Identification of a Highly Diverged Class of S-Adenosylmethionine Synthetases in the Archaea*

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S-Adenosylmethionine is the primary alkylating agent in all known organisms. ATP:L-methionine S-adenosyltransferase (MAT) catalyzes the only known biosynthetic route to this central metabolite. Although the amino acid sequence of MAT is strongly conserved among bacteria and eukarya, no homologs have been recognized in the completed genome sequences of any archaea. In this study, MAT has been purified to homogeneity from the archaeon *Methanococcus jannaschii*, and the gene encoding it has been identified by mass spectrometry. The peptide mass map identifies the gene encoding MAT as MJ1208, a hypothetical open reading frame. The gene was cloned in *Escherichia coli*, and expressed enzyme has been purified and characterized. This protein has only 22 and 23% sequence identity to the *E. coli* and human enzymes, respectively, whereas those are 59% identical to each other. The few identical residues include the majority of those constituting the polar active site residues. Each complete archaeal genome sequence contains a homolog of this archaeal-type MAT. Surprisingly, three bacterial genomes encode both the archaeal and eukaryal/bacterial types of MAT. This identification of a second major class of MAT emphasizes the long evolutionary history of the archaeal lineage and the structural diversity found even in crucial metabolic enzymes.

S-Adenosylmethionine (AdoMet)1 plays a central role in the metabolism of all known organisms (1, 2). AdoMet is the most widely used methyl group donor in cells (3). In another major role, decarboxylation of AdoMet generates the donor of the propylamine moiety employed in polyamine biosynthesis (4, 5). AdoMet has recently been found to serve as the precursor to the acyl-homoserine lactones that many bacteria use as a means of sensing cell density (quorum sensing) (6) as well as the precursors of the acyl-homoserine lactones that many bacteria use as a means of sensing cell density (quorum sensing) (6) as well as the precur-

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contained 385 mM NaCl, 38 mM MgCl₂, 6H₂O, 16.8 mM NH₄Cl, 4.1 mM KC1, 1.45 mM K₂HPO₄, 1.85 mM KH₂PO₄, 1.5 mM Na₂CO₃, 0.2% (w/v) yeast extract, 0.2% (w/v) tryptone, 73.4 mM nitritroacetic acid, 8.4 mM CaCl₂·6H₂O, 7.8 mM FeCl₃·6H₂O, 7.3 mM ZnCl₂, 5.1 mM MnCl₂·4H₂O, 4.5 mM CaCl₂·2H₂O, 4.2 mM MgCl₂·6H₂O, 4.1 mM Na₂MoO₄·2H₂O, 5.1 mM CuSO₄·5H₂O, and 2 mM EDTA, and was adjusted to pH 7.0 and maintained by the addition of sterile, anaerobic NaOH solution.

**MAT Purification from M. jannaschii**—Since we anticipated that this purification would not be repeated, no attempt was made to optimize any step. At each step, a portion of the protein was used in pilot chromatographic trials; therefore, it was not meaningful to calculate an overall yield. Purification was monitored by SDS gel electrophoresis on a Pharmacia Phast system using 8–25% gradient gels.

Cells (5 g) were suspended in 20 ml of 0.1 M Tris·Cl, 1 mM EDTA, 0.03 mM phenylmethylsulfonyl fluoride, 0.1% 2-mercaptoethanol, pH 8.0. Cells were lysed by a single pass through a French press at 15,000 p.s.i., followed by centrifugation at 15,000 × g for 30 min. The pellet was discarded.

The protein was loaded onto a 2.6 × 16 cm column of phenyl-Sepharose HR equilibrated in 1 mM ammonium sulfate, 10 mM Tris·Cl, 1 mM EDTA, 10% glycerol, 1 mM dithiothreitol, pH 8.0. The column was eluted at 2.5 ml/min with 160 ml of starting buffer followed by a 750-ml linear gradient to a final buffer lacking ammonium sulfate. Enzyme eluted near the baseline. The fractions containing activity were pooled and dialyzed to equilibrate with buffer A (50 mM Tris·Cl, 50 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, pH 8.0). The protein was then fractionated by ion exchange chromatography on a 2.6 × 16 cm column of Q-Sepharose equilibrated with buffer A and eluted at 4 ml/min with 150 ml of buffer A followed by a 650-ml gradient to 1 X 1 M KCl in buffer C. The fractions containing activity were concentrated using Centriprep concentrators (Amicon), and 1-ml aliquots were chromatographed on a 1.6 × 40 cm Superdex-200 gel filtration column that was equilibrated and eluted at 0.5 ml/min with buffer A. The column was calibrated with molecular mass markers (Bio-Rad) to allow estimation of the native size of the enzyme.

