

# Catabolism of 5-Aminolevulinic Acid to CO<sub>2</sub> by Etiolated Barley Leaves<sup>1</sup>

Received for publication May 19, 1981 and in revised form August 14, 1981

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## ABSTRACT

The *in vivo* oxidation of the C<sub>4</sub> and C<sub>5</sub> of 5-aminolevulinic acid (ALA) to CO<sub>2</sub> has been studied in etiolated barley (*Hordeum vulgare* L. var. Larker) leaves in darkness. The rate of <sup>14</sup>CO<sub>2</sub> evolution from leaves fed [4-<sup>14</sup>C]ALA is strongly inhibited by aminooxyacetate, anaerobiosis, and malonate. The rate of <sup>14</sup>CO<sub>2</sub> evolution from leaves fed [5-<sup>14</sup>C]ALA is also inhibited by these treatments but to a lesser extent. These results suggest that (a) one step in ALA catabolism is a transamination reaction and (b) the C<sub>4</sub> is oxidized to CO<sub>2</sub> via the tricarboxylic acid cycle to a greater extent than is the C<sub>5</sub>.

ALA<sup>4</sup> is a key precursor of porphyrins in plants, animals, and bacteria. The role of this compound as a rate-limiting precursor in Chl synthesis in angiosperm leaves is well documented (1, 2, 4, 10).

Until recently, ALA was thought to be the first metabolite specifically committed to the tetrapyrrole biosynthetic pathway (9). However, there is a growing body of evidence which indicates that ALA may be metabolized via nonporphyrin pathway(s) in a number of organisms (7, 13, 15). For example, etiolated barley leaves evolve <sup>14</sup>CO<sub>2</sub> when fed [4-<sup>14</sup>C]ALA or [5-<sup>14</sup>C]ALA in the dark (7). The nature of the metabolism of the C<sub>4</sub> and C<sub>5</sub> of ALA to CO<sub>2</sub> is the subject of these studies.

A preliminary report of this work has appeared (5).

## MATERIALS AND METHODS

**Growth and Manipulation of Plant Material.** Seeds of *Hordeum vulgare* L. var. Larker (Field Seed Farm, Byron, MN) were germinated in vermiculite in the dark at 23 ± 1°C. All operations were performed under a low-intensity green safelight (6). The apical 6 cm of the leaves of 7-day-old etiolated seedlings was excised and cut into 1-cm segments. Unless otherwise noted, 1 g

of tissue was preincubated for 30 min in a 125-ml Erlenmeyer flask containing distilled H<sub>2</sub>O plus the indicated additions. Radio-labeled substrate was then added, bringing the total volume to 1.0 ml. The flasks were then sealed with rubber stoppers and incubated at 22°C for up to 4 h in the dark.

**Measurement of <sup>14</sup>CO<sub>2</sub> Evolution *in Vivo*.** Respired <sup>14</sup>CO<sub>2</sub> was trapped on filter paper discs moistened with 2 N KOH and suspended from wire hooks attached to the rubber stoppers in the flask (3, 7). These wicks were replaced periodically for liquid scintillation analysis as described previously (3, 7). Values shown in the figures represent the mean of three determinations. The standard errors are indicated by bars.

**Radiochemicals and Reagents.** [4-<sup>14</sup>C]ALA and [5-<sup>14</sup>C]ALA (40-60 mCi/mmol), [1,4-<sup>14</sup>C]succinic acid (20.4 mCi/mmol), and [1-<sup>14</sup>C]DL-glutamic acid (42 mCi/mmol) were purchased from Research Products International Corp. (Mt. Prospect, IL). Scintillants, inorganic compounds, and solvents of reagent grade were purchased from Fisher Chemical Co. AOA, L-glutamic acid, and malonic acid were obtained from Sigma Chemical Co. PBG was a gift from Professor B. Frydman, University of Buenos Aires.

## RESULTS

**<sup>14</sup>CO<sub>2</sub> Evolution from [<sup>14</sup>C]ALA *in Vivo*.** The rate of <sup>14</sup>CO<sub>2</sub> evolution by etiolated barley leaves was examined as a function of [<sup>14</sup>C]ALA concentration during a 1-h incubation in darkness (Fig. 1). Increasing the concentration of both substrates produces an increase in the rate of <sup>14</sup>CO<sub>2</sub> evolution. Subsequent experiments, unless otherwise specified, used [<sup>14</sup>C]ALA at 5 μCi/ml.

