Processing of Mammalian and Plant S-Adenosylmethionine Decarboxylase Proenzymes*

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S-Adenosylmethionine decarboxylase (AdoMetDC) is a pyruvoyl enzyme, and the pyruvate is formed in an intramolecular reaction that cleaves a proenzyme precursor and converts a serine residue into pyruvate. The wild-type potato AdoMetDC proenzyme processed much faster than the human proenzyme and did not require putrescine for an optimal rate of processing despite the presence of three acidic residues (equivalent to Glu11, Glu178, and Glu256) that were demonstrated in previous studies to be required for the putrescine activation of human AdoMetDC proenzyme processing (Stanley, B. A., Shantz, L. M., and Pegg, A. E. (1994) J. Biol. Chem. 269, 7901–7907). A fourth residue that is also needed for the putrescine stimulation of human AdoMetDC proenzyme processing was identified in the present studies, and this residue (Asp174) is not present in the potato sequence. The site of potato AdoMetDC proenzyme processing was found to be Ser73 in the conserved sequence, YVLSESS, which is the equivalent of Ser68 in the human sequence. Replacement of the serine precursor with threonine or cysteine by site-directed mutagenesis in either the potato or the human AdoMetDC proenzyme did not prevent processing but caused a significant reduction in the rate. Although the COOH-terminal regions of the known eukaryotic AdoMetDCs are not conserved, only relatively small truncations of 8 residues from the human protein and 25 residues from the potato proenzyme were compatible with processing. The maximally truncated proteins show no similarity in COOH-terminal amino acid sequence but each contained 46 amino acid residues after the last conserved sequence, suggesting that the length of this section of the protein is essential for maintaining the proenzyme conformation needed for autocatalytic processing.

AdoMetDC1 is an essential enzyme for the biosynthesis of polyamines and is one of a small class of decarboxylases that uses a covalently bound pyruvate as a prosthetic group (1, 2). These pyruvoyl-dependent decarboxylases form amines such as histamine, decarboxylated S-adenosylmethionine, phosphatidylethanolamine (a component of membrane phospholipids), and β-alanine (a precursor of coenzyme A), which are all of critical importance in cellular physiology and provide an important target for drug design. The mechanism of formation of the prosthetic group has been studied extensively using histidine decarboxylase from Lactobacillus (1, 3–6), and more preliminary studies with other decarboxylases including AdoMetDC (7–9) suggest that the mechanism is similar (Fig. 1). In all cases, the enzyme is synthesized as a proenzyme that then undergoes an intramolecular cleavage reaction forming the two subunits and generating the pyruvate at the amino terminus of one of the subunits from a serine precursor residue. Cleavage takes place via the formation of an intermediate ester resulting from a nucleophilic attack of this serine residue at the amide carbonyl group of the preceding amino acid. This is followed by β-elimination to form the β subunit and the α subunit containing a dehydroalanine at its amino terminus. The dehydroalanine then loses ammonia and is converted to pyruvate via the formation of imine and carbinolamine intermediates (1–3). The initial rearrangement step of this reaction to form a peptide ester linked to the hydroxyl side chain of serine is identical to that involved in protein splicing reactions (10, 11). Further information on such cleavage reactions would be very useful in understanding this reaction in more detail and in producing useful drugs based on prevention of protein maturation.

Studies in which the yeast and the human AdoMetDC (hAdoMetDC) proenzymes were expressed have shown that the site of cleavage was contained in the sequence YVLSESS, with the underlined serine residue forming the pyruvate (8, 9). Apart from the obvious presence of a serine residue, this sequence has little or no resemblance to the cleavage sites in the proenzymes of histidine decarboxylase, phosphatidylserine decarboxylase, and aspartate decarboxylase (1, 2). Processing and activity of the mammalian and yeast AdoMetDCs is enhanced by the presence of putrescine (12–14). This provides an important physiological mechanism favoring the conversion of putrescine into higher polyamines, since it links the level of putrescine to the amount of active AdoMetDC that is needed for the conversion of putrescine to spermidine. Site-directed mutagenesis studies of the hAdoMetDC proenzyme have indicated that the interaction of putrescine with at least three acidic acid residues (Glu11, Glu178, and Glu256) is necessary for the acceleration of processing, since conversion of any of these residues to glutamine abolishes the effect (15, 16).

