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NON-ALLOSTERIC REGULATION OF THE URIDINE KINASE FROM SEEDS OF *ZEA MAYS*

QUEY-ING DENG* and DAVID H. IVES**
Department of Biochemistry, College of Biological Sciences, The Ohio State University, Columbus, Ohio 43210 (U.S.A.)
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Summary

Uridine kinase (ATP: uridine 5′-phosphotransferase, EC 2.7.1.48) has been partially purified from ungerminated hybrid corn seed. It is associated with a soluble high molecular weight fraction from which it apparently cannot be dissociated without loss of activity. The stability of the enzyme is enhanced by the addition of dithiothreitol, glycerol and nucleotide substrate. The nucleoside specificity of the enzyme is limited to nucleosides containing pyrimidine and ribose moieties, such as uridine and cytidine. High concentrations of nucleosides cause substrate inhibition, however. The K_m values for uridine and cytidine are 53 μM and 125 μM, respectively, and under subsaturating conditions uridine is phosphorylated about five times faster than cytidine. The reaction follows an ordered Bi Bi kinetic pattern, with ATP and ADP in competition for the free form of the enzyme. Purine, but not pyrimidine, nucleoside triphosphates serve as phosphate donors without regard to the sugar moiety. However, all of these triphosphates appear to compete for the same site on the enzyme. (K_m ATP = 590 μM, K_m (app) GTP = 61 μM, and CTP and UTP are linear competitive inhibitors against ATP, with K_i values of 60 μM and 240 μM, respectively.) Therefore, end product control of uridine kinase apparently does not involve allosteric sites, but instead is envisioned as simple competition between relatively effective or ineffective phosphate donors for a position on the enzyme.

Introduction

The occurrence of an enzyme which specifically catalyzes the phosphorylation of uridine or cytidine with ATP was first demonstrated in extracts from

* Present address: Stauffer Chemical Co., Bioengineering Laboratory, San Jose, Calif. 95112, U.S.A.
** To whom correspondence should be addressed.

mammalian liver [1] and Ehrlich ascites tumor [2] and later in extracts from a variety of different cells and tissues [3—10]. This enzyme has been partially purified from Ehrlich and Novikoff ascites tumors [5,9] and relatively more extensively purified from *Tetrahymena pyriformis* GL cells [8], and from the obligate thermophile, *Bacillus stearothermophilus* [10]. Many investigators believe that this enzyme catalyzes a rate-limiting step in the anabolism of uridine to the successive nucleotide derivatives. The terminal products, UTP and CTP have been shown to serve as potent and specific inhibitors of this enzyme from tumor cells [6], sea urchin embryos [7], human lymphocytes [11], *Tetrahymena* [8] and several bacterial strains [6,10].

The inhibition by CTP or UTP can be partially reversed by high concentrations of either uridine or ATP [6,8,9], or by GTP or dGTP [7]. However the possible modes of action of these modifiers are not entirely clear.

Although there are reports of an enzyme capable of phosphorylating uridine in several crude plant extracts [12—14], very little study has been given to the properties of this enzyme from plants. Wanka [14] and Wanka and Walboomers [15] found two species of uridine phosphorylating enzymes existing during germination of *Zea mays*, of which only one was active in vitro during the initial hours of germination. The early-appearing enzyme differed from the late-appearing enzyme in being associated with one protein component rather than two, and was less heat stable and of higher molecular weight.

In a previous report [16], we presented evidence that in later stages of corn germination uridine is phosphorylated by the combined actions of ATPase and a non-specific nucleoside phosphotransferase capable of transferring phosphate from AMP to uridine or other nucleosides, and that only the early-appearing uridine phosphorylating enzyme species is a true, regulatory, uridine kinase.

We have partially purified uridine kinase from ungerminated corn seed and have used this preparation to investigate a number of catalytic properties and the kinetic regulation of this enzyme by end products.

Materials and Methods

Materials

Corn seed was provided from hybrid stock maintained by the Ohio State University, Department of Agronomy. Unlabeled nucleosides and nucleotides were obtained from P-L Biochemicals, Sigma Chemical Co. and Calbiochem.

