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Arginine Decarboxylase of Oats Is Clipped from a Precursor into Two Polypeptides Found in the Soluble Enzyme[1]

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ABSTRACT

We have examined soluble oat (*Avena sativa*) arginine decarboxylase by probing its structure with polyclonal antibodies that separately recognize amino-terminal and carboxyl-terminal antigens and with a monoclonal antibody that immunoprecipitates enzyme activity. These experiments indicated that oat arginine decarboxylase is clipped from a 66,000-D precursor polypeptide into 42,000- and 24,000-D produce polypeptides. Both of these are found in the enzyme and may be held together by disulfide bonds. A full-length precursor protein could not be detected in plants but could be produced by expression of the cDNA in *Escherichia coli*. Analysis of the expression of the cDNA in *E. coli*, with antibodies and using pulse labeling with [^{35}S]methionine, indicated that the bulk of the expressed protein was the full-length 66,000-D form. Small amounts of 42,000- and 24,000-D polypeptides could also be detected. A reconstruction experiment, adding a radioactively labeled full-length protein isolated from *E. coli* to powdered oat leaves, supported the idea that the protein extraction method used for western blots was not likely to result in artifactual proteolytic degradation.

Putrescine synthesis in higher plants can proceed via two alternative pathways from ornithine via ornithine decarboxylase and from arginine via arginine decarboxylase (EC 4.1.1.19) through the intermediate agmatine. The arginine decarboxylase pathway is also present in some bacteria, including *Escherichia coli* (15), but is largely absent from other eukaryotic kingdoms. Animals and fungi rely predominately on ornithine decarboxylase as the initial, highly regulated, step in polyamine synthesis. In previous research, we (10, 11) described regulation of arginine decarboxylase at the enzyme activity level in tobacco suspension cultures, finding that there were differences in end product repression and in low pH induction between arginine decarboxylase and ornithine decarboxylase. Results from other investigators (reviewed in refs. 7 and 8) have also indicated that arginine decarboxylase, not ornithine decarboxylase, may be specifically regulated by environmental stresses and as part of fruit ripening and senescence.

Recently, we (1) isolated and characterized a cDNA clone for the oat leaf arginine decarboxylase. The cloning strategy involved purifying the arginine decarboxylase polypeptide, N-terminal amino acid sequencing, construction of a degenerate oligonucleotide probe, and screening of an oat leaf cDNA library. Extensive similarity exists between the oat and *E. coli* arginine decarboxylase amino acid sequences. We also generated an antibody (anti-C in Table I) to a carboxyl-end portion of the arginine decarboxylase polypeptide.

Analysis of the cDNA and oat leaf proteins indicated that arginine decarboxylase of oats might be proteolytically processed (1) based on the following considerations: The arginine decarboxylase cDNA contains an open reading frame that encodes a 66,000-D polypeptide; however, experiments probing oat leaf extracts with the anti-C antibody revealed 24,000- and 34,000-D polypeptides on western blots. The 24,000-D form was the same size as the polypeptide we (2) characterized via its ability to bind the enzyme-activated irreversible inhibitor DFMA[3]. The amino terminus of the 24,000-D DFMA-binding polypeptide we purified was subsequently found internally in the predicted arginine decarboxylase open reading frame, approximately two-thirds of the way from the amino to the carboxyl end. The results of both the purification of DFMA-binding activity and the probing of plant extracts with anti-C thus indicated that a 24,000-D polypeptide, derived from the carboxyl one-third of the full-length arginine decarboxylase polypeptide, could be found in plants.

These initial results concerning the structure of the oat arginine decarboxylase enzyme left many points unresolved. We did not know the fate of the amino two-thirds of the 66,000-D sequence, because the anti-C antibody did not provide a probe for it. The amino two-thirds of this sequence could conceivably be degraded or left intact; it could remain associated with arginine decarboxylase enzyme activity, or it could have some independent function. The research described in this report was directed toward learning more about the proteolytic cleavage of oat leaf arginine decarboxylase, to discovering the fate of the amino two-thirds of the enzyme, and to determining which of the polypeptide fragments were found in the soluble enzyme.

