The Bifunctional Active Site of S-Adenosylmethionine Synthetase
ROLES OF THE BASIC RESIDUES*

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 a unique two-step enzymatic reaction leading to formation of the primary biological alkylating agent. The crystal structure of Escherichia coli AdoMet synthetase shows that the active site, which lies between two subunits, contains four lysines and one histidine as basic residues. In order to test the proposed charge and hydrogen bonding roles in catalytic function, each lysine has been changed to an uncharged methionine or alanine, and the histidine has been altered to asparagine. The resultant enzyme variants are all tetramers like the wild type enzyme; however, circular dichroism spectra show reductions in helix content for the K245M and K269M mutants. (The asterisk denotes that the residue is in the second subunit.) Four mutants have kcat reductions of $-10^3$-10-fold in AdoMet synthesis; however, the kcat of K165*M variant is only reduced 2-fold. In each mutant, there is a smaller catalytic impairment in the partial reaction of triplyphosphate hydrolysis. The K165*A enzyme has a 100-fold greater kcat for triplyphosphate hydrolysis than the wild type enzyme, but this mutant is not activated by AdoMet in contrast to the wild type enzyme. The properties of these mutants require reassessment of the catalytic roles of these residues.

S-Adenosylmethionine (AdoMet) is a central metabolite in all cells (1–4). AdoMet plays a myriad of biological roles including acting as the primary methyl group donor in all organisms and as a precursor to the polyamines, as well as a precursor to the 5'-deoxyadenosyl radical used by some enzymes (1–4). S-Adenosylmethionine synthetase (ATP: L-methionine S-adenosyltransferase; EC 2.5.1.6) catalyzes the only known route of AdoMet biosynthesis (5–9). The synthetic reaction is composed of two sequential steps, AdoMet formation and the subsequent hydrolysis of triplyphosphate (PPP), which occurs prior to release of AdoMet from the enzyme, as depicted below.

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

The structural and mechanistic properties of AdoMet synthetase have been extensively studied in the Escherichia coli metK enzyme, which is a tetramer of identical 383-residue subunits (10–20). The crystal structure of E. coli AdoMet synthetase has been solved as the apoenzyme and in the presence of ADP and P, which arose from enzymatic ATP hydrolysis in the crystals) as well as with the bound products PP and P (17–19). A binding site for the product AdoMet has been proposed based on NMR studies; crystals of complexes with AdoMet or methionine have not been found. The polar active site residues are all conserved in the more than 30 reported AdoMet synthetase sequences. Although naturally occurring human variants have been recently discovered in patients with familial hypermethioninemia (21–23), the only variation in an active site residue is at an arginine whose crucial role in the E. coli enzyme has been characterized (15).

The active site of the enzyme resides between two subunits, with contributions from side chains of residues from both subunits (Fig. 1). The phosphate groups bind at the bottom of a deep cavity with the adenosyl group nearer the entrance (19). Within the active site are four lysines, two from each subunit, and a histidine residue (a residue from the second subunit is denoted by an asterisk; e.g. His-14*, Lys-165*, Lys-245*, Lys-265, Lys-269). When suggesting the possible roles of these residues, it must be recalled that the available crystal structures are of a product complex and the products of a side reaction; thus, the structures of the active complexes may differ in detail from the structures observed to date, particularly since one substrate (or product) is absent in each of the available structures. The side chain of lysine 269 appears to interact with the N-3 of the adenine ring; however, slight rotations of the side chain would bring it near O-5 to facilitate C-5'–O-5' bond cleavage during AdoMet formation. The other four side chains of interest are also positioned near the sites of catalytic action; Lys-165* may hydrogen-bond to one of the oxygens of the β-phosphoryl group, perhaps to facilitate the hydrolytic reaction. Lys-245* is near the P, which originates as the γ-phosphoryl group of ATP and thus could participate in catalysis of hydrolysis; Lys-265 lies intermediate between the α- and γ-phosphoryl groups and could be involved in either reaction. Histidine 14* is also near the β-phosphoryl group; it is unclear whether the imidazole of His-14* is protonated; however, the proximity to the positively charged lysines and the two active site Mg2+ ions suggests that it might not be protonated, in which case it could function to aid in deprotonation of the water that is involved in the hydrolytic reaction. Alternatively, it was noted that a slight rearrangement of the active site in the presence of ATP could make a protonated side chain of His-14* a proton donor to O-5' to facilitate C-5'–O-5' bond cleavage (19).

