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Structural Roles of Cysteine 50 and Cysteine 230 Residues in *Arabidopsis thaliana* S-Adenosylmethionine Decarboxylase

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The *Arabidopsis thaliana* S-Adenosylmethionine decarboxylase (AdoMetDC) cDNA (GenBank™ U63633) was cloned. Site-specific mutagenesis was performed to introduce mutations at the conserved cysteine Cys^{50}, Cys^{83}, and Cys^{230}, and lys^{81} residues. In accordance with the human AdoMetDC, the C50A and C230A mutagenesis had minimal effect on catalytic activity, which was further supported by DTNB-mediated inactivation and reactivation. However, unlike the human AdoMetDC, the Cys^{50} and Cys^{230} mutants were much more thermally unstable than the wild type and other mutant AdoMetDC, suggesting the structural significance of cysteines. Furthermore, according to a circular dichroism spectrum analysis, the Cys^{50} and Cys^{230} mutants show a higher a-helix content and lower coiled-coil content when compared to that of wild type and the other mutant AdoMetDC. Also, the three-dimensional structure of *Arabidopsis thaliana* AdoMetDC could further support all of the data presented here. Summarily, we suggest that the Cys^{50} and Cys^{230} residues are structurally important.

Keywords: AdoMetDC, *Arabidopsis thaliana*, Site-specific mutagenesis, CD, Chemical modification

Introduction

The aminopropyl groups in spermidine and spermine are obtained from S-adenosylmethionine (AdoMet) after its decarboxylation by the action of S-adenosylmethionine decarboxylase (AdoMetDC) (Tabor and Tabor, 1984). Under physiological conditions, the decarboxylated AdoMet is a limiting factor in polyamine synthesis. Although ubiquitous in eukaryotic cells, AdoMetDC constitutes only a minor fraction of intracellular proteins. This is partly due to its very short half-life. It is also partly due to the fact that the AdoMetDC expression is regulated at multiple levels-transcriptional, translational, as well as post-translational (Tabor and Tabor, 1984; Heby and Persson, 1990; Pegg and McCann, 1992; Marie *et al.*, 1992). AdoMetDC exhibits an unusually high molecular degradation/turnover rate with an *in vivo* half-life of 1-2 hours. The short half-life of AdoMetDC is thought to be related to the propensity for substance-mediated transamination of the enzyme (Pegg *et al.*, 1998). These molecular degradation rates are decreased significantly in the presence of most of the inhibitors of the enzyme. However, the mechanisms for the degradation of AdoMetDC and for the alteration in degradation rate in response to polyamines, inhibitors, and other stimuli are presently unknown. In plants, the enzyme has been cloned and characterized from wheat (Dresselhaus *et al.*, 1996), potato (Mad Arif *et al.*, 1994), periwinkle (Schroder and Schroder, 1995), carnation (Lee *et al.*, 1997), and *Arabidopsis thaliana* (Genbank™ U63633, Park and Cho, 1999). All of the known sequences, except that from *Escherichia coli*, show a significant degree of similarity with about 50 fully conserved residues in the core region of 300 amino acids. This includes the sequences (KTCGTT) that contain an essential active site lysine and cysteine residues (Stanley and Pegg, 1991; Stanley *et al.*, 1994; Xiong *et al.*, 1997). In spite of rather extensive studies on the functionally important residues of AdoMetDC, almost all of the studies were confined to the enzyme active site (Stanley and Pegg, 1991; Stanley *et al.*, 1994; Xiong *et al.*, 1999), except the essential Cys^{83} residue in the active site. There are presently no studies on the other conserved cysteine residues of plant AdoMetDC.

Since there is no report on the structurally important amino acid residues of plant AdoMetDC, we chose *Arabidopsis thaliana* AdoMetDC as a representative of the plant AdoMetDC for the present study. In this paper, we constructed and characterized the site-directed mutants to investigate the structural roles of conserved cysteine and lysine residues, Cys^{50}, Lys^{81}, Cys^{83}, and Cys^{230}.

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Materials and Methods

Materials Restriction enzymes and T4 DNA ligase were purchased from Promega. Isopropyl-1-thio-β-D-galactopyranoside and ampicillin were purchased from Sigma. Pfu DNA polymerase and the *Arabidopsis thaliana* cDNA library were purchased from Stratagene. DNA sequencing was performed with the Sequenase 2.0 system from United States Biochemical. The pGEX-2T vector, glutathione, Glutathione-*S*-transferase Sepharose 4B, thrombin, and *S*-adenosyl-[carboxy^{14}C]methionine were obtained from Amersham Pharmacia Biotech. All of the oligonucleotides were acquired from Koma Biotech. All other reagents were obtained from commercial sources.

