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ADENOSINE KINASE FROM HUMAN LIVER

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Adenosine kinase (ATP: adenosine 5′-phosphotransferase, EC 2.7.1.20) has been purified to homogeneity from human liver. The yield was 55% of the initial activity with a final specific activity of 6.3 μmol/min per mg protein. The molecular weight was estimated as about 40 000 by Sephadex G-100 gel filtration and polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS). The enzyme catalyzed the phosphorylation of adenosine, deoxyadenosine, arabinoadenosine, inosine and ribavirin. The activity of deoxyadenosine phosphorylation was 18% of that of adenosine. The pH optimum profile was biphasic; a sharp pH optimum at pH 5.5 and a broad optimum at pH 7.5–8.5. The K_m value for adenosine was 0.15 μM, and the activity was strongly inhibited at higher concentrations than 0.5 μM. ATP, dATP, GTP and dGTP were proved to be effective phosphate donors. Co^{2+} was more effective than Mg^{2+}, and Ca^{2+}, Mn^{2+}, Fe^{2+} and Ni^{2+} showed about 50% of the activity for Mg^{2+}. Some difference in structure between the adenosine kinase from human liver and that from rabbit or rat tissue, was observed by amino acid analysis and peptide mapping analysis.

Introduction

In mammalian cells, adenosine is removed by deamination to inosine or phosphorylation to AMP. Adenosine kinase (ATP: adenosine 5′-phosphotransferase, EC 2.7.1.20) catalyzes the phosphorylation of adenosine to AMP according to the following reaction;

$$\text{adenosine} + \text{ATP} \xrightarrow{Mg^{2+}} \text{AMP} + \text{ADP}$$

The enzyme has a broad substrate specificity [1–5] and exists at relatively high levels in tissue [6–8]. Decreased levels of adenosine kinase have been associated with resistance to the antiproliferative effects of a number of adenosine analogs [9], increased purine excretion [10], and primary gout [11]. The phosphorylation of adenosine and deoxyadenosine, the substrate of adenosine deaminase, is important in adenosine deaminase deficiency associated with immunodeficiency [12]. In this disease, dATP concentration was markedly increased in erythrocytes [13,14]. By the addition of deoxyadenosine to T lymphoblast in the presence of an inhibitor of adenosine deaminase, dATP accumulated in the cell and the levels of dATP correlated well with the cytotoxicity [15,16].

Adenosine kinase was first discovered in yeast [17,18] and mammalian tissue [17]. It has been purified to homogeneity from brewer's yeast [19] and recently from a number of mammalian sources [5,8,20–22] by affinity chromatography on AMP-Sepharose or AMP-agarose. We have obtained apparently homogeneous adenosine kinase from human liver by the same purification procedure as we described in a study on rat brain enzyme [5]. In this paper, we report several properties of human liver adenosine kinase and differences in structure of three enzyme from human, rabbit and rat.

Materials and Methods

Materials. [8-^{14}C]Adenosine was purchased from The Radiochemical Centre Amersham. Nucleosides, nucleotides, phospho*enol*pyruvate, NADH, pyruvate

kinase and lactate dehydrogenase were obtained from Sigma Chemical Co. and Boehringer Mannheim. AMP-Sepharose 4B and Sephadex G-100 were obtained from Pharmacia. *Staphylococcus aureus* V8 protease was purchased from Miles Laboratories. Other reagents were commercial preparations of the highest purity available.

Enzyme assay and protein determination. A standard radiochemical assay was prepared in a final volume of 0.1 ml and contained 64 mM Tris-HCl (pH 7.5)/1 μM [8-^{14}C]adenosine (59 Ci/mol)/40 mM KCl/1 mM ATP/0.5 mM $MgCl_2$/0.5 mM phospho*enol*-pyruvate/5 units/ml pyruvate kinase and appropriate amounts of adenosine kinase. After incubation of reaction mixtures for 2 min at 30°C, the reaction was initiated by addition of [8-^{14}C]adenosine. Reaction mixtures were incubated for 5 min at 30°C and then in a boiling water bath for 1 min. After cooling, 0.05 ml of the reaction mixtures was transferred to DEAE-cellulose (DE-81) discs. The discs were air-dried and washed several times in 2 mM ammonium formate (pH 7.0), to remove unphosphorylated nucleosides, and air-dried. The discs were counted in 5 ml toluene scintillation fluid which consisted of 0.4% 2,5-diphenyloxazole and 0.01% 2,2'-*p*-phenylene-bis(5-phenyl-oxazole), using an Aloka LSC-651 liquid scintillation spectrometer.

