NUCLEOSIDE MONOPHOSPHATE KINASES

II. TRANSPHOSPHORYLATION BETWEEN ADENOSINE MONOPHOSPHATE AND NUCLEOSIDE TRIPHOSPHATES*

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SUMMARY

1. A nucleoside monophosphate kinase has been fractionated from aqueous extracts of calf liver acetone powder. It catalyzes transphosphorylations between adenosine 5′-phosphate and any nucleoside triphosphate to yield adenosine 5′-diphosphate and another nucleoside diphosphate. The reactions are reversible.

2. The enzyme fraction has been freed of phosphatase activity and contains no nucleoside diphosphate kinase. It has also been separated from another nucleoside monophosphate kinase which is specific for ATP but reacts with any of several nucleoside monophosphates.

INTRODUCTION

In the preceding paper[4] an enzyme fraction purified from extracts of calf liver acetone powder was described, which catalyzed transphosphorylations between adenosine triphosphate and any of a number of nucleoside 5′-monophosphates. No other nucleoside triphosphate could replace ATP. This fraction has been designated as the ATP–nucleoside monophosphate kinase, to indicate that its specificity is not limited to a particular nucleoside monophosphate.

From the same extracts transphosphorylation activity of another type has been separated, free of the first enzyme system. In the present investigation this fraction has been shown to catalyze the following reactions:

$$ITP + AMP \rightleftharpoons IDP + ADP \quad (1)$$
$$UTP + AMP \rightleftharpoons UDP + ADP \quad (2)$$
$$CTP + AMP \rightleftharpoons CDP + ADP \quad (3)$$
$$GTP + AMP \rightleftharpoons GDP + ADP \quad (4)$$
$$ATP + AMP \rightleftharpoons 2\,ADP \quad (5)$$

The following abbreviations will be used: AMP, ADP, ATP = adenosine 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; IMP, IDP, ITP = inosine 5′-mono-, di- and triphosphates; EDTA = ethylene diamine tetraacetate; GSH = reduced glutathione; DPNH = reduced diphosphopyridine nucleotide; P_i = inorganic phosphate; IDPase = inosine diphosphatase.

* Part of this material has been presented in several preliminary reports[1,2,3].

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No other nucleoside monophosphate could replace AMP and, for convenience, this fraction has been designated as the nucleoside triphosphate–AMP kinase.

Reactions (1), (2) and (4) were followed by measuring the inorganic phosphate formed in a coupled reaction catalyzed by inosine diphosphatase:

$$IDP \longrightarrow IMP + P_i \qquad (6)$$

This enzyme was discovered simultaneously in several laboratories[1,5–7] and is active with IDP, UDP and GDP. Recently, it has also been shown to hydrolyze ribose 5'-pyrophosphate[8]. In the present communication a method is given for purifying this phosphatase to a point where it is free of both types of kinase activity, so that it could be used to follow the purification of the nucleoside triphosphate–AMP kinase.

EXPERIMENTAL

Materials. Nucleotides were obtained from sources mentioned in the preceding paper[4]. In addition, GTP, CTP, CDP, UDP and GMP were purchased from Sigma Chemical Company, and samples of chemically synthesized UDP, UTP and GMP were kindly provided by Dr. H. G. KHORANA.

Methods. Descending paper chromatography was carried out with the following solvent systems. Solvent 1: isobutyric acid, 100 ml; 1 *N* NH_4OH, 60 ml; 0.1 *M* EDTA, 1.6 ml[9]. Solvent 2: saturated ammonium sulfate–isopropanol–1 *M* sodium acetate (80:2:18, v/v/v)[10]. Solvent 3: 95 % ethanol, 750 ml; 1 *M* ammonium acetate, 300 ml[11]. For quantitative chromatography each ultraviolet-absorbing region was cut out, eluted with 0.1 *N* HCl for 24 h, and the absorbance of the eluate was determined at a wave length corresponding to the absorption maximum for the particular compound. A blank for the paper was subtracted.

