Rat Guanidinoacetate Methyltransferase

EFFECT OF SITE-DIRECTED ALTERATION OF AN ASPARTIC ACID RESIDUE THAT IS CONSERVED ACROSS MOST MAMMALIAN S-ADENOSYL METHIONINE-DEPENDENT MethylTransferases*

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Most mammalian S-adenosylmethionine (AdoMet)-dependent methyltransferases have a conserved aspartate residue in a sequence Odso (o denotes a hydrophobic amino acid and s denotes a small neutral amino acid). Rat guanidinoacetate methyltransferase has two aspartate residues (Asp-129 and Asp-134) conforming to the motif in close proximity to Tyr-136 that is photoaffinity-labeled by AdoMet (Takata, Y., and Fujioka, M. (1982) Biochemistry 31, 4369–4374). In order to investigate the role of these residues, we prepared variant forms of the enzyme by oligonucleotide-directed mutagenesis. Conversion of Asp-129 to asparagine or alanine resulted in a functional enzyme. Alteration of Asp-134 to glutamate (D134E) and asparagine (D134N) decreased activity, and replacement with alanine (D134A) led to inactivation. Decreases of 3- and 120-fold were found for $k_{cat}$ values of D134E and D134N, respectively. The $K_m$ values of D134E for AdoMet and those for guanidinoacetate were increased about 160- and 80-fold over the respective values of wild type. The corresponding increases in D134N were 800- and 50-fold, respectively. Conservative changes in the residues flanking Asp-134 had little effect on activity. Guanidinoacetate methyltransferase obeys an ordered Bi Bi mechanism in which AdoMet binds first. Thus, the large decreases in $k_{cat}/K_m$ values for AdoMet indicate that Asp-134 is crucial for binding AdoMet. Spectroscopic studies indicated that the amino acid substitutions of Asp-134 resulted in no significant changes in the secondary and tertiary structures, and urea denaturation experiments showed that the altered enzymes were not destabilized.

Guanidinoacetate methyltransferase (EC 2.1.1.12) catalyzes the S-adenosyl-t-methionine (AdoMet)-dependent methylation of guanidinoacetate to form creatine. The enzyme is found ubiquitously and in abundance in the livers of all vertebrates and is believed to be a major enzyme involved in the metabolic conversion of AdoMet to AdoHcy in these organisms. Rat liver guanidinoacetate methyltransferase is a simple, monomeric protein with $M_s$, 26,000 (Ogawa et al., 1983). The enzyme has been produced recombinantly in large amounts in Escherichia coli (Ogawa et al., 1988), and its structural and functional features have been studied using chemical modification, site-directed mutagenesis, and limited proteolysis. These studies have revealed that Cys-15, Cys-90, and Cys-219 occur spatially close together (Fujioke et al., 1988; Takata et al., 1991) and that the region around residues 19–24 is highly exposed to the solvent and flexible (Takata and Fujioka, 1990; Fujioke et al., 1991). Whereas disulfide cross-linking of Cys-15 with either Cys-90 or Cys-219 leads to a large loss of activity and removal of the N-terminal microapeptide results in an inactive enzyme, the portion comprising the 3 cysteines as well as the N-terminal region are apparently distant from the active site. The first indication of an active site residue has been obtained recently by photo-affinity labeling, UV irradiation of the enzyme in the presence of AdoMet resulted in covalent attachment of the compound to Tyr-136, showing characteristics of affinity labeling (Takata and Fujioka, 1992).

Ingrosso et al. (1989) showed that many methyltransferases as well as other AdoMet-utilizing enzymes shared three regions of sequence similarity (regions I, II, and III from the N-terminal side). Of these, region III, which has a sequence motif L(R/K)PGGX (X represents any amino acid), is unique to and is conserved among most mammalian methyltransferases (Gomi et al., 1992). A previous study, however, demonstrated that this region was not involved in binding of AdoMet in guanidinoacetate methyltransferase (Gomi et al., 1992). Region II, which is located 20–30 residues upstream of region III, has an aspartate residue preceded by a hydrophobic amino acid and followed successively by a small neutral and a hydrophobic residue (Table I). In guanidinoacetate methyltransferase, the sequences around Asp-129 and Asp-134 conform to the motif. Since these aspartates are close to Tyr-136, it may be considered that either one of the aspartate-containing segments forms part of the AdoMet-binding site. In order to test this possibility and to explore the role of the conserved aspartate residue, we introduced amino acid changes to Asp-129, Asp-134, and neighboring residues. In this article, we show that alteration of Asp-134 only exerts profound effects on the catalytic properties of the enzyme.

