Mechanistic Studies of the Processing of Human S-Adenosylmethionine Decarboxylase Proenzyme

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Human S-adenosylmethionine decarboxylase is synthesized as a proenzyme that undergoes an autocatalytic cleavage reaction generating the α and β subunits and forming the pyruvate prosthetic group, which is derived from an internal Ser residue (Ser-68). The mechanism of this processing reaction was studied using site-directed mutagenesis of conserved residues (His-243 and Ser-229) located close to the cleavage site. Mutant S229A failed to process, and mutant S229C cleaved very slowly, whereas mutant S229T processed normally, suggesting that the hydroxyl group of residue 229 is required for the processing reaction where Ser-229 may act as a proton acceptor. Mutant His-243A cleaved very slowly, forming a small amount of the correctly processed pyruvyl enzyme but a much larger proportion of the α subunit with an amino-terminal Ser. The cleavage to form the latter was greatly enhanced by hydroxylamine. This result suggests that the N-O acyl shift needed for ester formation occurs normally in this mutant but that the next step, which is a β-elimination reaction leading to the two subunits, does not occur. His-243 may therefore act as the basic residue that extracts the hydroxyl of the α-carbon of Ser-68 in the ester in order to facilitate this reaction. The availability of the recombinant His243A S-adenosylmethionine decarboxylase proenzyme provides a useful model system to examine the processing reaction in vitro and test the design of specific inactivators aimed at blocking the production of the pyruvyl prosthetic group.

S-Adenosylmethionine decarboxylase (AdoMetDC)† is an essential enzyme for the biosynthesis of polyamines (1–4) and is one of a small class of enzymes that use a covalently bound pyruvate as a prosthetic group (5, 6). Four of these enzymes, histidine decarboxylase (HisDC), phosphatidylserine decarboxylase, aspartate-1-decarboxylase (AspDC), and AdoMetDC are decarboxylases forming important biological amines. All of these enzymes are known to have the pyruvyl prosthetic group attached via an amide linkage to the amino terminus of the α subunit. Two other enzymes that contain a similar bound pyruvate prosthetic group are n-proline reductase and glycine reductase. Although these enzymes were originally reported to contain the pyruvate in an ester linkage, recent studies have also demonstrated its presence bound to the amino terminus of one of the subunits by an amide bond (7, 8).

All of the known pyruvoyl enzymes are synthesized in a proenzyme form. In the case of the four decarboxylases described above, it is clear that the source of the pyruvate is an internal Ser residue and that an autocatalytic cleavage reaction occurs to generate the pyruvate and the α and β subunits. Site-directed mutagenesis of this Ser residue in the proenzyme has shown that changing it to Ala completely prevents the processing and the formation of active enzyme (9–11). Replacement with Cys allows processing and pyruvate formation to occur although at a slower rate (11–13). Although it has been studied in less detail, it appears that the normal precursor of the pyruvate in the n-proline and glycine reductase enzymes is actually a Cys residue (7, 8).

As first proposed by Snell and van Poelje for HisDC (14), the first step in the processing reaction (Fig. 1a) is likely to be a rearrangement in which the hydroxyl side chain of the Ser residue attacks the carbonyl carbon of the preceding amino acid, forming an ester bond in the protein. The formation of such an ester is also the first step in protein splicing reactions, in which either Ser or Cys can take part (15–17). Studies of the mechanism of formation of pyruvoyl prosthetic groups are therefore important not only in understanding the biochemistry of enzymes and in the design of inhibitors of these key regulatory enzymes but also in understanding the mechanism of protein splicing that is essential for the generation of certain proteins.