The dependence of <sup>14</sup>CO<sub>2</sub> evolution on tissue sample size is shown in Figure 2. The rate of this activity was proportional to the amount of tissue added, and 1-g samples were chosen for subsequent experiments.

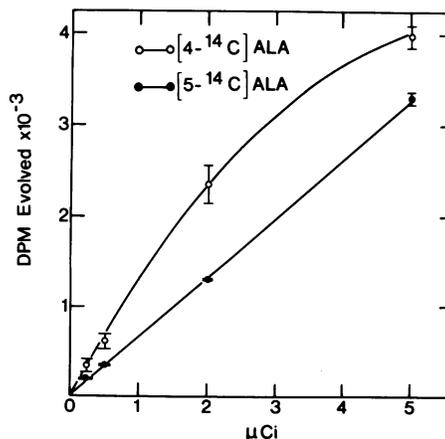


FIG. 1. The evolution of <sup>14</sup>CO<sub>2</sub> by etiolated barley leaves as a function of [<sup>14</sup>C]ALA concentration during a 1-h incubation in darkness.

<sup>1</sup> Supported by National Science Foundation Grants PCM 76-02308 and PCM 79-01605 and a grant from the University of Illinois at Chicago Circle Research Board (to M. L. G.). These studies formed a part of a thesis submitted (by J. X. D.) to the Graduate College of the University of Illinois at Chicago Circle in partial fulfillment of the requirements for the PhD degree.

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<sup>4</sup> Abbreviations: ALA, 5-aminolevulinic acid; PBG, porphobilinogen; DOVA, 4,5-dioxovaleric acid; C-4 activity, the rate of <sup>14</sup>CO<sub>2</sub> evolution from barley leaves fed [4-<sup>14</sup>C]ALA; C-5 activity, the rate of <sup>14</sup>CO<sub>2</sub> evolution from barley leaves fed [5-<sup>14</sup>C]ALA.

**Effect of AOA under Aerobic and Anaerobic Conditions.** The metabolic origin of  $\text{CO}_2$  from ALA could be the tricarboxylic acid cycle. One possible route by which ALA could reach the tricarboxylic acid cycle might be via a reversal of one of the postulated biosynthetic pathways. One of these pathways involves the transamination of DOVA to ALA (1, 2, 8). To test whether a trans-

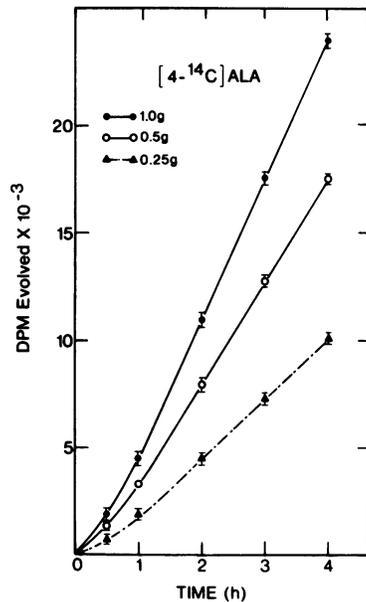


FIG. 2. The dependence of  $^{14}\text{CO}_2$  evolution from  $[4\text{-}^{14}\text{C}]\text{ALA}$  on the size of the tissue sample.

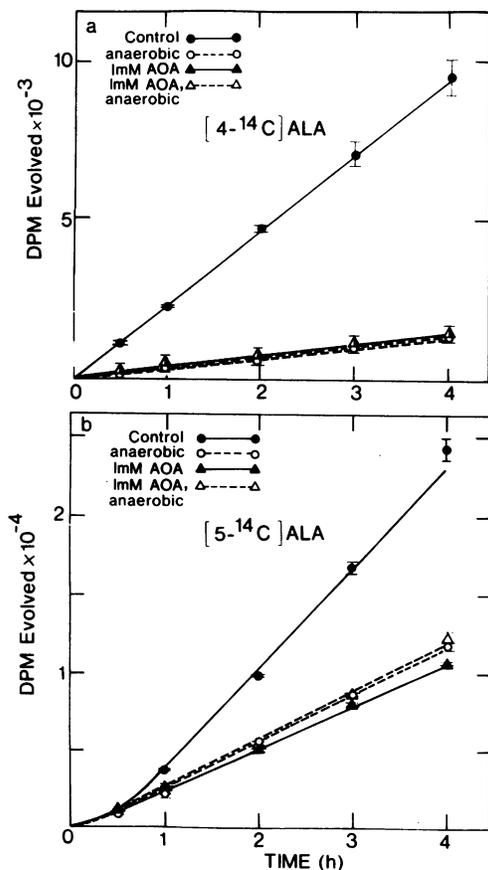


FIG. 3. The effect of AOA on C-4 and C-5 activities under aerobic and anaerobic conditions.

