Aminolevulinic Acid Dehydratase in Pea (Pisum sativum L.)
IDENTIFICATION OF AN UNUSUAL METAL-BINDING DOMAIN IN THE PLANT ENZYME*

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Aminolevulinic acid dehydratase (ALA dehydratase) catalyzes the second step of tetrapyrrole synthesis leading to the formation of heme and chlorophyll in higher plant cells. Antibodies elicited against spinach leaf ALA dehydratase were used to immunoscreen Xgt11 cDNA libraries constructed from etiolated pea (Pisum sativum L.) leaf poly(A)* RNAs. A set of overlapping cDNAs was characterized that encode the pea enzyme. The predicted amino acid sequence of the pea ALA dehydratase is similar to those reported for other eukaryotic and prokaryotic ALA dehydratases. The pea enzyme has an active site domain centered on lysine that is highly conserved in comparison to other known ALA dehydratases. Consistent with the previously reported requirement of Mg** for catalytic activity by plant ALA dehydratases, the pea enzyme lacks the characteristic Zn**-binding domain present in other eukaryotic ALA dehydratases, but contains a distinctive metal ligand-binding domain based upon aspartate. Northern blot analyses demonstrated that ALA dehydratase mRNA is present in leaves, stems, and to a lesser extent in roots. Steady state levels of mRNA encoding ALA dehydratase exhibit little or no change during light-induced greening.

Tetrapyrroles and their derivatives, notably the hemes, sirohemes, and chlorophylls, participate in many of the fundamental biosynthetic and catabolic processes of living organisms. Heme is present in all cells, as part of the membrane-bound proteins of mitochondrial- and chloroplast-localized electron transport chains or as cofactors in soluble redox enzymes (1, 2). Heme derivatives, like siroheme, serve as the prosthetic groups in nitrite and sulfite reductases and are used to form linear bilins such as the phytochrome chromophore. In contrast, the chlorophylls and their derivatives are only found in photosynthetic cells where they are involved in the trapping of light energy and its transduction into chemical energy (1, 3).

Studies beginning in the early 1950s resulted in the characterization of the biochemical events leading to the synthesis of tetrapyrrole compounds, and today there are few steps for which the reaction mechanism and biosynthetic intermediates are unknown. Aminolevulinic acid (ALA)** dehydratase (EC 4.2.1.24) (also known as porphobilinogen synthase) catalyzes the second step of tetrapyrrole formation condensing two molecules of ALA to form porphobilinogen, the first pyrrole compound in the pathway (4). This enzyme, first isolated from bovine liver by Gibson et al. (5), has been subsequently isolated from a variety of prokaryotic and eukaryotic cells (7–10).

The basic catalytic properties of all ALA dehydratases are similar, although differences in enzyme structure, metal ion requirements for catalysis, and sulfhydryl sensitivity have been observed between the enzymes purified from different organisms (11–12). ALA dehydratases range in size between 250–340 kDa. Those from animal cells and yeast consist of eight identical subunits of about 37 kDa each (8, 10, 13–14). In contrast, ALA dehydratases isolated from higher plants and bacteria are hexameric proteins consisting of six identical subunits of 50 and 40 kDa, respectively (4, 7, 9, 15–19).

ALA dehydratases from animal cells have a pH optimum between pH 6.3–7.1 and have been shown to require Zn** for maximal catalytic activity (8, 10, 20). The yeast enzyme also requires Zn** for activity but has a more alkaline pH optimum, pH 9.8, than its animal counterparts (21). Like yeast, the plant and bacterial ALA dehydratases have alkaline pH optima (pH 8.0 for the plant enzyme and pH 8.0–8.5 for the bacterial enzyme) (17, 22) but differ from other eukaryotic forms of enzyme in their metal ion requirement. The plant ALA dehydratase utilizes Mg** or Mn** instead of Zn**, whereas the enzyme from bacterial sources shows either no metal requirement or, as in the case of Rhodobacter sphaeroides, requires K** ions for activation (11).