^{3}H-labeled nucleosides and nucleotides were purchased from New England Nuclear Corp. or International Chemical and Nuclear Corp. ^{3}H-labeled UMP was prepared by the enzymatic phosphorylation of [5-^{3}H]uridine with uridine kinase, and purified by paper chromatography. The purities of radioactive compounds were checked periodically, and purified by paper chromatography with one or two solvent systems when the purity fell below 95%.

Tris, *N*-ethylmaleimide, β-mercaptoethanol, calcium phosphate gel, imidazole, EDTA, lysine, RNAase B (from bovine pancreas, type II-B, 100 Kunitz units/mg protein), Sepharose 4B-200, Sepharose 6B-100, were all obtained from the Sigma Chemical Co. DEAE-cellulose (DE 23), DEAE-cellulose paper (DE 81) and Amerlite ion-exchange resin-loaded paper sheets (grade SB-2) were

purchased from the Reeve Angel Co. Streptomycin sulfate, *p*-mercuribenzoate, and dithiothreitol were obtained from the Calbiochem. Co. Triton X-100, RNAase T_1 (550 000 units/mg protein), and crystalline bovine serum albumin were purchased from Rohm and Haas, Miles Laboratories and Pentex, respectively.

Preparation of crude enzyme extract

Corn seed was washed, dried and ground to a powder with a Wiley mill (Arthur H. Thomas Co.) using a 2 mm mesh screen, and then extracted with acetone chilled to −20° C. The residue was spread out on filter paper and dried at room temperature. The dried acetone powder can be stored in an air-tight jar at −20° C for several months without losing kinase activity.

Enzyme was extracted from 100 g of acetone powder by blending with 400 ml of cold 0.2 M sodium potassium phosphate buffer (pH 7.0), and the homogenate, after passing through double layers of cheese cloth, was centrifuged at 23 000 × *g* for 30 min. The supernate (Fraction I) was quick frozen and kept at −60° C until needed.

Enzyme assays

Uridine kinase was assayed by using the disc method of Ives et al. [17], by which ^{3}H-labeled UMP formed by the enzymatic reaction is adsorbed on discs of ion-exchange resin-impregnated paper, washed free of ^{3}H-labeled nucleoside, and eluted in situ in liquid scintillation vials and counted. In most cases, the following standard assay conditions were used, employing duplicate assay tubes from which duplicate discs were spotted. The final concentrations of reagents in the general assay were: ATP, 5 mM; $MgCl_2$, 6.25 mM; Tris—HCl, 50 mM (pH 8.0 at 4° C); dithiothreitol, 10 mM; nucleoside, 0.02 mM (0.5 μCi per assay mixture of 100 μl for all ^{3}H-labeled nucleosides and 0.1 μCi for ^{14}C-labeled nucleosides). Enzyme concentrations were adjusted to give not more than 20% conversion of substrate to product during the incubation time of 20 min at 30° C. The reaction velocities were shown to be constant for at least 30 min for the ranges of substrate concentrations described in this report.

Protein determination

Protein concentrations were determined by the method of Lowry et al. [18]. However, due to the high content of phenolic compounds in plants, as well as other interfering substances such as NH_4^+, β-mercaptoethanol and dithiothreitol in some enzyme fractions, protein was generally first precipitated and washed with cold 10% trichloroacetic acid. The pellets were taken up in 0.1 M NaOH before following the procedure of Lowry et al [18].

Sepharose gel filtration

Sepharose 6B-100 or Sepharose 4B-100 gel was equilibrated with buffer solution (50 mM Tris—HCl (pH 8.0 at 4° C)—10 mM β-mercaptoethanol—20% glycerol) and then packed in a 2.5 cm × 50 cm column. Fractions of 6 ml were collected.

Kinetic studies

$MgCl_2$ was always present in 25% excess over all nucleotide species pre-

sent to ensure that essentially all of the ATP could be considered as ATP—Mg^{2-}. The ADP used for product inhibition experiments was purified immediately before use by gel chromatography on Bio-Gel P-2 to remove contaminating ATP. Kinetic constants were obtained by graphical analysis. In this instance K_m values could be obtained directly from X-intercepts, and V and K_i values were obtained by intercept and slope replots, respectively.

Results

Purification of uridine kinase

All manipulations were carried out at 4°C and all centrifugations were done at 0°C at 23 000 × g. All Tris—HCl buffers used were pH 8.0 at 4°C. Enzyme stability was greatly enhanced by the presence of either 1 mM $(NH_4)_2SO_4$ or 20% glycerol in the buffers.