MATERIALS AND METHODS

Preparation of Oat Extracts and Enzyme Assays

Lyophilized leaf total protein extracts were prepared as described previously (1, 5). Leaves from 2-week-old oat (*Av-*

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[3] Abbreviations: DFMA, difluoromethylarginine; IPTG, isopropylthiogalactoside; Ig, immunoglobulin.

ena sativa cv Garry) plants were cut and then immediately frozen in liquid nitrogen. To prevent any chance of a thaw, the leaves were ground to a powder under liquid nitrogen, transferred to a lyophilizer while bathed in liquid nitrogen, and lyophilized for 2 d. For a western blot, 50 μg of lyophilized leaf powder was added to 1 mL of SDS sample buffer that had been preheated to 100°C. The sample was then boiled for 5 min and clarified by a 10-min spin in a microcentrifuge. Typically, 10 μL was loaded per lane in a miniprotein gel apparatus (Bio-Rad).

Soluble extracts were prepared by grinding oat leaves with mortar and pestle at 2 mL of homogenization buffer plus 0.5 g of sand per gram of leaves. All solutions contained the protease inhibitors iodoacetamide at 1.85 mg/mL and PMSF at 0.174 mg/mL. The homogenization buffer contained 100 mM Hepes (pH 7.5), 10 mM EDTA, 5 mM DTT, 0.05 mM pyridoxal-phosphate, plus protease inhibitors. A 20 to 70% ammonium sulfate-precipitated fraction was collected, resuspended, and then dialyzed against 25 mM Hepes (pH 7.5), 1 mM EDTA, 5 mM DTT, 0.05 mM pyridoxal-phosphate, plus protease inhibitors. This fraction was then clarified by centrifugation in a microcentrifuge for 10 min. Arginine decarboxylase assays were determined by the method of $^{14}CO_2$ release, as reported previously for plant extracts (11). Protein concentrations were determined by the method of Bradford (3).

Generation of Polyclonal Antibodies from Fusion Proteins

Portions of the arginine decarboxylase cDNA were used in *E. coli* expression vectors to generate antigens for polyclonal antibodies; the restriction sites used are diagrammed in Figure 4. The antigen for the anti-C antibody (Table I) was generated as described previously, by subcloning a 600-bp 3′ *Sal*I/*Eco*RI fragment of pADC2 into the *trp*E protein fusion expression vector pATH23, inducing and preparing protein as described before (13), and then immunizing egg-laying hens; the IgY fraction was collected from preimmune and immunized eggs.

The antigen for the anti-N antibody (Table I) was generated in several steps by transferring a 720-bp 5′ *Bal*I fragment into the same pATH23 expression vector. This *Bal*I fragment begins in the middle of the second amino acid of the reading frame and, hence, encodes a near amino-terminal peptide. After the *Bal*I fragment was isolated from pADC2, 8-bp *Eco*RI linkers were added and then cleaved with *Eco*RI; this fragment was then ligated into the *Eco*RI site of the vector pBluescript II SK− (Stratagene). Sequence analysis confirmed the correctness of the construct and reading frame. The (now) *Eco*RI fragment was then excised and transferred into the *Eco*RI site of pATH23; restriction analysis of this plasmid identified a construct in the proper orientation. Fusion protein generation and harvest, chicken immunization, and IgY purification were performed as described previously (1, 13, 16).

Generation of Monoclonal Antibody As-8B5

The protein purification strategy used previously (1) relied upon SDS gels as an important purification step. To generate an antibody that recognized nondenatured protein, we purified arginine decarboxylase using nondenaturing preparative PAGE and then sampled the enzyme activity in each gel slice. Mice were immunized with the partially pure extracts, and hybridomas were generated by standard methods (6, 9). Media from individual hybridomas were screened by an immunoprecipitation assay to test for monoclonal antibodies that would immunoprecipitate arginine decarboxylase activity; one line, As-8B5, was positive. These cells were cloned several times and retested, and then the spent media was used as a source of antibody to be purified. Purification was performed on goat anti-mouse IgG agarose (Sigma) affinity columns, producing a purified antibody at a concentration of 0.5 μg/mL.