Electrophoretic separation of nucleotides was performed according to MARKHAM AND SMITH[10] on strips (56 × 9 cm) of Whatman No. 3 MM paper saturated with 0.05 *M* ammonium formate–formic acid buffer pH 3.5 or pH 2.0, or with 0.05 *M* ammonium acetate–acetic acid buffer pH 4.5.

Assay of the nucleoside triphosphate–AMP kinase as routinely carried out depended on coupling reaction (1) with IDPase (reaction (6)); the sum of these reactions is equation (8):

$$ITP + AMP \longrightarrow IMP + ADP + P_i \qquad (8)$$

In the presence of excess IDPase, the kinase activity is directly proportional to the P_i formed. The incubation mixture contained 1 μmole of ITP, 0.5 μmole of AMP, 0.03 mg of crystalline bovine serum albumin, 1.65 μmoles of GSH, 0.01 μmole of EDTA, 1.5 μmoles of veronal–acetate buffer[12], 2 units of inosine diphosphatase and from 0.06 to 2.6 units of kinase enzyme in a volume of 0.1 ml. A control incubation mixture was routinely set up from which AMP was omitted; this was in order to correct for P_i present in the ITP. Enzymic activity with ITP as the only nucleotide present was negligible. The preparation of AMP was free of P_i and was not dephosphorylated by the enzymes. After 90 min at 37° the incubation mixture was analyzed for P_i by the method of FISKE AND SUBBAROW[13]. Deproteinization was not necessary except for the initial extract. A unit of activity was defined as that amount which catalyzed the formation of 1 μmole of P_i (derived from IDP) per h. Specific

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activity was units per mg of protein. Protein was determined by the method of LOWRY *et al.*[14].

Purification of IDPase. PLAUT[6] has reported on a highly purified preparation of IDPase and has noted its high substrate affinity for IDP and the absence of nucleoside diphosphokinase. We also purified this phosphatase, but our aim was to get a preparation free of both types of nucleoside monophosphate kinase activity. Such a fraction was needed for the assay of the nucleoside triphosphate–AMP kinase, and it was obtained as follows:

Extraction. Except as noted, all steps were carried out at 0–3°. Calf liver acetone powder was made as described earlier[4]. It could be used after storage for as long as six months at 3°. Thirty g of powder were extracted with 600 ml of cold distilled water for 30 min, with gentle stirring. The mixture was centrifuged for 7 min at 13,000 *g* and the precipitate discarded.

First acid ammonium sulfate step. The extract (535 ml) was adjusted to pH 5.0 with 100 ml of 0.1 *N* acetic acid, using mechanical stirring. A precipitate formed which was separated from the supernatant solution (solution A, 605 ml) by centrifugation. The precipitate was extracted by mixing with 0.05 *M* sodium acetate and adjusting the suspension to pH 7.0 with NH_4OH. It dissolved only partly, and insoluble material was removed by centrifugation and discarded. The extract (Fraction Ia) measured 104 ml.

The supernatant solution A (605 ml) was mixed with 100 g of ammonium sulfate (0.3 saturation). A precipitate formed which was collected by centrifugation and extracted with sodium acetate and NH_4OH, as described above, giving 72 ml of extract (Fraction Ib). Fractions Ia and Ib were combined (Fraction I, 176 ml).

Second acid ammonium sulfate step. 176 ml of Fraction I were mixed with 29 g of ammonium sulfate (0.3 saturation). An inactive precipitate was removed by centrifugation and discarded. The pH of the supernatant solution was adjusted to 4.6 with 13.8 ml of 1 *N* acetic acid. After 10 min a precipitate was collected by centrifugation. This precipitate was treated with 0.05 *M* sodium acetate and NH_4OH, as in step 2, yielding 30.4 ml of extract (Fraction II).