Despite the proposed similarity in the reaction forming pyruvate in the four decarboxylases described above, there is no obvious similarity in the sequence of amino acids surrounding the Ser that forms the pyruvoyl precursor, and there is little similarity between the subunit structure or the three-dimensional structure of the three enzymes for which the crystal structure is known. HisDC from Lactobacillus 30a is an (αβ)3 hexamer in which the αβ unit is a four-layer sandwich of a pair of three-stranded antiparallel β-sheets stacked face to face and flanked by α helices. Each pyruvate group is located in a loop connecting to separate β sheets (18, 19). Aspartate 1-decarboxylase from Escherichia coli has an (αβ)4 structure in which each αβ unit comprises a six-stranded β-barrel capped by small helices at each end. The pyruvates are each placed in a loop connecting two nonadjacent strands within the same β-sheet (20). Human AdoMetDC is an (αβ)4 tetramer with each αβ unit having a novel four-layer αβ sandwich fold made up of two antiparallel eight-stranded sheets flanked by several α and 310 helices. The pyruvate is located far away from the dimer inter-
Processing of a Pyruvoyl Enzyme

Fig. 1. Postulated mechanism of the processing of AdoMetDC proenzyme to form the α and β subunits and the pyruvoyl prosthetic group. Panel a shows the normal processing of the proenzyme that is thought to occur through the formation of an ester intermediate involving Ser-68 (step I). This intermediate then undergoes a β elimination reaction (step II) to form the β subunit and the α subunit containing a dehydroalanine residue at its amino terminus. This dehydroalanine is converted to pyruvate with the loss of ammonia (step III), completing the formation of the pyruvoyl prosthetic group on the α chain. Only the part of the proenzyme containing residues 67–68 (Glu) and 68 (Ser) is shown. Panel b shows the hydrolytic cleavage (step IV) described in the text in which the ester intermediate formed in step I is cleaved to yield the β subunit and the α subunit, with a Ser at the carboxyl end. Panel c shows the amino acid sequence surrounding the cleavage site.

A

B

C

Sequence of AdoMetDC proenzyme and cleavage products

-AYVLE$$\beta$$MFVSKR$$\alpha$$- $$\Rightarrow$$ -AYVLE$$\beta$$ subunit + Py$$\beta$$MFVSKR - (α subunit not sequencable directly but converted to ASMFVSKR by reductive amination)

Cleavage via pathway I and IV

-AYVLE$$\beta$$MFVSKR$$\alpha$$-$$\Rightarrow$$ -AYVLE$$(\beta$ subunit) + SSMFVSKR - (α subunit)

face within a loop connecting two adjacent strands within the same β sheet (21). The crystal structure of the AdoMetDC from E. coli is not yet known, but there is remarkably little similarity at the amino acid level between this protein, which has an (αβ)6 structure (22, 23), and all of the eukaryotic AdoMetDCs, which are similar to human AdoMetDC (6, 13).

Human AdoMetDC proenzyme consists of 334 amino acids and has a molecular mass of 38.4 kDa (24). The cleavage site of human AdoMetDC is between Glu-67 and Ser-68, with Ser-68 being converted to pyruvate during the proenzyme processing (Fig. 1c) (9). Processing of the proenzyme generates two non-identical subunits, termed α (having 266 amino acids plus the pyruvate derived from residues 68–334 of the proenzyme and a molecular mass 30.7 kDa) and β (having 67 amino acids derived from residues 1–67 of the proenzyme and a molecular mass of 7.7 kDa), which are both indispensable components of the mature enzyme (4, 25).

According to the model for pyruvate generation for HisDC (5, 6, 14), the cleavage of the AdoMetDC proenzyme may occur in three steps as shown in Fig. 1a. In the first step, an N-O acyl shift leads to the formation of an ester bond between Glu-67 and Ser-68. In the second step, a β-elimination reaction occurs, forming the β subunit and the α subunit, which contains a dehydroalanine residue at its amino terminus. The α subunit then undergoes a reaction converting the dehydroalanine to pyruvate with the release of ammonia. There is good experimental evidence supporting this mechanism for HisDC (reviewed by Hackert and Pegg (6)). Although no intermediates have been isolated for AdoMetDC, site-directed mutagenesis experiments showing that replacement of Ser-68 with either Cys or Thr still allow cleavage to generate an α subunit with an amino-terminal α-keto acid suggest that it also applies to AdoMetDC (13). Recently, the crystal structure of aspartate 1-decarboxylase has provided further support for this model since, in this case, one of the four active sites was identified not as the finished pyruvoyl enzyme but as the ester intermediate (20).

