Enzymatic Properties of S-Adenosylmethionine Synthetase from the Archaeon \textit{Methanococcus jannaschii}\textsuperscript{*}

Received for publication, October 31, 2001, and in revised form, February 25, 2002
Published, JBC Papers in Press, February 28, 2002, DOI 10.1074/jbc.M110456200

Zichun J. Lu and George D. Markham\textsuperscript{‡}

From the Fox Chase Cancer Center, Institute for Cancer Research, Philadelphia, Pennsylvania 19111

S-Adenosylmethionine synthetase \(\text{(ATP}:\gamma\text{-methionine S-adenosyltransferase, MAT)}\) catalyzes a unique enzymatic reaction that leads to formation of the primary biological alkylating agent. MAT from the hyperthermophilic archaeon \textit{Methanococcus jannaschii} (MjMAT) is a prototype of the newly discovered archaean class of MAT proteins that are nearly unrecognizable in sequence when compared with the class that encompasses both the eucaryal and bacterial enzymes. In this study the functional properties of purified recombinant MjMAT have been evaluated. The products of the reaction are AdoMet, PP\(_i\) and P\(_i\); >90\% of the P\(_i\) originates from the \(\gamma\)-phosphoryl group of ATP. The circular dichroism spectrum of the dimeric MjMAT indicates that the secondary structure is more helical than the \textit{Escherichia coli} counterpart (EcMAT), suggesting a different protein topology. The steady state kinetic mechanism is sequential, with random addition of ATP and methionine; AdoMet is the first product released, followed by release of PP\(_i\) and P\(_i\). The substrate specificity differs remarkably from the previously characterized MATs; the nucleotide binding site has a very broad tolerance of alterations in the adenosine moiety. MjMAT has activity at \(70 \, ^\circ\text{C}\) comparable with that of EcMAT at \(37 \, ^\circ\text{C}\), consistent with the higher temperature habitat of \textit{M. jannaschii}. The activation energy for AdoMet formation is larger than that for the \textit{E. coli} MAT-catalyzed reaction, in accord with the notion that enzymes from thermophilic organisms are often more rigid than their mesophilic counterparts. The broad substrate tolerance of this enzyme provokes routes to preparation of novel AdoMet analogs.

\textsuperscript{*}This work was supported by National Institutes of Health Grants GM31186 and CA06927 and by an appropriation from the Commonwealth of Pennsylvania. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\textsuperscript{‡}To whom correspondence should be addressed: Fox Chase Cancer Center, Inst. for Cancer Research, 7701 Burholme Ave., Philadelphia, PA 19111. Tel.: 215-728-2439; Fax: 215-728-3574; E-mail: gd_markham@fccc.edu.

\textsuperscript{1}The abbreviations used are: AdoMet, S-adenosyl-L-methionine; A\(\text{NH}\)\(\text{TP}\), 5’-amino-5’-deoxy-ATP; A\(\text{Si}\)\(\text{TP}\), 5’-mercapto-5’-deoxy-ATP; PNPNP, diimidotriphosphate (\(\text{O}_2\)\(\text{P}-\text{NH}-\text{PO}_3\)\(-\text{NH}-\text{PO}_3\)); PPP, triphosphate; MAT, S-adenosylmethionine synthetase; EcMAT, S-adenosylmethionine synthetase from \textit{E. coli}; MjMAT, S-adenosylmethionine synthetase from \textit{M. jannaschii}; DTT, dithiothreitol; MES, 4-morpholineethanesulfonic acid.

**Enzymatic Properties of S-Adenosylmethionine Synthetase from the Archaeon \textit{Methanococcus jannaschii}**

S-Adenosylmethionine (AdoMet)\textsuperscript{1} occupies a central role in the metabolism of all cells. The biological roles of AdoMet include acting as the primary methyl group donor, as a precursor to the polyamines, and as a progenitor of a 5 methyl group donor, as a precursor to the polyamines, and as a progenitor of a phosphoramidate chain.

1. \textit{Methanococcus jannaschii} synthetase from \textit{E. coli}; sticking (1–5).

2. Syl to the polyamines, and as a progenitor of a 5 phosphoramidate chain.

3. Include acting as the primary methyl group donor, as a precursor to the polyamines, and as a progenitor of a phosphoramidate chain.

4. Although the sequence of Syl is conserved, the catalytic mechanism operates with the archaeal enzyme, particularly at elevated temperature, is of interest in view of the unclear role of PPP, hydrolysis in the thermodynamics of AdoMet formation (12).

5. The present work has investigated the functional properties of MAT from \textit{M. jannaschii}, which is the first of the archaean class to be readily available in substantial amounts as a result of cloning and expression in \textit{E. coli} (11).

