The structure of S-adenosylmethionine synthetase (MAT, ATP:L-methionine S-adenosyltransferase, EC 2.5.1.6.) from Escherichia coli has been determined at 3.0 Å resolution by multiple isomorphous replacement using a uranium derivative and the selenomethionine form of the enzyme (SeMAT). The SeMAT data (9 selenomethionine residues out of 383 amino acid residues) have been found to have a sufficient phasing power to determine the structure of the 42,000 molecular weight protein by combining them with the other heavy atom derivative data (multiple isomorphous replacement). The enzyme consists of four identical subunits; two subunits form a spherical tight dimer, and pairs of these dimers form a peanut-shaped tetrameric enzyme. Each pair dimer has two active sites which are located between the subunits. Each subunit consists of three domains that are related to each other by pseudo-3-fold symmetry. The essential divalent (Mg\(^{2+}/Co^{2+}\)) and monovalent (K\(^+\)) metal ions and one of the product, P\(_i\), ions, were found in the active site from three separate structures.

In biological systems, there are a myriad of reactions in which methyl groups are transferred from a few types of methyl donors to a wide variety of methyl acceptors. Among biological methyl group donors, S-adenosylmethionine (AdoMet), discovered by Cantoni in 1953 (1), is the most widely used, while 5-methyltetrahydrofolic acid, methylcobalamin, and betaine are involved in far fewer methylation reactions. The activated methyl cycle involving AdoMet is illustrated below (Scheme 1).

AdoMet, synthesized from L-methionine and ATP, transfers a methyl group to a methyl acceptor molecule, yielding S-adenosylhomocysteine. S-Adenosylhomocysteine is subsequently hydrolyzed to adenosine and homocysteine, the latter of which can be metabolized to methionine.

Methylation reactions involving AdoMet are increasingly being recognized as significant control factors in the regulation of a variety of cellular functions. Methylation of nucleic acids is known to have regulatory effects on DNA replication and transcription, as well as the regulation of a variety of metabolic processes such as bacterial chemotaxis (2, 3), sperm mobility (4), and release of neurotransmitters (5). AdoMet is also the methyl donor to a vast number of small molecules (e.g., in the biosynthesis and/or metabolism of various catecholamine neurotransmitters). AdoMet is being used in clinical trials for treatment of depression (6), liver injury (7), and as a potential cancer chemopreventive agent (8). In addition to its role as a methyl donor, AdoMet undergoes decarboxylation to generate the propylamine donor in the biosynthesis of the polyamines, spermine and spermidine, which are widely distributed in nature and appear to be involved in cellular proliferation (9). Recently, inhibition of the metabolism of AdoMet has been found to be an important target for development of the chemotherapeutic agents for neoplastic and viral diseases (10, 11). Regland and Gottlieb (12) have proposed that slowed synthesis of AdoMet is a pathogenetic mechanism common to the dementia seen in Alzheimer’s disease, Down’s syndrome, and the acquired immunodeficiency syndrome.

The formation of AdoMet is catalyzed solely by AdoMet synthetase (MAT, ATP:L-methionine S-adenosyltransferase, EC 2.5.1.6). The synthesis of AdoMet occurs in an unusual two-step reaction in which the complete triplyphosphate chain is cleaved from ATP as AdoMet is formed, and the triplyphosphate is subsequently hydrolyzed to pyrophosphate (PP\(_i\)) and orthophosphate (P\(_i\)) before the sulfonium product (AdoMet) is released, giving the overall reaction shown below (13).

\[
\text{ATP} + \text{L-Met} + \text{H}_2\text{O} \rightarrow \text{AdoMet} + \text{PP}_i + \text{P}_i \quad (\text{Eq. 1})
\]

Thus, the enzyme uniquely catalyzes reactions at both ends of the triplyphosphate chain in a defined temporal sequence. AdoMet synthetase requires divalent metal ions for activity (e.g., Mg\(^{2+}\), Co\(^{2+}\)) and is greatly activated by certain monovalent cations such as K\(^+\).

The amino acid sequences of the enzyme from Escherichia coli (14, 15), Saccharomyces cerevisiae (16, 17), Arabidopsis thaliana (18, 19), rat liver (20), rat kidney (21), human liver (22), human kidney (23), and the leaf of Dianthus caryophyllus (24) have been reported deduced based on DNA sequences. In all organisms studied so far, sequence homologies show that AdoMet synthetase is an exceptionally well conserved enzyme through evolution. No biological data regarding the amino acid residues involved in the active site of MAT has been reported. The first crystallization of MAT was reported by Gilliland et al. (25); however, no subsequent work was reported. The determi-
nation of the three-dimensional structure of the enzyme is essential for elucidation of the unique catalytic mechanism and will also facilitate development of effective inhibitors of this enzyme. In the present paper, we describe the x-ray structures of three MAT complexes with the product Pi ions and various metal ions.