Cloning of AdoMetDC cDNA and site-directed mutagenesis The AdoMetDC cDNA was amplified by PCR using the *Arabidopsis thaliana* cDNA library. The oligonucleotides contained BamHI and SmaI sites, respectively, to facilitate cloning (Sambrook *et al.*, 1989). The PCR product was digested with BamHI and SmaI, and ligated to the BamHI-SmaI backbone fragment of pGEX-2T, which contained the T7lac promoter and Glutathione-*S*-transferase preceding the N-terminus of the recombinant protein. The identity of the cloned cDNA and the fidelity of the PCR were confirmed by DNA sequencing of the plasmid inserts by the dideoxy termination method (Sanger *et al.*, 1977). Mutagenesis of the AdoMetDC coding sequence was performed by sequential PCR (Dieffenbach and Dveksler, 1995). Ten oligonucleotides were designed to replace amino acid residues that are involved in the AdoMetDC activity (Table 1, Fig. 1). The conserved cysteines, Cys^{50} ,Cys^{83}, and Cys^{230}, were changed to alanine. The conserved lysine^{81} was also replaced by alanine. Terminal sense and antisense primers were designed to hybridize with the N-terminal and C-terminal regions, respectively, with the introduction of restriction sites (BamHI in primer sense and SmaI in primer antisense) in order to facilitate ligation and cloning. For each mutation, a set of internal primers was designed to hybridize with the regions that flank the mutation site. The internal antisense primer has a mutated codon sequence. More than half of the sequence of this primer is complementary to the internal sense primer. In the first reaction, either the terminal sense primer and internal antisense primer, or internal sense and terminal antisense primers, were used as a primer set. A recombinant pGEX-2T plasmid that contained the *Arabidopsis thaliana* AdoMetDC cDNA sequence was used as a template. In the second round, the terminal sense and terminal antisense primers were used as a primer set. Two PCR products from the first round of reactions were used as templates to produce a single annealed PCR product. The final PCR product was digested with BamHI and SmaI, and ligated to the pGEX-2T vector that was linearized with BamHI and SmaI. Verification of all of the mutants was analyzed by performing DNA sequencing of the plasmid inserts by the dideoxy termination method (Sanger *et al.*, 1977).

Expression and Purification of Wild Type and Mutant AdoMetDC *E. coli* BL 21 (DE3) was transformed with the pGEX2T-AdoMetDC plasmid. Transformants were tested for the AdoMetDC expression upon induction with isopropyl-1-thio-β-D-galactopyranoside (IPTG). *E.coli* BL 21 (DE3) cells that carry the expression plasmid containing the wild type or mutant AdoMetDC were grown overnight at 37ºC (Sambrook *et al.*, 1989; Kim *et al.*, 2001; Sohn *et al.*, 2001). The cells were diluted 100-fold into the same medium and allowed to grow until A_{600} reached 0.5. To induce the expression, 1 mM IPTG was added to the culture. The cells were harvested 4 h after induction by centrifugation ($5000 \times g$; 10 min) and sonicated in a Phosphate-buffered saline (PBS). The cell lysate was centrifuged at $13{,}000 \times g$ for 20 min. The resulting supernatant was used for purification on a Glutathione-*S*-transferase Sepharose 4B resin in a batch procedure according to the manufacturers recommendation. Recombinant mutated or wild type AdoMetDC was recovered from the fusion protein by thrombin cleavage. It was then purified according to the manufacturers recommendation. The purity of the recombinant protein was

Table 1. Mutagenic oligonucleotides for site-directed mutagenesis of AdoMetDC. Nucleotides that were exchanged in order to obtain the desired mutation are underlined and in bold letter. Numbers in superscript indicate the position of nucleotides in the coding region of the gene. Restriction sites are underlined (Sense; BamHI, Antisense; SmaI). The mutation at a specific residue number is indicated by one-letter amino acid abbreviation. The first letter is the wild type residue, and the last letter is the amino acid which it is changed by the mutated codon. WT, wild type; S, sense; AS, antisense