In the present studies, the active site histidine residue (His-14*) has been changed to asparagine to remove potential proton donor and electrostatic contributions to binding and catalysis while attempting to maintain steric (altered) hydrogen
bonding capabilities. The lysine residues (Lys-165*, Lys-245*, Lys-265, and Lys-269) have been changed to methionine to remove charge and hydrogen bonding contributions while maintaining steric bulk to as great an extent as possible; the K265M mutant was unstable, thus the residue was also changed to alanine so that potentially detrimental hydrophobicity of the side chain would be reduced. In a complementary approach, we have examined the activity of the enzyme with the ATP analogs A(NH)TP, which has a C-NH-P linkage and therefore would require a proton donor to be a substrate, and 3-deaza-ATP to provide a further probe of the importance of hydrogen bonding to N-3 of the purine. The properties of these mutants and the behavior of alternate substrates provide insight into the important side chain properties for both enzyme-catalyzed reactions.

**EXPERIMENTAL PROCEDURES**

Reagents were purchased from Sigma unless noted. AdoMet was purchased from Research Biochemicals International. L-[methyl-14C]Methionine was purchased from NEN Life Science Products. Ecoscint scintillation fluid was purchased from National Diagnostics.

**Syntheses**—A(NH)TP was synthesized by the reaction of 5′-amino-5′-deoxyadenosine with trimetaphosphate (24, 25). The nucleoside (0.22 meq of the tosylate salt) was incubated with sodium trimetaphosphate (0.88 meq) in 2.2 ml of water at pH 11 for 2 days at room temperature; the pH decreased during the reaction and was occasionally adjusted back by the addition of KOH. The reaction was diluted to 25 ml, and the product was purified by ion exchange chromatography on a 50-ml column of Source-Q15 resin (Amersham Pharmacia Biotech). The column was washed with three volumes of water and then eluted with a linear gradient to 1 M triethylamine bicarbonate, pH 9. The UV-absorbing peak that eluted near 0.35 M triethylamine bicarbonate was taken to dryness under vacuum, with additions of Tris base to maintain the pH above 8, since this type of compound readily reverts to the starting material below neutral pH (25). A(NH)TP was obtained in 45% yield based on the nucleoside. At pH 8.5, proton-decoupled 31P NMR spectra, not previously reported, showed peaks at −0.9 ppm (doublet, P1, JpP = 20 Hz), −6.1 ppm (doublet, P1, JpP = 20 Hz), and −21.6 ppm (triplet, P1).

3-Deaza-ATP was prepared by phosphorylation of the nucleoside with POCl3, as described by Sowa and Ouchii (26). The monophosphate with phosphorus was isolated by ion exchange chromatography as described for AdoMet/TP. The nucleotide (10 μmol) was converted to the triphosphate enzymatically using 50 units of adenylate kinase from Bacillus stearothermophilus and 100 units of rabbit muscle pyruvate kinase in 1 ml of 50 mM Hepes/KOH, 50 mM KCl, 20 mM MgCl2. A 4-fold molar excess of phospho(enol)pyruvate was used, and the reaction was primed with a 1% molar equivalent of A(S)TP (10), since A(S)TP is readily separated from ATP, and it is not a substrate for AdoMet synthetase. The reaction was monitored by ion exchange HPLC on Mono-Q (Amersham Pharmacia Biotech), and the 3-deaza-ATP was isolated as described for AdoMet/TP.