**EXPERIMENTAL PROCEDURES**

Crystallization—AdoMet synthetase (MAT) was prepared from E. coli strain DM50pK8, a strain which harbors the plasmid pK8 which contains the structural gene for the enzyme and produces the MAT as nearly 20% of the total cell protein (14). The enzyme was purified to electrophoretic homogeneity using methods described previously (26, 27). MAT, in which all methionine residues were replaced by selenomethionine (SeMAT), was prepared by transfer of pK8 into the methionine auxotrophic strain DL41, followed by growth of DL41pK8 on defined media containing selenomethionine in place of methionine as described by Hendrickson (28). The SeMAT protein was purified in the same fashion as the unsubstituted enzyme and displayed no alterations in physical properties. The degree of selenomethionine substitution was determined by an electrospray mass spectrometry as described below.

Crystals of the native enzyme (MAT) and SeMAT from E. coli were grown in a buffer containing 100 mM potassium phosphate (pH 7.0), 10 mM MgCl2, 33% (v/v) saturated ammonium sulfate with the protein concentration at 10 mg/ml. The hexagonal bipyramidal shaped crystals were grown at 26 °C for 2 weeks. Crystals of the native enzyme containing Co2+ ions in the active site (MAT-Co) were grown from a similar solution which contained 10 mM CoCl2 instead of 10 mM MgCl2. The uranium derivative crystals were prepared by the soaking method; native crystals were incubated in an artificial mother liquor containing 50 mM Tris-malate buffer (pH 7.0), 10 mM MgCl2, 40% saturated ammonium sulfate, and 5 mM UO2(NO3)2 for 3 h before they were mounted into the capillary.

Data Measurement—Preliminary precession photographs showed that the crystals belonged to the hexagonal crystal system with space group P6222 or its enantiomorph with cell dimensions a = b = 129 Å, c = 139 Å. As the crystals decayed very quickly under the x-ray exposure conditions required for data collection, the total intensity data were obtained from several fresh crystals. Diffraction data for the native enzyme (MAT) and the SeMAT were measured on a locally modified DIP100S, the diffraction data were measured at the University of Kansas using a DIP100S imaging plate x-ray diffractometer; MARK-III, the diffraction data were measured at the University of California at San Diego using the MARK-III multiwire area detector x-ray diffractometer.

**TABLE I**

| Source of data | MAT | SeMAT | MAT-Co | MAT-U |
|---------------|-----|-------|--------|-------|
| Data set     | DIP100S | DIP100S | MARK-III | MARK-III |
| Resolution (Å) | 10–3.0 | 10–3.0 | 10–3.0 | 10–3.0 |
| Number of crystals | 3 | 10 | 8 | 12 |
| Number of unique reflections | 13,223 | 13,507 | 13,378 | 13,135 |
| % complete | 95.7 | 97.7 | 96.8 | 95.0 |
| Rsym | 0.072 | 0.069 | 0.070 | 0.069 |
| I/σ(I) | 2.87 | 2.77 | 2.59 | 2.08 |
| Number of heavy atom sites | 9 | 2 | 2 | 2 |
| RFDa | 0.16 | 0.40 | 0.45 | 1.64 |
| Phasing power | 1.76 | 0.42 (0.78) | 0.33 (0.76) | 0.57 (0.71) |
| FOM of MIRb | 0.57 (0.81) | 0.57 (0.81) | 0.57 (0.81) | 0.57 (0.81) |

**Refinement parameters**

| Number of residues | 377 | 377 | 377 | 377 |
| Number of atoms | 2899 | 2899 | 2899 | 2899 |
| Number of water molecules | 0 | 0 | 0 | 0 |
| Contents of ions | (2PO4, 2Mg, 2K) | (2PO4, 2Mg, 2K) | (2PO4, 2Co, 2K) | (2PO4, 2Co, 2K) |
| R1 | 0.189 | 0.188 | 0.197 | 0.197 |
| Free R | 0.260 | 0.260 | 0.260 | 0.260 |
| Root mean square deviations | Bond (Å) | 0.014 | 0.015 | 0.015 |
| Angle (°) | 3.5 | 3.4 | 3.5 |
| Torsion angle (°) | 25.7 | 25.8 | 25.6 |
| Mean B values | Calculus | 34.2 | 36.1 | 32.1 |
| Main chain | 34.9 | 36.6 | 32.7 |
| All atoms | 37.9 | 39.2 | 35.3 |

