Conformational Stabilization of Rat *S*-Adenosylmethionine Decarboxylase by Putrescine

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The activity and processing of mammalian *S*-adenosylmethionine decarboxylase (AdoMetDC) is stimulated by putrescine. To obtain new insights into the mechanism through which putrescine stimulates AdoMetDC, we investigated conformational changes in rat prostate AdoMetDC in the presence or absence of putrescine. We examined the reactivity of purified rat prostate AdoMetDC to the SH-reagent iodoacetic acid (IAA) and its susceptibility to proteolysis in the presence or absence of putrescine using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The activity of AdoMetDC treated with IAA in the absence of putrescine was reduced, but about 80% of its activity remained after treatment with IAA in the presence of putrescine. In the presence of putrescine, IAA incorporation was 1.9 mol IAA/mol of AdoMetDC α-subunit, while there was no incorporation of IAA in the β-subunit of AdoMetDC. In the absence of putrescine, 5.0 mol of IAA/mol of α-subunit and 0.9 mol of IAA/mol of β-subunit were incorporated. Only Cys292 and Cys310 were carboxymethylated by IAA in the presence of putrescine. In contrast, in the absence of putrescine all cysteines were carboxymethylated by IAA. In addition, putrescine slowed the rate of AdoMetDC degradation by trypsin. These results demonstrate that the conformation of AdoMetDC purified from rat prostate is stabilized by putrescine.

Key words *S*-adenosylmethionine decarboxylase; putrescine; conformational stabilization; matrix assisted laser desorption ionization time-of-flight mass spectrometry; SH-reagent

S-Adenosylmethionine decarboxylase (AdoMetDC, EC4.1.1.50) is an essential enzyme for the biosynthesis of the polyamines spermidine and spermine, which are required for normal cell proliferation and differentiation.[1—3] AdoMetDC catalyzes the removal of the carboxyl group from *S*-adenosylmethionine (AdoMet) to form decarboxylated *S*-adenosylmethionine (deAdoMet). The propylamine group of deAdoMet is transferred to putrescine to form spermidine or from spermidine to form spermine. AdoMetDC is one member of a small class of decarboxylases that use a covalently bound pyruvate as a prosthetic group.[4,5] All known AdoMetDCs are synthesized in the proenzyme form which subsequently undergoes an intramolecular cleavage at a serine residue to generate two subunits.[6—8] In this process, the serine residue is converted to a pyruvoyl group. Proenzyme processing generates two non-identical subunits, termed α and β, which are both indispensable components of the mature enzyme.[8—10]

The first step of AdoMetDC catalysis is AdoMet binding through the pyruvate prosthetic group, which reacts to form a Schiff base adduct with the substrate.[2,11] It is postulated that Cys82 is the proton donor in the decarboxylation reaction catalyzed by AdoMetDC. The importance of Cys82 is illustrated by the fact that mutation of this residue to serine or alanine greatly reduces the enzyme's activity.[11,12]

The activity and processing of mammalian AdoMetDC is stimulated by putrescine.[13—15] Stimulation of AdoMetDC activity is believed to result from a conformational change in the protein that causes a reduction in the K_m for its substrate, AdoMet. Since stimulation of AdoMetDC activity is pH dependent and occurs to a greater extent the more acidic the pH, it has been proposed that the interaction of putrescine with acidic residues in human AdoMetDC causes this conformational change. Studies by site-directed mutagenesis demonstrated that substitutions resulting from mutation in any one of four acidic residues (Glu11, Asp174, Glu178, and Glu256) in human AdoMetDC abolishes the stimulation of activity and processing.[12,16,17] Structural studies of His-tagged human AdoMetDC revealed that the functional form of the enzyme is an $(\alpha\beta)_2$ dimer with one active site and one pyruvoyl cofactor per promoter.[18—20] The putrescine molecule is bound to the enzyme in an unusual cluster of buried, negatively charged residues located between the two β-sheets, away from active site.[19,20] Recently, crystal structures of putrescine-free His-tagged human AdoMetDC and the acid to amide mutants of Asp174, Glu178, and Glu256 have been determined.[21] The structure of putrescine-free His-tagged human AdoMetDC showed conformational differences compared to AdoMetDC complexed with putrescine. Although there is considerable literature pertaining to structural characteristics of recombinant AdoMetDC, no information regarding structural changes occurring upon putrescine binding in unmodified AdoMetDC purified from mammalian tissues has been reported.