Western Blots and Immunoprecipitations

Western blots were performed essentially as described previously and by others (6, 9). After the blots were transferred to nitrocellulose, they were blocked in 4% BSA plus 1% fish skin gelatin overnight at 4°C. Primary antibody was applied at concentrations from 5 to 10 μg/mL, with gentle shaking for 2 h at room temperature. Secondary antibody, goat anti-chicken IgY alkaline phosphatase conjugate (Sigma), was applied at a 1000-fold dilution. The detecting color reaction was the standard bromochloroindolylphosphate + nitrotetrazolium blue method (9). Standard controls for the chicken polyclonal antibodies included preimmune IgY (from the same chicken), and anti-*trpE* (because the immunizing protein was a fusion of *trpE* to oat arginine decarboxylase).

Immunoprecipitations with murine monoclonal As-8B5 were performed using minor modifications of standard techniques (9). Killed *Staphylococcus aureas* cells (Pansorbin, Calbiochem) were washed three times in 100 mM Hepes (pH 7.5) with 2% BSA (Hepes-BSA), then incubated on ice for at least 1 h in Hepes-BSA, and followed by a final resuspension in a volume equal to the original volume removed from the bottle. In a typical precipitation experiment, varying amounts of As-8B5 were diluted to 50 μL with Hepes-BSA, then mixed with 25 μL of soluble oat extract, and incubated on ice for 1 h. To this was added 1 μL of rabbit anti-mouse Ig (Sigma) diluted 10-fold in Hepes-BSA. The mixture was incubated for 1 h on ice, and 5 μL of washed pansorbin was added. Then, the mixture was again incubated for 1 h on ice. The mixture was pelleted and washed three times with Hepes-BSA, followed by a final washing in Hepes alone. The final pellet was resuspended in Hepes for performing enzyme assays; alternatively, the pellet was resuspended in sample buffer for SDS gels, boiled for 5 min, clarified, and then loaded onto SDS polyacrylamide gels for analysis.

Expression of Intact Reading Frame in *E. coli*

The three bases of the pADC2 sequence that immediately preceded the start codon ATG were changed to CAT using standard oligonucleotide-mediated in vitro mutagenesis procedures (14), with sequence analysis to confirm the nucleotide replacement. The resulting sequence CATATG is an *Nde*I site that was used in combination with the *Eco*RI site at the 3′ end of the cDNA to transfer the oat arginine decarboxylase reading frame to the *E. coli* expression vector pT7-7 (20) at a

position suitable for both efficient transcription from the T7 promoter and efficient translation with a suitable mRNA leader sequence and ribosome-binding site. This plasmid, named pANOK, was transformed into the host BL21 (DE3) (19), which contains an inducible *lac* operon-derived promoter fragment driving transcription of the T7 RNA polymerase gene. As a control, the pT7-7 vector without insert was also transformed into BL21 (DE3).

[^{35}S]Methionine-labeled protein was prepared essentially as described by Tabor (20), except that growth was always at 30°C and specific protein synthesis was induced by the addition of IPTG to 1 mM rather than by heat shock. We used rifampicin as recommended to reduce background protein synthesis, because the T7 promoter driving the expressed cDNA is insensitive to rifampicin. Pulse and pulse-chase experiments showed that a large percentage of the label in the pANOK-containing cells was being incorporated into the desired protein product and that this was stable, with a $t_{1/2}$ of more than 1 h.

For the preparative experiment, 200 μCi of [^{35}S]methionine (ICN) were added to 1 mL of bacteria, after IPTG and rifampicin treatments, for a 10-min pulse. The bacteria were then washed and boiled in SDS gel sample buffer, and the extract was clarified. The labeled protein was run on a 7.5% SDS polyacrylamide gel, with thioglycolic acid added to the gel reservoir buffer (12), and with prestained molecular weight markers flanking the sample (Sigma). Immediately after the gel was run, the sample region between the 58,000- and 84,000-D markers was cut into narrow gel slices, which were electroeluted in a standard apparatus (Bio-Rad). Further gel analysis of the elution fractions allowed us to determine which one contained the band corresponding to the oat arginine decarboxylase-encoded protein.