EXPERIMENTAL PROCEDURES

Materials—AdoMet (chloride salt), AdoHcy, and adenosine deaminase (type VI) were obtained from Sigma, and sinefungin was

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Table I

| Enzyme* | Sequence | Position | Ref. |
|---------|----------|----------|------|
| Guanidinoacetate MT (rat liver) | HFDGILY | 127-133 | Ogawa et al. (1988) |
| Hydroxyniolde MT (bovine pineal gland) | LYDTPY | 132-138 | |
| Phenylethanolamine MT (bovine adrenal medulla) | FKADPE | 238-244 | Ishida et al. (1987) |
| Glycine MT (rat liver) | PADAIVS | 174-180 | Baetge et al. (1986) |
| D-Asp/L-isoAsp MT (bovine brain) | GFLAVIC | 131-137 | Ogawa et al. (1997) |
| Catechol MT (rat liver) | PYDAHIV | 151-157 | Henzel et al. (1989) |
| Histamine TM (rat kidney) | DVTDLDM | 181-187 | Salminen et al. (1993) |
| | KWDHFM | 136-142 | Takeamura et al. (1992) |

*M, methyltransferase; D-Asp/L-isoAsp, aspartyl/L-isoaspartyl.

*Mutagenesis of Guanidinoacetate Methyltransferase—Wild type recombinant guanidinoacetate methyltransferase was expressed in E. coli JM109 (recA1, endA1, gyrA96, thi-1, hsdR17, supE44, relA1). A pUC118 plasmid containing each mutated sequence was grown, and the recombinant enzyme was purified by passage through a C-60 column (Sep-Pak; Waters Associates) as described previously (Fujio and Iabiguro, 1986), and iodooacetic acid (Merek) was recrystallized from hot chloroform. Other chemicals were of the highest purity available from commercial sources and were used without further purification.

Expression and Purification of Wild Type Recombinant Guanidinoacetate Methyltransferase—Wild type recombinant guanidinoacetate methyltransferase was produced in E. coli JM109 (recA1, endA1, gyrA96, thi-1, hsdR17, supE44, relA1, lacIq, proA2, lacIq, and ZAM15) (Yanosch-Perron et al., 1985) transformed with plasmid pUCGTA9-1 that contained the coding region of rat guanidinoacetate methyltransferase DNA linked to the lac promoter (Ogawa et al., 1988). The cells were grown in 2 Y T medium containing 36 mg/liter ampicillin at 36 °C. When the cell turbidity reached 600 nm reached an absorbance of about 0.2, isopropyl-1-thio-β-D-galactopyranoside was added to a concentration of 1 mM, and the culture was continued for an additional 16 h. Cells harvested by centrifugation were lysed by treatment with lysozyme, and the recombinant enzyme was purified to homogeneity by the procedure described previously (Ogawa et al., 1988). The enzyme was purified by chromatography on DEAE-cellulose paper (Whatman DE81) with 5 mM KH2PO4 as the solvent. AdoMet and AdoHcy were located by UV light. AdoHcy (RF = 0.95) was removed at the time intervals from each reaction mixture to obtain initial velocities.

HPLC Peptide Mapping—Wild type and variant guanidinoacetate methyltransferases were purified by gel filtration using a Sepharose 26,000 column (25 cm) using a gradient of CH3CN. The portion of the HPLC chromatogram corresponding to the AdoHcy spot was cut out, and the radioactivity was determined by scintillation counting. Several aliquots (25 μl) were removed at time intervals from each reaction mixture to obtain initial velocities.

Urea Denaturation—Stock urea solutions were prepared daily. The guanidinoacetate methyltransferase proteins were incubated in 0.1 M Tris-HCl (pH 7.5) containing 5 mM dithiothreitol and various concentrations of urea (substrate/protease ratio, 200:1, w/w) for 16 h at 27 °C. Each digest was fractionated on a TSK ODS 120T column (0.46 × 25 cm) using a gradient of CH3CN in 0.05% trifluoroacetic acid as described previously (Ogawa et al., 1988). Subdigestion of isolated peptides with chymotrypsin (substrate/protease ratio, 200:1, w/w) was carried out in 0.1 M NH4HCO3 for 5 h at 37 °C, and the resulting peptides were subjected to HPLC as above. Identification of peptides was carried out by amino acid analysis (Takata and Fujioka, 1992).

