Evolutionary Links as Revealed by the Structure of Thermotoga maritima S-Adenosylmethionine Decarboxylase*

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S-adenosylmethionine decarboxylase (AdoMetDC) is a critical regulatory enzyme of the polyamine biosynthetic pathway and belongs to a small class of pyruvoyl-dependent amino acid decarboxylases. Structural elucidation of the prokaryotic AdoMetDC is of substantial interest in order to determine the relationship between the eukaryotic and prokaryotic forms of the enzyme.

Although both forms utilize pyruvoyl groups, there is no detectable sequence similarity except at the site of pyruvoyl group formation. The x-ray structure of the Thermotoga maritima AdoMetDC proenzyme reveals a dimeric protein fold that is remarkably similar to the eukaryotic AdoMetDC protomer, suggesting an evolutionary link between the two forms of the enzyme. Three key active site residues (Ser55, His68, and Cys83) involved in substrate binding, catalysis or proenzyme processing that were identified in the human and potato AdoMetDCs are structurally conserved in the T. maritima AdoMetDC despite very limited primary sequence identity. The role of Ser55, His68, and Cys83 in the self-processing reaction was investigated through site-directed mutagenesis. A homology model for the Escherichia coli AdoMetDC was generated based on the structures of the T. maritima and human AdoMetDCs.

AdoMetDC belongs to a small class of decarboxylating enzymes that use a covalently bound pyruvate as a prosthetic group rather than the cofactor pyridoxal 5′-phosphate (PLP) typically employed in amino acid decarboxylation reactions (6, 7). All AdoMetDCs currently characterized are pyruvoyl enzymes but they can be divided into two classes. Class 1 enzymes are present in bacteria and Archaea, and class 2 enzymes are present in Eukarya (see Table I for representative members of each class). Formation of the active enzyme in both cases involves a self-maturation process in which the active site pyruvoyl group is generated from an internal serine residue via an autocatalytic post-translational modification. Two non-identical subunits are generated from the proenzyme in this reaction, and the pyruvate is formed at the N terminus of the α-subunit, which is derived from the carboxyl end of the proenzyme. The post-translational cleavage follows an unusual pathway, termed nonhydrolytic serinolysis, in which the side chain hydroxyl group of the serine supplies its oxygen atom to form the C terminus of the β-chain, while the remainder of the serine residue is converted to ammonia and the pyruvoyl group blocking the N terminus of the α-chain. Although all AdoMetDCs undergo the same self-maturation process, the class 1 and class 2 enzymes have almost no detectable sequence homology, and they do not share similarity to any of the other known pyruvoyl-dependent amino acid decarboxylases. The class 1 AdoMetDCs can be further divided into two groups. Class 1A AdoMetDCs are found primarily in Gram-negative bacteria as the speD gene product. For example, the Escherichia coli enzyme cleaves to give an 18-kDa α-chain and a 12-kDa β-chain (8, 9). The active form of the enzyme is an (αβ)4 tetramer and requires a divalent metal ion, such as Mg2+, for catalytic activity. Class 1B AdoMetDCs cleave to form α- and β-chains, each with a molecular mass of about 7 kDa. This class forms an (αβ)2 dimer and does not require Mg2+ or other activators. Class 1A and 1B enzymes show low levels of sequence similarity. These similarities are evident in the residues surrounding the probable key active site residues.
cleavage site and those surrounding a cysteine residue, which was identified as part of the active site from modifications that occur during substrate-mediated inactivation of the *E. coli* and *Salmonella typhimurium* enzymes (9, 12).

Crystal structures are now available for several pyruvoyl enzymes including histidine decarboxylase (HisDC) from *Lactobacillus* 30a (13, 14), aspartate decarboxylase (AspDC) from *E. coli* (15, 16), arginine decarboxylase (ArgDC) from *M. jannaschii* (17), and class 2 AdoMetDCs from human (18–20) and potato (21).

The three-dimensional structure of the human AdoMetDC demonstrated that its active site is located far from the interface between the two αβ protomers and that it utilizes residues from both the α- and β-chains (18, 20). The topology of each αβ protomer showed an internal structural duplication in which residues 4–164, which contain the pyruvoyl group, and residues 172–329 have similar topologies. The two halves of the human AdoMetDC protomer are similar in size to the class 1 protomer; however, this is the only indication that the two classes of pyruvoyl-dependent AdoMetDC might be structurally homologous.