Numerous cDNA sequences for eukaryotic AdoMetDC proenzymes have now been obtained, and at least 23 such sequences can be compared. These include five mammalian (human (17), mouse (18), rat (19), hamster (20), and cow (21)), amphibian (Xenopus laevis) (22), yeast (9), two protozoan parasites (Trypanosoma brucei (GenBank U20092) and Leishmania dono-
vanii (GenBank™ U20091), the parasitic worm Onchocerca volvulus, (23), and at least 13 plants including both monocotyledons such as wheat (24), maize (GenBank™ Y07767), and rice (GenBank™ Y07766) and dicotyledons such as potato (25, 26), tomato (GenBank™ Y07768), periwinkle (27), cabbage (GenBank™ X95729), arabidopsis (GenBank™ U63633), morning glory (GenBank™ U64927), and carnation (28). The derived amino acid sequences from these cDNAs show that there are some very highly conserved regions that include the sequence for proenzyme cleavage and the sequence (KTCTG) that converted the unique KpnI site was used, and for the pTUB13, a selection primer that destroyed a unique XbaI site (for mutations in pCM9) or with 

Studying the processing of AboMetDC have been hampered by the speed and apparently autocatalytic nature of the reaction. This has rendered it difficult to study the processing reaction, since significant processing occurs during the period of synthesis of the proenzyme needed to accumulate sufficient protein for analysis. In the present studies, we have investigated the effect of replacing the pyruvate-generating serine with two other amino acids, cysteine and threonine, which could also form a peptide ester. Studies were carried out with both the hAboMetDC and the pAdoMetDC proenzymes. The importance of the COOH-terminal region of the molecule has also been studied, since the largest region of differences in the known AboMetDC sequences occurs at the carboxyl end of the molecule, where there is no clear similarity after the sequence FXPXXF located at human residues 275–280 (Fig. 2). The potato and all of the other known plant AboMetDC sequences have a highly charged extension at the carboxyl terminus.
**Synthesis and Processing of AdoMetDC Proenzyme**—The TNT assay system (Promega) with T7 RNA polymerase was used for the coupled transcription and translation from the control and mutant pCM9 and pTUB13 plasmids. Reactions were carried out according to the manufacturer’s specifications with some modifications, as indicated below. A typical 12.5-μl reaction assay mix contained 0.2 μg of plasmid DNA, 10 units of RNasin, 1 unit of T7 RNA polymerase, 10 pmol of [35S]methionine (1 Ci/mmol), 20 μM of the other 19 amino acids, and 6.25 μl of rabbit reticulocyte lysate (nuclease-treated). The tubes were incubated at 30 °C for 30 min, and translation was stopped by adding cycloheximide to a final concentration of 200 μM. Aliquots (5 μl) were removed at this time and at various time points after continued incubation at 30 °C in order to measure the processing. The protein products in these aliquots were separated by SDS-PAGE using 12.5% gels. The gels were dried, and the radioactivity of the bands corresponding to proenzyme and mature enzyme were directly measured with a PhosphorImager 425E-120 (Molecular Dynamics, Inc.). In some experiments, 1 mM putrescine was included in the processing reaction mixes. The rates of processing were calculated as described previously (7).

**AdoMetDC Activity Assay**—For the AdoMetDC activity assays, 47 μM of unlabeled methionine was added to the TNT reactions in place of the [35S]methionine. A 5-μl aliquot of the reaction mix was then assayed for AdoMetDC activity by measuring the ability to convert [1,4-CO2]AdoMet into [14C]methionine by incubation at 37 °C (35). The assay mix consisted of 1.25 mM dithiothreitol, 50 mM sodium phosphate buffer (pH 8.0), 6 μM [1,4-CO2]AdoMet (32 mCi/mmol). In some experiments, putrescine was added to a final concentration of 1.9 mM as indicated to stimulate AdoMetDC activity. Specific activity was calculated as the cpm of [14C]methionine produced per 30 min divided by the band intensity corresponding to the 31- or 32-kDa processed AdoMetDC α subunit from a parallel synthesis with [35S]methionine.

