**Short Communication**

**Delta-Aminolevulinic Acid Dehydrase in Greening Bean Leaves**

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The enzyme δ-aminolevulinic acid dehydrase (E.C. 4.2.1.24) catalyzes the synthesis of porphobilinogen (PBG) from 2 molecules of δ-aminolevulinic acid (ALA). ALA has been shown to be a precursor of both heme and chlorophyll (2,14) and ALA dehydrase has been reported to bacteria, animals and plants (3,5,6,11). Laseles (6) reported that during adaptation to form bacteriochlorophyll in *Rhodopseudomonas spheroides*, ALA dehydrase activity increased but not as much as did ALA synthetase.

During the preparation of this report the paper of Stobart and Thomas (16) was published in which ALA dehydrase activity in greening tissue cultures of *Kalanchoe crenata* was studied. Here we report on ALA dehydrase activity in intact etiolated bean leaves during illumination and we obtain similar results. In addition the influence of red and blue light are discussed.

The experimental material was *Phaseolus vulgaris* cv. Resistant Asgrow Valentine (Charter Seed Company, Twin Falls, Idaho, Crop No. 1-5269). Growth and illumination conditions are reported fully elsewhere (15). Seeds were germinated in darkness and used on the seventh day. Continuous illumination (900 ft-c) was provided in a growth chamber at 26°.

The enzyme assay was based on the procedure of Loomis and Battaile (7). Polyclar AT (insoluble polyvinylpyrrolidone) was a gift of General Aniline and Film Corporation, Union, New Jersey, and was purified by boiling with 10% HCl, washing with water, then acetone and drying in air. Before use, it was hydrated by soaking overnight in water.

Leaf pairs (20–40) were removed to a dark cold room and ground in a mortar and pestle with 0.5 g Polyclar AT and 10 ml of a solution containing 200 mM tris HCl pH 7.2, 100 mM 2-mercaptoethanol, 100 mM MgCl₂, 100 mM sodium ascorbate and 3 mM o-phenanthroline. The homogenate was transferred to a glass homogenizer for further disruption and then was strained through 8 layers of cheesecloth. The filtrate was centrifuged at 100g for 5 min to precipitate the finer Polyclar AT particles and debris. The supernatant fraction was used for enzyme assays.

The effects of variations in the extraction procedure on the enzyme activity are shown in table I. The amount of total protein extracted in each case was similar. Freezing the leaves, grinding dry and adding the Polyclar AT and the solution so that the whole froze and then allowing it to thaw slowly resulted in less activity than if the grinding was carried out without freezing. Both ascorbate and o-phenanthroline were necessary for maximum activity but the use of Polyclar AT had little effect, nevertheless it was included in the routine procedure. The role of o-phenanthroline was not investigated further but it suggests that iron has an inhibitory effect upon the enzyme.

The assay system consisted of 100 μmoles tris HCl pH 7.8. 2 μmoles ALA and 0.5 ml enzyme in a final volume of 1 ml. The mixture was incubated in a closed tube at 25° for 1 hr; incubation in an evacuated Thunberg tube did not give higher measurable PBG. The reaction was linear for up to 2 hr. A control minus ALA was incubated similarly. The reaction was stopped by bringing the solution to 4% (w/v) with respect to trichloroacetic acid and 10 mM to HgCl₂ (13). After 30 sec the pH was adjusted to 5.0 with sodium acetate buffer and KOH, the precipitated protein was spun down and the supernatant assayed for PBG using the modified Ehrlich reagent: 0.1 g p-dimethylnitrobenzaldehyde in 1 ml 70% perchloric acid and 4.2 ml glacial acetic acid (17). Equal volumes of the reagent and the supernatant were mixed and the O.D.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Extraction conditions</th>
<th>Activity of ALA dehydrase (nmoles/hr·mg protein)</th>
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<tbody>
<tr>
<td>I</td>
<td>Complete</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>Minus Polyclar AT</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td>Minus Na ascorbate</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Minus o-phenanthroline</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Complete frozen</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Complete not frozen</td>
<td>0.84</td>
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1 Supported by funds from the National Science Foundation.
2 Present address: Department of Botany, The University, Bristol, Great Britain.
at 555 nm read 15 min after mixing. Experimental tubes were done in duplicate and the difference in optical density between the experimental and control (minus ALA) readings were used to calculate nmoles PBG present using a molar absorbancy of \(6.8 \times 10^4\) (17). Assay for porphyrins in both the supernatant and pellet showed that they could be considered negligible in calculating the PBG formed. In this system it appeared that very little PBG was metabolized.

Protein was measured by the Folin method (8) and chlorophyll in 80% aqueous acetone using the formula of Bruinsma (1).