**EXPERIMENTAL PROCEDURES**

Reagents were purchased from Sigma unless noted. AdoMet was purchased from Research Biochemicals International, 5-Mercapto-5-deoxy-ATP (A\(\text{Si}\)\(\text{TP}\)), 5-amino-5-deoxy-ATP (A\(\text{NH}\)\(\text{TP}\)), purine triphosphate, 3-deaza-ATP, and 7-deaza-ATP were synthesized as described previously (13–16). Diimidotriphosphate (\(\text{O}_2\)\(\text{P}-\text{NH}-\text{PO}_3\)\(-\text{NH}-\text{PO}_3\); PNPNP) was synthesized by the Organic Synthesis Facility at FCCC as
described (15, 17). l-[methyl-14C]Methionine and H335PO4 were purchased from PerkinElmer Life Sciences. [carboxy-14C]AdoMet, [8-14C]ATP, and [γ-32P]ATP were purchased from Moravek Biochemicals. Ecoscint scintillation fluid was purchased from National Diagnostics. l-cis-2-Amino-4-methoxybut-3-enoic acid (18) was a generous gift from Dr. Janice Sufrin (Roswell Park Cancer Institute, Buffalo, NY).

**MjMAT Purification—Recombinant M. jannaschii AdoMet synthetase** was prepared from the strain BL21(DE3)(codon plus)/pMJ1208-1. This protein has a decahistidine tag on the N terminus (11). Cells were prepared from the strain BL21(DE3)(codon plus)/pMJ1208-1.

**Cell lysis:** Cells were grown at 37 °C in LB medium containing 50 μg/ml carbenecillin. Induction of MjMAT expression was obtained by addition of 1 mM isopropyl-1-thio-β-D-galactopyranoside to a 20-fold dilution of an overnight culture and shaking for 3 h. Cells were harvested by centrifugation and stored at −80 °C until use.

**Cell lysis:** The cell pellet was suspended in 50 mM Tris-HCl, 1 mM DTT, 30 μM phenylmethylsulfonyl fluoride, pH 8, using 10 ml of buffer/g (wet weight) of cells. The cells were lysed by one pass through a French press at 10,000 p.s.i. Debris was removed by centrifugation at 13,000 × g for 30 min. Ammonium sulfate was added to the supernatant to 20% saturation, and the insoluble material was removed by centrifugation. Ammonium sulfate was then added to the supernatant to 80% saturation, and the pellet was collected by centrifugation.

The scale of following steps is described for a preparation from 25 g (wet weight) of cells. The ammonium sulfate pellet was dissolved in ~20 ml of Buffer A (containing 50 mM Tris-HCl, 1 mM DTT, pH 8) and dialyzed overnight at 4 °C against 2 liters of the same buffer. The protein was divided into 5-ml portions and heated in a 85 °C water bath for 30 min. Precipitated materials were removed by centrifugation.

**Chromatographic steps:** Chromatographic steps were performed at room temperature. The protein was dialyzed overnight into 50 mM Tris-HCl, pH 8, and then NaCl and imidazole were added to final concentrations of 300 and 5 mM, respectively. The sample was loaded onto a 15-cm column of Ni2⁺-His-Bind resin (Novagen) that was equilibrated with 50 mM Tris-HCl, 5 mM imidazole, pH 8. The column was washed with 80 ml of starting buffer (at a flow rate of 1.0 ml/min) and eluted with a 200-ml gradient from the starting buffer to a final buffer of 50 mM Tris-HCl, 300 mM NaCl, 5 mM imidazole, pH 8. The fractions containing activity were dialyzed overnight against 50 mM Tris-HCl, 1 mM DTT, pH 8, and stored at −80 °C.

Minor contaminants were subsequently removed by ion exchange and gel filtration chromatography. Before ion exchange chromatography, the protein was equilibrated in 25 mM MES, 1 mM DTT, pH 6, by dialysis. The protein was loaded onto a HiLoad Q-Sepharose (Amersham Biosciences) column (2.6 × 12 cm) equilibrated and washed with 100 ml of dialysis buffer (at a flow rate of 2.5 ml/min), and the protein was eluted with a 500-ml gradient of this buffer also containing 0.7 mM KCl. The active fractions were pooled and dialyzed against 50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, pH 8. The final protein displayed a single band on Coomassie Blue-stained 8–25% gradient polyacrylamide Phast gels (Amersham Biosciences) in the presence and absence of SDS. The concentration of purified MjMAT was estimated from the absorbance at 280 nm using an extinction coefficient of 1.0 (mg/ml) −1 cm −1 as calculated from the amino acid composition (19). Approximately 50 mg of enzyme were obtained from 15 g (wet weight) of cells.

**E. coli AdoMet synthetase (EcMAT)** was purified as described previously (20).

AdoMet synthetase activity was determined by a 14C/AdoMet cation exchange filter binding method (20). Assays were performed at 55 °C in 25 mM Hepes(CH3)4N+ at pH 8.0 with 50 mM KCl, and 10 mM MgCl2. Routine assays contained 9.5 mM ATP and 0.5 mM [methyl-14C]methionine. Substrate saturation data were evaluated using the kinetic equations of Cleland (21) or the kinetics module of Sigmaplot 2000 (SPSS Science), which utilizes the Segel formalism (22). All fits were consistent with linear dependence of activity on substrate and/or inhibitor concentration. Inhibition studies were conducted with substrate concentrations near their Km values.

