The Bifunctional Active Site of S-Adenosylmethionine Synthetase

ROLES OF THE ACTIVE SITE ASPARTATES*

(Received for publication, August 11, 1999, and in revised form, September 2, 1999)

John C. Taylor and George D. Markham‡

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

S-Adenosylmethionine (AdoMet) synthetase catalyzes the biosynthesis of AdoMet in a unique enzymatic reaction. Initially the sulfur of methionine displaces the intact triphosphate chain (PPPi) from ATP, and subsequently PPPi is hydrolyzed to PPi and Pi before product release. The crystal structure of Escherichia coli AdoMet synthetase shows that the active site contains four aspartate residues. Aspartate residues Asp-118 and Asp-238* are proposed to interact with methionine. Each aspartate has been changed to an uncharged asparagine, and the metal binding residues were also changed to alanine, to assess the roles of charge and ligation ability on catalytic efficiency. The resultant enzyme variants all structurally resemble the wild type enzyme as indicated by circular dichroism spectra and are tetramers. However, all have \( k_{\text{cat}} \) reductions of \( \sim 10^5 \)-fold in AdoMet synthesis, whereas the MgATP and methionine \( K_m \) values change by less than 3- and 8-fold, respectively. In the partial reaction of PPPi hydrolysis, mutants of the Mg\(^{2+}\) binding residues have \( >700 \)-fold reduced catalytic efficiency \( (k_{\text{cat}}/K_m) \), whereas the D118N and D238*N mutants are impaired less than 35-fold. The catalytic efficiency for PPPi hydrolysis by Mg\(^{2+}\) site mutants is improved by AdoMet, like the wild type enzyme. In contrast AdoMet reduces the catalytic efficiency for PPPi hydrolysis by the D118N and D238*N mutants, indicating that the events involved in AdoMet activation are hindered in these methionyl binding site mutants. Ca\(^{2+}\) uniquely activates the D271A mutant enzyme to 15% of the level of Mg\(^{2+}\), in contrast to the ~1% Ca\(^{2+}\) activation of the wild type enzyme. This indicates that the Asp-271 side chain size is a discriminator between the activating ability of Ca\(^{2+}\) and the smaller Mg\(^{2+}\).
hydrolysis of PPP to PP and P, a reaction that mimics the second part of the synthetase reaction (5, 23–25). The rate of PPP hydrolysis is greatly stimulated by AdoMet; the physical mechanism of this activation is as yet unknown but is probably due to local conformational adjustments. The features of AdoMet or the enzyme that contribute to this activation are unclear, although the sulfonium group is important since neither the adenosylhomocysteine nor methylthioadenosine fragments activate the rate of PPP hydrolysis (5).

In the present studies, the active site aspartate residues have been changed to asparagine to remove their electrostatic contributions to binding and catalysis while maintaining steric and (altered) hydrogen bonding capabilities. In addition, the metal binding aspartates have been changed to alanine to evaluate the role of steric effects in discrimination among cations of different sizes. The properties of these mutants provide insight into the important side chain properties for metal ion binding and into the basis of AdoMet activation of PPP hydrolysis.

**EXPERIMENTAL PROCEDURES**

L-Methionine, ATP, KCl, MgCl₂, (NH₄)₆Mo₇O₂₄·4H₂O, NaAsO₂, citric acid, ascorbic acid, acetic acid, malachite green, Tris, Hapes, 2-mercaptoethanol, and sodium tripolyphosphate were purchased from Sigma. AdoMet was purchased from Research Biochemicals International. Glycerol was purchased from Baxter Scientific. 2-Hydroxy-4-methylthiobutyric acid and 2-keto-4-methylthiobutyric acid were purchased from Aldrich. Glycerol was purchased from Baxter Scientific. Malachite green was purchased from Pel-Freeze Biologicals, Inc. L-Methionine was purchased from NEN Life Science Products. Ecoscint scintillation fluid was purchased from National Diagnostics. Phosphocellulose P81 filters (2.5 cm) were purchased from Whatman.