His-tagging is widely used in the purification of recombinant proteins. His-tags, which typically consist of a string of six or more consecutive histidine residues, are bound to the N- or C-terminus of a target protein, and the protein is then typically isolated by metal affinity chromatography. Since the sequence of the His-tag is small and simple, the procedure is thought to have minimal impact on protein function. However, this may not always be the case, as His-tags may have unexpected effects on protein conformation, depending on the type of protein targeted.[22—24] We recently presented the primary structure of purified rat prostate AdoMetDC, including post-translational modification, using matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) coupled with five specific cleavage methods (Fig. 1).[25] The results of that study demonstrated that the N-terminus of the β-subunit of AdoMetDC is acetylated. Since His-tags and post-translational modifications change

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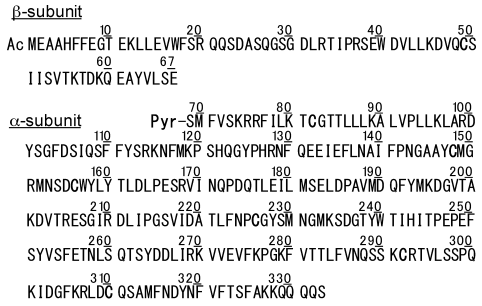

Fig. 1. Primary Structure of Rat Prostate AdoMetDC

the primary structure of a protein, they may induce changes in the conformation as well. It is therefore important to examine the effect of putrescine on intact AdoMetDC purified from animal tissues or cultured cells.

X-ray crystallography and NMR are the primary analytical tools used to obtain atomic level structural information relating to protein-ligand binding sites and protein conformation changes induced by complex formation. However, these methods require relatively large amounts of pure protein, and are therefore of limited value in analyzing relatively low-abundance proteins such as intact AdoMetDC. Recently, various methods involving side chain-specific chemical modification have been used to elucidate the role of specific amino acids in enzyme active site reactivity,[26,27] examine protein–protein interactions,[28] and conduct structural studies of protein conformation.[29] Mass spectrometric peptide mapping is an ideal method for the unequivocal characterization of chemical modifications in proteins. The combination of limited tertiary structure–selective chemical modification with mass spectrometric peptide mapping enables the molecular characterization of protein function when other methods are either unreliable or unavailable.[30,31] In addition, susceptibility to proteolysis has been shown to be a sensitive method for detecting subtle conformation changes in proteins.[32,33]

In this study, we examined the effect of putrescine on the conformation and activity of AdoMetDC purified from rat prostate using MALDI-TOF MS, and the susceptibility of AdoMetDC to trypsin following treatment with iodoacetic acid (IAA). We also examined the correlation between the presence of putrescine and stimulation of AdoMetDC activity and incorporation of IAA.

MATERIALS AND METHODS

Chemicals Dithiothreitol (DTT), adenosylmethionine chloride salt, 2,5-dihydroxybenzoic acid, 2-hydroxy-5-methoxybenzoic acid, bovine pancreatic insulin, substance P, angiotensin I, apomyoglobin, IAA, and cytochrome *c* were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). IAA was recrystalized prior to use. Putrescine dihydrochloride was purchased from Tokyo Chemical Industries (Tokyo, Japan). *S*-Adenosyl [carboxyl-^{14}C] methionine (57 mCi/mmol) was obtained from GE Healthcare (Little Chalfont, U.K.). *O*-Methylisourea hemisulfate and lysylendopeptidase (*Achromobactor* protease I) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Sequencing grade porcine trypsin was obtained from Promega (Madison, WI, U.S.A.). All other regents and organic solvents were of commercial analytical grade.

Purification of AdoMetDC from Rat Prostate Glands AdoMetDC was purified from rat prostate glands as described previously.[34,35] Purified AdoMetDC was stocked in 10 mM Tris–HCl buffer (pH 7.5) containing 0.1 mM ethylenediaminetetracetic acid (EDTA), 2.5 mM DTT, and 2.5 mM putrescine at 0 °C. Protein concentration was measured using the Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Hercules, CA, U.S.A.) using bovine serum albumin (Sigma-Aldrich, St. Louis, MO, U.S.A.) as the standard.

