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Molecular cloning and developmental expression of a human kidney *S*-adenosylmethionine synthetase

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cDNA clones encoding the human kidney *S*-adenosylmethionine synthetase (kidney-type isozyme) were isolated. The amino acid sequence deduced from the cDNA indicates that this enzyme contains 395 amino acids and has a molecular mass of 43,660 Da. The predicted amino acid sequence of this protein shares 84% similarity with that of human liver *S*-adenosylmethionine synthetase (liver-type isozyme). In addition, the developmental expression of these two isozyme mRNAs has been studied in the human liver using the reverse transcription–polymerase chain reaction (RT-PCR).

S-Adenosylmethionine; Isozyme; cDNA sequence; Human kidney

1. INTRODUCTION

S-Adenosylmethionine synthetase (MAT) (ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6)), which Cantoni [1] first reported, is the enzyme that catalyzes the formation of *S*-adenosylmethionine from methionine and ATP. *S*-Adenosylmethionine is an important methyl donor in most transmethylation reactions and is also the propylamino donor in the biosynthesis of polyamines. Mammalian MAT exists as three different isoforms, designated α (or I), β (or III), and γ (or II) [2,3]. The α and β forms are confined to the liver, whereas the γ form is widely distributed in extra hepatic tissues [2,4,5]. The α and β forms purified from rat liver have been shown to be composed of four and two identical subunits, respectively, of molecular mass 48 kDa on polyacrylamide gel electrophoresis [2,6]. Although the α and β forms of MAT differ in their properties, they are thought to be products of the same gene and, therefore, represent different forms of the same enzyme (liver-type isozyme). In contrast, the γ form (non-hepatic- or kidney-type isozyme) from human lymphocytes contained three polypeptide bands of 53, 51, and 38 kDa, and the 51 kDa protein appeared to be derived from the 53 kDa protein [5]. However, the function of each subunit remains to be established.

Recently, liver-type MAT cDNAs have been isolated from rat [7,8] and human [9] liver cDNA libraries. In addition, kidney-type isozyme cDNA has also been isolated from a rat kidney cDNA library [4]. Comparisons of amino acid sequences from these species revealed a significant degree of homology.

To study the regulation of the MAT gene in human cells it is necessary to know the sequences of these two mRNAs. We now report the cloning of a human kidney MAT (kidney-type isozyme) cDNA, the determination of the nucleotide sequence and derived amino acid sequence, and the sequence comparison of human kidney MAT with previously described human liver (liver-type) MAT. We have also investigated the developmental expression of the mRNAs for human isozymes.

2. MATERIALS AND METHODS

[α-^{32}P]dCTP, [α-^{35}S]dATP and Hybond-N nylon membranes were obtained from Amersham Corp. Restriction enzymes, DNA-modifying enzymes, a random-primed DNA labeling kit, and random hexamers ($(dN)_6$) were from Takara Shuzo Co. (Kyoto, Japan). Avian myeloblastosis virus reverse transcriptase XL was from Life Sciences. The sequenase DNA sequencing kit was from United States Biochemical Corp. A human kidney λgt11 cDNA library and fetal (21-week-old) human liver poly(A)$^+$ RNA were from Clontech Laboratories Inc. GeneAmp DNA amplification reagent kit was from Perkin Elmer Cetus. All other chemicals and reagents were of analytical grade and obtained from commercial suppliers.

2.1. *Cloning and sequencing of the cDNA encoding human kidney MAT*

A human kidney cDNA library in λgt11 plated on *Escherichia coli* LE392 was screened by standard plaque hybridization techniques [10] using the *Eco*RI insert of rat kidney MAT cDNA [4]. The probe was labelled using the random primer method [11]. Phage and plasmid DNA isolations, restriction analysis, subcloning, and subsequent recombinations were performed using standard procedures [10].

2.2. *Nucleotide sequence analysis*

DNA sequencing was carried out on both strands of isolated clones

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by the dideoxy chain-termination method [12], using [^{35}S]dATP and sequenase.

2.3. *RNA preparation*

Total RNA was prepared from human kidney and liver by the method of Chirgwin et al. [13]. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography [14].