The activation energy for AdoMet synthesis, Ea, was calculated from the slope of linear ranges of an Arrhenius plot of ln(kcat) versus 1/T (slope = −Ea/R). The free energy of activation, ΔG*, was calculated as

\[ \Delta G^* = RT \ln(k_{cat}/T) - \ln(k_{cat}) \]

where R = 1.9872 cal/mol-K, kcat is the Boltzmann constant, h is Planck’s constant, and kcat is the value measured at temperature T. The activation enthalpy, ΔH*, was calculated as

\[ \Delta H^* = (E_a - RT) \]

The activation entropy ΔS* was then obtained from

\[ \Delta S^* = \Delta H^*/T \]

A more complete analysis of the temperature dependence of

---

**Fig. 1.** Circular dichroism spectra of MAT from *E. coli* and *M. jannaschii*. Spectra were obtained at 0.3 mg/ml protein in 25 mM Tris-HCl, 25 mM KCl, pH 8.0, and were corrected for buffer contributions. Open circles, *E. coli* MAT; closed circles, *M. jannaschii* MAT.

**Fig. 2.** Potassium activation of AdoMet formation. A and B show activation by K+ at different methionine and ATP concentrations, respectively. Solutions contained 25 mM Hepes(CH3)4N+ and 10 mM MgCl2 at pH 8.0. When methionine was varied the ATP concentration was fixed at 0.5 mM, and when ATP was varied the methionine concentration was fixed at 0.5 mM. In A the KCl concentrations were 0, 5, and 100 mM (○, □, △), and in B 5, 25, and 100 mM (□, ○, △). Reactions were conducted at 55 °C.
FIG. 3. **Steady state kinetics of AdoMet formation.** A and B show substrate saturation at different ATP and methionine concentrations. C–L illustrate inhibition by A(S)TP, L-ethionine, AdoMet, PPi, and Pi, respectively. When methionine was varied the ATP concentration was fixed at 0.2 mM, and when ATP was varied the methionine concentration was fixed at 0.4 mM. The concentrations of inhibitors used were as follows: A(S)TP (C and D): 0, 0.3, and 0.6 mM; L-ethionine (E and F): 0, 20, and 40 mM; AdoMet (G and H): 0, 1.5, and 3.0 mM; PPi (I and J): 0, 0.5, and 1.5 mM; Pi (K): 0, 1.0, and 2.0 mM; and Pi (L): 0, 0.6, and 1.2 mM. Solutions contained 25 mM Hepes(CH3)2N+, 50 mM KCl, and 10 mM MgCl2 at pH 8.0. Reactions were conducted at 55 °C.
Fig. 3—continued
Characterization of Archaeal S-Adenosylmethionine Synthetase

reaction rates considers that nonlinearity of an Arrhenius plot may arise from heat capacity change during the reaction, $\Delta C_p^{\circ}$ (24). Thus, with respect to a reference temperature ($T_0$, defined as 37 °C), at a given temperature $T$, $\Delta H(T) = \Delta H(T_0) + \Delta C_p^{\circ}(T - T_0)$ and $\Delta S(T) = \Delta S(T_0) + \Delta C_v^{\circ}(T - T_0)$. These relationships were used in fitting the observed data for MJMAT via nonlinear least squares using the program Scientist (MicroMath Inc.).

The phosphorus-containing reaction products were identified by evaluating PPP, PP, P, and formation from $\gamma$-32P-ATP in the presence and absence of inorganic pyrophosphatase. Compounds were separated by thin layer chromatography on polyethyleneimine-cellulose anion exchange thin layer sheets (EM Science) developed in 0.9 M LCl, 50 mM EDTA, pH 7. The radiation was quantified using a Fuji phosphorimager. A $\gamma$-32P-PP, standard was prepared from the $\gamma$-32P-ATP by periodate oxidation followed by aniline cleavage (12, 25); $\gamma$-32P-PP, was prepared from $\gamma$-32P, using E. coli MAT. Solutions contained 5 mM ATP (4.4 $\times$ 10$^5$ cpm/mmol), 5 mM methionine, 1 mg/ml MJMAT, 100 mM Hepes, pH 8, 50 mM KCl, 10 mM MgCl$_2$, when present, 0.025 units/ml inorganic pyrophosphatase were added. Time points were taken from 1 to 60 min. ATP was removed from the reactions by adsorption to Norit before TLC (12). ATP, P, PP, and PPP, standards had R$_f$ values of 0.27, 0.8, 0.13, and 0.04, respectively. After 4 h, ~77% of the $\gamma$-32P was present as P.

The reverse reaction was examined in two ways: by looking for either the formation of $\gamma$-32C)methionine from $\gamma$-32C)AdoMet or the formation of ATP or $\gamma$-32P from $\gamma$-32P, (Fig. 2). In the first case, 0.2 mM $\gamma$-32C)AdoMet (56 Ci/mmol), 5 mM P, and 5 mM P, were incubated with 1 mg/ml enzyme in 0.1 mM TrisCl, 50 mM KCl, 10 mM MgCl$_2$ for up to 60 min at 55 °C. Compounds were separated by thin layer chromatography on cellulose sheets developed in n-butyl alcohol: acetic acid:water (25:4:10) in which AdoMet and methionine have R$_f$ values of 0.27 and 0.56, respectively. Radioactivity was quantified by phosphorimaging. In the latter case, 5 mM AdoMet, 5 mM PP, and 5 mM $\gamma$-32P (11 Ci/mmol) were incubated with 1 mg/ml enzyme for up to 60 min at 55 °C. Compounds were separated by thin layer chromatography on polyethyleneimine-cellulose sheets and radioactivity quantified as described above.

