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Cloning of human adenosine kinase cDNA: Sequence similarity to microbial ribokinases and fructokinases

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ABSTRACT **Adenosine kinase catalyzes the phosphorylation of adenosine to AMP and hence is a potentially important regulator of extracellular adenosine concentrations. Despite extensive characterization of the kinetic properties of the enzyme, its primary structure has never been elucidated. Full-length cDNA clones encoding catalytically active adenosine kinase were obtained from lymphocyte, placental, and liver cDNA libraries. Corresponding mRNA species of 1.3 and 1.8 kb were noted on Northern blots of all tissues examined and were attributable to alternative polyadenylylation sites at the 3′ end of the gene. The encoding protein consists of 345 amino acids with a calculated molecular size of 38.7 kDa and does not contain any sequence similarities to other well-characterized mammalian nucleoside kinases, setting it apart from this family of structurally and functionally related proteins. In contrast, two regions were identified with significant sequence identity to microbial ribokinase and fructokinases and a bacterial inosine/guanosine kinase. Thus, adenosine kinase is a structurally distinct mammalian nucleoside kinase that appears to be akin to sugar kinases of microbial origin.**

Adenosine kinase (AK; ATP:adenosine 5′-phosphotransferase, EC 2.7.1.20) is an abundant enzyme in mammalian tissues that catalyzes the transfer of the γ-phosphate from ATP to adenosine, thereby serving as a potentially important regulator of concentrations of both extracellular adenosine and intracellular adenine nucleotides. Adenosine has widespread effects on the cardiovascular, nervous, respiratory, and immune systems (1) and it has been postulated that inhibitors of AK could play an important pharmacologic role in increasing intravascular adenosine concentrations and acting as anti-inflammatory agents (2, 3). In addition, this enzyme is responsible for the phosphorylation and consequent clinical activity of several therapeutically useful nucleosides, including the antiviral drug ribavirin and the immunosuppressive drug mizoribine (4, 5). AK has been purified from a number of sources including rabbit liver (6), human placenta (7) and liver (5), murine leukemia cells (8), and *Leischmania donovani* (9) and extensively characterized at the kinetic level. To extend our knowledge of this important protein to the structural level, we have cloned and expressed the human adenosine kinase cDNA.

MATERIALS AND METHODS

Cell Lines. Wild-type and AK-deficient lymphoblastoid cell lines CEM and WI L2 were obtained from Buddy Ullman (Oregon Health Sciences University, Portland) and Michael S. Hershfield (Duke University, Durham, NC) (10). Cells were grown in RPMI 1640 medium/10% fetal calf serum and total RNA was purified by the acid guanidine isothiocyanate procedure (11).

AK Purification. AK was purified from the human T-lymphoblast cell line Molt-4 by a modification of the procedure previously described (7). Cytosol was passed over a 5′-AMP-Sepharose column equilibrated with 20 mM imidazole·HCl, pH 7.6, containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM EGTA. The column was eluted with buffer containing 5 mM adenosine and the peak fractions were pooled in 20% (vol/vol) glycerol. Several 2-ml fractions were loaded onto a Superose 12 column (50 × 1.6 cm) previously equilibrated with 50 mM imidazole·HCl, pH 7.2/50 mM KCl/5 mM dithiothreitol (DTT)/1 mM ATP/1 mM $MgCl_2$/5% glycerol. Fractions containing AK activity, assayed as described (7), were pooled, diluted into buffer containing 1 mM PMSF, and applied to a DE52 column. Fractions were eluted with a linear gradient of 0–200 mM KCl and peak fractions were pooled in 20% glycerol for SDS/PAGE and amino acid analysis.

Peptide Sequencing. Amino acid sequence analysis of the purified protein was performed after cyanogen bromide cleavage by Bill Henzel and Byron Nevins (Genetech). Peptides were separated by reverse-phase HPLC and amino acid sequence analysis was performed by gas-phase sequencing. The cleavage mixture was also run on SDS/polyacrylamide gels and three major bands were obtained. Bands were electroblotted and sequenced.