**Site-directed Mutagenesis**—Oligonucleotides used in mutagenesis were prepared in the Fannie Ripple Biotechnology Facility at the Fox Chase Cancer Center. Mutants were constructed using the Quikchange Site-directed Mutagenesis Kit (Strategene). The plasmid used was pT7K (14), which has the E. coli metK gene inserted between the PstI and EcoRI sites of plasmid pTT76. Plasmid DNA was extracted from ampicillin-resistant transformants using the Wizard Plus Miniprep DNA Purification System (Promega

**FIG. 1. Illustration of the AdoMet synthetase active site.** The positions of P1, P2, and the two Mg2+ are shown from the crystal structure (Ref. 19; Protein Data Bank code 1mxb), and the location of AdoMet is taken from the NMR model (20). The two subunits that contribute to the active site are shown in different shades of gray, and atoms are colored by the Corey-Pauling-Koltun scheme. The locations are shown for His-14*, Asp-16*, Lys-165*, Lys-245*, Lys-265, Lys-269, and Asp-271*; residues denoted by an asterisk come from a different subunit than the other residues. The Mg2+ are each ligated to all three phosphoryl groups and to one of the aspartates.
Fig. 2. Circular dichroism spectra of wild type and mutant AdoMet synthetases. Solutions contained 7 μg protein in 25 mM Tris/HCl, 25 mM KCl, pH 8.0; spectra were recorded from 200 to 260 nm and were corrected for buffer contributions. Spectra are the average of three scans.

Expression, Purification, and Characterization of Mutant AdoMet Synthetases—Plasmids were transformed into strain RSR15(DE3) for protein isolation. Cultures were grown in LB medium containing 50 μg/ml carbenicillin. Following overnight growth, protein expression was enhanced by the addition of 0.1 mM isopropyl-β-D-thiogalactopyranoside 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 (14). The procedure consisted of ammonium sulfate fraction followed by successive chromatography on the hydrophobic interaction matrix phenyl-Sepharose HR (Amersham Pharmacia Biotech) and then hydroxylapatite CHT-I (Bio-Rad), followed by weak anion exchange resin aminohexyl-Sepharose (EAH-4B; Amersham Pharmacia Biotech). All characterized AdoMet synthetases were electrophoretically homogeneous. AdoMet synthetases were analyzed for oligomerization state by native gel electrophoresis on 8–25% gradient gels (14). 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 were corrected for buffer contributions. When present, the AdoMet concentration was 0.1 mM.

AdoMet Synthetase Assays—AdoMet synthetase activity was determined by the [14C]AdoMet filter binding method (10). Assays were performed in the presence of 9.1–5.0 mM L-[methyl14C]methionine (1.9 mCi/mmol), 0.05–15 mM ATP (Tris form) in 50 mM Hepes (CH3)4N salt) at pH 8.0 with 50 mM KCl and 50 mM MgCl2. The triplyphosphatase activity was determined by quantifying orthophosphate production (27, 28). Assays contained 3–500 μg PPP, (Na+ salt) in 50 mM Hepes(CH3)4N at pH 7.8 with 10 mM KCl and 10 mM MgCl2. AdoMet synthetase-catalyzed hydrolysis of ATP to ADP was followed by coupling diphosphate formation to NADH oxidation using pyruvate kinase and lactate dehydrogenase (10); NADH oxidation was followed at 340 nm.

Substrate saturation data were evaluated using the kinetic equations of Cleland (29) as implemented in the program Scientist (MicroMath, Inc.) or the Enzfitter program (Elsevier Biosoft).

RESULTS AND DISCUSSION

Characterization of AdoMet Synthetase Mutants—The six mutants constructed have varying physical properties, ranging from indistinguishable from wild type in secondary and quaternary structure (H14*N, K165*M, K265A) to tetrameric but with reduced helical content (K245*M, K269*M) to unstable and refractory to purification (K265M). The variation in secondary structure is illustrated by the circular dichroism spectra shown in Fig. 2; the 220-nm trough reflective of helical content is substantially reduced in the K245*M and K269*M variants. This is consistent with locations observed in the crystal structure, where residues Lys-245* and Lys-269 are located at the beginning of helices, whereas residues His-14* and Lys-265 are located in coil regions, and Lys-165* is in a β-sheet. Each of the mutations affects the catalytic behavior of the enzyme, especially the AdoMet synthesis activity, in all but K165*M (Tables I and II; illustrated graphically in Fig. 3). As a result of the variation in physical properties, the mutants are discussed individually in terms of their catalytic properties and the functional implications. There is no obvious pattern in kcat, Km, or Kcat/Km alternations with respect to the proposed role of the residues or the subunit from which the residues originate.