Circular Dichroism (CD) Spectra—Protein secondary structure was assessed from circular dichroism spectra obtained on an Aviv model 62A spectropolarimeter. Samples (0.3 mg/ml protein in 25 mM Tris/HCl, 25 mM KCl, pH 8.0) were placed in 1-mm path length cells; spectra were recorded from 200 to 260 nm and were corrected for buffer contributions.

RESULTS

Recombinant MJMAT was purified to electrophoretic homogeneity from E. coli as described under "Experimental Procedures." The protein was allowed to retain an N-terminal decahistidine tag, because we had found that the properties of the recombinant protein were comparable with those of the protein isolated from M. jannaschii (11). Recombinant MJMAT chromatographed with a $V_m$ of 86,000 on gel filtration, consistent with its being a dimer of 45-kDa subunits (11). The purified enzyme readily crystallizes under a variety of common conditions, and attempts to obtain diffraction quality crystals are in progress.

CD spectra were compared for MJMAT and the E. coli enzyme to assess potential similarities in secondary structure (Fig. 1). MJMAT displays substantially larger ellipticity at 220 nm, a wavelength characteristic of the proportion of $\alpha$-helix. The crystal structures of the E. coli and rat liver enzymes show that they are composed of $-25\%$ $\alpha$-helix and $-20\%$ $\beta$-sheet (26, 27). The differences in CD spectra suggest that the overall secondary structure of MJMAT is more helical than the eu-

crysal or bacterial MAT-$\alpha$.

Origin of the P, Formed in the Reaction—A notable feature of MAT-$\alpha$-catalyzed reaction is that the products released from the enzyme are PP, and P, rather than PPP, initially formed in conjunction with AdoMet synthesis. The PPP, formed as an intermediate is primarily hydrolyzed even before it can reori-

...ent, with the result that >95% P, originates from the $\gamma$-phosphoryl group of ATP (6). Our previous studies of MJMAT dem-

...onstrated that PP, and P, were formed as products (11). When the products formed in the MJMAT-catalyzed reaction from $\gamma$-32P,ATP were analyzed, $\gamma$-32P, constituted 93% of the product and 7% was present in $\gamma$-32PP, $\gamma$-32PP, was not detected. Thus, even at the 55 °C temperature used in this experiment, the PPP, initially created upon AdoMet synthesis neither dissociates from the enzyme nor readily reorients before hydrolysis, and P, primarily originates from the $\gamma$-phosphoryl group of ATP (11).

Irreversibility of the Reaction—Attempts were made to measure the reverse reaction by conversion of [carboxy-$^{14}$C]AdoMet or $\gamma$-32P, to radiolabeled methionine or ATP, respectively (see "Experimental Procedures"). In neither case was any reaction observed, even using levels of enzyme and reaction times where 0.1% conversion could be measured; less than 0.1 eq of ATP, PPP, or AdoMet, per enzyme subunit, was formed in a 2500-fold longer time than that required for a single turnover in the forward direction. Thus, reversal of both the AdoMet forming and the PPP, hydrolytic steps appears to be unfavorable. This result is concordant with the established kinetic as well as thermodynamic irreversibility of AdoMet synthesis in reactions catalyzed by the $\gamma$-type enzyme (6, 12).

Cation Activation—No reaction was detected in the absence of Mg$^{2+}$, consistent with the divalent cation requirement of other MATs. K$^+$ both enhanced the $k_{cat}$ by 5-fold and decreased the $K_m$ values for both substrates, with half-maximal effect at 5 mM (Fig. 2). Saturating KCl decreased the $K_m$ for ATP from 1.4 to 0.22 mM and the $K_m$ for methionine from 1.1 to 0.3 mM. Plots of 1/v versus 1/K$^+$ at different substrate concentrations (and the converse) did not intersect on the 1/v axis, showing that the cation and substrate binding are not related by an equilibrium ordered binding process. The data indicate that K$^+$ is a stimulator rather than an essential activator, in common with the $\alpha$-type MATs.

Kinetic Mechanism—Substrate saturation kinetic studies of the AdoMet synthetic reaction show that MJMAT catalyzes a sequential reaction in which both ATP and methionine bind before products are released (Fig. 3, A and B). The $K_m$ values do not vary substantially with the concentrations of co-substrate, indicating little synergism in binding affinity.