**Cells and Plasmids—** E. coli strains XL1-Blue (an E. coli K12 derivative) or STR15(DE3) (an E. coli B derivative) were used. STR15(DE3) is a metK derivative of BL21(DE3); extracts of this strain have no detectable AdoMet synthetase activity (11). Site-directed Mutagenesis—Oligonucleotides used in mutagenesis were prepared in the Core Facility at the Fox Chase Cancer Center. Mutants were constructed using the Quickchange kit (Stratagene) on plasmid pT7K (11), which has the E. coli metK gene inserted between the PstI and EcoRI sites of plasmid pT7–6. Following transformation and selection for ampicillin resistance, plasmid DNA was extracted using the Wizard Plus Miniprep DNA Purification System (Promega Corp., Madison, WI). Mutants were identified directly by DNA sequencing on an ABI automated sequencer. In all cases complete DNA sequences confirmed that only the desired mutations were introduced.

**Expression, Purification, and Characterization of Mutant AdoMet Synthetases—** Plasmids were transformed into strain STR15(DE3). Cultures were grown in LB media containing 50 µg/ml carbenicillin. Following overnight growth, 0.1 ml isopropyl-1-thio-β-D-galactopyranoside was added to the cultures for 30 min prior to harvesting. Mutant proteins accounted for 10–20% of the total cellular protein.

A standard purification protocol was used to isolate both wild type and mutant AdoMet synthetases (11). Steps consisted of ammonium sulfate fraction followed by successive chromatography on the hydrophobic interaction matrix phenyl-Sepharose HR 4FF (Amersham Pharmacia Biotech), hydroxyapatite HTP-1 (Bio-Rad), and the weak anion exchange resin aminohexyl-Sepharose (EAH-4B, Amersham Pharmacia Biotech). Purifications were monitored by electrophoresis on 10–15% gradient gels containing SDS. All AdoMet synthetases were electrophoretically homogeneous. Following purification, AdoMet synthetases were analyzed for oligomerization state by native gel electrophoresis on 8–25% gradient gels (11). Secondary structure was assessed by circular dichroism spectra recorded 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 spectra were corrected for buffer contributions.

**AdoMet Synthetase Assays—** AdoMet synthetase activity was determined by the [14C]AdoMet filter binding method (11). Assays were performed in the presence or absence of AdoMet. Assays were performed in the presence of 0.01–0.5 mM [14C-methyl]methionine (1.9 mCi/nmol), 0.05–15 mM ATP (Tris form) in 50 mM Hapes (CH₃COONa) at pH 8.0 with 50 mM KCl, and 50 mM MgCl₂. The triphospho-phosphatase activity was determined in the presence or absence of AdoMet by quantifying orthophosphate production (26, 27). Assays contained 3–500 µM PPP, (Na⁺ salt) in 50 mM Hapes (CH₃COONa) at pH 7.8 with 10 mM KCl and 10 mM MgCl₂. Triphospho-phosphatase activity assays in the presence of CaCl₂ were performed in place of MgCl₂. CaCl₂ was present at 40 mM. It was not possible to perform triphosphophosphatase activity assays in the presence of Ca²⁺ due to the low solubility of the Ca²⁺ complexes.

In substrate tests for 2-hydroxy-4-methylthiobutyric acid and 2-keto-4-methylthiobutyric acid, potential products were separated by chromatography on a Mono-S cation exchange column developed in 0.6N HCl since the products would be uncharged at neutral pH and would not be retained in the filter binding assay. Reactions contained 10 mM ATP and 10 mM methionine or analog. Product formation was assessed from absorbance at 254 nm; the analogs yielded <1% of the amount of product obtained with methionine.

Substrate saturation data were evaluated using the kinetic equations of Cleland (28) as implemented in the program Scientist (MicroMath, Inc.), or the Enzfitter program (Elsevier Biosoft).

**RESULTS**

**Characterization of AdoMet Synthetase Mutants—** The six mutants constructed in this study are physically indistinguishable from the wild type enzyme. All the proteins behaved identically to the wild type enzyme through a purification procedure that exploits a variety of physical properties (in contrast, previously studied mutants at Cys-89 yielded rather differently behaving active dimeric enzymes; Ref. 11). These six mutant AdoMet synthetases are similar to the wild type enzyme in secondary structure as judged by indistinguishable circular
AdoMet synthesis activity was measured as described under "Experimental Procedures." Assays were performed in the presence of 0.01–0.5 mM l-[14C]-methylmethionine (1.9 mCi/mmol), 0.05–15 mM ATP (Tris·HCl pH 8.0) with 50 mM KCl, 50 mM MgCl₂.