Metal chelators, such as EDTA, or divalent heavy metal ions inhibit ALA dehydratase activity (10–11, 20). Additionally, the oxidation state of the enzyme’s sulfhydryl groups has been shown to be important in catalysis, since in the presence of O2 there is a rapid loss of activity (23–24). Maximal activity can be restored to the enzyme by treatment with exogenous thiol reagents. The importance of the reactive sulfhydryl groups appears to be in the binding of the metal ion by the enzyme (25). Subsequent studies have demonstrated, however, that Zn** is not essential for full enzymatic activity, but only when the enzyme is maintained under strict anaerobic conditions (24). These studies suggest that Zn** ions likely function to maintain the oxygen-sensitive thiol groups in a reduced state thus stabilizing enzyme activity and possibly quaternary structure (8, 17, 23, 26).

The isolation of cDNAs and/or genomic DNA fragments encoding ALA dehydratases from eukaryotic and prokaryotic cells (27–32) has provided considerable insight into the structure of the protein. Among the more significant observations

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1 The abbreviations used are: ALA, aminolevulinic acid, SDS, sodium dodecyl sulfate; kb, kilobase pairs; bp, base pairs.
is the identification of a putative Zn²⁺-binding domain similar to the consensus sequence of other Zn²⁺ metalloproteins (27). This domain contains four conserved cysteine residues, 2 of which have been proposed to be responsible for the O₂ sensitivity of the enzyme, as well as a conserved histidine residue that is thought to be involved in binding of the metal ion (24).

Although considerable information is available on the catalytic properties of plant ALA dehydratases, little is known about the primary structure of the enzyme and the genes that encode it. We report here the successful cloning of cDNAs encoding a pea ALA dehydratase. We show that while the plant enzyme is similar to its animal, fungal, and bacterial counterparts and contains a highly conserved active site domain, it lacks a well-defined Zn²⁺-binding domain. The availability of the cloned cDNA sequences for this enzyme has also permitted an analysis of the developmental regulation of ALA dehydratase gene expression and its response to light.

**Experimental Procedures**

**Plant Growth and Materials**—Pea seeds (Pisum sativum L. var. Progress No. 9 purchased from Burpee Seed Co., Inc.) were imbibed overnight in distilled water at room temperature and planted in moist vermiculite. Plants were grown under standard greenhouse conditions (25–28 °C). Leaves, stems, and roots were collected, frozen in liquid N₂, and either immediately or stored at −80 °C for later use. Leaves of etiolated peas, grown for 10–14 days in complete darkness at 28 °C, were harvested under dim green safelight (33). A portion of the etiolated plants were moved to a separate growth chamber and greened under continuous white light (approximately 850 microwatts/cm²) supplied by a bank of cool-white fluorescent bulbs for the time intervals specified in the text.

**Extraction of Total RNA and Preparation of Poly(A)⁺ RNA**—Total RNA was prepared from etiolated and greening pea leaf tissues and from light-grown leaves, stems, and roots as described by Cashmore (36). Poly(A)⁺ RNA was prepared from total RNA by chromatography on oligo(dT)-cellulose (37). The quantity of the total and poly(A)⁺ RNA recovered was determined spectrophotometrically (38).

**Construction and Screening of cDNA Libraries**—Double-stranded cDNA was synthesized from poly(A)⁺ mRNA isolated from etiolated pea leaves using the procedure described by Gubler and Hoffman (39) with minor modifications (40). Two independently prepared libraries were used in these studies. Library I was constructed using oligo(dT) as primer for first strand synthesis, whereas Library II was prepared using random hexanucleotides (Boehringer Mannheim) as primer. The cDNAs were cloned into the EcoRI site in Xgtll (41) and packaged in vitro according to the manufacturer's instructions (Stratagene, Inc.).

Approximately 5 × 10⁶ independent recombinant phage from Library I were immunoscreened (35) using polyclonal rabbit antiserum directed against spinach ALA dehydratase. A single, reproducibly immunoreactive recombinant phage (designated λ-pALAD 10) was isolated after two subsequent rounds of immunoscreening. The 1.5-kb EcoRI insert present in λ-pALAD 10 was purified following electrophoresis on a 1% low melting agarose gel (Bio-Rad) and ligated into the EcoRI site of pBluescript (Stratagene, Inc.). This plasmid is designated pALAD10.