5’	CG<u>GGATCC</u>TGTTCGCTCACACAACAAGG	Sense
5’	<u>CCCGGG</u>CTAGATTCCCTCGTCCTTCT	Antisense
5’	^{671}CACCTGCTGCATGCACGATCGTTTCATCTC	C50WT
5’	CACCTGCTGCA**<u>GC</u>**CACGATCGTTTCATCTC	C50A (S)
5’	GAGATGAAACGATCGTG**<u>GC</u>**TGCAGCAGGTG	C50A (AS)
5’	^{762}CAAAGTCATCATCAAGACTTGCGGTACCAC	K81WT
5’	CAAAGTCATCATC**<u>GC</u>**GACTTGCGGTACCAC	K81A (S)
5’	GTGGTACCGCAAGTC**<u>GC</u>**GATGATGACTTTG	K81A (AS)
5’	^{771}CATCAAGACTTGCGGTACCACTAAGCTCCT	C83WT
5’	CATCAAGACT**<u>GC</u>**CGGTACCACTAAGCTCCT	C83A (S)
5’	AGGAGCTTAGTGGTACCG**<u>GC</u>**AGTCTTGATG	C83A (AS)
5’	^{1208}TTGAATTCGAGCCCTGCGGCTACTCTATGA	C230WT
5’	TTGAATTCGAGCCC**<u>GC</u>**CGGCTACTCTATGA	C230A (S)
5’	TCATAGAGTAGCCG**<u>GC</u>**GGGCTCGAATTCAA	C230A (AS)

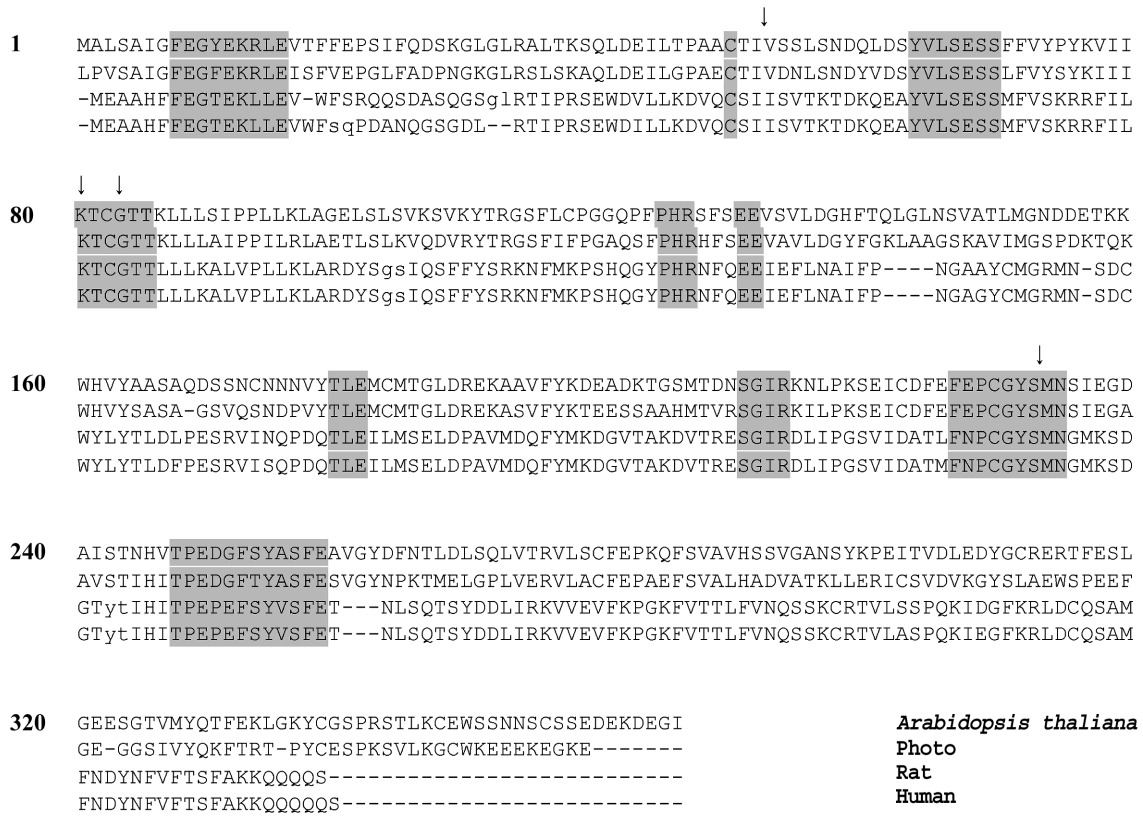

Fig. 1. Comparison of primary amino acid sequences of *Arabidopsis thaliana*, potato, rat, and human AdoMet decarboxylase. Sequences were deduced from the papers describing the *Arabidopsis thaliana* (GenBank™ U63633), potato (7), rat (GenBank™ M34464), and human (GenBank™ M21154). Regions of primary sequence similarity are shown in the shaded box. The arrows indicate the amino acid residues in the *Arabidopsis thaliana* sequence, which were mutated in the present experiments (detailed under "Materials and Methods").

assessed by a sodium dodecyl sulfate-polyacrylamide gel electrophoresis and polyacrylamide gradient gel electrophoresis.