Isolation and Characterization of cDNA Clones. A mixture of degenerate ^{32}P-labeled oligonucleotide probes encoding a 15-amino acid sequence from the CN3 peptide (VKRQRIVIFTQRDDT) was used to screen a Molt-4 cDNA library in λgt11 (12). Hybridizations were carried out at 42°C in 5× SSC/10 mM NaH_2PO_4/2.5 mM EDTA/1× Denhardt's solution containing 150 μg of herring testes DNA per ml. Filters were washed at 37°C in 2× SSC/0.1% SDS and autoradiographed. λ phage DNA from positive plaques was purified and the cDNA inserts were subcloned into pGEM-4Z (Promega) for sequencing.

Sequence Analysis. Data were analyzed using PUSTELL IBI and MAC VECTOR version 4.5 software and obtained sequences as well as amino acid sequence predicted by the isolated cDNA were searched against GenBank (release 90.0), Swiss-Prot (release 32.0), PIR (release 45.0), and EMBL (release 40.0) data bases using the Genetics Computer Group package (Madison, WI) and the Entrez (release 15.0) sequence retrieval system (National Center for Biotechnology Information). Similar sequences were retrieved and aligned using the

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Abbreviations: AK, adenosine kinase; DTT, dithiothreitol; IPTG, isopropyl β-D-thiogalactopyranoside.

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BLAST algorithm designed for finding ungapped local alignments (13).

Northern Blot Analysis. A commercial blot (human multitissue blot; Clontech) was used to assess AK mRNA levels in various tissues. Blots were probed with randomly primed, ^{32}P-labeled full-length cDNA using QuickHyb hybridization solution (Stratagene). Five micrograms of total RNA from wild-type and AK-deficient lymphoblast cell lines was run on a 1% agarose/1.1 M formaldehyde gel, transferred to nitrocellulose, and probed with β-actin or AK cDNA probes.

Expression of Recombinant AK in *Escherichia coli*. A 1.8-kb AK clone 911, obtained from the human liver cDNA λ ZAP library, was subcloned into the pET3a vector using the forward PCR primer 5′-AAACATATGACGTCAGTCAGAGAAA-AT-3′ and *Nde* I and *Xho* I restriction sites. The resulting 911/pET3a construct was transformed into *E. coli* host cell BL21[DE3]. Induction was performed for 4–6 hr in the presence of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG).

Purification and Characterization of Recombinant AK Protein. Recombinant AK protein was obtained from the BL21[DE3] cells by lysis of the bacterial pellet in 10 vol of 50 mM Tris·HCl, pH 7.5/1 mM EDTA/1 mM DTT/lysozyme (1 mg/ml) using three freeze–thaw cycles in liquid nitrogen. The lysate was treated with 5 mg of DNase I per ml in the presence of 10 mM $MgSO_4$ for 60 min on ice and centrifuged at 30,000 × *g* for 30 min at 4°C and the protein was precipitated with ammonium sulfate between 70% and 100% saturation. The precipitate was dissolved in 10 mM Tris·HCl, pH 7.5/1 mM DTT, and dialyzed overnight against 1000 vol of the same buffer. The sample was applied to a DEAE-Sephacel column and the bound protein was eluted with a linear gradient of 1–100 mM KCl in the same buffer. AK activity was eluted at ≈40 mM KCl concentration and characterized. Use of AMP-

