Some Properties of Adenosine Kinase from Ehrlich Ascites-Tumour Cells

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1. Adenosine kinase was measured in dialysed extracts from Ehrlich ascites-tumour cells by a chromatographic procedure. 2. In the absence of added Mg^{2+} the K_m values for ATP and adenosine were 0.22 mM and 2.8 μM respectively. 3. The maximum velocity of adenosine kinase with free ATP was about three times that with the Mg^{2+} -ATP complex. Free Mg^{2+} was a non-competitive inhibitor of the reaction. A small amount of added Mg^{2+} , Mn^{2+} or Ca^{2+} was required for maximum adenosine kinase activity after cation bound to the enzyme had been released by treatment with *p*-chloromercuribenzoate and then removed by dialysis. 4. GTP, ITP, deoxy-ATP, deoxy-GTP, CTP, xanthosine triphosphate, UTP and thymidine triphosphate could partially or completely replace ATP as a phosphate donor. 5. The reaction of ATP with adenosine kinase was competitively inhibited by AMP, GMP, IMP, ADP, deoxy-ADP and IDP (K_i 0.2, 1.1, 5.9, 1.2, 0.5 and 0.78 mM respectively). Enzymic activity was markedly affected by the relative concentrations of AMP, ADP and ATP in assay mixtures. 6. The results are discussed in terms of possible mechanisms regulating the rate of adenosine kinase *in vivo*.

Adenosine kinase (ATP-adenosine 5'-phosphotransferase, EC 2.7.1.20) has been shown to be present in extracts from yeast (Cuputto, 1951; Kornberg & Pricer, 1951; Kornberg, 1955) and mammalian tissues (Franz, Franz & Decker, 1962; Schnebli, Hill & Bennett, 1967). Caldwell, Henderson & Paterson (1966) have reported evidence that adenosine kinase catalyses phosphorylation of 6-methylthioinosine in Ehrlich ascites-tumour cells. Schnebli *et al.* (1967) purified the enzyme 175-fold from a line of human tumour cells in culture and showed that it could catalyse the phosphorylation of a wide range of analogues of adenosine. Unlike most kinases the enzyme did not appear to require the addition of Mg^{2+} for the phosphorylation of adenosine. The present paper describes some of the properties of adenosine kinase present in extracts from Ehrlich ascites-tumour cells. Although the extracts used contained contaminating enzymes, assay conditions could be arranged so that interference with adenosine phosphorylation was small. Crude extracts were used in these experiments as there is evidence that the regulatory properties of some enzymes involved in purine nucleotide metabolism can be altered during purification (see Hartman, 1963; Murray, 1967). From the results obtained it is suggested that the relative concentrations of ATP, Mg^{2+} -ATP complex and free Mg^{2+} , and the relative concentrations

of ATP, ADP and AMP, are important factors regulating the activity of this enzyme.

EXPERIMENTAL

Substrates and inhibitors. [8- ^{14}C]Adenosine was obtained from The Radiochemical Centre (Amersham, Bucks.). The material contained no contaminants that could be detected after chromatography in isobutyric acid-aq. 0.19 N-NH₃ (661:339, v/v) and scanning in a gas-flow counter (Actigraph III; Nuclear-Chicago Inc., Chicago, Ill., U.S.A.). This solvent separated adenosine (R_F 0.80) and inosine (R_F 0.51).

All nucleotides were obtained from the Sigma Chemical Co. (St Louis, Mo., U.S.A.) and contained no contaminants that could be detected after chromatography in the isobutyric acid-NH₃ system described above or on electrophoresis in 0.05 M-sodium citrate buffer, pH 4.2.

Assays of adenosine kinase activity

Separation of reaction products by chromatography. Extracts were prepared from Ehrlich ascites-tumour cells after 7-10 days of tumour growth as described by Murray (1966) but in 0.05 M-phosphate (K^+), pH 7.0. The extracts were dialysed against 200 vol. of 0.05 M-phosphate (K^+), pH 7.0, at 4° for 18 hr., and the dialysis residue was used as the source of adenosine kinase. Solutions containing 10 μ moles of phosphate (K^+), pH 7.0, and 0.5 μ mole of ATP were mixed with 0.05 ml. of the dialysed extract containing about 0.5 mg. of protein. After 2 min. at 37° a solution of [8- ^{14}C]adenosine (sp. radioactivity 1 $\mu C/\mu$ mole) in 0.05 ml.

was added to give an adenosine concentration of 0.3 mM in a final volume of 0.2 ml. After the required incubation time the reaction was stopped by the addition of 0.01 ml. of 10 N-HCl and the reaction mixture was cooled in ice. Portions (0.05 ml.) were chromatographed on Whatman 3MM paper, together with internal markers of 10 μ g. of adenosine and AMP, in butan-1-ol-acetic acid-water (20:3:7, by vol.) for approx. 4 hr. The areas containing the nucleotide products of the reaction (AMP plus some ADP and ATP) were cut from the dried paper and counted as described by Murray (1966) in a Nuclear-Chicago liquid-scintillation counter (mark I). Quenching corrections were made by the channels-ratio method.