**Table I. Kinetic parameters for AdoMet synthetase activity of aspartate mutants**

| Enzyme  | \( k_{\text{cat}} \) | \( K_{\text{m,ATP}} \) | \( K_{\text{m,Met}} \) | \( k_{\text{cat}}/K_{\text{m,ATP}} \) | \( k_{\text{cat}}/K_{\text{m,Met}} \) |
|---------|----------------------|------------------------|------------------------|-------------------------------|-------------------------------|
| Wild type | 1.5 0.11 0.08 14 × 10⁻³ | 19 × 10⁻³ | 0.12 |
| D16*N | 0.0007 0.17 0.01 4.1 | 70 1.9 |
| D16*A | 0.0013 0.041 0.031 32 | 42 1.3 |
| D118N | 0.0016 0.25 0.38 6.4 | 4.2 1.0 |
| D238*N | 0.0017 0.036 0.016 47 | 106 1.3 |
| D271N | 0.0036 0.13 0.067 28 | 53 1.2 |
| D271A | 0.0039 0.10 0.057 39 | 68 1.4 |

*Values from Markham et al. (5). Uncertainties in \( k_{\text{cat}} \) (moles of product formed/mol of enzyme active site(s)) are within 15%, and uncertainties in \( K_m \) or \( K_a \) are within 20%.

Dichroism spectra, and are tetrameric according to native polyacrylamide gel electrophoresis. However, the mutations have profound effects on the catalytic behavior of the enzyme (Tables I–III and illustrated graphically in Fig. 2).

**AdoMet Synthesis Activity**—In the presence of Mg²⁺ as activator, the \( k_{\text{cat}} \) for AdoMet synthesis by each of the six mutants is reduced by ~3 orders of magnitude with respect to the wild type enzyme (Table I). In contrast, there are only 3- and 8-fold alterations in the \( K_m \) values for MgATP and methionine, respectively. The kinetic properties of the D16A mutant show a 2-fold improved \( k_{\text{cat}} \) and different \( K_m \) values from the D16N mutant, being 4-fold diminished for ATP and 3-fold increased for methionine. In contrast, the kinetic parameters for the D271A and D271N mutants are not significantly different. In all six cases, the production of AdoMet is linear with time from 0.1% of the wild type level, while \( k_{\text{cat}} \) values decreased 11-fold and actually increased ~5-fold, respectively.

In common with the wild type enzyme, AdoMet dramatically enhances \( k_{\text{cat}}/K_m \) for the D16N, D16A, D271A, and D271N enzymes, although the AdoMet concentrations required are larger than the wild type levels. For the D271N and D271A mutants AdoMet causes ~200-fold and 20-fold reductions in the \( K_m \) of PPP, to approximately the wild type value; the \( k_{\text{cat}} \) value appears to decrease 1.5–2-fold, although since the value in the absence of AdoMet is an extrapolation this may not be significant. In the presence of AdoMet, the PPP, \( K_m \) for the D16N mutant is ~6-fold larger than the wild type value while the \( K_m \) for the D16A mutant is 77-fold larger. The \( k_{\text{cat}} \) values at saturating AdoMet for each of the Asp-16* and Asp-271 mutants are substantially larger than the rate of the overall reaction, consistent with an early rate-limiting step in the overall reaction.

The AdoMet effects on PPP, hydrolysis by the variants under consideration are summarized in Table II. In the absence of AdoMet, there is a dramatic effect on \( k_{\text{cat}} \) (moles of product formed/mol of enzyme active site(s)) in all six cases. After the AdoMet synthetase reaction and the PPPase reaction in the absence of MgCl₂. In contrast, there are only 3- and 8-fold increases for the D16*N and D16*A mutants is an estimate based on limits of detectability. B, comparison of the \( K_m \) values for ATP, methionine, PPP, PPP, in the presence of AdoMet, the concentration of AdoMet required for half-binding, the concentration dependence of Mg²⁺ concentrations for the D16*N and D271N mutants, respectively.

**Tripolyphosphatase Activity**—AdoMet synthetase also catalyzes the hydrolysis of added tripolyphosphate, and the reaction rate is stimulated by AdoMet. The kinetic parameters for PPP, hydrolysis by the variants under consideration are summarized in Table II. In the absence of AdoMet, there is a dramatic effect on \( k_{\text{cat}} \) (moles of product formed/mol of enzyme active site(s)) in all six cases.
the presence of Mg$^{2+}$.

