Structure and Regulation of Mammalian S-Adenosylmethionine Decarboxylase*

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Antti Pajunen†, Anne Crozat‡, Olli A. Janne§, Ritva Ihalainen‡, Paivi H. Laitinen†, Bruce Stanley¶, Rentala Madhubala¶, and Anthony E. Pegg∥

From the †Department of Biochemistry, University of Oulu, Linnanmaa, SF-90570 Oulu, Finland, the ‡Departments of Physiology and Pharmacology, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pennsylvania 17033, and §The Population Council and the Rockefeller University, New York, New York 10021

In order to understand the structure and regulation of S-adenosylmethionine decarboxylase, cDNA clones encoding this enzyme have been isolated from rat prostate and human fibroblast cDNA libraries. The authenticity of the cDNAs was verified by: (a) transfecting the Chinese hamster ovary cells with the human cDNA in the pCD vector which resulted in a transient 10–20-fold increase in S-adenosylmethionine decarboxylase activity in recipient cells; and (b) translating the mRNA formed by transcription of the cDNA insert in a reticulocyte lysate and recording an increase in S-adenosylmethionine decarboxylase activity. The amino acid sequences deduced from the cDNAs indicate that the human proenzyme for this protein contains 334 amino acids and has a molecular weight of 38,331 whereas the rat proenzyme contains 333 amino acid residues. The human and rat enzymes are very similar having only 11 amino acid differences and the cDNAs are also closely related showing over 90% homology in the 1617-nucleotide overlap which was sequenced. A further indication of the highly conserved nature of mammalian S-adenosylmethionine decarboxylases is that the amino acid sequences deduced from the human and the rat cDNAs contained peptide sequences identical to those previously reported for the purified bovine enzyme. In vitro transcription/translation experiments showed that the proenzyme is converted to two polypeptides of molecular weights about 32,000 and 6,000 in a processing reaction which generates the prosthetic pyruvate group and that the final enzyme contains both polypeptides. Two forms of S-adenosylmethionine decarboxylase mRNA (2.1 and about 3.4–3.6 kilobases) are present in human and rodent tissues and may originate from the utilization of two different polyadenylation signals. Southern blots of rat genomic DNA indicated that the S-adenosylmethionine decarboxylase gene belongs to a multigene family. Depletion of cellular polyamines by inhibitors of ornithine decarboxylase or the aminopropyltransferases led to an increased content of S-adenosylmethionine decarboxylase protein and mRNA, but the elevation in the mRNA was not sufficient to account for all of the change in the enzyme level, particularly in cells in which spermine was depleted.

Decarboxylated AdoMet¹ is an important intermediate in polyamine biosynthesis which serves as an aminopropyl donor for spermidine and spermine syntheses (1–3). It is formed by the action of AdoMet decarboxylase. This enzyme has been purified from several mammalian sources and is known to be activated by putrescine and to contain a covalently bound pyruvate prosthetic group (see reviews in Refs. 4–7). Purified preparations of AdoMet decarboxylases have been isolated from rat liver and prostate, mouse liver, and bovine liver. These studies suggested that the enzyme has a subunit M₁ of 32,000 and the native enzyme appeared to be a dimer which may aggregate to higher forms. More detailed investigation of the structure of this enzyme has been hampered by the lack of sufficient material since it is present in very small amounts but some partial peptide sequences from the bovine enzyme have been obtained (8).

Little is known about the derivation of the pyruvate prosthetic group in mammalian AdoMet decarboxylase. Experiments in which antibodies to it were used to isolate the newly synthesized enzyme after translation of prostatic mRNA in reticulocyte lysates, and the labeled immunoreactive translation products were separated by SDS-PAGE, indicated that it was formed as a precursor of approximate M₂, 37,000. This proenzyme was converted to the subunit M₁, 32,000 on further incubation (9). The conversion, which is accelerated by the presence of putrescine (10), presumably generates the pyruvate from an internal serine residue. Snell and colleagues (11, 12) have demonstrated that this mechanism leads to the formation of pyruvate in histidine decarboxylase from Lactobacillus 30a and related microorganisms, and a similar internal cleavage reaction generates pyruvate from serine in Escherichia coli AdoMet decarboxylase (13, 14) and in E. coli phosphatidylserine decarboxylase (15).

Mammalian AdoMet decarboxylase has a rapid rate of turnover and is regulated by a variety of stimuli including cellular polyamine levels (5–7). Preliminary experiments suggest that this regulation may occur at the level of mRNA synthesis or stability since treatment of rats with DFMO to reduce spermidine content led to an increased content of mRNA.

