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Review

Biosynthesis of the Tetrapyrrole Pigment Precursor, δ-Aminolevulinic Acid, from Glutamate[1]

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ABSTRACT

δ-Aminolevulinic acid (ALA), the common biosynthetic precursor of hemes, chlorophylls, and bilins, is synthesized by two distinct routes. Among phototrophic species, purple nonsulfur bacteria form ALA by condensation of glycine with succinyl-CoA, catalyzed by ALA synthase, in a reaction identical to that occurring in the mitochondria of animals, yeast, and fungi. Most or all other phototrophic species form ALA exclusively from the intact carbon skeleton of glutamic acid in a reaction sequence that begins with activation of the α-carboxyl group of glutamate by an ATP-dependent ligation to $tRNA^{Glu}$, catalyzed by glutamyl-tRNA synthetase. Glutamyl-tRNA is the substrate for a pyridine nucleotide-dependent dehydrogenase reaction whose product is glutamate-1-semialdehyde or a similar reduced compound. Glutamate-1-semialdehyde is then transaminated to form ALA. Regulation of ALA formation from glutamate is exerted at the dehydrogenase step through end product feedback inhibition and induction/repression. In some species, end product inhibition of the glutamyl-tRNA synthetase step and developmental regulation of $tRNA^{Glu}$ level may also occur.

The three major classes of tetrapyrrole pigments that function in photosynthesis, hemes, Chls, and bilins, arise from a single, branched biosynthetic pathway. The earliest well-characterized committed tetrapyrrole precursor is ALA.[2] The biosynthetic steps leading from ALA to the various end products are similar or identical in all species that have been examined. In contrast, two distinct mechanisms exist for ALA formation. In the mitochondria of animal cells, yeast, and fungi, and in some bacterial species (including the purple nonsulfur bacteria), ALA is formed from glycine and succinyl-CoA in a condensation reaction catalyzed by ALA synthase. In other bacteria (including cyanobacteria and most phototrophic bacterial groups) and in plants and algae, ALA is formed from the intact carbon skeleton of glutamic acid, in a process requiring three enzymatic reactions and $tRNA^{Glu}$ (Fig. 1). This article will focus on recent progress toward understanding the five-carbon route of ALA biosynthesis. Readers are referred to a recent review article (6) for a summary of earlier work and pre-1989 references.

OCCURRENCE OF THE TWO PATHWAYS FOR ALA BIOSYNTHESIS

Because the ALA synthase route of ALA formation from glycine and succinyl-CoA was the first to be described, and because it was found in bacteria, animals, and nonphototrophic lower eukaryotes, it was considered to be the universal route of ALA formation. Later, it was determined that not only higher plant cells, but also green and red algae, cyanobacteria, and some other bacterial groups form ALA from glutamate via the five-carbon route.

Recently, all of the phototrophic bacterial groups were surveyed for the mode of ALA formation (5). The results indicated that the pathway from glutamate is widely distributed among the bacterial groups and is probably the more primitive and evolutionarily earlier pathway. Species utilizing the five-carbon pathway include members of the green sulfur bacteria (*Chlorobium*), green nonsulfur bacteria (*Chloroflexus*), Gram-positive green bacteria (*Heliospirillum*), purple sulfur bacteria (*Chromatium*), and *Desulfovibrio* (which is not photosynthetic but may be closely related to photosynthetic groups). Of the phototrophic species examined, ALA synthase activity was found only in purple nonsulfur bacteria (*Rhodobacter spheroides, Rhodospirillum rubrum*). The ALA synthase route has also been reported in several strains of bacteria that form Bchl *a* under aerobic growth conditions.

It should be noted that the five-carbon ALA biosynthetic pathway is not restricted to Chl-containing organisms. This pathway operates *in vivo* and in extracts of *Clostridium thermoaceticum, Methanobacterium thermoautotrophicum, Escherichia coli* (2, 14, 17), and *Bacillus subtilis* (17).