The kinase activity for other nucleotides and the activity in the presence of high concentrations of adenosine were assayed by enzymatically coupling the formation of ADP to NADH oxidation. NADH oxidation was measured by following the decrease in absorbance at 340 nm ($\Delta\epsilon = -6\,220\ M^{-1} \cdot cm^{-1}$) minus 400 nm on a Hitachi 356 two-wavelength double-beam spectrophotometer at 30°C. The assay mixtures contained 64 mM Tris-HCl (pH 7.5)/180 mM KCl/1 mM ATP/0.5 mM $MgCl_2$/0.5 mM phospho*enol*pyruvate/0.1 mM NADH/5 units/ml pyruvate kinase/13.8 units/ml lactate dehydrogenase/appropriate nucleoside and the enzyme in a final volume of 1 ml. Reactions were initiated by addition of nucleoside. 1 unit of enzyme activity is defined as the amount catalyzing the phosphorylation of 1 μmol nucleoside for 1 min. Specific activity was defined as units/mg protein. Protein concentration was determined by the method of Lowry et al. [23], using bovine serum albumin as a standard.

Purification of enzyme. Human liver (80 g) obtained at autopsy and stored at −70°C was homogenized and centrifuged at 20 000 × *g* for 30 min. Adenosine kinase was purified to homogeneity from the supernatant by $(NH_4)_2SO_4$ fractionation (45–80%), affinity chromatography on AMP-Sepharose 4B, gel filtration with Sephadex G-100, and DEAE-cellulose (DE-52) column chromatography, as described in a study on rat brain enzyme [5]. The final enzyme preparation was stored at −70°C.

SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis was carried out by the method of Weber and Osborn [24], except for peptide mapping analysis. The samples, prior to electrophoresis, were incubated for 3 min in boiling water in 0.01 M sodium phosphate (pH 7.0)/1% SDS/5% 2-mercaptoethanol. Electrophoresis was performed in 7.5% polyacrylamide gel. Gels were stained in a solution containing final concentration of 0.25% Coomassie brillant blue R/50% methanol/10% acetic acid, and destained by diffusion in a solution of 5% methanol/7% acetic acid. As the protein standards, Pharmacia's electrophoresis calibration kit was used, which covered the molecular weight range 14 400–94 000; phosphorylase *b* (94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), soybean trypsin inhibitor (20 100) and α-lactalbumin (14 400).

Amino acid analysis. Purified enzyme (50 μg each) was hydrolyzed in 0.5 ml 6 N HCl in a vacuum hydrolysis-tube (Pierce Chem. Co., Rockford) at 110°C for 24, 48 and 72 h. The hydrolysate was analyzed with an automatic amino acid analyzer (Hitachi, model 835). Values for threonine and serine were obtained from the samples hydrolyzed for 24 h, and those for valine and isoleucine were from samples hydrolyzed for 72 h. For all other amino acids, values were obtained by averaging the results. Contents of half-cystine and tryptophan were not determined.

Peptide mapping analysis. Peptide mapping by limited proteolysis in SDS and analysis by gel electrophoresis were performed according to the method described by Cleveland et al. [25]. Purified enzymes were dissolved at approx. 0.2 mg/ml in the buffer containing 0.1 M Tris-HCl (pH 6.8)/0.5% SDS/10% glycerol. The samples were then heated at 100°C for 2 min. Proteolytic digestions were carried out at 37°C

for 150 min by addition of 0.5 or 1.0 μg *S. aureus* V8 protease. Following addition of 2-mercaptoethanol and SDS to final concentrations of 10 and 2%, respectively, proteolysis was stopped by boiling the samples for 2 min. About 50 μl (10 μg) of each sample were used for analysis by SDS-polyacrylamide gel electrophoresis. Slab gel electrophoresis was performed in 15% polyacrylamide using the system described by Laemmli [26]. Gels were stained with 0.1% Coomassie blue solution in 50% methanol/10% acetic acid.