Additional phage containing sequence encoding the ALA dehydratase were isolated by screening approximately 5 × 10⁷ phage from unsampled Library I using radiolabeled pALAD 10 insert as probe. Phage were grown in Y10-90 cells (41), and replica filters were prepared according to the manufacturer's instructions (MSI, Inc.). Filters were prehybridized in 50 mM Na-phosphate buffer, pH 7.2, 10 mM Na₂-EDTA, 7.0% (w/v) SDS, 1.0% (w/v) bovine serum albumin (42) and hybridized in the same buffer containing 1-2 × 10⁶ cpm/ml of a [α-³²P]dCTP-random primed labeled probe. The filters were washed twice for 30 min in 2.0 × SSC (1 × SSC = 0.15 M NaCl, 0.15 M Na₃citrate, pH 6.8), 0.1% SDS at 23 °C. Filters were blotted dry, wrapped in saran wrap and exposed to x-ray film with a Cronex intensifying screen at −80 °C for 24–36 h.

Phage DNA was plaque purified using the same protocol. Phage were grown in Escherichia coli strain Y10-90, and phage DNA was isolated from each independent recombinant (38). The λ-DNA was digested with EcoRI, and the inserts from each phage were subcloned into pBluescript.

**Nucleotide Sequencing and Analyses**—The DNA sequence of both strands of DNA in the individual cDNAs was determined by the dideoxy chain termination method (43) using Sequenase 2.0 (United States Biochemical Corp.) on double-stranded DNA templates. The overall sequencing strategy is shown in Fig. 1. Recombinant plasmids used as sequencing templates were generated by several different methods. Unique restriction fragments were generated from pALAD 10 by digestion with EcoRV and HindIII, purified, and ligated into pBluescript for subsequent sequencing. In addition, overlapping deletions were made across pALAD 10 using Exonuclease III digestion (44). Either the Universal and M13 reverse sequencing primers, or synthetic oligonucleotides were used as primers in the reactions. Synthetic oligonucleotide primers generated from the nucleotide sequences of pALAD 10 were used to sequence the remaining cDNAs.

**Northern Blot Analysis**—Aliquots of total and/or poly(A)⁺ RNA were prepared in 50% formamide, 6% formaldehyde sample buffer at 10 °C for 10 min, fractionated on agarose gels containing 6% formaldehyde (38), and subsequently transferred to nylon membrane (MSI, Inc.) for analysis. The filter was prehybridized in 5 × SSPE (1 × SSPE = 0.15 M NaCl, 0.25 M NaH₂PO₄, pH 7.4, 2.5 M Na₂-EDTA), 50% (v/v) formamide, 5 × Denhardt’s media, 0.5% (w/v) SDS, and 100 μg/ml denatured salmon sperm at 42 °C for 6–12 h. Hybridization was carried out in buffer containing 5 × SSPE, 50% (v/v) formamide, 5 × Denhardt’s media, 0.2% (w/v) SDS, 10% (w/v) dextran sulfate, and 1-2 × 10⁶ cpm/ml of [α-³²P]dCTP-random primed labeled probe generated using the EcoRI insert from pALAD 10. Following incubation for 12-18 h at 42 °C, the filters were washed twice in 5 × SSPE, 0.5% (w/v) SDS at 23 °C for 15 min each, twice in 1 × SSPE, 0.5% (w/v) SDS at 37 °C for 15 min each, and finally once in 0.1 × SSPE, 1.0% (w/v) SDS at 37 °C. The filters were subsequently wrapped in Saran Wrap and exposed to x-ray film using Cronex intensifying screens.