HPLC Peptide Mapping—Wild type and variant guanidinoacetate methyltransferases were treated with sialidase and then S-carboxymethylated with iodoacetate at pH 8.0 in the presence of 6 M guanidine hydrochloride (Darbre, 1986) prior to proteolytic digestion. The carboxymethylated proteins were dialyzed extensively against water and lyophilized. The lyophilized samples were suspended in 1 M NH4HCO3, and the resulting mixture was centrifuged to remove the precipitate. The supernatant was subjected to HPLC as above. Identification of peptides was carried out by amino acid analysis (Takata and Fujioka, 1992).

Urea Denaturation—Stock urea solutions were prepared daily. The guanidinoacetate methyltransferase proteins were incubated in 0.1 M Tris-HCl (pH 7.5) containing 5 mM dithiothreitol and various concentrations of urea for 30 min at 25 °C. Denaturation was followed by monitoring the change in intrinsic protein fluorescence. Wild type and altered guanidinoacetate methyltransferases had virtually identical fluorescence spectra with emission maxima at 357 nm (excitation at 280 nm). Upon denaturation with 8 M urea, the emission maximum changed to 361 nm with one dominant peak at 340 nm. A wavelength of 326 nm, which shows the greatest fluorescence...
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| Enzyme | Sequence |
|--------|----------|
| Gly His Phe | | |
| Asp Gly Ile | Leu Tyr |
| Asp Thr Tyr Pro Leu Ser Glu Glu Thr Trp |
| 5'-GGT CAC TTT GAT GGG ATT CTA TAC GAC ACA TAT CCA CTG TCT GAA GAC ACC TGC-3' |
| 3'-CCA GTC AAA CTA CCC TAA GAT ATG CTG TGT ATA GGT GAC AGA CTT CTC TGG ACC-5' |

D129N 3' GTG AAA TTA CCC TAA GA G 5'
D129A 3' A CTG AAA CCG CCC TAA G 5'
Y133F 3' TAAGAGCGTATGAT 5'
Y133F 3' TAATGCTTCTGTTATA GGT G 5'
D134E 3' TATGCTTCTGTTATA GG 5'
D134A 3' GAT CGG TGT ATA G 5'
T135A 3' ATG CTG GGT ATA GGT 5'
Y136F 3' TATGCTGTTAGGGCAGAGA G 5'

FIG. 1. Oligonucleotides used for mutagenesis. The sequences of synthesized oligonucleotides are aligned with the sequence of guanidinoacetate methyltransferase cDNA corresponding to amino acids 126–143. Asterisks indicate the base changes introduced, and arrows above the amino acid sequence show chymotrypsin cleavage sites.

| Table II | Amino acid compositions of selected chymotryptic peptides |
|----------|----------------------------------------------------------|
| Amino acid | Enzyme |
| Wild type | D134E | D134N | D134A |
| Asp | 0.93 | 0.98 | |
| Glu | 2.05 | 2.76 | 1.91 | 1.66 |
| Ser | 0.87 | 0.98 | 1.04 | 0.73 |
| Thr | 1.70 | 1.87 | 1.85 | 1.72 |
| Ala | 1.03 | 1.03 | 1.03 | 1.03 |
| Pro | 1.07 | 1.03 | 1.11 | 0.88 |
| Tyr | 1.00 | 1.00 | 1.00 | 1.00 |
| Leu | 0.84 | 0.86 | 0.84 | 0.94 |

Fluorescence emission spectra (λmax = 337 nm; excitation at 280 nm) were also identical except for D134A that had the same λmax but a 7.5% decreased fluorescence intensity.

RESULTS

Characterization of Variant Enzymes—Variant proteins were expressed in E. coli JM109 and purified to electrophoretic homogeneity as described under "Experimental Procedures." Yields of purified proteins ranged from ~10 mg/liter (D129A) to ~20 mg/liter (D129N, Y133F, D134E, D134N, D134A, T135A, and Y136F). Preliminary activity measurements under the standard assay conditions (20 mM AdoMet and 0.1 mM guanidinoacetate) indicated that the proteins with substitutions at positions 129, 133, 135, and 136 retained considerable activity, whereas little or no activity was found for Asp-134 variants. To ascertain that the latter proteins contain only the desired and no other substitutions, they were subjected to tryptic peptide mapping. The HPLC elution profiles of the tryptic digests from variants were identical to the profile of the wild type digest except for one peptide in each case (data not shown). The peptides differing in retention times were isolated and further digested with chymotrypsin.