**Purification of Potato AdoMetDC**—The potato AdoMetDC cDNA was inserted into the pQE31 expression vector. This construction replaced the first three residues of the amino terminus of the pAdoMetDC sequence (MEM) with the sequence MRGS(H10)TPD and allows the protein to be purified by immobilized metal affinity chromatography. Insertion was carried out by using PCR with pTUB13 as a template and the primers 5′-CTATAATGGATCCGGATTGCCAGTTTCTGCC-3′ and 5′-TCCAGACCCCTGGACAAAGGGG-3′ to generate a 1.4-kilobase fragment that was cut with BamHI and KpnI and inserted into pQE31 cut with the same enzymes, giving plasmid pHIS-PSAM. XL-1 Blue cells were transformed with pHIS-PSAM and grown to a density corresponding to A600 of 0.6. Expression of AdoMetDC was then induced by the addition of isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 0.3 mM, and after 3 h the cells were harvested and washed once with buffer X (10 mM Tris-HCl, pH 8.0, 200 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride) at 4 °C. The cell pellet was then resuspended in buffer X and homogenized with a French press. The suspension was centrifuged at 20,000 × g for 30 min, and the supernatant was applied to a 1-ml column of TalonTM resin pre-equilibrated with buffer X. The column was washed with buffer X containing 10 mM imidazole, and the protein was eluted with buffer X containing 200 mM imidazole. The eluted protein was immediately passed through a Sephadex G-25 column pre-equilibrated with 10 mM Tris-HCl, pH 8.0, 50 mM NaCl, 2.5 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride to remove the imidazole, and the protein was stored at −80 °C. The S73C mutant pAdoMetDC protein was prepared in the same way using the pTUB13 containing the S73C mutation for the PCR template.

**Sequencing of Potato AdoMetDC**—The pyruvate prosthetic group was converted to alanine by reductive amination by incubation at 37 °C in 2 mM ammonium acetate, pH 6.5, and 100 mM NaNBH4 (8, 9, 36), and the protein subunits were resolved by SDS-polyacrylamide gel electrophoresis. The protein bands were transferred to polyvinylidene difluoride membranes (Millipore), and the 32-kDa band was subjected to amino acid sequencing using an Applied Biosystems 477A protein sequencer.

**RESULTS**

**The Processing of AdoMetDC**—Previous studies have shown that the 38-kDa hAdoMetDC proenzyme is processed in reticulocyte lysates and that putrescine stimulates this reaction. The data for hAdoMetDC shown in Fig. 3a are in agreement with this. After a synthesis period of 30 min in a typical TNT reaction in the presence of putrescine, 35% of hAdoMetDC was in the processed 31-kDa form. After 60 min of further incubation plus putrescine, virtually all of the proenzyme was pro-

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that this is the case, the pAdoMetDC protein was expressed in *E. coli* and purified to homogeneity. The purified protein was treated by reductive amination using ammonium ions and NaCNBH₃ to convert the amino-terminal pyruvate into alanine and allow sequencing by Edman degradation. The reduced protein was separated by SDS-polyacrylamide gel electrophoresis, and the 32-kDa subunit was sequenced using a gas phase sequencer. The sequence obtained was Ala-Ser-Leu-Phe-Val-

### Table I

| AdoMetDC used | Specific activity | Processing |
|---------------|------------------|------------|
| Wild type human | 100 | >95 |
| S68T | <0.2 | 12 |
| S68C | 7 | 4 |
| S68A | <0.2 | <0.3 |
| Wild type potato | 100 | >95 |
| S73T | <1 | 10 |
| S73C | 90 | 4 |
| S74C | 98 | >95 |

![Figure 4](image-url)