**Results**

**Identification of a pea ALA Dehydratase cDNA Clone in λgt11**—Antiserum elicited against the spinach ALA dehydratase (9) was used to immunoscreen a cDNA expression library constructed in λgt11 from poly(A)⁺ RNA isolated from 10 to 14-day-old etiolated pea leaves. From an initial screening of approximately 5 × 10⁷ independent recombinants, a single immunoreactive phage (λ-pALAD 10) was isolated. λ-pALAD 10 was found to contain a 1346-bp EcoRI insert. The insert was purified and subcloned into pBluescript SK⁺ to yield plasmid designated pALAD 10.

The complete nucleotide sequence of both the antisense and sense strands of the insert in pALAD 10 was determined (see Fig. 1 and "Experimental Procedures"). The presence of a short (10 bp) poly(A) stretch at one end of the sequence made it possible to identify the orientation of the coding strand. Comparisons of the nucleotide sequence and predicted amino acid sequence of the longest open reading frame encoded by pALAD 10 with the GenBank and EMBL sequence data bases identified similarities between the pea sequences and those of other prokaryotic and eukaryotic ALA dehydratases.

Using the insert for pALAD 10 as a hybridization probe, we screened a second, independently constructed λgt11 library for additional hybridizing clones in an attempt to obtain the sequence of the full-length cDNA. Four additional clones, designated λ-pALAD 202, λ-pALAD 203, λ-pALAD 204, and λ-pALAD 209 were isolated. DNA was purified from each phage, digested with EcoRI, and the cloned cDNA inserts ligated into the pBluescript (yielding pALAD 202–209, respectively). DNA restriction and sequence analyses indicated that the cDNAs pALAD 202–209 were either partially identical and contained within pALAD 10 (pALAD 204) or included additional sequences to the 5' end of the original clone (pALAD 202, pALAD 203, and pALAD 209) (Fig. 1).

**Primary Structure of the ALA Dehydratase**—A composite nucleotide sequence of 1468 bp derived from the two longest cDNAs, pALAD 209 and pALAD 10, is shown together with...
FIG. 1. Partial restriction map and sequencing strategy for ALA dehydratase cDNAs. The upper portion of the diagram shows the protein coding (solid box) and 3'-untranslated regions (solid line) of the clones. In the lower portion of the diagram, the sequencing strategy for each clone is designated by arrows. Forward and reverse sequencing primers are designated by arrows attached to boxes ( ); synthetic oligonucleotide primers by arrows attached to circles (O).

FIG. 2. Nucleotide and predicted amino acid sequence of pea ALA dehydratase. The composite nucleotide sequence of pALAD 10-pALAD 209 and the predicted amino acid sequence derived from the largest open reading frame are given. The first in frame amino acid has been designated as position 1. The apparent polyadenylation signal in pALAD 10 is boxed.
its predicted amino acid sequence in Fig. 2. One long open reading frame encoding 398 amino acids is present in the sequence. The predicted primary structure of the pea ALA dehydratase has a high degree of similarity to the ALA dehydratases previously identified from animal, fungal, and bacterial sources (27–32) (Fig. 3). At least two types of evidence suggest that the composite sequence for ALA dehydratase comprised by the pALAD 10-pALAD 209 overlapping clones does not encode the full-length protein. First, the longest open reading frame derived from the nucleotide sequence does not initiate with a methionine codon and the protein encoded is smaller than the 50 kDa predicted for the mature plant ALA dehydratase. Second, hybridization of pALAD 10 to RNA isolated from developing leaf tissues (see Fig. 5) indicates a transcript size of approximately 1.6–1.7 kb for the ALA dehydratase, slightly larger than the composite sequence.

Within the coding portion of the pea protein, several regions highly conserved in relation to other known ALA dehydratases are easily recognized. The most striking similarity exists within the vicinity of the predicted active site region of the pea ALA dehydratase. Over a region of 20 amino acids (residues 311–330), the pea enzyme is identical at 15 out of 20 positions to the human, mouse, and rat proteins, and 12 out of 20 positions to the yeast and E. coli proteins (Fig. 3). This region includes the invariant lysine residue (position 319) proposed to be involved in Schiff base formation within the active site of mammalian ALA dehydratases (45) as well as highly conserved arginine residue (position 329) identified in biochemical modification studies of the spinach (22) and maize (46) enzymes and thought to be important in catalysis.
Wetmer and his colleagues (27) have previously noted a potential for ionic interactions due to charge complementarity in two hydrophilic regions immediately flanking the active site. By comparison, while preserving the general hydrophilicity of these regions, the predicted secondary structure within the pea enzyme shows a more limited region of charge complementarity.