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1     CGCCTTCCCT CCAATCAGCA CCGGGGCCGG CTAGCCAGGG GCCGGCCGCG CGGGGTGTGT GAGGACGCGC
71    TCCCAGTCGC TGAGTGCCTG AGCCGGGAAG CAGTTGCTGT GGTACCTGCG CTGCCCGAGC GGACGTAGAG
141   CATCGGACGC GGGCGCCGTG GCGTTGGGCA GGAGGCGAAG CCA ATG ACG TCA GTC AGA GAA AAT
                                                  M   T   S   V   R   E   N     7
205   ATT CTC TTT GGA ATG GGA AAT CCT CTG CTT GAC ATC TCT GCT GTA GTG GAC AAA GAT
       I   L   F   G   M   G   N   P   L   L   D   I   S   A   V   V   D   K   D    26
262   TTC CTT GAT AAG TAT TCT CTG AAA CCA AAT GAC CAA ATC TTG GCT GAA GAC AAA CAC
       F   L   D   K   Y   S   L   K   P   N   D   Q   I   L   A   E   D   K   H    45
319   AAG GAA CTG TTT GAT GAA CTT GTG AAA AAA TTC AAA GTC GAA TAT CAT GCT GGT GGC
       K   E   L   F   D   E   L   V   K   K   F   K   V   E   Y   H   A   G   G    64
376   TCT ACC CAG AAT TCA ATT AAA GTG GCT CAG TGG ATG ATT CAA CAG CCA CAC AAA GCA
       S   T   Q   N   S   I   K   V   A   Q   W   M   I   Q   Q   P   H   K   A    83
433   GCA ACA TTT TTT GGA TGC ATT GGG ATA GAT AAA TTT GGG GAG ATC CTG AAG AGA AAA
       A   T   F   F   G   C   I   G   I   D   K   F   G   E   I   L   K   R   K   102
490   GCT GCT GAA GCC CAT GTG GAT GCT CAT TAC TAC GAG CAG AAT GAG CAG CCA ACA GGA
       A   A   E   A   H   V   D   A   H   Y   Y   E   Q   N   E   Q   P   T   G   121
547   ACT TGT GCT GCA TGC ATC ACT GGT GAC AAC AGG TCC CTC ATA GCT AAT CTT GCT GCT
       T   C   A   A   C   I   T   G   D   N   R   S   L   I   A   N   L   A   A   140
604   GCC AAT TGT TAT AAA AAG GAA AAA CAT CTT GAT CTG GAG AAA AAC TGG ATG TTG GTA
       A   N   C   Y   K   K   E   K   H   L   D   L   E   K   N   W   M   L   V   159
661   GAA AAA GCA AGA GTT TGT TAT ATA GCA GGC TTT TTT CTT ACA GTT TCC CCA GAG TCA
       E   K   A   R   V   C   Y   I   A   G   F   F   L   T   V   S   P   E   S   178
718   GTA TTA AAG GTG GCT CAC CAT GCT TCT GAA AAC AAC AGG ATT TTC ACT TTG AAT CTA
       V   L   K   V   A   H   H   A   S   E   N   N   R   I   F   T   L   N   L   197
775   TCT GCA CCG TTT ATT AGC CAG TTC TAC AAG GAA TCA TTG ATG AAA GTT ATG CCT TAT
       S   A   P   F   I   S   Q   F   Y   K   E   S   L   M   K   V   M   P   Y   216
832   GTT GAT ATA CTT TTT GGA AAT GAG ACA GAA GCT GCC ACT TTT GCT AGA GAG CAA GGC
       V   D   I   L   F   G   N   E   T   E   A   A   T   F   A   R   E   Q   G   235
889   TTT GAG ACT AAA GAC ATT AAA GAG ATA GCC AAA AAG ACA CAA GCC CTG CCA AAG ATG
       F   E   T   K   D   I   K   E   I   A   K   K   T   Q   A   L   P   K   M   254
946   AAC TCA AAG AGG CAG CGA ATC GTG ATC TTC ACC CAA GGG AGA GAT GAC ACT ATA ATG
       N   S   K   R   Q   R   I   V   I   F   T   Q   G   R   D   D   T   I   M   273
1003  GCT ACA GAA AGT GAA GTC ACT GCT TTT GCT GTC TTG GAT CAA GAC CAG AAA GAA ATT
       A   T   E   S   E   V   T   A   F   A   V   L   D   Q   D   Q   K   E   I   292
1060  ATT GAT ACC AAT GGA GCT GGA GAT GCA TTT GTT GGA GGT TTT CTG TCT CAA CTG GTC
       I   D   T   N   G   A   G   D   A   F   V   G   G   F   L   S   Q   L   V   311
1117  TCT GAC AAG CCT CTG ACT GAA TGT ATC CGT GCT GGC CAC TAT GCA GCA AGC ATC ATA
       S   D   K   P   L   T   E   C   I   R   A   G   H   Y   A   A   S   I   I   330
1174  ATT AGA CGG ACT GGC TGC ACC TTT CCT GAG AAG CCA GAC TTC CAC TGA TGGAAGAGC
       I   R   R   T   G   C   T   F   P   E   K   P   D   F   H                   345
1231  TGAAAACACA AGCCCAGGAG TGCAGACACT GCCCTAATTG CTTCCTGACA ATTCCCATAT TAATAAAGAA
1301  GAAAATTATC TGCCATTTTT TCCTACTATA ATAATGCTGA ATCTTAATTT AGAGGGTACA AGGGTATGGT
1371  AATGCTTGTA GAATCTTTAT TATCTCAACA ATCTAAAAAA TGATGTTTAT TTCCATAGTT TGATAGTGCC
1441  ACTTAAATGC CAATTAAACA AGAATATAAC ATTTCAATAG AAATTTTTAT TTCATTTTCA ATTACTTTGT
1511  AAATTCGTGT GTATTTAGTA CACTGATTTG TTTTTTACAT TTCTGCTTTG AATGCAGATG CAATTTAATA
1581  TAATAGATTT TTTAATGAAT TAATCTTAAC ATAGTAATCT TTAGCTTTTT ATACAAATAT ATTTAATTTA
1651  GGAGTATATG TGTGTCTATA CACACACATA CATAAATATA CCACATATAC ACCTGATAGT CAAATAAGGT
1721  ACAGAAATTT TATCTTGTCA ATTATGCCAA ATAATCTCTT TAATGTGCAC TCAACATGTA ATAAACTTTG
1791  GATAATTAAA AAAAAAAAA
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FIG. 1. Nucleotide and deduced amino acid sequence of human AK. Amino acid sequences of peptides obtained from the purified protein and alternative polyadenylylation signals (aataaa) at bp 1292 and 1780 are underlined.

