Molecular Cloning and Nucleotide Sequence of cDNA Encoding the Rat Kidney S-Adenosylmethionine Synthetase*

(Received for publication, January 25, 1990)

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We previously reported the isolation of a cDNA encoding the liver-specific isozyme of rat S-adenosylmethionine synthetase from a λgt11 rat liver cDNA library. Using this cDNA as a probe, we have isolated and sequenced cDNA clones for the rat kidney S-adenosylmethionine synthetase (extrahepatic isozyme) from a λgt11 rat kidney cDNA library. The complete coding sequence of this enzyme mRNA was obtained from two overlapping cDNA clones. The amino acid sequence deduced from the cDNAs indicates that this enzyme contains 395 amino acids and has a molecular mass of 43,715 Da. The predicted amino acid sequence of this protein shares 85% similarity with that of rat liver S-adenosylmethionine synthetase. This result suggests that kidney and liver isozymes may have originated from a common ancestral gene. In addition, comparison of known S-adenosylmethionine synthetase sequences among different species also shows that these proteins have a high degree of similarity.

The distribution of kidney- and liver-type S-adenosylmethionine synthetase mRNAs in kidney, liver, brain, and testis was examined by RNA blot hybridization analysis with probes specific for the respective mRNAs. A 3.4-kilobase (kb) mRNA species hybridizable with a probe for kidney S-adenosylmethionine synthetase was found in all tissues examined except for liver, while a 3.4-kb mRNA species hybridizable with a probe for liver S-adenosylmethionine synthetase was only present in the liver. The 3.4-kb kidney-type isozyme mRNA showed the same molecular size as the liver-type isozyme mRNA. Thus, kidney- and liver-type S-adenosylmethionine synthetase isozyme mRNAs were expressed in various tissues with different tissue specificities.

S-Adenosylmethionine (AdoMet) synthetase (ATP:L-methionine S-adenosyltransferase (EC 2.5.1.6)) which Cantoni (1) first reported is the enzyme that catalyzes the formation of AdoMet from methionine and ATP. AdoMet is an important methyl donor for transmethylation and also the propylamino donor in the biosynthesis of polyamines. In eukaryotes, several isoenzymic forms have been identified. Chiang and Cantoni (2) first purified two forms of AdoMet synthetase from Saccharomyces cerevisiae. In mammalian tissues, three distinct forms of AdoMet synthetase have been identified and designated as AdoMet synthetase α (or I), β (or III), and γ (or II) (3–8), and all these forms have different biochemical properties (7, 9, 10). Two of the forms, AdoMet synthetase α and β, are expressed only in adult liver (7). On the other hand, the γ form is widely distributed in extrahepatic tissues including kidney, brain, and lymphocytes (7, 10, 11). In addition, the γ form predominantly exists in fetal rat liver and is progressively replaced by the α and β forms during development (7). Carcinogenesis apparently reverses this process (12). Thus, expression of AdoMet synthetase isozymes is tissue-specific and is developmentally regulated (7, 12).

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 (7, 9). Recently, Cabrero’s group (13) has shown that the peptide maps of tryptic digests of the two proteins, the α and β forms, are indistinguishable. Therefore, they have concluded that the α and β form enzymes are constructed of the same polypeptide chains. In contrast, the γ form has an apparent molecular mass of 160–190 kDa and has been reported to consist of two or three distinct subunits (7, 10, 11). The γ form purified from bovine brain was composed of two distinct subunits with molecular masses 48 and 38 kDa (10), whereas the enzyme from human lymphocytic leukemia cells contained three polypeptide bands of 53, 51, and 38 kDa (11). Therefore, comparison of the primary structures of the two isozymes, AdoMet synthetase α/β and γ, is of great interest and may be useful in deducing the sequences responsible for the catalytic properties. Recently, we have determined the primary structure of rat liver AdoMet synthetase (liver-specific isozyme) from the nucleotide sequence of the cDNA (14).

In this paper, we report the isolation and sequences of rat kidney AdoMet synthetase (γ form or extrahepatic isozyme) cDNA clones from a λgt11 rat kidney cDNA library. Alignment of the deduced amino acid sequence of rat kidney AdoMet synthetase with that of rat liver enzyme indicates a high degree of sequence similarity between the two isozymes. We have also determined the tissue-specific expression of the mRNAs for rat enzymes.