¹The abbreviations used are: AdoMet, S-adenosylmethionine; DFMO, o-difluoromethylornithine; AdoMetS(CH₃)₂, dimethyl-(5'-adenosynyl)sulphonate perchlorate salt; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; bp, base pair(s); kb, kilobase(s); Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; CHO, Chinese hamster ovary.

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†To whom correspondence should be addressed: Dept. of Physiology, P.O. Box 850, Milton S. Hershey Medical Ctr., Hershey, PA 17033.
mRNA for AdoMet decarboxylase as determined by translation in a reticulocyte lysate. However, evidence has also been presented that the translation of mRNA for AdoMet decarboxylase is inhibited by spermidine or spermine.

Mach et al. (8) have reported a cDNA clone for bovine AdoMet decarboxylase, but this clone contained only a small portion of the amino acid coding sequence. In order to provide more information on the structure and regulation of this enzyme, we have isolated and sequenced cDNA clones from rat prostate and human fibroblasts. Our results indicate that the rat and human AdoMet decarboxylase are very similar to each other and to the bovine enzyme. They also demonstrate that the enzyme consists of two nonidentical subunits and that the smaller subunit may have been overlooked in earlier studies. Depletion of spermidine by pretreatment with inhibitors of polyamine biosynthesis lead to an increased content of AdoMet decarboxylase mRNA.

**EXPERIMENTAL PROCEDURES**

**Materials**—The antiserum to rat prostate AdoMet decarboxylase was raised in rabbits as described previously (17). AdoMet decarboxylase was purchased from Sigma, Pharmacia LKB Biotechnology Inc., and from Bethesda Research Laboratories. A 180-base pair (bp) fragment from the bovine cDNA for AdoMet decarboxylase (8) was a generous gift from D. R. Morris (Department of Biochemistry, University of Washington, Seattle, WA). DFMO was kindly provided by Dr. P. P. McCann (Merrell Dow Research Institute, Cincinnati, OH). S-Adenosyl-l,3-diamino-3-carboxysteine, and [3H]methylamine were obtained from Amersham Corp. Biochemical reagents were purchased from Sigma, Pharmacia LKB Biotechnology Inc., and from Bethesda Research Laboratories.

A 180-base pair (bp) fragment from the bovine cDNA for AdoMet decarboxylase was isolated by the alkaline lysis method (23) and hybridized with two different [32P]cDNA probes: a 55-bp EcoRI fragment complementary to the poly(A) RNA-enriched fraction (8) and a synthetic oligonucleotide (45-mer) complementary to the poly(A) RNA containing the 5'- terminal 90 bases of the mRNA (45). The same labeled oligonucleotide primer was used for determination of the distance of the 5'- end of the mRNA from the 5'- end of the cDNA insert of pSAM1 by primer extension carried out as described by Grunenberg and Mason (46). Both 5' and 3' ends of the rat mRNA sequence corresponding to positions 639-683 in the human AdoMet decarboxylase cDNA sequence was also used for direct sequencing of part of the rat prostate AdoMet decarboxylase mRNA by the method described above.

**Construction of Vectors for RNA Synthesis**—Plasmid pSAM1 was cleaved with PstI and XbaI to yield a 1313-bp fragment containing the entire protein coding region. After isolation by gel electrophoresis this was inserted into the pTT7/T3-19 vector (Bethesda Research Laboratories) cleaved by the same enzymes. The [32P]-labeled complementary and sense RNAs were synthesized using T3 and T7 RNA polymerases in the presence of [32P]UTP (37) and the PstI and XbaI linearized templates. Synthesis of the sense AdoMet decarboxylase RNA for cell-free translation studies was carried out under the same conditions but in the absence of [35S]methionine and in the presence of 0.5 mM 5'-mGppG-3' (38).

**In Vitro Translation of Hybridization-selected RNA**—Ten µg of plasmid DNA from pSAM1 or other plasmids was denatured and bound to 0.5-cm square nitrocellulose filters (Schleicher & Schuell), and hybridization selection was performed according to Parnes et al. (27). The selected RNA was translated in a rabbit reticulocyte lysate system (Bethesda Research Laboratories) using [35S]methionine as the labeled amino acid.

**Nucleotide Sequence Determination**—Sequencing of the human AdoMet decarboxylase cDNA from pSAM1 was performed by the shotgun sequencing method described by Bankier and Barrell (28). Briefly, the plasmid was linearized with BamHI, the insert isolated by gel electrophoresis, self-ligated with T4 DNA ligase, and sheared by sonication. The sheared DNA was fractionated and cloned into the Smal site of M13mp8 as described previously (29). Buffer gradients (30) were used during electrophoresis. Specific regions of the sequence were confirmed using the chemical degradation method (31).

