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NUCLEOSIDE MONOPHOSPHATE KINASES

I. TRANSPHOSPHORYLATION BETWEEN ADENOSINE TRIPHOSPHATE AND NUCLEOSIDE MONOPHOSPHATES

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SUMMARY

1. The nucleoside monophosphate kinase catalyzing the ATP–UMP reaction has been purified 40–45-fold from an extract of calf liver acetone powder. The ATP–CMP kinase followed the fractionation closely but the ATP–AMP kinase was partially removed while the ATP–GMP kinase and the kinases for reactions between nucleoside triphosphate and AMP were completely removed.

2. Some of the properties of this ATP–nucleoside monophosphate kinase preparation are presented.

INTRODUCTION

The synthesis of nucleoside di- and triphosphates from nucleoside monophosphates is catalyzed by enzymes which transfer phosphate from one nucleotide to another.

The following terminology will be used throughout the paper: AMP, ADP, ATP = adenosine 5′-mono; di- and triphosphates; IMP, IDP, ITP = inosine 5′-mono, di- and triphosphates; GMP, GDP, GTP = guanosine 5′-mono, di- and triphosphates; UMP, UDP, UTP = uridine 5′-mono-, di- and triphosphates; CMP, CDP, CTP = cytidine 5′-mono, di- and triphosphates; EDTA = ethylene diamine tetraacetate; DPNH = reduced diphosphopyridine nucleotide.

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Adenylate kinase which catalyzes the transfer of phosphate from ATP to AMP was the first observed reaction of this kind[1]. The transfer of phosphate from a nucleoside triphosphate to a nucleoside diphosphate, in which nucleotides containing two different bases may be involved, is catalyzed by enzymes called nucleoside diphosphokinases[2,3].

Nucleoside-A triphosphate + Nucleoside-B diphosphate ⇌
Nucleoside-A diphosphate + Nucleoside-B triphosphate

Most recently, the transfer of phosphate between a nucleoside triphosphate and a nucleoside monophosphate, catalyzed by enzymes termed nucleoside monophosphate kinases, was observed simultaneously in several laboratories[4–9].

Nucleoside-A triphosphate + Nucleoside-B monophosphate ⇌
Nucleoside-A diphosphate + Nucleoside-B diphosphate

A detailed study of some yeast nucleoside monophosphate kinases has been published by LIEBERMAN, KORNBERG AND SIMMS[10].

In the present paper the purification, partial separation and properties of nucleoside monophosphate kinases from calf liver which catalyze transphosphorylations between adenosine triphosphate and a number of nucleoside monophosphates (equations 1–4) will be described.

$$ATP + UMP \rightleftharpoons ADP + UDP \quad (1)$$
$$ATP + CMP \rightleftharpoons ADP + CDP \quad (2)$$
$$ATP + GMP \rightleftharpoons ADP + GDP \quad (3)$$
$$ATP + AMP \rightleftharpoons 2\ ADP \quad (4)$$

These have been separated from enzymes which catalyze transphosphorylation between various nucleoside triphosphates and AMP (equations 4–8) which will be the subject of the following paper[11].

$$UTP + AMP \rightleftharpoons ADP + UDP \quad (5)$$
$$CTP + AMP \rightleftharpoons ADP + CDP \quad (6)$$
$$GTP + AMP \rightleftharpoons ADP + GDP \quad (7)$$
$$ITP + AMP \rightleftharpoons ADP + IDP \quad (8)$$

A separation of these enzymes has not been reported previously. These transphosphorylation reactions were encountered during a study of the dephosphorylation of nucleoside triphosphates, and several preliminary accounts have appeared[9].

MATERIALS

Nucleotides. AMP, IMP, GMP, ADP, IDP, ATP (crystalline) and ITP were obtained from Sigma Chemical Company, St. Louis, Mo. UMP, CMP, UTP, and CTP were obtained from Pabst Laboratories, Milwaukee, Wisc. The UTP was separated from impurities of both UDP and ATP by ion exchange chromatography in the presence of $AT^{32}P$ (to insure freedom from trace amounts of ATP) by Dr. H. M. KALCKAR (who generously gave this material). UDP was prepared from UTP by means of the phosphorylation of glucose with a crude yeast hexokinase, followed by ion exchange chromatography of the products. GDP was prepared by hydrolysis of guanosine diphosphomannose from hen oviduct[12] and later was obtained from Sigma Chemical Co. GTP was prepared by phosphorylation of GDP in the pyruvate phosphokinase system[13].