Alkaline ammonium sulfate step. Fraction II was mixed with 71 ml of distilled water and 16.7 g ammonium sulfate (0.3 saturation). The pH was adjusted to 8.0 by the addition of 1 ml of a 1 *N* NH_4OH solution which also contained 16.7 g of ammonium sulfate per 100 ml. Then 15.2 ml of saturated ammonium sulfate were introduced (0.39 saturation). The saturated ammonium sulfate solution contained 3.7 ml of concentrated NH_4OH per l and its pH, after 5-fold dilution with water, was 8.0. After 15 min a precipitate was removed by centrifugation and discarded. To the supernatant solution were added 165 ml of saturated alkaline ammonium sulfate (0.75 saturation). This time the precipitate was collected by centrifugation, dissolved in 0.05 *M* acetate buffer pH 6, and dialyzed against running distilled water for 6 h (Fraction III, 71 ml).

Ethanol step. Fraction III was diluted with distilled water to 113 ml and its pH adjusted to 5.2 with 0.5 ml of 0.1 *N* acetic acid. A precipitate formed which was collected by centrifugation and dissolved in 0.05 *M* sodium acetate (Fraction IV, 71 ml). The supernatant solution was mixed with 1.7 ml of 2 *M* acetate buffer pH 5.2, followed by 13.9 ml of absolute ethanol (11 %). The temperature was allowed to fall to —4° during the addition of ethanol. After 10 min, the mixture was centrifuged

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at 13,000 *g* for 3 min. The precipitate was allowed to drain at —8° and was dissolved in 0.05 *M* sodium acetate (Fraction V, 71 ml). The results of purification are summarized in Table I. Both Fractions IV and V were free of kinase activity.

TABLE I

PURIFICATION OF IDPASE

Fraction	*Total units*	*Over-all yield %*	*Specific activity U/mg protein*	*Ratio of: ITP–AMP kinase / IDPase*
Initial extract	354,000	100	61	0.075
I. Ammonium sulfate, pH 5	172,800	49	114	0.032
II. Ammonium sulfate, pH 4.6	103,000	29	330	0.006
III. Alkaline ammonium sulfate	67,500	19	520	< 0.001
IV. Precipitate, pH 5	30,000	8.5	570	
V. 0–11 % Ethanol	33,500	9.5	1630	

Assay of IDPase involved an incubation mixture containing 1.5 μmoles of veronal–acetate buffer[12] pH 7.0, 1.6 μmoles of GSH, 0.8 μmole of $MgCl_2$, 0.01 μmole of EDTA, 0.03 mg of crystalline serum bovine albumin and 0.7 μmole of IDP in a total volume of 0.08 ml. This was incubated at 37° for 30 min, together with a control mixture lacking enzyme. After addition of 0.2 ml of 2.5 % perchloric acid, analysis for P_i was carried out. A unit of activity corresponds to the formation of 1 μmole of P_i per h.

Enzyme fractions could be stored at any stage for several weeks at —15° before proceeding to the next step. Fraction V showed no loss of activity after being stored for three years at —15°. As other workers have also reported[5,6], the enzyme was found to require Mg^{++} and the pH optimum was approximately 7.0. A two-fold stimulation of the purified fractions by albumin was noted. Fraction V had a specific activity of 1,630 for IDP, 1,000 for UDP, 1,300 for GDP and 1,030 for ribose 5-pyrophosphate. There was no hydrolysis of CDP nor of ADP. Also, none of the nucleoside 5'-triphosphates or monophosphates were split, in agreement with other reports[5,6]. Enzyme Fraction II was free of ATP–nucleoside monophosphate kinase after storage for three weeks at —15°. The nucleoside triphosphate–AMP kinase was absent when the purification had been carried up to the isolation of Fraction III (Table I).

RESULTS

Fractionation of the nucleoside triphosphate–AMP kinase

The following procedure resulted in only a 4-fold purification with respect to protein, but the final preparation of the nucleoside triphosphate–AMP kinase was free of ATP–nucleoside monophosphate kinase activity and was also devoid of IDPase.

Step 1, extraction. This step was the same as for the purification of IDPase.