The rat AdoMet decarboxylase cDNA sequences were determined by the dideoxynucleotide chain termination method (32) using recombinant M13 templates (33) and synthetic oligodeoxynucleotide primers (15-mers). Relevant regions of the XLASM10 subclones were sequenced directly from double-stranded DNA with T7 and SP6 polymerases as described by Chen and Seeburg (34). In order to obtain additional sequence corresponding to the mRNA nucleotides beyond the 5' end of pSAM1, direct RNA sequencing (35) was used. Poly(A)RNA was isolated from rat tissues with DFMO (9), and a synthetic oligonucleotide (45-mer) complementary to the rat cDNA was used as a primer. The 32P-labeled 1313-bp fragment was isolated by preparative 1% agarose gel electrophoresis and labeled by nick-translation. The distance of the 5' end of the mRNA from the 5'- end of the cDNA insert in pSAM1 (from positions 963 to 1207 in the cDNA sequence) was labeled at the 5' end and used as the primer. The mixture of labeled oligonucleotide primer (5 µg) and poly(A) RNA (10 µg) was heated in a boiling water bath for 3 min in annealing buffer (10 mM Tris-HCl, pH 8.5, 25 mM KCl) and allowed to anneal for 40 min at 80°C. After cooling to room temperature, sequencing reactions were carried out as described by Gelebter et al. (35).

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RESULTS

Cloning of cDNA for Rat Prostate AdoMet Decarboxylase—In order to prepare a cDNA library enriched for AdoMet decarboxylase cDNA sequences, rats were treated with DFMO for 3 days to increase the content of its mRNA in the prostate. Poly(A) RNA isolated from these polysomes contained a high content of AdoMet decarboxylase mRNA, which was much greater than that present in the mRNA isolated from polysomes which did not bind to the immunoadsorbent column (Fig. 1A). At least 400-fold purification of AdoMet decarboxylase mRNA was achieved by the protein A-Sepharose immunoadsorbent chromatography, and the final purity of this preparation was about 10% based on results of in vitro translation assays in which the labeled bands corresponding to AdoMet decarboxylase were determined by scanning with a densitometer as described (9).

An aliquot of 0.2 μg of the immunopurified mRNA was used as a template for reverse transcriptase to synthesize cDNA inserts in the plasmid vector described by Okayama and Berg (21) using the methods indicated in this paper. Transformation of E. coli with an aliquot corresponding to 1% of the resulting plasmids yielded 576 transformants. One-fifth from these was subjected to differential colony hybridization as described under "Experimental Procedures." Approximately 20% of the colonies showed a positive reaction with the (+) cDNA probe and about one-half of these gave a much weaker signal with the (−) cDNA probe. About 40 colonies including those which appeared most promising in the first screening were further analyzed by an additional colony hybridization using the same probes and also a nick-translated 180-bp DNA fragment representing the 5′ end of the bovine cDNA clone isolated by Morris and colleagues (8). From these screenings, two plasmids were selected for further analysis and designated pSAMr1 and pSAMr2. Plasmid pSAMr1 hybridized with the bovine cDNA clone but pSAMr2 did not. These plasmids were used for hybridization selection using rat prostate mRNA. As shown in Fig. 1B cell-free translation of mRNA selected by hybridization to either of these plasmids produced peptides corresponding to AdoMet decarboxylase. The inserts in pSAMr1 and pSAMr2 did not hybridize with each other and there was no similarity in their restriction maps. Plasmid pSAMr2 was found to contain an insert of approximately 475 bp, whereas the insert in plasmid pSAMr1 was 1258 bp.

Northern blot analysis of prostate mRNA was carried out with nick-translated pSAMr1 and pSAMr2 and the 180-bp

![Fig. 1. Analysis of the translation products of immunopurified AdoMet decarboxylase mRNA and in vitro translation of hybridization-selected RNA. In the experiment shown in A, poly(A) RNA was translated in a rabbit reticulocyte lysate with [35S]methionine as the labeled amino acid, and the translation products (before and after immunoprecipitation) were analyzed on a 10% polyacrylamide gel containing 0.1% SDS. Lanes 1 and 2 show results after immunoprecipitation when 18 and 36 ng of immunopurified poly(A) RNA were used in the cell-free translation. Lane 3 shows results after immunoprecipitation for translation of the poly(A) RNA (1 μg) that failed to bind to the protein A-Sepharose. Lanes 4 and 5 show results after immunoprecipitation when no message was added to the in vitro translation reaction. In the experiment shown in B, 10 μg of plasmid DNA was desalted and bound to a 0.5-cm square of nitrocellulose, and hybridization-selection was performed according to Parnes et al. (27) using poly(A) RNA from prostates of DFMO-treated rats. The selected RNA was translated, and immunoprecipitation of the radiolabeled translation products was performed with anti-AdoMet decarboxylase antisemur (lanes 1 to 4). Lane 5 shows a radiolabeled marker of AdoMet decarboxylase. Results are shown for plasmids pSAMr1 (lane 1), pSAMr2 (lane 2), a plasmid containing the 180-bp fragment from bovine AdoMet decarboxylase cDNA (8) (lane 3), and pBR322 (lane 4). In both figures, the positions of standard proteins are indicated by arrows and the Mn (×10^3).](image)