AdoMetDC activity assay The AdoMetDC activity was assayed at 37°C for 60 min by monitoring the liberation of $^{14}CO_2$ from S-Adenosyl-[carboxy^{14}C]methionine as a substrate (Yang and Cho, 1991). One unit of the enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 nmole $^{14}CO_2$ per h. The AdoMetDC activity was a linear function of both incubation time and concentration under these conditions.

Chemical modification of AdoMetDC with group-specific reagents The wild type AdoMetDC was incubated with sulfhydryl group modification reagents, such as DTNB, under appropriate conditions (Lee and Cho, 1993a, b; Park and Cho, 1995). Before the sulfhydryl group modification reaction, DTT was removed from the enzyme solution. A reactivation experiment was also carried out by adding DTT to the inactivated enzyme, respectively.

Thermostability of the AdoMetDC mutants Enzymes were diluted to a final concentration of 1 mg/ml with PBS and incubated at 50, 60, and 70°C. At various times, the aliquots were removed and assayed for AdoMetDC activity (Segel, 1975).

Circular dichroism measurements CD spectra in the far-ultraviolet range, 190-250 nm, were recorded at room temperature on a Jobin Yvon CD6 spectrometer. Signal averaging during accumulation of the five scans was performed automatically. The path length of the cells used was 1 mm. The buffer was a 5 mM sodium phosphate buffer, pH 7.0. The data were corrected by subtracting the spectrum of the buffer from the sample spectrum. Molecular ellipticity values, [θ], were measured as described previously (Provencher and Glockner, 1981; Menavalen and Johnson, 1987; Yin and Jing, 2000).

Prediction of the secondary and tertiary structures The sequence of the *Arabidopsis thaliana* AdoMetDC was submitted to PSIpred (Jones, 1999) (various protein structure prediction methods at the University of Warwick) on the Internet to prove the predicted secondary structure. For a 3-D structure prediction with the human AdoMetDC as a template, the *Arabidopsis thaliana* AdoMetDC sequence was sent via the Internet to Swiss-Model, "an Automated Comparative Protein Modeling Server" (http://www.expasy.ch/swissmod/SWISS-MODEL.html), which runs on the software ProMod II and Gromos96 (Peitsch, 1996; Guex and Peitsch, 1997; Nomura *et al.*, 1999; Odani *et al.*, 2000; Odani *et al.*, 2001). The tertiary structure data of the human AdoMetDC (PDB, 1 JENB, 1 JENA, 1 JEND, 1 JENC; 30) were used as the template of

prediction. The e-mail files of atomic coordinates for the predicted 3-D structure from the SWISS-Model were combined and made into a single text file. The 3-D structure was visualized with the software POV-Ray for windows and the Swiss-Pdb Viewer 3.51 that was downloaded via the Internet. They have characteristic functions of their own. Various display modes of the 3-D structure-Ribbons, Strands, Backbone-as well as the assignments of each residue to α-helix or β-sheet are available on the Swiss-Pdb Viewer.

Results

Expression and purification of wild type and mutant AdoMetDCs The cDNA encoding AdoMetDC was amplified by PCR from the *Arabidopsis thaliana* cDNA library. The 1.1 kb PCR product was cloned into an expression vector-pGEX-2T. In order to identify structurally important amino acid residues, we constructed a series of site-directed mutants of AdoMetDC. However, there are no reports on the structurally important amino acid residues of the *Arabidopsis thaliana* AdoMetDC. Therefore, possible candidate residues for mutagenesis were selected from the human AdoMetDC crystal structure (Ekstrom *et al.*, 1999) and previous study (Park and Cho, 1999). Mutagenesis of the AdoMetDC coding sequence was performed by sequential PCR. Ten oligonucleotides were designed to replace the conserved amino acid residues (Table 1, Fig. 1). The conserved cysteines (Cys^{50}, Cys^{83}, and Cys^{230}) were changed to alanine. The conserved $lysine^{81}$ was also replaced by alanine. The pGEX-2T/Glutathionine-S-transferase(GST) fusion vector that contained the stringer promoter was utilized. After lysis of the cells and clarification by centrifugation, we were able to obtain high levels of AdoMetDC in an essentially soluble form. Therefore, we could easily purify the GST-AdoMetDC fusion protein to near homogeneity by using a GST-Sepharose 4B affinity column. Treatment of the purified GST-AdoMetDC fusion protein with thrombin and subsequently with a GST-sepharose 4B affinity column yielded significant quantities of homogeneous AdoMetDC protein (Park and Cho, 1999, 2000).