a DIP100S, the diffraction data were measured at the University of Kansas using a DIP100S imaging plate x-ray diffractometer; MARK-III, the diffraction data were measured at the University of California at San Diego using the MARK-III multiwire area detector x-ray diffractometer.
b Rsym = Σ[I - (I)]/ΣI.I.
c I/σ(I) in 3.0–3.1 Å resolution range.
d RFD = Σ|Fobs - Fcalc|/ΣFobs. In the phase determination, the SeMAT and MAT-U data were cut off at 3.5 Å and 4.0 Å resolution, respectively.
e R = Σ|Fobs - Fcalc|/ΣFobs.
f Phasing power = (Fobs - Fcalc)/ΣFobs.
g FOM of MIR = figure of merit of multiple isomorphous replacement. The value after solvent flattening phase refinement is given in parentheses. The phasing statistics listed in the table are for 4.0 Å resolution data. The completeness of the data at 4.0 Å resolution level is more than 98.7% for all data sets.
h FOM of MIR = figure of merit of single isomorphous replacement (Se and U derivatives). The value after solvent flattening phase refinement is given in parentheses.

**SCHEME 1. Activated methyl cycle.**
DIP100S imaging plate x-ray diffractometer (MAC Science, Japan), while data for the uranium derivative (MAT-U) and the cobalt derivative (MAT-Co) were measured on the MARK III multiwire area detector x-ray diffractometer at University of California at San Diego (29); CuKα radiation was used in both cases. The data of the native enzyme and the SeMAT were processed with the program ELMS (30), whereas the data of the MAT-U and the MAT-Co were processed by the software associated with MARK III (29). Integrated reflections were scaled and reduced with locally developed programs (31). The data statistics are given in Table I.

### Structure Determination

The uranium positions (two sites) were located in a difference Patterson map and were refined by the locally developed program HEVY. The positions are listed in Table II. Initial protein phases were determined from the single isomorphous replacement (SIR) data in conjunction with solvent flattening (32). The phases of the 4.0 Å resolution isomorphous data were refined and gradually extended to 3.0 Å resolution by solvent flattening. The space group, P6222, was selected by reference to the observed α-helix configuration. Although the electron density map calculated with the initial phases showed the molecular boundaries and all α-helices and β-sheets, it was difficult to trace the main chain correctly and to assign the side chain groups.

The difference electron density map calculated with coefficients ($F_{\text{SeMAT}} - F_{\text{MAT}}$) and the phases obtained from the SIR described above showed nine peaks (>7σ) centered on the selenium atoms of the nine selenomethionine residues (Fig. 1). The positions of the selenium atoms were refined by using the centric reflections (Fig. 1). The positions of the selenium atoms were refined by using the centric reflections. The relatively high B-values of selenium atoms refined by using the centric reflections might be due to some systematic errors introduced by the many crystals used to collect each data set, but all SeMet residues in SeMAT structure are well-ordered in the final structure. The calculated phasing power of the SeMAT data (1.76) was as strong as that of the MAT-U derivative data (1.64). Therefore, the protein phases were determined by the SeMAT isomorphous replacement data in conjunction with solvent flattening, x-ray diffractometer at University of California at San Diego (29); CuKα radiation was used in both cases. The data of the native enzyme and the SeMAT were processed with the program ELMS (30), whereas the data of the MAT-U and the MAT-Co were processed by the software associated with MARK III (29). Integrated reflections were scaled and reduced with locally developed programs (31). The data statistics are given in Table I.

### Table II

| Site | x | y | z | Occupancy | B<sup>a</sup> | Binding site<sup>b</sup> |
|------|---|---|---|-----------|---------|-------------------|
| MAT-U | | | | | | |
| Site 1 | 0.162 | 0.324 | 0.000 | 0.500 | 39.7 | Glu<sup>113</sup> |
| Site 2 | 0.047 | 0.400 | 0.117 | 0.567 | 15.5 | Glu<sup>42</sup> |
| SeMAT | | | | | | |
| Site 1 | −0.079 | 0.184 | 0.092 | 1.000 | 14.5 | Met<sup>134</sup> |
| Site 2 | 0.065 | 0.298 | 0.090 | 1.000 | 26.5 | Met<sup>122</sup> |
| Site 3 | −0.041 | 0.456 | 0.177 | 1.000 | 39.9 | Met<sup>49</sup> |
| Site 4 | −0.201 | 0.330 | 0.149 | 1.000 | 37.7 | Met<sup>82</sup> |
| Site 5 | −0.143 | 0.389 | 0.209 | 1.000 | 45.9 | Met<sup>236</sup> |
| Site 6 | −0.271 | 0.115 | 0.193 | 1.000 | 69.3 | Met<sup>203</sup> |
| Site 7 | 0.026 | 0.242 | −0.003 | 1.000 | 62.0 | Met<sup>143</sup> |
| Site 8 | −0.070 | 0.149 | 0.157 | 1.000 | 57.9 | Met<sup>254</sup> |
| Site 9 | 0.064 | 0.207 | 0.122 | 1.000 | 56.1 | Met<sup>311</sup> |

<sup>a</sup> Occupancy on the absolute scale referred to the SeMAT data.

