Catalytic Properties of the Archaeal S-Adenosylmethionine Decarboxylase from Methanococcus jannaschii*

Zechun J. Lu and George D. Markham‡

From the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111-2497

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S-Adenosylmethionine decarboxylase (AdoMetDC) is a pyruvoyl cofactor-dependent enzyme that participates in polyamine biosynthesis. AdoMetDC from the Archaea Methanococcus jannaschii is a prototype for a recently discovered class that is not homologous to the eucaryotic enzymes or to a distinct group of microbial enzymes. M. jannaschii AdoMetDC has a $K_m$ of 95 $\mu$M and the turnover number ($k_{cat}$) of 0.0075 s$^{-1}$ at pH 7.5 and 22 °C. The turnover number increased 38-fold at a more physiological temperature of 80 °C. AdoMetDC was inactivated by treatment with the imine reductant NaCNBH$_3$ only in the presence of substrate. Mass spectrometry of the inactivated protein showed modification solely of the pyruvoyl-containing subunit, with a mass increase corresponding to reduction of a Schiff base adduct with decarboxylated AdoMet. The pseudo-first order rate constant for reaction reversion was determined to be 80 s$^{-1}$, with $k_{cat}/K_m$ of 1.6; there was not a significant $D_2$O kinetic isotope effect on $k_{cat}$, suggesting that product release is rate-limiting in turnover. The pH dependence of the steady state rate showed participation of acid and basic groups with $pK$ values of 5.3 and 8.2 for $k_{cat}$ and 6.5 and 8.3 for $k_{cat}/K_m$, respectively.

The decarboxylation of S-adenosylmethionine (AdoMet)$^1$ occurs at a metabolic branch point at which AdoMet becomes committed to the synthesis of the ubiquitous polyamines rather than partaking in methylation or its many other roles (1–3). S-Adenosylmethionine decarboxylase (AdoMetDC) is one of a small group of decarboxylases that use a covalently attached pyruvoyl group rather than pyridoxal phosphate to form a Schiff base with the substrate during catalysis (4, 5). The pyruvoyl-containing enzymes are initially synthesized as preenzymes that self-cleave at an internal serine residue to yield two polypeptides, one of which (designated the $\alpha$ subunit) has the serine transmogrified into a pyruvoyl group at the new N terminus, a reaction in which the formation of an ester intermediate is analogous to intein processing (6).

A remarkable feature of AdoMetDC is that the enzyme from all studied eucarya, bacteria, and Archaea contains the pyruvoyl cofactor, whereas for other decarboxylases for which pyruvoyl-containing enzymes are known there are often pyridoxal-dependent enzymes with the same function (4, 7–11). Three distinct classes of AdoMetDC have been described, with little sequence similarity among them. The enzymes from eucarya are well conserved within this kingdom and are typically composed of $\sim$8-kDa ($\beta$) and 32-kDa ($\alpha$) polypeptides; in some organisms these associate to an ($\alpha\beta$)$_2$ heterotetramer, whereas in others the functional form is the $\alpha\beta$ heterodimer. Many eucaryotic AdoMet decarboxylases are activated by the polyamine putrescine, which is a substrate for the next metabolic reaction that uses decarboxylated AdoMet (dcAdoMet) as an alkyl group donor to synthesize spermidine (12); however, some plant AdoMetDCs are unaffected by this amine (cf. Bennett et al. (13)). The human and potato enzymes have been characterized by crystallographic and functional studies, which have revealed much about the protein structure and active site, as well as the allosteric nature of putrescine activation (13–18). A second class of AdoMet decarboxylase with a distinct sequence is typified by the Mg$^{2+}$-dependent Escherichia coli enzyme, which is composed of 12-4 and 18-kDa subunits in an ($\alpha\beta$)$_2$ arrangement (7, 15, 19–23). Less is known about the structure or mechanism of this protein; in particular, the role of the Mg$^{2+}$ has not been elucidated. The third class is a metal-free, activator-independent enzyme recently described in the Archaea Methanococcus jannaschii and the Gram-positive bacterium Bacillus subtilis (24, 25). This protein has an ($\alpha\beta$)$_2$ arrangement with subunit sizes of 61 and 63 residues (6.9 and 7.0 kDa, respectively) in M. jannaschii and subunits of comparable size in its homologs. A phylogenetic analysis showed that although this third class is the only type present in archaenal genomes, representatives are also found in many bacteria including the human pathogens Bacillus anthracis and Pseudomonas aeruginosa (24). In light of the established importance of polyamines in cell growth, this class may represent a newly identified target for the design of anti-bacterial agents. In the present study, we have undertaken an investigation of the functional properties of the M. jannaschii enzyme with the goals of understanding the catalytic process of this small enzyme and of identifying additional distinguishing properties for use in the design of selective inhibitors.
EXPERIMENTAL PROCEDURES