With one exception, no species has been convincingly shown to have both ALA biosynthetic routes. The exception is the green photosynthetic phytoflagellate *Euglena gracilis*. Both ALA synthase activity and enzymes of the five-carbon pathway have been detected in extracts of this species. An examination of the physical and kinetic properties of the *Euglena* ALA synthase and the regulation of its activity indicated that in *Euglena*, ALA synthase functions exclusively

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[2] Abbreviations: ALA, δ-aminolevulinic acid; Bchl, bacteriochlorophyll; Chlide, chlorophyllide; gabaculine, 3-amino-2,3-dihydrobenzoic acid; GSA, glutamate-1-semialdehyde; PALP, pyridoxal phosphate, Pchlide, protochlorophyllide.

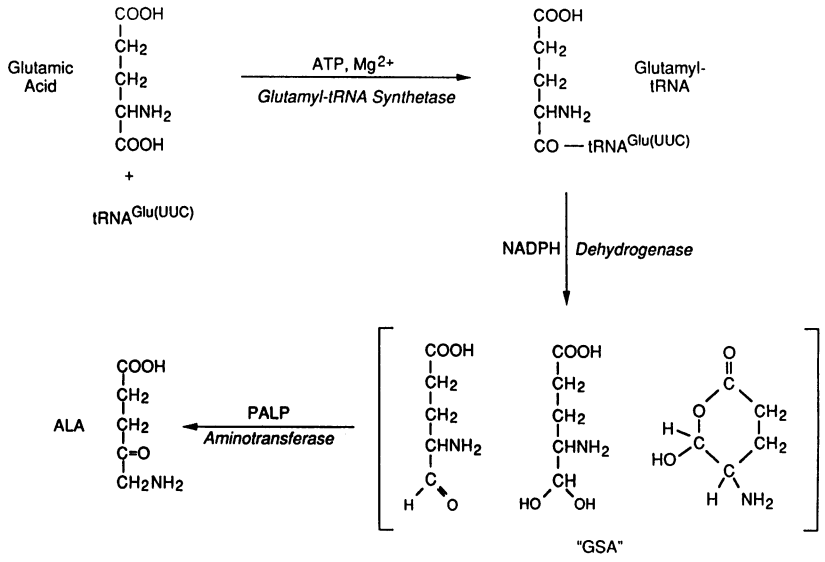

Figure 1. Current scheme for the formation of ALA from glutamate. Glutamate is activated by ligation to $tRNA^{Glu}$ in an ATP- and Mg^{2+}-requiring reaction catalyzed by glutamyl-tRNA synthetase. The activated glutamate is reduced by the pyridine nucleotide-linked dehydrogenase, and the reduction product is rearranged by a PALP-requiring aminotransferase to form ALA. The structures of three proposed forms of the dehydrogenase reaction product are shown: GSA; the hemiacetal of GSA; and the cyclized internal ester between the γ-carboxyl and hydrated aldehyde functional groups of GSA.

to provide precursors for nonplastid tetrapyrroles. This conclusion was subsequently proven directly by measuring incorporation of labeled precursors into isolated tetrapyrroles specific to plastids and mitochondria. Under all growth conditions examined, heme *a* of mitochondrial Cyt *c* oxidase was formed from glycine, even while in cells growing under conditions permitting Chl formation, the plastid pigments were formed exclusively from glutamate. In contrast, heme *a* of the red alga *Cyanidium caldarium* and etiolated maize leaves is derived from ALA that is formed from glutamate. Thus, unlike higher plants and other algae examined, *Euglena* has both ALA-forming pathways, each separately compartmented and responsible for synthesizing distinct pools of precursors for different classes of tetrapyrrole end products.