Sepharose affinity chromatography instead of DEAE-Sephacel chromatography yielded enzyme with comparable specific activity.

Enzyme Assay and Protein Determination. Standard radiochemical assay was prepared in a final vol of 200 μl, which contained 50 mM Tris malate (pH 7.0), 1.25 mM $MgCl_2$, 0.1% bovine serum albumin, 1.25 mM ATP, 1.2 μM [U-^{14}C]adenosine (0.05 mCi/ml; 1 Ci = 37 GBq) and an appropriate amount of AK. Reaction mixtures were incubated for 10 min at 37°C and then heated at 100°C for 1 min. Aliquots of 30 μl from each tube were transferred to DE-81 discs (Whatman) and the discs were washed several times in 2 mM ammonium formate (pH 7.0) and placed in scintillation vials for counting. One unit of activity was defined as μmol of used substrate per min. Protein concentration was determined by the method of Bradford using IgG as a standard (14).

Materials. Radionuclides were obtained from Amersham. pET3a vector was obtained from Novagen and IPTG and 5-bromo-4-chloro-3-indolyl β-D-galactoside from GIBCO/BRL. Random labeling Prime-A-Gene system and restriction enzymes were from Promega. 5′-AMP-Sepharose was purchased from Sigma. All other materials and reagents were of the highest quality available.

RESULTS

Analysis of the peptides generated by cyanogen bromide cleavage of purified AK revealed the following sequences: CN1, LVEKARVCYIAGFFLTVSPEXVLXVA; CN2, IQQP(A)KA(A)TFFGXIGIDKFG(E)ILKXKAAEXXV(D)AXYYEQN; CN3, X(V)KRQRIVIFTQRDDT(N).

A degenerate oligonucleotide derived from CN3 (residues 2–16) was used to screen a λgt11 Molt-4 cDNA library. A 600-bp cDNA clone was obtained in this initial screen, subcloned into pGEM4Z (Promega), and subsequently used as a probe to obtain a 900-bp cDNA clone that contained nucleotide sequences encoding the amino acids in CN1, CN2, and CN3 peptides. To obtain a full-length clone the 900-bp fragment was used to screen a breast cDNA library constructed in pCDM8 (courtesy of Michael Clark, University of Michigan, Ann Arbor) and a commercial human liver cDNA library constructed in λ ZAP (Stratagene). Two 1.3-kb and one 1.8-kb cDNA clones, all containing poly(A) tails, were obtained and the DNA sequence of each insert was determined for both strands.

The full-length cDNA sequence of human AK, obtained from clone 911 (human liver, 1.8 kb) and encoding all of the peptide sequences identified in the native protein, is shown in Fig. 1. The sequence includes 183 bp of 5′ untranslated sequence, a 1035-bp open reading frame that encodes a protein of 345 amino acids with a compositional molecular mass of 38.7 kDa, and 579 bp of 3′ untranslated sequence with two polyadenylylation signals at bp 1292 and 1780. Plasmids with shorter inserts (clones 711 and 811) each contained a poly(A) tail 3′ to the first polyadenylylation sequence, whereas the insert in clone 911 utilized the second polyadenylylation signal.