**FIG. 4.** The processing of mutants at the cleavage site of human and pAdoMetDC. Processing was measured as described in Fig. 3. Panel a shows the time course of processing in the presence of 1.0 mM putrescine after a 30-min synthesis reaction for the hAdoMetDC and its mutants in which the Ser⁶⁸ residue was changed to threonine, cysteine, or alanine as shown. Panel b shows results for the pAdoMetDC in which residues Ser⁷³ and Ser⁷⁴ were changed to cysteine or Ser⁷³ was changed to threonine as shown. Samples were taken at the end of the 30-min synthesis reaction. Panel c shows the effect of putrescine on the processing of S73C and S73T pAdoMetDC mutants. Processing was measured after a 30-min synthesis period in the presence or absence of putrescine (Put.) as shown. Aliquots of the synthesis reaction for the S73C mutant were also precipitated with 2 M (NH₄)₂SO₄ (A.S.), at 4 °C, redisolved and desalted, and then incubated in the presence or absence of reticulocyte lysate as indicated. W.T., wild type.

37), neither the human S68T nor the potato S73T AdoMetDCs had any detectable activity (Table I). Replacement of the serine precursor with cysteine has no effect on the prothetic group formed on cleavage, which is still pyruvate but produces a thio-carboxylate group at the carboxyl terminus of the β chain in the mutant (1, 4). Within the limits of experimental error, the plant S73C mutant appeared to be fully active (Table I). The human S68C AdoMetDC was also active, but the specific activity was considerably less than that of wild type (Table I). The correct processing/cleavage of the pAdoMetDC S73C mutant was confirmed by expressing the protein in *E. coli* and sequencing the large subunit after reductive amination as described above.

### Putrescine Effect on the Processing and Activity of AdoMetDC—Putrescine had absolutely no stimulatory effect on the slower rate of processing of the potato S73C or S73T mutants (Fig. 4c). This is not due to the presence of sufficient putrescine in the rabbit reticulocyte lysate used in the TNT reactions to stimulate the processing of pAdoMetDC. The concentration of putrescine was investigated by high performance liquid chromatography and found to be < 1 mM. Furthermore, treatment of the samples with 2 M (NH₄)₂SO₄ at 4 °C to precipitate the AdoMetDC proenzyme protein and remove putrescine did not slow the rate of processing and re-addition of untreated lysate did not stimulate it (Fig. 4c). The addition of Mg²⁺ also did not influence the processing of the pAdoMetDC proenzyme (results not shown). Activity assays with the wild type and S74C mutant pAdoMetDC also indicated that the activity of pAdoMetDC was not affected by putrescine or by Mg²⁺ at concentrations of up to 8 mM (data not shown).

Three acidic residues have been found to be essential for the stimulation of the processing of mammalian AdoMetDC proenzyme by putrescine. Two of these acid residues are the cationic putrescine and the protein changes its configuration to facilitate processing (7, 15, 16). A simple model for this interaction would involve two putrescine molecules, each forming a bridge between two acidic sites, and would suggest that a fourth site also exists. Previous studies have eliminated all of the other conserved glutamic acid residues (15, 16), but comparisons of the amino acid sequences of the putrescine-activated AdoMetDCs revealed two conserved aspartic acid residues that were
possible candidates for a fourth site. These two residues, Asp174 and Asp266, were therefore mutated separately in the hAdoMetDC to Asn, and the effect of putrescine on the processing and activity was studied. As shown in Fig. 5, the processing of the D174N mutant proenzyme was not stimulated by putrescine, whereas the processing of the D266N proenzyme was the same as wild type AdoMetDC. Therefore, Asp174 appears to be the fourth site of interaction of putrescine with the human AdoMetDC. An acidic residue at this site is absent from the pAdoMetDC (Fig. 2) and from all other plant AdoMetDC sequences.

The enzymatic activity of the processed D174N mutant hAdoMetDC was not stimulated by putrescine (Table II). Although the activity was slightly reduced compared with that of the wild type or the D266N mutant, most of this reduction was due to the lack of putrescine stimulation.