Measurement of AdoMetDC Activity AdoMetDC activity was measured using *S*-adenosyl [carboxyl-^{14}C] methionine as a substrate.[35] The standard assay medium contained 1.25 mM DTT, 3 mM putrescine, 0.2 mM AdoMet, 0.95 μM *S*-adenosyl [carboxyl-^{14}C] methionine, and 50 mM sodium phosphate buffer (pH 7.4). In case of the experiment shown in Fig. 5, 50 mM sodium phosphate buffer (pH 6.8) was used for the measurement of AdoMetDC activity instead of 50 mM sodium phosphate buffer (pH 7.4).

Removal of Putrescine from Purified Rat AdoMetDC Purified rat AdoMetDC solution (100 μl, 24 μg protein) was applied to a Sephadex G-25 column (6 mm×15 cm) at a flow rate of 0.03 ml/min. The column was equilibrated at 4 °C with a three-component buffer (AMT buffer) containing 0.05 M sodium acetate, 0.05 M 2-(*N*-morpholino)-ethanesulfonic acid, and 0.1 M triethanolamine, pH 9.0. Fractions of 250 μl each were collected and those containing AdoMetDC were pooled.

Inhibition and Carboxymethylation by IAA Putrescine-free AdoMetDC solution (250 μl) was treated with 4 mM IAA in the presence or absence of 0.3 mM putrescine for 60 min in AMT buffer at pH 9.0. The reactions were carried out in the dark at 37 °C and were terminated by the addition of an excess of DTT. The remaining enzymatic activity of IAA-treated AdoMetDC was measured using the standard assay described above.

Lysylendopeptidase Digestion IAA-treated AdoMetDC was desalted by extensive dialysis against 10 mM Tris–HCl buffer (pH 8.5), 1 mM EDTA, and 5% (v/v) acetonitrile, and then digested for 16 h at 37 °C by lysylendopeptidase. The molar ratio of AdoMetDC to lysylendopeptidase was 200 : 1.

Modification of Digested Peptides by *O*-Methylisourea[36] After lysylendopeptidase digestion, an equal volume of 2 M *O*-methylisourea in 200 mM Na_2CO_3, adjusted to pH 10, was added and the peptide mixture was incubated at 37 °C.

Trypsin Digestion in the Presence or Absence of Putrescine[32] Putrescine-free AdoMetDC was digested with trypsin in the presence or absence of 0.3 mM putrescine in 0.05 M Tris–HCl buffer, pH 8.0, at 37 °C. The molar ratio of AdoMetDC to trypsin was about 50 : 1. The mixture was analyzed by MALDI-TOF MS at various reaction times. Proteolysis was stopped by addition of 4 μl 10% trifluoroacetic acid (TFA) to the 20 μl reaction mixture.

Preparation of Samples for MS Proteins and peptides were extracted for MS analysis using $ZipTip_{C4}$ and $ZipTip_{C18}$ (Millipore, Bedford, MA, U.S.A.), respectively. $ZipTip_{C4}$ and $ZipTip_{C18}$ were wetted with 10 μl of 70% acetonitrile, followed by an equilibration with 10 μl of 0.1% TFA. Ten microliter aliquots of proteins and peptides solutions were then loaded onto the $ZipTip_{C4}$ and $ZipTip_{C18}$ by performing 10 aspirate-dispense cycles with these aliquots. These columns

were washed by 10 cycles applying 10 μl of 5% acetonitrile containing 0.1% TFA. The retained proteins and peptides were eluted with 5 μl of 70% acetonitrile containing 0.1% TFA.