Table I. *Plasmid, Antibody, and Strain List*

Name	Description or Origin
pADC2	Oat arginine decarboxylase cDNA clone in the vector pTZ19R (U.S. Biochemicals) (1).
As-8B5	Murine monoclonal antibody that immunoprecipitates oat arginine decarboxylase enzyme activity but does not work well on western blots.
anti-C	Chicken polyclonal antibody raised against the carboxyl 22% of oat arginine decarboxylase expressed as a fusion protein in *E. coli* in the plasmid pATH23-ADCCSE. The sequences used were entirely carboxyl to the described clipping site (1). The pATH vectors are described by Koerner et al. (13).
anti-N	Chicken polyclonal antibody raised against the amino 39% of oat arginine decarboxylase expressed as a fusion protein in *E. coli* in the plasmid pATH23-ADCNB. The sequences used were entirely amino to the described clipping site.
pANOK	Plasmid containing the full-length, intact, reading frame of oat arginine decarboxylase inserted into the expression vector pT7-7 (20) and grown in the *E. coli* strain BL21(DE3) (19).

RESULTS

The Amino-Terminal Two-Thirds of the Arginine Decarboxylase Sequence Can Be Detected in Oat Leaves

The antibody anti-C (Table I) provides a probe for the carboxyl 22% of the arginine decarboxylase sequence, including the C terminus. To provide a probe for the amino portion of the arginine decarboxylase sequence, we generated a polyclonal antibody by immunizing chickens with a portion of the amino-terminal sequence. A 720-bp *Bal*I cDNA fragment that begins within the second amino acid of the sequence was transferred to the expression vector pATH23 (13), resulting in a *trpE*-arginine decarboxylase fusion protein that was purified and used as an antigen. The antibody that resulted, anti-N (Table I), could recognize epitopes in the amino 39% of the arginine decarboxylase sequence, excluding the first two amino acids.

In all of our chicken antibody preparations, we noticed a significant reaction in the preimmune IgY from the chickens we immunized; the preimmune IgY reacted to a variety of polypeptides on western blots of plant and bacterial proteins. All of the western blot analysis, therefore, compares preimmune with postimmunization reactivity.

Total protein from oat leaves was prepared for western blots using a lyophilized leaf protocol that was designed to yield accurate molecular mass estimates free from protease activity (5). Anti-N reacted to a polypeptide of 42,000-D (Fig. 1, first two lanes). We also tested anti-N against oat leaf preparations that were identical except that the β-mercaptoethanol reductant was excluded from the sample buffer. Anti-N reacted to a polypeptide of 60,000 D (Fig. 1, right two lanes). In both cases, there was no evidence of a second band of greater or lesser molecular mass.

As reported previously (1), anti-C yields a 24,000-D polypeptide and also a 34,000-D polypeptide in the same type of western blot in the presence of β-mercaptoethanol in the sample buffer. In sample buffers without β-mercaptoethanol, anti-C yielded no reaction at all (data not shown). This suggests that the anti-C antibody recognizes epitopes that require reduction for proper presentation to the antibody. This may not be surprising, because the antigen was purified from normal SDS protein gels, after treatment with sample buffer containing β-mercaptoethanol.

Soluble Arginine Decarboxylase Enzyme Contains Both Parts of the Molecule

The reaction of anti-N with a 42,000-D band on lyophilized leaf extracts demonstrates that the amino portion of the full-length polypeptide can be detected in plants. It does not provide evidence that the amino 42,000 D is associated with arginine decarboxylase enzyme activity. Conceivably, the amino 42,000-D portion could have another function after processing, with the carboxyl 24,000-D polypeptide being the active form of oat arginine decarboxylase.