**Effect of Carboxyl Truncation of AdoMetDC Proenzyme on Processing**—The hAdoMetDC and the pAdoMetDC differ most significantly at the COOH terminus, where there is little similarity after human residue Phe280 (equivalent to potato residue Phe283) and the potato protein contains a very acidic 17-amino acid extension that is not present in the human. These differences raised the possibilities that (a) part of the carboxyl domain may not be necessary for activity or processing and (b) that the COOH-terminal region of the pAdoMetDC is responsible for the very rapid processing of this proenzyme. To determine the minimum size of the AdoMetDC proenzyme compatible with processing, a series of deletion mutations were made in both enzymes (Fig. 6).

Removal of eight residues from the COOH-terminal of hAdoMetDC proenzyme had no effect on processing in the presence of putrescine and only a slight effect on processing in the absence of putrescine. Removal of one more residue (mutant A326Stop) greatly reduced the rate of processing, and any additional truncations (mutants F325Stop, S324Stop, V321Stop, and C310Stop) reduced processing to undetectable levels (>98% reduction) (Fig. 6, a and b). These results indicate that the minimal size for normal processing of the hAdoMetDC is 326 amino acids, with the protein extending for 46 residues beyond the Phe280 residue. Similarly, truncations of the pAdoMetDC proenzyme that still included Thr329 (which is 46 residues beyond Phe280) still processed at wild type rates, but truncations to 334 or fewer residues produced essentially complete inhibition of processing (Fig. 6c).

Although their removal prevented proenzyme processing, the nature of the COOH-terminal residues in the smallest processable truncated proenzymes (K327Stop, human; R336Stop, potato) seemed to make little difference to the processing rate. Thus, both potato COOH-terminal mutants T335A/R336Stop, T335S/R336Stop, T335K/R336Stop, and T335E/R336Stop (Fig. 6c) and, in the presence of putrescine, human COOH-terminal mutants A326G/K327Stop, A326K/K327Stop, and A326E/K327Stop (results not shown) still processed at the same rate as their respective wild type proenzymes. However, when assayed in the absence of putrescine, the human K327Stop mutant processed somewhat slower than longer proenzymes, and whereas the double mutation of A326G/K327Stop did not slow down the processing rate further, A326K/K327Stop and A326E/K327Stop mutants did process still more slowly than K327Stop.

To determine whether the pAdoMetDC proenzyme processes so much faster than the human because of the highly charged COOH-terminal sequence, the final six residues of the human sequence (QQQQQS) were replaced by a sequence similar to the potato (EEEEKE or QEQEEE). However, these alterations had no effect on the processing rate in the absence or presence of putrescine (results not shown).

**Effect of Carboxyl Truncation of AdoMetDC Proenzyme on Activity**—Deletions up to K327Stop had only slight effects on the specific activity of processed hAdoMetDC assayed in the absence of putrescine, a series of deletion mutations were made in both enzymes (Fig. 6).

| AdoMetDC used | Putrescine | Specific activity |
|---------------|------------|------------------|
| hAdoMetDC     | 1.9        | 100              |
| hAdoMetDC     | 0          | 5.2 ± 1.2        |
| D174N hAdoMetDC | 1.9      | 5.4 ± 1.5        |
| D174N hAdoMetDC | 0          | 5.3 ± 0.7        |
| D266N hAdoMetDC | 1.9       | 82 ± 3           |
| D266N hAdoMetDC | 0          | 4.9 ± 0.3        |
| Q329Stop hAdoMetDC | 1.9  | 102              |
| K327Stop hAdoMetDC | 1.9  | 83               |
| A326E/K327Stop hAdoMetDC | 1.9  | 42               |
| A326K/K327Stop hAdoMetDC | 1.9  | 65               |
| A326E/K327Stop hAdoMetDC | 1.9  | 53               |
| A326Stop hAdoMetDC | 1.9  | 7                |
| pAdoMetDC     | 0          | 100              |
| T335Stop pAdoMetDC | 0        | 78               |
| R336Stop pAdoMetDC | 0       | 40               |
| T335A/R336Stop pAdoMetDC | 0       | 51               |
| T335S/R336Stop pAdoMetDC | 0      | 39               |
| T335K/R336Stop pAdoMetDC | 0      | 43               |
| T335E/R336Stop pAdoMetDC | 0      | 46               |
| T335Stop pAdoMetDC | 0      | 0                |