Results

Purification of enzyme

The affinity chromatography on AMP-Sepharose 4B, was the most effective purification procedure of adenosine kinase [5,8,21,22]. In this step, the specific activity was increased about 120-fold over $(NH_4)_2SO_4$ fraction. Most of proteins in the $(NH_4)_2SO_4$ fraction passed through AMP-Sepharose 4B, but the enzyme activity was adsorbed. After the elution of the enzyme with 5 mM adenosine the column was further eluted with 2 M KCl. Some proteins were eluted in this fraction but no kinase activity was detected.

The stepwise purification of adenosine kinase is summarized in Table I. The specific activity of the final preparation is about 6.3 μmol/min per protein and represents a 3 300-fold purification over the original supernatant and a recovery of 55% of the initial activity. The final preparation was tested for purity and appeared to be homogeneous, since only a single protein band was observed by polyacrylamide gel electrophoresis in the presence of SDS.

Molecular weight determination

When the native enzyme preparation was applied to a column of Sephadex G-100, enzyme activities toward adenosine and deoxyadenosine were eluted from the column as a single peak and a molecular weight of 38 000 was estimated. The molecular weight of the enzyme was also determined by electrophoresis in 7.5% polyacrylamide gel carried out in the presence of 0.1% SDS. The enzyme migrated as a single electrophoresis species and a molecular weight of 41 000 was obtained. These results indicate that adenosine kinase has monomeric structure.

Enzyme properties

The ratios of the ability to phosphorylate deoxyadenosine to adenosine remain constant at a value of around 0.18 after the AMP-Sepharose step (Table I). When the final preparation was assayed for the phosphorylation of other nucleosides, arabinoadenosine, inosine and ribavirin were also phosphorylated (Table II). The maximum activities of arabinoadenosine, inosine and ribavirin phosphorylations were 7, 9 and 49%, respectively, of adenosine phosphorylation. Guanosine, deoxyguanosine, cytidine, deoxycytidine, uridine and deoxythymidine were not phosphorylated.

Effects of pH on the phosphorylation of adenosine

TABLE I

PURIFICATION OF HUMAN LIVER ADENOSINE KINASE

1 unit equals 1 μmol [8-^{14}C]AMP formed/min under standard assay conditions. The ratio of deoxyadenosine phosphorylation to adenosine phosphorylation was calculated from the respective maximum activities.

Fraction	Total volume (ml)	Total activity (units)	Total protein (mg)	Specific activity (units/mg protein)	Deoxyadenosine /adenosine
Crude extract	250	9.68	5 220	0.0019	
$(NH_4)_2SO_4$ (45–80%)	50	10.26	1 975	0.0052	0.16
AMP-Sepharose 4B	5	9.59	15.67	0.612	0.18
Sephadex G-100	30	7;16	3.06	2.339	0.18
DE-52 cellulose	15	5.31	0.84	6.321	0.18

TABLE II

SUBSTRATE SPECIFICITY

The enzyme activity was assayed at 1 mM ATP and 0.5 mM $MgCl_2$ by enzymatically coupling the formation of ADP to NADH oxidation, except at 0.0005 mM adenosine which was assayed radiochemically.

Substrate	Concentration	Relative activity
Adenosine	0.0005 * mM	100
	0.025	30
	1.0	13
Deoxyadenosine	0.025	2
	1.0 *	18
Arabinoadenosine	0.025	1
	1.0 *	7
Inosine	0.025	1
	1.0 *	9
Guanosine	1.0	0
Deoxyguanosine	1.0	0
Cytidine	1.0	0
Deoxycytidine	1.0	0
Uridine	1.0	0
Deoxythymidine	1.0	0
Ribavirin	0.025	1
	1.0	14
	10.0 *	49

* Optimal concentration to obtain the maximum activity.

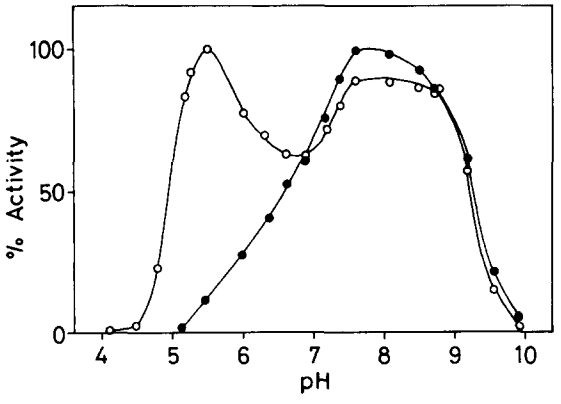
and deoxyadenosine are shown in Fig. 1. When optimal concentration of adenosine was used to obtain maximum activity, the pH optimum profile was biphasic; a sharp pH optimum at pH 5.5 and a broad pH optimum at pH 7.5–8.5. The pH optimum profile, using deoxyadenosine as a substrate, was monophasic with a broad peak at pH 7.5–8.5.