amination is involved in this oxidative metabolism of ALA, etiolated barley leaves were fed  $[^{14}\text{C}]\text{ALA}$  in the darkness in the presence and absence of AOA, an inhibitor of transamination (Ref. 16; Fig. 3). While AOA inhibited the evolution of  $^{14}\text{CO}_2$  from both substrates, its effect upon C-4 activity was greater than that upon C-5 activity.

Figure 3 also shows the effect of  $\text{O}_2$  on C-4 and C-5 activities. As was observed with AOA, the inhibition of C-4 activity by anaerobiosis was greater than that of C-5 activity. Each treatment yielded the same degree of inhibition on the respective activity, alone or in combination with the other. The inhibitory effect by AOA is probably greater than that shown by the figure since AOA was found to increase the uptake of  $[^{14}\text{C}]\text{ALA}$ . Anaerobiosis inhibited the uptake of  $[^{14}\text{C}]\text{ALA}$  by about 20%.

To assess the specificity of AOA on this system, the effect of this compound was determined on a reaction known not to involve transamination. The oxidation of succinic acid to  $\text{CO}_2$  was chosen; and, for comparative purposes, the effects of malonate, a competitive inhibitor of succinate oxidation, and anaerobiosis, were also examined. The results of an experiment in which  $[1,4\text{-}^{14}\text{C}]\text{succinic acid}$  was administered to etiolated barley leaf segments in darkness, with or without  $\text{O}_2$ , 1 mM AOA, or 10 mM malonate, are shown in Figure 4. Succinate respiration was substantially in-

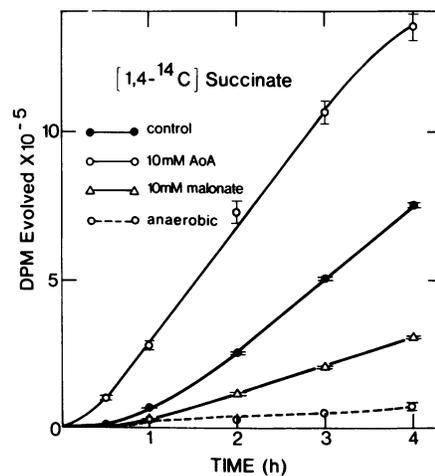


FIG. 4. The effect of anaerobiosis, AOA, and malonate on  $^{14}\text{CO}_2$  evolution from  $[1,4\text{-}^{14}\text{C}]\text{succinate}$ . Etiolated barley leaves were incubated in darkness with  $3.0 \mu\text{Ci/ml}$  of  $[1,4\text{-}^{14}\text{C}]\text{succinate}$ .

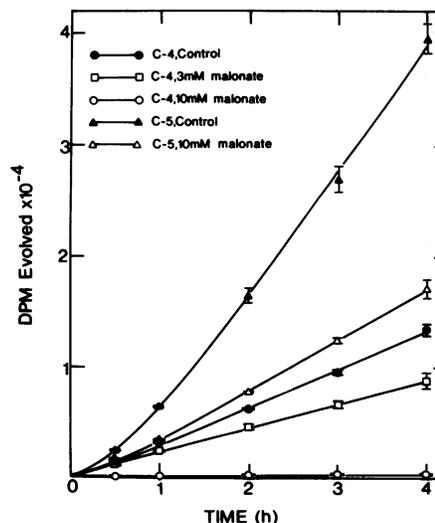


FIG. 5. The effect of malonate on C-4 and C-5 activities.

hibited by anaerobiosis and by malonate. The rate of <sup>14</sup>CO<sub>2</sub> evolution from this substrate was substantially greater in AOA-treated tissue than in control tissue. These results are consistent with the proposition that AOA is inhibiting transamination reactions which would divert labeled intermediates out of the tricarboxylic acid cycle into amino acid biosynthesis. The consequence would be that more labeled carbon would remain in the cycle for oxidation to <sup>14</sup>CO<sub>2</sub>. These data also suggest that AOA-elicited inhibition of C-4 and C-5 activities cannot be attributed to non-specific effects on respiratory metabolism.