INTERMEDIATES IN THE TRANSFORMATION OF GLUTAMATE TO ALA

Glutamyl-tRNA

In vitro ALA-forming activity in extracts from plastids and algal cells is blocked by preincubation of the extracts with RNase A. Subsequent addition of RNase inhibitor plus low molecular weight RNA obtained from the same species restores activity. RNA has been similarly found to be required for ALA formation from glutamate in extracts of cyanobacteria, a prochlorophyte, and several phototrophic bacteria.

Activity of barley $tRNA^{Glu}$ in ALA formation required the presence of the 3′-terminal CCA, suggesting that the tRNA functions as a glutamate acceptor in the ALA-forming system analogous to its role in protein synthesis. Glutamyl-tRNA served as a substrate for ALA formation in *Chlamydomonas* extracts. Although it was concluded that glutamyl-tRNA was a true intermediate in ALA formation, the efficiency of incorporation of label from ^{14}C-glutamyl-tRNA into ALA was low, and the extracts to which the ^{14}C-glutamyl-tRNA was added still had the ability to form ALA from glutamate. More direct proof for the intermediacy of glutamyl-tRNA was obtained with fractionated *Chlorella* extract. Glutamyl-tRNA, but not free glutamate, served as precursor to ALA in a partially reconstituted system lacking one fraction. The missing enzyme fraction had glutamyl-tRNA synthetase activity.

Although tRNA has been found to function in several biochemical processes in addition to protein synthesis, this appears to be the only example of the participation of an aminoacyl-tRNA in a reaction that does not result in peptide bond formation.

In the barley chloroplast system, the required RNA was purified, sequenced, and characterized as $tRNA^{Glu}$, bearing the UUC glutamate anticodon. Two of the anticodon bases bear modifications: the first U is 5-methylaminomethyl-2-thiouridine and the second is pseudouridine. Although the extent of the modifications in the anticodon region is greater than previously reported for any $tRNA^{Glu}$, this tRNA is the only $tRNA^{Glu}$ found in barley chloroplasts, and thus it must take part in both protein synthesis and ALA formation.

In all plant and algal species examined, the tRNA required for ALA formation has been found to contain the UUC glutamate anticodon. This was determined by affinity purification using an affinity ligand directed against the UUC glutamate anticodon. The purified UUC anticodon-bearing tRNA from *Synechocystis* was then further fractionated into two components by mixed mode (anion exchange and hydrophobic interaction) HPLC. Each homogeneous tRNA was tested for the ability to be charged with glutamate and to participate in ALA formation and protein synthesis in extracts derived from *Synechocystis*. The results indicated that both tRNAs can participate in both processes. The two *Synechocystis* $tRNA^{Glu}$s were reported to have the same nucleotide sequence and to differ by some unspecified base modification.

GSA

A two-electron reduction of the α carboxyl group of glutamate would formally yield GSA. GSA was chemically synthe-

sized and used as a substrate for the *in vitro* enzyme system from barley. The conversion required only the enzyme system plus GSA, and was inhibited by the aminotransferase inhibitors, aminooxyacetate and cycloserine. Although the proposed structure of chemically synthesized GSA has been questioned, newer synthetic methods and structure analysis (9) appear to have unambiguously identified GSA, its hydrate, or possibly a cyclic internal lactone form (Fig. 1), as the correct structure for the reduced form of ALA precursor. Material identical to chemically synthesized GSA was reported to accumulate in greening barley leaves and extracts of *Scenedesmus* cells when treated with gabaculine, which is a mechanism-based inhibitor of ω-aminotransferases that blocks Chl synthesis.

Jordan (13) has proposed that the compound synthesized by the chemical procedure of Kannangara and Gough is neither GSA nor its hemiacetal, but the cyclic ester between the γ-carboxyl group and the hydrated aldehyde group. The cyclic structure does not contain free aldehyde or carboxylic acid functions, and is more compatible with previously reported properties of the chemically synthesized product (stability in aqueous solution, heat stability) than the free or hydrated α-aminoaldehyde. The cyclic compound, and not GSA, was reported to be the product of the dehydrogenase enzyme and the substrate of the barley aminotransferase (13).