MALDI-TOF MS Analysis MALDI mass spectra were collected in reflector mode on a Finnigan MAT Vision 2000 TOF mass spectrometer (ThermoQuest, San Jose, CA, U.S.A.) using a mixture of 2,5-dihydroxybenzoic acid and 2-hydroxy-5-methoxybenzoic acid as the matrix. Ionization was achieved by irradiation with a nitrogen laser at 337 nm. The mass spectra were calibrated with angiotensin I (monoisotopic mass: *m*/*z* 1296.7), substance P (monoisotopic mass: *m*/*z* 1347.7), insulin B (average mass: *m*/*z* 5734.5), and horse cytochrome *c* (average mass: *m*/*z* 12362.0). The calculated masses and the observed masses are listed as monoisotopic (the molecular weight range of 1—2500 Da) or average (the molecular weight higher than 2500 Da) *m*/*z* values. The theoretical mass was calculated using EXPASy-PeptideMass.

RESULTS

Putrescine and the reducing agent DTT are necessary for the maintenance of mammalian AdoMetDC activity. In addition, Cys82 is essential for catalytic activity of human AdoMetDC.[11,12] Therefore, we examined how putrescine prevents inactivation of purified rat AdoMetDC using the SH-reagent, IAA (Fig. 2). The activity of AdoMetDC incubated at 37 °C for 60 min in the absence of putrescine was decreased by 55%. In contrast, the activity of AdoMetDC incubated at 37 °C for 60 min in the presence of putrescine was not diminished. After treatment with IAA at 37 °C for 60 min, the activity of AdoMetDC in the absence of putrescine was almost completely inhibited, while activity in the presence of putrescine remained at about 80% of the control. Putrescine therefore protects AdoMetDC from inhibition by IAA modification.

The MALDI-TOF MS spectra of purified rat AdoMetDC treated with IAA in the presence and absence of putrescine are shown in Fig. 3. In the absence of IAA, the higher *m*/*z* values observed were close to the calculated mass of the α-subunit of rat AdoMetDC in which Ser68 has been converted into a pyruvate residue (average mass: *m*/*z* 30503.9). The lower *m*/*z* value approximated the calculated mass of the β-subunit of rat AdoMetDC in which the N-terminus has been acetylated (average mass: *m*/*z* 7678.6).[25] The mass for the α-subunit of AdoMetDC treated with IAA in the presence of putrescine was 108.7 Da greater than the calculated mass, while there was no change in the β-subunit mass. In contrast, the mass for the α-subunit of AdoMetDC treated with IAA in the absence of putrescine was 292.6 Da greater than the calculated mass, whereas the peak representing the β-subunit was 53.7 Da greater than its calculated mass. The conversion of cysteine to carboxymethylated cysteine results in a mass increase 58 Da per cysteine residue. These results suggest that in the presence of putrescine, 1.9 mol of IAA was incorporated per mol of AdoMetDC α-subunit, while no IAA was incorporated into the β-subunit. Considerably more IAA was incorporated into both subunits in the absence of putrescine (5.0 mol of IAA/mol of α-subunit and 0.9 mol of IAA/mol of β-subunit).

Rat AdoMetDC has six cysteine residues (Cys82, Cys148, Cys156, Cys226, Cys292, and Cys310) in the α-subunit and one cysteine residue (Cys49) in the β-subunit. To examine which cysteine residues are labeled by IAA in the presence or absence of putrescine, we analyzed the peptides obtained by lysylendopeptidase-digestion of IAA-treated rat AdoMetDC by MALDI-TOF MS (Table 1). In the absence of putrescine, the following IAA-labeled peptides were observed: peptide D46-K56, containing Cys49; peptide D202-K234, containing Cys226; peptide C292-K301, containing Cys292; and peptide R307-K327, containing Cys310. A doubly derivatized peptide (P120-K195) containing two cysteines (Cys148 and Cys156) was also observed by MALDI-TOF MS. Only two IAA-labeled peptides (C292-K301 containing Cys292, and peptide R307-K327 containing Cys310) were detected in spectra of AdoMetDC digested in the presence of putrescine. In contrast, peptide D202-K234 (containing Cys226) and peptide P120-K195 (containing Cys148 and Cys156) were present as unmodified peptides. Peptide T81-K89 (containing Cys82) was undetectable in digests of AdoMetDC treated with IAA in either the presence or absence of putrescine. Peptide D46-K56 (containing Cys49) could not be detected in digests of AdoMetDC treated with IAA in the presence of putrescine.