Effects of adenosine, deoxyadenosine and ribavirin concentrations on the kinase activity are shown in Fig. 2. An apparent K_m value for adenosine at pH 7.5 was 0.15 μM and the maximum activity was observed at 0.5 μM. At higher concentrations of adenosine, the activity was strongly inhibited; 50 and 80% inhibition at 5 μM and 50 μM adenosine, respectively. Apparent K_m values for deoxyadenosine and ribavirin at pH 7.5 were 0.48 and 3.7 mM, respectively, and the substrate inhibition was not observed.

Effects of ATP and Mg^{2+} concentrations on enzyme activity are shown in Fig. 3. Palella et al. [27] had reported that $MgATP^{2-}$ was the true substrate of adenosine kinase. Effects of $MgATP^{2-}$ concentration on the enzyme activity, using adenosine as a substrate, were studied under the conditions of fixed ATP (1 mM) or $MgCl_2$ (0.5 mM) concentration and of the constant ratios of ATP/Mg^{2+}. $MgATP^{2-}$ concentrations were calculated from the pH-dependent equilibrium,

$$MgATP^{2-} \overset{K}{\rightleftharpoons} Mg^{2+} + ATP^{4-} \quad (K = 0.0143 \text{ mM}) .$$

Free Mg^{2+} strongly inhibited the activity of adenosine kinase. When the ratio of ATP/Mg^{2+} was equal to 1 or 2, an apparent K_m value for $MgATP^{2-}$ was 73 μM.



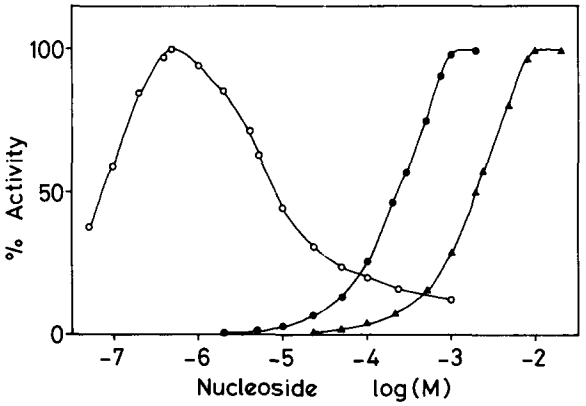


Fig. 1. Effects of pH on the phosphorylation of adenosine and deoxyadenosine. Acetate buffers at pH 4.1–5.2, cacodylate buffers at pH 5.3–7.2, Tris-HCl buffers at pH 7.4–8.8, and carbonate-bicarbonate buffers at pH 8.9–9.9 were used. o———o, 1 μM adenosine; •———•, 1 mM deoxyadenosine.

Fig. 2. Effects of adenosine, deoxyadenosine and ribavirin concentrations on the kinase activity. o———o, adenosine; •———•, deoxyadenosine; ▲———▲, ribavirin.