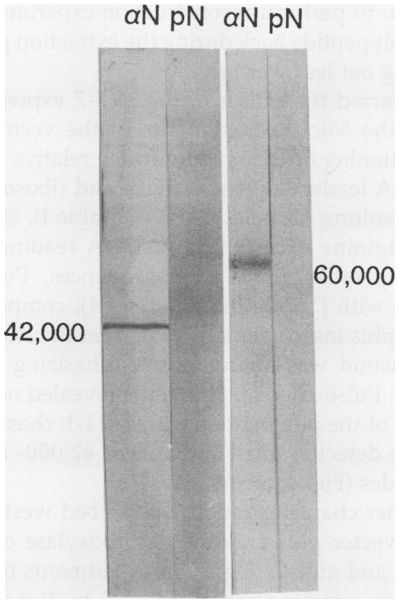

Figure 1. Oat leaf extracts probed with anti-N. Lyophilized oat leaf powder was boiled in SDS sample buffer either with (left two lanes) or without (right two lanes) β-mercaptoethanol. The proteins were resolved on a polyacrylamide gel, then transferred to nitrocellulose, and analyzed with anti-N (αN) or with preimmune IgY from the same chicken (pN).

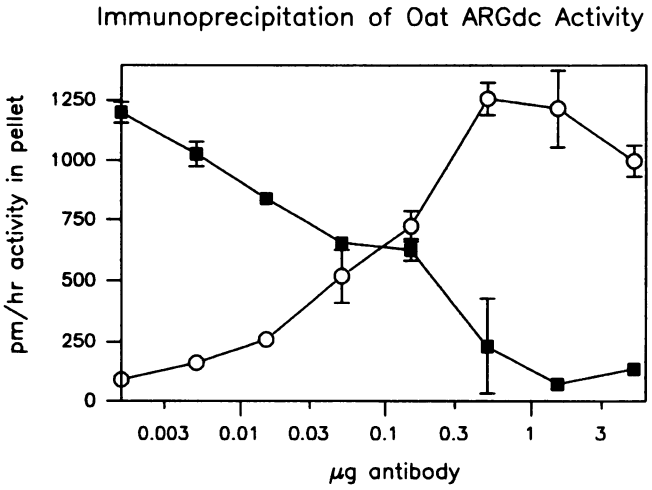

Figure 2. Immunoprecipitation of arginine decarboxylase by monoclonal antibody As-8B5. Enzyme activity was measured both in the resuspended Pansorbin pellets (O) and in the original treated supernatants (■). Data shown are the average ± SE of four independent experiments.

To help test these possibilities, we partially purified enzymatically active oat arginine decarboxylase. This was used as an antigen in mice to generate hybridomas, resulting in one monoclonal antibody, As-8B5 (Table I), that immunoprecipitates arginine decarboxylase enzyme activity from soluble oat extracts. A dose-response curve for the immunoprecipitation is shown in Figure 2. The antibody detected no band on western blots of oat extracts; apparently, it does not react to the form of arginine decarboxylase that is displayed on nitrocellulose after gel electrophoresis. We do not know the location on the arginine decarboxylase molecule of the epitope to which As-8B5 reacts.

The immunoprecipitation property of As-8B5 allows us to test whether both portions of the molecule, detected by anti-N and anti-C, respectively, are present in the enzyme found in the antibody/*S. aureas* pellets. To perform this test, we prepared a soluble oat leaf extract and concentrated the protein with a 20 to 70% saturated ammonium sulfate fractionation. The protease inhibitors PMSF and iodoacetamide were present throughout the procedure. The soluble oat leaf extract was then precipitated with As-8B5, the presence of arginine decarboxylase activity was verified, and the polypeptides in the sample were resolved on an SDS polyacrylamide gel. Western blots of the immunoprecipitated arginine decarboxylase yielded a 42,000-D polypeptide when probed with anti-N and a 24,000-D polypeptide when probed with anti-C (Fig. 3). We saw no evidence of a 34,000-D polypeptide in the immunoprecipitated enzyme, or in other soluble extracts, using anti-C as a probe.

We do not view the soluble extract plus protease cocktail preparation as being as convincing as the lyophilized leaf