The AK cDNA was used to probe a Northern blot containing total cellular RNA from a variety of tissues. As shown in Fig. 2, at least two distinct and somewhat diffuse bands were observed in all lanes, a result that is consistent with two distinct mRNA species of 1.3 and 1.8 kb, differing by the 500 bp separating the two polyadenylylation signals. Hybridization of the same blots with cDNA probes for adenosine deaminase and deoxycytidine kinase revealed consistently compact bands, indicating that the diffuse AK bands did not result from the electrophoresis, transfer procedure, or quality of RNA (data not shown). Of note is the relatively low level of AK mRNA in brain and lung, while the placenta, liver, muscle, and kidney have the highest levels. Intermediate levels are present in the heart and pancreas (Fig. 2*A*). WI L2 B-lymphoblast and CEM

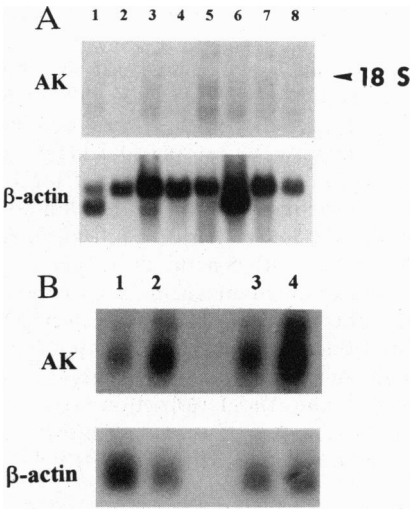

FIG. 2. Northern blot analysis of AK and β-actin levels in total cellular RNA. (*A*) From human tissues. Lanes: 1, heart; 2, brain; 3, placenta; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, pancreas. (*B*) From lymphoblast cell lines. Lanes: 1, WI L2 AK deficient; 2, WI L2 wild type; 3, CEM AK deficient; 4, CEM wild type.

T-lymphoblast cell lines demonstrate variably higher levels of 1.3-kb transcript, whereas AK-deficient subclones of these cells, selected by virtue of resistance to 6-methylmercaptopurine ribonucleoside, show a modest reduction in the level of the AK mRNA (Fig. 2*B*).

To verify that the cDNA clone encoded a protein containing AK activity, we expressed the protein in *E. coli* strain BL21[DE3]. Expression of the insert from clone 911 in pET3a resulted in 100-fold increase in AK activity. Purification of the recombinant AK activity from BL21[DE3] cells resulted in a protein band of ≈43 kDa on SDS/PAGE, equivalent to that of the native protein (Fig. 3). This protein, purified 46-fold from the crude extract, had a specific activity of 3.36 units per mg of protein and a K_m for adenosine of 41 nM, equivalent to values obtained for the native human heart enzyme (3.68 units/mg and 57 nM, respectively; Fig. 4) and with the purified human Molt-4 T-lymphoblast and pig liver enzymes (K_m values of 75 and 46 nM, respectively). From these studies, we conclude that the cDNA encodes a protein that is catalytically equivalent to the human enzyme and that the full coding region of AK has been identified.

The protein sequence was used to search for regions of amino acid sequence identity or similarity to the consensus

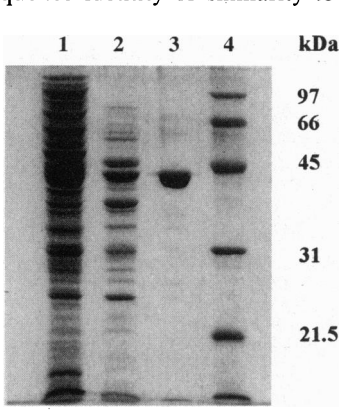

FIG. 3. Purification of recombinant human AK from *E. coli*. Protein was purified from BL21[DE3] cells after an 8-hr IPTG induction as described, separated on a SDS/10% polyacrylamide gel, and stained with Coomassie blue. Lanes: 1, *E. coli* lysate; 2, ammonium sulfate (70–100%) fraction; 3, DEAE eluate; 4, molecular size markers.