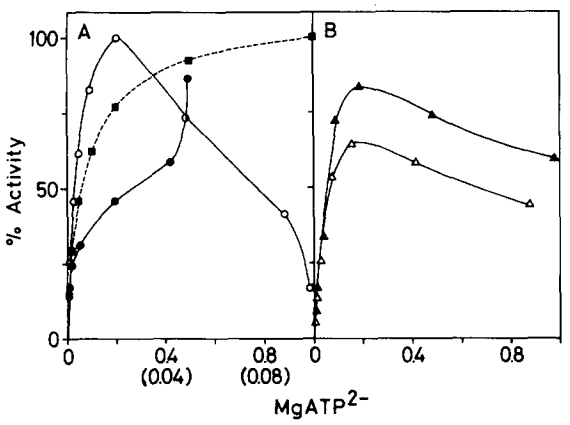


Fig. 3. Effects of ATP and Mg^{2+} concentrations on enzyme activity. A: Effects of $MgATP^{2-}$ concentration when $MgCl_2$ or ATP concentration was fixed ○———○, $MgCl_2$ concentration ranging from 0 to 2 mM at 1 mM ATP, using 1 μM adenosine as a substrate; ●———●, ATP concentration ranging from 0 to 2 mM at 0.5 mM $MgCl_2$, using 1 μM adenosine as a substrate; ■———■, ATP concentration ranging from 0 to 0.1 mM at 0.5 mM $MgCl_2$, using 1 mM deoxyadenosine as a substrate. Concentration of $MgATP^{2-}$ were shown in parentheses. B: Effects of $MgATP^{2-}$ when the ratio of ATP/Mg^{2+} was equal to 1 (△———△), or equal to 2 (▲———▲), using 1 μM adenosine as a substrate.

Using deoxyadenosine as a substrate, an apparent K_m value for $MgATP^{2-}$ was 6 μM under the condition of fixed $MgCl_2$ concentration (0.5 mM), since free Mg^{2+} did not inhibit the activity.

Adenosine kinase had a fairly broad specificity for the naturally occurring nucleoside triphosphate. ATP, dATP, GTP and dGTP proved to be effective phosphate donors (Table III). GTP and dGTP were more effective than ATP. In general, the pyrimidine nucleoside triphosphates were less effective than the purine, and the deoxyribonucleotides were less than the ribonucleotides. AraATP was not very effective.

Divalent metal ion requirement is summarized in Table IV. Co^{2+} was more effective than Mg^{2+}. Ca^{2+}, Mn^{2+}, Fe^{2+} and Ni^{2+} were also capable of replacing Mg^{2+} in the reaction mixture but resulted in a 50% reduction in reaction rate. Cr^{2+}, Zn^{2+} and Ba^{2+} were not effective and in the presence of Cu^{2+} the activity was inhibited. In the absence of added metal ion, the reaction proceeded at 22% of the rate observed in the presence of an optimal Mg^{2+} concentration. However, by preincubation of the enzyme preparation with EDTA, the activity in the absence of added metal ion was reduced; 80% reduction at 0.5 mM (Table IV). These results indirectly suggest that the adenosine kinase from human liver contains a bound metal ion.

The enzyme was stable for at least 1 year when stored at −70°C, and also stable when thawed and refrozen. At 4°C, it was relatively stable in the presence of dithiothreitol or a high concentration of salt; e.g., 0.15 M KCl. Under the condition of low concentration of salt at 4°C, the enzyme was rapidly

TABLE III

NUCLEOSIDE TRIPHOSPHATE DONOR SPECIFICITY

The enzyme activity was assayed at 1 μM [8-^{14}C]adenosine and 0.5 mM $MgCl_2$

Nucleoside triphosphate (1 mM)	Relative activity
ATP	100
dATP	93
araATP	17
GTP	142
dGTP	135
CTP	64
dCTP	13
UTP	55
dTTP	16

TABLE IV

METAL ION REQUIREMENT

The enzyme activity was assayed at 1 μM [8-^{14}C]adenosine and 1 mM ATP. Enzyme was pretreated with EDTA for 5 min at 30°C and 10 μl were used for assay. Reaction was carried out in the absence of added metal ion.

Divalent metal ion (0.05 mM)	Relative activity	EDTA treatment	Relative activity
None	22	None	22
$MgCl_2$	100	EDTA 0.1 mM	14
$CaCl_2$	53	0.5	4
$CrCl_2$	19	2.5	0
$MnCl_2$	59		
$FeSO_4$	69		
$CoCl_2$	144		
$NiCl_2$	68		
$CuSO_4$	0		
$ZnSO_4$	18		
$BaCl_2$	25		

inactivated. But the activity was recovered thoroughly when it was redialyzed against the buffer containing dithiothreitol.

Differences of the liver enzymes from the three species

Only small differences were observed between adenosine kinase from human liver and that from rat brain [5] or liver [21], with respect to molecular weight, kinetic properties and the elution profile from AMP-Sepharose 4B and DE-52 cellulose. In order to examine differences in structure of the three enzymes from human, rabbit and rat liver, obtained from the same purification procedure, amino acid analysis and peptide mapping analysis were carried out. The amino acid compositions of three enzymes are summarized in Table V. The amino acid compositions of three enzymes were alike, and especially those of human and rabbit resemble each other closely. The difference between human and rabbit was observed only in the number of proline residues, 6 and 9, respectively. The difference between rat and others was observed in the number of proline, valine, isoleucine, lysine and histidine residues.