2.4. *Reverse transcription–polymerase chain reaction (RT-PCR)*

Poly(A)$^+$ RNA (1 μg) was incubated for 60 min at 42°C in a reaction mixture (50 μl) containing 50 mM Tris·HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 5 nmol of each deoxynucleotide triphosphate (dNTP), 5 U of ribonuclease inhibitor from human placenta, 100 pmol of random hexamer primers, and 20 U of Avian myeloblastosis virus reverse transcriptase. 1 μl of the product was subsequently subjected to the PCR using a GeneAmp DNA Amplification kit in a total volume of 50 μl. The mixture was incubated for 25 cycles: denaturation, 1 min at 94°C; annealing, 1 min at 54°C; and polymerization, 2 min at 72°C. The last cycle ended with 7 min at 72°C and gradually cooled to ambient temperature. The sizes of the resulting PCR products were analyzed by electrophoresis in a 2% agarose gel. The PCR product was purified on agarose gel, phosphorylated with ATP and T4 polynucleotide kinase, and cloned into pUC119 plasmid at the *Sma*I site. The nucleotide sequences were determined as described.

2.5. *Northern blot analysis*

1 μg of poly(A)$^+$ RNA from human kidney was denatured and

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-65                                  TTTCGCAGCCGCTGCCGCCTCGCCGCTGCTCCTTCGTAAGGCCACTTCCGCACACCGACACCAAC

  1  ATGAACGGACAGCTCAACGGCTTCCACGAGGCGTTCATCGAGGAGGGCACATTCCTTTTCACCTCAGAGTCGGTCGGGGAAGGCCAC
  1  MetAsnGlyGlnLeuAsnGlyPheHisGluAlaPheIleGluGluGlyThrPheLeuPheThrSerGluSerValGlyGluGlyHis

 88  CCAGATAAGATTTGTGACCAAATCAGTGATGCTGTCCTTGATGCCCACCTTCAGCAGGATCCTGATGCCAAAGTAGCTTGTGAAACT
 30  ProAspLysIleCysAspGlnIleSerAspAlaValLeuAspAlaHisLeuGlnGlnAspProAspAlaLysValAlaCysGluThr

175  GTTGCTAAAACTGGAATGATCCTTCTTGCTGGGGAAATTACATCCAGAGCTGCTGTTGACTACCAGAAAGTGGTTCGTGAAGCTGTT
 59  ValAlaLysThrGlyMetIleLeuLeuAlaGlyGluIleThrSerArgAlaAlaValAspTyrGlnLysValValArgGluAlaVal

262  AAACACATTGGATATGATGATTCTTCCAAAGGTTTTGACTACAAGACTTGTAACGTGCTGGTAGCCTTGGAGCAACAGTCACCAGAT
 88  LysHisIleGlyTyrAspAspSerSerLysGlyPheAspTyrLysThrCysAsnValLeuValAlaLeuGluGlnGlnSerProAsp

349  ATTGCTCAAGGTGTTCATCTTGACAGAAATGAAGAAGACATTGGTGCTGGAGACCAGGGCTTAATGTTTGGCTATGCCACTGATGAA
117  IleAlaGlnGlyValHisLeuAspArgAsnGluGluAspIleGlyAlaGlyAspGlnGlyLeuMetPheGlyTyrAlaThrAspGlu

436  ACTGAGGAGTGTATGCCTTTAACCATTGTCTTGGCACACAAGCTAAATGCCAAACTGGCAGAACTACGCCGTAATGGCACTTTGCCT
146  ThrGluGluCysMetProLeuThrIleValLeuAlaHisLysLeuAsnAlaLysLeuAlaGluLeuArgArgAsnGlyThrLeuPro

523  TGGTTACGCCCTGATTCTAAAACTCAAGTTACTGTGCAGTATATGCAGGATCGAGGTGCTGTGCTTCCCATCAGAGTCCACACAATT
175  TrpLeuArgProAspSerLysThrGlnValThrValGlnTyrMetGlnAspArgGlyAlaValLeuProIleArgValHisThrIle

610  GTTATATCTGTTCAGCATGATGAAGAGGTTTGTCTTGATGAAATGAGGGATGCCCTAAAGGAGAAAGTCATCAAAGCAGTTGTGCCT
204  ValIleSerValGlnHisAspGluGluValCysLeuAspGluMetArgAspAlaLeuLysGluLysValIleLysAlaValValPro

697  GCGAAATACCTTGATGAGGATACAATCTACCACCTACAGCCAAGTGGCAGATTTGTTATTGGTGGGCCTCAGGGTGATGCTGGTTTG
233  AlaLysTyrLeuAspGluAspThrIleTyrHisLeuGlnProSerGlyArgPheValIleGlyGlyProGlnGlyAspAlaGlyLeu