Zn$^{2+}$ ions are known to be essential for catalytic activity in other eukaryotic ALA dehydratases and the presence of a metal (Zn$^{2+}$)-binding domain is another highly conserved feature among ALA dehydratases examined thus far (27–30, 32). ALA dehydratases from plants have been documented to require Mg$^{2+}$ or Mn$^{2+}$ for activity (22). By comparison to published protein sequences for other ALA dehydratases, a metal-binding domain would be contained within the region extending from amino acid residues 186–206. Although there is a strong similarity among the plant, mammalian, and yeast enzymes within this region, a Zn$^{2+}$-binding domain corresponding to the consensus sequence for the so-called zinc fingers (47) in other zinc-binding proteins including ALA dehydratase (27) is not present in the pea enzyme.

Cysteine and histidine residues implicated in chelating Zn$^{2+}$ by the mammalian enzyme (27) have been replaced by threonine (at position 187), by alanine (at position 190), or aspartate (at positions 197 and 200) in the pea enzyme (Fig. 4). An invariant cysteine residue in the mammalian, yeast, and bacterial enzymes has been replaced by aspartate (at position 192) in pea. Within the metal-binding domains of ALA dehydratases, there also appears to be several invariant residues of yet unknown function: aspartate at position 188, tyrosine at position 194, serine at position 196, histidine at position 199, and glycine at positions 198 and 201.

In addition to these previously well-characterized regions within the ALA dehydratase protein, several other regions also exhibit considerable sequence conservation among enzymes isolated from various organisms. These regions lie between residues 232–250, 258–280, and 374–388. The functional significance of these regions, if any, remain to be elucidated.

The greatest amount of divergence among the proteins is observed within the amino-terminal portions of the molecule. This is not unexpected, since there is a considerable difference of molecular weight between the plant enzyme and those from other organisms (e.g. pea (50 kDa) versus human (35 kDa)). Since both domains important to catalytic function are located in the more carboxyl-terminal regions of the molecule, the additional sequences within the pea enzyme are of as yet unknown functional/structural significance. The amino-terminal region is relatively hydrophobic and has characteristics of a membrane-spanning domain. In this regard it is interesting to note that Nasri et al. (48) have suggested that a portion of the ALA dehydratase activity appears to be transiently bound to the thylakoid membrane. Since most of the nuclear encoded, cytoplasmically synthesized proteins destined for incorporation into the chloroplast stroma/thylakoid membrane are synthesized as higher molecular weight precursors with amino-terminal extensions of varying length a portion of this region may comprise part of the transit peptide for chloroplast import. Additional experiments will be required to determine the amino terminus of the pea ALA dehydratase protein and the role of these additional sequences in the pea enzyme.

The pea cDNA (pALAD 10) contains 290 bp of 3′-untranslated sequence prior to the poly(A)$^+$ tail. Based upon its location 19 nucleotides upstream from the poly(A) addition site as well as its similarity to known polyadenylations signals (49), we can identify a putative polyadenylation signal (AATTGAA) in this region.

**Analysis of ALA Dehydratase mRNA Levels during Light-regulated Development**—Amounts of steady state mRNA encoding the pea ALA dehydratase were determined in various tissues and in greening leaves by Northern blot analyses (Fig. 5). In all of the tissues analyzed, the pALAD 10 probe hybridized to a single size message, approximately 1.6–1.7 kb. The levels of ALA dehydratase mRNA were highest in the leaves of light-grown plants, low but detectable in stem tissues, and undetectable in the root tissue. Even under prolonged exposure, only a faint signal could be detected in root tissues.