Separation of reaction products by butan-1-ol extraction. The assay described above was generally used during these studies. However, the following assay procedure was used to provide a rapid estimate of adenosine kinase activity in stored extracts.

Assays were carried out as described above but were stopped by the addition of 1 ml. of butan-1-ol saturated with water at room temperature; 0.3 ml. of water was added and the aqueous phase was extracted six times with 1 ml. of water-saturated butan-1-ol (see Ellis & Scholefield, 1962). This procedure removed all of the residual adenosine, and the radioactivity associated with the nucleotide product in the aqueous phase was measured by liquid-scintillation counting. Assays carried out by this method and by the chromatographic separation agreed to within 5%.

Estimation of nucleotide kinases and of ATP-degrading enzymes in ascites-tumour-cell extracts. Routine assays were carried out as described above but omitting adenosine and ATP and including 0.5 μ C of [8-¹⁴C]ATP (0.5 μ mole), 0.5 μ C of [8-¹⁴C]AMP (0.2 μ mole) or 0.5 μ C of [8-¹⁴C]ATP + 0.2 μ mole of IDP. After the required time at 37° the reactions were stopped with 0.01 ml. of 10 N-HCl and 0.025 ml. portions were spotted on to polyethyleneimine paper with internal markers of AMP, ADP and ATP (see Gilliland, Langman & Symons, 1966). The chromatograms were developed in 0.2 M-NH₄HCO₃ (ascending; 1.5 hr.) and the radioactivity associated with AMP, ADP and ATP was measured by liquid-scintillation counting as described above.

Nature of the products of the adenosine kinase reaction. Routine assays of adenosine kinase activity were carried out as described above. The areas corresponding to residual adenosine and to the nucleotide products were cut from the chromatograms and material was eluted from the paper with 0.1 N-HCl. After evaporation to dryness the residues were dissolved in a small volume of 1 N-HCl and hydrolysed for 1 hr. at 100° in a sealed glass tube. After chromatography in 5% (w/v) Na₂HPO₄, areas corresponding to adenine and hypoxanthine were cut from the paper and radioactivity was measured by liquid-scintillation counting. Alternatively, the reaction products were separated by electrophoresis in 0.05 M-sodium citrate buffer, pH 4.2.

RESULTS

Assay of adenosine kinase. Under standard assay conditions the rate of phosphorylation of adenosine was constant for up to 30 min.; a 5 min. incubation time was used as a routine in these studies. Enzyme activity was also proportional to concentration of enzyme with concentrations up to 1 mg. of protein/

assay. A small residual rate of nucleotide formation (less than 5% of the maximum rate) was observed in the absence of added ATP; this was presumably due to traces of ATP present in the dialysed extracts. Added Mg²⁺ was not required for the reaction with dialysed crude extracts (see below).

Analysis of the reaction products (see the Experimental section) showed that approx. 6% of the added adenosine was converted into inosine, indicating some adenosine deaminase activity. However, all of the radioactivity of acid hydrolysates of the nucleotide products was associated with adenine; under the conditions described there was no deamination of AMP or phosphorylation of inosine. The addition of 1 mM-inosine to the assays had no inhibitory effect, so the small amount of inosine formed by deaminase activity would not affect the reaction rate. In addition about 5% of the added adenosine was converted into adenine in the routine assay. The adenine could have arisen by hydrolytic cleavage of adenosine (Wang, 1955a) or by the action of an adenosine phosphorylase (Korn & Buchanan, 1955; Ott & Werkman, 1957). The routine assay procedure separated adenine from nucleotide material and the addition of 0.5 mM-adenine to reaction mixtures only inhibited the rate of the adenosine kinase reaction by 9%.