784  ACTGGACGGAAAATCATTGTGGACACTTATGGCGGTTGGGGTGCTCATGGAGGAGGTGCCTTTTCAGGAAAGGATTATACCAAGGTC
262  ThrGlyArgLysIleIleValAspThrTyrGlyGlyTrpGlyAlaHisGlyGlyGlyAlaPheSerGlyLysAspTyrThrLysVal

871  GACCGTTCAGCTGCTTATGCTGCTCGTTGGGTGGCAAAATCCCTTGTTAAAGGAGGTCTGTGCCGGAGGGTTCTTGTTCAGGTCTCT
291  AspArgSerAlaAlaTyrAlaAlaArgTrpValAlaLysSerLeuValLysGlyGlyLeuCysArgArgValLeuValGlnValSer

958  TATGCTATTGGAGTTTCTCATCCATTATCTATCTCCATTTTCCATTATGGTACCTCTCAGAAGAGTGAGAGAGAGCTATTAGAGATT
320  TyrAlaIleGlyValSerHisProLeuSerIleSerIlePheHisTyrGlyThrSerGlnLysSerGluArgGluLeuLeuGluIle

1045  GTGAAGAAGAATTTCGATCTCCGCCCTGGGGTCATTGTCAGGGATCTGGATCTGAAGAAGCCAATTTATCAGAGGACTGCAGCCTAT
349  ValLysLysAsnPheAspLeuArgProGlyValIleValArgAspLeuAspLeuLysLysProIleTyrGlnArgThrAlaAlaTyr

1132  GGCCACTTTGGTAGGGACAGCTTCCCATGGGAAGTGCCCAAAAAGCTTAAATATTGAAAGTGTTAGCCTTTTTTCCCCAGACTTGTT
378  GlyHisPheGlyArgAspSerPheProTrpGluValProLysLysLeuLysTyr *
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of the human kidney *S*-adenosylmethionine synthetase. Nucleotides are numbered beginning with the predicted N-terminal residue. The asterisk indicates a translation termination codon.

separated on a 1.0% agarose gel as described by Thomas [15], then transferred to Hybond-N nylon membranes. Prehybridization and hybridization of the membranes were performed as described [4]. HKSAM1 cDNA probe was labelled by the random primer DNA labeling kit.

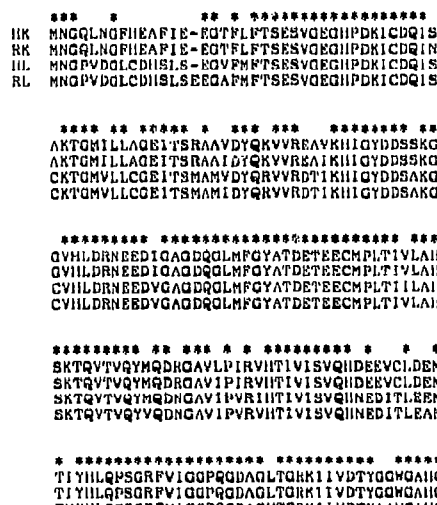
2.6 *Polyacrylamide gel electrophoresis*

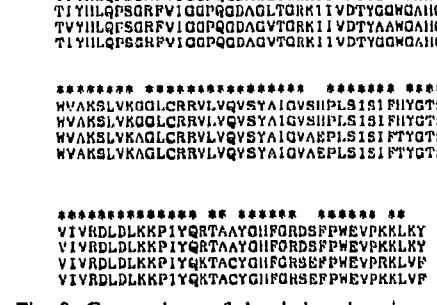
A sample of the PCR mixture (5 μl) was added to 5 μl of loading buffer (50% glycerol, 0.23% Bromophenol blue, xylenecyanol FF) and size-fractionated by electrophoresis in a 1-mm thick non-denaturing 6% polyacrylamide gel. Gels were stained in ethidium bromide (0.5 μg/ml), de-stained in water, and examined on a ultraviolet trans-illuminator. Gels were photographed using an orange filter and Polaroid 665 positive/negative film.