In order to study the effects of light on steady state levels of ALA dehydratase mRNA, total RNA were extracted from 10- to 14-day-old etiolated pea leaves and leaves illuminated for 6, 12, 24, and 48 h with white light. Little or no change was observed in the steady state levels of the ALA dehydratase mRNA during greening in leaf tissues.

**Fig. 4.** Comparison of the putative metal-binding domain of the pea ALA dehydratase with those observed in other ALA dehydratases. A comparison of the metal-binding domains proposed in other eukaryotic and prokaryotic ALA dehydratases is shown along with the consensus sequence for Zn$^{2+}$-binding proteins (47). Conserved residues referred to in the text are _backshadowed_; the proposed Zn$^{2+}$-binding domain is _boxed._

**Fig. 5.** Northern blot analysis of ALA dehydratase expression in various tissues during light-induced development. Equivalent amounts of total RNA (25 μg/sample) were fractionated on formaldehyde-agarose gels, transferred to nylon membranes, and hybridized to $^{[32P]}$dCTP-labeled insert from pALAD 10. *Lanes 1–5* are RNA extracted from etiolated leaf tissue subject to: *lane 1*, 0 h of illumination; *lane 2*, 6 h of illumination; *lane 3*, 12 h of illumination; *lane 4*, 24 h of illumination; *lane 5*, 48 h of illumination; *lanes 6–8* are RNA prepared from tissues of light-grown plants: *lane 6*, root; *lane 7*, stem; *lane 8*, leaf. The positions of the 18 S and 25 S ribosomal RNA of pea are indicated for size estimates.
Molecular Cloning of Pea ALA Dehydratase

DISCUSSION

We present here the first description of the primary structure of ALA dehydratase from higher plants. We show that overall, plant ALA dehydratase bears a remarkable degree of similarity to those previously described from bacterial, yeast, and mammalian cells. Our analysis of the pea enzyme supports the previous observations (27–32) suggesting that among the most highly conserved structural features of all ALA dehydratases are the presence of two domains, an active site domain containing a catalytically active lysine residue and a metal-binding domain. Our studies show, however, that unlike ALA dehydratases from other eukaryotic cells, the plant ALA dehydratase contains a metal-binding domain of unusual structure.

Previous workers (7, 11, 50) have proposed that the binding of substrate by ALA dehydratase involves the formation of a Schiff base intermediate between the enzyme and one of the two ALA molecules. Enzyme inactivation studies employing various protein modification reagents have implicated a lysine residue within the active site of animal, plant, and bacterial ALA dehydratases as being important in substrate binding (45, 51–52). Subsequent studies of the spinach (22) and maize (46) ALA dehydratases have also suggested that an arginine residue in or near the active site is necessary for catalysis. Consistent with these biochemical analysis, both a lysine (position 319) and an arginine (position 329) residue can be located within the active site domain of the pea enzyme. The likely importance of these residues in catalysis is underscored by the fact that they are invariant in all ALA dehydratases thus far examined, even in organisms as evolutionarily divergent as E. coli, plants, and humans.

In contrast to the high degree of similarity observed within the active site domain of various ALA dehydratases, the pea enzyme differs from other eukaryotic ALA dehydratases in the structure of its putative metal-binding domain, lacking the cysteine and histidine residues characteristic of a Zn"+ binding site. Previous studies have demonstrated a lack of activation of plant enzyme by Zn"+ and a requirement for Mg"+ (22). Plant ALA dehydratase have also been shown to be relatively insensitive to treatment with iodoacetamide (22) suggesting that cysteine residues do not actively participate in catalysis. Marahalli et al. (52) have reported, however, that diethylpyrocarbonate treatment of maize ALA dehydratase resulted in the complete inactivation of this enzyme suggesting that histidine residues are essential for catalysis. In light of the results of these previous studies, our structural observations are not surprising. What is noteworthy, however, is the fact that within the pea enzyme, the overall structure of the putative metal-binding domain has not been greatly altered, but rather there has been a replacement of the cysteine and histidine residues making up the Zn"+ finger by either negatively charged aspartate residues or the neutral residues alanine and threonine.