H14*N—The AdoMet synthetase activity of the H14*N mutant is reduced by 104-fold, with less than a 50% change in substrate Km values. In the PPP, hydrolysis reaction, the kcat in the absence of AdoMet is reduced 16-fold, with a 55-fold increase in Km. The presence of AdoMet results in a 6-fold decrease in the Km and a 10-fold increase in kcat for PPP; hydrolysis, yielding a much more efficient enzyme; this kcat value is 165-fold higher than the kcat for AdoMet synthesis, showing that the first step in the overall reaction has been preferentially impaired. The AdoMet-induced 10-fold increase in kcat is comparable with the 20-fold increase seen with the wild type enzyme and appears to be a property shared by mutants that do not affect the protein interactions with AdoMet. However, the affinity for AdoMet is decreased 35-fold; this is the only mutation that we have found to have an effect on AdoMet affinity; from the crystal structure, this would appear to be an indirect effect. Since the change corresponds to only 2.1 kcal/mol, a modest change in hydrogen bonding or environmental polarity could be responsible. The interpretation of the observations in terms of the crystal structure is facilitated, since the physical properties of this mutant are indistinguishable from the wild type enzyme. The much larger effect on AdoMet formation than PPP, hydrolysis supports the suggestion that ATP binds slightly deeper in the active site than is seen in the ADP complex, and H14* acts as a proton donor to O-5′ during AdoMet formation. A protonated imidazole side chain is consistent with the inability to inactivate the enzyme with histidine-selective chemical modifying reagents such as diethylpyrocarbonate (10).

In order to independently test the interactions with O-5′ proposed from the crystal structures, we examined the behav-
Active Site Mutants of AdoMet Synthetase

**Kinetic parameters for AdoMet synthetase activity**

AdoMet synthetase activity was measured as described under “Experimental Procedures.” Assays were performed in the presence of 0.01–0.5 mm L-[methyl-14C]-methionine (1.9 mCi/mmol), 0.05–15 mM ATP (Tris+ form) in 50 mM Hepes · (CH3)2N+ at pH 8.0 with 50 mM KCl, 50 mM MgCl2. Uncertainties in $k_{cat}$ (mol of product formed/mol of enzyme active site/s) are within 15%, and uncertainties in $K_m$ or $K_a$ are within 20%.

| Enzyme | $k_{cat}$ | $K_m$ (MgATP) | $K_m$ (Met) | $k_{cat}/K_m$ (MgATP) | $k_{cat}/K_m$ (Met) |
|---|---|---|---|---|---|
| Wild type | 1.5 | 0.11 | 0.08 | $14 \times 10^3$ | $19 \times 10^3$ |
| H14*N | $2.3 \times 10^{-4}$ | 0.07 | 0.11 | 3.3 | 2.0 |
| K165*M | 0.60 | 0.066 | 0.24 | $9.1 \times 10^3$ | $2.5 \times 10^3$ |
| K245*M | $3.6 \times 10^{-5}$ | 0.082 | 0.095 | 0.45 | 0.34 |
| K265A | $2.0 \times 10^{-4}$ | 5.2 | 0.54 | $0.037$ | 0.37 |
| K269M | $7.1 \times 10^{-4}$ | 0.16 | 7.1 | 4.4 | 0.10 |

*Values from Markham et al. (10).*

**Triplyphosphatase activity**

The crystallographic data show that Asp-16* is spatially adjacent to the substrate active site of the C-S-P-linked compound A(S)/TP, although in that case the lack of activity might be attributed to the poor hydrogen bonding ability of the sulfur (10). In the absence of methionine, A(NH)TP was a substrate for the ATPase activity of the enzyme, with a $K_m$ of 0.14 mM and a $k_{cat}$ of 0.03 s−1; the rate was not altered by the presence of 1 mM methionine. This $k_{cat}$ is larger than that for hydrolysis of ATP (10−3 s−1, $K_m = 1.3$ mM) but less than the value of 2.2 s−1 for A(S)/TP ($K_m = 0.6$ mM). Thus, for AdoMet synthetase, A(NH)TP is a better analog of ATP than A(S)/TP. However, the combined mutagenesis and substrate analog results do not resolve whether 0-5′ must be protonated for AdoMet synthesis.

