Proc. Nat. Acad. Sci. USA
Vol. 72, No. 7, pp. 2719–2723, July 1975
Cell Biology

Biosynthesis of δ-aminolevulinic acid from the intact carbon skeleton of glutamic acid in greening barley

(chlorophyll/photosynthesis/porphyrins/levulinic acid/plastids)

SAMUEL I. BEALE, SIMON P. GOUGH*, AND S. GRANICK

The Rockefeller University, New York, N.Y. 10021

Contributed by S. Granick, April 24, 1975

ABSTRACT **The customary route in animals and bacteria for δ-aminolevulinic acid biosynthesis is from glycine and succinyl CoA, catalyzed by the enzyme δ-aminolevulinic acid synthetase [succinyl-CoA:glycine *C*-succinyltransferase (decarboxylating), EC 2.3.1.37]. Attempts to demonstrate this route in plants have been unsuccessful. Evidence is given for a new enzymic route of synthesis of δ-aminolevulinic acid in plants. This route involves the incorporation of the intact five-carbon skeleton of glutamic acid into δ-aminolevulinic acid. Demonstration of the new pathway in plants has been made by feeding specifically labeled [^{14}C]glutamic acid to etiolated barley shoots greening in the light. In the presence of levulinate, a competitive inhibitor of δ-aminolevulinic acid dehydratase [porphobilinogen synthase; δ-aminolevulinate hydro-lyase (adding δ-aminolevulinate and cyclizing); EC 4.2.1.24], δ-aminolevulinate accumulates. The δ-aminolevulinate formed was chemically degraded by periodate to formaldehyde and succinic acid. The C_5 (formaldehyde) fragment was separated, as the 5,5-dimethyl-1,3-cyclohexanedione (dimedone) derivative, from the C_1–C_4 (succinic acid) fragment. The C_5 atom contained radioactivity predominantly derived from C_1 of glutamic acid. Conversely, the labeled C_3 and C_4 atoms of glutamic acid were found primarily in the succinic acid (C_1–C_4) fragment of δ-aminolevulinate. This labeling pattern for δ-aminolevulinic acid is consistent with a biosynthetic route utilizing the intact five-carbon skeleton of α-ketoglutarate, glutamate, or glutamine, and is inconsistent with the δ-aminolevulinic acid synthetase pathway utilizing glycine and succinyl CoA as precursors.**

The formation of δ-aminolevulinic acid (ALA) is the first identified step of the tetrapyrrole biosynthetic pathway leading to heme, chlorophyll, vitamin B_{12}, and other specialized plant tetrapyrroles (1). In photosynthetic (2) and nonphotosynthetic bacteria (3), yeast (4), avian erythrocytes (5), and liver (1), and mammalian tissues (6, 7), ALA has been shown to be formed by the enzyme ALA synthetase [succinyl-CoA:glycine *C*-succinyltransferase (decarboxylating), EC 2.3.1.37] postulated to initially yield the unstable product α-amino-β-ketoadipic acid, which then decarboxylates to yield ALA. The released carbon is derived from the carboxyl carbon of glycine (8).

ALA synthetase has not been detected in extracts from any greening plants, including algae. Treatment of plants with [^{14}C]glycine or [^{14}C]succinate has shown the relative inability of these compounds to serve as precursors of ALA in these tissues. Rather it was found that in plants the five-carbon compounds glutamic acid, α-ketoglutarate, and glutamine were more effective in labeling the ALA (9, 10). Therefore, an alternative route of synthesis of ALA in plants appeared to be indicated.

In this paper we report the labeling of specific carbon atoms of ALA by specifically labeled glutamate, clearly indicating that the five-carbon skeleton of glutamate is incorporated intact into ALA. We discuss possible biochemical routes of the labeling pattern observed.

MATERIALS AND METHODS

Materials

Levulinic acid (Lev) and ALA were purchased from Sigma Chemical Co.; [*1*-^{14}C]glutamic acid and [*3,4*-^{14}C]glutamic acid were from ICN Pharmaceuticals, Inc.; [*4*-^{14}C]ALA, [*5*-^{14}C]ALA, and Aquasol were from New England Nuclear; and Dowex 50W-X8, 200–400 mesh was from Bio-Rad Labs.

Methods

Plant Materials. Barley (*Hordeum vulgare* L.cv. Svalof's Bonus) seeds were sown in vermiculite saturated with the nutrient solution of Huffaker *et al.* (11) modified by the omission of KNO_3 and the addition of 1 μM $CuSO_4$. After 7 days of growth in complete darkness at 25°, the terminal 80 mm of the leaves were excised with a razor blade and placed in 10 ml (25 mm diameter) beakers containing 5 ml of distilled water. Samples comprising 5 g of leaves (approximately 60 leaves) per beaker were then subjected to experimental treatment.