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Barium phosphopyruvate was kindly given by Mr. W. E. PRICER, Jr.
Pyruvate phosphokinase was prepared by the procedure of KORNBERG AND PRICER[14].
Crystalline lactic dehydrogenase[15] was the gift of Dr. A. MEISTER.
DPNH was prepared essentially as described by OHLMEYER[16].
Hexokinase was obtained from Sigma Chemical Co.
Glucose-6-phosphate dehydrogenase was prepared according to KORNBERG[17].

METHODS

Assays for nucleoside monophosphate kinases

It has been necessary to employ several different assays in order to investigate the variety of reactions (equations 1–8) which occur in an aqueous extract of calf liver acetone powder.

Assay (a). Measurement of nucleoside diphosphate formation kinetically by coupling the reaction to pyruvate phosphokinase and lactic dehydrogenase[14]. This assay can be employed for the ATP–UMP and ATP–CMP transphosphorylations (reactions 1 and 2). The assay system contained, in a final volume of 0.5 ml: 0.1 *M* potassium phosphate buffer, pH 7.3, 0.38 ml; 0.1 *M* $MgCl_2$, 0.02 ml; 0.004 *M* DPNH, 0.02 ml; 0.1 *M* ATP, 0.01 ml; 0.05 *M* UMP, 0.01 ml; 10% glutathione in 0.002 *M* EDTA, 0.025 ml; lactic dehydrogenase, 0.5 mg and pyruvate phosphokinase, 0.01 ml. Interference with this assay was encountered from a blank oxidation of DPNH by the assay enzymes (lactic dehydrogenase and pyruvate phosphokinase) with ATP alone. It was not due to impurities in the substrates. After 10–15 min incubation this blank oxidation of DPNH ceased, and for this reason the assay system was always preincubated for 15 min before the addition of nucleoside monophosphate kinase. Similarly, the nucleoside monophosphate kinase preparations, particularly the cruder fractions, cause some oxidation of DPNH with ATP alone. It is, therefore, necessary to subtract a blank rate, obtained with ATP alone, from the rate of oxidation in the presence of ATP and UMP (or CMP). The oxidation of DPNH (measured as the decrease in optical density per minute at 340 mμ) usually started at a low rate and reached a maximum after 3 to 5 min. This maximum rate was proportional to the amount of nucleoside monophosphate kinase added; *e.g.*, in one experiment with 1.8, 5.3, and 10.7 μl of enzyme the optical density decreases per minute at 340 mμ were 0.009, 0.022 and 0.045. It was found desirable to adjust the amount of enzyme so that the rate fell in the range 0.010 to 0.040 per minute*.

*Assay (b). Measurement of the total amount of nucleoside diphosphate formed as a function of time***. Reactions 3–8 were measured by removing aliquots at intervals and analyzing for their content of nucleoside diphosphate as indicated below. The aliquot removed for analysis was greatly diluted in the assay so that the nucleoside monophosphate kinase no longer had any significant effect. The incubation mixture

* It is not certain that the formation of both nucleoside diphosphates is measured in the assay. On the one hand the rates of phosphorylation of UDP and CDP in the pyruvate phosphokinase reaction are only about 3 and 2%, respectively, of the rate of phosphorylation of ADP[13]. Secondly, the crude fractions contain a very active nucleoside diphosphatase[9] which hydrolyzes all of the diphosphates except ADP and CDP.

** This procedure is a variation of assay (a), in which the incubation and analysis for diphosphate are carried out in two separate stages. It is necessitated by a high rate of phosphorylation of AMP[18] under the conditions of assay (a). This difficulty is overcome by carrying out the procedure in two stages.