Both the specific activity (nmoles per hr per mg protein) of ALA dehydrase and \(\mu g\) chlorophyll per mg protein increased in a similar manner when etiolated seedlings were exposed to continuous white light (Fig. 1). They showed little further change after 75 hr although chlorophyll per leaf pair (shown) and ALA dehydrase activity per leaf pair (not shown) continued to increase as leaf growth continued. The increase in specific activity of the enzyme was linear with respect to \(\mu g\) chlorophyll per mg protein (Fig. 1 inset). This constant relationship between ALA dehydrase activity and chlorophyll content suggested a common intracellular site and common control over development for the 2 systems.

Fractionation of leaves using a number of aqueous preparative techniques always resulted in all the ALA dehydrase activity being recovered in the supernatant after removal by differential centrifugation of a chloroplast and mitochondrial fraction. This pattern of distribution has been reported for the enzyme from beef liver (12) and from rat liver (3) where by far the greatest activity was in the particle-free supernatant. An investigation of the enzyme in wheat leaves showed that chloroplasts and the mitochondrial containing fraction had activity but no value for the supernatant was reported for comparison (11). In addition Granick (4) reported that isolated plastids of tobacco, bean and barley would metabolize added ALA to various end products but in all cases the results were consistent with an active ALA dehydrase in the plastids. While the present results support the scheme of Sano and Granick (12) that envisages a cytoplasmic site for ALA dehydrase it seems likely that the enzyme was very readily lost from the organelles during preparation even though the chloroplasts appeared intact by microscopic observation.

Chlorophyll synthesis in etiolated leaves exhibits, in many cases, a lag of 2 to 3 hr. Elimination of the lag by light treatment in the preceding dark period is phytochrome controlled (10,18). To see if ALA dehydrase activity responded to short irradiations of colored light, seedlings were exposed to 10 min red or 15 blue and then returned to the dark. Enzyme activity plotted against mg protein per leaf pair is presented in Fig. 2. Included for comparison is the response to continuous white light taken from the experiment of Fig. 1. After all 3 treatments, leaf growth continued at the dark rate for about 20 hr. After blue irradiation, it stopped at the end of that time as shown by the 2 experimental points at 9.4 mg protein per leaf pair. The fall in specific activity was due solely to growth of the leaf, after 20 hr it remained more or less constant. The initial fall in activity after red light may also be ascribed to continued leaf growth at the dark rate. The rise after 29 hr was concurrent with the leaf growth induced by red irradiation and presumably was associated with the production of new proplastids. No indication of an increase in ALA dehydrase activity after a short red or blue light irradiation was found.

![Fig. 1. ALA dehydrase activity and chlorophyll content during continuous white illumination of etiolated leaves. \(\mu g\) chlorophyll/mg protein \(x-x\); \(\mu g\) chlorophyll/leaf pair \(O-o\); specific activity of ALA dehydrase \(\Delta--\Delta\). Inset: ALA dehydrase activity (nmoles PBG per hr per mg protein) as a function of chlorophyll/mg protein during the continuous white illumination of etiolated leaves.](image-url)

![Fig. 2. ALA dehydrase activity as a function of protein content of leaves after different light treatments. x-x 15 min blue (100 Kergs/cm²) followed by darkness. ●—● 10 min red (1160 Kergs/cm²) followed by darkness. △——△ continuous white light. The figures adjacent to the experimental points are the time in hr after the beginning of the treatment at which the activity was measured.](image-url)
Meglio and Jagendorf (9) have distinguished 2 phases in the development of chloroplasts: one is induced by short exposures to red or white light and is far-red reversible. Synthesis of plastid proteins and lipids occurs in the subsequent dark period together with an increase in plastid diameter. The second phase is dependent upon extensive illumination and controls grana formation, extensive chlorophyll synthesis and the change of plastid shape from spherical to lens shaped. Our experiments suggest that changes in ALA dehydrase activity may be included in the second system. In continuous light, activity increased from the beginning of illumination and it paralleled the chlorophyll content. After red illumination no net synthesis of the enzyme occurred for 29 hr and the increase in specific activity after that time was concomitant with the growth of the leaf. The difference in response to continuous illumination and to short exposure to red light is most convincingly seen during the first 20 hr when both sets of leaves continued to grow at the dark rate. Yet, an increase in specific activity occurred in continuous white light but after red the activity declined. The absence of a rapid response to red irradiation suggests that changes in ALA dehydrase activity are not concerned in the phyochrome controlled elimination of the lag phase in chlorophyll synthesis.

In conclusion, the parallel increase in ALA dehydrase activity and chlorophyll content during continuous illumination suggested common controls over development. The lack of response to short exposures to red or blue irradiation suggested that extensive illumination was required for the increase in ALA dehydrase activity being in this respect similar to grana formation.

**Literature Cited**