To the crude extract from acetone powder, Fraction I, (see Materials and Methods), 0.1 vol. of streptomycin sulfate (adjusted to pH 7.0 with NaOH) was added. After a further 20 min of stirring, the supernatant solution (Fraction II) was cleared by centrifugation.

To each 100 ml of Fraction II 20.8 g of solid $(NH_4)_2SO_4$ was then added. The solution was stirred in the cold for 40 min more and the precipitate was collected by centrifugation. The precipitate was then dissolved in a minimum volume of 50 mM Tris—HCl—10 mM β-mercaptoethanol buffer (Fraction III) and stored at —20°C until used. The apparent increase in total activity is thought to be due to removal of much of the interfering phosphatase by $(NH_4)_2SO_4$ fractionation.

Enzyme Fraction III, after being diluted 10-fold with the buffer to reduce the $(NH_4)_2SO_4$ concentration, was applied to a column of DEAE-cellulose (Whatman DE-23) equilibrated with 50 mM Tris—HCl (pH 8.0) containing 10 mM β-mercaptoethanol and 1 mM $(NH_4)_2SO_4$. After the column had been washed with 50 ml of the equilibrating buffer, the enzyme was eluted by a linear $(NH_4)_2SO_4$ concentration gradient extending between 1 and 250 mM, 500 ml total volume. The $(NH_4)_2SO_4$ solutions were made up in a buffered solution containing 2.5 mM ATP, 10 mM β-mercaptoethanol and 50 mM Tris —HCl. 15 ml fractions were collected, and the enzyme protein was precipitated from the peak fractions by adding solid $(NH_4)_2SO_4$ to a final concentration of 45% saturation. The pellet was dissolved in 10 mM Tris—HCl—10 mM β-mercaptoethanol—50% glycerol (Fraction IV).

Fraction IV was applied to a Sepharose 4B-100 column (2.5 cm × 50 cm) equilibrated with 50 mM Tris—HCl buffer containing 1 mM $(NH_4)_2SO_4$, 10 mM β-mercaptoethanol and 20% glycerol and 6-ml fractions were collected. The elution profile obtained is shown in Fig. 1. The fractions corresponding to the enzyme peak were pooled and concentrated by $(NH_4)_2SO_4$ precipitation as described above, and redissolved in 10 mM Tris—HCl—10 mM β-mercaptoethanol—50% glycerol (Fraction V).

Fractions IV and V were stable for months when kept unfrozen at —20°C. The summary of the results of the individual purification steps is presented in Table I.

Freedom from contaminating enzyme activities. The final preparation,

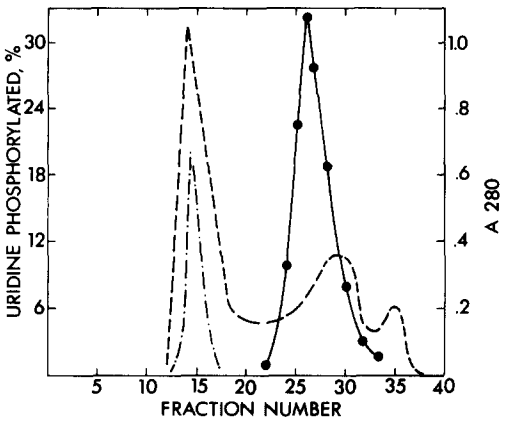

Fig. 1. Chromatography of uridine kinase on Sepharose 4B-200. The conditions for running the column are described in Materials and Methods,— · — ·, Blue Dextran 2000; - - - - - -, protein; ●———●, enzymatic activity.

Fraction V, appeared to be free of ATPase, uridine phosphorylase, cytidine deaminase, nucleoside monophosphate kinase and non-specific phosphotransferase activities, as determined by chromatography after reaction with the appropriate radioactive substrate. The amount of nucleoside phosphomonoesterase contamination was also insignificant when assayed in the presence of 0.2 mM UMP, the highest concentration which is likely to be produced by the uridine kinase reactions carried out here. Measurement of the $A_{280\ nm}/A_{260\ nm}$ ratio suggested the presence of a trace of RNA, perhaps 0.4%. However, the spectral ratio was not altered by treatment of the enzyme with either RNAase B or RNAase T_1.