**K245*M**—This variant has the lowest AdoMet synthetase activity of any mutant that we have studied, 42,000-fold lower than the wild type enzyme. The altered CD spectrum of the enzyme demonstrates that the protein is conformationally modified and makes interpretation of the kinetic data in terms of residue function ambiguous. Nevertheless, the $K_m$ values for both ATP and methionine are within 20% of the wild type values, suggesting that the binding sites are not grossly disrupted. The $k_{cat}$ for AdoMet hydrolysis is reduced by a relatively meager 155-fold in the presence of AdoMet, and the $K_m$ is 24-fold increased. In the absence of AdoMet, the PPPi hydrolysis reaction is undetectable, <0.1% of the wild type activity. Although AdoMet activates the enzyme for PPPi hydrolysis, it does not alter the circular dichroism spectrum of the protein, indicating that activation does not result from a large structural change. The large decrease in the $k_{cat}$ for PPPi hydrolysis in the absence of AdoMet and the recovery of activity in the presence of AdoMet are reminiscent of the behavior of mutants at the Mg2+-binding residue Asp-16* (D16*A and D16*N (16)). The crystallographic data show that Asp-16* is spatially adjacent to Lys-245* (Fig. 1); mutations at Asp-16* cause a 1000-fold reduction in the $k_{cat}$ for AdoMet synthesis, with less than 1.5-fold increase in $K_m$ values; these mutations do not cause alterations in CD spectra. The effects of mutations at Lys-245* and Asp-16* may both reflect the impaired ability to neutralize the negative charge that develops during hydrolysis (with Asp-16 acting through positioning a Mg2+-binding site). The role of Lys-165* and His-14* would appear similar based on the crystallographic data alone; however, the mutations at these residues have drastically different effects, suggesting that in the truly functional structures these amino acids are in slightly different positions than observed to date.
with the P₇. The R244*L mutation causes a 1000-fold decrease in $k_{cat}$ and -10-fold increases in $K_m$ for both ATP and methionine, whereas the K245*M has a 40-fold greater impairment of $k_{cat}$ without changes in $K_m$ values. Furthermore, the effects on $k_{cat}$ and $K_m$ for PPP hydrolysis are more than 10-fold larger for the K245*M mutant. Thus, the detailed roles of these residues in AdoMet synthetase appear distinct, with Lys-245* having a primary role in transition state stabilization and Arg-244* having a role in both ground state and transition state interactions.

**K265M and K265A**—The K265M mutant was unstable and lost at different stages of several independent purifications. Although this protein appears to have trace activity, the specific activity could not be reliably quantified. Since Lys-265 lies at the beginning of a helix, we constructed the K265A mutant to maintain helix forming propensity while decreasing the bulk hydrophobicity of the side chain. The circular dichroism spectrum of the K265A mutant shows no difference from the wild type enzyme. This mutant had a 10,000-fold decrease in $k_{cat}$ for AdoMet synthesis and increases in the $K_m$ values for ATP and methionine of 47- and 7-fold. In contrast, the PPP hydrolytic rate in the absence of AdoMet is 3.5-fold greater than the wild type enzyme, although the $K_m$ is increased 75-fold. The $k_{cat}$ does not increase in the presence of AdoMet, although the $K_m$ decreases 7-fold. While these properties resemble those of the K165*M mutant and the previously studied methionine binding site mutants, this residue is not near the proposed methionine binding site. In the crystal structures, Lys-265 is hydrogen-bonded to Asp-271, a ligand to a Mg²⁺ ion. The D271N and D721A mutants have 20-fold less impairment of the $k_{cat}$ for AdoMet synthesis than the K265A mutant and no changes in $K_m$ values; however, these were 4–5-fold more impaired in the
k_{\text{cat}} for PPP, hydrolysis in the presence and absence of AdoMet than is the K265M mutant. These comparisons suggest that the increased $K_m$ values for the K265A mutant may reflect minor conformational alterations rather than changes in specific enzyme-substrate interactions.