<sup>b</sup> B, isotropic temperature factor (Å²).

<sup>c</sup> Binding site or corresponding site.

<sup>d</sup> Special site, i.e. on the 2-fold axis.

### Figure 1

The difference electron density map calculated with the coefficients of ($F_{\text{SeMAT}} - F_{\text{MAT}}$) and the phase angles obtained by the single isomorphous replacement (SIR) using the UO<sub>2</sub> derivative data. Nine peaks (>7σ) centered on the selenium atoms of the nine selenomethionine residues are visible. The map was calculated with 10–4.0 Å resolution data, and the contour was drawn at 5.0 s level.

### Figure 2

Electron density maps of the B5 β-strand (119QGLMF-GYATN<sup>128</sup>) containing various sized residues. A, SIR map phased by the MAT-U derivative; B, SIR map phased by the SeMAT; C, MIR map phased by the MAT-U and SeMAT; D, final (2F<sub>c</sub>−F<sub>c</sub>) map; E, omit map. The SIR and MIR maps were calculated with the phases refined and extended by solvent flattening. The omit map was calculated after 30 cycles of the positional refinement by X-PLOR. The contours are drawn at 1.5σ level for map A, B, C, and D and 3.0σ for map E. The final model is superimposed in the maps.
in order to test whether the SeMAT data have enough phasing power as indicated by the calculation and whether the positions of the selenium atoms are reliable. As shown in Fig. 2, the map calculated using the phases determined from the SeMAT data had the similar electron density distribution seen in the map calculated from MAT-U data. Thus, the MIR phases (4.0 Å resolution) were obtained by combining the phase distribution functions of uranium and selenium derivatives. The phases were refined and extended from 4.0 Å to 3.0 Å resolution by solvent flattening. It is noted that the SeMet forms of enzymes have recently been used successfully for one of the MIR derivatives (33, 34). The map calculated with the new phases was improved (Fig. 2). The entire main chain except for Ala1-His3, Ile102-Asp107, and Gly381-Lys383 was traced without ambiguity, and the side chain groups of 343 out of 383 residues were placed in the electron density peaks contoured at 1.1σ level. The initial model was built on an IRIS work station using the program TOM/FRODO (35–36). The model was refined with the positional protocol and then the simulated annealing procedure of X-PLOR (37). The model was rebuilt where necessary, and previously undefined residues were built into the electron density map.

During later stages of refinement, difference maps calculated with coefficients of \(F_o - F_c\), and calculated phases showed several significant residual electron density peaks in the region of active site (Fig. 3). The electron density peaks were assigned to two P, one K+, and two Mg2+ ions based on the size of the electron density, refined temperature factors, and polar environment, and those ions were introduced into the refinement. Assignment of the divalent ions (Mg2+) was confirmed with the structure analysis of MAT-Co which is described below. The additional residual electron density peak found near the center of tight dimer and on the 2-fold axis was significant and assigned to a K+ ion based on the size of the electron density peak and polar environment. Refinement of isotropic temperature factors for individual atoms was carried out by the individual B-factor refinement procedure of X-PLOR using bond and angle restraints. The thermal parameters of the two P, and metal ions were relatively large in comparison with those of the amino acid residues constituting the active site, suggesting that the ion sites are partially occupied. After four cycles of model building and refinement, all residues from 1 to 101 and 108 to 383 were built into the electron density map. The residues 102 to 107, which are outside the active site, were not visible in the electron density maps and were assumed to be heavily disordered. Residues 102 to 107 were arbitrarily placed using a TOM/FRODO routine; however, those residues were not included in the refinement.

### Table III

| Resolution | MAT | SeMAT | MAT-Co |
|------------|-----|-------|--------|
| Å          | No. reflections | R value | No. reflections | R value | No. reflections | R value |
| 10.00–5.66 | 1746 | 0.196 | 1810 | 0.204 | 1793 | 0.207 |
| 4.64–5.66  | 1691 | 0.150 | 1728 | 0.152 | 1706 | 0.159 |
| 4.10–4.64  | 1699 | 0.141 | 1699 | 0.144 | 1697 | 0.150 |
| 3.75–4.10  | 1650 | 0.165 | 1684 | 0.167 | 1678 | 0.173 |
| 3.49–3.75  | 1628 | 0.189 | 1665 | 0.191 | 1642 | 0.191 |
| 3.29–3.49  | 1616 | 0.224 | 1654 | 0.216 | 1654 | 0.227 |
| 3.13–3.29  | 1617 | 0.267 | 1663 | 0.252 | 1637 | 0.284 |
| 3.00–3.13  | 1576 | 0.300 | 1604 | 0.279 | 1571 | 0.302 |

![MAT(2PO₄, 2Mg, K)](image1)

![MAT-Co (2PO₄, 2Co, K)](image2)

**Fig. 3.** Difference electron density maps calculated after 30 cycles of positional refinement of the protein structure (not including the P, and metal ions) by X-PLOR. The contours are drawn at 3σ level. The final model is superimposed on the map. The Mg²⁺/Co²⁺ ions (X) and the P ions are shown in the center of maps. The residual electron density peak of the K⁺ ion (X) is visible in the left side of the maps.