An ATP analog that contains a C5'-S-P linkage was not a substrate for AdoMet formation, but was a competitive inhibitor with respect to ATP and noncompetitive with respect to methionine (Fig. 3, C and D), with a $K_i$ comparable with the $K_m$ for ATP (Table I). The alternate substrate GTP was also a competitive inhibitor with respect to ATP and noncompetitive with methionine (AdoMet formation was measured with [8-$^{14}$C]ATP in these inhibition experiments). A nonreactive analog of methionine that has high affinity was not identified. Cycloleucine, 1-amino-1-carboxy-cyclopentanone, is a commonly used dead-end inhibitor of the $\alpha$-type enzymes (28). However, 20 mM cycloleucine gave no detectable inhibition of MJMAT, even with both the substrates present at their $K_m$ values. Furthermore, MJMAT was not significantly inhibited (<10%) by 10 mM concentrations of L-homocysteine, L-norleucine, or L-cis-2-amino-4-methoxy-3-butenonic acid (the most potent methionine analog inhibitor of MAT-$\alpha$ (Ref. 18)). The alternate substrates L-threonine and L-methionine methyl ester were competitive inhibitors with respect to methionine for AdoMet formation from [methyl-$^{14}$C]methionine, and noncompetitive inhibitors with respect to ATP (cf. Fig. 3, E and F). The observed pattern of inhibition by nonreactive compounds and alternate substrates indicates that substrate binding is random. Both ethionine and methionine methyl ester have much smaller $K_m$ values than their $K_i$ values for inhibition of the reaction with methionine (6- and 70-fold, respectively), sug-

---

2 Z. J. Lu and G. D. Markham, unpublished results.
gesting the presence of substantial kinetic contributions to the $K_{in}$. The noncompetitive inhibition toward methionine by non-reactive ATP analogs reflects formation of dead-end enzyme-substrate-inhibitor complexes (21, 22).

Product inhibition studies showed that AdoMet is a noncompetitive inhibitor with respect to both methionine and ATP with $K_i$ values near 2 mM (Fig. 3, G and H). This high $K_i$ contrasts with that of most $\alpha$-type MATs for which AdoMet is a potent inhibitor with $K_i$ values in the $10^{-5}$ M range. Pyrophosphate is a competitive inhibitor with respect to ATP ($K_i = 0.83$ mM) and noncompetitive with respect to methionine (Fig. 3, I and J). Phosphate is a competitive inhibitor toward both ATP and methionine, with $K_i$ values comparable for the $K_i$ for PPi (Fig. 3, K and L). The product inhibition results indicate that product release is ordered with AdoMet dissociating before PPi and P, which subsequently dissociate randomly. Apparently, P binding prevents access of both ATP and methionine to their binding sites. This kinetic mechanism is illustrated in Scheme I. The finding that none of the inhibition patterns are uncompetitive, and that at least one product is a competitive inhibitor with each substrate, in conjunction with the dead-end and alternative substrate inhibition data, shows that the only compatible kinetic scheme for the forward reaction has random addition of ATP and methionine and partially random product release (22). The noncompetitive inhibition by AdoMet toward both substrates reflects formation of dead-end enzyme-substrate-AdoMet complexes with both ATP and methionine, whereas the noncompetitive inhibition by PPi, with respect to methionine reflects formation of a dead-end enzyme-methionine-PPi complex. Because the kinetics of the reverse reaction could not be studied, preferential binding order in that direction could not be further evaluated.

The nonhydrolyzable PPP, analog diimidotriphosphate (O,P-NH-PO2-PO3-NH-PO3) is a potent inhibitor of the $\alpha$-type EcMAT with a $K_i$ of 2 nM (17). Approximately 50% inhibition of MjMAT was observed at 2 nM PNPNP when enzyme, ATP, and methionine were present at 5 μM, 0.5 mM, and 2.4 mM, respectively (at 55 °C). The same result was obtained when the reaction was initiated by addition of enzyme or by addition of substrates to a mixture of enzyme and PNPNP. Clearly PNPNP has high affinity with a $K_i$ of less than 2 μM. Because of the complexities of quantitative analysis of tight binding inhibition (29), the details of the inhibition have not yet been pursued.

The AdoMet metabolites S-adenosylhomocysteine (20 mM) and 5’-methylthioadenosine (5 mM) produced less than 10% inhibition when ATP and methionine were present at 0.2 and 0.5 mM, respectively, suggesting that these compounds are unlikely to be significant physiological regulators of enzyme activity. The AdoMet analog sinefungin was also a poor inhibitor, with 12 mM sinefungin giving less than 25% inhibition under these conditions.

**Substrate Specificity**—Because AdoMet is the substrate of a large number of enzymes, the ability to prepare a variety of AdoMet analogs would be a valuable tool. EcMAT has been extensively used to prepare isotopically labeled AdoMet and some analogs despite limitations from severe product inhibition and significant substrate selectivity (14, 30, 31). The potential of MjMAT as a tool for synthesis of AdoMet analogs was assessed. Tables II and III compare the results for the use of ATP and methionine analogs by MjMAT and the E. coli enzyme. The tolerance of the ATP site of MjMAT is substantially greater than the bacterial enzyme, and MjMAT readily accepts modifications in both the adenine and ribose moieties. For example, GTP, UTP, and CTP are substrates for MjMAT but not for EcMAT. Both 2’-deoxy- and 3’-deoxy-ATP are substrates for MjMAT, whereas only the latter is accepted by EcMAT.