Under standard assay conditions about 2–6% of the added adenosine was converted into nucleotide, and electrophoretic separation showed that radioactivity was associated with ADP and ATP as well as AMP. The resulting concentrations of ADP and AMP in the assay mixtures were about 2 and 9 μ M respectively. Incubation of [8-¹⁴C]ATP with the enzyme extract showed the presence of an adenosine triphosphatase-type activity. In the presence of 0.5 μ mole of ATP about 0.02 μ mole of ADP was formed in 10 min. and 4 μ moles were formed in 2 min. at 37°. Addition of 1 mM-IDP to a reaction mixture containing [8-¹⁴C]ATP resulted in formation of only 0.4 μ mole of ADP through the action of nucleoside diphosphokinase. As indicated above, the extracts contained a nucleotide kinase activity even in the absence of added Mg²⁺. Assay systems containing 1 mM-[8-¹⁴C]AMP showed considerable incorporation of radioactivity into ADP and ATP after 10 min. incubation at 37°. However, after 2 min. incubation about 5 and 7 μ moles of ATP and ADP were formed respectively from [8-¹⁴C]AMP; these concentrations of nucleotides did not interfere with the adenosine kinase assay (see below). In all assays in which the effects of added nucleotides on the adenosine kinase reaction were studied, incubations were carried out for 2 min. at 37°.

Under standard assay conditions the dialysed extracts catalysed the conversion of between 0.5 and 1.6 μ moles of adenosine into nucleotide/min./mg. of protein. When stored at –15° enzyme

Fig. 1. Variation of adenosine kinase activity with concentration of adenosine. Assays were carried out for 1 min. at 37° with dialysed crude extract from ascites-tumour cells (0.06 mg. of protein; see the Experimental section).

Fig. 2. Effect of ATP concentration on the activity of adenosine kinase in the presence and absence of Mg^{2+} . Assays were carried out as described in the Experimental section in the absence of added Mg^{2+} (○) and in the presence of 1 mM- $MgCl_2$ (●) or 2 mM- $MgCl_2$ (Δ).

activity was stable for at least 4 weeks with repeated thawing and freezing.

Characteristics of the adenosine kinase reaction

Nucleoside triphosphate specificity. Adenosine kinase was found to be relatively non-specific for the triphosphate acting as a phosphate donor. The

following relative rates were found with a range of triphosphates: ATP, 100; GTP, 112; ITP, 94; deoxy-ATP, 78; deoxy-GTP, 69; CTP, 68; xanthosine triphosphate, 65.5; UTP, 57.5; thymidine triphosphate, 29.5. All triphosphates were tested at a final concentration of 2.5 mM in the standard assay system. Schnebli *et al.* (1967) reported that GTP, ITP and deoxy-ATP can replace ATP as a phosphate donor with purified adenosine kinase from human tumour cells.

At a concentration of 2.5 mM, adenylyl methylene diphosphonate (the $\beta\gamma$ -methylene analogue of ATP) and the isomeric $\alpha\beta$ -methylene analogue did not react with adenosine kinase but, in the presence of 0.53 mM-ATP, either of the phosphonate analogues at 2.5 mM inhibited adenosine kinase by about 45%. It has recently been shown that the $\beta\gamma$ -methylene phosphonate analogue of ATP can replace ATP as an allosteric activator of adenylylate deaminase from Ehrlich ascites-tumour cells (Atkinson & Murray, 1967).

Effect of pH. The effect of pH on the reaction rate was determined at eight pH values between 5.9 and 8.0 in 0.05 M-phosphate (K^+). There was a broad pH optimum over the range 6.2–7.3. This is similar to the optimum range reported by Schnebli *et al.* (1967); rates at pH 5.9 and pH 8.0 were 89% and 49% respectively of the maximum rate.

Effect of variation of the substrate concentrations in the presence and absence of Mg^{2+} . In the absence of added Mg^{2+} , plots of rate against the concentration of ATP or adenosine were rectangular hyperbolas (see Figs. 1 and 2). With either substrate the reciprocal of the rate of conversion of adenosine into nucleotide material was a linear function of the reciprocal of the concentration of ATP or adenosine. Values of the Michaelis constant (K_m) were 0.22 mM for ATP and 2.8 μM for adenosine. An ATP concentration of 6.3 mM inhibited the reaction by about 20%; concentrations of adenosine up to 0.6 mM did not inhibit the reaction rate. Kornberg (1955) reported that the rate of phosphorylation of adenosine by ATP with yeast adenosine kinase remained maximal at 0.5 mM-ATP and 0.2 mM-adenosine, and Schnebli *et al.* (1967) reported a K_m of 1.8 μM for adenosine with tumour-cell adenosine kinase. In Fig. 2 the effect of increasing concentrations of ATP in the presence of 1 or 2 mM-magnesium chloride on the rate of adenosine kinase is shown. Initially the rate was much slower in the presence of Mg^{2+} but increased as the concentration of ATP was increased, eventually reaching the same maximum velocity as that obtained in the absence of added Mg^{2+} . These results suggested that a Mg^{2+} -ATP complex was not the most effective substrate for adenosine kinase and that the rate of reaction remained low up to ATP concentrations where all the Mg^{2+} formed a complex and free ATP