**TABLE III**

**Distribution of R factor**

| Resolution | MAT | SeMAT | MAT-Co |
|------------|-----|-------|--------|
| Å          | No. reflections | R value | No. reflections | R value | No. reflections | R value |
| 10.00–5.66 | 1746 | 0.196 | 1810 | 0.204 | 1793 | 0.207 |
| 4.64–5.66  | 1691 | 0.150 | 1728 | 0.152 | 1706 | 0.159 |
| 4.10–4.64  | 1699 | 0.141 | 1699 | 0.144 | 1697 | 0.150 |
| 3.75–4.10  | 1650 | 0.165 | 1684 | 0.167 | 1678 | 0.173 |
| 3.49–3.75  | 1628 | 0.189 | 1665 | 0.191 | 1642 | 0.191 |
| 3.29–3.49  | 1616 | 0.224 | 1654 | 0.216 | 1654 | 0.227 |
| 3.13–3.29  | 1617 | 0.267 | 1663 | 0.252 | 1637 | 0.284 |
| 3.00–3.13  | 1576 | 0.300 | 1604 | 0.279 | 1571 | 0.302 |
The structure determination has been verified by the following procedures. The R-factor distributions in several resolution shells are reasonably uniform (Table III), and the free R (38) calculated using 10% reflections selected randomly is relatively low (0.26). The structure was also checked by the computer program PROFILE 3D (39), and no unusual connectivity has been found in the protein structure (Fig. 4). Also, a standard protein structure verification program, PROCHECK (40), does not indicate any unusual feature in the structure. The Ramachandran plot (41) indicates that nearly all residues are in allowed conformations (Fig. 5). The root mean square deviations of bond distances, angles, and torsion angles from the ideal geometry data are relatively small (Table I). As shown in Fig. 2, an omit map calculated after 30 cycles of the positional refinement reproduces the similar electron density distribution seen in the final (Fo − Fc) map. Finally, the correctness of the main chain connectivity is strongly supported by the difference electron density map calculated with the coefficients of (FSeMAT − FSeMAT) and the phases obtained by the SIR using the UO2 derivative data showed nine peaks (>7σ) at the positions of the selenium atoms of nine selenomethionine residues (Fig. 1). There is little chance of incorrectly tracing the peptide chain and still correctly locating the nine methionine sites. The parameters for the structure determination and refinement are listed in Table I. The coordinates have been deposited in the Protein Data Bank (code numbers 1XRA, 1XRB, and 1XRC).

The structures of MAT-Co and SeMAT were initially refined with the coordinates of the protein structure described above. The difference map for SeMAT calculated after 30 cycles of the positional protocol, showed the same residual electron densities in the active site as were observed in the MAT structure. The residual electron density peaks in the MAT-Co structure were also quite similar to those of the MAT structure. However, the electron density peaks at the positions where the Mg2+ ions were assigned in the MAT structure were significantly higher in the MAT-Co structure (Fig. 3). On the basis of this difference in the residual electron density peaks, the binding locations for the divalent ions (Mg2+ or Co2+) in the active site were deduced.

### RESULTS AND DISCUSSION

New Amino Acid Sequence—As described above, the difference electron density map calculated with the coefficients of (FSeMAT − FSeMAT) and the phases obtained by the SIR using the UO2 derivative data showed nine peaks (>7σ), which agrees with nine methionine residues in the sequence determined previously (14). However, amino-terminal sequences of both enzyme forms (MAT and SeMAT) showed alanine 2 as the terminus, indicating that the amino-terminal methionine encoded by the gene had been removed. Thus, the nine methio-
nine residues found by x-ray analysis did not agree with the eight deduced from the published gene sequence (14). Therefore, the number of selenomethionine residues incorporated into the enzyme was determined independently by mass spectrometry. Molecular masses of the Met-enzyme and the SeMet-enzyme were determined by an electrospray-mass spectrometry at the Protein and Carbohydrate Structure Facility at the University of Michigan. Masses of 41,843.0 ± 10.5 and 42,249.7 ± 9.6 were obtained for the unsubstituted enzyme and the SeMet-enzyme, respectively. The mass difference indicates that 8.7 ± 0.3 SeMet residues are incorporated per subunit, which agrees with the value of nine predicted from the x-ray data. Therefore, the metK gene was completely resequenced using the Sequenase polymerase (U. S. Biochemical Corp.) rather than the Klenow polymerase used previously. Several errors in the original sequence were found, apparently resulting from the inability of the Klenow fragment to correctly read some regions of the DNA. The revised sequence will be deposited in GenBank. The new sequence of metK gene indeed contains 10 methionine residues including the amino-terminal residue. The molecular weight (41,835.1) calculated from the new sequence is in much better agreement with the molecular weight (41,843.0) obtained by an electrospray-mass spectrometry than the molecular weight (41,935.6) calculated from the earlier sequence. The structure has been solved based on the new sequence as listed in Table IV.