**Effect of Malonate on CO<sub>2</sub> Evolution from ALA.** To examine the role of the tricarboxylic acid cycle in the respiration of ALA, barley leaf segments were treated with malonate (Fig. 5). At 3 mM,

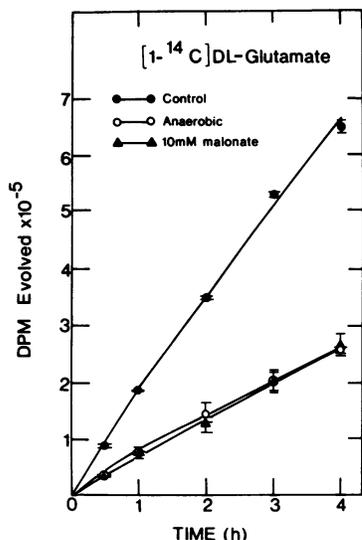


FIG. 6. The effect of anaerobiosis and malonate on <sup>14</sup>CO<sub>2</sub> evolution from [1-<sup>14</sup>C]DL-glutamic acid. Etiolated barley leaves were incubated in darkness with 2.5 μCi/ml of [1-<sup>14</sup>C]DL-glutamic acid.

this compound caused 30% inhibition of C-4 activity and, at 10 mM, almost complete inhibition. C-5 activity was less sensitive to the effect of this inhibitor. Malonate, at 2 mM, did not affect [<sup>14</sup>C]ALA uptake. Malonate does indeed interfere with tricarboxylic acid cycle activity in these tissues since, at 10 mM, it causes a considerable reduction in the rate of <sup>14</sup>CO<sub>2</sub> evolution from [1,4-<sup>14</sup>C]succinate (Fig. 4).

**<sup>14</sup>CO<sub>2</sub> Evolution from [1-<sup>14</sup>C]DL-Glutamate.** If the oxidation of ALA proceeds via glutamate through the tricarboxylic acid cycle, then the sensitivity of C-5 activity to respiratory inhibitors should show similarities to that of the C<sub>1</sub> of glutamate, assuming reversal of the 5-carbon pathway for ALA biosynthesis (1, 2). The kinetics of <sup>14</sup>CO<sub>2</sub> evolution from [1-<sup>14</sup>C]DL-glutamate administered to etiolated barley leaves in the dark are shown in Figure 6. Anaerobiosis and 10 mM malonate both caused 62% inhibition, suggesting that a considerable amount of the CO<sub>2</sub> lost from the C<sub>1</sub> of glutamate is dependent upon respiratory activity (cf. Figs. 3B and 5).

**Effect of PBG.** If C-4 and C-5 activities are the result of the degradation of [<sup>14</sup>C]PBG, formed within the tissue from [<sup>14</sup>C]ALA, then the treatment of leaves with unlabeled PBG might be expected to cause reduction of <sup>14</sup>CO<sub>2</sub> evolution by isotopic dilution. The application of 2 mM PBG caused no change in either C-4 or C-5 activity (data not shown). The lack of effect may also be due to difficulty in PBG reaching the site of oxidation within the cell.

DISCUSSION

These results confirm earlier studies which show that ALA can be catabolized to CO<sub>2</sub> (7). The catabolism of ALA depends upon respiratory activity (Figs. 3 and 5) and a pyridoxal phosphate-dependent reaction (Fig. 3). Furthermore, we have provided evidence that the metabolic fate of the C<sub>5</sub> of ALA differs from that of the C<sub>4</sub>. The stronger dependence of C-4 activity upon respiration can be deduced from its greater sensitivity to anaerobiosis and to malonate (Figs. 3 and 5).

The inhibitory effects of anaerobiosis and AOA are not compounded when the two treatments are administered simultane-