Here we report the structures of the wild-type proenzyme and S63A mutant of a class 1B AdoMetDC from *T. maritima* determined to 1.55 and 1.7 Å resolution, respectively, using selenomethionine multiwavelength anomalous diffraction (MAD) phasing methods. The class 1B TmAdoMetDC structure provides striking evidence of an ancient gene duplication event resulting in the class 2 AdoMetDC enzymes. TmAdoMetDC is synthesized as a 14.8 kDa proenzyme, which after processing contains two chains, α and α, of molecular mass 7.0 and 7.8 kDa, respectively. The active form of TmAdoMetDC is an αβ dimer. Each protomer contains one active site that occurs at the dimer interface and also requires residues from the adjacent protomer. Several key active site residues involved in substrate binding, catalysis, or proenzyme processing that were identified in the human and potato class 2 AdoMetDCs are readily recognized in the class 1B TmAdoMetDC despite the overall low primary sequence homology. A sequence alignment of EcAdoMetDC with TmAdoMetDC shows 13% identity. Homology modeling was carried out for the core structure of EcAdoMetDC based on the limited sequence homology and a structural superposition of the *T. maritima* and human AdoMetDCs.

**EXPERIMENTAL PROCEDURES**

Cloning of *T. maritima* speD—PCR was performed using *T. maritima* genomic DNA (purchased from ATCC) as the template with the following primer pair: TmSpeDF: TAG TAG CAT ATG AAG GTG CTG GGA AGG CAC (inserts an Ndel site at the start of the gene) and TmSpeDR: TAG TAG CTC GAG TCA GAC GCC GCC GGC CTT GTG CCG (inserts an XhoI site after the stop codon of the gene). The amplified PCR product was purified (QiAquick PCR purification kit from Qiagen), and ligated into pET-28a. A representative clone was sequenced and named pTmSpeD28.

**Mutagenesis of *T. maritima* speD—**Site-directed mutagenesis with the QuikChange kit was performed according to the manufacturer’s guidelines. The following complementary primer pair was used: TmSpeDASAP: GGT GGT GAT ATC TGA GAC GCC GCC GGC CTT GTG CCG and TmSpeDASAP: GCC CAG GTC TGA ATG GTC AGG TGA GCT TCA GAT ATC ACC and C. The amplified PCR product was purified (QiAquick PCR purification kit from Qiagen), and ligated into pET-28a. A representative clone was sequenced and named pTmSpeD28.

**Protein Expression and Purification—**For the production of native S63A protein, the pTmSpeD28 S63A plasmid was transformed into the BL21Star(DE3)pLys strain of *E. coli* (Invitrogen). A 5-ml saturated starter culture was used to inoculate 1 liter of LB supplemented with 15 μg/ml chloramphenicol and 30 μg/ml kanamycin. The cells were grown at 37 °C until they reached an OD600 of 0.4, at which point the temperature was shifted to 15 °C. The cells were induced with 1 mM isopropyl β-D-thiogalactoside (IPTG) at an OD600 of ~0.6. After induction for 16 h the cells were spun down at 5,000 rpm for 10 min and stored at −80 °C.

For production of the S63A TmAdoMetDC mutant protein with selenomethionine (SeMet) incorporated, the methionine auxotrophic strain of *E. coli*, B834(DE3) (Novagen), was transformed with pTmSpeD28 S63A. The growth medium contained M9 salts supplemented with all amino acids (40 μg/ml each) except l-methionine, which was replaced with L-SeMet, 0.4% (w/v) glucose, 2 mM MgSO4, 25 μg/ml FeSO4·7H2O, 1 mM CaCl2, 35 μg/ml canavanine, and a 1% BME vitamin solution (Invitrogen). The cells from the initial 5-ml starter culture were washed with the above medium, and used to start a 1-liter culture. This culture was washed again to an OD600 ~0.6, at which point the temperature was lowered to 25 °C, and the cells were induced with 500 μM IPTG. After induction for 5 h, the cells were centrifuged, and the cells were centrifuged, and the resulting supernatant was dialyzed against 10 mM Tris-HCl, pH 8.0, and stored at −80 °C.

For production of the TmAdoMetDC proenzyme, pTmSpeD28 was transformed into the B834(DE3) strain of *E. coli* (Novagen). The cells were grown using LB media supplemented with 35 μg/ml kanamycin. The remainder of the growth protocol was as described for the SeMet S63A protein.