The ester formation in step I requires a nucleophilic attack by the primary hydroxyl of Ser-68 at the Glu-67–Ser-68 bond. A proton acceptor is likely to be needed to extract the hydroxyl proton of the cleavage-site Ser to facilitate this reaction. In aspartate 1-decarboxylase, a triad of residues Tyr-58, Lys-9, and His-11 has been postulated to be involved in relaying the hydroxyl proton (20). The second step in the processing reaction requires a base to facilitate the breakdown of the ester intermediate. It has been suggested that either the phenolate of Tyr-58 or the hydroxylate of Thr-57 may be needed for this reaction in the aspartate 1-decarboxylase proenzyme, and the presence of the ester linkage in one of the four sites is due to the increased distance of these residues from the ester bond when the other three sites have been processed completely (20).

In the current experiments we have examined the processing of AdoMetDC proenzyme using mutants produced by site-directed mutagenesis of residues that are located close to the active-site pyruvate of the protein in the crystal structure (21). Residues essential for processing were isolated, and one of these mutants allowed the isolation of an uncleaved ester intermediate, providing experimental support for the processing mechanism shown in Fig. 1.

EXPERIMENTAL PROCEDURES

Materials—The pQE plasmids were obtained from QIAgen (Chatsworth, CA), and the Talon™ metal affinity resin was purchased from CLONTECH (Palo Alto, CA). The Chameleon™ site-directed mutagenesis kit and E. coli competent cells were purchased from Stratagene (La Jolla, CA). The T7-coupled Transcription and Translation kit (TNT) and RNasin™ were purchased from Promega. The restriction enzymes used were from Life Technologies, Inc., Promega, and New England Biolabs (Beverly, MA). Polyvinylidene fluoride membranes were purchased from Millipore (Bedford, MA). AdoMet was purchased from Aldrich. [14C]AdoMet was from Amersham Pharmacia Biotech. All other chemicals were from Fisher. The [35S]AdoMet was prepared enzymat-
A single degenerate oligonucleotide was designed to generate H243F and H243Y. W in the primer represents an equal mixture of A and T.

### Table I

| Mutant | Primer |
|--------|--------|
| S229A  | 5′-CATTCCTCATGCATACCGACAGG-3′ |
| S229T  | 5′-CCATTCATGATACCCACAGG-3′ |
| S229C  | 5′-CCATTCATGATACCCACAGG-3′ |
| H243A  | 5′-CTGAGGTAGGCAATGTCACGATG-3′ |
| H243E  | 5′-CTGAGGTACATGTCACGATG-3′ |
| H243FYY | 5′-CTGAGGTAGGCAATGTCACGATG-3′ |

* A single degenerate oligonucleotide was designed to generate H243F and H243Y. W in the primer represents an equal mixture of A and T.

### Processing of a Pyruvoyl Enzyme

**AdoMetDC Synthesis and Processing in Vitro**—Wild type and mutant AdoMetDC proteins were synthesized in vitro using the T7-coupled TNT reactions from the control or mutant pCM9 plasmids as described (13) with minor modifications. For processing experiments, [35S]methionine was added to the reaction mix. Processing of the labeled proenzyme was measured by further incubation followed by gas-phase peptide sequencing. AdoMetDC Activity Assay—The activity of AdoMetDC was determined from the amount of [14C]CO2 released from [14COOH]AdoMet (24). Each activity assay contained 50 μM sodium phosphate buffer, pH 6.8, 1.25 μM dithiothreitol, 40 μM AdoMet, 9.6 μM [14COOH]AdoMet (52 μCi/μmol), and 1 mM putrescine. Purified AdoMetDC proteins or AdoMetDC synthesized in the TNT system was added to the reaction mixture in a total volume of 250 μl. The reactions were incubated at 37 °C for 15 min and terminated by addition of 0.3 ml of 5 mM H2SO4.