Neither MjMAT nor EcMAT catalyzes AdoMet formation from analogs in which the scissile C-O bond is replaced by a C-S

---

**Table I**

| Substrates | $K_m$ (mM) | $K_i$ (mM) |
|------------|------------|------------|
| ATP        | 0.29       | 0.26       |
| l-Methionine | 0.24       | 0.22       |

**Product and dead-end inhibition**

| $K_{in}$ vs. ATP | $K_{in}$ vs. methionine |
|------------------|-------------------------|
| AdoMet          | Inhibition type $K_{in}$, $K_i$ |
| NC, $K_i = 1.2$, $K_{in} = 2.5$ | NC, $K_i = 2.2$, $K_{in} = 3.0$ |
| PP              | $C, K_i = 0.83$ \n| P               | $C, K_i = 0.65$ \n| PPP             | $C, K_i = 0.12$ \n| ATP             | $C, K_i = 0.32$ \n| GTP             | $C, K_i = 1.5$ \n| l-Ethionine     | $NC, K_i = 31$, $K_{in} = 140$ \n| -Methionine     | $NC, K_i = 46$, $K_{in} = 25$ |

---

*a* C, competitive; NC, noncompetitive.

*b* $K_{in}$, $K_i$ value from slope; $K_{in}$, $K_i$ value from intercept (for non-competitive inhibitors). Reactions were conducted in 25 mM Hepes-(CH$_3$)$_2$N$^+$ at pH 8.0 with 50 mM KCl and 10 mM MgCl$_2$ at 55 °C. When methionine was varied the ATP concentration was fixed at 0.5 mM, and when ATP was varied the methionine concentration was fixed at 0.4 mM.

*c* When the methionine concentration was fixed at 5 mM, the inhibition was noncompetitive with $K_{in} = 2.9$ mM, $K_i = 6.5$ mM.

*d* When the ATP concentration was fixed at 5 mM, the inhibition was noncompetitive with $K_{in} = 5.2$ mM, $K_i = 8.1$ mM.
Characterization of Archaeal S-Adenosylmethionine Synthetase

TABLE II
MAT specificity at the ATP site
Reactions were conducted in the presence of 0.5 mM [methyl-14C]l-methionine in 25 mM Hepes · (CH3)4N+ at pH 8.0 with 50 mM KCl and 10 mM MgCl2. Data for MjMAT and EcMAT were obtained at 55 and 22 °C, respectively. EcMAT data from Refs. 14 and 16 except for Ki values for A/NH/TP and A/S/TP, which are from the present work. Compounds listed in the literature as inactive with EcMAT but which were found to be substrates for MjMAT were verified as inactive with EcMAT but which were found to be substrates for MjMAT were verified as inactive with EcMAT in the present study.

| Substrates                     | M. jannaschi | E. coli |
|-------------------------------|--------------|---------|
|                               | Vmax | Km   | Ki   | Vmax | Km   | Ki   |
| ATP                           | 100  | 0.26 |      | 100  | 0.11 |      |
| Adenosine-modified            |      |      |      |      |      |      |
| GTP                           | 68   | 0.62 | N    | 3    | N    | ND*  |
| CTP                           | 85   | 2.2  | N    | 0.10 | N    | ND   |
| UTP                           | 74   | 0.43 | N    | >8   | N    | ND   |
| Purine ribosome-TP            | 36   | 0.22 | 31   | 0.25 | 81   | 0.42 |
| 1,6-Etheno-ATP                | 15   | 0.42 | N    | >4   | N    | ND   |
| 2-Methylthio-ATP              | 69   | 0.50 | N    | N    | N    | ND   |
| 3-DeazaATP                    | 94   | 0.48 | 0.2  | 0.29 | 33   | 0.11 |
| 7-Deaza-ATP                   | 150  | 0.44 | 0.3  | 0.03 | 37   | 0.13 |
| 8-Bromo-ATP                   | 37   | 0.13 |      |      |      |      |
| Ribose-modified               |      |      |      |      |      |      |
| 2'-Deoxy-ATP                  | 81   | 0.42 | N    | >2   | N    | ND   |
| 3'-Deoxy-ATP                  | 97   | 0.52 | 62   | 0.10 |      |      |
| Phosphate-modified            |      |      |      |      |      |      |
| A/NH/TP                       | N    | 0.10 | N    | 0.096| N    | 0.22 |
| A/S/TP                        | N    | 0.10 | N    | >10  | N    |      |
| ADP                           | N    | 5.5  | N    |      | N    |      |

* N, no substrate activity was detected, <0.1% of ATP.
** ND, not determined.
* Ks as a competitive inhibitor with respect to ATP.

TABLE III
MAT specificity at the methionine site
Reactions were conducted in the presence of 0.5 mM [8-14C]ATP in 25 mM Hepes · (CH3)4N+ at pH 8.0 with 50 mM KCl and 10 mM MgCl2 at 55 °C for MjMAT and at 22 °C for EcMAT.

| Substrates | M. jannaschi | E. coli |
|------------|--------------|---------|
|            | Vmax | Km   | Ki   | Vmax | Km   | Ki   |
| l-Methionine| 100  | 0.24 | 0.08 | 100  | 0.08 |      |
| l-Ethionine| 37   | 0.74 | 9    | 8.7  |      |      |
| l-Methionine-methyl ester | 12   | 2.6  | 90   | 0.5  |      |      |
| d-Methionine| 13   | 3.5  | 14   |      |      |      |
| Active      |        |      |      |      |      |      |
| 3-Methylthiopropionaldehyde | N    | N    |      |      |      |      |
| 2-Keto-γ-methylthiobutyrate | N    | N    |      |      |      |      |
| 3-Methylthiopropylamine | N    | N    |      |      |      |      |
| l-Methioninol| N   | N    |      |      |      |      |

* <0.2% of the Vmax for methionine when present at 10 mM.
** <5% inhibition at 10 mM.

or C-N bond. The C5'-S and C5'-N containing compounds are good competitive inhibitors with respect to ATP, with Ki values near the Km for ATP for both enzymes.