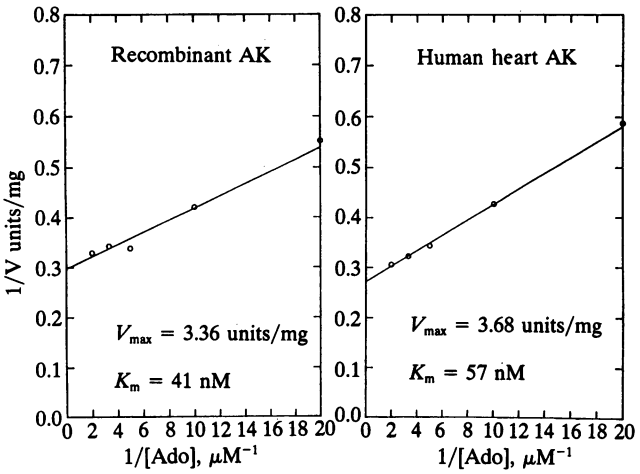

FIG. 4. K_m and V_{max} determinations of purified human recombinant AK and human heart AK.

motifs for ATP or GTP binding. The P-loop (15) and kinase motifs 1–3 (16) that have been found in a wide variety of kinases available for comparison, including deoxycytidine, uridine, and thymidine kinases, were not definitively identified in the AK sequence. Although the AK sequence GSTQNSIK (aa 64–71) has the flanking G and basic amino acid (K) found in the kinase 1a motif (16), there is a distinct lack of conservation of the internal amino acids. Furthermore, a homology search performed using the National Center for Biotechnology Information BLAST procedure did not reveal significant amino acid sequence similarity with other nucleoside kinases, including deoxycytidine and thymidine kinases, or with other adenosine or adenylate metabolizing enzymes such as adenosine deaminase, *S*-adenosylhomocysteine hydrolase, or AMP deaminase. Of interest, however, was the demonstration of two regions of striking similarity to a number of plant and microbial sugar kinases including ribokinase from yeast and several bacterial fructokinases (Table 1). In the region from aa 86 to 111 of AK, 9 of 24 amino acids (37%) are identical to those in a corresponding N-terminal region of yeast ribokinase (17). A second region from aa 294 to 305 of AK contains a 12-aa consensus sequence (DTXGAGDAFXGG, where the second A may be substituted by T or S and the last 2 G may be substituted by A) that is found in all microbial ribokinases and fructokinases. Combined with the flanking sequences, the second region exhibits 44% identity (20 of 45 residues) with *E. coli* ribokinase. Highly similar sequences corresponding to the second motif also occur in other microbial and plant sugar-related enzymes such as *E. coli* deoxygluconokinase (3-deoxy-2-oxo-D-gluconate kinase) and inosine kinase, potato fructokinase, and *Streptomyces* α-amylase (23).

DISCUSSION

The role of AK in the regulation of adenosine concentrations (1, 3, 25) and its importance in the phosphorylation of several pharmacologically relevant adenosine analogs have focused considerable attention on this enzyme. Adenosine has been demonstrated to have anti-arrhythmic effects on the heart and potent vasodilatory effects on the vascular system (26). In addition, there is strong evidence for both immunosuppressive and anti-inflammatory effects of this molecule. It has been demonstrated to inhibit lymphocyte-mediated cytolysis (27) and proliferation (28), interleukin 2 production (29), and regulated capping of surface molecules by a cAMP-dependent pathway (30). In human neutrophils, adenosine has been demonstrated to decrease generation of toxic oxygen metabolites and both promote (A_1 adenosine receptors) and inhibit (A_2 receptors) neutrophil adherence to endothelium (31, 32). In addition, it has been postulated that the anti-inflammatory effects of methotrexate in disorders such as rheumatoid arthritis are mediated, at least in part, by stimulation of adenosine release from connective tissue cells (33). Since the therapeutic benefits of systemic adenosine administration are limited by its short half-life and cardiovascular side effects (26, 34), it has been postulated that targeting the enzymes involved in adenosine metabolism might prove an effective approach to increasing endogenous adenosine concentrations. AK, by virtue of its pivotal role in adenosine phosphorylation, has been

Table 1. Alignments for maximal amino acid homologies among human AK and microbial pentose and hexose kinases