### FIG. 2. Structure of the active-site region surrounding the pyruvyl group of AdoMetDC

The representation of the region of the AdoMetDC protein containing residues Phe-7, Glu-11, Glu-67, the pyruvoyl group of human AdoMetDC, and shown in the enol form, Cys-82, Ser-229, and His-243 of the AdoMetDC crystal structure was generated using Insight II software package (Molecular Simulations Inc., San Diego, CA) using coordinates provided by (21).

### RESULTS

Amino acid residues His-243 and Ser-229 are located in close proximity to the pyruvyl prosthetic group of human AdoMetDC (Fig. 2). To test if the side chains of these residues were involved in the processing reaction that converts the proenzyme into mature AdoMetDC and/or the activity of the enzyme, these residues were mutated to Ala. The AdoMetDC proenzyme was then synthesized labeled with [35S]methionine in a coupled TNT system, and the cleavage of the 38-KDa proenzyme was measured by further incubation followed by analysis by SDS-PAGE. As shown in Fig. 3, the rates of cleavage of the two mutants, H243A and S229A, were very slow. More of the wild type AdoMetDC was in the cleaved form within 1 h. On the other hand, only about 15% of S229A mutant was in the cleaved form after a 6-h incubation at 37 °C (Fig. 3). The cleavage of H243A was even slower, only about 5% at 6 h, the subunits from purified H243A were resolved by SDS-PAGE, electrophoretically transferred onto polyvinylidene fluoride membranes, and visualized by staining with staining with 0.1% Ponceau S, and the a subunit was excised and subjected to gas-phase peptide sequencing.
were carried out in the presence of 1 mM putrescine. At the time points
the total plasmid content remained constant. Both processing reactions
were established in the TNT reactions as described under “Experimental Procedures.” In
the co-translation experiments with both H243A and S229A, half the
normal amounts of both plasmids were added to the reactions, so that
the total plasmid content remained constant. Both processing reactions
were carried out in the presence of 1 mM putrescine. At the time points
indicated, aliquots of 5 μl of the reaction mixture were taken and
separated by SDS-PAGE (panel a). Panel b shows quantification of these
results using a PhosphorImager SI. The results are expressed as the
percentage of AdoMetDC proenzyme cleaved in the time shown. Results
are shown for wild type AdoMetDC (triangles), mutant S229A (squares),
mutant H243A (circles), and for mutants S229A and H243A translated
together (inverted triangles).

The processing of the proenzyme proteins for pyruvoyl en-
zymes is thought to occur in several steps, the first of which is
the rearrangement of the protein such that an ester is formed
between the Ser precursor of the pyruvate and the carboxyl
group of the preceding amino acid (Fig. 1, panel a). This ester
intermediate should be labile to cleavage by hydroxylamine.
Therefore, to test if the mutants were able to carry out the first
step in the conversion of the proenzyme, the labeled products
synthesized in the TNT reactions resulted in a cleavage rate
that was very similar to the average rate of the two mutations
alone, both in the presence and absence of putrescine (Fig. 3).
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subunit was sequenced using Edman chemistry performed on a gas-phase sequencer. Previous studies (9, 31) have shown that the pyruvate present at the amino terminus of correctly processed wild type AdoMetDC blocks this sequencing and that the pyruvate present at the amino terminus of correctly processed enzyme by approximately two-thirds. The enzymatic activity of the H243A mutant AdoMetDC was greatly decreased. Although small, this activity could be measured reasonably accurately by using large amounts of the purified protein for assay. The specific activity of the H243A mutant was reduced to 1.6% that of wild type (Table II). Much of this decrease is likely to be due to the reduction in the amount of the correctly processed α subunit. Although the absolute amount of the α subunit present was 32% that expected, as described above, only approximately 4% of the AdoMetDC appears to contain pyruvate at the amino terminus of the α subunit.