Final purification was obtained by chromatofocusing on a Mono-P column (0.5 × 20 cm). Protein was dialyzed into 25 mM imidazole, 1 mM dithiothreitol, pH 6.8. The sample was then loaded onto the column, which was equilibrated with the same buffer. After washing with 10 ml of equilibration buffer, the column was eluted with a solution of a 1:8 dilution of Polybuffer (Amersham Pharmacia Biotech), pH 4.0. Enzyme activity eluted at pH 4.2. The protein showed a single band after silver staining a 8–25% gradient SDS gel and a 8–25% nondenaturing gel. The total protein was estimated as 5 µg using the Coomassie Plus Protein Assay Kit (Fierce) with bovine serum albumin as a standard.

**Mass Spectrometry**—Mass spectrometry was performed in the Fanny Rippel Biotechnology Facility at the Fox Chase Cancer Center. Approximately 1 µg of purified protein was electrophoresed on an 8–25% Phast gel. The Coomassie-stained protein band was excised, reduced, alkylated, and proteolytically cleaved with trypsin. Peptide mass maps comprising the ensemble of tryptic peptides were obtained by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Voyager DE, Perceptive Biosystems) (25–27).

**Cloning**—The MJ1208 gene was amplified from M. jannaschii genomic DNA by polymerase chain reaction using the primers MJ1208FWD (GGATCCTTAGAATGTAGTTACTTTTCC) and MJ1208REV (GGATCCTTAGAATGTAGTTACTTTTCC). The MJ1208FWD (GCATATGAGAAACATAATTGTAAAAAAATTAG) and MJ1208REV (GGATCCTTAGAATGTAGTTACTTTTCC) primers contained the BstN I and BamHI sites introduced into the gene through the primers were used to clone the DNA into the pET19b vector (Novagen Inc.). The recombinant clone was used to transform E. coli BL21(DE3) (26) for protein expression.

Expression of Recombinant MAT from E. coli—Cells were suspended in 50 mM Tris·Cl, 300 mM NaCl, 0.03 mM phenylmethylsulfonyl fluoride, pH 8.0, at a density of 4 ml of buffer/g of cells. Cells were lysed by a sonication for three 30-s cycles, followed by centrifugation at 15,000 × g for 30 min. The pellet was discarded. Imidazole was added to the supernatant to a final concentration of 5 mM. The solution was purified by chromatography on a nickel-chelate column (2 × 8 cm; Novagen). The column was eluted at 0.75 ml/min with 50 mM Tris·Cl, 300 mM NaCl, 5 mM imidazole followed by a 150-ml gradient of 500 mM CuSO₄, 2H₂O, followed by elution with 0.3 M KCl. The elution volume of the native protein was 160 ml, followed by a 750-ml column of Q-Sepharose.

Sequence data from partial genome sequences was obtained from TIGR (available on the World Wide Web) for Chlorobium tepidum, the University of Utah (available on the World Wide Web) for Pyrococcus furiosus, Caltech (available on the World Wide Web) for Pyrococcus aerophilum, and the University of Oklahoma (available on the World Wide Web) for Streptococcus pyogenes. Sequence data for Methanococcus maripaludis 3J were from unpublished data.2 Taxa are identified using Swiss-Prot five-letter tags. Amino acid sequences were aligned using the CLUSTALW (version 1.7.4) program. From the alignment, 426 positions defined to be confidently aligned were analyzed by protein maximum parsimony methods using a heuristic search algorithm (PAUP* version 4 beta 2; D. Swofford, Sinauer Associates, Inc). The 500 shortest trees were evaluated by maximum likelihood criteria using the PROTML program (version 2.2) in the MOLPHY package (29) with the JTT model for amino acid substitutions. The TreeCons program (version 1.0) (30) was used to standardize and exponentially weight the trees using maximum likelihood scores and the Kishino-Hasegawa test for significance (p ≤ 0.01). The CONSENSE program (J. Felsenstein, PHYLP (phylogeny inference package), version 3.5c, Department of Genetics, University of Washington) constructed a consensus tree from the weightings.

**RESULTS AND DISCUSSION**

Purification of AdoMet synthetase from M. jannaschii yielded a protein that chromatographed as an 86-kDa species on gel filtration and gave a single band upon SDS gel electrophoresis with an apparent molecular mass of 44 kDa. Thus, the native protein appears to be a dimer. The specific activity of the purified enzyme was 0.26 µmol of AdoMet formed/min/mg of protein at 58 °C. A portion of the protein was eluted from a SDS gel and digested with trypsin, and the resultant peptides were analyzed by mass spectrometry. The masses of the peptides were compared with those predicted from the entire M. jannaschii genome. All of the masses were found in the open reading frame containing the MJ1208 gene (Table 1). MJ1208 encodes a 406-amino acid protein of 45,252 daltons; we designate this gene as MAT. The peptides identified covered 65% of the protein (264 residues) ranging from residue 3 to 397.