ENZYMES CATALYZING ALA FORMATION FROM GLUTAMATE

Cell-free extracts capable of converting glutamate to ALA have been obtained from many sources including higher plants, *Euglena,* green and red algae, cyanobacteria, a prochlorophyte, and bacteria. Similar or identical reaction mechanisms appear to operate in all cases, and reaction components from some heterologous sources can be mixed to reconstitute activity in fractionated systems.

According to the current scheme (Fig. 1), a minimum of three enzyme reactions are required for transformation of glutamate to ALA. In the first reaction, glutamate is ligated to tRNA by an enzyme identical or very similar to the glutamyl-tRNA synthetase that functions in protein biosynthesis. Like aminoacyl-tRNA formation in general, this reaction requires ATP and Mg^{2+}. Next, the tRNA-bound glutamate is converted to a reduced form in a reaction that requires a reduced pyridine nucleotide. Finally, the positions of the nitrogen and oxo atoms of the reduced five-carbon intermediate are interchanged to form ALA.

Consistent with the above scheme, the ALA-forming systems extracted from barley, *Chlorella,* and *Synechocystis* have each been separated into four macromolecular components, all of which must be present to catalyze *in vitro* ALA formation from glutamate. Three of these are enzymes and the fourth is glutamyl-tRNA.

Glutamyl-tRNA Synthetase

A single glutamyl-tRNA synthetase enzyme was detected in barley chloroplasts. The synthetase was purified by immunoaffinity chromatography. The purified enzyme was capable of linking glutamate to the $tRNA^{Glu}$ and both $tRNA^{Gln}$ species present in chloroplasts. The aminoacylation reaction required ATP. The glutamyl-tRNA synthetase in *Chlorella* extracts that participates in ALA formation was separated from the other enzyme components by serial affinity chromatography on Blue-Sepharose and ADP-agarose. In the absence of this enzyme fraction, ALA was formed from glutamyl-tRNA, but not from free glutamate. Molecular weight values of 54,000, 73,000, and 63,000 were reported for the enzymes from barley, *Chlorella,* and *Synechocystis* (18), respectively.

Dehydrogenase

In unfractionated or reconstituted ALA-forming systems, a reduced pyridine nucleotide is required for activity. In *Chlorella* extracts, NADH was about half as effective as NADPH, in *Euglena* extracts the two reduced pyridine nucleotides were about equally effective, and in *Synechocystis* extracts NADPH was more effective at low concentrations but became inhibitory above 1 mM, and NADH was more effective at 5 mM. The ALA-forming system from *Chlorobium vibrioforme* had a nearly exclusive preference for NADPH (19).

The *Chlorella* and *Synechocystis* enzyme components that utilize the pyridine nucleotide cofactor were physically separated from the other enzyme components by affinity chromatography. When incubated along with the aminotransferase-containing fraction, this fraction was active in ALA formation from glutamyl-tRNA in the absence of glutamyl-tRNA synthetase. Barley dehydrogenase was also separated from glutamyl-tRNA synthetase by immunoaffinity chromatography, and found to regain ALA-forming activity when recombined with the glutamyl-tRNA synthetase and aminotransferase fractions. Earlier, aminotransferase activity was physically separated from the other enzymes in barley extracts by Blue-Sepharose affinity chromatography. The product formed by incubation of the other enzymes with glutamate, ATP, Mg^{2+}, and NADPH copurified with chemically synthesized GSA.

Aminotransferase

Enzymes capable of converting chemically synthesized GSA to ALA have been purified by affinity chromatography from extracts of barley chloroplasts and cells of *Chlamydomonas, Chlorella, Synechocystis,* and *Synechococcus.* The transamination reaction requires no added substrate or cofactor other than GSA. Native aminotransferases from barley, *Chlorella* and *Synechocystis* have mol wts of 80,000, 60,000, and 99,000, respectively (1, 18). Purified aminotransferase from both barley and *Synechococcus* has a mol wt of 46,000 on denaturing SDS-PAGE (10).