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contained: 0.035 ml of 0.03 *M* veronal buffer, pH 7.3, containing 0.004 *M* $MgCl_2$, 0.005 ml of 10% glutathione in 0.002 *M* EDTA, 0.5 μmole nucleoside triphosphate, 0.5 μmole nucleoside monophosphate, nucleoside monophosphate kinase and water to 0.1 ml. After incubation for varying times at 38°, 0.005 to 0.002 ml aliquots were removed and added to 0.5 ml of a mixture with the following composition: 4.7 ml of 0.1 *M* potassium phosphate buffer, pH 7.0 which contained 0.004 *M* $MgCl_2$; 0.15 ml of 0.02 *M* potassium phosphopyruvate; 0.05 ml of 0.01 *M* DPNH; 0.2 ml of crystalline lactic dehydrogenase (1:50 dilution); 0.1 ml of pyruvate phosphokinase. The amounts of lactic dehydrogenase and pyruvate phosphokinase used were sufficient to cause, in the presence of excess ADP, a complete oxidation of DPNH in less than 2 min. This mixture was preincubated for about 15 min at 23° and an initial reading was taken before an aliquot of a kinase incubation was added. Thereafter, readings were taken every few minutes until the rate of decrease of optical density at 340 mμ reached a low blank rate of about 0.002 per min. The total decrease in optical density was corrected for a blank value obtained from an incubation with nucleoside triphosphate alone.

In the initial extracts, the nucleoside diphosphatase present also interferes with this assay, but this contamination is removed by the first ammonium sulfate fractionation.

Assay (c). Measurement of the amount of nucleoside diphosphate formed by coupling the reaction to dephosphorylation by nucleoside diphosphatase. This assay which can be employed for reactions 1, 3, 5, 7 and 8 will be described in detail in the following paper[11].

Assay (d). Measurement of the ATP–AMP kinase. In this assay the ATP formed from ADP was measured by the phosphorylation of glucose with hexokinase coupled to glucose-6-phosphate dehydrogenase[19]. The assay mixture contained 0.47 ml of 0.1 *M* potassium phosphate buffer, pH 7.0, with 0.007 *M* $MgCl_2$, 0.01 ml of 0.02 *M* triphosphopyridine nucleotide, 0.03 ml of 0.5 *M* ADP, 0.02 ml glucose-6-phosphate dehydrogenase (10 mg/ml), and 0.01 ml hexokinase (50 mg/ml).

Paper electrophoresis was carried out as described by BERG AND JOLIK[2].

Protein was determined with the modified Folin phenol reagent[20].

RESULTS

Purification of the ATP–UMP kinase

Purification of a kinase active with ATP and UMP was followed with assay (a). All fractionation steps were carried out at 0–3°.

Step 1. Extraction and acid precipitation. 100 g of calf liver acetone powder[21] were extracted by slowly adding 1000 ml of 0.0001 *M* EDTA with gentle grinding in a mortar and pestle. After 30–45 min the mixture was centrifuged. The supernatant solution (fraction A, 1630 ml) was brought to pH 5.0 by the dropwise addition of about 2 ml of 5 *N* acetic acid with continuous stirring. After 15 min, the preparation was centrifuged and the supernatant solution brought to pH 5.5 with 1 ml of 5 *N* NH_4OH (fraction B, 1550 ml).

Step 2. First ammonium sulfate. Fraction B was mixed with 525 g of ammonium sulfate (34 g/100 ml, 0.57 saturation). After 30 min the precipitate was removed by centrifugation and discarded. To the supernatant solution 200 g of ammonium sulfate (13 g/100 ml of fraction B, 0.75 saturation) were added. After 30 min the precipitate

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was collected and dissolved in 400 ml of 0.025 *M* sodium acetate in 0.0001 *M* EDTA (hereinafter called acetate–EDTA) to give fraction D. This fraction contained 5% ammonium sulfate (measured by conductivity using a Barnstead purity meter). The saturation was brought to 0.55 by the addition of 118 g of ammonium sulfate (29.6 g/100 ml), and the precipitate discarded. To the supernatant solution were added 59 g of ammonium sulfate (14.8 g per initial 100 ml, 0.75 saturation). After 30 min the precipitate was collected and dissolved in 100 ml of acetate–EDTA (fraction G). This was dialyzed for 5 h against 0.0001 *M* EDTA (fraction H, 125 ml).