The methionine site is rather restrictive in both MjMAT and EcMAT, but it is quantitatively different for the two enzymes (Table III). For both enzymes, the ethyl analog of l-methionine (i.e. l-ethionine) is a substrate, as is l-methionine methyl ester, but which is the better substrate differs for MjMAT and EcMAT. d-Methionine is also a substrate for both MjMAT and EcMAT. No activity was seen with either enzyme with the aldehyde methional, the alcohol methinol, or 3-methylthiopropylamine, all of which lack one of the polar attachments present in methionine.

Temperature Dependence of Kinetic Parameters—Fig. 4 illustrates an Arrhenius plot of the temperature dependence of kcat for MjMAT and EcMAT. The temperature dependence of kcat for MjMAT is nonlinear. Because the enzyme was stable at elevated temperatures and care was taken to measure initial reaction rates and verify substrate saturation, the change in slope of the Arrhenius plot apparently reflects a negative heat capacity (ΔCp) of activation (24). Analysis of the temperature dependence of kcat using ΔH‡ and ΔCp as parameters yielded values of 23 kcal/mol, +1 entropy units, and −0.3 kcal/mol-K, respectively, and the fit shown in Fig. 4. The negative heat capacity change is consistent with a protein conformational alteration during catalysis that results in a net decrease in exposure of nonpolar surface area (32). Interestingly, the MjMAT and EcMAT have comparable kcat values at approximately 37 and 70 °C, consistent with the optimal growth temperature for M. jannaschii of 87 °C. The ΔH‡ for EcMAT is 16
kcal/mol, lower than that for MjMAT. The activation entropy for the EcMAT-catalyzed reaction, $\Delta S^\circ_{\text{vap}}$, is $\sim\!7$ entropy units. The $K_m$ values vary less than 3-fold over the ranges studied for both enzymes, increasing with temperature in both cases.

**DISCUSSION**

Despite the widely different sequences of the $\alpha$- and $\gamma$-types of MAT, substantial similarities are present. Both classes of MAT require $\text{Mg}^{2+}$ for activity and are activated by $K^+$, although the monovalent cation is not essential. The enzymes hydrolyze the PPP$_i$ initially formed from ATP, to yield PP$_i$ and P$_i$; the P$_i$ largely originates as the $\gamma$-phosphoryl group of the nucleotide, indicating that motion of P$_i$ is restricted within the active site. Consistent with the tightly bound nature of the PPP$_i$ intermediate, the nonhydrolyzable analog diimidotriphosphate is a potent inhibitor of both MAT classes.

The steady state kinetic mechanism of MjMAT shows random substrate binding and ordered product release, AdoMet dissociating before PP$_i$ or P$_i$. Random substrate binding was previously found for MAT from *E. coli*, whereas some eucaryal MAT have ordered binding wherein ATP associates before methionine (8). The order of product release in the MjMAT reaction is AdoMet before PP$_i$ and P$_i$; with other MATs, both the same and different orders of product release have been observed. A remarkable property of MjMAT is the low affinity for AdoMet, with millimolar $K_m$ values, which contrasts with the $\alpha$-type MATs for which AdoMet typically inhibits with a $K_m$ in the physiologically significant $10^{-3}$ M range.

The substantial substrate activity of common naturally occurring nucleotides other than ATP, such as 2'-deoxy-ATP, GTP, CTP, and UTP, is surprising. Although the higher $K_m$ values compared with ATP and the probable lower intracellular concentrations of the other triphosphates may render the in vivo synthesis of large amounts of the corresponding sulphonium ions unlikely, whether physiologically significant quantities are made in vivo is as yet unclear. Thus, it is possible that there is an additional in vivo role of this enzyme in producing histidine unknown sulphonium metabolite. Such an as yet unidentified enzymatic role might rationalize why the primitive bacterium *Aquifex aeolicus*, which has one of the smallest genomes known for a free living organism, harbors genes for both classes of MAT (11).

The comparable activity of the *M. jannaschii* and *E. coli* enzymes near their physiological temperatures reflects the "principle of corresponding temperature" (33) and suggests a similar extent of utilization of AdoMet in these organisms. The thermal lability of AdoMet, both in chirality at the sulfonium center and in covalent bonding (34), requires a substantial metabolic flux through the synthetic reaction to maintain a pool of substrate for further metabolic requirements. The larger apparent activation energy for the MjMAT in the 0–37°C range reflects an increase in the apparent enthalpy of activation, whereas there is a more favorable entropy of activation. These observations are consistent with the notion that enzymes from organisms that inhabit warmer environments are "stiffer" than those from cooler habitats (23).