It is interesting to speculate that as a result of these amino acid replacements within the putative metal-binding domain in the pea ALA dehydratase, there now exists an altered arrangement of negative charges that might be responsible for conferring Mg"+ dependence on the enzyme. There is currently little information available on the exact nature of Mg"+ binding sites. However, the metal-binding domain in pea ALA dehydratase is most reminiscent of the metal-binding domains in Ca"+ and Mg"+ binding proteins (e.g. calmodulin, troponin C, phospholipase D, etc.) containing EF hand consensus sequences (57). Further studies employing both biochemical and molecular approaches are therefore needed to demonstrate the involvement of these residues in Mg"+ binding by the pea enzyme, as well as to identify other critical residues involved in catalysis in the plant enzyme.

Among the features of the metal-binding domain most conserved among various ALA dehydratases are invariant aspartate, tyrosine, and histidine residues (residues 188, 194, and 199, respectively, in the pea enzyme). From their analysis of the role of Zn"+ in catalysis by the bovine ALA dehydratase, Dent et al. (26) have suggested the existence of two possible sites for Zn"+ binding, a site that binds Zn"+ for catalytic activity, and a second site that is not required directly for activity, but stabilizes the native protein and protects it from denaturation. The former site in the bovine enzyme would involve 2/3 histidine residues, 1/2 aspartate and/or tyrosine or a water molecule, and 1 cysteine to coordinate the Zn"+; in the latter, 4 cysteines would provide the points of coordination. The exact function of the conserved aspartate, tyrosine, and histidine residues in either catalysis or enzyme stabilization is unknown and requires further investigation.

Previous studies in a number of plants have shown that the levels of ALA dehydratase protein and enzyme activity depend upon the tissue and stage of plant development (53–56). The molecular cloning of an ALA dehydratase cDNA allowed us to examine the extent to which changes in ALA dehydratase gene expression contribute to the regulation of enzyme protein and activity levels during development. ALA dehydratase activity appears to be present in all tissues tested within the pea plant. Significant differences were observed in the steady state mRNA levels for ALA dehydratase in these various tissues, with mRNA accumulation being highest in photosynthetically active leaves and stems. This is not unexpected since all cell types presumably require a basal level of pyrrolic compounds, whereas there would be a much larger requirement for these compounds (e.g. in chlorophyll formation) in photosynthetically active cells.

In contrast to the dramatic differences in tissue-specific accumulation of ALA dehydratase mRNA, we observed little to no change in the steady state levels of ALA dehydratase mRNA upon greening of etiolated leaf tissues even after 48 h of illumination. In both mustard and radish cotyledons, enzyme protein and activity increase with developmental age reaching a maximum 60–72 h after germination (53–55). Enzyme activity and amount could be enhanced in these tissues by treatment of dark-grown seedlings with far red light (55), suggesting phytochrome involvement in controlling levels of enzyme accumulation. Levels of ALA dehydratase protein and activity have also been reported to increase 2–3-fold in leaves undergoing light-induced development (56). It has been also been suggested that regulation of enzyme activity may involve both de novo synthesis of the enzyme as well as enzyme activation (54).

The lack of significant change in mRNA levels encoding the ALA dehydratase suggests that light regulation of ALA dehydratase activity likely occurs at the post-transcriptional level, either at the levels of translation or enzyme activation. This suggestion would consistent with our recent observation showing that porphobilinogen deaminase, the subsequent step in the pathway, is also constitutively expressed in pea leaves during greening, but shows similar increases in activity (34).

The information resulting from this study should provide a basis for more in depth studies of structure/function relationships in ALA dehydratase proteins in plants and other organisms. They also should allow for future studies to probe the regulation of ALA dehydratase gene expression in plants, the role of transcriptional and post-transcriptional processes in regulating enzyme activity, the signals which determine its localization, and the characteristics which set this enzyme
and the tetrapyrrole biosynthetic pathway apart from the pathway in non-photosynthetic organisms.

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