Step 3. Calcium phosphate gel. Fraction H was diluted to 730 ml in 0.0001 *M* EDTA and 250 ml of aged calcium phosphate gel[22] (17.9 mg/ml, 3 years old) were added. The appropriate amount of gel was established by a small-scale trial for each batch of enzyme. After 10 min, the gel was centrifuged off, washed with 100 ml of 0.0001 *M* EDTA and eluted two times with 100 ml of 0.1 *M* potassium phosphate, pH 5.2. In order to concentrate the protein, 103 g of ammonium sulfate were added (51.6 g/100 ml, 0.80 saturation). The precipitate was collected by centrifugation and dissolved in 20 ml of acetate–EDTA (fraction J).

Step 4. Second ammonium sulfate. Fraction J was diluted to 125 ml and then was found to be 0.026 saturated with ammonium sulfate. 17.8 g of ammonium sulfate were added (14.2 g/100 ml, 0.3 saturation) and the pH brought to 4.1 by the addition of 3.5 ml of 1 *N* acetic acid. A further 7.3 g of ammonium sulfate were added (5.9 g per initial 100 ml, 0.40 saturation), and a precipitate was collected by centrifugation (fraction K_1). Fractions K_2 (0.40–0.50 saturation), K_3 (0.50–0.60 saturation), K_4 (0.60–0.70 saturation) and K_5 (0.70–0.90 saturation) were collected similarly by the successive addition of 7.6, 7.8, 8.1 and 17.5 g of ammonium sulfate to the supernatants after each precipitation. Each precipitate was dissolved in about 25 ml of acetate–EDTA. This procedure yielded 40–45-fold purified material with an overall yield of 14% (Table I).

TABLE I

PURIFICATION OF ATP–UMP KINASE

Fraction	*Volume ml*	*Units* ($\times 10^{-6}$)	*Overall yield %*	*Specific activity Units/ug protein*	*Purification*
A. Extract	1630	24.6	(100)	1.6	—
B. Acid supernatant	1550	22.7	92	2.1	1.3
D. Am. sulf. 57–75%	400	16.6	68	5.5	3.3
G. Am. sulf. 55–75%	100	17.9	73	10.0	6.0
H. Dialysis	125	13.9	57	9.3	5.6
J. Calcium phosphate gel eluate	20.5	5.6	23	34	20
K_4 Acid am. sulf. 60–70%	33	1.8	14	68	41
K_5 Acid am. sulf. 70–90%	24	1.5		73	44

Specificity

In addition to the ATP–UMP reaction, the purified enzyme fraction also catalyzed reactions between ATP and CMP and between ATP and AMP. The ATP–UMP reaction was about twice as rapid as the ATP–CMP reaction in the extract and this ratio was maintained throughout the purification (Table II)*.

* However, in one aged preparation the ATP–CMP reaction was found to be 1.5 times as fast as the ATP–UMP reaction. This 3-fold change in ratio suggests that these two reactions may be catalyzed by different proteins.

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TABLE II

OCCURRENCE OF ATP–CMP AND ATP–AMP KINASES IN FRACTIONS DURING PURIFICATION OF ATP–UMP KINASE

	*ATP–UMP kinase**	*ATP–CMP kinase**	*Ratio***	*ATP–AMP kinase****	*Ratio***
Extract (A)	15.1	8.6	1.8	11.4	1.3
Gel eluate (J)	272	149	1.8	25.0	10.8
Acid am. sulf. (K_5)	63	24	2.6	1.5	42

* Units per ml in assay (a).
** The ratio of the ATP–UMP kinase to the ATP–CMP or the ATP–AMP kinase.
*** Units per ml in assay (d). Units per ml of the ATP–AMP kinase in Fraction A are about 75 by assay (a).

The ATP–AMP reaction was about five times as fast as the ATP–UMP reaction in the extract (both measured in assay (a)), but with the purified enzyme the ATP–UMP reaction was almost six times as fast as the ATP–AMP reaction. This 30-fold change in ratio of activities indicates that some protein catalyzing the ATP–AMP reaction was removed during the preparation. However, as the ATP–AMP reaction might be catalyzed by several different proteins, it is not possible to state at the present time whether or not catalysis of the ATP–AMP transphosphorylation is a property of the ATP–UMP enzyme.

The catalyst for the ATP–GMP reaction which was present in the extract was completely removed during the purification (Table III). Similarly the ATP–UMP kinase (fraction K_5) did not catalyze to an appreciable extent the UTP–AMP, CTP–AMP, ITP–AMP or GTP–AMP reactions, all of which occur in the extract (Table III). No reaction was found to occur between ATP and IMP in the calf liver acetone powder extract (Table III). This transphosphorylation reaction is also absent in extracts of yeast[10] and brain[8].