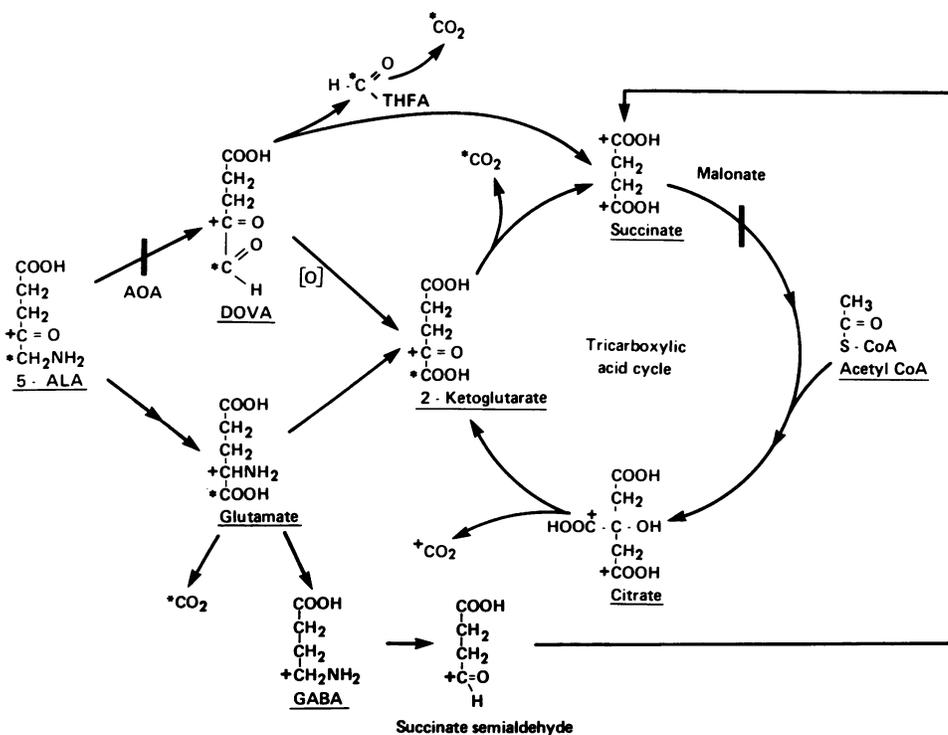


FIG. 7. A scheme for the catabolism of ALA. THFA, tetrahydrofolic acid.

ously (Fig. 3). It is interesting that the inhibitory effect of AOA on both activities is the same in the presence or absence of O<sub>2</sub>. These results suggest the following interpretation: one step in the catabolism of ALA involves a transamination and is independent of O<sub>2</sub>. Following this step, the C<sub>4</sub>—C<sub>5</sub> bond would be cleaved. The C<sub>4</sub> of ALA would then be oxidized via (an) O<sub>2</sub>-sensitive reaction(s), while the C<sub>5</sub> would be oxidized via both O<sub>2</sub>-sensitive and O<sub>2</sub>-insensitive reactions.

The 5-carbon skeleton of ALA is biosynthetically derived from 5-carbon compounds such as glutamate or  $\alpha$ -ketoglutarate in greening leaves (1–3, 10). The conversion of these compounds to ALA is thought to proceed through an internal transamination of glutamate-1-semialdehyde (1, 10, 12) or via transamination of DOVA (1, 2, 8, 10). It is possible that the biosynthetic route is reversible. ALA might then be metabolized to glutamate and/or  $\alpha$ -ketoglutarate, the C<sub>5</sub> and C<sub>4</sub> becoming the C<sub>1</sub> and C<sub>2</sub>, respectively, of these dicarboxylic acids (Fig. 7). The sensitivity of C-4 and C-5 activities to AOA and to anaerobiosis would be consistent with this explanation. The synthesis of [<sup>14</sup>C]ALA from [<sup>14</sup>C]glutamate and [<sup>14</sup>C] $\alpha$ -ketoglutarate by barley chloroplasts is inhibited by AOA (11).

C-5 activity is less sensitive to inhibition by anaerobiosis or by malonate than is C-4 activity (Figs. 3 and 5). In addition, the evolution of <sup>14</sup>CO<sub>2</sub> from [1-<sup>14</sup>C]glutamate was inhibited by anaerobiosis to about the same extent as was C-5 activity (Figs. 3b and 6). The metabolism of the C<sub>1</sub> of glutamate to CO<sub>2</sub> via glutamate decarboxylase (Fig. 7) would account for the incomplete inhibition by anaerobiosis on <sup>14</sup>CO<sub>2</sub> evolution from [1-<sup>14</sup>C]glutamate (Fig. 6). These data tend to support the suggestion that glutamate is an intermediate in ALA catabolism.

The catabolism of ALA might be initiated by a transamination to DOVA followed by the oxidation of the latter compound via the succinate-glycine cycle proposed by Shemin and Russell (14). If ALA breakdown proceeds through this cycle, carbons 1 to 4 would become succinate, while C<sub>5</sub> would become a formyl group carried by tetrahydrofolic acid into the 'C<sub>1</sub>' pool (Fig. 7). The studies reported here further establish that the metabolic fate of

ALA is not exclusively associated with porphyrin biosynthesis and that this amino acid can be respired to CO<sub>2</sub>.

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