The experiment was carried out as in Fig. 3 with no putrescine (open symbols) or 1.0 mM putrescine (closed symbols) present during the synthesis and processing reactions as indicated.
FIG. 6. Effects of carboxyl truncation of hAdoMetDC and pAdoMetDC proenzyme on processing. Truncations were made by converting codons corresponding to the residue indicated to stop codons as described under “Experimental Procedures.” Processing was then measured using a 30-min synthesis period and a 1-h processing period at 30 °C for the hAdoMetDC and a 30-min synthesis period for the pAdoMetDC. Panel a shows the gels of the wild-type (W.T.) and truncation mutants of hAdoMetDC assayed in the presence or absence of 1.0 mM putrescine. The numbers below the lanes are the total number of residues in the corresponding AdoMetDC mutants. Panel b shows the percentage of the processed hAdoMetDC proenzymes in the presence (complete bar) or absence (shaded bar) of putrescine. Panel c shows the percentage of processed pAdoMetDC found after a 30-min period at 30 °C. The mutants are indicated by the amino acid residue whose position corresponds to the residue indicated to stop codons on processing. Truncations were made by converting codons corresponding to the residue indicated to stop codons as described under “Experimental Procedures.” Processing was then measured using a 30-min synthesis period and a 1-h processing period at 30 °C for the hAdoMetDC and a 30-min synthesis period for the pAdoMetDC. Panel a shows the gels of the wild-type (W.T.) and truncation mutants of hAdoMetDC assayed in the presence or absence of 1.0 mM putrescine. The numbers below the lanes are the total number of residues in the corresponding AdoMetDC mutants. Panel b shows the percentage of the processed hAdoMetDC proenzymes in the presence (complete bar) or absence (shaded bar) of putrescine. Panel c shows the percentage of processed pAdoMetDC found after a 30-min period at 30 °C. The mutants are indicated by the amino acid residue whose position corresponds to the residue indicated to stop codons on processing. Truncations were made by converting codons corresponding to the residue indicated to stop codons as described under “Experimental Procedures.” Processing was then measured using a 30-min synthesis period and a 1-h processing period at 30 °C for the hAdoMetDC and a 30-min synthesis period for the pAdoMetDC. Panel a shows the gels of the wild-type (W.T.) and truncation mutants of hAdoMetDC assayed in the presence or absence of 1.0 mM putrescine. The numbers below the lanes are the total number of residues in the corresponding AdoMetDC mutants. Panel b shows the percentage of the processed hAdoMetDC proenzymes in the presence (complete bar) or absence (shaded bar) of putrescine. Panel c shows the percentage of processed pAdoMetDC found after a 30-min period at 30 °C. The mutants are indicated by the amino acid residue whose position corresponds to the residue indicated to stop codons on processing.

DISCUSSION

Our results confirm that the residue forming the pyruvate moiety after cleavage of the pAdoMetDC proenzyme is Ser73. This is as expected since it is the equivalent residue to Ser68, which is known to form the pyruvate in the hAdoMetDC proenzyme (8), and the sequence surrounding these residues, YVELSESS, is totally conserved in all of the known eukaryotic AdoMetDC sequences. The equivalent residue has also been identified as the source of pyruvate in AdoMetDCs from Saccharomyces cerevisiae (9) and Catharanthus roseus (27). Cleavage of the proenzyme of either the human or the plant AdoMetDC to form the α and β subunits was not prevented when either threonine or cysteine was substituted for this serine, but the rate of processing was greatly reduced. This finding is similar to that found for two other pyruvoyl enzymes, histidine decarboxylase (4) and phosphatidylserine decarboxylase (37), and suggests that all of these proenzymes undergo a similar type of reaction in generating the two subunits and the pyruvoyl prosthetic group needed for activity.