The digestion yielded six major peptides in each case, five of which showed identical chromatographic behaviors. Table II shows amino acid compositions of the peptides unique to each enzyme. The composition is compatible with residues 134–142 with or without amino acid substitution. The presence of asparagine rather than aspartate in the peptide from D134N was confirmed by sequence analysis (data not shown). These results establish that single amino acid alterations at position 134 lead to inactivation.

All variant guanidinoacetate methyltransferases prepared had UV absorption spectra and far- and near-UV CD spectra indistinguishable from those of wild type (data not shown).

Fluorescence emission spectra (λmax = 337 nm; excitation at 280 nm) were also identical except for D134A that had the same λmax but a 7.5% decreased fluorescence intensity.

Kinetic Mechanism of Guanidinoacetate Methyltransferase—To facilitate interpretation of effects of amino acid changes on kinetic parameters, we first determined the kinetic mechanism of guanidinoacetate methyltransferase. Double-reciprocal plots of initial velocities against concentrations of AdoMet or guanidinoacetate at different fixed levels of the other substrate gave a series of straight lines intersecting to the left of the vertical axis (Fig. 2). The initial velocity patterns exclude the ping-pong or equilibrium ordered mechanism and indicate either a steady-state ordered or an equilibrium random mechanism for the guanidinoacetate methyltransferase-catalyzed reaction. To distinguish between ordered and random mechanisms, it is necessary to perform product and/or dead-end inhibition studies. Guanidinoacetate methyltransferase is not inhibited by creatine and analogues of guanidinoacetate (guanidinopropionate, guanidinosuccinate, glycine, β-alanine, and γ-aminobutyrate) (Fujikawa et al., 1988), and compounds to be used as inhibitors are limited to AdoHcy and AdoMet analogues. The product inhibition by AdoHcy was linear competitive versus AdoMet and linear noncompetitive versus guanidinoacetate (not shown). Sinefungin, an analogue of AdoMet, gave the same inhibition patterns (not shown). The observed patterns are consistent with an ordered Bi Bi mechanism in which AdoMet binds first. If the reaction
follows a rapid equilibrium random mechanism, AdoHcy and sinefungin should be competitive inhibitors with respect to both substrates. In this mechanism, however, a noncompetitive pattern with respect to variable guanidinoacetate could be observed if E\textsubscript{-}AdoHcy\textcentering guanidinoacetate or E\textsubscript{-}sinefungin\textcentering guanidinoacetate complex is formed. It was shown previously using equilibrium dialysis that free guanidinoacetate methyltransferase bound AdoMet, but guanidinoacetate became bound only in the presence of sinefungin (Konishi and Fujikura, 1991). Thus, consistent with the available data is an ordered steady-state mechanism in which AdoMet is the first reactant to bind. The values of kinetic constants obtained from initial velocity and inhibition studies are shown in Table III.

**Kinetic Properties of Variant Enzymes**—Table IV lists the values of apparent kinetic constants determined by varying the concentration of one substrate at a constant concentration (at least 5 times the $K_m$ value) of the other. All variants except D134A showed activity. Conservative changes at residues 129, 133, 135, and 136 produced only minor effects on both $k_{cat}$ and $K_m$. Conversion of Asp-134 to glutamate decreased $K_m$ about 3-fold and increased $K_m$ for AdoMet and guanidinoacetate 160- and 80-fold, respectively. Alteration of this residue to asparagine resulted in further reduction in $k_{cat}$, but the $K_m$ values were not greatly different from those of D134E. Binding of AdoMet to D134A was not detected by equilibrium dialysis study.