All purification steps were carried out at room temperature. The cells were resuspended in 35 ml of binding buffer (50 mM Tris-HCl, pH 8.0, 10 mM imidazole, 500 mM NaCl) and lysed using a French press. The crude extract was centrifuged, and the resulting supernatant was stirred for 1 h with 1.5 ml of Ni-NTA beads (Novagen) equilibrated with the binding buffer. The resin was then loaded into a polypropylene column and washed with binding buffer (100 ml) followed by 50 ml of wash buffer (50 mM Tris-HCl, pH 8.0, 35 mM imidazole, 500 mM NaCl). TmAdoMetDC was eluted from the column with 15 ml of elution buffer (50 mM Tris-HCl, pH 8.0, 150 mM imidazole, 500 mM NaCl). After dialysis with 20 mM Tris-HCl, pH 8.0 and 1 mM dithiothreitol, the protein was concentrated to 6 mg/ml, frozen, and stored at −80 °C. The purification protocol for the wild-type TmAdoMetDC was as described.
for the S63A protein. The wild-type TmAdoMetDC was found to be greater than 95% unprocessed as determined by Coomassie-stained SDS-PAGE (data not shown).

**Processing of TmAdoMetDC**—The proenzyme derived from the TmspeD gene was synthesized in vitro using the Promega Tnt system (catalog L4610). Protein was translated from the TnT mRNA using the Promega Tnt system and purified by a Biocube 2000M concentrator (13). A concentrated solution of protein was incubated for 2 h at 20 °C in a cold block to induce dimer formation. The proenzyme was further purified by gel filtration on Superose 12 to remove uncleaved proprotein and further resolved by Tricine-SDS-PAGE (data not shown).

**Table II**

| Wild type | S63A | Peak | Inflection | Remote |
|-----------|------|------|------------|--------|
| Wavelength (Å) | 0.9786 | 0.9641 | 0.9792 | 0.9793 | 0.9841 |
| Space group | R3 | R3 | R3 | R3 | R3 |
| a (Å) | 104.90 | 104.97 | 104.90 | 104.94 | 104.99 |
| c = (Å) | 69.69 | 69.52 | 69.58 | 69.60 | 69.63 |
| Resolution (Å) | 1.55 | 1.7 | 2.0 | 2.1 | 2.1 |
| Unique reflections | 40567 | 31186 | 19288 | 19307 | 16863 |
| Redundancy | 3.7 (3.4) | 5.7 (5.6) | 7.5 (7.1) | 7.5 (6.3) | 7.5 (6.9) |
| % complete | 98.2 (98.2) | 98.6 (100) | 99.9 (99.9) | 99.9 (99.9) | 99.9 (99.9) |
| Io | 29.3 (3.1) | 30.7 (4.7) | 29.8 (4.5) | 28.3 (5.2) | 29.5 (1.5) |
| R
\text{sym, b} | 4.0 (33.9) | 4.8 (38.5) | 5.5 (42.0) | 6.1 (38.9) | 6.2 (42.5) |

\text{Values for the outer resolution shell are given in parentheses.}

\text{a} R
\text{sym} = \sum \text{I}(h) - \langle \text{I}(h) \rangle, \text{where I(h)} \text{is the mean intensity of N reflections with intensities I}_h \text{and common indices h, k, and l.}

The highest sequence similarity with EcAdoMetDC was 19% for the S68A human AdoMetDC structure (PDB code 1MSV) was separated from the EcAdoMetDC sequence (13% identity). The best SeMet-S63A protein crystals grew under similar conditions with an alkaline shift to pH 8.6 and an increase in precipitant concentration to 3.2 M. Under these conditions S63A was separated into two templates corresponding to the N- and C-terminal domains. The highest sequence similarity with EcAdoMetDC was 19% for the TmAdoMetDC sequence (33% identity). The β-sheet domains of the TmAdoMetDC and the N- and C-terminal halves of the S63A human AdoMetDC were structurally superimposed using LSO (28), and a ClustalW alignment (29). The sequence alignment of the three templates with EcAdoMetDC were manually adjusted based on the structural superposition. Ten homology models were built from the three template structures using Modeler version 6 (30, 31) with 2 cycles of slow MD annealing (MD LEVEL = refine_4, LIBRARY_SCHEDULE =...
The region between the sheet. The Arg 112 NH1 nitrogen atom interacts directly with stabilizes the dimer interface through hydrogen bonding. The two active site serine residues (Ser63) are separated by against the outer face of the homology model on the TmAdoMetDC dimer. The topology of a four layer all subunits; no disulfide bonds are observed. The nine and the C terminus, spanning residues 119 through a water molecule to Asp79, His64 directly hydrogen bonded to His68, His110 and the 2-fold related His110, also found near the charged cluster, form a hydrogen bond further stabilizing the dimer interface.