At high concentrations, GSA is spontaneously transformed to ALA in solution at physiological pH. The reaction rate is highly dependent on GSA concentration and the reaction has been proposed to proceed through a bimolecular aminohem-

iacetal intermediate (9). A bimolecular reaction would result in intermolecular transfer of the amino nitrogen during the conversion of GSA to ALA. The question of whether the enzymatic reaction involves intramolecular or intermolecular transfer was examined in *Chlamydomonas* extracts by the use of ^{13}C- and ^{15}N-labeled glutamate. When the heavy isotope labels were present on separate substrate molecules, a significant proportion of the ALA product molecules contained two heavy atoms, suggesting that the conversion occurs by intermolecular nitrogen transfer. However, the isotopic stability of ALA in the incubations was not measured, leaving open the possibility that the observed redistribution of heavy isotopes among product molecules occurred after the initial formation of ALA.

Postulated enzyme-bound intermediates in the aminotransferase reaction have included γ,δ-dioxovaleric acid and γ,δ-diaminovaleric acid. Dioxovaleric acid was earlier proposed to be a free intermediate. Cell extracts do contain enzyme activity that is capable of transaminating dioxovaleric acid to ALA. However, this activity appears to be a side-reaction catalyzed by glyoxylate aminotransferase, and neither free dioxovaleric acid nor dioxovaleric acid aminotransferase are now considered to participate in the transformation of glutamate to ALA.

Early evidence for the involvement of PALP as a cofactor for the aminotransferase step consisted of the observation that *in vitro* ALA formation was inhibited by compounds such as aminooxyacetate and gabaculine, which are considered to be PALP antagonists. Direct proof for the involvement of PALP in the *Chlorella* aminotransferase reaction was obtained by carrying out the cell disruption and enzyme fractionation in the absence of added PALP, and adjusting the relative proportion of the enzymes in the reconstituted ALA-forming assay so that the aminotransferase was rate limiting. In these experiments, a strong PALP dependence of ALA formation was observed (1). Moreover, gabaculine-inactivated *Chlorella* aminotransferase could be reactivated by the addition of PALP after passing the enzyme over Sephadex to remove free gabaculine and gabaculine-PALP adducts.

Spectral evidence for the presence or participation of PALP could not be obtained with the purified barley aminotransferase (10). Thus, the seemingly unlikely possibility remains open that the aminotransferase reaction is catalyzed by different mechanisms in different plant and algal species.

All of the aminotransferases that have been examined are inhibited by very low concentrations of gabaculine, both *in vitro* and *in vivo,* as indicated by inhibition of Chl formation. Gabaculine-resistant mutants of *Chlamydomonas* contain elevated levels of aminotransferase activity that is still sensitive to gabaculine *in vitro*. In contrast, gabaculine-resistant mutants of *Synechococcus* 6301 were recently reported to contain a variant aminotransferase that is relatively insensitive to gabaculine (7).

In addition to gabaculine, other GSA analogs have been found to be powerful inhibitors of the aminotransferase. In contrast to the reversibility of gabaculine inhibition of the *Chlorella* enzyme described above, irreversible inhibition was obtained with 4-amino-5-hexynoic acid (3). This result suggests that 4-amino-5-hexynoic acid may react to become covalently bound to the protein at the active site.

REGULATION

Early ALA administration experiments demonstrated that ALA formation is the rate-limiting step for tetrapyrrole formation, at least in greening etiolated plant tissues. Models for the feedback regulation of pigment formation have proposed end product modulation of the rate of ALA synthesis via allosteric inhibition of enzyme activity and/or regulation of the rate of enzyme synthesis and turnover.