Nucleoside specificity. Table I also reveals a constant ratio of specific activities for uridine and cytidine phosphorylation during purification. The corn seed kinase showed a very high specificity for these two substrates. Indeed, they were the only two nucleosides phosphorylated among all eight of the common nucleoside nucleic acid precursors, plus deoxyuridine and 5-methyluridine. The strict structural demands of the enzyme for the phosphate

TABLE I

PURIFICATION OF URIDINE KINASE FROM CORN SEED

1 unit of the enzyme is defined as the quantity of enzyme that catalyzes the formation of 1 nmole of UMP in 30 min at 30°C.

Fraction	Volume (ml)	Total protein (mg)	Total activity (units)	Spec. act. (units/mg)	Uridine cytidine ratio*	Yield (%)
I. Crude extract	250	1050	2520	2.4	4.12	100
II. Streptomycin sulfate	270	850	2240	2.8	—	89
III. $(NH_4)_2SO_4$	25	195	3939	20.2	4.69	156
IV. DEAE-cellulose	5	37.5	2738	73.0	4.66	108
V. Sepharose 4B-200	4	9.0	1539	171.0	4.65	61

* Relative specific activities for uridine and cytidine phosphorylation.

acceptor were reflected in its complete inability to phosphorylate either 2′-deoxyuridine or 2′-deoxycytidine.

Cytidine and uridine probably compete for a common site on the enzyme since cytidine has been found to be a competitive inhibitor of uridine phosphorylation; (K_m uridine = 53 μM, K_i cytidine = 210 μM, data not shown), but uridine is the preferred substrate.

Requirements and characteristics of the uridine kinase

A number of properties of this enzyme will be described below, but without a display of data in the interest of saving journal space.

The corn seed enzyme showed an absolute requirement for divalent metal ions, and although Mg^{2+} was preferred, it could replaced by Mn^{2+} (64% of activity with Mg^{2+}), Fe^{2+} (as SO_4^{2-} salt, 52%), Ca^{2+} (13%), and Co^{2+} (8%), all tested at 10 mM.

When ATP and Mg^{2+} concentrations were varied independently, optimal activity resulted from a Mg^{2+} to ATP ratio of 1.25, if the fixed component was present at 10 mM. If both components were varied at a ratio fixed at 1.0, optimal enzyme activity was reached at about 10 mM. The enzyme activity gradually decreased as the ATP-Mg concentration was increased above 10 mM.

The effect of pH on the enzyme activity was studied over a range of 5.0 to 10.2 using six different buffers at a constant ionic strength of 0.04. The curve obtained was biphasic, with distinct pH optima at 7.5 and 9.0.

All of the naturally occurring nucleoside triphosphates have been tested for their ability to phosphorylate uridine in the uridine kinase reaction. Among those tested only ATP, dATP, GTP and dGTP gave significantly phosphorylation activity, being about equally efficient at a concentration of 10 mM (saturating levels). Conversely, the end products, UTP and CTP, showed almost no ability at all to serve as phosphate donors. ADP and AMP gave less than 0.5% of the activity of ATP, and that was probably due to slight ATP contamination in preparations of these nucleotides.

The uridine kinase from corn is quite sensitive to sulfhydryl group inhibitors. The enzyme was inhibited 83% by 10 μM $HgCl_2$, 51% by 10 μM *p*-mercuribenzoate and 31% by 1 mM *N*-ethylmaleimide. The effect of *p*-mercuribenzoate was completely reversed by 0.4 mM dithiothreitol. The enzyme loses most of its activity after 3 days at 4° C in the absence of thiol reagents. However, most of the lost activity can then be recovered by incubation with 50 mM dithiothreitol for 10 min at room temperature. Although the enzyme normally was stored in β-mercaptoethanol, it was briefly preincubated with 50 mM dithiothreitol before conducting kinetic experiments.