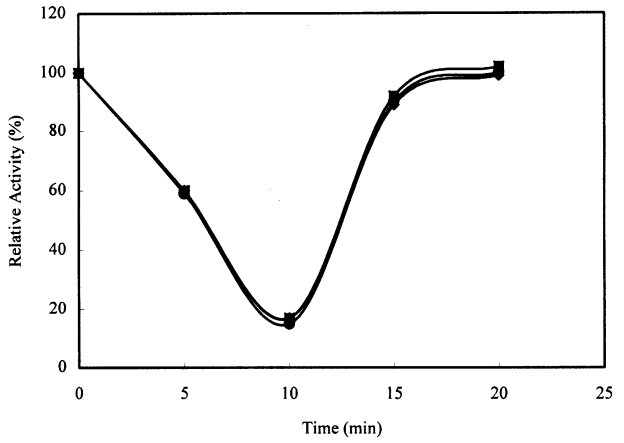

Fig. 2. Reactivation of the DTNB-inactivated AdoMetDCs by DTT. The purified enzyme was incubated with 1 mM DTNB at 25°C for 10 min. The wild type (●), C50A (◆), and C230A (×) mutants are presented as representatives. By adding excess DTT to the DTNB-inactivated enzyme, reactivation of the AdoMetDC was performed. At various times, the aliquots (1 μg) were removed and assayed for AdoMetDC activity at 37°C for 1 h.

Table 2. Half-life ($t_{1/2}$) for the thermal inactivation of wild type and mutant AdoMetDCp at 50, 60 and 70°C. Enzyme was present at a concentration of 1 mg/ml with PBS. Samples were incubated at 50, 60 and 70°C, respectively. At various times aliquots(1 μg) were removed and assayed for residual AdoMetDCp activity at 37°C for 1 h. t1/2 for loss of activity as a function of time.

Constructs	$t_{1/2}$		
	50°C	60°C	70°C
	min	min	min
Wild Type	8.01 ± 0.4	1.15 ± 0.2	1.01 ± 0.1
C50A	3.67 ± 0.3	0.76 ± 0.1	0.4 ± 0.2
K81A	7.39 ± 0.5	0.99 ± 0.1	0.87 ± 0.1
C83A	7.49 ± 0.3	0.98 ± 0.2	0.89 ± 0.2
C230A	4.19 ± 0.3	0.8 ± 0.1	0.53 ± 0.1

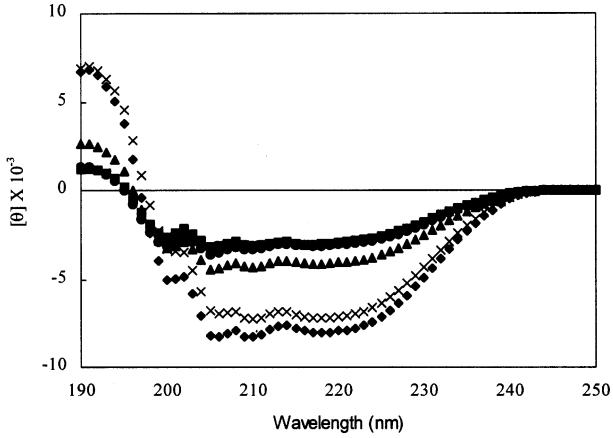

Fig. 3. Far ultraviolet CD spectra of AdoMetDC and its mutants. The CD spectra of AdoMetDC and its mutant proteins were measured as described in "Materials and Methods". The CD spectra of Wild Type (●), C50A (◆), K81A (■), C83A (▲), and C230A (×) mutants are presented as representatives.

Table 3. Circular dichroism analysis of wild type and mutant AdoMetDCp. The percentages of various secondary structures obtained from variable selection analysis of the circular dichroism spectra. The spectrum was taken at 25°C using 0.05 mm quartz cell containing 1 mg/ml enzyme in PBS.

	Wild Type (%)	C50A (%)	K81A (%)	C83A (%)	C230A (%)
α-helix	22 ± 0.5	24 ± 0.5	22 ± 0.4	22 ± 0.5	24 ± 0.7
β-sheet	29 ± 0.7	28 ± 1	29 ± 0.6	29 ± 0.5	28 ± 0.8
Random Coil	49 ± 1	48 ± 0.9	49 ± 1.2	49 ± 1	48 ± 1.5

Site-directed mutagenesis of cysteine and lysine residues of AdoMetDC C50A and C230A had activity, which was inactivated by DTNB. Their activities were restored by the addition of DTT (Fig. 2). Therefore, these results suggest that the Cys^{50} and Cys^{230} residue may not be involved in the active site.

Thermal stability of wild type and mutant AdoMetDC To ascertain whether there are any differences in thermalstability, the thermal stability of wild type and mutant AdoMetDC was evaluated at temperatures of 50°C, 60°C, and 70°C, respectively (Table 2). Thermal inactivation of the wild type and mutant AdoMetDC followed pseudo-first order kinetics. Although all enzymes lose their activities, half time of the thermal inactivation of K81A and C83A mutants was similar to that of the wild type AdoMetDC; whereas half time of the thermal inactivation of the C50A and C230A mutants was shorter than that of the wild type, K81A and C83A mutant AdoMetDC.