Exogenous heme administered to intact barley shoots and to cultures of *Chlamydomonas* inhibited Chl formation *in vivo,* although in both cases the concentration of exogenous heme was extremely high. Increased ALA synthesis occurs in intact tissues treated with aromatic Fe chelators, and this may be due to interference with the formation of heme, causing a decrease in the concentration of this feedback inhibitor. Similar effects were observed in intact plastids, where the Fe chelator blocked the inhibition of ALA synthesis caused by added protoporphyrin. It has been shown that chloroplasts are capable of heme biosynthesis and that heme undergoes significant turnover *in vivo.* If heme breakdown is an ongoing aerobic process, then anaerobic conditions might be expected to prevent normal heme breakdown and thereby inhibit ALA biosynthesis. This effect has been observed in isolated plastids. Light-driven ALA formation in plastids isolated from cucumber cotyledons requires O_2. Although most of the O_2 requirement could be overcome by addition of ATP and NADPH, there was still a 30% inhibition of ALA formation under anaerobic conditions in the light or the dark. It is possible that the residual inhibition under anaerobic conditions was due to the block in O_2-dependent heme breakdown, which allowed the accumulation of inhibitory concentrations of heme.

Regulation of Enzyme Activity

Heme is a potent inhibitor of ALA formation in intact plastids and most of the soluble enzyme systems which have been characterized. Heme concentrations which inhibited *in vitro* activity by 50% ranged from 0.05 μM in partially purified *Chlamydomonas* extracts to 1.2 μM in *Chlorella* extracts. In contrast to most of the species examined, high concentrations (25 μM) of heme were required for 50% inhibition in *Euglena* cell extracts, and it is therefore unlikely that heme plays a significant role in regulating ALA formation from glutamate by modulating enzyme activity in this species.

The heme concentrations required for 50% inhibition appear to be higher in the more highly purified preparations than in unpurified cell extracts. This difference suggests that another factor may be required to confer maximum sensitivity to heme. In preliminary studies, it was found that reduced glutathione decreased by a factor of eight to ten the heme concentration required to inhibit ALA formation by 50% in unfractionated *Chlorella* extract (20). The effect was obtained with both reduced and oxidized glutathione, but not with other sulfhydryl-containing compounds.

Although Mg-protoporphyrin inhibits ALA synthesis in intact plastids, relatively little inhibition, and only at high concentrations, was observed in solubilized systems. Other potential feedback inhibitors (protoporphyrin, Pchlide, and Chlide) were not effective, except at very high concentrations. Cucumber cotyledons were exposed to various light regimes to alter their Pchlide content. The rate of ALA formation in subsequently isolated intact plastids did not appear to correlate with the Pchlide content (11). It was concluded that Pchlide does not act as a feedback inhibitor of ALA formation in the plastids.

In extracts of *Chlamydomonas,* it was earlier reported that of the three enzyme activities required for ALA formation from glutamate, heme exerts its effect only on the dehydrogenase that reduces glutamyl-tRNA. However, more recent work with separated *Chlamydomonas* enzyme fractions revealed that both the glutamyl-tRNA synthetase and the dehydrogenase activities were inhibited by heme, with 50% inhibition occurring at 2 and 10 μM, for the synthetase and dehydrogenase steps, respectively (15). Heme appears to bind to a protein that was identified as glutamyl-tRNA synthetase in nondenaturing gel electrophoresis (W-Y Wang, personal communication). Glutamyl-tRNA synthetase was also reported to be subject to feedback inhibition in *Scenedesmus obliquus* extracts, but in this case, the inhibitor was Pchlide (8). Half-maximal inhibition was achieved at approximately 1 μM Pchlide, and more than 80% inhibition was reached at Pchlide concentrations above 10 μM. In view of the absence of effects of Pchlide on ALA synthesis in the *in vivo* cases described above, the relative ineffectiveness of Pchlide as an inhibitor of *in vitro* ALA synthesis, and the generally nonrate-limiting activity of glutamyl-tRNA synthetase in the systems examined thus far, it is premature to conclude that the inhibition of *Scenedesmus* glutamyl-tRNA synthetase by Pchlide is of physiological significance.