Comparison of the TmAdoMetDC Proenzyme and S63A Mutant Structures—The overall structures of the TmAdoMetDC proenzyme and the non-processing S63A mutant are very similar with an r.m.s.d. of 0.35 Å for 236 superimposed Ca atoms. In the wild-type proenzyme structure, residues Glu62 and Ser63 at the cleavage site are connected by continuous electron density (Fig. 1), with their Ca atoms separated by 3.8 Å. The electron density and stereochemical constraints indicate that these atoms are connected through an amide linkage and that the wild-type enzyme has not undergone partial processing. Residues near the Glu62-Ser63 Cleavage Site—Residues 60–63 form a type II β-turn that connects the ends of two adjacent antiparallel β-strands, β3 and β4. The B-factors for the main chain atoms in this region do not deviate significantly from the average values reported in Table III. However, some mobility for the side chain of Ser61 was observed and the residue was modeled with two conformations in final model. The S63A mutation of TmAdoMetDC traps the enzyme in the proenzyme form by removing the hydroxyl moiety needed for the N → O acyl rearrangement. Both the wild-type and S63A AdoMetDC structures reveal many of the interactions that promote the N → O acyl rearrangement leading to the formation of the pyruvoyl group. These interactions involve the sulf-hydryl of Cys83, which is 3.7 Å from the carboxyl oxygen atom of Glu62, and the hydroxyl oxygen atom of Ser65, which is 4.2 Å from the carboxyl oxygen atom of Glu62 (Fig. 5). In the wild-type AdoMetDC proenzyme structure the hydroxyl oxygen atom of Ser65 is 3.5 Å from the carboxyl carbon atom of Glu62. The Ser65 hydroxyl oxygen atom is also in position to donate a hydrogen bond to the carbonyl group of Ser61.

Homology Modeling of E. coli AdoMetDC—The results of the homology modeling indicate that the core structure of the E. coli enzyme will likely be a 6 stranded anti-parallel β-sheet with at least two flanking α-helices. The creation of the active site requires that two EcdAdoMetDC monomers associate via a face-to-face β-sheet interaction in a manner analogous to the TmAdoMetDC dimer. There are a number of insertions in the E. coli enzyme with respect to TmAdoMetDC for which there is no significant degree of sequence homology in any of the other classes of AdoMetDC. These insertions comprise residues 1–12, 29–38, 85–111, 124–131, and 189–264 (Fig. 6). The 27 amino acid insertion, residues 85–111, is proline-rich and contains a high concentration of negatively charged residues and immediately precedes the serine residue, Ser112, involved in the nonhydrolytic serinolysis and pyruvoyl moiety formation. The high concentration of negatively charged residues and proxim-

| Table III | Refinement statistics and model building | Wild type | S63A |
|-----------|----------------------------------------|-----------|------|
| Resolution (Å) | 1.55 | 1.7 |
| Total no. of non-hydrogen atoms | 2,112 | 1,997 |
| No. of protein atoms | 1,950 | 1,884 |
| No. of water oxygen atoms | 162 | 115 |
| No. of reflections in refinement | 39,872 | 27,853 |
| No. of reflections in the test set | 3,832 | 2,954 |
| R-factor | 15.0 | 1.8 |
| R_factor^2 (% | 18.5 | 18.5 |
| R_factor | 0.28 | 0.273 |
| R.m.s. deviation from ideal geometry | 0.006 | 0.006 |
| Angles (%) | 1.200 | 1.285 |
| Ramachandran plot | 92.9 | 91.9 |
| Most favored (%) | 7.1 | 8.1 |
| Allowed (%) | 0.0 | 0.0 |
| Generously allowed (%) | 0.0 | 0.0 |
| Disallowed (%) | 0.0 | 0.0 |
| Average B-factors (Å^2) | 27.3 | 28.6 |
| Main chain | 25.3 | 25.3 |
| Side chain | 27.8 | 29.2 |
| Solvent | 39.6 | 42.9 |

a = R-factor = Σ ||Fobs|| - k||Fcac|| / Σ ||Fobs||, where Fobs and Fcalc are the observed and calculated structure factors, respectively.

b For R_factor, the sum is extended over a 10% subset of reflections excluded from all stages of refinement.

2. An EcdAdoMetDC dimer was built by superimposing the monomer homology model on the TmAdoMetDC dimer.

RESULTS

Overall Structure—The structure of the TmAdoMetDC S63A non-processing mutant was solved by MAD phasing using SeMet-containing crystals. The wild-type proenzyme structure was solved by molecular replacement. Both the proenzyme and the S63A mutant crystallize in the trigonal space group R3 with unit cell dimensions a = 104.9 Å and c = 69.5 Å. The asymmetric unit contains one complete dimer with overall dimensions of ~42 Å × 33 Å × 25 Å. Crystallographic data and refinement statistics are shown in Tables II and III. A representative section of the electron density is shown in Fig. 1.