Reagents were obtained from Sigma unless otherwise noted. Purified decarboxylated S-adenosylmethionine was a gift from Dr. David Anton, DuPont.

Improving the Expression of M. jannaschii AdoMetDC in E. coli—E. coli strain AM1A262, which contains the M. jannaschii AdoMetDC gene (MJ0315) cloned into pUC18, was purchased from the American Type Culture Collection as part of the collection from The Institute for Genome Research. The M. jannaschii DNA fragment cloned in this plasmid encodes the complete MJ0315 gene flanked by parts of the coding regions of the adjacent MJ0314 and MJ0316 genes (24).

The MJ0315 gene was subcloned into pET3a using NdeI and BamHI sites that were created by PCR at the start and end of the coding region, respectively. The DNA sequence confirmed that the desired coding region was obtained, and the mass spectrum of the purified protein showed the same ions reported previously (24). This plasmid, denoted pMJDCase-1, was transformed into strain BL21(DE3)-codon-plus-RIL (Stratagene) for protein purification. The MJ0315 gene has numerous codons for arginine and isoleucine that are rare in E. coli, and the additional TRNAS, provided by the Codon Plus system, were found to substantially increase the protein yield.

AdoMetDC Purification—A small culture of BL21(DE3)-codon-plus-RIL(pMJDCase-1) was grown overnight at 37 °C in LB medium containing 50 μg/ml carbenicillin. Larger cultures of LB media were then inoculated with 1:20 dilution (v/v) of the overnight culture and grown at 37 °C for 4 h. Induction of AdoMetDC expression was obtained by the addition of 1 mM isopropyl-1-thio-(β-D-galactopyranoside) followed by growth for an additional 3 h. Cells were harvested by centrifugation and stored at −80 °C until use.

The steps below reflect a preparation from 30 g (wet weight) of cells. The cell pellet was suspended in 50 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, 30 μM phenylmethylsulfonyl fluoride, pH 8, using 10 ml of buffer/g (wet weight) of cells. The cells were lysed by one pass through a French press at 10,000 p.s.i. Debris was removed by centrifugation at 13,000 × g for 30 min. The ammonium sulfate was then added to the supernatant to 20% saturation, and the insoluble material was removed by centrifugation. Ammonium sulfate was then added to the supernatant to 80% saturation, and the pellet was collected by centrifugation. The ammonium sulfate pellet was dissolved in 50 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, 0.5 M KCl, pH 8. The fractions containing activity were collected and dialyzed overnight against 50 mM MOPS, 1 mM EDTA, 1 mM DTT, pH 7. The protein was then loaded onto a ceramic hydroxyapatite (Type I, 200 μm, Bio-Rad) column (2.5 × 15 cm) and washed with 200 ml of 50 mM MOPS, 1 mM EDTA, 1 mM DTT, pH 7, at a flow rate of 2 ml/min. The protein was eluted with a 500-ml gradient from the wash buffer to the same buffer containing 0.5 M phosphate.

Final purification was obtained by gel filtration chromatography on a 2.6 × 100-cm Sephacryl S-100 column (Amersham Biosciences) that was equilibrated and eluted at 0.75 ml/min with 50 mM KH2PO4, 1 mM EDTA, pH 7.5. The final protein displayed a single band after native electrophoresis on a 8–25% gradient polyacrylamide Phast gel that was stained with Coomassie Blue (Amersham Biosciences). The very small size of the two subunits results in protein bands that run near the front even on high density SDS gels (Amersham Biosciences), and the proteins stain poorly, rendering this an insensitive method. The overall protein yield was 8 mg of protein/g of cells. Protein concentrations were calculated from the absorbance at 280 nm using an extinction coefficient of 12330 M⁻¹ cm⁻¹ as calculated by the program PeptideSort (26).