Fig. 3. Reaction of Mg^{2+} -ATP with adenosine kinase. Extracts were made from Ehrlich ascites-tumour cells in 0.05 M-*N*-ethylmorpholine buffer (pH 7.5) and routine assays were carried out with this buffer (for details see the text). Assays were carried out in the absence of Mg^{2+} (O), in the presence of equimolar Mg^{2+} and ATP (●) or in the presence of equimolar Mg^{2+} and ATP plus 1 mM- MgCl_2 (Δ).

was available to the enzyme. The results were checked with *N*-ethylmorpholine buffer as this does not form a complex with bivalent metal ions (Morrison, O'Sullivan & Ogston, 1961). Extracts were made from ascites-tumour cells in 0.05 M-*N*-ethylmorpholine (adjusted to pH 7.5 with hydrochloric acid) and dialysed against 200 vol. of the same buffer for 18 hr. at 4°. Assays were carried out as before but with 10 μmoles of *N*-ethylmorpholine (pH 7.5) replacing the phosphate buffer. Fig. 3 shows the results of assays carried out in the absence of added Mg^{2+} , in the presence of equimolar ATP and magnesium chloride or in the presence of equimolar ATP and magnesium chloride plus 1 mM-magnesium chloride. Because of the high stability constant (73 mM^{-1}) for the Mg^{2+} -ATP complex (O'Sullivan & Perrin, 1964), it can be calculated that within experimental error all of the Mg^{2+} and ATP was present as a Mg^{2+} -ATP complex; reaction mixtures with the additional 1 mM-magnesium chloride should therefore contain this concentration of free Mg^{2+} (see also Jackson, 1962; Keech & Barritt, 1967). The results clearly indicate that Mg^{2+} -ATP is a poorer substrate than free ATP, and also suggest that free Mg^{2+} is a non-competitive inhibitor of adenosine kinase. Similar results were obtained with the *N*-ethylmorpholine salt of ATP, prepared as described by Keech & Barritt (1967), or when the experiments were carried out in phosphate buffer. Although the stability constant for magnesium hydrogen phosphate is low relative to that for Mg^{2+} -ATP (approx. 0.17 mM^{-1} ; see Martell, 1964), the presence of phosphate in the assay mixtures would be expected to have some effect on the concentration of Mg^{2+} -ATP present.

Fig. 4. Effect of free Mg^{2+} on the reaction of adenosine kinase. Routine assays were carried out (see the Experimental section) in the presence of 0.375 mM-ATP and 0.375 mM- MgCl_2 and with increasing concentrations of additional MgCl_2 .

However, the close correspondence of the results of assays containing *N*-ethylmorpholine or phosphate suggested that interference due to formation of a Mg^{2+} -phosphate complex was negligible. The inhibitory effect of free Mg^{2+} was further shown in experiments in which increasing concentrations of magnesium chloride were added to assays containing 0.37 mM-ATP and magnesium chloride (see Fig. 4). The inhibition obtained with 5 mM-magnesium chloride was not overcome by increasing the concentrations of ATP and Mg^{2+} to 1.7 mM, indicating that the inhibition is not competitive in nature.

As mentioned above, the addition of further Mg^{2+} to assays with dialysed extract inhibited the adenosine kinase reaction. However, the addition of 1 mM-EDTA to assays resulted in 80–90% inhibition of the enzyme activity and this activity could be partially restored by the addition of 1 mM-magnesium chloride, 1 mM-manganese chloride or 1 mM-calcium chloride. Dialysed extract was further dialysed for 18 hr. at 4° against 1000 vol. of 0.05 M-phosphate (K^+), pH 7.0, and found to retain 71% of its activity in the absence of added Mg^{2+} ; the activity was completely restored by 0.1 mM-magnesium chloride, higher concentrations of Mg^{2+} being inhibitory. These results suggested that a small amount of bound bivalent cation may be required for the adenosine kinase reaction. *p*-Chloromercuribenzoate strongly inhibited the enzyme (see below), and to test the possibility that binding of the thiol reagent at or near the active