Circular dichroism analysis We performed a CD spectral analysis of the wild type and mutant AdoMetDC. The spectra of the C50A and C230A mutant AdoMetDC shows different

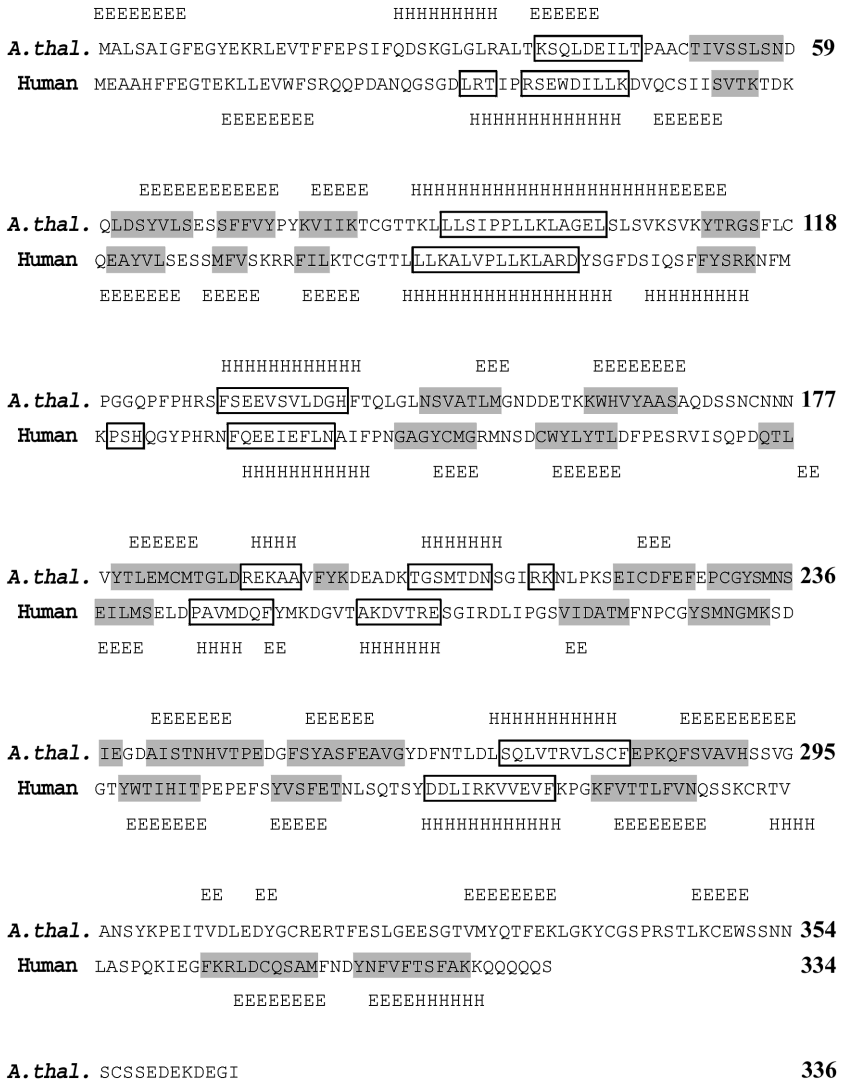

Fig. 4. Comparison of the secondary structures predicted by PSIpred (*Arabidopsis thaliana* AdoMetDC and human AdoMetDC) and Swiss-Model (*Arabidopsis thaliana* AdoMetDC), and determined by X-ray crystallography (human AdoMetDC). Sequences of *Arabidopsis thaliana* AdoMetDC (top) and Human AdoMetDC (bottom) are aligned, and the secondary structures predicted by PSIpred are shown above (*Arabidopsis thaliana* AdoMetDC) and below (human AdoMetDC) the sequences by the symbols H (α-helix) and E (β-sheet). The secondary structure of *Arabidopsis thaliana* AdoMetDC predicted by the Swiss-Model, and that of the human 8AdoMetDCp that was determined by X-ray crystallography are shown in their respective sequences by a box (α-helix) and a shaded box (β-sheet). Residue alignment for α-helices and β-sheet in *Arabidopsis thaliana* AdoMetDC was performed on the Swiss-Pdb Viewer control panel, and for those on the human AdoMetDC by the description in the PDB file. Functionally important amino acid residues of *Arabidopsis thaliana* and human AdoMetDC are shown as bold letters.

overall ellipticity compared to that of the wild type AdoMetDC (Fig. 3). An analysis of the far ultraviolet CD spectra in terms of secondary structures, according to the variable selection procedures of Provencher and Glockner (1981), revealed that the wild type, K81A, and C83A mutant AdoMetDC showed similar ratios of α-helix (22%), β-sheet (29%), and random coil (49%) contents (Table 3). However, the mutation of the Cys^{50} and Cys^{230} residue to alanine resulted in a slight increase in the a-helix structure. The CD spectra of the C50A and C230A mutant AdoMetDC was different than those of the wild type, K81A and C83A mutant AdoMetDC.