Uncertainties in $k_{cat}$ values are within 15% and $K_m$ or $K_a$ values within 20% unless noted.

### Active Site Aspartate Mutants of AdoMet Synthetase

**Table II**

| Enzyme | $k_{cat}$ | $K_m$ | $k_{cat}/K_m$ |
|--------|-----------|-------|--------------|
| Wild type | 0.06 s$^{-1}$ | 3 μM | 20 s$^{-1}$ μM$^{-1}$ |
| D16*N | $<6 \times 10^{-5}$ | $<6 \times 10^{-5}$ | $<6 \times 10^{-5}$ |
| D18*A | $<6 \times 10^{-5}$ | $<6 \times 10^{-5}$ | $<6 \times 10^{-5}$ |
| D118N | 0.0048 | 1.8 | 2.6 |
| D238*N | 0.30 | 5.6 | 53 |
| D271N | 0.06 | >2000 | 0.03 |
| D271A | 0.06 | 350 | 0.17 |

| Enzyme | $k_{cat}$ | $K_m$ | $k_{cat}/K_m$ |
|--------|-----------|-------|--------------|
| Wild type | 1.2 s$^{-1}$ | 13 μM | 92 s$^{-1}$ μM$^{-1}$ |
| D16*N | 0.014 | 82 | 0.17 |
| D18*A | 0.24 | 1000 | 0.24 |
| D118N | 0.0013 | 4.1 | 0.32 |
| D238*N | 0.26 | 77 | 3.4 |
| D271N | 0.029 | 11 | 2.6 |
| D271A | 0.040 | 18 | 2.2 |

*a* Concentration of AdoMet producing half-maximal change in the rate hydrolysis at 0.1 mM PPP.

*b* Data from Raczkowski et al. (12).

*c* No reaction (<0.1% of the wild type rate) was observed with up to 2 mM PPP, preventing kinetic studies.

*d* This is an extrapolation. Saturation was not achieved up to the highest concentration of PPP, feasible, 2 mM. The uncertainty is ±50%.

while the $k_{cat}$ actually decreased 4-fold.

As a complementary approach to mutagenesis, we evaluated interactions of the wild type enzyme with two methionine anlogs that lack the o-amino group, i.e., 2-hydroxy-4-methylthio-butryic acid and 2-keto-4-methylthiobutyric acid. Neither was a substrate, and concentrations up to 10 mM did not inhibit the rate of AdoMet formation at 0.1 mM methionine, illustrating the importance of the amino group in the affinity for the substrate.

Ca$^{2+}$ Activation of Metal Binding Mutants—Since previous studies suggested that one of the two metal sites was more tolerant of replacement of Mg$^{2+}$ by the larger Ca$^{2+}$ ion (7), we tested Ca$^{2+}$ activation of the isosteric Asp→Asn mutants and the side chain size reduced Asp→Ala mutants. Of all the mutants, activity could only be detected for the D271A enzyme (Table III). For the D271A enzyme, the $k_{cat}$ is only 7-fold reduced from Mg$^{2+}$, in contrast to the wild type enzyme, where $k_{cat}$ is reduced 78-fold in the presence of Ca$^{2+}$. The $K_m$ for Ca$^{2+}$ is 20 mM for the D271A mutant, and 15 mM for the wild type enzyme. The $K_m$ values for ATP are similar and are 8- and 5-fold larger than with Mg$^{2+}$ for the mutant and wild type enzyme.

For the wild type enzyme, the methionine $K_m$ is 53-fold increased in the presence of Ca$^{2+}$; this may reflect that in the presence of Mg$^{2+}$ the $K_m$ may be decreased from the dissociation constant due to contributions from steps subsequent to binding which would be reduced in the slower Ca$^{2+}$-activated reaction (29). In the D271A mutant the $K_m$ for a methionine is 2.6-fold larger in the presence of Ca$^{2+}$ than Mg$^{2+}$, which may more clearly reflect the changes in dissociation constants. In all, the poor ability of Ca$^{2+}$ to activate the wild type enzyme appears to be largely due to the bulk of the Asp-271 side chain.