The architecture of each protomer (designated A and B) is a two-layer αβ-sandwich with an anti-parallel β-sheet flanked by two α-helices and one short 3_10-helix (Fig. 2). The β-sheet consists of six strands, with a strand order of β23/β4/β5/β6. The two α-helices (α2, α3) are amphipathic and pack tightly against the outer face of the β-sheet. The N-terminal methionine and the C terminus, spanning residues 119–130, are disordered in all monomers. Pro31 adopts a cis conformation in all subunits; no disulfide bonds are observed.

The Dimer Interface of TmAdoMetDC Proenzyme—The dimer interface of TmAdoMetDC forms the putative active sites and buries ~3100 Å^2 of surface area. The monomers are related by noncrystallographic 2-fold symmetry and interact via a face-to-face β-sheet interaction resulting in an overall topology of a four layer αββα sandwich fold (Fig. 3, A and B). The region between the β-sheets contains several pockets of bound water and a grouping of charged residues including His7, Glu11, His64, His68, Asp79, His110, and Arg122, several of which are conserved in the class 1B AdoMetDC enzymes (Fig. 4). The two active site serine residues (Ser63) are separated by 16 Å and are connected by a hydrogen-bonding network involving His68, His64, His68, His68, and a water molecule (Fig. 4; the primed residues refer to the B protomer). The charged cluster stabilizes the dimer interface through hydrogen bonding. The Ne of Arg122 forms a hydrogen bond with Glu11 in the adjacent sheet. The Arg122 NH1 nitrogen atom interacts directly with His7 and Asp79. Asp79 is hydrogen bonded to His7 and through a water molecule to Asp79, His64, and His64. His64 is
ity to the cleavage site indicates that it may play a role in binding Mg$^{2+}$, which stimulates the decarboxylation reaction in the class 1A AdoMetDCs. Weak secondary structural predictions combined with poor sequence homology to any known structure for residues 189–264 prevent useful homology modeling in this region.

Effects of Mutations of Conserved Residues on Processing—The processing of the TmAdoMetDC was studied by in vitro translation at 30 °C followed by heating at 65 °C. There was virtually no processing (less than 1%) of the proenzyme during the 30 min synthesis period at 30 °C but substantial conversion to the form at 65 °C. As expected, the mutation S63A, which removes the source of the pyruvate, completely prevented processing. Mutant S55A was cleaved more rapidly than the wild type as was mutant C83A (although slower than S55A). The mutant H68A was cleaved much more slowly than the wild type but the addition of hydroxylamine which is known to cleave ester bonds led to the cleavage of this mutant (Fig. 7) and the conversion was then equivalent to that of the wild type, which was not affected by hydroxylamine.

DISCUSSION

Comparison with Class 2 AdoMetDCs—The TmAdoMetDC proenzyme monomer was submitted to the DALI server (32) to identify other proteins of similar structure. The most significant similarity found was to the C-terminal domain of the human AdoMetDC structure (18). A comparison of the dimeric TmAdoMetDC and the human ($\alpha\beta$) protomer reveals remarkably similar topologies (Fig. 3, A and B). Fig. 3C shows a sequence alignment according to a structural superposition of TmAdoMetDC and the N- and C-terminal halves of the human AdoMetDC. Although TmAdoMetDC shows less than 8% sequence identity to either half of the human AdoMetDC, the secondary structures show strong correlation. The TmAdoMetDC dimer can be superimposed on the human AdoMetDC S68A proenzyme monomer (PDB code 1MSV) (33) with an r.m.s.d. of 2.0 Å for 156 aligned C atoms. The main differences are a result of several insertions in the human AdoMetDC accounting for 74 additional residues relative to the TmAdoMetDC dimer. The insertions occur primarily relative to the C terminus of each TmAdoMetDC monomer. The insertions form two additional $\beta$-strands in both N- and C-terminal halves of the human AdoMetDC ($\beta_7$, $\beta_8$, $\beta_15$, and $\beta_16$), an additional $\alpha$-helix in the N-terminal half ($\alpha_3$), as well as a loop region that connects the N- and C-terminal halves of the human structure. The strands $\beta_7$ and $\beta_15$ are involved in the ($\alpha\beta$)$_2$ dimer interface of the human AdoMetDC.

Key differences between the T. maritima and human structures are associated with the removal of the second active site of the human AdoMetDC. Overall the T. maritima structure is in better agreement with the C-terminal half of the human enzyme than with the N-terminal half. The $\beta_2$-$\beta_3$ and $\beta_4$-$\beta_5$ loops of the TmAdoMetDC are considerably longer than in the N-terminal.
domain of the human AdoMetDC. The corresponding loops in the C-terminal half of the human AdoMetDC (β10-β11 and β12-β13) overlap well and are involved in forming the substrate binding pocket in the human enzyme (20). Also found in this region of the human structure are the catalytically important residues Ser229 and His243 (19, 34). In TmAdoMetDC, the structurally equivalent residues are Ser55 and His68, which are highly conserved in all class 1 AdoMetDCs.