Secondary and tertiary structure of AdoMetDC The secondary structure of *Arabidopsis thaliana* and human AdoMetDCp that was predicted by PSIpred (Jones, 1999) (various protein structure prediction methods at the University of Warwick) on the Internet are indicated in Fig. 4 above and below their sequences, respectively, by the symbols H (α-helix) and E (β-sheet). *Arabidopsis thaliana* AdoMetDC was predicted to have 6 α-helices with length 4-22 residues and 16 b-sheets with length 2-12 residues for the monomer. Human AdoMetDC has 9 α-helices of 4-18 residues and 15 β-sheets of 2-8 residues (Ekstrom *et al.*, 1999). The PRIpred-predicted secondary structure of *Arabidopsis thaliana* AdoMetDC is generally similar to that of human AdoMetDC with a few different structures due to the sequence variation (Fig. 4).

In another method of prediction, the secondary structure assignment of each residue in *Arabidopsis thaliana* AdoMetDC, based on the 3-D structure predicted by the Swiss-Model, was obtained from the Swiss-Pdb Viewer. In it, each residue in the human AdoMetDC was obtained from the description in its PDB file [PDB, 1 JENB, 1 JENA, 1 JEND, 1 JENC] based on the report by Ekstrom, J.L. *et al.* (1999). The α-Helices and β-sheets are shown in their respective sequences by a box and a shaded box, respectively, as described in the legend to Fig. 4. The *Arabidopsis thaliana* AdoMetDC secondary structure with its predicted 3-D structure is quite similar to the human AdoMetDC crystal structure. The secondary structural elements of *Arabidopsis thaliana* AdoMetDC were determined to be 7 α-helices and 14 β-sheets, including many turns (Fig. 4). The core activity region was found in the N-terminus of the a subunit (residue 69-366) near a wide cleft, which is formed by the α (residue 69-366) and β subunits (residue 1-68) (Fig. 5). The sequence alignment of the representative eukaryotic AdoMetDC (Fig. 1) reveals this pocket as one of several areas of high sequence conservation. This pocket is surrounded by residues from 5β-sheets and the loop between β-sheets (Fig. 5), which contains the Lys^{81} and Cys^{83} residues. However, the Cys^{50} and Cys^{230} residues seem to be far away from the active site.

Discussion

It was suggested that AdoMetDC requires disulfide bond for activity, therefore implies the necessity for two cysteine

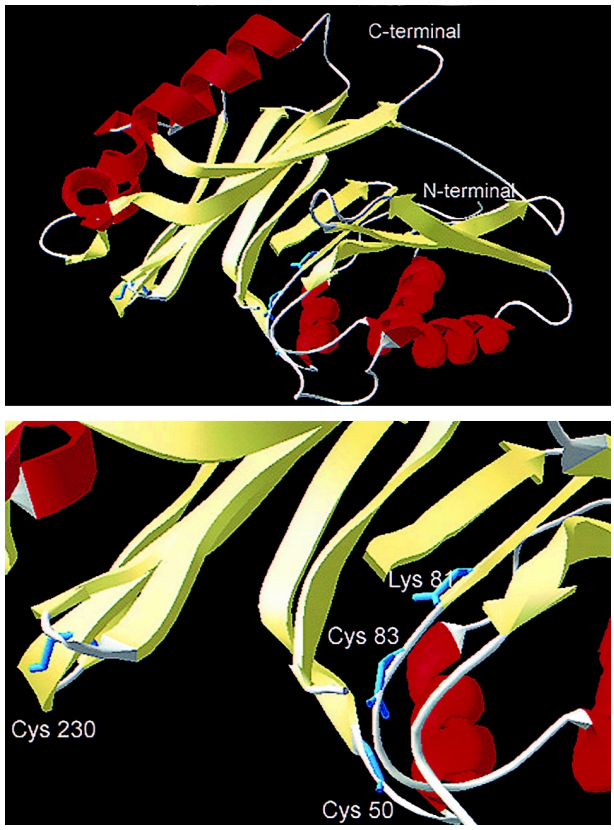

Fig. 5. The overall and active site structure of *Arabidopsis thaliana* AdoMetDC generated by molecular modeling. (A) The overall three-dimensional structure of *Arabidopsis thaliana* AdoMetDC is illustrated as ribbon diagrams. The helices and strands are indicated in the red and yellow, respectively. (B) Catalytically important residues (Lys^{81} and Cys^{83}) and conserved Cys^{50} and Cys^{230} residues were drawn in stick models (light blue). These figures were drawn by the SWISS-MODEL, An Automated Comparative Protein Modeling Server.