### DISCUSSION

The mutants reported here were constructed to clarify the roles of the conserved active site aspartate residues, two of which are known from crystallography to be involved in Mg$^{2+}$ binding and two that are proposed to be involved in methionine binding based on NMR and molecular modeling. All six mutants were tetrameric with no obvious change in secondary structure, which facilitates interpretation of the results of the kinetic studies illustrated in Fig. 2. For the Asp-16*, Asp-238*, and Asp-271 mutants, the rate of PPP$^3$ hydrolysis in the presence of Mg$^{2+}$ was 2.6-fold larger in the presence of Ca$^{2+}$ than Mg$^{2+}$, which may more clearly reflect the changes in dissociation constants. In all, the poor ability of Ca$^{2+}$ to activate the wild type enzyme appears to be largely due to the bulk of the Asp-271 side chain.

**Table III**

| Enzyme | $k_{cat}$ | $K_m$ (mM ATP) | $K_m$ (L-Met) | $k_{cat}/K_m$ ATP | $k_{cat}/K_m$ L-Met | $K_m$ (ATP)$^a$ | $K_m$ (L-Met)$^a$ |
|--------|-----------|---------------|--------------|-----------------|-------------------|---------------|-----------------|
| Wild type | 0.02 s$^{-1}$ | 0.51 | 4.2 | 39 | 4.7 | 15 |
| D16*A | NR$^a$ | NR$^a$ | NR$^a$ | NR$^a$ |
| D18*N | 0.00057 | 0.32 | 0.14 | 1.8 | 4.020 |

*a* No product formation was detected (<0.1% of wild type) at 0.05--2.5 mM ATP, 0.05--0.5 mM methionine, 40 mM CaCl$_2$. Uncertainties in $k_{cat}$ values are within 15% and $K_m$ or $K_a$ values within 20%.

The lack of a dramatic effect on the concentration dependence for Mg$^{2+}$ activation of the Asp-16* and Asp-271 mutations was initially surprising. However, a survey of the literature finds precedent in studies of inorganic pyrophosphatase, alkaline phosphatase, and T7 RNA polymerase, all of which trans-

2 For example when the non-essential K$^+$ activator is omitted in the Mg$^{2+}$-activated reaction, the methionine $K_m$ is 1.0 mM; under these conditions the $k_{cat}$ is 0.02 s$^{-1}$, comparable to Ca$^{2+}$-activated reaction (10).

3 Ongoing studies of mutants at other active site residues such as His-14 and Lys-245 are consistent with this trend.
decreases in $k_{\text{cat}}$ (31, 32). In the case of T7 RNA polymerase, single Asp $\rightarrow$ Asn mutations decreased the affinity of the Mn$^{2+}$ probe only 2–5-fold, but inactivated the enzyme (33). In contrast, a single Asp $\rightarrow$ Asn mutation in alkaline phosphatase dramatically decreased Mg$^{2+}$ affinity, with the result that Mg$^{2+}$ no longer bound at that site but activated by slowly replacing Zn$^{2+}$ at an adjacent site (34). For AdoMet synthetase, the lack of a dramatic alteration in the concentration dependence of Mg$^{2+}$, unlike Mg$^{2+}$ replacing Zn$^{2+}$ in the D271A mutant is 15-fold greater than this ratio for the wild type. There is no lysine side chain near the Mg$^{2+}$ phosphoryl groups in complexes with PPi plus Pi, or ADP plus Pi (13, 14). Although the available crystal structures show that the environment of Asp-16$^*$ is less restricted by other protein groups than is the environment of Asp-271, it appears that the substrate independent site that selectively binds VO$^{2-}$ is near Asp-271, which allows Ca$^{2+}$ to bind at Asp-16$^*$ and activate the enzyme in the mixed VO$^{2-}$/Ca$^{2+}$ complex to a $\sim$100-fold higher level than the Ca$^{2+}$/Ca$^{2+}$ complex (7). This is consistent with spectroscopic studies showing a lysine amino group ligand to VO$^{2-}$ (8), and analysis of the crystal structure, which shows the amino group of Lys-265 within 3 $\AA$ of the Mg$^{2+}$ ligated to Asp-271; there is no lysine side chain near the Mg$^{2+}$ ligated to Asp-16$^*$. Thus, it appears that the divalent metal ion that binds in conjunction with the substrate migrates to the bottom of the active site near Asp-16$^*$. Although the available crystal structures show both metal ions coordinated to all three phosphoryl groups in complexes with PPi plus P$_0$, or ADP plus P$_i$ (13–15), molecular models indicate that doubly tridentate coordination of a triphosphate chain in ATP or PPPi is geometrically unlikely. Thus, the metal ion coordination scheme in the substrate complexes may not be well represented by the available structures of the product complexes. It is possible that a metal ion coordinated water is the source of the oxygen that hydrolyzes the polyphosphate chain, and thus the different properties of Ca$^{2+}$ and Mg$^{2+}$ coordinated water could be significant. However, since Ca$^{2+}$ impairs the AdoMet forming step (7), there is an early event that is less efficient with the larger cation.