The presence of pyruvate was analyzed by covalently labeling the protein with \(^{35}S\)dcAdoMet and sodium cyanoborohydride. This procedure specifically labels AdoMetDC by reducing the Schiff base formed between the substrate and the pyruvoyl prosthetic group (26). Wild type AdoMetDC incorporated 187 ± 23 cpm/μg of protein, but the H243A mutant incorporated only 7.5 ± 1 cpm/μg of protein (in control experiments, essentially no radioactivity (<10 cpm/μg) was incorporated into the S68A mutant of AdoMetDC, which lacks the pyruvate group). These results suggest that, at most, only 4% of the mutant protoenzyme α subunit is correctly processed. Since 35% of the protein was cleaved (Fig. 6), it appears that the H243A protoenzyme is cleaved by hydrolysis much more readily than it is correctly processed to form pyruvate and that only about 11% of the cleaved protein actually contains a pyruvate.

The enzymatic activity of the H243A mutant AdoMetDC was measured as described in the legend to Fig. 4 but in the presence of various concentration of hydroxylamine. The cleavage time course for mutant H243A AdoMetDC was measured as described in the legend to Fig. 4, and 50-μg aliquots were incubated in 20 mM Tris, pH 7.6, 50 mM NaCl, 1 mM putrescine, 2.5 mM dithiothreitol, and 1 mM EDTA at 37 °C in the presence or absence of 0.5 mM hydroxylamine in a total volume of 225 μl. At the times indicated, 45-μl aliquots were taken and subject to SDS-PAGE. The Coomassie Blue-stained SDS-PAGE gel of the cleavage is shown in panel a. The percentage of the cleavage was quantified using a PhosphorImager SI as described under “Experimental Procedures” and plotted in panel b.

Subunit present was 32% that expected, as described above, only approximately 4% of the AdoMetDC appears to contain pyruvate at the amino terminus of the α subunit. These results suggest that His-243 does not play a critical role in catalysis but may play some part since its mutation to Ala appears to reduce activity in the small fraction of correctly processed enzyme by approximately two-thirds.

Several other mutations at the His-243 or Ser-229 sites were also examined for their effect on the rate of cleavage of the 38-kDa proteoglycan using the coupled TNT system in vitro. Changing His-243 to Phe or Tyr completely prevented the formation of the 30.7-kDa α subunit within the 6-h incubation period, but the proteoglycan from H243F or H243Y was cleaved.
FIG. 7. Effect of mutations at His-243 on cleavage of AdoMetDC proenzyme. Proenzymes from each of the four His-243 mutants tested were synthesized in TNT reactions. Panel a shows results for processing reactions carried out as described in Fig. 3. Panels b and c show the effect of hydroxylamine on the cleavage. Labeled proenzyme was synthesized in the TNT reaction carried out for 30 min. After the addition of cycloheximide, the reactions were incubated at 30 °C for 2 h before passing through a small G-50 column pre-equilibrated with buffer Y (see “Experimental Procedures”). The samples were then incubated at 37 °C for various time periods in the presence (open symbols) or absence (filled symbols) of 0.5 mM hydroxylamine. Aliquots were taken at the time points indicated, and the cleavage was measured as described in the legend to Fig. 5. Results are shown for wild type (WT) AdoMetDC (circles) and mutants S229T (triangles), S229C (inverted triangles), and S229A (squares).

FIG. 8. Effect of mutations at Ser-229 on cleavage of AdoMetDC proenzyme. The mutant and wild type AdoMetDC proenzymes were synthesized in the TNT reactions, and the cleavage was studied as described in the legend to Fig. 3. Results are shown for wild type (WT) AdoMetDC (circles) and mutants S229T (triangles), S229C (inverted triangles), and S229A (squares).

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by hydroxylamine (Fig. 7). When His-243 was changed to Glu (mutant H243E), the cleavage was considerably faster than with mutant H243A but was not accelerated by hydroxylamine (Fig. 7). About 70% of the H243E proenzyme was cleaved within the incubation period used, but there was no detectable AdoMetDC activity (Table II). The limit of detection in the in vitro system is about 1%. Less than 10% of the processed H243E α subunit contained pyruvate.