**K269M**—This mutant at an adenine binding residue was structurally altered from the wild type enzyme; circular dichroism spectra indicated a reduction in a-helix content, consistent with its position at the beginning of helix. The kinetics of the AdoMet synthetase reaction show not only a 2100-fold decrease in $k_{\text{cat}}$ but an 89-fold increase in the $K_m$ for methionine with only a 1.5-fold increase in the $K_m$ for ATP. This is the first mutant that we have found that has a selectively increased $K_m$ for methionine. In the PPP, hydrolytic reaction, while the $k_{\text{cat}}$ is reduced 4-fold in the absence of AdoMet (and the $K_m$ is increased 7-fold), the notable feature is that the $k_{\text{cat}}$ is only enhanced 3-fold by AdoMet, in contrast to the 20-fold for the wild type enzyme. The modest AdoMet enhancement of the $k_{\text{cat}}$ is reminiscent of mutants at other residues proposed to be near the methionine binding site, Asp-118 and Asp-238* (12). However, the affinity for AdoMet is only decreased 2.5-fold by the mutation. The NMR model for AdoMet binding does not indicate a role for the e-amino group of Lys-269 in binding, since the methionyl side chain points away from any position readily occupied by Lys-269. While the size of smaller Met-269 side chain could reduce favorable interactions, it as likely that the effects are indirect and result from the conformational alterations. Like the wild type enzyme, AdoMet does not prompt alterations in the circular dichroism spectrum of the protein, showing that catalytic function is not trivially related to this measurement of protein structure.

In order to further test the importance of hydrogen bonding to N-3 of ATP, we synthesized 3-deaza-ATP and characterized its activity as a substrate, since it cannot form the putative hydrogen bond with Lys-269. With the wild type enzyme, 3-deaza-ATP was a poor substrate, with a $K_m$ of 0.29 mM, or 2.6-fold higher than the $K_m$ for ATP, and a $k_{\text{cat}}$ 468-fold lower than found with ATP. Thus, the data do not support an important role for the amino group of Lys-269 in binding the adenine ring in enzyme-substrate complexes, although an interaction is apparently important in catalysis.

**Conclusions**—The mutants reported here were constructed to clarify the functions of the conserved active site basic residues, roles that were unclear based on the crystallographic and NMR data. The preferential impairment of AdoMet formation seen in most of these mutants is common with the seven of the eight other residues that we have studied by mutagenesis: the K+ binding glutamate 42 residue (13), the reactive cysteines, Cys-89 and Cys-239 (14), the active site arginine 244* (15), the two Mg2+ binding aspartates, and the aspartate 238* that interacts with the e-amino group of methionine/AdoMet (16). The K165*M mutant resembles the D118N mutant, which affects a side chain that is proposed to interact with the sulfur of methionine/AdoMet, in comparably reducing $k_{\text{cat}}$ values for both AdoMet synthesis and AdoMet-activated PPP, hydrolysis, and this is due to AdoMet inhibition rather than activation of the PPP, hydrolysis $k_{\text{cat}}$ (16). Nevertheless, all of the mutations thus far studied have had effects on AdoMet synthesis despite varying locations in the protein structure. The most remarkable change in catalytic capability found in this set of mutants is the 100-fold increase in PPP, hydrolytic activity seen with the K165*M enzyme; however, the $K_m$ value is increased more than 600-fold, rendering it ineffective at low PPP concentrations. While it is unlikely that there was evolutionary effort to optimize the PPP, hydrolytic reaction, it is interesting to find that the maximal catalytic activity of the enzyme can be enhanced in at least one reaction.

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. It is apparent from the sum of our mutagenesis studies that the catalytic efficiency in AdoMet synthesis arises from a highly coordinated active site. The precise roles of the active site amino acids cannot be interpreted definitively from their locations in the available crystal structures, probably because the available structures are of product complexes and one ligand is absent in each case. Thus, these studies continue to show that understanding catalysis relies upon the interplay of functional and structural studies (30, 31).