The tolerance of MjMAT for chemical alterations of both the methionine and ATP moieties, the low affinity for the product AdoMet, in conjunction with the stability of the protein, suggest that this enzyme may be a useful synthetic tool for preparation of AdoMet analogs. In view of the myriad of roles of AdoMet in metabolism, and the paucity of available analogs, the feasibility of these syntheses are being explored.

**Acknowledgments**—We thank John C. Taylor for aid throughout this project and the Fox Chase Organic Synthesis Facility for preparation of the diimidophosphate.

**REFERENCES**

1. Tabor, C. W., and Tabor, H. (1984) *Annu. Rev. Biochem.* 53, 749–790
2. Cantoni, G. L. (1975) *Annu. Rev. Biochem.* 44, 435–451
3. Chiang, P. K., Gordon, R. K., Tal, J., Zeng, G. C., Doctor, B. P., Pardhasaradhi, K., and McCann, P. P. (1996) *FASEB J.* 10, 471–480
4. Frey, P. A. (2001) *Annu. Rev. Biochem.* 70, 121–148
5. Sofia, H. J., Chen, G., Hetzel, B. G., Reyes-Spindola, J. F., and Miller, N. E. (2001) *Nucleic Acids Res.* 29, 1097–1106
6. Mudd, S. H. (1973) in *The Enzymes* (Boyer, P. D., ed) 3rd Ed.,Vol. 8, pp. 21–54, Academic Press, New York
7. Matsu, M. I., Alvarez, L., Ortiz, P., and Pajares, M. A. (1997) *Pharmacol. Ther.* 73, 263–280
8. Koth, M., and Geller, A. M. (1993) *Pharmacol. Ther.* 59, 125–143
9. Tabor, C. W., and Tabor, H. (1984) *Adv. Enzymol.* 56, 251–282
10. Kotb, M., Mudd, S. H., Matsu, M. I., Geller, A. M., Kredich, N. M., Chou, J. Y., and Cantonio, G. L. (1997) *Trends Genet.* 13, 51–52
11. Graham, D. E., Bock, C. L., Schalk-Hibi, C., Lu, Z. J., and Markham, G. D. (2000) *J. Biol. Chem.* 275, 4055–4059
12. McQueney, M. S., Anderson, K. S., and Markham, G. D. (2000) *Biochemistry* 39, 4443–4454
13. Trowbridge, D. B., Yamamoto, D. M., and Kenyon, G. L. (1972) *J. Am. Chem. Soc.* 94, 3816–3824
14. Markham, G. D., Hafner, E. W., Tabor, C. W., and Tabor, H. (1980) *Biochemistry* 19, 3816–3824
15. Ma, Q. F., Kenyon, G. L., and Markham, G. D. (1990) *Biochemistry* 29, 1412–1416
16. Taylor, J. C., and Markham, G. D. (2000) *J. Biol. Chem.* 275, 4060–4065
17. Reczekowski, R. S., and Markham, G. D. (1999) *Biochemistry* 38, 9063–9068
18. Sufrin, J. R., Lombardini, J. B., and Keith, D. D. (1996) *Biochem. Biophys. Res. Commun.* 215, 211–215
19. Genetix Computer Group (1999) *Peptideisor*, Version 10, Genetics Computer Group, Madison, WI
20. Reczekowski, R. S., Taylor, J. C., and Markham, G. D. (1998) *Biochemistry* 37, 13499–13506
21. Cleland, W. W. (1979) *Methods Enzymol.* 63, 103–138
22. Segel, I. H. (1975) *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems*, Wiley-Interscience, New York
23. Lohmann, T., Gerday, C., and Feller, H. (2000) *Biochem. Biophys. Acta* 1543, 1–10
24. Cooper, A., Johnson, C. M., Lakey, J. H., and Nollmann, M. (2001) *Biophys. Chem.* 95, 215–230
25. Dunaway-Mariano, D., and Cleland, W. W. (1980) *Biochemistry* 19, 1496–1505
26. Takusagawa, G., Kamitori, S., Misaki, S., and Markham, G. D. (1996) *J. Biol. Chem.* 271, 136–147
27. Gonzalez, B., Pajares, M. A., Hermosa, J. A., Alvarez, L., Garrido, F., Sufrin, J. R., and Sanz-Aparicio, J. (2000) *J. Mol. Biol.* 300, 363–375
28. Coutler, A. W., Lombardini, J. B., Sufrin, J. R., and Talalay, P. (1974) *Mol. Pharmacol.* 10, 319–334
29. Szelueczek, S. E., and Duggleby, R. F. (1995) *Methods Enzymol.* 249, 144–180
30. Park, J., Tal, J., Roessner, C. A., and Scott, A. I. (1996) *Bioorg. Med. Chem.* 4, 2179–2185
31. Kumar, I. L., Kladanova, D. M., Van Kirk, E. A., and Halsey, B. E. (1983) *J. Biol. Chem.* 258, 1747–1751
32. Murphy, K. P., and Freire, E. (1992) *Adv. Protein Chem.* 43, 313–361
33. Somero, G. N. (1995) *Annu. Rev. Physiol.* 57, 43–68
34. Hoffman, J. L. (1990) *Biochemistry* 28, 4444–4449