Step 2, first ammonium sulfate. The extract (535 ml) was brought to pH 5 by the introduction of 40 ml of 0.5 *N* acetic acid with mechanical stirring. After removal of a precipitate by centrifugation, the supernatant solution (570 ml) was treated with 93.5 g of solid ammonium sulfate (0.3 saturation). A precipitate formed which was removed by centrifugation and discarded. The supernatant solution (531 ml) was mixed with 78.6 g of ammonium sulfate (0.55 saturation). The resultant precipi-

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tate was collected by centrifugation and dissolved in 0.05 *M* sodium acetate (Fraction A, 81.5 ml).

Step 3, second ammonium sulfate. Fraction A was mixed with an equal volume of distilled water. Its saturation with respect to ammonium sulfate was found to be 0.02, from measurement of its conductivity with a Barnstead purity meter. After the addition of 26.8 g of ammonium sulfate (0.3 saturation), the pH was adjusted to pH 4.6, using 4.9 ml of 2 *N* acetic acid. A precipitate formed which was removed by centrifugation, and the supernatant solution (169 ml) was treated with 14.5 g of ammonium sulfate (0.45 saturation). This time the precipitate was collected by centrifugation and dissolved in 0.05 *M* sodium acetate (64 ml, Fraction B). A second fraction was obtained by the further addition of 44 g of ammonium sulfate to the supernatant solution (0.85 saturation). It was also dissolved in 0.05 *M* sodium acetate (Fraction C, 71 ml, pH 6.5–7.0).

Step 4, heat treatment. Fraction C was heated at 65° for 6 min and then rapidly cooled in an ice bath. A precipitate appeared. After centrifugation the precipitate was discarded, and full activity was recovered in the supernatant solution (Fraction D). This treatment removed the last traces of IDPase activity, which is completely destroyed by heating to 55° for 5 min*. Fraction B could also be carried through a heat step but the results were less satisfactory, for about half of the AMP-kinase was destroyed and traces of IDPase remained. Most of the results reported below were obtained with Fraction D. The results of purification are outlined in Table II.

TABLE II

FRACTIONATION OF THE NUCLEOSIDE TRIPHOSPHATE–AMP KINASE

Fraction	*ITP–AMP kinase**			*ATP–UMP kinase*	*Ratio: IDPase / ITP–AMP kinase*
	U/ml	*Total U*	*U/mg protein*	*U/ml*	
Initial extract	80	24,000	4.0	57	16.7
A (1st Ammonium sulfate)	139	9,300	11.5	5.5	1.0
B (2nd Ammonium sulfate, 0.3–0.45)	115	7,100	15.0	0.0	0.4
C (2nd Ammonium sulfate, 0.45–0.85)	24	1,540	8.5	0.0	0.1
D** Heat step	24	1,540	17.0	0.0	0.0

* In following the fractionation by coupling to IDPase it is better to use the pair ITP–AMP rather than any of the others. This is because the product of the coupled reaction is IMP which is inert in the ATP–kinase system (see ref. [1]). With a pair such as UTP–AMP, on the other hand, first UDP and then UMP are formed in the coupled system. As a result, UDP will be regenerated if the nucleoside triphosphate–AMP kinase is still contaminated with ATP–UMP kinase activity. Thus, only one kinase is measured with the pair ITP–AMP, whereas both kinases become involved with the pair UTP–AMP.

** Fraction D results from heating Fraction C to 65° for 6 min and removing an inert precipitate by centrifugation.

Properties of the nucleoside triphosphate–AMP kinase

Stability. Fractions B and C (Table II), when tested with the combinations ITP–AMP and UTP–AMP, showed no loss of activity after being stored for three years at —15°. Material that had been heated (Fraction D) lost one-third of its activity after several weeks of storage; for this reason the heat step was carried out

* A similar heat step was used by GIBSON, AYENGAR AND SANADI[15] to remove IDPase.