Kinetic Analyses of AdoMetDC Activity—AdoMetDC activity was assayed either by measuring the production of 14CO2 from [carboxy-14]AdoMet (Moravek Biochemicals) (24) or by HPLC analysis. Routine reaction mixtures contained 50 mM Hepes-KOH, 5 mM EDTA, pH 7.5, in a volume of 100 μl. Reactions using [carboxy-14]AdoMet were stopped by the addition of 100 μl of 4N HCl to sealed reaction vials containing Ba(OH)2-soaked filters in the caps; after the samples were shaken for 30 min the 14CO2 was determined by scintillation counting of the filters.

For HPLC analysis the reactions were terminated by the addition of 25 μl 1.6 N of HClO4. The reaction mixture was then centrifuged at 13,000 × g for 2 min. Substrate and product were quantified by cation exchange HPLC on a VYDAC 400VHP575P column (0.75 × 5 cm) eluted with 25 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, 0.5 M KCl, pH 8. The fractions containing activity were collected and dialyzed overnight against 50 mM MOPS, 1 mM EDTA, 1 mM DTT, pH 7.
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70 °C and then the eluant was changed to 70% buffer, 30% H2O; decarboxylated-AdoMet eluted after 10 min. The HPLC system consisted of a Waters 600E equipped with Waters 994 programmable photodiode array detector. The integrated peak areas for AdoMet and dcAdoMet were determined at 280 nm, and the data were analyzed to obtain the relative amounts of the remaining substrate and the product.

To determine the kinetic parameters, kcat and KM, initial velocities were measured for at least five AdoMet concentrations. Product formation was linear with time under the conditions used. The data were fitted to the Michaelis-Menten equation, and kinetic parameters were calculated using the Levenberg-Marquardt method of nonlinear least squares regression using equal weighting in the kinetics module of SigmaPlot (SigmaPlot 2000, SPSS Science). The pH dependencies of the reaction rates were analyzed using the equation,

\[ \frac{v_{\text{observed}}}{v_{\text{max}}(1 + 10^{pK_a - pH}) + 10^{pH - pK_c}} + v_{\text{pH-independent}} \]  

(Eq. 1)

because the observed rates approach a non-zero limiting value at the limiting pH values compatible with protein and substrate stability.

Kinetic studies in D2O were performed at room temperature as described above except that the reaction solutions contained 90% D2O. Solutions for solvent deuterium isotope effect measurements were prepared from a stock solution of buffer that was dried and redissolved in D2O; this method maintains a constant ratio of acidic and basic buffer components and, thus, an equivalent pL (L = H or D) (27). Rates were measured at pL of 7.5 and 8.0, and no differences in kinetic isotope effects were found. The kinetic isotope effects were determined by fitting data in H2O and D2O using the VVKSISOT program (28).

Temperature and pH Effects on AdoMetDC Activity—AdoMetDC activity of the purified enzyme was studied by varying reaction conditions including temperature and pH. Effects of temperature on AdoMetDC activity were analyzed by HPLC analyses. Reactions were initiated by the addition of enzyme to reaction mixtures that were preequilibrated at desired temperatures from 15 to 90 °C; solutions contained 50 mM Hepes-KOH, 5 mM EDTA, pH 7.5. Care was taken to ensure substrate saturation at each temperature. The pH of the reaction solutions did not vary significantly over the range studied.