The MJ1208 gene was cloned into an E. coli expression system with an N-terminal decahistidine tag and an inducible T7 RNA polymerase promoter. The recombinant enzyme was then purified using nickel-chelate chromatography and gel filtration. The enzyme is quite thermonaly stable, losing no activity during incubation for 30 min at 85 °C, conditions under which the E. coli MAT has a half-life of less than 1 min (31). The enzyme activity continued to increase at least 90 °C.

Functional properties of MAT were assessed at 70 °C. No activity was detectable in the absence of Mg²⁺, and maximal activity was observed at 10 mM MgCl₂ when ATP was present at 5 mM, indicating that MgATP is probably the true substrate and suggesting involvement of additional Mg²⁺ beyond the Mg²⁺-ATP complex as is the case for the E. coli and human isozymes (14, 20). MAT activity was stimulated 10-fold by the addition of KCl to a reaction mix to which only large organic

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2 P. Haney and D. E. Graham, unpublished data.
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Table I

| m/z submitted | MH+ matched | Start | End | Peptide sequence |
|---------------|-------------|-------|-----|------------------|
| 713.97        | 714.93      | 3     | 8   | NHIVKK           |
| 2053.05       | 2053.33     | 8     | 24  | KLDVPEIEPTVEIVER |
| 2053.05       | 2053.33     | 9     | 25  | LDVEPIEERTVEIKER |
| 1899.04       | 1999.22     | 25    | 43  | KGLPHDSICDGIAESVR |
| 1857.71       | 1871.04     | 26    | 43  | GLHPHSIDCGIAESVR |
| 2434.48       | 2434.73     | 53    | 74  | FGTIILHNTDQVELVGHHAYPK |
| 1766.64       | 1767.15     | 75    | 91  | FGGGGMVSPYIILLGSR |
| 515.28        | 515.68      | 123   | 126 | KVLR             |
| 1561.21       | 1561.76     | 127   | 139 | NVDDKVDVLDCR     |
| 1566.09       | 1566.78     | 140   | 153 | IGQGSMIDLVDFER   |
| 2695.96       | 2695.95     | 154   | 175 | QKNEVPLANDSFGOVAPLST |
| 2132.18       | 2132.38     | 186   | 204 | FLNSDELKNEIPAVIDEK |
| 592.19        | 591.75      | 205   | 209 | VMLGRF           |
| 1448.02       | 1447.78     | 213   | 225 | KTIIAMAVDVR      |
| 1394.11       | 1394.57     | 229   | 239 | NIEFKEYEVK       |
| 2244.33       | 2244.35     | 251   | 269 | IADGYEVEHINTADDYER |
| 2352.63       | 2352.53     | 250   | 269 | KIADGYEVEHINTADDYER |
| 2023.06       | 2023.39     | 295   | 313 | VNGLITTFRPSMSMKEAAOSK |
| 865.08        | 864.98      | 314   | 321 | NPVNHVVK         |
| 1659.99       | 1659.97     | 322   | 336 | IYNILANLIADIK    |
| 1437.73       | 1437.73     | 347   | 359 | ILISQGKPINPKE    |
| 2321.47       | 2321.59     | 360   | 379 | ALDIEIITEDSYDIKDIEPK |

The methionines in these peptides were present as methionine sulfoxide.

Table II

| Organism       | Vmax (μmol/min/mg) | Km (ATP) | Km (methionine) |
|----------------|--------------------|----------|-----------------|
| M. jannaschii  | 30                  | 0.25     | 0.14            |
| E. coli        | 12                  | 0.11     | 0.08            |
| S. cerevisiae  | Isozyme 1          | 0.06     | 0.08            |
|                | Isozyme 2          | 0.05     | 0.14            |
| H. sapiens     | Rat                | 0.2      | 0.03            |
|                | Rat                | 0.02     | 0.15            |

The extreme sequence divergence of the archaeal-type MAT proteins from the bacterial/eukaryal-type MAT proteins cannot be entirely due to requirements for thermal stability. The bacterial-type MAT protein from the hyperthermophilic bacterium A. aeolicus (95 °C optimum growth temperature) is 50% identical to that of the mesophilic E. coli (37 °C). Furthermore the sequence of the archaeal-type MAT protein from the hyperthermophilic M. jannaschii (87 °C) is 72% identical to that of the mesophilic archaeon M. maripaludis (37 °C).