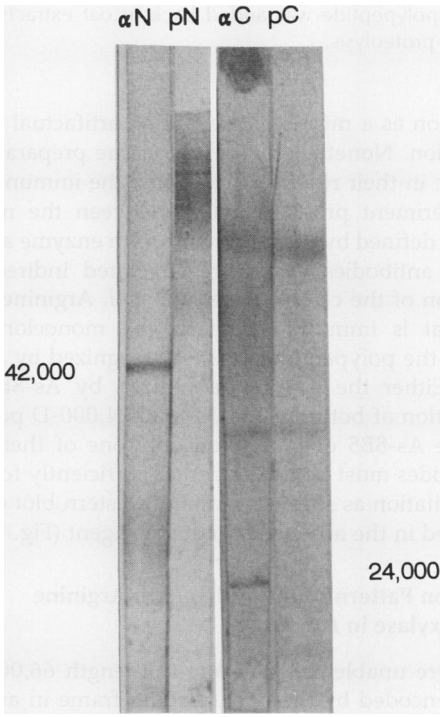

Figure 3. Immunoprecipitated arginine decarboxylase probed with anti-N and anti-C. Arginine decarboxylase activity was immunoprecipitated with As-8B5 and then analyzed via western blots probed with anti-N (αN), preimmune IgY for N (pN), anti-C (αC), and preimmune IgY for C (pC).

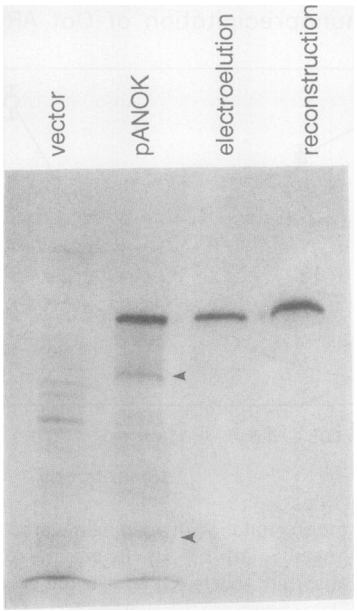

Figure 4. Radiolabeled oat arginine decarboxylase protein from *E. coli* used in a reconstruction test for proteolysis. The vector pT7-7 and its oat arginine decarboxylase derivative, pANOK, in the host strain BL21(DE3) were labeled by an [^{35}S]methionine pulse. Proteins were harvested, used in experiments, resolved by polyacrylamide gels, and then visualized by fluorography. vector, Radiolabeled proteins from pT7-7. pANOK, Radiolabeled proteins from pANOK. The arrowheads indicate faint 42,000- and 24,000-D bands. electroelution, Sample of electroeluted polypeptide isolated from gels resolving pANOK/BL21(DE3) proteins. reconstruction, The electroeluted polypeptide was added back to oat extracts to test for artifactual proteolysis.

preparation as a means of preventing artifactual proteolytic degradation. Nonetheless, the two tissue preparation methods agree in their results. In addition, the immunoprecipitation experiment provides a link between the monoclonal antibody defined by its relationship with enzyme activity and the two antibodies that were generated indirectly, using expression of the cDNA clone in *E. coli*. Arginine decarboxylase that is immunoprecipitated by monoclonal As-8B5 contains the polypeptide epitopes recognized by anti-N and anti-C. Either the epitope recognized by As-8B5 is on a combination of both the 42,000- and 24,000-D polypeptides or, if the As-8B5 epitope is on only one of them, the two polypeptides must be held together sufficiently for coimmunoprecipitation as suggested by the western blot experiment performed in the absence of reducing agent (Fig. 1).

Expression Pattern of the Full-Length Arginine Decarboxylase in *E. coli*

We were unable to detect the full-length 66,000-D polypeptide encoded by the open reading frame in any oat leaf preparation. To have a source of the full-length form, we decided to express the cDNA in an *E. coli* vector that would result in the full reading frame being transcribed and translated, without fusion to any extra amino acids. This would allow us to examine the expression pattern obtained in *E. coli*, and also to perform reconstruction experiments, adding 66,000-D polypeptide back during the extraction process used for preparing oat leaf western blots.

We transferred the cDNA to the pT7-7 expression vector (20), using the *Nde*I restriction site of the vector to permit precise positioning of the reading frame relative to optimum *E. coli* mRNA leader sequence length and ribosome-binding sites. The resulting plasmid, pANOK (Table I), expresses the entire oat arginine decarboxylase cDNA reading frame not fused to any other amino acid sequences. Pulse-labeling experiments with [^{35}S]methionine (Fig. 4), comparing vector with vector plus insert, demonstrated that the strain with the pANOK plasmid was abundantly synthesizing a 66,000-D polypeptide. Pulse-chase experiments revealed no detectable degradation of the polypeptide within a 1-h chase. The pulse labeling also detected small amounts of 42,000- and 24,000-D polypeptides (Fig. 4, arrowheads).