Uridine kinase proved to be fairly labile at elevated temperatures. It has a half-life of 10 min when incubated at 45° C with Tris—HCl (pH 7.2). A series of supposed "protective agents" were added along with the buffer and the enzyme was incubated at 45° C for up to 60 min. Aliquots were taken at intervals and assayed for enzyme activity. In order of effectiveness the most useful combination of stabilizing agents were: dithiothreitol + ATP—Mg > dithiothreitol > 20% glycerol > GTP. The respective residual enzyme activities after 60 min were 65%, 58%, 47% and 27%. Bovine serum albumin, which stabilizes such labile enzymes as thymidine kinase [19] and deoxycytidine kinase [20] had no

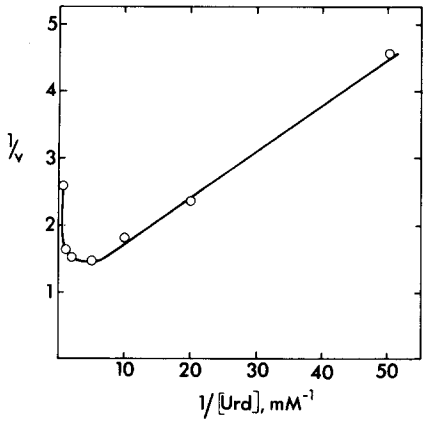

Fig. 2. Substrate inhibition of uridine kinase, with ATP—Mg^{2-} saturating. Conditions were as described in Materials and Methods, except that the uridine concentration was varied as indicated. The enzyme was 2.2 μg of Fraction V protein. Velocity units are nmoles per 20 min.

effect, while the end product inhibitor, CTP, actually accelerated the rate of decay slightly, also in contrast to calf thymus deoxycytidine kinase which is substantially stabilized by its inhibitory end product, dCTP [20].

Substrate inhibition

In general, this enzyme gives simple linear and uncomplicated kinetics, with no indication of multiple interacting homotropic or heterotropic sites, as will be shown below. It does appear to be subject to substrate inhibition, however, at concentrations of uridine exceeding 200 μM, as seen in Fig. 2. To rule out the possibility of a trace contaminant causing this effect several lots of uridine from different suppliers were checked chromatographically and com-

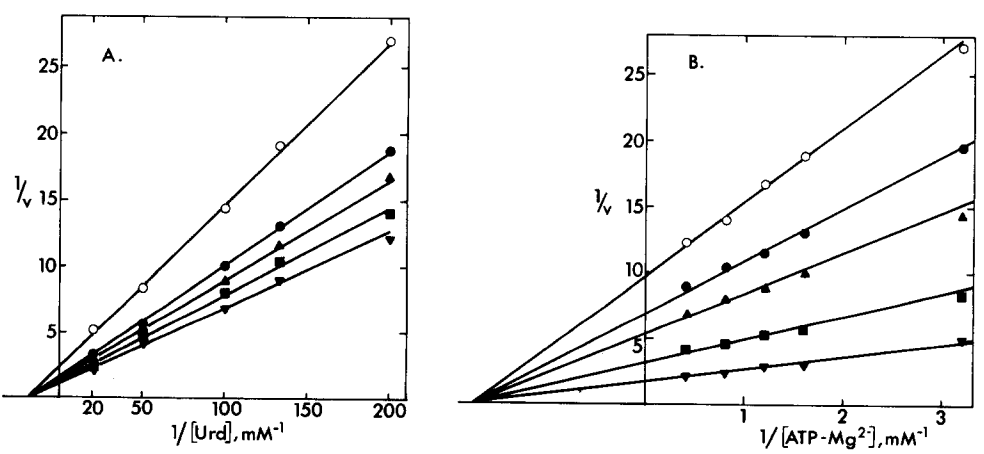

Fig. 3. Effect of varying uridine and ATP—Mg^{2-} concentrations on the reaction rate of uridine kinase. Conditions were as described in Materials and Methods, except that the uridine and ATP—Mg^{2-} concentrations were varied, concentration was as detailed below. The enzyme was 2.2 μg of Fraction V protein and velocity units are nmoles per 20 min. (A) ATP—Mg^{2+} concentrations: ○———○, 0.312 mM; ●———●, 0.625 mM; ▲———▲, 0.833 mM; ■———■, 1.25 mM; ▼———▼, 2.50 mM. (B) Uridine concentrations: ○———○, 5 μM uridine; ●———●, 7.5 μM uridine; ▲———▲, 10 μM uridine; ■———■, 20 μM uridine; ▼———▼, 50 μM uridine.

pared in repetitions of this experiment; in each case the pattern shown in Fig. 2 was observed. Accordingly, it was not feasible to carry out kinetic studies at saturating uridine concentrations.

