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Some additional properties of partially purified mammalian adenosine kinase

The partial purification and some properties of adenosine kinase (ATP:adenosine 5'-phosphotransferase, EC 2.7.1.20) from rabbit liver and from Ehrlich ascites tumor cells have previously been published[1]. Since then SCHNEBLI *et al.*[2] have reported the isolation of a similar enzyme from human tumor cells of the type H.Ep.2.

In addition to the adenosine analogs previously investigated, eleven more analogs have been examined with the adenosine kinase from rabbit liver. Four compounds were found to serve as phosphate acceptors in the kinase reaction. The effect of using Mn^{2+} instead of Mg^{2+} as the divalent cation in the assay has been investigated, and a modified purification procedure has been established.

Table I presents the K_m and V values obtained from the regression lines in the LINEWEAVER–BURK[3] plots of the experimental data with the 4 nucleoside analogs found to be substrates for the adenosine kinase. All investigations were performed with the enzyme obtained from rabbit liver and with the enzyme assay A previously published[1]. Mg^{2+} was used as the cofactor in all assays. The ratio between the total amount of NADH consumed and the amount of formycin (8-aza-9-deaza-adenosine) added to the reaction mixture was found to be about 3, suggesting that this compound is converted to the corresponding triphosphate in the reaction mixture. With the other 3 nucleosides, no definite results with regard to this point were obtained. N^6-Methyl-3'-amino-3'-deoxyadenosine and formycin are relatively good substrates for the kinase. In agreement with previous findings, the K_m value for N^6-methyl-3'-amino-3'-deoxyadenosine is very close to that for 3'-amino-3'-deoxyadenosine, and the K_m value for formycin is about 100 times that for adenosine. In both 3-isoadenosine and 3'-deoxy-3-isoadenosine, the pentose is bound to N^3 instead of to N^9 in the adenine ring. These compounds are substrates for the kinase, although the K_m values are about 2000 times that for adenosine. In contrast to previous results, where the replacement of the 3'-hydroxyl group with a hydrogen atom increased the K_m value by about 200, the K_m values for 3-isoadenosine and 3'-deoxy-3-isoadenosine are very similar. On the other hand, the replacement decreases the V value by about 10. This result might explain why 2'-deoxy-3-isoadenosine is found not to be a substrate for the kinase, since a decrease in the V value by a factor of 20 or more relative to 3-isoadenosine would make it impossible to detect any activity with the assay used here.

The 7 nucleoside analogs found not to be substrates for the kinase were 2-aminopurine riboside; 2',3'-dideoxy-2',3'-didehydroadenosine; 3'-*S*-ethyl-3'-thio-2',3'-dideoxy-xylofuranosyle adenine; 2'-deoxy-3-isoadenosine; 5-amino-imidazole-4-carboxamide ribotide (Sigma); 2',3'-isopropylidine adenosine (Calbiochem); and cytidine (Calbiochem). The finding that cytidine is not phosphorylated in the assay indicates that 3-isoadenosine and 3'-deoxy-3-isoadenosine, in which the pentose is bound to N^3 instead of to N^9 in the adenine ring, were phosphorylated by the adenosine kinase and not by a contaminating cytidine kinase.

HOLMSEN AND ROZENBERG[4] have recently shown that the phosphorylation of adenosine by dialyzed blood platelet lysates is dependent on either Mg^{2+} or Mn^{2+}.

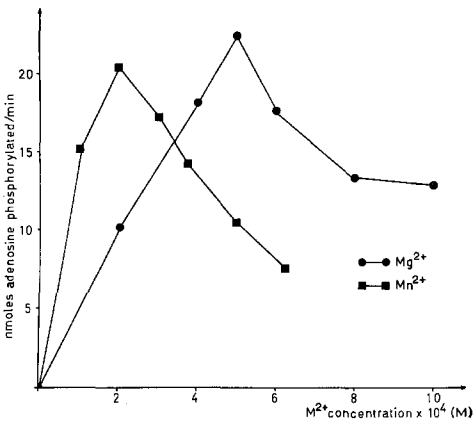

Fig. 1. Effect of Mg^{2+} and Mn^{2+} concentration on the adenosine kinase activity.