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on 0.1 ml or less of enzyme solution, as it was needed. Fraction C could be heated to 65° for 10 min without loss of activity, but after 10 min at 80° two-thirds of the kinase was destroyed.

Effect of pH and Mg^{++}. The pH optimum was approximately 7.5, as measured with the standard assay system using ITP and AMP. A broad peak was observed, and the activity at pH 9 and at pH 6 was over half of that measured at pH 7.5. There was an absolute requirement for magnesium ion, and a concentration of from 0.005 *M* to 0.01 *M* appeared to be most favorable.

Specificity. The purified fractions (B, C, D) were active with the following combinations of nucleotides (Table III): ITP–AMP, UTP–AMP, CTP–AMP, GTP–AMP and ATP–AMP. There was no activity when a nucleoside monophosphate other than

TABLE III

SPECIFICITY OF THE AMP-KINASE

Combination	*U/ml enzyme*
ITP–AMP	24
GTP–AMP	17
UTP–AMP	24
CTP–AMP	6

The enzyme was Fraction D and it was assayed by coupling to IDPase where possible (see METHODS). For nucleotide pairs involving cytidine derivatives, the formation of ADP was looked for, using the pyruvic kinase assay[4].

The following combinations were negative (rates less than 1/200 of that for ITP–AMP): ATP–GMP, GTP–IMP, GTP–CMP, GTP–UMP, GTP–GMP, ATP–UMP, ATP–CMP, and ribose-5′-triphosphate[8] –AMP.

TABLE IV

TRANSPHOSPHORYLATION REACTIONS

Expt.	*Nucleotides added*				*Reaction products*					
	μmoles		*μmoles*		*μmoles*		*μmoles*		*μmoles*	
1	UTP	0.7	AMP	0.6	UDP	0.17	ADP	0.14	ATP	0.03
2	ITP	1.0	AMP	0.6	IDP	0.36	ADP	0.20	ATP	0.10
3	CTP	0.8	AMP	0.6	CDP	0.12	ADP	0.14	ATP	0.04
4	UDP	2.0	ADP	1.0	UTP	0.20	AMP	0.40	ATP	0.16
5	IDP	0.8	ADP	0.9	ITP	0.23	AMP	0.50	ATP	0.20
6	GDP	0.7	ADP	0.9	GTP	0.20	AMP	0.50	ATP	0.20
7	GTP	0.7	AMP	0.6	GDP	0.18	ADP	0.15	ATP	0.06
8	IDP	1.3	ADP	0.28	ITP	0.20	AMP	0.20	ATP	0.02
9	UDP	2.0	ADP	0.28	UTP	0.20	AMP	0.20	ATP	0.03

The reaction mixture (0.1 ml), contained 0.03 mg crystalline bovine serum albumin, 1.65 μmoles of GSH, 0.01 μmole of versene, 1.5 μmoles of veronal–acetate buffer, pH 7.0[12], 0.9 μmole of $MgCl_2$, nucleotides, and from 0.1 to 0.4 unit of nucleoside triphosphate–AMP kinase. In a control incubation the enzyme was omitted. After 1 h at 37° the reaction mixture was chromatographed in solvent 1, using Whatman No. 1 paper. Development for 48 h was required to obtain adequate separation. With cytidine derivatives, the nucleotide zones were eluted and rechromatographed in solvent 2. Nucleotides were determined spectrophotometrically after elution from paper. No reaction was observed with the pairs ATP–CMP, ATP–GMP, ATP–UMP and ATP–IMP. In solvent 1, the following values were noted for the ratio of R_P of the nucleotide to that of AMP: ITP, GTP, UTP, 0.34; IDP, GDP, UDP, 0.44; IMP, GMP, UMP, 0.58; CTP, 0.51; CDP, ATP, 0.65; CMP, ADP, 0.80.