Kinetic mechanism of the phosphorylation reaction

Initial velocity and product inhibition patterns were studied to determine the reaction mechanism of the enzyme. As shown in Fig. 3 changing the concentration of one substrate does not affect the apparent K_m of the other. Therefore, the intercepts with the abscissa yield the limiting K_m values. Based on repeated experiments, the average constants are: K_m uridine = 53 ± 16 μM; K_m ATP = 590 ± 120 μM. The V, obtained from an intercept replot of Fig. 3B, is 25.2 nmoles/min per mg enzyme protein.

The converging initial velocity patterns of Fig. 3 suggest that uridine kinase has one of the sequential mechanisms in which both substrates must bind to the enzyme before either product is released. Product inhibition patterns were carried out to determine whether or not there is an obligatory order of addition or release of reactants. As revealed by Fig. 4, UMP is non-competitive with

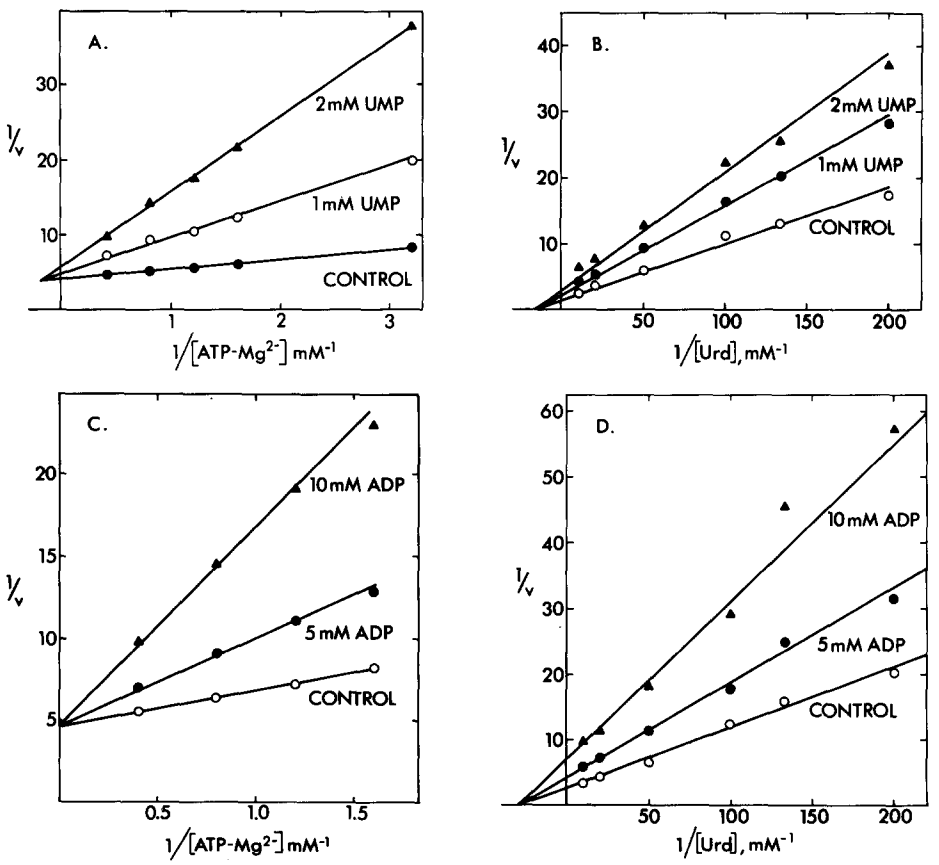

Fig. 4. Product inhibition of uridine kinase. Conditions were as described in Materials and Methods, except as detailed below. The enzyme was 1.8 μg of Fraction V protein, and velocity units are nmoles per 20 min. (A) Nonsaturating uridine concentration fixed at 20 μM (1.25 mCi/mmole); inhibition by UMP. (B) Nonsaturating ATP concentration fixed at 0.833 mM; inhibition by UMP. (C) Nonsaturating uridine concentration fixed at 20 μM; inhibition by ADP. (D) Nonsaturating ATP—Mg^{2-} concentration fixed at 0.833 mM; inhibition by ADP.

both substrates, while ADP is non-competitive with uridine but is competitive with ATP. Therefore, by applying "Cleland's rules" [21], corn seed uridine kinase is seen to have an ordered Bi Bi reaction mechanism, with ATP—Mg^{2-} and ADP as the leading or obligatory reactants.