centre would release any bound cation from the enzyme surface a small portion (1ml.) of dialysed extract was made 0.1mM with respect to *p*-chloromercuribenzoate and then dialysed against 1l. of 0.05M-phosphate (K^+), pH 7.0, for 18hr. This extract contained no adenosine kinase activity, and only 20% of the original activity was restored in the presence of 0.5mM-dithiothreitol or 0.1mM-magnesium chloride. However, the addition of both 0.5mM-dithiothreitol and 0.1mM-magnesium chloride to assays completely restored the original adenosine kinase activity; higher concentrations of Mg^{2+} were inhibitory. The addition of 0.1mM-manganese chloride or 0.1mM-calcium chloride together with 0.5mM-dithiothreitol gave 86% and 80% respectively of the rate obtained with 0.1mM-magnesium chloride and 0.5mM-dithiothreitol. Thus it seems that cation bound to the enzyme is released by treatment with *p*-chloromercuribenzoate and can then be removed by dialysis. The resulting enzyme is essentially inactive even after removal of *p*-chloromercuribenzoate with dithiothreitol and requires the addition of low concentrations of cation for maximum adenosine kinase activity. The purified adenosine kinase obtained by Schnebli *et al.* (1967) did not require added Mg^{2+} but had been exposed to solutions containing Mg^{2+} during the purification procedure. As suggested by the authors it is likely that this enzyme also contained bound Mg^{2+} or other bivalent cation.

Inhibition of adenosine kinase. AMP, GMP and IMP were found to compete with ATP in the reaction catalysed by adenosine kinase. Values of the inhibitor constant (K_i) were 0.20, 1.1 and 5.9mM for AMP, GMP and IMP respectively. In the presence of 0.5mM-ATP, 1mM-thymidine monophosphate inhibited the reaction by 25% and both deoxy-AMP and deoxy-GMP inhibited by 15%. Under these conditions less than 5% inhibition was obtained with 1mM-3',5'-(cyclic)-AMP, -2'- and -3'-AMP (mixed isomers), -xanthosine monophosphate, -UMP, -CMP, -deoxy-UMP and -deoxy-CMP. In addition, adenosine kinase was found to be weakly inhibited by 6-methylthioinosine 5'-phosphate (prepared as described by Caldwell *et al.* 1966). At ATP concentrations of 0.22 and 1.11mM, 1.66mM-6-methylthioinosine 5'-phosphate inhibited the reaction by 43% and 14% respectively. Several nucleoside diphosphates were also found to inhibit adenosine kinase from ascites-tumour cells. ADP, deoxy-ADP and IDP were competitive inhibitors with respect to ATP and the values of K_i found were 1.2, 0.5 and 0.78mM respectively. In the presence of 0.5mM-ATP, inhibition by 1mM-GDP, -thymidine diphosphate, -xanthosine diphosphate, -UDP and -CDP was 37, 24, 15, 10 and 4% respectively. The action of nucleotide inhibitors could not be tested in the

presence of Mg^{2+} because of the interference caused by nucleotide kinase and nucleoside diphosphokinase activities in the crude cell extracts (see above).

An experiment was carried out to determine the effect of ATP 'charge' on the rate of adenosine kinase (see Atkinson, 1966; Atkinson & Walton, 1967; Atkinson & Fall, 1967). It was not possible to obtain the correct proportions of ATP, ADP and AMP by equilibration with adenylate kinase because of the interference caused by Mg^{2+} ; concentrations were calculated by using an equilibrium constant of 0.8 (see Atkinson & Walton, 1967) and ATP, ADP and AMP were added to assay mixtures to give an ATP 'charge' of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 [ATP 'charge' is defined as $([ATP] + \frac{1}{2}[ADP])/([ATP] + [ADP] + [AMP])$]. The results of such an experiment are shown in Fig. 5; when establishing the ATP 'charge' the total adenylate nucleotide concentration was kept constant at 1mM. ATP 'charge' has a marked effect on the rate of phosphorylation of adenosine between 0.4 and 0.7; over this range the ratio $[ATP]/([ATP] + [ADP])$ changes from about 0.27 to 1.3.