Overall Structure of the Tetrameric Enzyme—The structures of the MAT protein in the three individual crystal structures described here are essentially indistinguishable. The root mean square deviations among the three MAT structures (MAT, SeMAT, and MAT-Co) are summarized in Table V. Particularly the structures of MAT and SeMAT are equivalent within experimental uncertainty, suggesting that replacement of the methionine residues with selenomethionine does not change the structure of the protein. On the other hand, the MAT-Co structure deviates significantly from the other two. As will be discussed below, these differences might be due to the different coordination schemes around the divalent metals. The other possible explanation for the deviation is due to different data sources, i.e. the MAT and SeMAT data were measured on a DIP100S imaging plate diffractometer, whereas the MAT-Co data were measured on the MARK-III multwire detector x-ray diffractometer.

MAT consists of four identical subunits related by 222 symmetry (Fig. 6). The four subunits are denoted subunits A, B, C, and D. Pairs of subunits (A and B or C and D) strongly interact with each other to form a spherical tight dimer, and these tight dimers associate to a peanut-shaped tetrameric enzyme. The interactions between the tight dimers appear to be much weaker than the subunit-subunit interactions within the tight dimer. Each tight dimer has two active sites located between subunits A and B or C and D, with amino acid residues from both subunits contributing to each active site (Fig. 6). We use the following convention to distinguish between residues in subunits A and B. A residue name with no added symbol refers to a residue of subunit A; a residue name with an appended asterisk refers to a residue of subunit B. For example, Glu42 means Glu42 of subunit A, and Arg244* means Arg244 of subunit B. The convention also applies to elements of secondary structure (α-helix: H1, H1*; β-strand: B1, B1*, etc.).

Overall Structure of the Subunit—Each subunit of MAT is composed of three domains, with the peptide chain organized into nine α-helices, eleven β-strands, and five 3_10 one-turn helices assigned by the program PROCHECK (40) (Figs. 7 and 8). The three domains are denoted the amino-terminal domain (residues 1–12 and 129–233), the central domain (residues 13–101 and 234–268), and the carboxyl-terminal domain (residues 108–128 and 269–383) are related to each other by pseudo-3-fold symmetry (Fig. 7). In each domain, two α-helices are located on a β-sheet formed by three or four β-strands. A long α-helix, followed by a pair of anti-parallel β-strands and a
short α-helix is a common secondary structural motif in each domain (H3-B6-B7-H4 in the amino-terminal domain, H1-B2-B3-H2 in the central domain, and H6-B10-B11-H7 in the carboxyl-terminal domain) (Fig. 8). The amino-terminal domain consists of two α-helices and four β-strands in which B1, B6, and B7 compose an anti-parallel β-sheet and the edge β-strand, B8, runs parallel to B7. Similarly the central domain has two α-helices and four β-strands, but a β-strand B9 corresponding to B1 in the amino-terminal is relatively short. The missing portion of the β-strand is replaced with the end of the large core loop (Gly264-Ser268); these regions interact with B2 as if to form an anti-parallel β-sheet. In the carboxyl-terminal domain, the fourth β-strand, which is missing, and the corresponding polypeptide chain forms two additional α-helices. To our knowledge, this secondary structure topology represents a novel protein fold. The electron density map of six amino acid residues (102–107) which are near to, but outside of, the active site are too weak to trace correctly. Since the distance between the α-carbons of Asp103 and Arg108 is 16 Å, the peptide chain of the missing six residues should be relatively extended and apparently has variable, multiple conformations. These undefined residues might form a "flexible loop" over the active site cleft, comparable to the flexible loop found in triosephosphate isomerase (42) and lactate dehydrogenase (43).

Three crystal structures of methyltransferases with bound AdoMet, catechol O-methyltransferase (44), HhaI DNA methyltransferase (45), and TaqI DNA methyltransferase (46) have recently been reported. The AdoMet binding domains of these methyltransferases are strikingly similar to each other, indicating that many methyltransferases may have a common structure (47). However, the AdoMet binding pocket of the E. coli methionine repressor (48), the only other AdoMet binding protein with a structure determined by x-ray analysis, has no resemblance to the AdoMet-binding pockets found in the methyl-
transferases. MAT has no structural similarity to either of these methyltransferases or to the repressor.