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**REFERENCES**

1. Usdin, E., Borchardt, R. T., and Creveling, C. R. (1982) Biochemistry of S-Adenosylmethionine and Related Compounds, MacMillan Press, London
2. Chiang, P. K., Gordon, R. D., Tal, J., Zeng, G. C., Doctor, B. P., Pardhasaradhi, K., and McCann, P. P. (1996) FASEB J. 10, 471–480
3. Tabor, C. W., and Tabor, H. (1984) Annu. Rev. Biochem. 53, 749–790
4. Frey, P. A., Ballinger, M. D., and Reed, G. H. (1998) Biochem. Soc. Trans. 26, 304–310
5. Mudd, S. H. (1973) in *The Enzymes*, 3rd Ed., Vol. 8, pp. 121–154, Academic Press, Inc., New York
6. Cantoni, G. L. (1975) *Annu. Rev. Biochem.* 44, 435–451
7. Tabor, C. W., and Tabor, H. (1984) *Adv. Enzymol.* 56, 251–282
8. Roth, M., and Geller, A. M. (1993) *Pharmacol. Ther.* 59, 125–145
9. Mats, J. M., Alvarez, L., Ortiz, P., and Pazare, M. A. (1997) *Pharmacol. Ther.* 73, 265–280
10. Markham, G. D., Hafner, E. W., Tabor, C. W., and Tabor, H. (1980) *J. Biol. Chem.* 255, 9082–9092
11. Markham, G. D., Parkin, D. W., Mentch, F., and Schramm, V. L. (1987) *J. Biol. Chem.* 262, 5609–5615
12. Reczkowski, R. S., and Markham, G. D. (1999) *Biochemistry* 38, 9063–9068
13. McQueney, M. S., and Markham, G. D. (1995) *J. Biol. Chem.* 270, 19277–19284
14. Reczkowski, R. S., and Markham, G. D. (1995) *J. Biol. Chem.* 270, 18484–18485
15. Reczkowski, R. S., Taylor, J. C., and Markham, G. D. (1996) *Biochemistry* 37, 13499–13506
16. Taylor, J. C., and Markham, G. D. (1999) *J. Biol. Chem.* 274, 32909–32914
17. Takusagawa, F., Kamitori, S., Misaki, S., and Markham, G. D. (1996) *J. Biol. Chem.* 271, 136–147
18. Fu, F., Wu, Y., Markham, G. D., and Takusagawa, F. (1996) *J. Biomol. Struct. Dyn.* 13, 727–739
19. Takusagawa, F., Kamitori, S., and Markham, G. D. (1996) *Biochemistry* 35, 2586–2596
20. Schalk-Hihi, C., and Markham, G. D. (1999) *Biochemistry* 38, 4433–4440
21. Chamberlin, M. E., Ubagai, T., Mudd, S. H., Levy, H. L., and Chou, J. Y. (1997) *Am. J. Hum. Genet.* 60, 540–546
22. Chamberlin, M. E., Ubagai, T., Mudd, S. H., Wilson, W. G., Leonard, J. V., and Chou, J. Y. (1996) *J. Clin. Invest.* 98, 1021–1027
23. Hazeldine, S., Bernardini, I., Shotelersuk, V., Tangerman, A., Guo, J., Mudd, H., and Gahl, W. A. (1997) *Am. J. Med. Genet.* 75, 385–400
24. Trowbridge, D. B., Yamamoto, D. M., and Kenyon, G. L. (1972) *J. Am. Chem. Soc.* 94, 3816–3824
25. Letsinger, R. L., Wolfs, J. S., and Dumas, L. B. (1972) *J. Am. Chem. Soc.* 94, 292–293
26. Sowa, T., and Ouchi, S. (1975) *Bull. Chem. Soc. Jpn.* 48, 2084–2090
27. Chifflet, S., Torriglia, A., Chiesa, R., and Tolosa, S. (1986) *Anal. Biochem.* 168, K–4
28. Baykov, A. A., Evtushenko, O. A., and Avaseva, S. M. (1988) *Anal. Biochem.* 171, 266–270
29. Cleland, W. W. (1979) *Methods Enzymol.* 63, 103–138
30. Tian, G., Yan, H., Jiang, R.-T., Kishi, F., Nakasawa, A., and Tsai, M.-D. (1990) *Biochemistry* 29, 4296–4304
31. Fersht, A. R., Knill-Jones, J. W., Bedouelle, H., and Winter, G. (1988) *Biochemistry* 27, 1581–1587