Protein	Ref.	Motif 1	Motif 2
AK (human)		^{86}FFGCIGIDKFGEILKRKAAEAHVD^{111}	^{294}DTNGAGDAFVGG^{305}
Ribokinase (yeast)	17	^{62}MIGNVGNDTFGKQLKDTLSDCGVD^{85}	^{279}DTTGAGDTFLGG^{291}
Ribokinase (*E. coli*)	18	^{60}FIACTGDDSIGESVRQQLATDNID^{83}	^{251}DTIAAGDTFNGA^{262}
Fructokinase (*Klebsiella*)	19	^{47}FIGRVGDDPFGRFMRHTLAQEQVD^{71}	^{245}DTTGAGDAFVAG^{258}
Fructokinase (*Salmonella*)	19	^{47}FIGAVGGDPFGRYMRHTLQQEQVD^{71}	^{245}DTTGAGDAFVAG^{258}
Fructokinase (*Vibrio*)	20	^{46}FFGRVGDDPFGRFMQSILDQEGVC^{70}	^{243}DTTGAGDAFVGG^{256}
Fructokinase (*Rhizobium*)	21	^{48}FFTGIADDMMGEILLETLKASNVD^{71}	^{243}DTVGAGDTFDAG^{254}
1-Phosphofructokinase (*E. coli*)	22		^{249}STVGAGDSMVGG^{260}
α-Amylase (yeast)			^{248}DSNGAGDAFAAA^{259}
Inosine kinase (*E. coli*)	21		^{352}NTNGAGDGALAA^{363}
Deoxygluconokinase (*E. coli*)	23		^{258}DTTAAGDSFSAG^{269}
Fructokinase (potato)	24	^{62}FVGKLGDDEFGHMLAGILKTNGVQ^{85}	^{252}DTTGAGDSFVGA^{263}
Consensus		$F^{F}_{I}GxV_{I}GxDxFGxxxxxxxxxxxVD$	$DTxGAGDxFx^{GG}_{AA}$

Residues in boldface indicate regions of amino acid identity. Consensus sequence was based on 70% or more occurrence among sequences. Sequences were obtained from Swiss-Prot or PIR data base.

felt to be an excellent candidate enzyme for the development of inhibitors. Recent data have supported the hypothesis that such an inhibitor can exhibit anti-inflammatory effects by enhancing adenosine concentrations at the site of inflammation and decreasing adhesion of activated neutrophils to endothelial cells (35). Knowledge of the structure of AK and the ability to express catalytically active protein are the critical first steps in the development of such inhibitors.

Our data strongly support the view that among nucleoside kinases, AK has distinct structural and kinetic properties. The several mammalian nucleoside kinases that have been characterized to date are dimers with subunit molecular sizes in the range of 26–31 kDa with higher substrate specificity toward 2′-deoxynucleosides (24, 36–41). In addition, all nucleoside kinases that have been cloned contain kinase 1a, kinase 2, and kinase 3a nucleotide binding motifs, as defined by Traut (16). In contrast, the amino acid sequence we have obtained from the AK cDNA does not contain the classic N-terminal P-loop sequence motifs that function to bind either ATP or GTP, although these two nucleotides have been identified as the preferred phosphate donors for adenosine phosphorylation by AK in a number of studies (5, 8). The majority of kinases containing these motifs simultaneously bind the NTP and the acceptor substrate to form a ternary complex, facilitating the direct transfer of phosphate between the two substrates (16), and such enzymes are highly prevalent in eukaryotic cells, encompassing proteins like those in the ras family, elongation factors, and phosphoglycerate kinase, as well as nucleoside kinases and adenylate kinase. In contrast, the microbial sugar kinases do not contain these motifs and have recently been subdivided into three distinct nonhomologous families (hexokinase, ribokinase, and galactokinases) based on differences in conserved sequence patterns (21). Published aligned conserved regions for 13 members of the ribokinase family include the motif 2 found in AK (Table 1), but motif 1 has not been previously identified (21). Conversely, a number of additional aligned sequences in microbial enzymes are not present in AK (21).