![Fig. 2. Northern blot hybridization of poly(A) RNA from prostates of difluoromethylornithine-treated rats. Samples of poly(A) RNA from rat prostate were electrophoresed on 1% agarose gel containing 2.2 M formaldehyde, blotted onto nitrocellulose, and hybridized to nick-translated PstI/PstI fragment (894 bp) of pSAMr1 (lanes 1 and 4) or PstI/HindIII fragment (345 bp) of pSAMr2 (lanes 2 and 5) or the 180-bp fragment from the bovine AdoMet decarboxylase cDNA (lane 3). Lanes 1 and 2 show results for 5 μg of RNA and lanes 3–5 show results with 10 μg of RNA. The size of the bands (in kilobases × 10^-3) is indicated. These were determined from the positions of a marker RNA ladder containing sequences corresponding to λ DNA and developed with a nick-translated λ probe.](image)

### Table I

<table>
<thead>
<tr>
<th>Transfection</th>
<th>AdoMet decarboxylase activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
</tr>
<tr>
<td>Mock transfection</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>pSVneo</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>pSAMh1</td>
<td>23.2 ± 3.6</td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
</tr>
<tr>
<td>pSVneo</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>pSAMh1</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td><strong>Experiment III</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Mock transfection</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Vector plasmid</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>pSAMh1</td>
<td>11.0 ± 0.6</td>
</tr>
</tbody>
</table>
bovine AdoMet decarboxylase insert (Fig. 2). Both pSAMr1 and the bovine cDNA hybridized to two mRNA species corresponding to approximate sizes of 2.1 and 3.4 kb. These are in good agreement with previous reports of the size of the AdoMet decarboxylase mRNA in bovine, mouse, and human cells (7, 8). Only the larger mRNA was detected with pSAMr2 (Fig. 2). These results suggest that pSAMr2 contained a cDNA sequence corresponding to part of the 3' region of the larger mRNA for AdoMet decarboxylase which does not overlap with sequence of the shorter mRNA.

A 495-bp internal fragment from the insert of pSAMr1 was used to screen a human fibroblast cDNA library in the pcD expression vector (24). A clone, pSAMh1, with a 1.8-kb insert was identified. This pSAMh1 plasmid was then used to transfect CHO cells and the transient expression of AdoMet decarboxylase activity examined 50 h later. As shown in Table I, there was a substantial increase in the enzyme activity in cells receiving pSAMh1 but not in cells transfected with other plasmids used as controls. These results confirm that pSAMh1 contains an insert corresponding to the human AdoMet decarboxylase and suggest that it encompasses the entire protein coding sequence.

Additional proof that pSAMh1 contains all of the coding sequence for human AdoMet decarboxylase was obtained by subcloning a 1313-bp PstI/XbaI fragment into the pT3/T7-19 vector that was used for the production of RNA by T7 RNA polymerase. The mRNA was translated in a reticulocyte lysate and the characteristic M, 37,000 precursor and M, 32,000 subunit bands were observed (Fig. 3, A–D). Continued incubation led to the conversion of the precursor into the subunits and both of these polypeptides formed during the translation of the synthetic mRNA made from plasmid pSAMh1 did react with the antibodies to AdoMet decarboxylase. Previous results (not shown) using mRNA isolated from