Regulation of uridine kinase from corn seed

In experiments similar to those performed on uridine kinase from other sources the end products UTP and CTP were found to give linear non-competitive inhibition with uridine as the variable substrate, but showed linear competitive inhibition against ATP, the phosphate donor. The data for CTP against variable ATP is shown in Fig. 5. Replots of the slopes from the two competition experiments vs inhibitor concentrations gave straight lines yielding the K_i values: K_i CTP = 60 μM and K_i UTP = 240 μM.

To determine whether the inhibition can be complete, or is only partial, the effects of increasing concentrations of CTP and UTP were analyzed in the presence of fixed concentrations of ATP—Mg^{2-} (5 mM) and uridine or cytidine (20 μM). In each case, uridine and cytidine phosphorylation was found to be totally inhibited when the UTP or CTP concentration reached about 10 mM. In view of these observations, it seems likely that high concentrations of these end products can completely displace ATP from the triphosphate site on the enzyme and inhibit it because they are ineffective as phosphate donors.

In contrast to UTP and CTP, the purine nucleoside triphosphates GTP and dGTP stimulated uridine kinase activity at sub-saturating concentrations of ATP—Mg^{2-}. As can be seen in Fig. 6 both classes of nucleotides interact com-

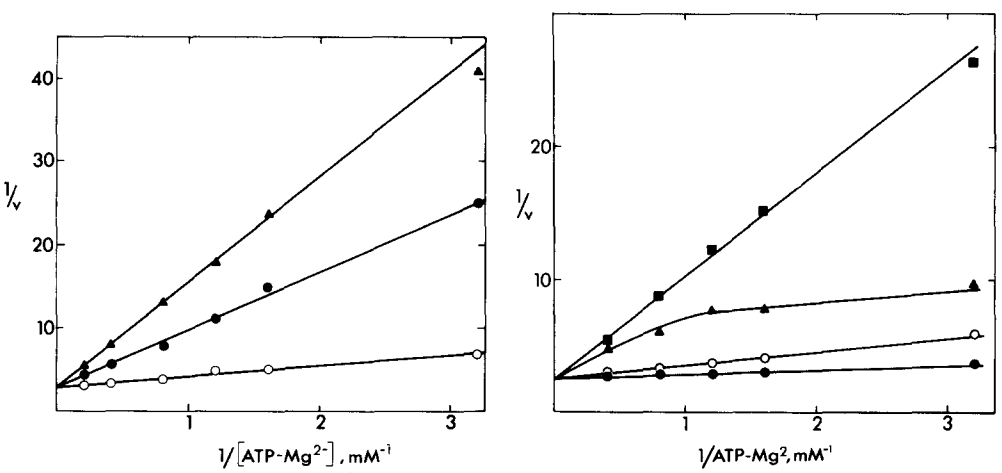

Fig. 5. Effect of varying ATP—Mg^{2-} on the rate of uridine phosphorylation; inhibition by CTP. The uridine concentration was fixed at 20 μM (1.25 Ci/mole), ATP—Mg^{2-} was varied as shown, and CTP was added as detailed below. Other conditions were as described in Materials and Methods. The enzyme used was 2.2 μg Fraction V protein, and velocities are expressed as nmoles per 20 min. ○———○, control; ●———●, 0.25 mM CTP; ▲———▲, 0.5 mM CTP.

Fig. 6. Effect of varying ATP—Mg^{2-} concentrations on the reaction rate of uridine phosphorylation; stimulation by GTP; inhibition by CTP and reversal of CTP inhibition by GTP. The uridine concentration was fixed at 20 μM (125 Ci/mole), ATP—Mg^{2-} was varied as shown, GTP and CTP were added as detailed below. Other conditions were described in Materials and Methods. The enzyme used was 2 μg of Fraction V protein. ○———○, control; ●———●, 0.15 mM GTP; ■———■, 0.5 mM CTP; ▲———▲, 0.15 mM GTP + 0.5 mM CTP.