The conservative replacement of Ser-229 with Thr had little effect on the cleavage of the proenzyme (Fig. 8) but greatly affected the activity, which was reduced to only 4.4% that of wild type (Table II). Thus, the side chain of Ser-229 appears to be involved both in the formation of the pyruvoyl prosthetic group and in the enzymatic activity of the mature AdoMetDC. Replacement of the Ser by Thr can substitute effectively in the processing reaction but not for the enzymatic activity. The replacement of Ser-229 with Cys had a more drastic effect on processing than the S229T change but was less inhibitory to processing than S229A. About 70% of the S229C mutant was cleaved by 6 h (Table II). No enzymatic activity was detectable for mutant S229C even when processing was allowed to take place for this 6-h period. The loss of activity of wild type AdoMetDC over this 6-h time period was negligible under these conditions (results not shown), so this result is not an artifact due to the long period needed for cleavage of the S229C mutant AdoMetDC proenzyme. Both of the mutant S229T and S229C AdoMetDCs did contain pyruvate at the amino terminus of the processed α chain, so it appears that Ser-229 is critical not only for processing but also for catalysis.

DISCUSSION

There are now four amino acids in the AdoMetDC proenzyme that have been firmly implicated in the processing reaction to form the two subunits and the pyruvate prosthetic group. The reason for the complete abolition of this process in the S68A mutant (9) is obvious since only a residue with an OH or SH side chain that can take part in the N-O acyl shift (step I, Fig. 1a) is compatible with the reaction (13). The other previously known mutation abolishing processing completely is E11K (27). As shown in Fig. 2, this Glu residue is located very close to the Glu-67–Ser-68 bond that is cleaved and to the new residues that appear to play essential roles in the processing identified in the present study, Ser-229 and His-243. All three of these residues are fully conserved in all known eukaryotic AdoMetDCs, where they are present in three highly conserved motifs (FEGXER(L/R)LE, FCGYGXXN, and TIH(I/V)TPE).

The role played by Glu-11 is unclear. Mutation to Gln has no apparent effect on basal rates of processing but abolishes the stimulation by putrescine (29), and mutation to Asp also has little effect but causes putrescine to become inhibitory to the reaction (32). Both of these mutations of Glu-11 reduce AdoMetDC enzymatic activity by more than 99% (29, 32). Thus, the carboxyl group of the side chain of this residue is clearly very important for the interaction of the protein with putrescine and also for the catalytic activity. However, the result with the E11Q mutant shows that this carboxyl group is not essential for processing. Although the more drastic change to lysine did block processing, in the absence of a crystal structure for the E11K mutant, we cannot rule out the possibility that the introduction of a basic Lys in place of Glu-11 at the active-site
cleft causes a major distortion of the AdoMetDC proenzyme structure.

Although the structure of AdoMetDC has only been determined for the processed enzyme (21), the close proximity of Glu-67 to the pyruvate residue derived from Ser-68 and Ser-69, suggests that this protein resembles other pyruvoyl enzymes (18, 20) in that it does not undergo a major change in conformation after processing and cleavage to form the two subunits. Therefore, it is likely that residues forming the active-site pocket for catalysis are also involved or permissive for the processing reaction. Mutations of several residues that are involved in the catalytic activity of AdoMetDC such as Glu-11, Cys-82, Lys-80, Tyr-112, Asp-174, Glu-178, and Glu-256 do reduce the processing rate (27–29), but these alterations are much less effective in preventing processing than the mutants of Ser-229 and His-243 identified in the present study. It therefore appears that the latter two residues are critically involved in the processing reaction.

The ability of hydroxylamine to cleave the proenzyme isolated from the H243A mutant is consistent with this mutant readily undergoing the N-O acyl shift generating the intermediate ester form of the proenzyme (Fig. 1a, step I). Although hydroxylamine can also cleave Asn-Gly bonds in proteins (33), the conditions for this cleavage are more harsh than those used in our experiments, and the only such bond in AdoMetDC proenzyme occurs at residues 144–145. Such cleavage would generate fragments entirely different from those seen. The size of the cleaved products and the result of the amino-terminal amino-sequence reactions show clearly that the H243A proenzyme is cleaved by hydroxylamine between residues 67 and 68. It is therefore very likely that the bond in the H243A mutant proenzyme between Glu-67 and Ser-68 is an ester bond. The S229A mutant AdoMetDC proenzyme is not cleaved by hydroxylamine, showing that the Glu-67–Ser-68 peptide bond is not unusually sensitive to this reagent. These results indicate that the His-243 side chain is needed for the β-elimination reaction that occurs to form the α subunit with an amino-terminal dehydroalanyl residue (Fig. 1a, step II). This step requires a basic residue that extracts the hydrogen of the α-carbon of Ser-68 in the ester and His-243 could fulfill this role. Alteration of His-243 to Ala, Phe, or Tyr prevents step II from occurring. Interestingly, alteration to Glu gives rise to a mutant AdoMetDC proenzyme that cleaved more slowly but did not accumulate the ester (Fig. 7). Ester hydrolysis may be facilitated by the proximity of the Glu acid residue.