Urea Denaturation of Asp-134 Variants—The difference in conformational stability between wild type and Asp-134 variants was assessed by analyzing urea denaturation curves. The reversible unfolding of the proteins in the presence of urea was followed by the decrease of intrinsic protein fluorescence. Between urea concentrations of 2.5 and 4 M, large changes in fluorescence were observed. The urea denaturation curves were similar to each other but were not superimposable. Assuming that no intermediate exists in the transition from native to denatured state, the free energy change of unfolding ($\Delta G_D$) at each urea concentration was calculated. As shown in Fig. 3, plots of $\Delta G_D$ versus urea concentrations were linear, and linear extrapolation to zero urea concentration (Pace, 1986) gave $\Delta G_D^{*0}$ values of 7.5, 7.0, 10.0, and 10.7 kcal/mol for wild type, D134E, D134N, and D134A, respectively.

**DISCUSSION**

With an exception of mouse erythroleukemia cell DNA methylase, all mammalian AdoMet-dependent methyltransferases sequenced to date have a conserved aspartate residue in the $L(R/K)PGGXL$ motif (Table I). The three-dimensional structure is not available for any methyltransferase, and relevance of the conserved aspartates in the structure and function of methyltransferases remains to be explored. In the present study we probed the role of the conserved aspartate of guanidinoacetate methyltransferase by site-directed mutagenesis. Guanidinoacetate methyltransferase has two aspartates satisfying the sequence and positional requirements at positions 129 and 134. Conversion of Asp-129 to asparagine or alanine resulted in a functional enzyme, whereas alteration of Asp-134 exerted dramatic effects on catalytic properties (Table IV). Amino acid changes introduced to the residues flanking Asp-134 have minor effects on activity. Asp-134 is in the immediate vicinity of Tyr-136 that is photolabeling labeled with AdoMet (Takata and Fujikura, 1992). Thus, it appears that Asp-134 lying within the AdoMet-binding site plays a specific and crucial role in the function of guanidinoacetate methyltransferase.

The simplest kinetic mechanism for the guanidinoacetate methyltransferase reaction may be represented as,

$$k_1[\text{AdoMet}] \to E \cdot \text{AdoMet} \to E \cdot \text{AdoMet} \cdot \text{GAA}$$

$$k_2 \to E \cdot \text{AdoHcy} \cdot \text{Cr} \to E$$

where GAA is guanidinoacetate and Cr is creatine. The steady-state kinetic parameters can be expressed in terms of individual rate constants as,

$$k_{cat} = \frac{k_2k_6}{k_2 + k_6} \text{ (Eq. 2)}$$

$$K_m(\text{AdoMet}) = \frac{k_5k_6}{k_5(k_6 + k_6)} \text{ (Eq. 3)}$$

$$K_m(\text{GAA}) = \frac{k_5(k_6 + k_6)}{k_6(k_6 + k_6)} \text{ (Eq. 4)}$$

In this mechanism, $k_{cat}/K_m(\text{AdoMet})$ represents the association rate constant of AdoMet and guanidinoacetate methyltransferase. Thus, addition of only one extra methylene to or deprivation of negative charge from the residue at position 134 strongly interferes with the interaction between guanidinoacetate methyltransferase and AdoMet. As calculated from the magnitudes of changes in $k_{cat}/K_m$ values, a change of Asp-134 to glutamate is accompanied by a loss of binding energy of about 7 kcal/mol. Guanidinoacetate methyltransferase would provide multiple sites of interaction, and these values suggest that something more than merely a salt bridge or hydrogen bonding interaction is lost when Asp-134 is changed to glutamate or asparagine.

Site-specific alteration of Asp-134 also reduced the $k_{cat}/K_m$.
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TABLE III

| Kinetic constant          | Substrate or inhibitor       |
|---------------------------|------------------------------|
|                           | AdoMet | GAA | AdoHcy | Sinefungin |
| Michaelis constant (µM)   |        |     |        |           |
|                           | 6.57 ± 0.74 | 11.40 ± 1.71 | 0.60 ± 0.05 | 3.52 ± 0.17 |
| Dissociation constant (µM)| 135.1 ± 16.8* |     |        |           |

*From $K_v$, †From $K_i$.