3. RESULTS AND DISCUSSION

3.1. *Isolation and sequence analysis of cDNA clones*

Using a rat kidney MAT cDNA as a probe, we screened about 200,000 clones from a human kidney cDNA library and obtained several clones. The purified clones contained cDNA inserts of sizes ranging over 1.0–1.8 kbp. The largest cDNA clone, HKSAM1, was subjected to further analysis. The cDNA was 1,840 bp in length and, when compared with the cDNA encoding the rat kidney counterpart, it seemed to be derived from an incompletely processed mRNA, containing a short unexcised intron of 635 bp in length (between nucleotide residues 1,085 and 1,086 in Fig. 1). To ensure that the intron-like sequence of 635 bp was absent in the mature mRNA, PCR was performed with primers that allowed amplification of the sequence across the intron-like segment. Poly(A)$^+$ RNA from human kidney was isolated and first strand cDNA was synthesized by random hexamer-primer reverse transcription. Such single-stranded cDNA was used as a template for DNA amplification by PCR using two primers (nucleotide residues 879–898 and 1,210–1,229) synthesized according to the nucleotide sequence of human kidney MAT. An amplified product of 351 bp in length was only detected by agarose gel electrophoresis. This result showed that the intron-like segment observed in HKSAM1 was completely excluded from the human kidney MAT mRNA sequence. This PCR product was subcloned into the pUC119 vector and the DNA sequence of the insert was determined from a purified clone. The nucleotide sequence of the insert cDNA was found to be 100% identical to the corresponding region of the HKSAM1 cDNA except for the intron-like sequence.





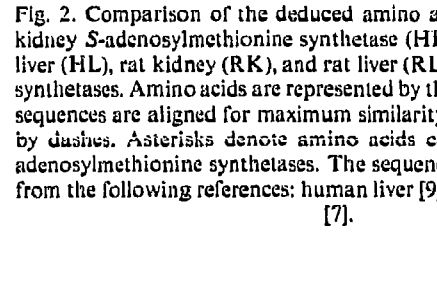


Fig. 2. Comparison of the deduced amino acid sequence of human kidney *S*-adenosylmethionine synthetase (HK) with those of human liver (HL), rat kidney (RK), and rat liver (RL) *S*-adenosylmethionine synthetases. Amino acids are represented by the single-letter code. The sequences are aligned for maximum similarity and gaps are indicated by dashes. Asterisks denote amino acids conserved in all four *S*-adenosylmethionine synthetases. The sequence data have been taken from the following references: human liver [9]; rat kidney [4]; rat liver [7].

The nucleotide sequence of the coding region and deduced amino acid sequence is shown in Fig. 1. The open reading frame, starting with the 5'-most ATG codon at position 1 through 3, and terminating with a TGA codon at 1,186 through 1,189, encoded a 395 amino acid polypeptide, giving a calculated molecular mass of 43,660 Da. The codon specifying the N-terminus of the protein was inferred by aligning the deduced amino acid sequence of human MAT with the published sequence of rat kidney counterpart [4]. The initiator methionine is localized at the same position as the rat sequence. The ACCAACATG sequence around this putative initiation site is in reasonable agreement with the consensus sequence for translation [16]. Thus, it seems likely that this ATG is the translational initiation codon.

3.2. *Comparison of human kidney MAT protein sequence with other MAT*

A comparison of the predicted amino acid sequence of human kidney MAT with those of human liver [9], rat liver [7], and rat kidney [4] isozymes is presented in Fig. 2. The human kidney enzyme showed 84, 84, and 99% similarity with human liver, rat liver, and rat kidney isozymes, respectively. The amino acid sequences of human kidney and liver MAT proteins are 84% identical throughout their length. This level of sequence similarity suggests that our cloned human kidney MAT protein seems to be a catalytic subunit.

3.3. *Northern blotting*

Northern blot analysis was performed using the entire insert of HKSAM1 cDNA as a probe. Probing of

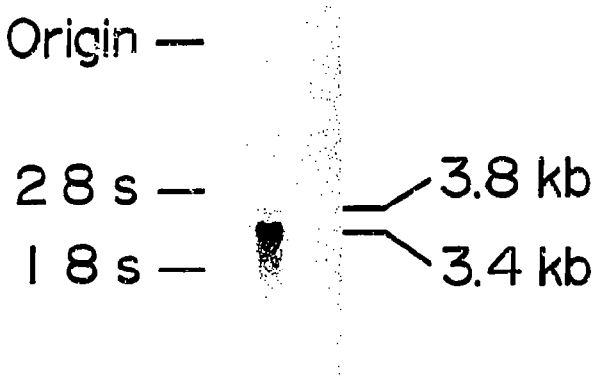


Fig. 3. RNA blot analysis. Poly(A)$^+$ RNA extracted from adult human kidney was hybridized to the HKSAM1 probe. 28 S (4.8 kb) and 18 S (1.9 kb) human kidney rRNA were used as size markers. RNA sizes in kb are indicated on the right.