TABLE V

AMINO ACID COMPOSITION OF ADENOSINE KINASE FROM LIVER

The values (number/mol) were calculated from a molecular weight of about 40 000 by averaging two series of experiments. Contents of half-cystine and tryptophan were not determined.

Amino acid	Human	Rabbit	Rat
Aspartic acid	32	31	35
Threonine	16	16	18
Serine	18	18	17
Glutamic acid	37	39	35
Proline	6	9	12
Glycine	20	20	21
Alanine	29	28	29
Valine	16	16	19
Methionine	6	5	5
Isoleucine	19	18	14
Leucine	21	20	21
Tyrosine	7	7	7
Phenylalanine	18	18	19
Lysine	26	26	22
Histidine	8	7	5
Arginine	10	8	11

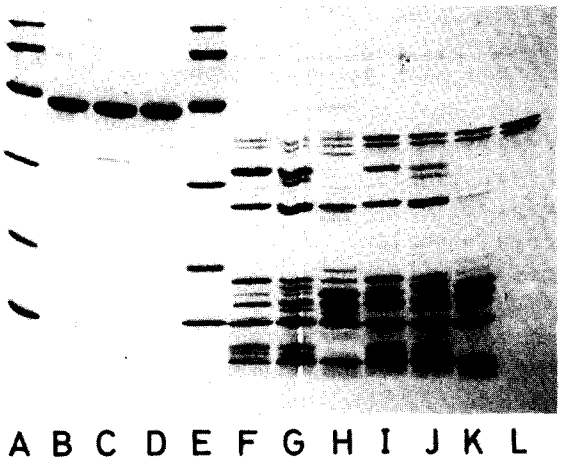


Fig. 4. Peptide maps of liver adenosine kinase from human, rabbit and rat. A and E, standard proteins described under Materials and Methods; B, C, and D, undigested liver adenosine kinase from human, rabbit, and rat; F, G, and H, digestions of human, rabbit, and rat liver enzyme with 0.5 μg *S. aureus* V8 protease; I, J, and K, digestions of human, rabbit, and rat liver enzyme with 1.0 μg of the protease; L, 1.0 μg of the protease.

Peptide mapping of the three enzymes by limited proteolysis in SDS was performed. The enzymes were digested with the *S. aureus* V8 protease, and the results of peptide mapping of three enzymes are shown in Fig. 4. In this analysis, peptide maps of human and rabbit were alike, but some extra bands were observed in the rabbit enzyme. The peptide map of the rat enzyme was apparently different from the other two, but some peptide bands were common to the three enzymes.

Discussion

Adenosine kinase was purified from human liver to a specific activity of 6.3 μmol/min per mg protein. The pure enzyme from rat liver or brain has a specific activity of 7.8 μmol/min per mg protein [5], which is about 20% higher than the human liver enzyme. This difference might be dependent on species. As described in previous studies [5,8,21,22], the affinity chromatography of human liver enzyme on AMP-Sepharose 4B was a very effective purification step, in which the

specific activity increased to about 120-fold. When the affinity chromatography was conducted at an earlier purification step, the enzyme preparation was concentrated and stabilized, and the final yield resulted in 55% of the initial activity.

The purified human liver adenosine kinase is a monomer of molecular weight of about 40 000 from results obtained by Sephadex G-100 gel filtration and SDS-polyacrylamide gel electrophoresis. The value of the molecular weight was similar to that of the enzyme from human placenta [8], rat brain [5] and liver [21], mouse erythrocytes [28] and yeast [19], but different from the value of 51 000 for the rabbit liver [20]. However, the rabbit liver adenosine kinase obtained by our purification procedure, had a molecular weight of about 40 000 by SDS-polyacrylamide gel electrophoresis (Fig. 4).