FIG. 3. Structural and sequence comparison of TmAdoMetDC with the N- and C-terminal domains of human AdoMetDC. A, ribbon diagrams of the TmAdoMetDC proenzyme dimer (left) and the human AdoMetDC dimer (right). B, topology diagrams of the TmAdoMetDC (left) and human AdoMetDC (right). Helices are represented by circles; β-strands are represented by triangles. The breaks between the α- and β-chains are indicated by stars. C, structure-based sequence alignment of TmAdoMetDC (residues 1–116) with the N-terminal domain of human AdoMetDC (residues 1–115) and C-terminal domain of human AdoMetDC (residues 171–287). Key active site residues are highlighted in yellow or highlighted in red if conserved in all three sequences. The numbering corresponds to the T. maritima sequence, with the last digit of the number over the corresponding residue. This figure was prepared with Molscript (41, 43), Raster3D (42), and EPSript (44).
Comparison with other Pyruvoyl-dependent Enzymes—The TmAdoMetDC structure was compared with the three other published pyruvoyl-dependent enzyme structures, those of Lactobacillus 30a HisDC (13, 14), E. coli AspDC (15, 16), and M. jannaschii ArgDC (17). None of these proteins show similarity in sequence, structure, or oligomeric state with TmAdoMetDC and only ArgDC and HisDC are homologous to each other. Although the monomers show little structural similarity, a few common features can be noted. AdoMetDC, HisDC, AspDC, and ArgDC are α/β proteins that catalyze an amino acid decarboxylation reaction (reviewed in Ref. 17). All four enzymes undergo an autocatalytic, intramolecular self-cleavage reaction that generates a pyruvoyl group in a loop between two β-strands. In AdoMetDC and AspDC the pyruvoyl group is generated between strands from the same β-sheet, whereas in HisDC and ArgDC the strands are from different β-sheets. The absence of any clear structural similarity between AdoMetDC, HisDC, and AspDC supports the hypothesis of convergent evolution in the creation of their similar catalytic functions.

Implications for Processing—The mechanism of pyruvoyl group formation is well established (13, 15) (Fig. 8). The self-processing reaction of pyruvoyl-dependent enzymes is initiated through an internal serinolysis in which the serine hydroxyl group participates in a nucleophilic attack at the carbonyl carbon atom of the preceding residue. The resulting oxyoxazolidine intermediate then undergoes a rearrangement to the ester form (34). Models of the human proenzyme AdoMetDC (33), strongly suggests that the serine hydroxyl is not activated by base catalysis in the first step of the self-processing reaction.

Mechanical strain in the loop preceding the cleavage site has been implicated in the self-processing reactions of HisDC and AspDC (14, 16). In contrast to the conformational changes observed for the wild-type and S25A AspDC proenzyme structures (16), the structures of human AdoMetDC processed enzyme, ester intermediate and proenzyme structures (18, 19, 33) showed little evidence for conformational change on the region of pyruvoyl formation. The TmAdoMetDC proenzyme and S63A mutant structures resemble the human AdoMetDC S68A proenzyme structure. In these structures, the cleavage site is preceded by a standard type II β-turn with no evidence of strain before or after the turn. These observations suggest that strain may play a lesser role in the processing of AdoMetDCs than in AspDC and HisDC.