The influence of pH on AdoMetDC activity was carried out in 50 mM buffer (Mes, pH 4.4–6.8; Hepes, pH 7.0–9.0; Chex and Hepes, pH 9.5–10.0) and 5 mM EDTA; the pH was adjusted by the addition of KOH as required. The 14CO2 production assay was used. Inhibitors of AdoMetDC Activity—Compounds tested as inhibitors included the product decarboxylated-AdoMet, the classic AdoMet decarboxylase inhibitor methyglyoxal bis(guanylhydrazone) (MGBG) (29), 1-methionine, S-adenosyl-L-homocysteine, S-methyl-L-methionine, and sinefungin, a natural product AdoMet analog in which the S\(^{-}\)-CH3 is replaced by a CH-NH\(^{+}\) group. Survey inhibition studies were conducted with an AdoMet concentration near the KM of 95 μM. The effect of pH on inhibition by MGBG and decarboxylated-AdoMet was studied by variation of both AdoMet and inhibitor concentrations at each pH value. Data were evaluated using the kinetics module of SigmaPlot 2000 (SPSS Science).

Substrate-mediated Inactivation—The possibility of substrate-mediated inactivation was measured by incubating AdoMetDC with or without 0.5 mM AdoMet at room temperature for various time periods (15–60 min) in 50 mM Hepes-KOH, 5 mM EDTA, at either pH 7.5 or 9. After preincubation, [carboxy-\(^{14}\)C]AdoMet was added to the mixtures, and the enzyme activity was measured in the standard CO2 formation assay. The same conditions with the addition of 0.1 mM NaCNBH\(_3\) were used for Schiff base trapping experiments. After incubation the samples were mixed with an equal volume of 20 mg/ml sinapinic acid in 1% trifluoroacetic acid, 50% acetonitrile and analyzed by matrix-assisted laser desorption-time of flight mass spectrometry on a Bruker instrument.

NMR Studies—\(^{1}H\) NMR spectra were obtained on a Bruker DRX-300 spectrometer operating at 300 MHz. Data were collected at 25 °C using a standard one-pulse sequence.

UV Spectroscopic Studies—UV spectra were obtained on a Hewlett-Packard 8453 instrument with a resolution of 1 nm. Spectra were obtained with a 30-s integration time to reduce noise. Spectra of the free enzyme and free MGBG were subtracted from the spectrum of the E plus MGBG mixture to reveal the spectral perturbations on complex formation. Solutions contained 100 μM (αβ) protein and various concentrations of MGBG between 25 and 600 μM in 25 mM Tris-Cl, 0.2 mM DTT, pH 7.5. The decrease in absorbance at 325 nm between the protein?
rate-limiting step over this temperature range and the absence of a significant heat capacity of activation, implying that a large protein conformational change does not accompany the reaction (31).

Several compounds were tested as potential inhibitors of AdoMetDC in the presence of AdoMet at a concentration corresponding to its $K_m$ value. No inhibition was observed with 4 mM S-adenosyl-L-homocysteine, 10 mM L-methionine, 25 mM S-methyl-L-methionine, or 20 mM sinefungin. Inhibition by the product dcAdoMet and by methylglyoxal bis(guanylylhydrazone) is described below.

**pH Dependence**—The pH dependence of the reaction rate was characterized at 22 °C. The pH-rate profiles shown in Fig. 2 are bell-shaped for both $k_{cat}$ and $k_{cat}/K_m$, but the rates approached non-zero limiting values, as is particularly apparent for $k_{cat}/K_m$. The $k_{cat}$ profile showed pK values for a group that must be deprotonated, with a pK$_a$ of 5.3 ± 0.1, and a group that must be protonated, with a pK$_a$ of 8.2 ± 0.1; a limiting pH-independent rate was ~2% of the maximal rate (Fig. 2A). The $k_{cat}$ profile reflects the ionization states of acidic and basic groups that are required for the enzyme-AdoMet complex to form products with maximal efficiency. The pH dependence of $k_{cat}/K_m$ slopes in the pH 5.5–9 region revealed a pK$_a$ value of 6.5 ± 0.3 and a pK$_s$ value of 8.3 ± 0.3 (Fig. 2B); this pH profile reflects the ionization of the free enzyme and free AdoMet as well as the enzyme-AdoMet complex. These pK values are not closely related to that of the substrate (the only AdoMet ionization in this range is that of the amino group, which is 7.8 (32)), and thus, these ionizations reflect groups on the protein.