Preincubation of partially purified *Chlorella* extracts with ATP stimulated ALA formation upon subsequent addition of the remaining substrates and cofactors. This behavior is consistent with protein kinase/phosphoprotein phosphatase-mediated modulation of activity. Because ATP did not stimulate ALA formation from glutamyl-tRNA, it can be concluded that if any enzymatic step leading from glutamate to ALA is activated by a protein kinase, it would be the glutamyl-tRNA synthetase. Although no direct evidence was obtained to support kinase-mediated activation of *Chlorella* glutamyl-tRNA synthetase, other aminoacyl-tRNA synthetases, including rabbit reticulocyte glutamyl-tRNA synthetase, have been reported to undergo reversible phosphorylation. However, in these cases, phosphorylation caused inactivation.

Regulation of Enzyme Synthesis and Turnover

Direct measurement of synthesis and turnover of the proteins catalyzing ALA synthesis has not yet been accomplished. The most reliable method for determining the level of enzyme protein is by immunochemical techniques, which require purified protein to elicit the antibodies. Although antibodies to barley aminotransferase (10) and glutamyl-tRNA synthetase from barley chloroplasts and *Chlamydomonas* have been prepared, their use for turnover studies has not yet been reported. However, there have been several studies based on the effects of inhibitors of protein synthesis on ALA accumulation *in vivo,* and on the levels of extractable ALA-forming activity during greening, which have been interpreted in terms of expression and turnover of enzyme protein. It should be emphasized that with either of these approaches, changes in ALA accumulated *in vivo* or changes in extractable activity *in vitro* can be the result of processes other than the turnover of new enzyme (*e.g.* changes in substrate supply or effector concentration *in vivo,* or covalent modification *in vitro*).

When etiolated barley leaves were first illuminated and then placed back in the dark and infiltrated with levulinic acid, the rate of ALA accumulation decayed during the dark period. ALA accumulation increased when the leaves were brought back into the light. Cycloheximide did not block the increase in ALA accumulation upon re-illumination. These results indicate that, after the ALA-synthesizing enzymes are formed during the initial illumination, the decrease in ALA formation during the dark period and the subsequent increase upon reillumination, are mediated by modulation of enzyme activity rather than rates of enzyme synthesis and turnover.

Levulinic acid-dependent accumulation of ALA in *Chlorella* was inhibited by treatment with the cytoplasmic protein synthesis inhibitor, cycloheximide. Inhibition was complete after 30 min, suggesting that continuous synthesis of enzyme is required in this organism. The phytochrome-mediated elimination of the lag phase in Chl synthesis in etiolated leaves is also thought to involve new enzyme synthesis. Thus, the normally rapid accumulation of ALA that occurs upon illumination in levulinic acid-treated, dark-germinated maize or beans is prevented by application of cycloheximide 2 h prior to light exposure. A half-life of 80 min for the activity was estimated by returning greening maize leaves to the dark and relating the length of the dark period to the rate of ALA accumulation when the plants were subsequently returned to the light.

The influence of light on the capacity of plants to form ALA could be measured during the later stages of greening by using isolated chloroplasts (11, 12). The ability of isolated cucumber chloroplasts to synthesize ALA from exogenous substrates was dependent on the light regime immediately preceding organelle isolation. Chloroplasts isolated from cotyledons of plants that were germinated in the dark and greened for 24 h synthesized ALA to an extent greater than that required for Pchlide regeneration (11). If the seedlings were returned to darkness prior to chloroplast isolation, the capacity for ALA synthesis by the subsequently isolated chloroplasts diminished threefold. The decrease to the basal ALA-synthesizing capacity was time dependent and nearly complete within 1 h in the dark. ALA-forming capacity recovered in a time-dependent manner when the dark-treated seedlings were reilluminated. The *in organello* activity was independent of endogenous Pchlide level, even in chloroplasts that were isolated from dark-treated seedlings. The light-dependent recovery of ALA-forming capacity was shown to be a phytochrome-mediated response, by the criteria of far-red light

reversibility and the effectiveness of red, but not blue light in modulating the recovery of activity (12). Light-induced recovery of ALA-forming capacity was blocked by prior administration of cycloheximide. In these studies it was apparent that the basal level of ALA-forming activity was not influenced by the light treatment.