As a further characterization, we probed western blots of vector and vector plus arginine decarboxylase cDNA insert with anti-N and anti-C (Fig. 5) in experiments in which the *E. coli* protein extracts were prepared 2 h after induction of the T7 RNA polymerase with IPTG. Both antibodies predominately detected the expected 66,000-D polypeptide. Anti-N showed some reaction in the vector control lane at a position slightly above the 66,000-D band; this suggests that anti-N has some cross-reactivity with the *E. coli* arginine decarboxylase, which is slightly larger than the plant protein (15). Anti-N also detected several fainter bands of smaller size, including one at 42,000 D (Fig. 5, arrowhead). Anti-C showed no cross-reactivity with the vector alone extract. We were unable to determine whether anti-C detected any 24,000-D polypeptide, owing to the presence of a cross-reacting material at about 25,000 D that is present in all lanes, vector, and vector plus insert, whether probed with anti-N or anti-C.

The pulse labeling of the 66,000-D oat arginine decarboxylase polypeptide in *E. coli* (Fig. 4) allowed us to perform a reconstruction test of the oat leaf extraction method we were using for western blots, in which lyophilized leaf powder is added directly to sample buffer that is already at 100°C (as used in Fig. 1). A sample of the [^{35}S]methionine-labeled 66,000-D polypeptide was isolated from *E. coli* and then

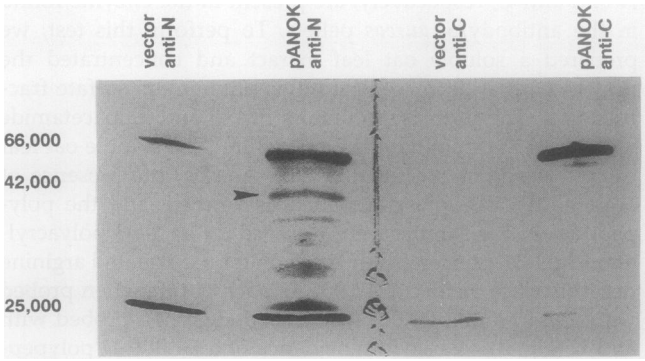

Figure 5. Western blot of *E. coli* extract containing vector or vector plus oat arginine decarboxylase cDNA. Left lanes, Probed with anti-N; right lanes, probed with anti-C. The arrowhead indicates a 42,000-D band.

prepared by electroelution from an SDS polyacrylamide gel (Fig. 4, lane "electroelution"). We mixed the electroeluted, ^{35}S-labeled 66,000-D polypeptide with lyophilized oat leaf powder and then added this to already heated SDS sample buffer, as we did for preparing western blots. The reconstruction sample was then analyzed by SDS-PAGE and autoradiography (Fig. 4, lane "reconstruction"). We detected no degradation of the labeled polypeptide.

This experiment supports the lyophilized leaf, preheated buffer protocol of preparing protein for western blots as a method that minimizes artifactual proteolytic degradation. If any degradation were occurring, we would have expected some lower molecular mass polypeptides to appear in the reconstruction lane. The absence of proteolysis was expected because the method was originally designed to give an accurate molecular mass estimate for phytochrome (5), a protein extremely sensitive to proteolysis. This result supports the measured molecular masses, 42,000 D detected by anti-N and 24,000 D detected by anti-C, as being good estimates of the sizes in vivo and, hence, also agrees with our suggestion that arginine decarboxylase is clipped into two pieces in vivo.