The unit slopes in the pH 5.5–9 region of both the $k_{cat}$ and $k_{cat}/K_m$ profiles reflect the involvement of single acidic and basic groups. The pK near 8.2 is in the normal range of a cysteine sulfhydryl in peptides (33). By analogy to previous studies of eucaryotic AdoMetDC, this pK likely reflects the

**Fig. 4. Interaction of AdoMetDC with MGBG.** Panel A illustrates the structure of the inhibitor MGBG. Panel B shows the UV difference spectrum for complex formation between AdoMetDC and MGBG. Solutions contained 100 µM ($\alpha$β) protein and 100 µM MGBG in 25 mM Tris-Cl, 0.2 mM DTT, pH 7.5. Panel C shows a Scatchard plot for MGBG binding as determined from diminution in the 325-nm absorbance in the presence of protein. Solutions contained 100 µM ($\alpha$β) protein and various concentrations of MGBG between 25 and 600 µM in 25 mM Tris-Cl, 0.2 mM DTT, pH 7.5.
ionization of cysteine in the conserved Thr-Cys-Gly sequence located 19 residues C-terminal to the pyruvoyl group in the *M. jannaschii* protein and in similar locations in all other characterized AdoMet decarboxylases (17, 34). The tendency of *k*<sub>cat</sub>/\(K_m\) to approach limiting values at the pH extremes emphasizes decreases in the \(K_m\) value; because the \(K_m\) is a complex kinetic constant, the molecular origin of this decrease is unclear at present. The *k*<sub>cat</sub>/\(K_m\) value approaches a limiting value of \(\sim 7\%\) of the maximal rate at high pH and tends toward value near 2% of the maximal rate at the low pH extreme. It is noteworthy that site-directed mutagenesis studies of the eucaryotic AdoMetDC have found residual activity after the removal of candidate acid/base catalytic groups, suggesting that the groups required for maximal activity are not absolutely essential for catalysis (17, 34) (see "Discussion").

The product dcAdoMet was a competitive inhibitor with respect to AdoMet; thus, product release is either random or CO<sub>2</sub> is released first (Fig. 3A). The pH dependence of inhibition by dcAdoMet was determined in the range of 6.0–10.0 (Fig. 3B); the \(K_i\) increased substantially below pH 6.8 but did not significantly vary between pH 8 and 10. The \(K_i\) increase at low pH may reflect the same enzyme group that has a pK of 6.5 in the \(k_{cat}/K_m\) profile. The pH dependence does not reflect the ionization of the amino group of dcAdoMet, which was determined to have a pK of 9.2 from the pH dependence of the \(^1H\) NMR resonances of the \(\alpha\) protons.

**Inhibition by MGBG—**MGBG (the structure is illustrated in Fig. 4A) is a well characterized inhibitor of AdoMet decarboxylases (35). MGBG has been the parent of a multitude of analogs that have been prepared in searches for compounds of increased efficacy as cancer chemotherapeutic agents (36, 37). Immobilized MGBG has been commonly used for affinity chromatographic purification of the enzymes (38) and was used for the initial purification of the *M. jannaschii* enzyme (24). MGBG has a pK of 7.9, above which the compound displays a bright yellow color with an absorption maximum at 325 nm. A neutron diffraction study of the colorless MGBG-2HCl crystals showed that the dication is protonated on all four of the terminal nitrogens (39). Presumably, one of these terminal NH<sub>2</sub> groups loses a proton with a pK of 7.9, creating an extended conjugated structure that absorbs at 325 nm.

MGBG is a competitive inhibitor of *M. jannaschii* AdoMetDC with a \(K_i\) of 25 \(\mu\)M. The \(K_i\) did not vary in the pH range from 6.5 to 9.0 but decreased \(\sim 2\)-fold at pH 6.0. To probe the ionization state of MGBG bound to AdoMetDC, the perturbation of the UV spectrum upon formation of the enzyme-MGBG complex was determined by difference spectroscopy (Fig. 3B). Upon complex formation at pH 7.5 the 325 nm absorption of the +1 ionization state disappears, indicating that MGBG is bound as the dication. The difference in 325 nm absorption at various MGBG concentrations yielded a dissociation constant of 23 ± 3 \(\mu\)M and binding site stoichiometry of 1.1 ± 0.1 per (\(\alpha\))<sub>β</sub> heterodimer (Fig. 3C).