Results with the recombinant H243A AdoMetDC proenzyme slow that cleavage of this protein occurs predominantly via the hydrolysis of the ester (Fig. 1b, step IV), since it generates much larger quantities of the α subunit with an amino-terminal Ser than with an amino-terminal pyruvyl. This indicates that the hydrolytic cleavage is much more rapid than the β-elimination step, but even the former occurs more rapidly than would be expected for ester hydrolysis at 30 °C and neutral pH; probably, the influence of other residues close to the active site affects this rate. The fact that very little of the hydroxyl group may act as the proton acceptor to enhance the nucleophilicity of Ser-68 needed for this step (Fig. 1). Ser-229 is also clearly involved in the catalytic activity of the processed AdoMetDC, since the S229T mutant has very little activity although it processes at a normal rate. Residue Cys-82 has been identified as the proton donor needed for catalysis by AdoMetDC (28). In this study, an unidentified residue that is involved in the product release was indicated, and Ser-229 is a good candidate for this.

Our results strongly support the concept that the processing of AdoMetDC proenzyme occurs via a similar pathway to that of the other pyruvoyl proenzymes but indicate that quite different residues are involved in bringing about the processing reaction. In aspartate I-decarboxylase, step I is modulated by Tyr-58, and a Ser residue is not involved. Step II appears to require Tyr-58 and/or Thr-57 rather than a His residue (20). Although the crystal structure of HisDC is known, there are no clear indications as to which residues play an essential role in the processing reaction, and it has been postulated that tension at the cleavage site of the proenzyme might play some role (18). Mutation of the Ser residue in the HisDC proenzyme that forms the pyruvate, changing it to Ala, prevents correct processing but allows a very slow cleavage reaction at the adjacent peptide bond (6, 10). It seems unlikely the local conformation and strain on the cleavage site is so important in AdoMetDC. No indication of any cleavage of the S68A mutant of AdoMetDC proenzyme has been obtained in our experiments (9, 29). Alterations of other residues around the cleavage site of AdoMetDC, namely S66A, E67Q, and S69A do not significantly interfere with either the processing or the activity of the enzyme (9, 29).

Studies of hydroxylamine-mediated cleavage of a mutant form of the HisDC proenzyme from Lactobacillus 30a (which accumulates when the protein is expressed in E. coli and can be isolated and shown to activate within a few hours of incubation in the presence of monovalent cations) show that this protein is also readily cleaved by hydroxylamine and, thus, may contain or generate an ester intermediate (34). A striking difference between this HisDC proenzyme and the proenzyme from the AdoMetDC H243A mutant isolated in our experiments is that in the absence of hydroxylamine, the HisDC proenzyme cleaves correctly via pathway II/III of Fig. 1a to generate an α subunit with pyruvate rather than incorrectly via pathway IV of Fig. 1b to form an α subunit with Ser.

The polyamine biosynthetic pathway is a valid target for the design of therapeutic agents (25, 35–38). Agents interfering with polyamine synthesis have been shown to have promise both as antineoplastic and cancer chemopreventive agents and as potent antiprotozoal parasitic targets. The processing step for the AdoMetDC proenzyme is an attractive site for the design of such agents. The availability of the intermediate ester form of the AdoMetDC proenzyme from the H243A mutant, which can be purified in large quantities from the E. coli cultures expressing the recombinant protein, provides a useful model system in which the ester cleavage steps of this process can be studied.

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