DISCUSSION

The data we have presented are summarized in a model shown in Figure 6. We propose that the full-length 66,000-D arginine decarboxylase polypeptide is synthesized in oats but is then cleaved to produce a 42,000-D polypeptide containing the original amino terminus and a 24,000-D polypeptide containing the original carboxyl terminus. The mature soluble enzyme contains both of these processed polypeptides held together by disulfide bonds, as suggested by the apparent increase in molecular mass of the polypeptide detected by anti-N when reducing agent was omitted and by the coimmunoprecipitation by As-8B5. The clipping of the 66,000-D polypeptide might be either a self-catalyzed reaction or the result of a maturase activity. The original amino acid sequencing of the 24,000-D DFMA-binding polypeptide begins with the threonine at amino acid position 424 (1). This suggests that the site of the clipping is either at or quite close to this amino acid. In the deduced sequence, threonine-424 is immediately preceded by another threonine, as indicated in Figure 6. Our original arginine decarboxylase protein purification (1) provided evidence that DFMA binds to the carboxyl 24,000-D fragment and, hence, that at least part of the active site of the enzyme must reside on this fragment.

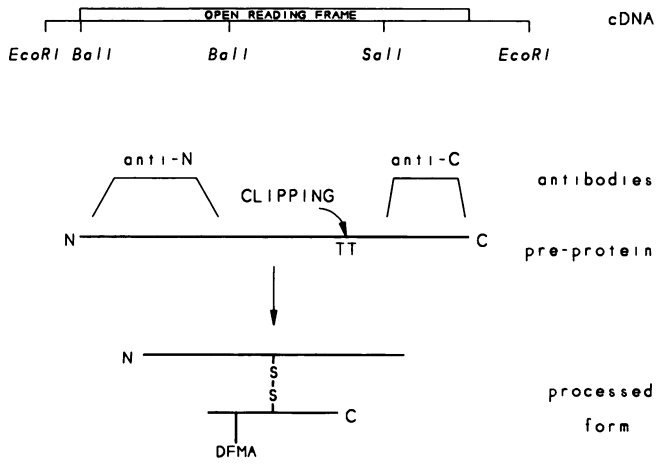

Figure 6. Model of oat arginine decarboxylase clipping and structure. This shows the proposed structure of oat arginine decarboxylase, the clipping site deduced from the original amino acid sequencing, and the relationships between the cDNA clone and the restriction fragments used to generate the antigens for anti-N and anti-C via expression in *E. coli*.

The appearance of faint 42,000- and 24,000-D bands in the *E. coli* expressing the full-length cDNA (Figs. 4 and 5) suggests either that the clipping reaction is a self-proteolysis or that *E. coli* contains a protease that recognizes a site quite close to the authentic clipping site. The host strain BL21(DE3) contains the *lon* mutation, which eliminates a major protease but certainly not all of the *E. coli* proteases. An in vitro reaction system that faithfully converts the 66,000-D polypeptide into the two smaller pieces will probably be needed to resolve the mechanism of clipping and to determine whether or not clipping is an activation step.

Our model provides no explanation for the 34,000-D band previously detected by anti-C in the lyophilized leaf total protein extracts (1) but not detectable in the soluble extracts tested in this report. We can speculate that possibly there are alternative forms of arginine decarboxylase that are solubilized by SDS but are not solubilized in the aqueous preparation used for the immunoprecipitation experiments.

Smith (18) previously purified oat arginine decarboxylase enzyme activity 3500×. Gel chromatography provided evidence for two molecular mass forms of the native enzyme, of 195,000 and 118,000 D. These two size estimates might be consistent with homotrimers and homodimers made up of monomers that have the form we postulate in Figure 4, i.e. containing both 42,000- and 24,000-D fragments. Smith did not report subunit sizes after denaturing SDS-PAGE. Ramakrishna and Adiga (17) studied arginine decarboxylase, in extracts containing activity that had been purified 977-fold, from *L. sativus*, providing evidence that it has a molecular mass of 220,000 D and is a hexamer; this configuration seems to be a completely different form than the one we are studying in oats.

Biosynthetic arginine decarboxylase of *E. coli* (the *speA* gene) is a tetramer of 280,000 D. The monomers are synthesized first as 74,000-D precursor proteins that are then post-translationally processed to a 70,000-D mature form localized in the inner periplasmic space (4). The gene for this enzyme has been cloned and sequenced (15) and, as we noted previously (1), contains multiple regions of amino acid sequence similarity to the oat enzyme.

We would like to know whether the clipping of the oat arginine decarboxylase we detect is associated with enzyme activation and whether it is a self-catalyzed or enzyme-mediated event. We hope to determine this in the near future.

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