**D<sub>2</sub>O Kinetic Isotope Effects and Presteady State Kinetic Studies—**The chemical mechanism for a decarboxylation reaction involving a Schiff base has numerous proton transfers to and from nitrogen and carbon atoms; hence, a significant solvent kinetic isotope effect might be anticipated. Steady state kinetic measurements for *M. jannaschii* AdoMetDC in 90% D<sub>2</sub>O showed a negligible kinetic isotope effect on *k*<sub>cat</sub> of 1.1 ± 0.2 and an isotope effect of 1.6 ± 0.3 on \(k_{cat}/K_m\) (Fig. 5). The observation of an isotope effect on \(k_{cat}/K_m\) requires that AdoMet be able to dissociate from the E-AdoMet-H<sup>+</sup> complex at a rate at least comparable with the rate of conversion of the complex to products (40).

Presteady state kinetic measurements were conducted with AdoMet concentrations less than that of the enzyme as well as in excess of both the enzyme concentration and the \(K_m\) value. The time courses for the reactions with excess substrate showed that an initial burst of product formation is followed by a slower steady state reaction rate (Fig. 6A). The amount of CO<sub>2</sub> formed in the initial burst was measured at 25, 50, 125, and 250 \(\mu\)M AdoMet and maximized at 0.7 equivalents/subunit. Interestingly, this stoichiometry is the same as that reported by Kinch and Phillips for the *Trypanosoma cruzi* enzyme (34). The solvent deuterium isotope effect on the burst rate was obtained at limiting substrate (25 \(\mu\)M AdoMet, 37 \(\mu\)M enzyme) in 90% D<sub>2</sub>O; fitting the data to a single exponential yielded a H<sub>2</sub>O/D<sub>2</sub>O rate ratio of 1.9 (Fig. 6B). The rate of the initial burst phase was 0.6 min<sup>-1</sup> at 25 \(\mu\)M AdoMet, providing a lower limit for the second order rate constant for AdoMet association of 400 M<sup>-1</sup> s<sup>-1</sup>; this is \(\sim 5\)-fold larger than the lower limit on the binding rate constant estimated from the value of \(k_{cat}/K_m\).

**Schiff Base Trapping—**Catalysis-dependent inactivation of AdoMet decarboxylase has been described both *in vitro* and *in vivo* and has been instructive in establishing a Schiff base mechanism (15, 17, 19, 21, 41). Inactivation results from incorrect decomposition of a Schiff base intermediate, either leading to transamination of the catalytic pyruvate to alanine or by a
more complex mechanism involving modification of the catalytic cysteine after elimination of methylthioadenosine from the Schiff base (21, 22, 42). Mass spectrometry of proteins modified by the latter pathway revealed adducts with mass increases of +57 and +75 daltons resulting from the addition of CH$_2$-CH$_2$-CHO and the hydrate thereof (15). In contrast the mass spectrum of the \textit{M. jannaschii} decarboxylase as isolated from \textit{E. coli} showed that this protein is not detectably modified in vivo when expressed either at low levels (24) or at 40-fold greater levels of expression obtained in the present work (Fig. 7A). The possibility of catalysis-dependent inactivation of the purified protein was investigated by a room temperature incubation with 0.5 mM substrate for 5 min at pH 7.5 before determination of enzyme activity. The specific activity of the enzyme was unchanged even after a 30-min incubation with AdoMet. However mass spectrometry showed a decrease on the \(\alpha\beta\) subunit ratio (Fig. 7, A and B), suggesting protein modification during sample preparation, perhaps by trapping of a Schiff base in the acidic conditions (43).