residues (Zhang *et al.*, 1991). To test this possibility, Stanley and Pegg (1991) changed the Cys^{49}, Cys^{82}, and Cys^{226} residues in the human AdoMetDC to alanine, which are known to be conserved residues. Changing Cys^{49} and Cys^{226} to alanine had minimal effect on activity, whereas changing Cys^{82} to alanine completely eliminated catalytic activity. They suggest that the disulfide-bond formation between the larger and smaller subunits is unnecessary for maintenance for catalytic activity since Cys^{49} is the only conserved cysteine residue in the smaller subunit. Analysis of the crystal structure of the human AdoMetDC also shows that there is no chance in such a disulfide bond. Therefore, it appears that the disulfide bond is not essential, as claimed by Eksrtrom *et al* (1999). However, to confirm whether *Arabidopsis thaliana* Cys^{50}, Cys^{83}, and Cys^{230} residues are involved in the disulfide-bond formation, which are counterparts of Cys^{49},Cys^{83}, and Cys^{226} of the human AdoMetDC, we changed these residues to alanine. The same was true for *Arabidopsis thaliana* AdoMetDC: C83A was inactive, whereas C50A and C230A were active.

Wild type and mutant AdoMetDC (C50A and C230A) were inactivated by DTNB and were reactivated by DTT (Fig. 2). Cumulative results suggest that only one of the three cysteine residues, Cys^{83}, appears to be catalytically important in the plant enzyme. However, Cys^{83} appears to be uninvolved in disulfides bonds since no cysteine residues, other than those tested here, are conserved in human, rat, yeast, and *Arabidopsis thaliana* sequences. However, not all of the cysteine residues in *Arabidopsis thaliana* AdoMetDC have been tested. Such cumulative evidence strongly suggests that inactivation of AdoMetDC can be attributed not just to change in the conformation of the enzyme, but to modification of the active site amino acid residue. Our results from *Arabidopsis thaliana* AdoMetDC are in agreement with that of human AdoMetDC (Ekstrom *et al.*, 1999). Because of the instability of C50A and C230A AdoMetDC mutants that were encountered during experimental manipulation, the possibility that the mutations may have altered thermal stability was examined. The half time of thermal inactivation of K81A and C83A mutants was similar to that of the wild type AdoMetDC. In contrast, the half time of the thermal inactivation of the C50A and C230A mutants was shorter than that of the wild type, K81A, and C83A mutant AdoMetDC (Table 2). Furthermore, in order to rationalize whether or not the thermal destabilization of mutants AdoMetDC resulted from a conformational change, we performed a CD spectral analysis of the enzyme and its mutants. Table 3 demonstrates that the C50A and C230A mutant AdoMetDC show different overall ellipticity compared to the wild type AdoMetDC. The physical basis for the thermal destabilization that was observed for the C50A and C230A mutants is presently unclear. However, there were a few reports regarding thermal stability. The replacement of the amino acid residue with alanine within α-helix of T4 lysozyme was shown to introduce various degrees of enhanced stability (Zhang *et al.*, 1991). On the contrary, site-directed mutagenesis of alanine-lysine residues of inorganic pyrophosphatase decreased the thermal stability (Satoh *et al.*, 1999). According to human AdoMetDC crystallographic studies, the Cys^{49} and Cys^{226} residues, counterparts of Cys^{50} and Cys^{230} in *Arabidopsis thaliana*, were not a component of α-helix within AdoMetDC, but are rather at the beginning of the β-sheet, strand β2, and strand β11, respectively (Ekstrom *et al.*, 1999).

A three-dimensional structure of *Arabidopsis thaliana* AdoMetDC from molecular modeling resembles the general fold of the human AdoMetDC crystal structure. Also, our model is in agreement with all mutagenesis results presented here. Our results show that the Lys^{81} and Cys^{83} residues are functionally important in the catalytic activity. According to the secondary and tertiary structure of *Arabidopsis thaliana* AdoMetDC, the Lys^{81} and Cys^{83} residues are located in the active site, which is similar to the human AdoMetDC structure. The Cys^{50} and Cys^{230} residues are located in the loop between strand β-2 and 3, strand β-9 and 10, respectively. Since the loops are located near the surface of AdoMetDC, we could not rule out the possibility that the Cys^{50} and Cys^{230} residues might be involved in the formation of disulfide bonds with non-conserved cysteine residues in the large and small subunits that are important to the structural integrity of AdoMetDC.

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