However, there are several differences between the responses of the AdoMetDC proenzyme mutants and those of these other proenzymes. First, the processing was more rapid when threonine was substituted rather than cysteine, whereas with histidine decarboxylase (4), the reverse was the case, and with phosphatidylserine decarboxylase (37), the rates were equal. Second, the serine-to-threonine change reduced AdoMetDC activity by >99%, whereas both histidine decarboxylase and phosphatidylserine decarboxylase mutants did retain some enzymatic activity with this substitution (1). This indicates that α-ketobutyrate cannot substitute for pyruvate in the AdoMetDC reaction. One possible reason for this might be that the larger prosthetic group is not compatible with substrate binding.

Third, although the replacement of the critical serine with cysteine in the hAdoMetDC greatly decreased the activity, which is similar to the result with the equivalent histidine decarboxylase and phosphatidylserine decarboxylase mutants (4, 37), this substitution caused little or no reduction of the activity of the pAdoMetDC. There are two possible reasons for the loss of activity with the hAdoMetDC. One is that the mutant proenzyme containing cysteine actually undergoes an abortive cleavage reaction that fails to generate pyruvate (1, 3). Such a reaction is known to occur with a mutant histidine decarboxylase (4) and is facilitated by the greater nucleophilicity of the thiol group of cysteine compared with the hydroxyl group of serine. A similar reaction occurs when protein splicing intermediates are produced with cysteine in place of serine at the upstream splice junction (11). These reactions may be stimulated by exogenous reagents such as thiol reactants or hydroxylamine, but the rate of cleavage of the mutant hAdoMetDC was not increased by the addition of excess dithiothreitol (results not shown). It is therefore more likely that any incorrect cleavage is spontaneous and is mediated by residues in the hAdoMetDC sequence. This interpretation would be consistent with the fact that the pAdoMetDC formed from the S73C mutant was fully active, indicating that incorrect cleavage does not occur with this protein. An alternative but less likely explanation is that the presence of the thioicarboxylate group at the carboxyl terminus of the β subunit reduces the activity in the hAdoMetDC.

The most striking difference in the processing of the hAdoMetDC and pAdoMetDC proenzymes is that the formation of the mature pAdoMetDC occurs much more rapidly and is not affected by putrescine. Since the potato proenzyme processes so rapidly, any effect of putrescine would be of minimal physiological significance and might be missed in our in vitro experiments. However, our results with the S73C and S73T mutants show unequivocally that putrescine does not accelerate the reaction.

Previous site-directed mutagenesis studies of the hAdoMetDC indicated that the acceleration of processing mediated by putrescine requires an interaction with three Glu residues located at positions 11, 178, and 256 (15, 16). The identification of Asp174 as an additional residue essential for acceleration of processing by putrescine provides support for the suggestion that the binding of two putrescine molecules is necessary for the change in conformation that favors the processing reaction (7, 38). Since all four acidic residues must be present for putrescine activation of the human enzyme, the absence in the potato enzyme of an acidic residue equivalent to human Asp174 may explain the lack of putrescine activation in the potato enzyme, despite the presence of acidic residues equivalent to Glu11, Glu178, and Glu256. It is noteworthy that all of the known putrescine-activated mammalian, parasite, and yeast AdoMetDC sequences contain an acidic residue in a position equivalent to human Asp174, whereas none of the
known plant AdoMetDC sequences do and none of the plant enzymes appear to be activated by putrescine. The mechanism of activation of the hAdoMetDC is postulated to involve a conformational change in response to the binding of putrescine to these acidic residues (16, 39). It therefore appears that other residues present in the pAdoMetDC proenzyme ensure the formation of a fully active configuration without the need for the binding of putrescine.

Although the totally conserved residue nearest to the carboxyl terminus of the hAdoMetDC proenzyme is Phe280, only a very limited truncation of eight of the COOH-terminal residues was compatible with processing. A somewhat greater deletion of 25 residues from the longer potato protein was possible. The COOH-terminal regions of the resulting active maximally truncated proteins are the same length, with 46 residues after this section of the protein forms an essential part of the structure, placing the key residues in position to facilitate the processing.

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