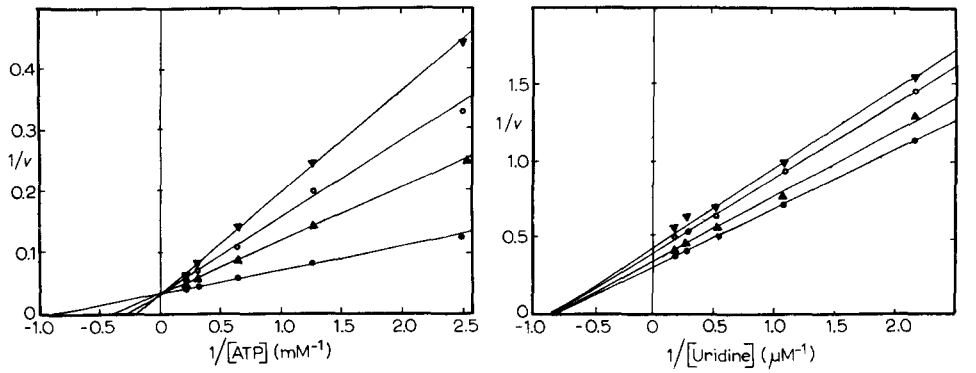


Fig. 7. CTP inhibition of uridine kinase activity when the ATP concentration is varied. Standard assays with ATP as the variable substrate were carried out at fixed concentrations of CTP: 0.3 mM CTP (▼), 0.2 mM CTP (○), 0.1 mM CTP (▲), no addition (●). The initial reaction velocity, v, was determined by the filter disk method and is expressed as μmoles [2-^{14}C]uridine phosphorylated per min per mg protein Fraction V.

Fig. 8. CTP inhibition of uridine kinase activity when the uridine concentration is varied. Standard assays with uridine as the variable substrate were carried out at fixed concentrations of CTP: 0.3 mM CTP (▼), 0.2 mM CTP (○), 0.1 mM CTP (▲), no addition (●). The initial reaction velocity, v, was determined and expressed as in Fig. 7.

stimulated upon the addition of dUTP or dCTP to the assay mixture (Figs. 6a and 6b, respectively). This latter effect was probably due to dUTP and dCTP acting as phosphate donors.

Finally, CTP inhibition of uridine kinase appeared to be competitive with respect to the phosphate donor, ATP (Fig. 7), and noncompetitive with respect to the phosphate acceptor, uridine (Fig. 8). Apparent K_m values for ATP (1.1 mM) and uridine (13 μM) were calculated from the uninhibited reactions shown in Fig. 7 and 8, respectively.

DISCUSSION

Eucaryotic cells in general have not displayed the stringent feedback regulation of pyrimidine ribonucleotide synthesis found in bacteria. YIP AND KNOX[38] have recently observed that the modulation of carbamyl phosphate synthetase activity by nucleotides is less prominent in mammalian cells than in bacteria, and have suggested that regulation of enzyme concentration, rather than the direct regulation of enzyme activity, may be important in controlling tissue growth rate. *In vitro* investigation of aspartate transcarbamylase from various eucaryotic sources[39,40] indicate that the activity of this enzyme is not regulated by nucleotides as it is in bacteria[41]. Thus the salvage enzymes may serve as regulatory sites in pyrimidine nucleotide synthesis, particularly in rapidly growing cells. Activities of the salvage enzymes thymidine kinase[24], uridine–cytidine kinase[14–17], deoxycytidine kinase[42] and UMP pyrophosphorylase[12,13] from eucaryotic cells have all been shown to be sensitive to modulation by pyrimidine nucleoside triphosphates. As *Tetrahymena* has no capacity for *de novo* pyrimidine formation, salvage enzymes catalyze and may regulate the synthesis of pyrimidine ribonucleotides. One such enzyme, uridine–cytidine kinase, is active in *Tetrahymena* and this study suggests a regulatory function for it.

The kinase exhibits strict stereospecific requirements for the phosphate acceptor. Results presented in Fig. 5 indicate that of the nucleosides tested, only cytidine competes with uridine for the phosphate acceptor site on the enzyme. The enzyme shows a specificity for pyrimidine nucleosides possessing a 2′-hydroxyl group since neither 2′-deoxyuridine nor 2′-deoxycytidine nor 5-fluoro-2′-deoxyuridine was phosphorylated. In the latter case, it is probable that inactivity was based on the absence of the 2′-hydroxyl group rather than the presence of the fluorine in the 5 position of the pyrimidine ring, since the uridine kinase of Ehrlich ascites tumor cells, an enzyme with preferences similar to those of the *Tetrahymena* enzyme for phosphate acceptors, was capable of phosphorylating both 5-fluorouridine and 5-fluorocytidine[34]. Both the kinase from *Tetrahymena* and that of Ehrlich ascites cells[34] are unable to utilize 5-methyluridine, indicating an intolerance for the bulky methyl group.

In contrast, a low specificity of the enzyme for the phosphate donor is suggested by the results in Table III. Although ATP is thought to be the phosphate donor *in vivo*, dATP gives comparable activity at all concentrations employed. In addition, GTP, dUTP, and dCTP yield 90% or greater activity at 1.0 mM and 0.5 mM. Hence purine and pyrimidine deoxynucleoside triphosphates and purine ribonucleoside triphosphates are able to function in uridine phosphorylation while pyrimidine ribonucleoside triphosphates show no activity.

As shown in Fig. 6, formation of UMP is inhibited by UTP and CTP, the end products of pyrimidine ribonucleotide synthesis. Similar inhibition of uridine kinase activity has been reported for partially purified enzymes from Novikoff ascites tumor cells[16] and sea urchin[15], and in extracts of human epidermoid carcinoma and mouse mast-cell neoplasm[14] as well as phytohemaglutinin-stimulated human lymphocytes[17]. The inhibition of uridine kinase by CTP appears to be competitive with the phosphate donor (Fig. 7) and noncompetitive with the phosphate acceptor (Fig. 8). A similar pattern of inhibition was found in the uridine–cytidine kinase of Novikoff ascites cells[16]. The specific structural requirements for inhibitory action are reflected by the results in Fig. 6. Neither dUTP nor dCTP show any inhibitory action on UMP formation. In addition, when UDP or CDP were added to the assay mixture at concentrations that gave 50% inhibition with the corresponding triphosphates, the reaction was inhibited only 16% and 23% respectively. Thus, a high degree of specificity exists for the inhibitor, maximal inhibitory action being rendered by UTP and CTP, end products of the pathway irreversibly initiated by uridine–cytidine kinase. This reguation may allow the organism to salvage the pyrimidines necessary for its nucleic acid requirement while limiting pyrimidine ribonucleotide triphosphate production to a rate commensurate with their utilization.

Other workers[11,20] have been unable to detect uridine–cytidine kinase activity in *T. pyriformis* strain W. Subsequently, several schemes summarizing the metabolism[11,21] and regulation[20] of pyrimidine nucleotide synthesis in *Tetrahymena* have omitted the kinase. It is possible that the high activities of uridine phosphorylase and the phosphatases[27] found in extracts of *Tetrahymena* obscured the activity of the kinase. However, using the growth conditions and assay procedures described herein, we found uridine–cytidine kinase activity in strain W to be comparable to that described here for strain GL in both crude extracts and partially purified preparations[36]. It is suggested that existing schemes of pyrimidine ribonucleotide metabolism for *Tetrahymena* be reconsidered in the light of these findings.

ACKNOWLEDGEMENT

This work was supported in part by National Institutes of Health Predoctoral Fellowship 5 F01 GM 37986-03 and National Science Foundation grant GB 6233.

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