The conversion of lysine into homoarginine by *O*-methylisourea dramatically increases the sensitivity of MALDI-TOF MS detection of lysine-containing peptides.[36] Therefore, lysylendopeptidase digests of rat AdoMetDC

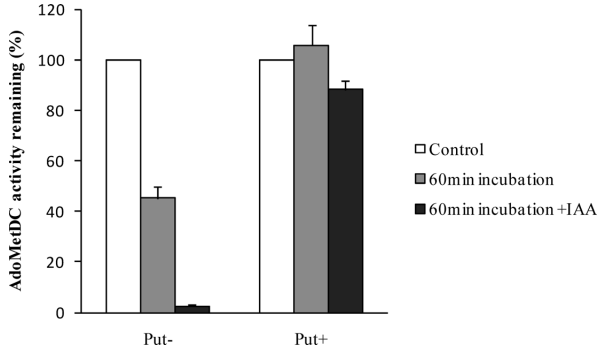

Fig. 2. Effect of Putrescine on the Inactivation of Rat AdoMetDC by IAA
The results are expressed as the means±S.D. (n=3).

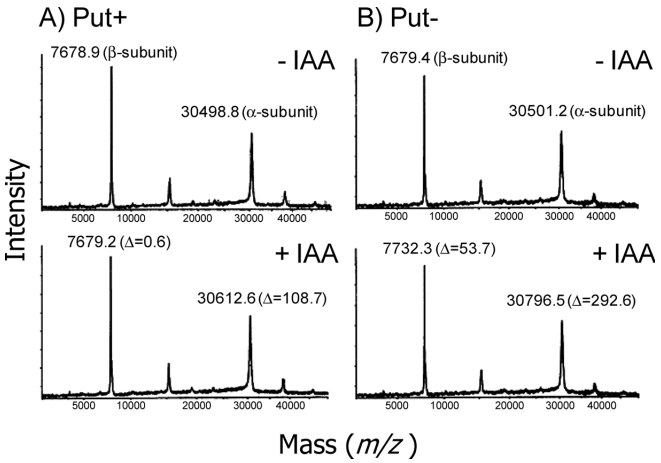

Fig. 3. MALDI-TOF MS Spectra of AdoMetDC Treated with IAA in the Presence of Putrescine (A) and in the Absence of Putrescine (B)

Table 1. MALDI-TOF MS Data for Peptides Obtained by Lysylendo-Peptidase-Digestion of IAA-Treated Rat AdoMetDC in the Presence or Absence of Putrescine

Cysteinylresidue	Fragment containing Cys	$[M+H]^+_{calc}$	Put− $[M+H]^+_{obs}$	Put+ $[M+H]^+_{obs}$
Cys49	D46-K56	1234.6[a]	—	1234.9[b]
	D46-K56+CM	1250.6	1250.3	—
	D46-K56+CM	1292.5[a]	1292.2[b]	—
Cys82	T81-K89	991.5[a]	—	991.5[b]
	T81-K89+CM	1049.8[a]	1049.2[b]	—
Cys148	P120-K195	8936.1	—	8937.1
	P120-K195+2CM	9052.1	9052.9	—
Cys156	P120-K195	8936.1	—	8937.1
	P120-K195+2CM	9052.1	9052.9	—
Cys226	D202-K234	3559.1	—	3561.0
	D202-K234+CM	3617.1	3617.1	—
Cys292	C292-K301	1118.6	—	—
	C292-K301+CM	1176.6	1176.8	1176.3
Cys310	R307-K327	2505.8	—	—
	R307-K327+CM	2563.9	2564.2	2564.3

a) The calculated as the C-terminal homoargnyrated rat sequence. *b*) After treatment with *O*-methylisourea.

treated with IAA in the presence or absence of putrescine were incubated with *O*-methylisourea to enhance peptide detection. The lysine to homoarginine conversion results in a mass increase of 42 Da per lysine residue. Following *O*-methylisourea treatment, the previously undetectable peptides T81-K89 and D46-K56 were detected as IAA-labeled peptides in digests of AdoMetDC treated in the absence of putrescine. In contrast, these peptides were found to be unmodified in digests of AdoMetDC treated with IAA in the presence of putrescine.