Adenosine, deoxyadenosine, arabinoadenosine, inosine and ribavirin were phosphorylated by the adenosine kinase (Table II). These results were also similar to results for the enzyme from rat liver and brain [5] or rabbit liver [4], and strongly suggest that adenosine and deoxyadenosine are phosphorylated by the same enzyme. However, in a human thymic-cell extract, adenosine kinase and deoxyadenosine kinase activities were separated on DE-52 cellulose, and their activities were found to vary widely in different tissues [12]. Furthermore, more than 90% of the deoxyadenosine kinase in human placenta did not co-purify with adenosine kinase [8]. Deoxyadenosine is also phosphorylated by deoxycytidine kinase in calf thymus [29]. Kinetic data from wild type and variants of S-49 lymphoma cells are consistent with the existence of two deoxyadenosine phosphorylating activities, one being associated with adenosine kinase and the other with deoxycytidine kinase [15]. Therefore, we are interested in whether or not deoxyadenosine is phosphorylated by two enzymes in human liver as well as in placenta. Unlike human placenta, the ratios of the ability to phosphorylate deoxyadenosine to adenosine remained constant (Table I) during the purification of liver adenosine kinase, and the activity for deoxyadenosine was not separated from that for adenosine. Deoxycytidine kinase is confined primarily to tissues of lymphoid origin, and there is little or no activity in liver, brain and erythrocyte [30]. We propose that all or most of the deoxyadenosine is phosphorylated by adenosine kinase in human liver.

The pH optimum profile was biphasic using adenosine as a substrate and monophasic with deoxyadenosine (Fig. 1). These phenomenon were observed in a previous study on rat brain adenosine kinase, and it was suggested that adenosine kinase had two active sites [5]. The optimal pH reported for the enzyme from a number of sources [1,3,8,20,31] was around pH 5.5, and that for the enzyme from Ehrlich ascites cells was pH 6.2–7.3 [32]. However, the values cannot be simply compared, since the pH optimum is affected by the adenosine concentration [5] and by the ratio of ATP/Mg^{2+} [20]. The adenosine kinase activity is complicated in that it is influenced by pH and by concentrations of nucleosides, ATP and Mg^{2+}. $MgATP^{2-}$ was the true substrate of adenosine kinase [27]. Free Mg^{2+} was a powerful inhibitor of adenosine kinase [1,3,5,19,20,27,32,33], and free ATP^{4-} inhibited the activity of the enzyme from human placenta [27], human erythrocyte [34] and rabbit liver [20]. The activity of human liver adenosine kinase was inhibited by free Mg^{2+}, and the effect of free ATP^{4-} was unclear (Fig. 3). The K_m value of 73 μM for $MgATP^{2-}$ was similar to that of adenosine kinase from human placenta [27]. The requirements for optimal activity are quite different for the phosphorylation of adenosine and deoxyadenosine, as described in a study on the enzymes from rat brain [5] and liver [21].

The phosphate donor specificity of adenosine kinase appears to vary from source to source. ATP, dATP, GTP and dGTP are effective phosphate donors for the human liver enzyme (Table III). For the rat brain enzyme [5] GTP, dGTP and dATP were more effective than ATP, and UTP was as effective as ATP. With both the rabbit liver enzyme [20] and the human erythrocyte enzyme [33] GTP could substitute for ATP with no apparent loss of enzyme efficiency, but dATP and dGTP were not as effective. The yeast enzyme has been reported to use ATP and dATP with equal efficiency [19]. On the other hand, the enzyme from Ehrlich acites cells is relatively nonspecific with regard to the phosphate donor [32].

Effects of divalent metal ions on the activity of adenosine kinase from human liver were similar to that from rat brain [5] and rabbit liver [20]. In the absence of added metal ion, the human liver enzyme

had appreciable catalytic activity (Table III), similar to that for the enzymes from rat brain [5] and rabbit liver [20]. As shown in previous studies [2,5,20,32], it was suggested indirectly that the enzyme from human liver contained a bound metal ion, since treatment with EDTA reduced the enzyme activity.

To structurally compare the adenosine kinases from liver of three species, human, rabbit and rat, amino acid analysis was carried out. As a common feature of three enzymes, the acidic amino acids, aspartic and glutamic acid, were present at higher levels than the basic amino acids, lysine, histidine and arginine (Table V). These data support studies indicating that the isoelectric pH of adenosine kinase is pH 5.9 [8] or pH 5.5 [21]. The difference between rat and others was clear, but that between human and rabbit was unclear. Therefore, peptide maps of the three enzymes were compared. In this analysis, some differences were observed between peptide maps of human adenosine kinase and those of rabbit (Fig. 4). Structural differences exist, therefore, between human and rabbit liver enzyme. We hope to examine whether adenosine kinases from human liver and other tissues are identical or not in future studies.

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