MATERIALS AND METHODS

RESULTS AND DISCUSSION

Isolation of cDNAs Encoding Rat Kidney AdoMet Synthetase—Approximately 2 × 10^6 independent recombinants were

* Portions of this paper (including “Materials and Methods” and Figs. 1–3) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full-size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
Screened by plaque hybridization with a $^{32}$P-labeled EcoRI insert from the rat liver AdoMet synthetase cDNA RLS1 (14) as a probe under weakly stringent conditions. Two overlapping clones were isolated. Agarose gel electrophoresis revealed that the clones RKSAM1 and RKSAM2 had a length of 1.2 and 2.0 kb cDNA inserts, respectively.

**Nucleotide and Amino Acid Sequences of the Rat Kidney AdoMet Synthetase**—Sequence analysis was performed on both strands of the coding region of the cDNAs. The nucleotide sequence of the coding region and deduced amino acid sequence are shown in Fig. 1. The two overlapping clones encompassed one complete open reading frame. The 9-base pair sequence, GCAGCCATG, including the ATG codon at nucleotide position 1 9 is in good agreement with the GCCA(A/G)CATT consensus sequence of eukaryotic initiation sites proposed by Kozak (15), suggesting that this ATG is indeed the initiation codon. As shown in Fig. 1, the amino acid sequence deduced from the cDNA indicates that the rat kidney enzyme for this protein contains 399 amino acids and has a molecular mass of 43,715 Da.

**Sequence Similarities**—The rat kidney AdoMet synthetase subunit consists of 395 amino acids, which is 2 residues less than those in the rat liver enzyme subunit (14). In addition, the molecular mass of the rat kidney AdoMet synthetase subunit, calculated on the basis of the deduced amino acid sequence, 43,715 Da, is nearly identical to the calculated molecular mass of rat liver AdoMet synthetase, 43,697 Da (14). The amino acid sequence similarity of rat kidney AdoMet synthetase with rat liver enzyme was estimated as 85% (Fig. 2). The nucleotide sequence of the coding region of rat kidney AdoMet synthetase is 73% identical to that of rat liver enzyme. Furthermore, the deduced amino acid sequence of the rat kidney AdoMet synthetase was compared with those of AdoMet synthetases encoded by the yeast genes SAM1 (16) and SAM2 (17), and Escherichia coli MetK (18), which is the structural gene for AdoMet synthetase in E. coli (Fig. 2). AdoMet synthetases in various species contain nearly similar number of amino acid residues as shown in Fig. 2. Of the positions aligned, identical amino acids occupy 67% of rat kidney AdoMet synthetase/yeast SAM1-encoded AdoMet synthetase, 68% of rat kidney AdoMet synthetase/yeast SAM2-encoded AdoMet synthetase, and 52% of rat kidney AdoMet synthetase/E. coli MetK encoded AdoMet synthetase.

The rat kidney AdoMet synthetase polypeptide contains an ATP-binding site, Gly-Xaa-Gly-Xaa-Gly and Lys in amino acid residues 131-136 and 159. This sequence (Gly-Ala-Gly-Asp-Gln-Gly and Lys) is perfectly conserved in the other AdoMet synthetases except that the lysine of the ATP-binding site in the E. coli enzyme is replaced by arginine as shown in Fig. 2. Therefore, the ATP-binding site of rat kidney AdoMet synthetase seems to be located at Lys-159. These results indicate that the AdoMet synthetase protein sequence has been well conserved through evolution. Our results also suggest that the two rat AdoMet synthetase genes may be evolved from a common ancestral gene.

**Northern Blot Analysis**—To examine tissue distribution of kidney- and liver-type AdoMet synthetase mRNAs in rat, 10 µg each of total RNAs isolated from various tissues were subjected to RNA blot analysis using two distinct probes, one for kidney AdoMet synthetase and the other for liver AdoMet synthetase as shown in Fig. 3. Rat kidney AdoMet synthetase cDNA was first hybridized to total rat RNAs isolated from kidney, brain, testis, and liver under strongly stringent conditions (Fig. 3A). This experiment identified two mRNA species of 3.4 and 3.8 kb (as determined by comparison to size markers) which were present in kidney, brain, and testis. The 3.4-kb band was the most abundant. These two mRNA species observed with rat kidney AdoMet synthetase cDNA probe may arise from transcriptional initiation at different promoters, alternative RNA splicing, and/or polyadenylation at different sites. Consistent with the tissue distribution of AdoMet synthetase γ activity, the mRNA for kidney-type enzyme occurred most abundantly in the kidney but was not detectable in the liver. On the contrary, when total RNAs from liver, kidney, brain, and testis were analyzed using the probe specific for liver AdoMet synthetase, a single band (3.4 kb) was detected only in RNA from liver but not from kidney, brain, and testis (Fig. 3B). The probes for kidney- and liver-type enzymes hybridized with the same molecular size, but distinct mRNA species of 3.4 kb, respectively. Thus, the mRNAs for kidney- and liver-type isozymes appears to be expressed in rat with different tissue specificities.