TABLE III

OCCURRENCE OF ATP–GMP, ITP–AMP AND ATP–IMP KINASES IN FRACTIONS DURING PURIFICATION OF ATP–UMP KINASE

Incubations were carried out with 35 μl $M/35$ Veronal–$MgCl_2$ buffer, 5 μl 10% glutathione in 0.002 M EDTA, 10 μl 0.1 M ATP (1 μmole), 10–20 μl nucleoside monophosphate (0.4–1 μmole), 5 μl nucleoside diphosphatase (600 units/ml) and 10 μl enzyme. At the end of 1 h the reaction was stopped by adding 0.9 ml 6% trichloracetic acid and 0.7 ml of the supernatant was analyzed for inorganic phosphate (Assay (c)). Units are μmole phosphate liberated per h and ml enzyme.

	ATP–UMP kinase	*ATP–GMP kinase*	*Ratio**	*ITP–AMP kinase*	*Ratio**	*ATP–IMP kinase*
Extract (A)	58	15	3.9	19.7	2.9	0.0
Am. sulf. I (D)	93	2	47	—	—	—
Gel eluate (J)	460	0 ($\pm$ 0.5)	>500	**	**	0.0
Acid am. sulf. (K_5)	75	—	—	0 ($\pm$ 0.5)***	>150	—

* Ratio of the ATP–UMP kinase to the ATP–GMP or ITP–AMP kinase.
** The ITP–AMP kinase was present in this fraction but a reliable assay was not carried out (*cf.* also Table IV).
*** The UTP–AMP, GTP–AMP, and CTP–AMP kinases were similarly absent from this fraction.

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Nucleoside monophosphate kinase reactions not involving adenosine nucleotides

Although extracts of calf liver acetone powder catalyze some transphosphorylation reactions in which neither substrate is an adenosine nucleotide, purified preparations do not catalyze such reactions (Table IV). In fractions less purified than Fraction J the following combinations were negative, tested by assay (a) or assay (b): UTP–UMP, UTP–IMP, CTP–IMP, CTP–UMP and CTP–CMP. The occurrence of enzymes in the acetone powder extract catalyzing such reactions could only be proven by purification, since their demonstration in the extract might be due to coupled reactions[9] involving the participation of catalytic amounts of adenosine nucleotides. In yeast, however, a UTP–UMP kinase has been demonstrated[10] *.

TABLE IV

NUCLEOSIDE MONOPHOSPHATE KINASE REACTIONS IN FRACTION PARTIALLY PURIFIED FOR ATP–UMP KINASE ACTIVITY

Fraction J was employed, and contained both ATP–nucleoside monophosphate kinases and nucleoside triphosphate–AMP kinases. All kinase reactions present included an adenosine nucleotide as one substrate. Assays were carried out by method (c) and data are given as μmoles inorganic phosphate formed. For each experiment the nucleoside monophosphate added is indicated at the top of the column of figures and the nucleoside triphosphate in the left hand column.

	AMP	*IMP*	*UMP*	*CMP*	*GMP*
ATP	*	0.00	0.12	*	0.00
ITP	0.15	0.00	0.00	0.00	—
UTP	0.13	—	0.01	0.00	—
CTP	*	0.00	0.00	*	—
GTP	0.11	0.00	0.00	0.00	0.00

* The reactions cannot be assayed by method (c) since neither of the products would be a substrate for nucleoside diphosphatase.

Nucleoside diphosphokinase in enzyme preparations. The purified fractions (J or $K_{4.5}$) were examined for nucleoside diphosphokinase activity by incubating ATP with UDP or IDP. The incubation mixtures were subjected to paper electrophoresis at pH 3.5 and 5.0. As ATP and UDP or IDP were the only nucleotides found, it was concluded that nucleoside diphosphokinase was absent from these preparations.

Stability to heat, acid and storage. The ATP–UMP kinase was completely (> 99 %) inactivated at pH 7.2, 5.2, or 1.3 by heating to 80° for 3 min. At pH 1.3 the enzyme was more than 50 % inactivated at 2° in a few minutes.