When the enzyme was incubated with the imine-reducing agent NaCNBH$_3$ (at 100 mM for 30 min at room temperature), no loss in activity was observed, and there was no alteration in the mass spectrum of the protein (Fig. 7C). However, when 0.5 mM AdoMet was also present, >75% of the enzyme activity was lost. The mass spectrum of the inactivated protein showed selective diminution of the peak from the pyruvoyl-containing \(\alpha\beta\) subunit (Fig. 7D) with the appearance of new peaks of higher mass/charge ratio. The mass species of the largest species was increased by 340 daltons, a mass change corresponding to that expected for the presence of a reduced dcAdoMet adduct. Other ions of lower mass correspond to the loss of CH$_3$ (m/z = 7316) and deoxyadenosine (m/z = 7081). An adduct with AdoMet was not detected. Other new peaks are plausibly due to additional Schiff base decomposition products.

**DISCUSSION**

The results of this study provide the initial glimpses into the mechanism of a recently discovered class of AdoMetDCs. It is
particularly noteworthy that the αβ heterodimer of the class of AdoMetDC for which the M. jannaschii protein is a prototype is less than one-half the size of the other microbial class, for which the Mg²⁺-dependent E. coli enzyme is the best studied, and one-third the size of the well characterized eucaryotic class. Thus, in addition to the diverged sequences, the proteins must have a different three-dimensional structures. Nevertheless, the use of the pyruvoyl group as a catalytic device is common to all three classes even though the sequence variation extends to the residues proximal to the serine that is the pyruvoyl precursor. A downstream sequence encompassing the presumed catalytic cysteine in the Thr-Cys-Gly sequence is the sole recognizable conserved region.

Despite the differences in size and sequence, the functional properties of the various AdoMetDC have substantial similarities. In light of the present results at least one enzyme from each class has been shown to catalyze a reaction in which a Schiff base is formed as an intermediate; in each case studied, only the Schiff base with the product is trapped by NaCNBH₃, implying that a step after CO₂ formation is rate-limiting in turnover (Scheme 1) (17, 23, 34). This result differs from the aspartate α-decarboxylase, where both substrate and product were trapped (44), indicating that the rate-limiting steps or enzyme-bound equilibrium constants differ for the aspartate and AdoMet decarboxylases.

Pyruvoyl group catalysis of decarboxylation via Schiff base formation involves both acid and basic catalytic groups (Scheme 2). Comparison of the pH dependence and solvent kinetic isotope effects of the reaction with those previously reported for the human, T. cruzi, and E. coli AdoMetDCs illustrates both similar and distinct features (Table I) (17, 23, 34). Each enzyme displays a bell-shaped pH dependence for kₐₙ/Kₘ and, where studied, for kₐₙ. None of the observed pK values corresponds to an ionization of AdoMet, and thus, they reflect either the enzyme or enzyme-substrate complex. The group that must be protonated in each case has a pK near 8 in the kₐₙ profile, i.e. 7.8 (human), 8.7 (E. coli), and 8.2 (M. jannaschii); in the kₐₙ/Kₘ profile the corresponding pK values are 8.9, 8.7, 8.3, and 8.7 for the enzyme T. cruzi (the T. cruzi enzyme was studied under single turnover conditions, and a dependence on two protons was noted for both the acidic and basic pK values (i.e. a dependence on [H⁺]²) (34)). Mutagenesis data for the eucaryotic enzymes and the observation of an inverse solvent kinetic isotope effect on the initial turnover of the T. cruzi protein have indicated that the pK of the acid is attributable to the sulphydryl of the reactive cysteine that is situated C-terminal to the pyruvoyl group in a conserved sequence (17, 34). Nevertheless, mutants with alanine in place of cysteine retained 0.3 and 0.04% activity for the T. cruzi and human AdoMetDC, showing that the sulphydryl moiety is not abso-

![Scheme 1. Minimal reaction sequence for AdoMet decarboxylase. Covalent enzyme ligand complexes are denoted by E-AdoMet, etc. The three final steps each represent a conglomerate of reactions including the formation and hydrolysis of Schiff base intermediates. The rate-limiting step in turnover is after decarboxylation. Proton transfer(s) is partially rate-limiting on steps up to and including decarboxylation.](image)