Structural and mutagenesis studies of the human AdoMetDC have identified several residues important in its processing reaction. These include Cys62, His243, and Ser229. The S229A mutant does not process, indicating that the hydroxyl group of residue 229 is required for processing (34). The H243A mutant processes very slowly, but the proenzyme from this mutant is readily split by hydroxylamine, indicating the proenzyme in the ester form (34). Models of the human proenzyme and the oxyoxazolidine intermediate structures suggest that Cys62 may initially assist the nucleophilic attack and that after the oxyoxazolidine ring forms the Cys62 hydrogen bond is replaced with a Ser229 hydrogen bond (33). Because the ester is trapped in the H243A mutant, the side chain of His243 was identified as the most likely candidate involved in the proton abstraction of the β elimination step (19, 34). Key resi-
The catalytic pyruvoyl group in TmAdoMetDC is generated by the $\beta$-chain forms a hydrogen bond to the amino group of the substrate. The resulting electron sink facilitates the removal of the $\alpha$-carboxylate. After loss of $\text{CO}_2$, the Schiff base is hydrolyzed to yield the decarboxylated product. In the absence of a processed TmAdoMetDC structure, a structural superposition of the TmAdoMetDC proenzyme and the human AdoMetDC complexed with the methyl ester of MeAdoMet (PDB code 1I7B) provides some insight into the residues involved in substrate binding and catalysis in the T. maritima enzyme. The pyruvoyl group in the human AdoMetDC is located in a cleft containing a cluster of highly conserved amino acids and is highly shielded from the solvent. The structure of the human MeAdoMet complex revealed key active site residues involved in substrate binding and catalysis (20). Three residues, Cys$^{82}$, Ser$^{229}$, and His$^{243}$, that are known to be important for either the processing or decarboxylation reaction are located near the 5′-linker that joins the ribosyl 5′-position to the pyruvoyl group through the $\text{C}=\text{N}$ Schiff base linkage. The absolutely conserved glutamate residue, Glu$^{347}$, forms two hydrogen bonds to the ribosyl group and the phenyl groups of Phe$^7$ and Phe$^{223}$ are involved in stabilizing the purine ring of the adenine moiety (20). The terminal carboxylate of the $\beta$-chain forms a hydrogen bond to the amino group of the adenine moiety further stabilizing substrate binding. In the complex, Cys$^{82}$ is located 3.6 Å from the $\alpha$-carbon of the substrate analog, making it the closest residue capable of protonating the decarboxylated Schiff base intermediate and regenerating the pyruvate during normal catalysis.

The pyruvoyl group in TmAdoMetDC is generated from Ser$^{85}$, which is found near the dimer interface in the loop.

**Implications for Activation of Processing**—Wild-type TmAdoMetDC exists in the crystal structure as the proenzyme form and cleaves only slowly with heating. It is not currently known if heating the enzyme results in formation of the pyruvoyl moiety or just results in enzyme cleavage. Heating of TmAdoMetDC in the presence of E. coli cell lysate (results not shown) results in an increase in the rate of cleavage. The optimal growth temperature for T. maritima is 80°C (36), making heat-induced activation a biologically reasonable possibility for TmAdoMetDC. However, the class 1B B. subtilis enzyme is also found in a primarily unprocessed state and heat-induced activation is not biologically relevant in this case (10). Studies of TmAdoMetDC processing resulted in several conflicting observations. The mutation of Ser$^{85}$ or Cys$^{83}$ to alanine, which by analogy to human AdoMetDC is important for processing, results in faster cleavage and the S55A mutant cleaves even without heating. The observations could be explained by the requirement of a yet to be identified processing factor. Efforts to identify a processing factor are ongoing.

**Implications for the Mechanism of Decarboxylation**—In the catalytic decarboxylation reaction of AdoMetDC, the pyruvoyl moiety functions as an electron sink in a manner similar to that of the cofactor PLP. The decarboxylation reaction begins with the formation of a Schiff base between that pyruvoyl cofactor and the $\alpha$-amino group of the substrate. The resulting electron sink facilitates the removal of the $\alpha$-carboxylate. After loss of $\text{CO}_2$, the Schiff base is hydrolyzed to yield the decarboxylated product. In the absence of a processed TmAdoMetDC structure, a structural superposition of the TmAdoMetDC proenzyme and the human AdoMetDC complexed with the methyl ester of MeAdoMet (MeAdoMet; PDB code 1I7B) provides some insight into the residues involved in substrate binding and catalysis in the T. maritima enzyme. The pyruvoyl group in the human AdoMetDC is located in a cleft containing a cluster of highly conserved amino acids and is highly shielded from the solvent. The structure of the human MeAdoMet complex revealed key active site residues involved in substrate binding and catalysis (20). Three residues, Cys$^{82}$, Ser$^{229}$, and His$^{243}$, that are known to be important for either the processing or decarboxylation reaction are located near the 5′-linker that joins the ribosyl 5′-position to the pyruvoyl group through the $\text{C}=\text{N}$ Schiff base linkage. The absolutely conserved glutamate residue, Glu$^{347}$, forms two hydrogen bonds to the ribosyl group and the phenyl groups of Phe$^7$ and Phe$^{223}$ are involved in stabilizing the purine ring of the adenine moiety (20). The terminal carboxylate of the $\beta$-chain forms a hydrogen bond to the amino group of the adenine moiety further stabilizing substrate binding. In the complex, Cys$^{82}$ is located 3.6 Å from the $\alpha$-carbon of the substrate analog, making it the closest residue capable of protonating the decarboxylated Schiff base intermediate and regenerating the pyruvate during normal catalysis.
connecting strands β3 and β4. The pyruvoyl group would thus be located on the edge of a large pocket between the sheets of the (αβ)2 dimer interface. Glu72, an absolutely conserved residue, is the structural equivalent of Glu247 in the human AdoMetDC and is expected to stabilize binding of the substrate by hydrogen bonding to the ribose hydroxyl atoms. Phe48 in the TmAdoMetDC (highly conserved as Phe or Tyr) is analogous to Phe229 in human AdoMetDC. Trp70 is located near the active site pocket and might be homologous to human AdoMetDC Phe8 following a conformation change. Cys83, the structural equivalent of the human Cys88, is located near the active site and in addition to its role in processing likely plays a role as a proton donor for catalysis.