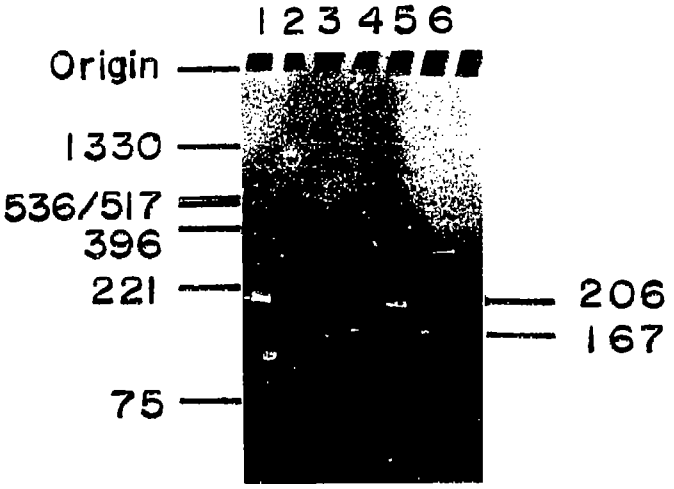


Fig. 4. Ethidium bromide-stained polyacrylamide gel of RT-PCR products. cDNAs from adult human kidney (lanes 1 and 2), adult human liver (lanes 3 and 4), and fetal human liver (lanes 5 and 6) were amplified using primer pair K (lanes 1, 3 and 5) or primer pair L (lanes 2, 4 and 6). Size markers (pUC118/*Hin*fI fragments) in bp are indicated on the left. PCR products in bp are indicated on the right.

poly(A)$^+$ RNA derived from human kidney revealed two mRNA species of 3.4 and 3.8 kb in length (Fig. 3). The 3.4 kb band was the most abundant.

3.4 *Developmental expression of MAT isozyme mRNAs*

We have previously shown, by determining the enzyme activities, that the kidney-type MAT predominantly existed in fetal rat liver and was progressively replaced by the liver-type isozyme during development [17]. However, the availability of these two human MAT isozyme cDNA sequences allowed us to examine the expression of both isozyme mRNAs in the fetal and adult human livers using the more sensitive RT-PCR method. Poly(A)$^+$ RNAs extracted from human kidney, fetal (21-week-old) human liver, and adult (78-year-old) human liver were reverse transcribed to obtain complementary DNAs for PCR amplification. Primers used in the PCR amplification were designed to amplify the human kidney-type (primer pair K: nucleotide residues 24–43 and 210–229 in Fig. 1) and liver-type (primer pair L: nucleotide residues 24–43 and 171–190 in [9]) MAT sequences of 206 bp and 167 bp, respectively. When cDNA derived from adult human kidney RNA was amplified, a single band was only detected at the size (206 bp) of the product expected from the kidney-type MAT using primer pair K (Fig. 4, lane 1). However, no PCR product was observed using primer pair L (lane 2). In contrast, the PCR products derived from adult human liver contained a major band (167 bp) of the liver-type MAT (lane 4), whereas the kidney-type MAT was faintly detected (lane 3). This experiment clearly showed that the kidney-type MAT mRNA was expressed at very low levels in the adult liver. These results indicated that the RT-PCR analysis accurately amplified the target regions, and the products were not the results of amplification of genomic DNAs. Furthermore, we have examined the expression of MAT isozyme mRNAs in the fetal human liver. The results showed that both kidney- and liver-type MAT mRNAs were expressed in fetal human liver and the kidney-type MAT mRNA was relatively abundant (lanes 5 and 6). It is considered to be somewhat difficult to derive absolute quantitative conclusions from PCR. However, we suggest that the differences in kidney-type MAT product levels within each sample reported here are likely to accurately reflect relative differences in mRNA levels. Our results are generally consistent with previous studies on the developmental expression of MAT isozymes in the rat liver [9]. These findings suggest that, from fetal to adult, the expression of these two MAT isozymes is developmentally regulated.

This cDNA will be a useful tool for future studies to understand the mechanisms of the tissue- and stage-specific gene regulation.

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