petitively with ATP: CTP as an inhibitor and GTP in a stimulatory fashion. GTP tended to overcome inhibition by CTP. From data not shown here, the apparent K_m of GTP is 61 μM, an order of magnitude lower than that of ATP. Thus, the reversal of CTP inhibition by GTP in Fig. 6 can be explained in terms of a very good phosphate donor, GTP, displacing a very poor donor, CTP, from the triphosphate-binding site. The curved line is to be expected, since at limiting ATP concentrations the reaction velocity is determined by the concentration ratio of the alternative phosphate donor, GTP, and the competitive inhibitor, CTP. Since both are present at fixed concentrations, the velocity approaches a constant value at low ATP concentrations.

Discussion

Although uridine kinase from animal [6,9], bacterial [10], and protozoan sources [8] have been described, the mechanism of end product control of the enzyme has not been made clear previously. While sharing a number of properties with uridine kinases from other sources, the plant enzyme we have described possesses several unique features. Its molecular weight appears to be several million, as seen by its exclusion from Bio-Gel P-300 and Sepharose 6B-100 but not from Sepharose 4B-100. For this reason it has not been feasible to use standard polyacrylamide gel electrophoresis to assess the degree of purity of the enzyme. Such a high molecular weight is, of course, unusual for most enzymes, let alone for uridine kinase which has been estimated to have a molecular weight considerably greater than 160 000 in Novikoff hepatoma [9], 195 000 in *Tetrahymena pyriformis* [8] and 120 000 and 30 000 for two species recently detected in normal or neoplastic rat liver [22]. Since the enzyme survives for years in dry seed stored at room temperature, it is conceivable that it is part of a stable complex of early-germination or late-maturation enzymes. However, enzyme extracted from seeds germinated for 24 h still has the high molecular weight, so the active enzyme is not released from such a complex upon germination. Moreover, various attempts to dissociate the putative complex in our hands invariably resulted in inactivation of the kinase. After about 2 days of germination this uridine kinase seems to decay and is replaced by a non-specific phosphotransferase by which AMP may donate its ester phosphate to a nucleoside [16].

Another unusual feature of the uridine kinase from corn seed is substrate inhibition by uridine levels in excess of about 200 μM. Such inhibition does not seem improbable, however, in view of the evidence presented here for an ordered Bi Bi sequential mechanism (Figs 3 and 4). As suggested by Cleland [21], the second substrate to bind (uridine), may combine in a dead end fashion with a complex consisting of the enzyme and the final product to leave (ADP). The mechanism we report here is in apparent contrast to that of the enzyme from Novikoff hepatoma [9], but in a recent preliminary report [23], a sequential mechanism has been proposed for uridine kinase from a murine mast cell tumor.

The mechanism for end product control of uridine kinase from corn seems refreshingly simple and uncomplicated. The end products CTP and UTP compete with ATP for the phosphate donor site of the enzyme, and this appears to

be true also for the enzyme from other sources [8,9]. CTP and UTP are normally unable to serve as phosphate donors and, therefore, form unproductive complexes with the enzyme. Their K_i values are of the same order of magnitude as the K_m values of GTP and ATP, respectively, so complete inhibition could be achieved only when their concentrations are relatively high or when the ATP + GTP energy charge is low. Thus, only partial inhibition might normally be expected, a situation which affords maximum sensitivity of control. On the other hand, end product inhibition tends to be reversed by GTP, which at limiting concentrations is a much more effective donor than ATP, owing to its lower K_m. In this way, a balance between purine and pyrimidine nucleotide nucleic acid precursors may be maintained. Reversal of UTP or CTP inhibition by GTP has also been seen in the case of the uridine kinase from sea urchin embryos [7], although a mechanism for this effect was not elucidated. Based apparently on the observations that UTP inhibited the enzyme from Novikoff hepatoma while dUTP was a very good phosphate donor (even though the enzyme failed to distinguish other ribonucleoside and deoxynucleoside pairs), Orengo [9] proposed that the enzyme has a separate binding site for inhibitor molecules. In the case of the uridine kinase from corn, none of the pyrimidine nucleotides are good phosphate donors. Therefore, there seems to be no reason to propose more than simple competition between phosphate donors of relative degrees of efficiency as a mechanism for the control of uridine kinase activity from corn.

Acknowledgements

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