The location of the methionine binding site of the enzyme proposed in the NMR-based docking model is consistent with the results of this study (16). The increases in $K_{\text{m}}$ for methionine and $K_{\text{cat}}$ for AdoMet in the D118N mutant are consistent with the loss of an energetically favorable electrostatic interaction between the sulfur and the carboxylate, the larger effect being seen with the positively charged sulfur of AdoMet. Favorable electrostatic interactions between sulfur atoms with a full or partial positive charge have been documented in studies of the crystallographic literature (37) and supported by theoretical studies (38, 39). The decreases in $K_{\text{m}}$ and $K_{\text{cat}}$ for methionine and AdoMet in the D238$^*$N mutant may reflect the inability of the mutant to exploit binding energy in catalysis as manifest in the failure of AdoMet to activate PPPi hydrolysis; thus, favorable binding interactions are reflected in the affinity. The present results indicate that interactions with both the sulfonium and $\alpha$-amino groups of AdoMet are important in activation, consistent with studies using AdoMet analogs (5). The inability of AdoMet to activate the $k_{\text{cat}}$ for PPPi hydrolysis in the D118N mutant is consistent with the requirement of enzyme interaction with the sulfonium center since neither the fragments adenosylhomocysteine nor methylthioadenosine activate (5). Furthermore, the methionine analogs having the $\alpha$-amino group replaced by an $\alpha$-hydroxy or an $\alpha$-keto group are neither substrates nor inhibitors of the wild type enzyme, showing the importance of specific recognition of the $\alpha$-amino group in substrate recognition. In contrast, methionine methyl ester is a good substrate, indicating that interactions between the carboxylate and the enzyme are less important (6).

The active site of the enzyme contains contributions from two subunits; however, the results of these and previous mutagenesis studies indicate that the residues from a particular subunit do not have a uniformly greater or lesser contribution to any property measured. Further studies combining mutant enzymes, AdoMet analogs, and structural data are ongoing in order to pinpoint the structural basis of the AdoMet induced activation of PPPi hydrolysis. It is apparent from the sum of the observations of this study that the catalytic efficiency in AdoMet synthesis arises from a highly coordinated active site, which can easily be perturbed to impair the enzyme in unexpected, albeit informative, ways.

Acknowledgments—We thank Kristen Ahern for numerous enzyme purifications, Dr. Z. Lu for testing the activity of methionine analogs, and Drs. R. S. Reczkowski and C. Schalk-Hihi for helpful discussions.