**Fig. 3. Polypeptides formed in cell-free translation of RNA corresponding to the 1313-bp PstI/XbaI sequence of pSAMh1.** Subcloning of the PstI/XbaI fragment into the pT3/T7-19 vector and synthesis of the capped RNA corresponding to the AdoMet decarboxylase mRNA was performed as described under "Experimental Procedures." Aliquots of the mRNA were translated in a rabbit reticulocyte lysate with either [3H]leucine (A and B), [35S]methionine (C), or [36S]cysteine (D) as the labeled amino acid for different periods of time. Panels A and B, the translation products were analyzed by SDS-PAGE on 10% polyacrylamide gels before (A) and after immunoprecipitation with anti-AdoMet decarboxylase antiserum (B). The translation reaction proceeded for the indicated time periods. Panels C and D, the translation products were analyzed by Tricine-SDS-PAGE on 10% polyacrylamide gels (48) before and after immunoprecipitation with anti-AdoMet decarboxylase. In each case, lane 1 shows results for no exogenous mRNA, lane 2 for translation of rabbit reticulocyte mRNA, and lanes 3 and 4 correspond to results for translation of AdoMet decarboxylase mRNA for 30 and 90 min, respectively. 0, no exogenous mRNA added to the translation reaction; GL, 1 μg of rabbit reticulocyte mRNA added to the translation reaction; ST, molecular weight standards.
HeLa cells had indicated that the human AdoMet decarboxylase precursor has approximately the same size as the rat. It also can be seen clearly in Fig. 3B that in parallel to the production of the $M_0$, 32,000 subunit there is also formation of a smaller peptide with $M_0$ of about 6,000. The size of this polypeptide was determined more accurately to be about 6,000 using the Tricine-SDS-PAGE system (48) (Fig. 3, C and D). The smaller polypeptide was most easily detected when [3H]leucine was used as the labeled precursor (Fig. 3B) but it was also formed and labeled when the radioactive amino acid was either [35S]methionine (Fig. 3C) or [35S]cysteine (Fig. 3D). This provides evidence, which is confirmed by the sequencing data given below, that the cleavage of the precursor occurs at an internal site and generates two peptides which form the final AdoMet decarboxylase.

Translation of synthetic mRNA by the reticulocyte lysate system in the presence of unlabeled amino acids also led to the formation of detectable AdoMet decarboxylase activity. This activity increased from 0.03 units in a lysate supplemented with 2 $\mu$g of control mRNA (prepared from a plasmid containing an insert corresponding to ornithine decarboxylase) to 4.8 units in a lysate to which 2 $\mu$g of mRNA prepared from the subcloned human AdoMet decarboxylase cDNA sequence was used for translation in a 1-h period.

**Sequences of Human and Rat AdoMet Decarboxylase cDNA Clones and Their Deduced Amino Acid Sequence**—The partial restriction maps and sequencing strategies for pSAMh1, pSAMr1, and $\lambda$SAMr10 are shown in Fig. 4, and the nucleotide sequences of the AdoMet decarboxylase cDNAs are shown in Fig. 5. Plasmid pSAMh1 contains an 1806-bp insert (excluding the poly(A) region), and there is an open reading frame beginning at position 249 and ending at 1253. The polypeptide of 334 amino acids specified by this sequence (Fig. 5), which corresponds to the AdoMet decarboxylase proenzyme, has a calculated molecular weight of 38,331, which is in reasonable agreement with the estimates of the AdoMet decarboxylase proenzyme based on SDS-PAGE of the product made in reticulocyte lysates (9, 17). The sequence containing the proximal ATG triplet is CGG(TG)G which corresponds to only the most conserved features of the Kozak rules (60, 61). There is a long 3′-noncoding region of 557 nucleotides before the start of the poly(A) segment. The sequence ATTAAA presumably serves as a poly(A) addition signal (52). The clone contains 245 bases of 5′-noncoding sequence but this may not represent the entire 5′ end (see below). Northern blotting of the human, mouse and rat poly(A) RNA samples with a [32P]cRNA corresponding to pSAMh1 indicated the presence of two bands in each case (Fig. 6A). The sizes of the two rodent AdoMet decarboxylase mRNA species were identical with those detected with pSAMr1 as the hybridization probe (cf. Fig. 3 and Fig. 6B), whereas the longer mRNA was slightly longer in the human than in the rodent tissues. The cDNA in pSAMh1 therefore appears to correspond to the shorter mRNA species.

The insert in pSAMr1 corresponding to the rat AdoMet decarboxylase was 1258 bp long and shows a close homology to the human sequence beginning at position 348 (Fig. 5). However, this rat cDNA contained only 297 bases before the poly(A) in the 3′-noncoding region which is considerably shorter than the human clone. It is likely that the rat cDNA clone does not represent the entire 3′ region because of internal priming from an A-rich sequence. This notion is supported by the sequence of $\lambda$SAMr10 which has a 3′-noncoding region almost identical in length to that of the
human sequence, including the polyadenylation signal AT-
TAAA (Fig. 5).