Overall, in the absence of putrescine, all cysteines were carboxymethylated by IAA. In contrast, only Cys292 and Cys310 were carboxymethylated in the presence of putrescine. These results indicate that putrescine changes the accessibility to solvent of the regions around Cys49, Cys82, Cys148, Cys156, and Cys226. In the absence of putrescine, 5.0 mol of IAA were incorporated per mol of AdoMetDC α-subunit, but lysylendopeptidase digestion indicated that six α-subunit cysteine residues were modified (Table 1). This may be due to some peptides remaining in the unmodified form but being present in a concentration too low for MALDI-TOF MS detection.

Susceptibility to proteolysis has been shown to be a sensitive method to detect subtle conformational changes in proteins.[32] In order to examine the effect of putrescine on the susceptibility of AdoMetDC to trypsin, purified rat AdoMetDC was digested with trypsin in the presence or absence of putrescine and analyzed by MALDI-TOF MS at various time intervals (Fig. 4). Before trypsin digestion, peaks corresponding to the α- and β-subunits of AdoMetDC were clearly observed in samples prepared both in the presence and absence of putrescine. Peptides corresponding to T294-S333 (calculated average mass: *m*/*z* 4635.2) and S68-R293 in which Ser68 has been converted to pyruvate (calculated average mass: *m*/*z* 25888.7) were observed within the first 5 min of the digestion in samples both with and without putrescine. A group of peptide fragments was detected in the lower mass region that increased in abundance as digestion time increased. There was little difference between the type and the

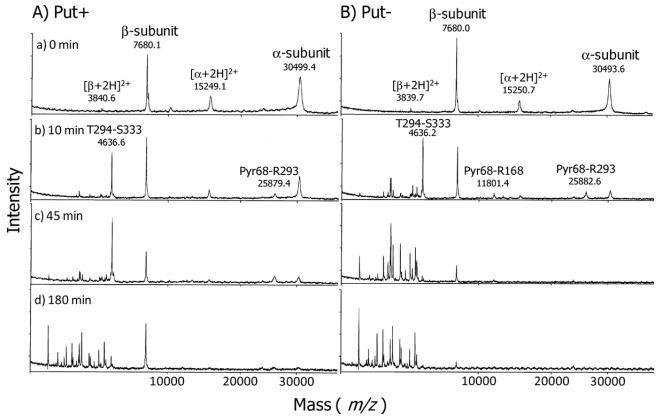

Fig. 4. MALDI-TOF MS Spectra of AdoMetDC Digested with Trypsin for (a) 0 min, (b) 10 min, (c) 45 min, and (d) 180 min in the Presence of Putrescine (A) and in the Absence of Putrescine (B)

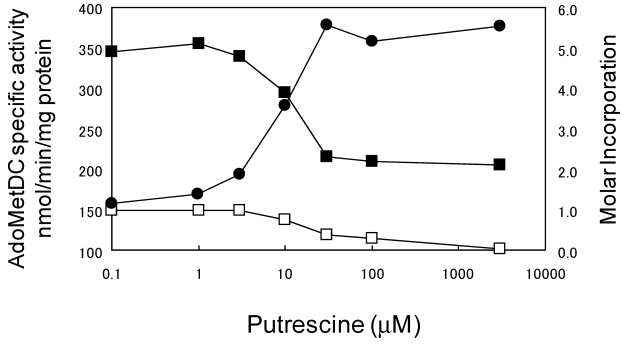

Fig. 5. Effect of Putrescine Concentration on AdoMetDC Activity and Total Incorporation of IAA

The symbols indicate AdoMetDC activity (●), incorporation of IAA into the α-subunit (■), and incorporation of IAA into the β-subunit (□). Data are expressed as the mean of duplicate assays.

detection order of observed peaks in the presence or absence of putrescine. However, in samples with putrescine these peptides were detected only after a much longer digestion time (Fig. 4). In the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of trypsin digested AdoMetDC as preliminary experiment, putrescine delayed the observed disappearance of the α-subunit in the digestion (data not shown). There results indicate that putrescine stabilizes the conformation of AdoMetDC.