REFERENCES

1. Mudd, S. H. (1973) in The Enzymes (Boyer, P. D., ed) 3rd Ed., Vol. 8, pp. 121–154, Academic Press, New York.
2. Tabor, C. W., and Tabor, H. (1984) Adv. Enzymol. 56, 251–282
3. Cantoni, G. L. (1975) Annu. Rev. Biochem. 44, 435–451
4. Markham, G. D. (1984) Biochemistry 23, 9082–9092
5. Markham, G. D., Hafner, E. W., Tabor, C. W., and Tabor, H. (1980) J. Biol. Chem. 255, 9062–9069
6. Markham, G. D. (1981) J. Biol. Chem. 256, 1903–1909
7. Markham, G. D. (1984) Biochemistry 23, 470–478
8. Zhang, C., Markham, G. D., and LoBrutto, R. (1993) Biochemistry 32, 9866–9873
9. Markham, G. D., and Leyh, T. S. (1987) J. Am. Chem. Soc. 109, 599–600
10. McQueney, M. S., and Markham, G. D. (1995) J. Biol. Chem. 270, 18277–18284
11. Reckzowski, R. S., and Markham, G. D. (1995) J. Biol. Chem. 270, 18484–18490
12. Reckzowski, R. S., Taylor, J. C., and Markham, G. D. (1998) Biochemistry 37, 13499–13506
13. Takanagawa, F., Kamitori, S., Misaki, S., and Markham, G. D. (1996) J. Biol. Chem. 271, 136–147
14. Zhang, C., Hu, Y., Markham, G. D., and Takanagawa, F. (1996) J. Biol. Chem. 271, 136–147
15. Takanagawa, F., Kamitori, S., Misaki, S., and Markham, G. D. (1996) Biochemistry 35, 2586–2596
16. Schalk-Hihi, C., and Markham, G. D. (1999) Biochemistry 38, 4433–4440
17. Chamberlin, M. E., Ubags, T., Mudd, S. H., Levy, H. L., and Cherny, J. Y. (1997) J. Am. Chem. Soc. 50, 540–546
18. Chamberlin, M. E., Ubags, T., Mudd, S. H., Wilson, W. G., Leonard, J. V., and Cherny, J. Y. (1996) J. Clin. Invest. 98, 1021–1027
19. Hazlewood, S., Bernardini, I., Skotelski, V., Tangerman, A., Gao, J., Mudd, S. H., and Gahl, W. A. (1997) J. Am. Med. Genet. 75, 395–400
20. Kraulis, P. J. (1991) J. Appl. Crystlogr. 24, 946–950
21. Sträter, N., Lipscomb, W. N., Klabunde, T., and Krebs, B. (1996) Angew. Chem. Int. Ed. Engl. 35, 2024–2055
22. Carrell, C. J., Carrell, H. L., Erlebacher, J., and Glusker, J. P. (1988) J. Am. Chem. Soc. 100, 8651–8656
23. Mudd, S. H. (1963) J. Biol. Chem. 238, 2156–2163
24. Mudd, S. H. (1983) J. Biol. Chem. 258, 2146–2170
25. Chiang, P. K., and Cantoni, G. L. (1977) *J. Biol. Chem.* **252**, 4506–4513
26. Chifflet, S., Torriglia, A., Chiesa, R., and Tolosa, S. (1986) *Anal. Biochem.* **168**, 1–4
27. Baykov, A. A., Evtushenko, O. A., and Avaeva, S. M. (1988) *Anal. Biochem.* **171**, 266–270
28. Cleland, W. W. (1979) *Methods Enzymol.* **63**, 103–138
29. Markham, G. D., Parkin, D. W., Mentch, F., and Schramm, V. L. (1987) *J. Biol. Chem.* **262**, 5609–5615
30. Bishop, E. O., Kimberly, S. J., Orchard, D., and Smith, B. E. (1980) *Biochim. Biophys. Acta* **635**, 63–72
31. Avaeva, S. M., Rodina, E. V., Kurilova, S. A., Nazarova, T. I., and Vorobyeva, N. N. (1996) *FEBS Lett.* **392**, 91–94
32. Avaeva, S. M., Ignatov, P., Kurilova, S. A., Nazarova, T. I., Rodina, E., Vorobyeva, N. N., Organessyan, V., and Harutyunyan, E. (1996) *FEBS Lett.* **399**, 99–102
33. Moon-Woody, A. Y., Eaton, S. S., Osumi-Davis, P. A., and Woody, R. W. (1996) *Biochemistry* **35**, 144–152
34. Tibbits, T. T., Murphy, J. E., and Kantrowitz, K. (1996) *J. Mol. Biol.* **257**, 700–715
35. Katz, A. K., Glusker, J. P., Markham, G. D., and Bock, C. W. (1998) *J. Phys. Chem. B* **102**, 6342–6350
36. Katz, A. K., Glusker, J. P., Beebe, A. A., and Bock, C. W. (1996) *J. Am. Chem. Soc.* **118**, 5752–5763
37. Burling, F. T., and Goldstein, B. M. (1992) *J. Am. Chem. Soc.* **114**, 2313–2320
38. Markham, G. D., and Bock, C. W. (1997) *J. Mol. Struct. (Theochem)* **418**, 139–154
39. Markham, G. D., and Bock, C. W. (1996) *Struct. Chem.* **7**, 281–300

---

*Active Site Aspartate Mutants of AdoMet Synthetase*