Functional Alignment of M. jannaschii MAT to the Crystal Structure of the E. coli Homolog—An alignment of the M. jannaschii MAT sequence on the E. coli structure was facilitated by the present of short sequences in which active site residues were conserved at similar spacing in the two sequences (Fig. 2). In this alignment all of the insertions and deletions in the M. jannaschii protein fall between elements of the E. coli structure. In surface loop regions. Relative to the E. coli MAT, this protein lacks one surface helix at its carboxyl terminus but has an amino-terminal extension; the latter is also present in numerous eukaryal MAT isoforms. All known MATs are homodimers or tetramers. This association is important for catalytic activity, since the E. coli MAT crystal structure shows both subunits of the dimer contributing functional groups to the interfacial active site (20).

Based on the alignment of the E. coli MAT sequence with all known archaeal-type MAT sequences, we observe that predicted active site residues are disproportionately conserved. Polar amino acids within 3 Å of the ligands observed in the crystal structure of the E. coli MAT (crystallized with ADP and
PO₄) are categorically conserved, suggesting that the two different classes of MAT share a common mechanism. A constellation of amino acids contacting Mg²⁺ and polyphosphate is evolutionarily conserved in the archaeal-type MAT sequences: His-14*, Asp-16*, Lys-165*, Arg-244* and Lys-265 (position numbers refer to the E. coli amino acid sequence and an asterisk indicates amino acids contributed by the second subunit of the homodimeric protein). Two other essential residues in the active site, phosphate-binding Lys-245* and Mg²⁺-binding Asp-271, are both replaced by Gly. These replacements are particularly surprising because Lys-245 → Met and Asp-271 → Ala mutations in the E. coli MAT reduce activity by nearly 10⁵- and 10³-fold, respectively (37). It is noteworthy that in a linear sequence alignment there are no nearby residues that can replace the missing functional groups, suggesting that the surrogate residues arise from elsewhere in the linear sequence but are brought into the active site in the three-dimensional structure.

In contrast, amino acids responsible for binding the adenine moiety of the substrate and product are less stringently conserved. In the crystal structure of the E. coli enzyme, the side chain amide of Gln-98 hydrogen-bonds to the exocyclic N-6 amine of ATP. Thus, the replacement of Gln-98 with a hydrophobic amino acid (Leu) may explain why ITP is a substrate for the M. jannaschii enzyme (Kₘ = 1.4 mM, V_max = 2% of ATP) but neither a substrate nor inhibitor of the E. coli enzyme. Similarly, Lys-269 may recognize the N-3 atom of ATP; Lys-269 → Met mutations in the E. coli MAT reduce enzyme activity by 10³-fold. The replacement of Lys-269 with His in the archaeal-type MAT, however, may be a conservative substitution, with no detrimental effect on activity. Least conserved are the residues responsible for K⁺ activation and dimerization of the E. coli MAT. The carboxylate of Glu-42, which is required for K⁺ activation of the E. coli enzyme (38), is replaced by more hydrophobic residues (Leu or Met), and Ser-263 is sometimes replaced by Ala. The predicted hydrogen bond between Ser-80 and Ser-80ʷ would be also lost. These changes in interfacial amino acids suggest that the interactions between M. jannaschii subunits may differ from those of the E. coli subunits.

Of the additional 68 residues that are conserved in the sequence alignment, 13 (17%) are surface residues in the E. coli structure, and the rest appear to be randomly distributed throughout the protein, suggesting that the majority of the matches may be coincidental. This analysis begs the possibility that the protein fold of the archaeal-type MAT is different from the bacterial enzyme; however, the apparent constraints of active site residues distributed through more than 250 residues in the linear sequence makes the possibility of a common topology seem plausible.

**Conclusions—** This study has identified the methionine adenosyltransferase gene from the hyperthermophilic archaeon M. jannaschii. This sequence has close homologs in the other archaea for which genome sequences have been reported. In contrast, the sequence is highly diverged from the majority of the bacterial and eukaryal MAT proteins, which are similar to...
each other. We anticipate that this archaeal class of MAT proteins will bolster future work in elucidating the complete mechanism of MAT catalysis. The identification of the sequence of \textit{M. jannaschii} MAT has also led to the finding that several bacteria, including the pathogenic organism \textit{S. pyogenes}, contain both bacterial- and archaeal-type MAT genes. The existence of two diverged classes, bacterial/eukaryal and archaeal versions, of a critical metabolic enzyme is reminiscent of previous observations for thymidylate synthase and dihydropyroate synthase (39). In aggregate, these differences emphasize the profound evolutionary events that accompanied the formation of modern archaeal metabolism. The apparent conservation of active site amino acids between these two diverse classes of MAT focuses our attention on functionally important residues that could not have been identified from alignments of bacterial and eukaryal forms due to the high level of overall sequence identity among those enzymes. Determination of the three dimensional structure of the archaeal enzyme will be of substantial interest.

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