![Figure 7. Mass spectra of AdoMetDC illustrate Schiff base trapping by NaCNBH₃. Panel A, AdoMetDC alone. The peak at 6794 daltons arises from the β subunit, and the peak at 6991 daltons arises from the pyruvoyl group-containing α subunit. Panel B, AdoMetDC incubated with 0.1 M NaCNBH₃. Panel D, AdoMetDC incubated with NaCNBH₃ and 0.1 M NaCNBH₃. Solutions contained with 50 mM Hepes-KOH, 5 mM EDTA, pH 7.5, at 22 °C. Thirty-minute incubations preceded the addition of an equal volume of 20 mg/ml sinapinic acid in 1% trifluoroacetic acid, 50% acetonitrile for mass spectrometry.](image)

![Table I. pH dependence of kₐₙ and kₐₙ/Kₘ for AdoMet decarboxylases from different organisms](image)
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Scheme 2. Steps in the AdoMet decarboxylase reaction. Specific amino acid residues are not illustrated. A Schiff base forms with the residue pyruvate 64. Cysteine 84 is likely to be a proton donor based on analogy to the eucaryotic enzymes for which this role has been shown for the homologous residue; the catalytic base(s) remain undefined (17, 34).

Solvent kinetic isotope effects can be informative in elucidating the contribution of proton transfer reactions to the rate-limiting steps. The solvent kinetic isotope effects are quite variable among AdoMetDC. The E. coli enzyme was reported to have a tritium kinetic isotope effect of 4.5 on $k_{\text{cat}}/K_m$ (45), whereas the T. cruzi enzyme had an inverse deuterium solvent kinetic isotope effect of 0.53 on the initial turnover (34), and the M. jannaschii enzyme had a deuterium solvent isotope effect value of 1.6 on $k_{\text{cat}}/K_m$. The observed kinetic isotope effect with the M. jannaschii AdoMetDC is much less than that expected for a fully rate-limiting proton transfer and may reflect attenuation by a AdoMet commitment to catalysis or by other partially rate-limiting steps. The similar solvent kinetic isotope effect of 1.9 on the initial turnover of the M. jannaschii enzyme indicates a partially rate-limiting proton transfer in a step that includes or precedes the first irreversible step, which is presumably decarboxylation. Presteady state bursts of CO$_2$ formation with both the M. jannaschii and T. cruzi AdoMetDC show that a late step in the reaction is rate-limiting, and the lack of a D$_2$O isotope effect on $k_{\text{cat}}$ suggests that product release rather than Schiff base hydrolysis is rate-limiting.

MGBG is a common inhibitor of AdoMetDC from all organisms tested, albeit with up to 1000-fold higher affinity for the eucaryotic enzymes. Our data show that MGBG binds as the dication, which is consistent with the recent crystal structure of the MGBG complex with the human AdoMetDC, which revealed MGBG bound in a sandwich between aromatic side chains (15) in an apparent example of cation-π interactions (46). If the micromolar affinity of the M. jannaschii AdoMetDC for MGBG is representative of this class of microbial AdoMetDC, the testing of MGBG analogs for selective inhibition of polyamine biosynthesis in pathogenic bacteria having homologs of the M. jannaschii enzyme, such as B. anthracis and P. aeruginosa, may be warranted.

An intriguing question that remains unresolved is why AdoMet decarboxylase remains the only known enzyme in which the pyruvoyl group is conserved as the catalytic device throughout evolution, particularly since the reminder of the protein is so widely diverged. The urban legend that the pyruvoyl group is a more economical (i.e. primitive) cofactor is unlikely to be the underlying cause because the in vivo catalysis-dependent inactivation of the enzyme cannot be repaired and, consequently, requires the energetically costly replacement of the entire protein; in contrast, inactivating side reactions of pyridoxal enzymes typically require only the exchange of the cofactor. Studies of the pyruvoyl- and pyridoxal-containing histidine decarboxylases have shown them to have comparable catalytic prowess (47, 48). Computational results support the ability of a pyruvoyl group to function in decarboxylation chemistry with comparable efficiency to a pyridoxal cofactor (49, 50). As additional pyruvoyl enzymes are discovered and characterized (cf. Ref. 11) presumably the underlying theme for their continued existence will become clear.

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