TABLE IV

| Enzyme            | $k_{cat}$ (min⁻¹) | $K_m$ (µM) | $k_{cat}/K_m$ | $K_m$ (µM) | $k_{cat}/K_m$ |
|-------------------|-------------------|------------|---------------|------------|---------------|
| Wild type         | 4.86 ± 0.21       | 6.76 ± 0.59| 1.22          | 25.14 ± 2.32|
| D129N             | 4.33 ± 0.16       | 4.92 ± 0.73| 0.63          | 29.05 ± 1.30|
| D129A             | 2.71 ± 0.04       | 9.94 ± 0.26| 1.19          | 14.72 ± 0.50|
| Y133F             | 3.92 ± 0.08       | 4.59 ± 0.31| 2.12 × 10⁻³  | 12.58 ± 1.96|
| D134E             | 1.68 ± 0.05       | 1.10 ± 0.11 × 10³ | 1.94 ± 0.16 × 10³ | 4.49 × 10⁻³|
| D134N             | 0.04 ± 0.00       | 5.71 ± 0.69 × 10⁸ | 9.74 × 10⁻⁶  | 1.22 ± 0.11 × 10⁹ | 1.70 × 10⁻⁴|
| T135A             | 3.54 ± 0.04       | 5.41 ± 0.21 | 0.91          | 10.75 ± 1.17 | 1.71          |
| Y136F             | 5.73 ± 0.06       | 6.47 ± 0.17 | 0.57          | 61.22 ± 0.39 | 0.49          |

*Relative to the wild type enzyme.

Fig. 3. Plots of $\Delta G_D$ as a function of urea concentration. Wild type and variant forms of guanidinoacetate methyltransferase (0.1 mg/ml) were incubated in 0.1 M Tris-HCl (pH 7.5), containing 5 mM dithiothreitol and various concentrations of urea as described under "Experimental Procedures." Values of $\Delta G_D$ were calculated from the equation, $\Delta G_D = -RT \ln\ K_D$, using values of $K_D$ (the equilibrium constant between the folded and unfolded states) obtained at each urea concentration.

$K_m^{GAA}$ value greatly. The degree of reduction is comparable with that of $k_{cat}/K_m^{AdoMet}$ and far exceeds that of $k_{cat}$. The expression for $k_{cat}/K_m^{GAA}$ includes the unimolecular rate constant for isomerization of the central complex ($k_5$). We do not know which of the chemical step or product release step is normally rate-limiting and which of these steps is affected by amino acid substitutions. If $k_5$ is much greater than $k_0$ and the amino acid change affects only the product release step, the decrease in $k_{cat}/K_m^{GAA}$ is due to a decreased affinity of guanidinoacetate to the E-AdoMet complex. On the other hand, when the effect of change is on the methyl transfer step (and this step now becomes rate-limiting), a large decrease of $k_{cat}/K_m^{GAA}$ relative to $k_{cat}$ would be observed depending on the degree to which $k_5$ is normally greater than $k_0$, even if binding of guanidinoacetate to the binary complex is not affected. If the chemical step determines the rate of catalytic turnover and is influenced by amino acid substitution, the observed changes in $k_{cat}$ and $k_{cat}/K_m^{GAA}$ suggest that the amino acid alterations exert large effects on $k_5$ and/or $k_4$ and decrease affinity. Thus, in the absence of knowledge about the rate-determining step in wild type and variants, it is not possible to determine whether alteration of Asp-134 to glutamate or asparagine interferes with binding of guanidinoacetate. The most straightforward way to examine this would be to compare the values of inhibition constants of competitive inhibitors of guanidinoacetate between wild type and variants. Such compounds are not available at present, however.

Electronic absorption, fluorescence, and CD spectra of the Asp-134 variants were virtually indistinguishable from those of wild type. Also, the urea denaturation curves were similar for all enzymes. These results indicate that the amino acid substitutions do not result in significant changes in the secondary and tertiary structures and do not destabilize the enzyme. Thus, Asp-134 appears to be a critical residue for the catalytic functioning of guanidinoacetate methyltransferase. The weakening of binding of AdoMet by amino acid substitutions, which is more than can be accounted for by loss of a single hydrogen bond or electrostatic interaction, suggests that the presence of an aspartate at this position is important in forming an adequate structure for substrate binding and catalysis.

Note Added in Proof—The cDNA-deduced amino acid sequences of phosphatidylethanolamine N-methyltransferase of rat liver (Cui, Z., Vance, J. E., Chen, M. H., Voeikker, D. R., and Vance, D. E. (1993) J. Biol. Chem. 268, 16655-16663) and Rhodobacter sphaeroides (Arondel, V., Benning, C., and Somerville, C. R. (1993) J. Biol. Chem. 268, 16002-16008) have recently been reported. The bacterial enzyme, but not the mammalian enzyme, contains the $O$-Deo motif and a variation of the L(R/K)PGGXL motif.

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