The insert in pSAMr1 lacked the 21-base sequence corre-
sponding to positions 573–593 in the human cDNA. However, 
other rat AdoMet decarboxylase cDNA clones including 
ASAmr10, which were sequenced in this region, did contain 
this sequence in a form identical to the human sequence (Fig. 
5), and it was also found in the rat prostate mRNA by direct 
sequencing using a 45-mer primer complementary to positions 
233–278. These alterations include the 5′-noncoding region of 
the human sequence shows only 11 differences out of 334  when compared 
with the rat mRNA corresponding in the 5′ region. This indicates that  the 5′-noncoding region was 
formed, This indicates that the 5′-noncoding region of 
the human cDNA, and its first open reading frame. In each nucleotide sequence, * indicates a padding character inserted to 
maximize its alignment with the other sequence.

The differences in the rat nucleotide and amino acid sequences 
are depicted under the human sequences:

**Fig. 5. Nucleotide sequence of human and rat AdoMet decarboxylase cDNAs, and the deduced amino acid sequence of the AdoMet decarboxylase proenzyme.** The reference sequences are those for the human 
cDNA and its first open reading frame. In each nucleotide sequence, * indicates a padding character inserted to 
maximize its alignment with the other sequence. The differences in the rat nucleotide and amino acid sequences 
are depicted under the human sequences: * indicates no difference; XXX shows the amino acid missing in the rat 
sequence; (u) refers to the poly(A) sequence. The lines above the sequences indicate the peptides of the bovine 
AdoMet decarboxylase determined by Mach et al. (8) either from a Lys-C digest of the bovine enzyme or deduced 
from a partial bovine cDNA clone.
in the two independently isolated cDNA clones, pSAMr1 and λSAMr10. Out of the 10 amino acid changes, only 4 are nonconservative replacements.

The mRNA sequences are also very similar at the nucleotide level with more than 90% homology overall in the determined sequences and 94% homology in bases corresponding to the coding sequence. (The 21-base sequence missing from pSAMr1 was ignored in this calculation since it is present in other rat cDNA clones and in the mRNA and is identical to the human sequence.) It is striking that even the 3’-noncoding regions exhibit over 85% sequence identity between the two species.

The distribution of hydrophobic and hydrophilic sections along the predicted AdoMet decarboxylase proenzyme amino acid sequence is shown in Fig. 7. The more hydrophilic regions of the molecule are located in the amino-terminal half and these include a region which may be close to the site of cleavage.

Changes in AdoMet Decarboxylase mRNA Content after Spermidine Depletion—Treatment of rats with DFMO (2% in the drinking water for 3 days) is known to lead to an increased content of AdoMet decarboxylase mRNA (6, 17). As shown in Table II, this increase was accompanied by a substantial increase in the amount of mRNA as measured by dot blot analysis. Northern blots indicated that both mRNA species were increased, although the smaller mRNA went up by a greater extent (Fig. 6B). The mRNA content was enhanced by about 7-fold which is somewhat less than the 9.5-fold increase in the AdoMet decarboxylase protein. These changes could be reversed by administration of spermidine to the DFMO-treated rats, indicating that the reduction in spermidine concentration brought about by...
but only a 2.9-fold increase in the mRNA.

restriction enzymes, and the fragments separated on agarose gel, blotted onto nitrocellulose, and hybridized to a [32P]cDNA probe prepared by nick translation of the 5'-terminal standards, which were HindIII cleaved fragments of

**TABLE III**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Cells and treatment</th>
<th>AdoMet decarboxylase activity</th>
<th>AdoMet decarboxylase mRNA content*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment I</td>
<td>L1210</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>L1210 + 5 mM DFMO</td>
<td>3.9</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>L1210 + 5 mM DFMO + 10 μM spermidine</td>
<td>0.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Experiment II</td>
<td>SV-3T3</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>SV-3T3 + 5 mM DFMO</td>
<td>2.2</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>SV-3T3 + 10 μM spermidine</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>SV-3T3 + 5 mM DFMO + 10 μM spermidine</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>SV-3T3 + 0.7 mM AdoS+(CH3)2</td>
<td>9.5</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>SV-3T3 + 0.1 mM AdoDatO</td>
<td>1.7</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*Results were expressed as densitometer units/μg RNA and then divided by the value for the untreated cells. It should be noted that the densitometer calibration was not the same in both experiments and these results are not comparable between the cell lines. The results shown represent the mean of at least triplicate observations which agreed within ± 15%.

DFMO is likely to be responsible for the increased mRNA level.