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AMP was paired with any nucleoside triphosphate. These results were obtained, where possible, by means of assays which involved coupling to IDPase. The results were confirmed by paper chromatography of the reaction mixtures in solvent 1 (Table IV). Because this solvent system does not resolve the pairs CMP–ADP and CTP–ADP, appropriate regions of the paper were eluted and rechromatographed in solvent 2. In considering the stoichiometric relationships for experiments 1, 2, 3 and 7 (Table IV) one must remember that the primary reaction forming ADP and some other nucleoside diphosphate is followed by reaction (5), which proceeds to a measurable extent in the reverse direction to form ATP and AMP.

Another assay procedure depended on measurement of ADP using pyruvic kinase, phosphopyruvate, lactic dehydrogenase and DPNH (see preceding paper[4] for details and discussion). In this way it was shown that 0.15 μmole, or more, of ADP was formed from the combinations ATP–AMP, ITP–AMP, UTP–AMP and CTP–AMP. Incubation conditions were as described in Table IV. There was no significant formation of ADP with the pairs ATP–CMP or ATP–IMP. In addition to all of the quantitative observations listed above, qualitative evidence for reactions involving the pairs ATP–AMP, ITP–AMP, GTP–AMP and UTP–AMP was obtained by paper electrophoresis.

In several experiments CTP labeled with ^{32}P in the stable phosphate group* was incubated with AMP and heated enzyme (Fraction D), under conditions similar to those described in Table IV. The reaction products were separated in solvent 3 and located by radioautography. A semiquantitative estimation of the amounts of CTP, CDP and CMP was obtained by means of a Geiger tube placed over the radioactive spot. Counts in the CTP and CDP areas were: at 0 time, 820 and 80; after incubation, 400 and 480. Thus, over half of the CTP was converted to CDP. Only a trace of radioactivity was found in the region corresponding to CMP.

Reversibility. The reversibility of reactions (1)–(4) has been tested with the following results: (1) After incubation of GDP and ADP with enzyme, the mixture was resolved in solvent 1; the products were AMP, ATP and GTP (Table IV). With a three-fold excess of GDP over ADP one could suppress reaction (5), the adenylate kinase reaction, so that only a faint density for ATP appeared and ADP was nearly completely utilized with the formation of AMP and GTP. (2) Similar results were obtained from incubation of the pairs UDP–ADP and IDP–ADP (Table IV). In other experiments these results were confirmed by separating the reaction products using paper electrophoresis at pH 4.6, 3.5 or 2.0. Again, the occurrence of ATP in addition to the expected products from the reverse of reactions (1) and (2) was due to the adenylate kinase reaction (5), and this could be greatly suppressed by using an excess of UDP or IDP over ADP (experiments 8 and 9, Table IV).

Absence of nucleoside diphosphokinase in purified nucleoside triphosphate–AMP kinase preparation. Incubations were carried out for 0, 45 and 90 min at 37° with ATP and UDP or with UTP and AMP as substrates. The incubation mixtures were then subjected to paper electrophoresis in 0.05 *M* formate buffer, pH 3.4, for 120 min at 20 V/cm**. With UTP and AMP the appearance of both ATP and ADP was maximal

* Prepared by an unpublished method by Dr. JERARD HURWITZ. We are very grateful to him for supplying this substrate.

** Mobilities for the various nucleotides were as follows: UTP, 41 cm; UDP, 39 cm; ATP, 29 cm; ADP, 26 cm; AMP, 13 cm.

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at 45 min, indicating that equilibrium had already been reached at that time. With ATP and UDP no reaction was observed*, even after 90 min of incubation. These data indicate that the UTP–AMP kinase reaction was occurring at a rapid rate under conditions where no nucleoside diphosphokinase reaction (ATP–UDP) could be detected.

Coupled dephosphorylation of nucleoside triphosphate. Fig. 1 is an example of a dephosphorylation resulting from the coupling of nucleoside triphosphate–AMP kinase to IDPase (equation 8). This is a major mechanism for the dephosphorylation of nucleoside triphosphates by extracts of calf liver acetone powder, as was brought out in a preliminary communication[1]. Thus, formation of P_i from UTP by such extracts was very low, but was greatly stimulated by addition of AMP. Also, what appeared to be a large loss in "UTPase" activity was encountered in the first fractionation steps, but this apparent loss could be restored by combining a fraction high in UDPase (but low in nucleoside triphosphate–AMP kinase) with a fraction high in kinase (but low in UDPase).