The ATP–AMP activity was similarly completely lost at pH 5.2 at 80° in 3 min. At pH 7.2 2–3 % of the activity remained under these conditions and at pH 1.3 about 10 % remained. These findings could indicate either that the catalyst for the ATP–AMP kinase has a slightly greater stability at pH 1.3, for example, than the ATP–UMP kinase, or that there are several proteins in liver which catalyze the ATP–AMP reaction, one of which is relatively stable to heating at pH 1.3.

A preparation of the ATP–UMP kinase (fraction J) which was stored for 7 weeks

* JOKLIK[26] has also reported the existence of an ITP–IMP kinase in yeast extracts. However, the reaction observed might have been the sum of several kinase reactions catalyzed by adenine nucleotide impurities in the crude yeast extract employed and the reported instability to purification, despite stability during extraction to 60° for 5 min in 0.1 *N* HCl, might be due to removal of contaminating nucleotides during purification.

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at — 15° lost no activity in this period. Another aliquot which had been repeatedly thawed and frozen lost 50 % of its acticity in this time. The most purified preparation (fraction $K_{4,5}$) seemed to be much more labile to storage, *e.g.*, one preparation lost 75 % of its activity in 3 weeks at —15°.

In extract and early fractions the ATP–UMP kinase was exceedingly labile, losses of more than 50 % being encountered overnight. For this reason the purification has been carried out as rapidly as possible. In the preparation described in Table I the preparation was carried through the dialysis (fraction H) in one day and was completed the next day.

Activation by glutathione and EDTA. The degree of activation of various preparations by glutathione and EDTA has been variable. With one preparation these substances stimulated activity more than 300 % (Table V). With another preparation, however, activities were, with glutathione 105 %, with EDTA 100 %, and with both 128 % of the activity without additions. A successful fractionation of the enzyme was

TABLE V

ACTIVATION OF ATP–UMP KINASE BY DIVALENT CATIONS AND BY GLUTATHIONE–EDTA

Assays were carried out by method (b), except that a divalent cation was added to the incubation only as indicated. This assay does not interfere with the requirement of pyruvate phosphokinase for divalent cations. 0.01 ml of Fraction K_5 (Table I) was employed, and the incubations were for 30 min at 38°.

Additions	*None*	*Mg^{++} 0.0004 M*	*Mg^{++} 0.004 M*	*Mg^{++} 0.04 M*	*Mn^{++} 0.004 M*	*Ca^{++} 0.004 M*	*Mg^{++} 0.004 M*
μmoles Nucleoside diphosphate formed	0.00	0.05	0.42	0.44	0.41	0.21	0.12*

* Glutathione and EDTA omitted.

not carried out until glutathione and versene were included in the assay mixture, and versene was added to the solutions employed in the fractionation.

pH optimum. At pH 5.3 the activity was 10 % and at pH 8.0 45 % of that observed in the optimal range, pH 6.5 to 7.5.

Activation by divalent cations. Mg^{++} and Mn^{++} were equally effective in activating the enzyme, and Ca^{++}, at the same concentration, was one-half as active as these. 0.004 *M* Mg^{++} seemed to saturate the enzyme (Table V).

Isolation of reaction products and equilibrium constants

In order to isolate the reaction products an incubation was carried out with ATP and CMP as substrates as follows: 6.5 ml 0.0001 *M* EDTA, 0.5 ml 0.1 *M* $MgCl_2$, 0.5 ml 0.077 *M* CMP (38 μmoles), 0.5 ml 0.094 *M* ATP (47 μmoles) and 2.8 ml fraction K_5. 8-μl aliquots were removed periodically and assayed for nucleoside diphosphate with the pyruvate phosphokinase system. At the end of 2.5 h at room temperature (at which time the total diphosphate had reached a constant value of about 35 μmoles) the entire incubation was run onto a column of Dowex-1 formate (X 10), 1 cm^2 × 6.5 cm. The column was eluted successively* with (A) 100 ml H_2O, (B) 100 ml 0.05 *N* formic

* We wish to thank Drs. E. HERBERT and V. R. POTTER for providing the details of their procedure before publication.