Comparison with the E. coli AdoMetDC Homology Model—The EcAdoMetDC proenzyme is longer than TmAdoMetDC by −130 residues. The additional residues form several insertions but the majority of these residues are contained in a C-terminal extension of about 70 residues. The E. coli enzyme has a tetrameric structure and the residues found in the C-terminal extension could form additional β-strands and helices that may be involved in intrasubunit interactions analogous to the human AdoMetDC dimer. Based on a structural superposition Cys84, Ser77, His117 in EcAdoMetDC align with active site residues Cys82, Ser229, and His243, respectively, in human AdoMetDC and Cys83, Ser56r, and His68, respectively, in TmAdoMetDC.

Implications for Protein Evolution—The human AdoMetDC (αβ)2 dimer contains two active sites, each comprised of residues from both chains (α and β) of one protomer, with the two active sites being separated by 54 Å. Structural homology between the N- and C-terminal halves was observed, each being comprised of an eight-stranded anti-parallel β-sheet with flanking helices. The two halves of the protomer are related by an approximate 2-fold symmetry axis and are connected by a single covalent link. This suggested that the class 2 AdoMetDCs evolved from an oligomer of identical chains and that the gene was duplicated and fused to form a single polypeptide chain. Gene duplication has become a well accepted mechanism for protein evolution, with numerous similar examples, such as arginine kinase (37) and PLP-independent amino acid racemases (38). The TmAdoMetDC structure provides further evidence for gene duplication and fusion in the evolution of class 2 AdoMetDCs. In the (αβ)2 dimer of TmAdoMetDC, the two active sites are comprised of residues from the α- and β-chains of two protomers. Superposition of the human and TmAdoMetDCs reveals an insertion of a glycine residue between Asp236 and Thr238 in the human enzyme (see Fig. 3C), which otherwise would align with the Glu282-Ser31 cleavage site, thus altering the conformation of the β11-β12 loop and resulting in loss of the processing site in the C-terminal half of the structure. The loops in this region are also shortened, further obliterating the second active site.

The structures of the human and potato AdoMetDCs, along with sequence alignments of the class 2 enzymes, suggest that AdoMetDCs from higher organisms have an unusual site containing numerous buried charges. This site is located between the two central β-sheets. In the human enzyme, this site binds putrescine, which stimulates both processing and decarboxylation reactions. Two mechanisms for putrescine stimulation have been proposed (19). The binding of putrescine may serve to properly orient the β-sheets by balancing the large number of negative charges in the buried charge site. The conformational changes could be propagated to the active site, which is located at the opposite end of the β-sheets. Alternatively, the observed hydrogen-bonding network between the charged site and the active site in the human structure could be altered by putrescine binding and could affect the relative orientation of critical residues in the active site. The stimulation of processing and activity of the potato enzyme is not affected by putrescine, but has basul levels of activity similar to the putrescine-activated human enzyme (39). The crystal structure of the tomato enzyme revealed the addition of three positively charged residues not present in the human charged site, Arg18, Arg114, and His294 (21). The arginine residues presumably play a similar role to putrescine by balancing charge and maintaining the hydrogen-bonding network that leads to the active site (Fig. 4).

The structure of the T. maritima enzyme and sequence alignments of the bacterial AdoMetDCs (class 1A and 1B) reveal an analogous site containing a high concentration of charged residues. This site is located between β-sheets at the dimerization interface and forms a hydrogen-bonding network that may allow communication between the two active sites through a charge relay.

Finally, a recent bioinformatics study of the enzymes involved in the plant polyamine biosynthetic pathway revealed bacterial origins for all of the enzymes involved with one exception, AdoMetDC (40). The plant S-adenosylmethionine decarboxylase was identified as a potentially eukaryote-specific enzyme form, showing a high degree of sequence homology to other class 2 AdoMetDCs. As noted previously, the class 1 AdoMetDCs found in bacteria have almost no detectable sequence homology to the eukaryotic form of the enzyme. The TmAdoMetDC structure provides evidence of a bacterial ancestor for the eukaryotic AdoMetDCs, thus demonstrating that all enzymes involved in the plant polyamine biosynthetic pathway have bacterial origins.
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