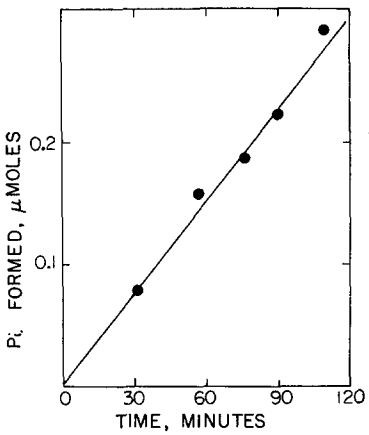

Fig. 1. Time course of inorganic phosphate formation in a coupled reaction with purified nucleosidetriphosphate–AMP kinase and excess IDPase (2 units). The reactants were 1 μmole of ITP and 0.5 μmole of AMP, and the resultant IDP was hydrolyzed to give phosphate. The incubation mixture (0.08 ml) also contained 1.2 μmole of veronal–acetate buffer pH 7.0, 1 μmole of GSH and 0.01 μmole of EDTA. Control mixtures were prepared omitting: (a) AMP; (b) ITP; (c) kinase enzyme; (d) IDPase. None of these showed progressive liberation of P_i with time.

DISCUSSION

The data presented here, taken in conjunction with results presented in the accompanying paper[4], demonstrate that two separable nucleoside monophosphate kinases occur in calf liver acetone powder extracts. One of these, called the ATP–nucleoside monophosphate kinase, reacts with ATP and any nucleoside monophosphate other than IMP. The second fraction, the nucleoside triphosphate–AMP kinase, reacts with AMP and any nucleoside triphosphate. Similar transphosphorylation reactions have been found by other investigators[15–17], but a separation of this kind has not been described. It is not known whether or not the adenylate kinase activity[18] represents another enzyme distinct from these kinases.

The ATP–nucleoside monophosphate kinase was unstable to storage at —15°, especially when present as an ammonium sulfate fraction. By contrast, the nucleoside triphosphate–AMP kinase remained fully active after being kept at —15° for three years. At an acid pH, the nucleoside triphosphate–AMP kinase is precipitated by

* Here, not only ADP and UTP, but also AMP, were looked for. Had ADP been formed by a nucleoside diphosphokinase, it would rapidly form AMP by the adenylate kinase reaction. AMP is especially well separated from the other compounds and its absence from electrophoresis strips was the best evidence that nucleoside diphosphokinase was absent.

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much less ammonium sulfate than is required for the ATP–nucleoside monophosphate kinase. Thus far, the nucleoside triphosphate–AMP kinase has not been further separated into a family of enzymes with similar properties.

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ALTERATIONS IN NUCLEIC ACID TURNOVERS IN SUBCELLULAR COMPONENTS DURING TRYPTOPHAN PEROXIDASE INDUCTION

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SUMMARY

Parenteral tryptophan administration to the rat leads to tryptophan peroxidase induction and to acceleration in the turnover rates of the RNA of the various liver subcellular fractions. The kinetics of these processes indicate that the stimulation in RNA turnover does not precede or accompany but, rather, is subsequent to the period of active enzyme protein synthesis. The maximum percentage increases in RNA turnover rates are: mitochondria and microsomal RNA, 260 %; soluble RNA, 154 %; nuclear RNA, 60 %. However, due to the markedly different basal turnover rates of RNA in the various subcellular organelles, the absolute increase in total amount of RNA synthesized as a result of enzyme induction are in the order: nuclear > soluble = microsomal > mitochondrial. The kinetic data are compatible with the hypothesis that enzyme induction results in extra nuclear RNA utilization with subsequent resynthesis largely in the nucleus.

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