Interaction between Subunits—A tight dimer is composed of subunits A and B or C and D, as described above. The contact surfaces between the subunits are mostly flat, and thus the subunit boundary is clearly distinguishable (Fig. 6). The major intersubunit interaction is a hydrophobic interaction between three sets of \( \beta \)-sheets (Fig. 9A). In the central region of the tight dimer, the core loop and short \( \alpha \)-helix (H5) in the central domain of subunit A interact quite extensively with the analogous regions of subunit B through mainly polar interactions. Glu\(^{44}\) and Thr\(^{242}\) especially are both involved in a salt bridge with Arg\(^{344}\), whereas Gly\(^{243}\) and Gly\(^{243}\) are coordinated to the K\(^+\) ion located on the 2-fold axis. These polar interactions in the central region of the tight dimer appear to construct a rigid framework for the active sites.

Interactions between the tight dimers appear to be less extensive. There is only one residue, Ser\(^{93}\) in the B4 \( \beta \)-strand, that is involved in polar interactions between subunit A and subunit C. Ser\(^{80}\) is the only residue which participates in the 2-fold intersubunit hydrogen bonds between subunits A and D. However, the measured dissociation constant for the equilibrium between the tetramer and two dimers is less than 10\(^{-10}\) M (49). It is noteworthy that upon modification of the enzyme with the sulfhydryl reagent N-ethylmaleimide, which reacts with both Cys\(^{89}\) and Cys\(^{239}\) in each subunit, the tetramer dissociates to two dimers (49). Furthermore, a site-directed mutagenesis change of Cys\(^{89}\) to Ala\(^{89}\) yields an enzyme that exists as a mixture of dimers and tetramers, while changing Cys\(^{239}\) to Ala\(^{239}\) has no effect on the aggregation state (50). Although Cys\(^{89}\) is located on the border between dimers, no strong interaction is observed (Fig. 9B). However, Cys\(^{89}\) is a conserved amino acid residue of MAT in various organisms suggesting that this residue plays an important role in dimer formation of the enzyme. Thus, the structure of the interface between the dimers might be changed substantially by replacing Cys\(^{89}\) with Ala\(^{89}\).

Active Site—It should be noted that this is the first study describing the amino acid residues involved in the active site of MAT. There is a large, deep cleft between the two subunits (Figs. 6 and 10). As shown in Figs. 3 and 10, the electron density maps show clearly two P\(_i\) ions (one of the products) and three metal ions (one K\(^+\) and two Mg\(^{2+}\)/Co\(^{2+}\)) in the cleft. Thus, this region must be the active site of the enzyme. Possible interactions between amino acid residues and the P\(_i\) and metal ions are illustrated in Fig. 11. Although several interactions appear to be slightly different among the three structures (especially coordination schemes of the Mg\(^{2+}\) and Co\(^{2+}\) ions), these differences are insignificant at the 3.0 Å resolution of these structures.

His\(^{245}\)(N\(_2\)) and Lys\(^{256}\)(N\(_1\)) hydrogen-bond to one P\(_i\), whereas Lys\(^{265}\)(N\(_1\)) and Lys\(^{245}\)(N\(_2\)) hydrogen-bond to the other P\(_i\). Lys\(^{245}\)(N\(_2\)) appears to participate by providing the bridging hydrogen bonds between the two P\(_i\) ions. Interactions between these positively charged residues and the negatively charged oxygen atoms of the P\(_i\) ions appear to neutralize the individual charges. Some of these positively charged residues may be involved in either the displacement of the triphosphate chain from ATP during AdoMet formation or the subsequent hydrolysis of the triphosphate to PP\(_i\) and P\(_i\).

Two Mg\(^{2+}\) (or Co\(^{2+}\)) ions are separated by 5 Å, and those ions bridge two P\(_i\) ions in a trigonal pyramidal fashion, i.e. each divalent ion is coordinated to two oxygen atoms of one P\(_i\) ion and to one oxygen atom of the other P\(_i\) ion. The negatively charged Asp\(^{42}\) and Asp\(^{16*}\) residues each coordinate to one of the two Mg\(^{2+}\) (or Co\(^{2+}\)) ions from the side opposite to the P\(_i\) ions. Additionally, two oxygen atoms (O\(_{\text{ex}}\) of Glu\(^{89}\) and O\(_{\text{ax}}\) of Asp\(^{118}\)) are located within 4.0 Å from the Co(1) ion. Similarly, the Co(2) ion is surrounded by two additional oxygen atoms (O\(_{\text{ax}}\) of Glu\(^{89}\) and O of Cys\(^{239}\)). These oxygen atoms may be involved in coordination bonds to the Co\(^{2+}\) ions. A similar environment is observed around the Mg\(^{2+}\) ions. It is not clear whether these divalent ions bind to the enzyme in the same fashion when ATP binds at the active site.