We also studied the correlation between the stimulation of purified rat AdoMetDC activity by putrescine and the incorporation of IAA in the presence of putrescine (Fig. 5). The incorporation of IAA into the α-subunit was approximately 5.0 mol of IAA/mol of α-subunit, while incorporation into the β-subunit was approximately 1.0 mol of IAA/mol of subunit at putrescine concentrations of 0—3 μM. The incorporation of IAA decreased at putrescine concentrations greater than 10 μM. Putrescine concentrations of 0—3 μM had little influence on AdoMetDC activity and the activity increased at putrescine concentrations of more than 10 μM, a result that is consistent with reports that putrescine at a concentration of several μM stimulates the activity of AdoMetDC.[15,37] Our results indicate that the observed stimulation of AdoMetDC activity is associated with conformational stabilization of the enzyme by putrescine.

DISCUSSION

This study clearly demonstrates that the conformation of AdoMetDC purified from rat prostate is stabilized by putrescine.

Putrescine protected AdoMetDC from irreversible inhibition by IAA (Fig. 2). The incorporation of IAA in the presence of putrescine (1.9 mol of IAA/mole of $\alpha\beta$ dimer) was much lower than it was in the absence of putrescine (5.9 mol of IAA/mole of $\alpha\beta$ dimer), suggesting that putrescine induced an alteration in the conformation that precluded IAA from gaining access to Cys residues (Fig. 3). In a previous study, it was shown that His-tagged human AdoMetDC could be inhibited by IAA without removing putrescine.[11] The maximal incorporation of IAA in this case was 2.7 mol of IAA/mol of the $\alpha\beta$ dimer (95% confidence interval 2.3—3.0 mol of IAA/mol) for the enzyme.[11] The difference in reactivity with IAA could be explained by the difference in the amino acid sequences of the rat and human forms of AdoMetDC or/and the differences in the existence of His-tagged modification.[38]

In this study, all cysteine were carboxymethylated by IAA in the absence of putrescine, but only Cys292 and Cys310 were carboxymethylated in the presence of putrescine (Table 1). Our results were consistent with the crystal structure study for His-tagged human AdoMetDC by Ekstrom *et al.* showing that Cys292 and Cys310 were exposed on the surface on the enzyme.[18—20] Our results also indicated that putrescine changes the accessibility to the solvent of the regions around Cys49, Cys82, Cys148, Cys156 and Cys226. In comparing the crystal structures of putrescine-free and putrescine-bound His-tagged AdoMetDC, Bale *et al.*[21] reported a local rearrangement of four aromatic residues near the putrescine binding site (Phe285, Phe315, Tyr318, and Phe320) and a conformational change in the S312-F320 loop, suggesting that binding of putrescine induces a closure of the S312-F320 loop that shields putrescine from external solvent.[21,39] It is likely that this closure of the loop would affect the accessibility of the solvent to the regions around the reactive cysteine residues in the protein. However, in order to obtain more detail information on the conformational change by putrescine, further studies would be needed using the enzyme purified from tissue or cultured cells.

Peptide S68-R293, in which Ser68 has been converted into pyruvate (calculated average mass: *m*/*z* 25888.7), as well as peptide T294-S333 (calculated average mass: *m*/*z* 4635.3), were both observed in samples examined during the early stages of trypsin digestion of AdoMetDC (Fig. 4). These results indicate that the initial trypsin degradation site is Arg293. Structural analysis indicates that the R293-S298 loop containing Arg293 is disordered in His-tagged human AdoMetDC.[18] Therefore it is thought that the R293-S298 loop structure is unstable and may freely move. In addition, Cys292, which is adjacent to Arg293, is carboxymethylated by IAA in the presence or absence of putrescine (Table 1). Therefore, the region around Arg293 must be accessible to external solvent and trypsin regardless of putrescine's presence.

Since AdoMetDC is a control point within the polyamine metabolic pathway, the enzyme has been targeted in the effort to design inhibitors as chemotherapeutic drugs for treating various cancers and parasitic infections.[6,40—44] Our study suggests that the conformation—and thus activity—of AdoMetDC is controlled by the low molecular weight polyamine putrescine. Therefore, new inhibitors might be developed that target the putrescine binding site and destabilize the conformation of AdoMetDC to disrupt its activity. The method we employed—MALDI-TOF MS coupled with reactivity to IAA—would be useful for screening such inhibitors.

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