Treatment of mouse L1210 cells and SV-3T3 cells and hamster CHO cells with DFMO also led to an increase in the concentration of AdoMet decarboxylase mRNA which was abolished by provision of spermidine (Table III and Fig. 6C). In the transformed mouse cell lines, about 90% of the AdoMet decarboxylase mRNA was the larger size of 3.4 kb (Fig. 6C, lanes 3-6). The increase in mRNA content with DFMO treatment was, however, less than the increase in enzyme activity (Table III). This discrepancy was also seen in SV-3T3 cells treated with DFMO where the enzyme activity increased by about 3.8-fold and the mRNA by 2.6-fold. Similarly, treatment with S-adenosyl-1,8-diamino-3-thiooctane, a spermidine synthase inhibitor (3), increased the AdoMet decarboxylase activity by 2.9-fold and the mRNA content by 2-fold. The difference was even bigger when SV-3T3 cells were exposed to AdoS+(CH3)2 which depletes spermine selectively (3). In this case, there was a 16-fold rise in the enzyme activity but only a 2.9-fold increase in the mRNA.

**DISCUSSION**

Although there is some evidence that phosphopantetheinylycysteine decarboxylase from horse liver may contain a pyruvate prosthetic group (53), AdoMet decarboxylase is the only mammalian enzyme for which this is firmly established. At least four different bacterial enzymes, histidine decarboxylase, AdoMet decarboxylase, phosphatidylserine decarboxylase, and phosphopantetheinylycysteine decarboxylase, have this co-factor (11-15, 54). With the exception of the E. coli phosphopantetheinylycysteine decarboxylase in which the pyruvate appears to be bound via an ester linkage and may be introduced by a post-translational modification (54), in each case, the pyruvate is joined via an amide linkage at the amino terminus of one of the enzyme subunits (11). In these enzymes, the pyruvate is formed from a serine residue within the amino acid sequence of a precursor molecule as the result of an intramolecular cleavage which results in the production of two polyptides, both of which remain as enzyme subunits (12, 13, 15). Our results suggest that the same process occurs in the mammalian AdoMet decarboxylase. It is synthesized as a preenzyme of Mr38,300, and this is cleaved to two subunits of about 6,000 and 32,000 which make up the enzyme.

The smaller subunit clearly is formed from the precursor in the reticulocyte lysates (see Fig. 3), but it has been overlooked previously both in experiments on the synthesis of AdoMet decarboxylase from mRNA in vitro (9, 10, 16) and in studies of the purified enzyme (reviewed in Refs. 5-7). The reasons for this are the small size, which would lead to its being lost from gels or present at the dye front, and its relatively poor labeling with methionine since it probably contains only a single methionine residue. The peptide was readily seen when leucine is used as the labeled precursor in the reticulocyte translation system. AdoMet decarboxylase is a minor protein in mammalian cells, and it has therefore been difficult to obtain sufficient enzyme for detailed studies of subunit composition and structure. The availability of the cDNA clones containing the entire protein coding region should enable the protein to be made in much larger amounts by recombinant DNA techniques, and these structural studies
will be possible. However, the evidence that the smaller polypeptide produced in the cleavage reaction remains in the enzyme is quite firm since the polypeptide reacts with antibodies made to the purified enzyme (see Fig. 3), and, as described below, it contains a peptide sequence previously derived by direct amino acid sequencing of bovine AdoMet decarboxylase.

Because the amino terminus of the M, 32,000 subunit is blocked by the pyruvate, we have not been able to obtain its amino acid sequence which would unequivocally establish the site of cleavage. Unblocking this residue by treatment with ammonium acetate and sodium cyanoborohydride which was used for the bacterial decarboxylases (12, 14) is not practical because the reaction goes in very poor yield, and, as described above, only very small amounts of material are available. Based on the apparent size of the polypeptides formed from the proenzyme, as determined by SDS-PAGE, the likely cleavage sites are those between the cysteine and serine residues at positions 49 and 50 or between the isoleucine and serine residues at positions 52 and 53. However, cleavages adjacent to the serine residues at positions 66, 68, and 69 are also possible. These sites are too close for the peptide products to be distinguished clearly on the basis of the Mr of the subunits as measured on SDS-PAGE. (Sites nearer to the amino terminus of the proenzyme than residue 49 are eliminated by the finding that the smaller fragment does contain cysteine (Fig. 3). Since the cysteine at position 49 is the first cysteine residue in the molecule.) The cleavage of the cysteine-serine bond would generate subunits of 32,600 and 5,687. The known sites for cleavage of the bacterial precursors to pyruvate enzymes are serine-serine for histidine decarboxylase (11), lysine-serine for AdoMet decarboxylase (13), and glycine-serine for phosphatidylserine decarboxylase (15) so there is no obvious pattern. Further study of the processing of the mammalian AdoMet decarboxylase will be facilitated by the availability of the cDNA sequences containing site-specific mutations and cloned into vectors from which mRNA can be transcribed in large amounts such as that used in the experiment shown in Fig. 3.