We have examined the molecular characteristics of two functionally identical but structurally different forms of AdoMet synthetase. The idea that the rat kidney- and liver-type AdoMet synthetases are evolutionally related enzymes is supported by both biochemical and nucleotide sequence evidence.

The finding of a single polypeptide in the derived structure of the kidney enzyme was unexpected in view of the previous results showing different subunits in the mature γ enzyme. Further work is needed to characterize the isolated protein subunits more completely and to explain their relationship to the coded sequences.

**REFERENCES**

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Molecular Cloning and Nucleotide Sequence of CRRA Encoding the Rat Kidney S-Adenosylmethionine synthetase

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Materials and Methods

**Cloning:**

DNA ligase (E. coli) and the nick-translation kit were obtained from Amerham Corp. Restriction endonucleases, DNA modifying enzymes, and 3' 5' sequencing primers were from Takara Shuzo Co (Kyoto, Japan). Octyl A cytochrome P-450 microsome fraction was from Mercia Girona S (Girona, Spain). Octyl A cytochrome P-450 membrane filter was from Bell Scientific Corp. Octyl A cytochrome P-450 was from Collaborative Research.

**Isolation of cDNA for Adenyl Synthetase:**

Total RNA was isolated from rat kidney by the guanidinium thione/cesium chloride method (21,22), and purified by oligo(dT)-cellulose chromatography (22). Double stranded cDNA was synthesized from rat kidney poly(A)+RNA as described by Gubler and Hoffman (23). Double stranded cDNA was cloned in M13 and was labeled with [32P]dCTP by nick-translation. Hybridization with plaque screening was carried out at 68°C for 15-20 h in a solution containing 5 x SSC (i.e., 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and 6 x Denhardt's solution (22) and 0.2% SDS. The filters were washed twice with 5 x SSC (i.e., 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and 0.2% SDS. The final wash was for 1-2 h at 55°C with 0.2 x SSC, 0.1% SDS.

**Northern Blot Analysis:**

Recombinant plasmid were propagated from positive clones by the plaque lysate method (22). The DNA was isolated by digestion with EcoRI and subcloned into the plasmid pBR322. Recombinant plasmids were isolated into lambda phages, as described by Hinnen et al. (23). Recombinant plasmids were isolated by the calcium phosphate method (24). The excised cDNA inserts were treated with EcoRI and subcloned into the plasmid pBR322. Recombinant plasmids were amplified by the deoxyribonuclease digestion/terminal transferase method of Sanger (25).

**Northern Analysis:**

Equal amounts of RNA were denatured with 1 M glyoxal and 50 g diethylpyrocarbonate, and electrophoresed on a 1% agarose gel as described by Thomas et al. (26). Blots were hybridized with 32P-labeled cDNA probes in 50% formamide, 5 x SSC, 1 x Denhardt's solution, 50% normal saline, pH 6.5, 0.1% SDS, and 0.25 mg/ml heat-denatured salmon testes DNA at 65°C for 18-20 h. The blots were washed with 5 x SSC at 55°C and were exposed to x-ray film at 4°C.
Molecular Cloning of Rat Kidney S-Adenosylmethionine Synthetase

![Diagram](image)

Fig. 1. RNA blot analysis. Total RNA (15 μg) from rat kidney (lane 1), brain (lane 2), testis (lane 3), and liver (lane 4) were subjected to RNA blot analysis using the EcoRI insert of 1183 bp containing nucleotide residues 171 to 1864 of rat kidney adenosyl synthetase cDNA (GenBank:U11016) and the EcoRI insert of rat liver AdoMet synthetase cDNA (M81185) as probes. The size markers used were 28S and 18S rat rRNA (indicated in the left). Sizes in kb are indicated on the right.

![Alignment of amino acids](image)

Fig. 2. Alignment of deduced amino acid sequence of the rat kidney (AdoMet synthetase) with the deduced amino acid sequences encoding rat liver (AdoMet synthetase), yeast SAM-I, and E. coli SAM-I-dependent AdoMet synthetase. Amino acids are represented by single-letter codes. The N-terminals are included in the sequence numbering. Dashes were inserted to maximize homology. Identical amino acids between rat kidney and liver enzymes are indicated by dots. Asterisks indicate the amino acids conserved in all five AdoMet synthetases. The sequence data have been taken from the following references: rat liver (14); yeast SAM-I (15); yeast SAM-II (16); E. coli (GenBank:U11016).