A comparison among the *E. coli* and *Pseudomonas* fructokinases, each containing 307 aa residues with 69% identity (19, 21), the *E. coli* ribose kinase, a protein of 309 aa with 39% identity to the fructokinases (18), and several other eukaryotic sugar kinases revealed a putative substrate binding site of hTcGA**GD**XmVGALf, identified by sequence alignment and corresponding to motif 2 in Table 1, in which identical amino acids are in boldface and strictly conserved amino acids (underlined) are flanked by less-conserved residues (h, hydrophilic or neutral; c, m, and f, hydrophobic or neutral) (21). Given the lack of structural information on the microbial ribokinase family, it is difficult to ascribe specific function to the motifs identified. However, since motifs 1 and 2 from all ribokinases and fructokinases exhibit similar relative positioning within the respective protein sequences and are consistently separated by 175–194 aa residues, it is certainly conceivable that they contribute to a conserved substrate binding site.

The commonality of a furanose moiety as a substrate for the enzymes listed in Table 1, as well as the inclusion in the list of an amylase without kinase function, make it likely that motif 2 is involved in binding to the sugar moiety of adenosine. This possibility is further strengthened by the observation that the substrate requirement of adenosine kinase includes a 2′-hydroxy group in trans to the purine ring. Data to support this observation came from a comparison of the relative AK activities toward the substrates 9-β-D-arabinosyl-8-azaadenine (cis orientation, no activity) and 9-α-D-arabinosyl-8-azaadenine (trans orientation, 16% activity) (4, 42). In the structure of β-D-fructofuranoside, which is a substrate for fructokinase, the trans orientation of the 1′-C in relation to the 3′-hydroxyl group simulates this stereochemical orientation and suggests that there may be a common structure of the pentose recognition site in both AK and the fructokinase/ribokinase family of enzymes. Whether AK originated evolutionarily as a sugar, rather than nucleoside, kinase or acquired similar ribose and/or ATP binding sites by convergence remains an open question. It is clear from our data that the monomeric structure of the enzyme, lack of "classic" ATP binding motif, and its relative specificity for adenosine set it apart both structurally and kinetically from the nucleoside kinases that have been characterized to date.

AK has also been implicated in the physiologic phosphorylation of 2′-deoxyadenosine (43). Although purified AK has a >1000-fold higher K_m for 2′-deoxyadenosine than for adenosine (44), studies with AK-deficient cell lines demonstrated that it accounts for a significant portion of total 2′-deoxyadenosine phosphorylation in lymphoblast cell lines (43, 45). While 2′-deoxycytidine kinase plays a critical role in the phosphorylation of deoxyadenosine at low concentrations, AK plays a major role at concentrations above 20 μM (10, 46). We have demonstrated that CEM and WI-L2 lymphoblast cell lines, selected for resistance to 6-methylmercaptopurine ribonucleoside and shown to be deficient in AK activity, have diminished levels of AK mRNA. Despite some overlap in the function of AK and deoxycytidine kinase, there is no structural similarity between the two enzymes based on primary amino acid sequence. It will therefore be of considerable interest to compare the relative three-dimensional structures of these proteins once their crystal structures become available.

AK is known to be widely distributed among mammalian cells. A comprehensive survey of Rhesus monkey tissues (47) demonstrated the highest level of AK activity in liver, 2- to 3-fold less activity in kidney, lung, and erythrocytes, and significantly lower levels in brain, heart, and skeletal muscle. A more limited survey of human tissues isolated from autopsy specimens demonstrated roughly equivalent levels of AK activity in liver, kidney, pancreas, and brain cortex, with lower levels in the lung (48). The mRNA levels of AK appear to correlate partially with these findings, with significantly lower levels of mRNA in the brain and lung and an intermediate level in the heart. However, the high AK activity in human brain and low activity in skeletal muscles do not correlate with the low level of mRNA in brain and intermediate level in skeletal muscle. Although the reasons for this deviation are unclear, they may involve other cellular factors regulating protein synthesis or enzymatic activity. Nevertheless, the expression of the gene appears to have some degree of tissue specificity that may account for variable levels of overall enzyme expression. The role of this variability, in conjunction with the variable expression of the opposing activities of the catabolic enzyme adenosine deaminase (EC 3.5.4.4) and adenosine-producing enzyme 5′-nucleotidase (EC 3.1.3.5), in influencing the fate of adenosine and in maintaining adenosine concentrations within specific tissues requires further attention.

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