The amino acid sequences for the rat and the human AdoMet decarboxylase contain all five of the peptides found by Mach and colleagues (8) to be present in Lys-C digests of bovine AdoMet decarboxylase. The sequences, which are indicated in Fig. 5 by a line above the amino acid sequences, begin at the lysine residues present at positions 12, 201, 233, 291, and 301 of the human enzyme and agree with the published bovine sequences in the human and show only 1 change with the rat. The additional 18 amino acids of bovine sequence predicted from the nucleotide sequence of the partial cDNA fragment (8) are also identical with the human and show only one change with the rat. These results emphasize the striking conservation of the amino acid sequence for mammalian AdoMet decarboxylase. The bovine AdoMet decarboxylase contained a peptide C"TVL where * represents a modified amino acid which could not be identified (8). In both the human and the rat sequence, this position is occupied by arginine. This implies that the enzyme contains a modified arginine residue, and methylation is perhaps the most likely modification which would permit the sequencing reaction to proceed but generate a product which does not chromatograph with the standard amino acids. The presence of the peptide with sequence LLEVWFSFS-RQQ among the products from the digestion of bovine AdoMet decarboxylase (8) is in agreement with our contention that the smaller fragment generated by cleavage of the AdoMet decarboxylase precursor remains as part of the enzyme. This sequence is located from residues 13 to 22 from the amino terminus of the precursor and must be located in the smaller fragment after this cleavage.

In contrast to the striking conservation of the amino acid sequence between the mammalian AdoMet decarboxylases there was little or any similarity between the sequence of the mammalian enzyme and that of the E. coli AdoMet decarboxylase reported by Taber and Tabor (13). The most similar region appears to be that of amino acids 81-91 in the human proenzyme (TCGTTLIIKAL) and 139-149 in the E. coli proenzyme (TCGVISPLKAL) which has 7 identical residues out of 11. The significance of this similarity is not obvious at present, and it should be stressed that E. coli AdoMet decarboxylase consists of four pairs of nonidentical subunits of Mr 12,400 and 18,000 with the larger subunit containing the pyruvate group. The mammalian and E. coli enzymes, therefore, differ strikingly in their subunit structure and in their activation by cations, since the former are activated by putrescine while the bacterial enzyme requires Mg2+ (4-6), but some similarity corresponding to the active site might be expected.

AdoMet decarboxylase has a rapid rate of turnover with a half-life of about 1 h or less in many mammalian cells. It has been suggested that certain regions of amino acids described as PEST sequences are important in the degradation of rapidly turning over proteins (55, 56). The sequence of AdoMet decarboxylase does contain such a sequence from residues 242 to 269 (namely HITPEPESYVSFETNLSQTSYDDLIR), and modification of this sequence by site-specific mutagenesis should provide a powerful approach to investigate its importance in protein degradation.

The AdoMet decarboxylase mRNA appears to have a long 3’-nontranslated sequence. This sequence as derived from the cDNA represents 557 nucleotides plus the poly(A) region and appears to represent the shorter mRNA species. The longer mRNA may have another 1.3-kb extension at the 3’ end. Additional clones which cover this region have been obtained and partially sequenced, and the sequences support this interpretation. It should be pointed out that there is another open reading frame in the cloned cDNAs which commences 9 bases after the termination codon. This reading frame could code for a protein of 124 amino acids in the human mRNA and 125 amino acids in the rat mRNA, but is not known at present whether it is translated in vivo. However, the sequence AAAAA(ATG)A that contains its putative initiation codon in both species concurs, at least in part, with the Kozak consensus rules (51).

In the rat prostate, and in cultured mouse cells, the content of AdoMet decarboxylase mRNA was affected by the cellular polyamine concentration. Depletion of polyamines by DFMO or by aminopropyltransferase inhibitors led to a significant increase in the mRNA content. This confirms and extends previous work in which the amount of mRNA giving rise to AdoMet decarboxylase protein after translation in a reticulocyte lysate was measured (17, 18). However, the spermine synthase inhibitor, AdoS’(CH3)2, was less effective than DFMO in increasing the mRNA content but produced a larger increase in enzyme activity. Also, in no case was the increase in mRNA sufficient to account for all of the increase in activity. These results suggest that polyamines also regulate AdoMet decarboxylase at the levels of mRNA translation or protein turnover and that spermine may be more active at these steps than spermidine.

A. Crozat, O. A. Jänne, B. Stanley, and A. E. Pegg, unpublished observations.
REFERENCES