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acid, (C) 200 ml 0.2 *N* formic acid, (D) 100 ml 0.25 *M* ammonium formate (pH 4.9), (E) 150 ml 0.3 *M* ammonium formate (pH 4.9), (F) 200 ml 3 *N* formic acid, (G) 120 ml 0.5 *M* ammonium formate (pH 4.9), (H) 170 ml 0.67 *M* ammonium formate and finally (I) 200 ml 1 *M* ammonium formate. The six cleanly separated ultraviolet-absorbing peaks which were obtained were pooled and the amount of nucleotide in each estimated from ultraviolet absorption: from elution (B) CMP (10.8 μmoles); from (C) AMP (6.1 μmoles); from (D) CDP (17.3 μmoles); from (F) ADP (12.1 μmoles); from (H) CTP (0.5 μmoles); and from (I) ATP (19.4 μmoles). Identification of the peaks was based upon their position on anion exchange chromatography, their ultraviolet absorption spectra, and their chromatographic position in neutral ethanol–ammonium acetate[23].

As both of reactions 2 and 4 were known to occur in the preparation, the expected products were CMP, AMP, CDP, ADP and ATP. The small amount of CTP which was found presumably came from the reverse of reaction 6, despite the fact that this reaction was not detected in this preparation by the usual assay (Table II). In the present experiment a very large amount of enzyme and a long time of incubation were employed in an attempt to insure equilibrium of reactions 2 and 4.

From the data presented, the following equilibrium constants have been calculated:

$$\text{Reaction 2} \quad \frac{(\text{CMP})\,(\text{ATP})}{(\text{CDP})\,(\text{ADP})} = 0.99$$

$$\text{Reaction 4} \quad \frac{(\text{AMP})\,(\text{ATP})}{(\text{ADP})^2} = 0.81$$

These values compare to values of about 0.9 reported for the UTP–UMP reaction[10] and 0.5 for the adenylate kinase reaction[24].

DISCUSSION

It has been concluded on the basis of purification of the enzyme and isolation of the reaction products that the mechanism of the reaction studied here is analogous to the reaction catalyzed by muscle adenylate kinase (myokinase)* [1], *i.e.*, the transfer of the terminal phosphate from a nucleoside triphosphate to a nucleoside monophosphate with formation of two nucleoside diphosphates. Stoichiometric studies have shown that two moles of nucleoside diphosphate appear for each mole of nucleoside triphosphate which disappears[9]. However, other possible mechanisms which might lead to such stoichiometry and to the observed reaction products were considered, for example (illustrated with reactions between cytidine and adenosine nucleotides):

$$\text{ATP} + \text{CDP} \rightleftharpoons \text{CTP} + \text{ADP}$$
$$\text{CMP} + \text{CTP} \rightleftharpoons 2\,\text{CDP}$$

of which the sum is reaction 2. This sequence, which might be initiated by catalytic amounts of CDP in either substrates or enzymes, has been ruled out by the failure to detect either a nucleoside diphosphokinase or a cytidylate kinase in these enzyme preparations. Furthermore, isotopic evidence for a direct transfer of a single phosphate from ATP to a nucleoside monophosphate, has been obtained by MUNCH-PETERSON[6].

The nucleoside monophosphate kinases from acetone powder extracts of calf liver

* A myokinase preparation, kindly supplied by Dr. S. COLOWICK, has been found to be completely devoid of the ATP–UMP kinase.

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have been separated into two groups. The preparation reported here catalyzes reactions between ATP and several nucleoside monophosphates (UMP, CMP, or AMP). The complete removal of the ATP–GMP kinase during the fractionation and the partial removal of the ATP–AMP kinase suggest that further fractionation may yield enzymes with even greater specificity. A second preparation which catalyzes transphosphorylations between ATP, UTP, CTP, or ITP and AMP will be described in the following paper[11].

It is noteworthy that no reaction has been found between ATP and IMP. The only known route, therefore, for the synthesis of IDP from hypoxanthine is *via* adenosine diphosphate deaminase[25]. Neither were any transphosphorylation reactions found in fractionated preparations in which neither of the reactants was an adenosine nucleotide. This fact might place adenosine nucleotides in a central role in distributing "high energy" phosphate among the nucleotides. Similarly, HERBERT, POTTER AND TAKAGI[5] have concluded that synthesis of all of the nucleoside di- and triphosphates during oxidative phosphorylation may proceed *via* adenosine nucleotides.

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