Cantoni (1) reported that divalent metal ions are essential for MAT activity. An EPR spectroscopic study with Mn\(^{2+}\) has indicated that one metal ion binds to the enzyme in the absence of substrates and a second metal ion binds as a complex with the substrate chain from ATP (27). When Mn\(^{2+}\) ions occupy both sites, the metal ions have been observed to interact magnetically by spin exchange, suggesting that a common ligand, such as a phosphoryl group, bridges the metal ions. In this x-ray study, in spite of the absence of substrates, two divalent metal ions are found in the active site, suggesting that the binding mode of the two P\(_i\) ions at the active site is somehow similar to that of the triphosphate group of ATP. In fact, the coordination scheme between the P\(_i\) ions and the divalent metal ions found in this study is consistent with the EPR observation described above.

Two divalent ions are found at the active site in structures of the 3',5'-exonuclease domain of DNA polymerase I (51). In the absence of substrate or product, the exonuclease domain of DNA polymerase I has one bound divalent metal ion (site A), whereas complexes with deoxynucleoside monophosphates show a second divalent metal ion (site B) separated by 4 Å from that in site A. Thus, MAT and DNA polymerase I may belong to an emerging group of structurally determined mem-

![Figure 8](https://example.com/fig8.png)
bers of a family of nucleotide-utilizing enzymes that bind multiple divalent metal ions.

The active site $K_1$ ion is surrounded by three oxygen atoms within coordination distance (Oe1 and Oe2 of Glu42 and O of Ser263), but there is no interaction with the $P_i$ ions. Thus, the monovalent cation appears to aid in construction of the framework of the active site, a situation analogous to that recently found for $K_1$ ion pyruvate kinase (52). Although there is no significant electron density peak which might indicate water molecules around the $K_1$ ion, several water molecules should be coordinated to the $K_1$ ion since there are two large open spaces around the $K_1$ ion and the coordination number of $K_1$ is often as large as 10 (53). The binding site of the $K_1$ ion is the same as that of one of the $UO_2$ binding sites in the heavy atom derivative, consistent with $UO_2$ being an inhibitor of the enzyme (54). On the basis of this crystal structure, a site-directed mutagenesis change of Glu42 to Gln42 has been carried out by McQueney and Markham (54). The mutation abolished the monovalent cation activation and produced an enzyme which has an activity virtually identical with that of $K_1$-free wild type MAT in both the overall AdoMet synthesis reaction and in the hydrolysis of tripolyphosphate, indicating that the monovalent and divalent metal ions in the active site were assigned correctly. It should be noted that all amino acid residues involved in either hydrogen bonds or coordination bonds with either metal ions or $P_i$ ions are conserved in the 12 reported sequences of MAT.

In summary, this crystallographic study on MAT from E. coli has revealed the active sites of the tetrameric enzyme which are located between the subunits forming a spherical tight dimer. Two divalent metal ions ($Mg^{2+}$) and one monovalent metal ion ($K^+$) have been found along with two $P_i$ ions (one of the products of the reaction catalyzed by MAT) in the active site. The $Mg^{2+}$ binding sites have been confirmed from the
cobalt atom position found in the MAT-Co derivative. The two Mg\(^{2+}\) ions bridge two \(\text{Pi}\) ions, and Mg(1) and Mg(2) ions are surrounded by Glu\(^{86}\), Asp\(^{118}\), and Asp\(^{271}\) residues, and Asp\(^{168}\), Glu\(^{42}\), and Cys\(^{239b}\) residues, respectively. The binding site (Glu\(^{42}\)) of the K\(^{+}\) ion is the same as that of one of the UO\(_2^+\) ion binding sites in the heavy atom derivative. Consequently, UO\(_2^+\) ion is an inhibitor of the enzyme. The K\(^{+}\) ion bound on Glu\(^{42}\) has been confirmed by a site-directed mutagenesis (E42Q). The mutation abolished the monovalent cation activation and produced an enzyme which has an activity virtually identical with that of K\(^{+}\)-free wild type MAT. The positively charged amino acid residues, His\(^{14}\), Lys\(^{165}\), Lys\(^{245}\), and Lys\(^{265}\) interact strongly with the \(\text{Pi}\) ions, suggesting that some of those amino acid residues are involved in the unusual two-step catalytic reaction. These amino acid residues as well as the amino acid residues interacting with the monovalent and divalent metal ions in the active site are all conserved in the 12 reported sequences of MATs from various species, indicating that the
FIG. 11. The active site geometry with $P_i$ and metal ions. Possible polar interactions (hydrogen and coordinate bonds) are indicated by thin lines. $P_i$ and metal ions are illustrated by the solid bonds and filled circles, respectively. The hydrogen and coordination bonds are defined as donor-acceptor distance to be less than 3.3 Å and metal-oxygen distance to be less than 2.5 Å, respectively.
MATs assume quite similar three-dimensional structures including the active site geometry. Therefore, the central features of the mechanism of the catalytic reactions are probably identical in the enzymes from a wide range of organisms.

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