With the rabbit liver enzyme, it has been found that except for a slight difference in the optimum concentrations ($5 \cdot 10^{-4}$ M for Mg^{2+} and $2 \cdot 10^{-4}$ M for Mn^{2+}) these ions are equaly potent in stimulating the enzyme activity when enzyme assay A is used. The curve for Mn^{2+} dependency is shown in Fig. 1 with the curve for the Mg^{2+} dependency published earlier. Since adenosine kinase is stored in 1 mM EDTA, the reaction mixture always contains about $5 \cdot 10^{-5}$ M EDTA, and the difference in optimal concentrations might be due only to the fact that Mg^{2+} forms more stable complexes with EDTA than Mn^{2+} does.

A modified purification procedure for the kinase has been worked out. It was possible to obtain further purification by omitting the dialysis step originally included in our purification procedure. Omission of the dialysis results in a specific activity and an enzyme yield 1.8-fold higher than previously obtained. Furthermore, chromatography on DEAE-Sephadex and on hydroxylapatite columns are included in the purification procedure. Chromatographic conditions similar to those used by SCHNEBLI *et al.*[2] in their purification of adenosine kinase from H.Ep.2 cells were employed. The total result of the chromatography on DEAE-Sephadex and on hydroxylapatite columns was a 2.8-fold purification with a 12% yield as compared to the 11-fold purification with a 21% yield obtained by Schnebli and coworkers on the two similar columns. Assuming that the yield after the Sephadex G-100 column is 100%, the inclusion of the chromatography on DEAE-Sephadex and on hydroxylapatite columns will give a total purification of 56-fold with a 12% yield. However, myokinase still contaminates the enzyme, and the enzyme activity deteriorates rapidly; about 50% of the activity was lost during storage 24 h at $-18°$ in a solution containing 20% glycerol.

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TABLE I

SUBSTRATES FOR ADENOSINE KINASE

Nucleoside	K_m *(M)*	*V (nmoles monophosphate per unit of enzyme per min)*
N^6-Methyl-3'-amino-3'-deoxyadenosine	$1.2 \cdot 10^{-4}$	0.07 (0.02)
Formycin (8-aza-9-deaza-adenosine)	$2.2 \cdot 10^{-4}$	0.34
3-Isoadenosine	$8.6 \cdot 10^{-3}$	2.40 (0.80)
3'-Deoxy-3-isoadenosine	$3.0 \cdot 10^{-3}$	0.27 (0.09)

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Purification of bovine renin

The present study is the first to describe the purification of renin (EC 3.4.4.15) from beef kidneys. The methods include many used in early efforts to purify pig renin[1–5], human renin[6–8] as well as those used in later efforts[9–15]. The purified enzyme was characterized by electrophoresis on polyacrylamide gel.

An aqueous (2 l/kg) extract of kidney tissue was purified by a $(NH_4)_2SO_4$ fractionation at 4° as follows: 0.8 M $(NH_4)_2SO_4$ (pH 2.6) for 20 min; pH 3.6 for 16 h; the supernatant to 2.5 M and the resulting precipitate diluted to 1.3 M (pH 6.5); the resulting supernatant to 2.3 M and the final precipitate diluted to 0.8 M before application to the Sephadex G-25 column. Because of the easy measurement, Na^+ from the tissue was used to follow the desalting process. The batch step with CM-Sephadex (C-50), equilibrated with 0.1 M (pH 5.0), entailed centrifugation of the gel after elution and washing with 1.1 M ammonium acetate (pH 6.8). Preparative multiphasic zone electrophoresis on polyacrylamide gels was performed essentially with the apparatus and method described by JOVIN *et al.*[16]. The buffer system was slightly modified as previously

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