Adenosine kinase activity was inhibited 78% and 100% by 0.01mM- and 0.1mM-*p*-chloromercuribenzoate respectively. Addition of 1mM-iodoacetate had no effect on the enzyme activity, and

Fig. 5. Effect of variation of the relative concentrations of ATP, ADP and AMP on the activity of adenosine kinase. Concentrations of ATP, ADP and AMP were added to assay mixtures to give the values of ATP 'charge' shown in the Figure [ATP 'charge' is defined as $([ATP] + \frac{1}{2}[ADP])/([ATP] + [ADP] + [AMP])$; for details see the text]. The total adenylate nucleotide concentration was kept constant at 1mM and the systems were at the equilibrium value for adenylate kinase. The graph shows the results of assays carried out in the presence of ATP alone (○), and as the value of the ATP 'charge' is varied (●).

1 mM-iodoacetamide inhibited the reaction by only 17%. Addition of 1 mM-GSH to assays completely overcame the inhibition by 0.01 mM-*p*-chloromercuribenzoate and restored 33% of the activity in the presence of 0.1 mM-*p*-chloromercuribenzoate, but 1 mM-GSH by itself had no effect on the activity of adenosine kinase. The inhibition by both 0.01 mM- and 0.1 mM-*p*-chloromercuribenzoate was completely overcome by 0.5 mM-dithiothreitol. Addition of 0.5 mM-dithiothreitol alone resulted in 72% stimulation of the rate of the adenosine kinase reaction. Thus it appears that adenosine kinase from Ehrlich ascites-tumour cells, in common with other kinases (see Atkinson & Morton, 1960), has sensitive thiol group(s) at or near the active centre.

DISCUSSION

The adenosine kinase from Ehrlich ascites-tumour cells has been shown to function at its maximum rate in the presence of very low concentrations of Mg^{2+} . In this respect the enzyme differs from most other kinases, which usually require considerably higher concentrations of Mg^{2+} for optimum activity (for references see Atkinson & Morton, 1960). It has frequently been proposed that a Mg^{2+} -ATP complex is the true substrate for many kinases and other enzymes requiring ATP and Mg^{2+} , or alternatively that Mg^{2+} attached to the enzyme molecule is required to bind ATP at the active site (Griffiths, Morrison & Ennor, 1957; Jackson & Atkinson, 1966; Keech & Barritt, 1967). Mg^{2+} -ATP is not an efficient substrate for adenosine kinase from ascites-tumour cells and bound cation may be required to bind ATP or to bring the enzyme into a catalytically active state by some other mechanism. The essentially sigmoidal responses to increasing ATP concentration that were found in the presence of Mg^{2+} (see Fig. 2) may represent an important mechanism controlling the activity of adenosine kinase *in vivo*. However, if there was sufficient intracellular Mg^{2+} to form a complex with all of the ATP, the rate of reaction with Mg^{2+} -ATP as phosphate donor would represent the situation *in vivo*; under these conditions adenosine kinase would be strongly inhibited by free Mg^{2+} . Fluctuations in intracellular Mg^{2+} and ATP concentrations resulting in varying concentrations of free Mg^{2+} or ATP would lead to a sensitive regulation of enzyme activity.

The inhibitions of adenosine kinase by nucleoside mono- and di-phosphates would be expected on the basis of results that have been obtained with other kinases. Thus several kinases are inhibited by ADP (Bublitz & Kennedy, 1954; Crane & Sols, 1955; Griffiths *et al.* 1957; Bessman, 1963) and AMP is a competitive inhibitor of flavokinase from yeast (Kearney, 1955) and also inhibits pigeon liver NAD

kinase (Wang, 1955b). These inhibitions and those reported in this study are consistent with the concept of regulation by ATP 'charge' as proposed by Atkinson (1966), Atkinson & Walton (1967) and Atkinson & Fall (1967), which provides for ATP conservation within the cell so that the rate of reactions utilizing ATP is decreased when the ratio of $[ATP]/([AMP] + [ADP])$ is low. Such an effect would be expected for any reaction utilizing ATP that is competitively inhibited by AMP and ADP.

6-Methylthioinosine 5'-phosphate had some inhibitory effect on adenosine kinase from Ehrlich ascites-tumour cells. Evidence has been obtained by other workers that the antitumour agent 6-methylthioinosine is converted into its 5'-phosphoribosyl derivative by adenosine kinase (Bennett, Schnebli, Vail, Allan & Montgomery, 1966; Caldwell *et al.* 1966; Schnebli *et al.* 1967). However, it does not seem likely that the relatively weak inhibition observed could significantly modify the rate of adenosine kinase *in vivo*.

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