In cell-free ALA-forming systems derived from plant and algal species that require light for greening, a basal level of activity is present in extracts of dark-grown cells or plants. The level of activity is three to four fold higher in extracts of plants or algae obtained after 2 to 4 h after light exposure. Barley aminotransferase is severalfold higher in greening plastids than in etioplasts or mature chloroplasts. Also, mutant strains of *Chlamydomonas* that are resistant to gabaculine appear to gain the resistance by having higher cellular levels of aminotransferase activity. It is not clear how many of the enzyme components increase(s) upon light exposure, but in *Chlorella* and barley, the tRNA component does not increase during greening. In contrast, the levels of both the tRNA and the enzymes are responsive to light in *Euglena* (16). Light-grown *Euglena* cells contain approximately three times more of the tRNA required for ALA synthesis than dark-grown cells. Extracts of dark-grown *Euglena* cells are deficient in the dehydrogenase and aminotransferase enzymes, but contain sufficient glutamyl-tRNA synthetase to catalyze ALA formation at about half the rate of extracts of light-grown cells, when supplemented with the other two enzyme fractions (16).

Regulation of ALA synthesis in *Euglena* is a particularly complex problem. *Euglena* is the only organism known in which both routes to ALA exist and operate simultaneously, albeit in separate compartments. In the dark, the level of ALA synthase is relatively high and decreases drastically when cells are brought into the light. ALA synthase activity is low in light-grown cells and increases four fold after 4 h in the dark. Activity of the five-carbon route is absent in dark-grown cells and high in light-grown cells. The interplay between the two biosynthetic routes was demonstrated by treatment of light-grown cells with gabaculine, the inhibitor of the glutamate pathway. The gabaculine treatment caused a two-fold increase in extractable ALA synthase activity in the light. Pretreatment with gabaculine also prevented the decrease in extractable ALA synthase activity which normally occurs upon transfer of dark-grown cells to the light. Thus it is clear that the cells have some mechanism to maintain a supply of porphyrin precursors and that this mechanism is not solely a light-dependent phenomenon. To complicate the matter further, both ALA synthase and the five-carbon route to ALA are insensitive to heme inhibition *in vitro*. Finally, the enzymes, but not the tRNA, of the five-carbon pathway are present in aplastidic cells that are devoid of all chloroplast components including DNA (16). Although the aplastidic cells do not contain the required tRNA and do not form ALA from glutamate *in vivo*, the enzymes are active *in vitro* and their accumulation in the cells is under light control (16). These results indicate that the genes for the enzymes, but not for the tRNA, of the five-carbon ALA biosynthetic pathway are encoded in the nucleus of *Euglena*, and that their expression is under the control of a nonplastid photoreceptor system.

The recent discovery that *Escherichia coli* utilizes the five-carbon pathway for ALA formation (14) has opened the door to molecular genetic studies of this pathway. Current efforts toward cloning of genes coding for the enzymes in phototrophic species have yielded genomic DNA from *Chlorobium* that is capable of complementing an *E. coli* mutant which is defective in ALA formation (4). It was found earlier that the *E. coli* mutant is defective in dehydrogenase activity (2). By comparing the properties of the dehydrogenase in extracts of the transformed *E. coli* cells with those of *Chlorobium* and wild-type *E. coli* dehydrogenases, it was established that the transforming DNA encodes a structural component of the dehydrogenase enzyme. The cloned DNA will aid in ongoing studies of the structure of this enzyme, its catalytic mechanism, and the regulation of its expression.

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