Incubation Conditions. The leaf samples were exposed to 400 μW/cm^2 of cool white fluorescent light as measured with a YSI model 65 radiometer (350 lux as measured with a General Electric DW58 exposure meter) at 25° for 3 hr. Then the water from the beakers was removed and replaced with incubation solutions consisting of the appropriate [^{14}C]glutamic acid (5 Ci/mol) and, in some cases, 25 mM levulinic acid, pH adjusted to 6.5 with KOH. In the presence of levulinic acid, ALA-dehydratase [porphobilinogen synthase; δ-aminolevulinate hydro-lyase (adding δ-aminolevulinate and cyclizing); EC 4.2.1.24] is inhibited, thus permitting the accumulation of ALA. Total volume of the incubation solutions was 2.0 ml in all cases. The leaf samples were placed back in the same light and in the air current of a fume hood to facilitate uptake of the solution into the leaves via transpiration. After 2 hr, the incubation solutions were almost completely taken up by the leaves, and at this time the incubation was terminated by placing the leaves in stoppered plastic vials and then freezing them in powdered dry ice.

Abbreviations: ALA, δ-aminolevulinic acid; ALA-pyrrole, 2-methyl-3-carbethoxy-4-(3 propionic acid)-pyrrole; Lev, levulinic acid.

* On leave from: Department of Physiology, Carlsberg Laboratory, Gl. Carlsbergvej 10, DK-2500 Copenhagen Valby, Denmark.

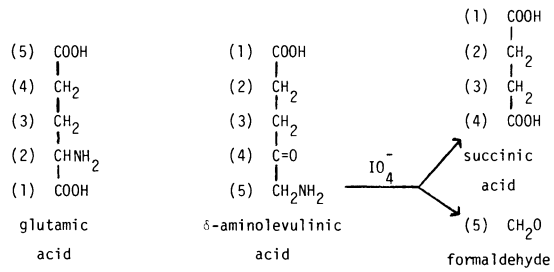

FIG. 1. Illustration of the carbon numbering system for glutamic acid, δ-aminolevulinic acid, and the periodate cleavage products, succinic acid and formaldehyde.

Extraction of ALA. The frozen leaf samples were ground in 10 ml of ice-cold 0.5 M $HClO_4$ in a VirTis homogenizer run at full speed for 2 min. The homogenates were centrifuged at 0° for 15 min at 12,000 × *g* (in a Sorvall RC2-B centrifuge). The supernatants were decanted and the pH was adjusted to 2.0 with 5 M KOH. (The ALA is stable in dilute acid.) After standing overnight at 4°, they were centrifuged again at 0° for 15 min at 12,000 × *g* to remove the precipitated $KClO_4$.

Chromatography on Dowex 50. Columns of Dowex 50W-X8, 200–400 mesh, 9.5 mm in diameter and 35 mm high were washed with 1 M NaOH and then with Buffer A (0.2 N in Na^+, pH adjusted to 3.07 with citric acid) until the effluent pH was about 3. The supernatant solutions from the $KClO_4$ precipitation step were applied to the columns, followed by 20 ml of Buffer A. This elutes organic acids and other materials which are not cationic at pH 3.07. Then the columns were eluted with buffer B (0.2 N Na^+, pH adjusted to 5.10 with citric acid). The first 5 ml were discarded and the next 10 ml, containing the ALA, were collected and subjected to further analysis.

Purification of ALA as a Pyrrole. Two milliliter portions of the ALA-containing fractions from the Dowex chromatography were brought to pH 2.5 with HCl, then extracted three times with 2 ml portions of diethyl ether. Next, the pH of the aqueous phase was raised to 6.8 with 0.5 M Na_3PO_4, then 0.1 ml of ethylacetoacetate was added and the contents were heated at 90° for 15 min in a water bath, to form 2-methyl-3-carbethoxy-4-(3-propionic acid)-pyrrole (ALA-pyrrole) (12). After cooling, the pH was again lowered to 2.5 with HCl, and three more ether extractions were performed. These three extracts containing the pyrrole were combined and backwashed once with 1 ml of water, and after evaporation of the ether the residue was assayed for radioactivity. This method is modified from one previously reported (9).

Periodate Cleavage of ALA. Portions of the ALA-containing fractions from the Dowex chromatography were treated with periodate under alkaline conditions (fully described in the *Results* section). This method has been reported to be successful for the cleavage of aminoethanol (13) and ALA (8). The cleavage products of ALA are formaldehyde and succinic acid (8).

Purification of Formaldehyde As the 5,5-dimethyl-1,3-cyclohexanedione (Dimedone) Derivative. The formaldehyde formed on periodate oxidation of ALA was precipitated as the dimedone derivative, and purified by reprecipitation (fully described in the *Results* section).

Purification of Succinic Acid Produced by the Periodate Reaction. After precipitation of the dimedone derivative from the reaction mixture, the supernatant was extracted three times with ether at pH 6 to remove any dimedone or its derivative in solution. Then the pH was lowered to 3 with HCl, and the succinic acid was extracted five times with ether. These ether extractions were combined, the ether was evaporated, and the residue was analyzed for radioactivity.

Radioactive Analysis. All samples were dissolved or suspended in Aquasol and counted in a Searle Isocap 300 liquid scintillator.

Determination of ALA. ALA was condensed with ethylacetoacetate by the method of Mauzerall and Granick (12). The absorption of light at 553 nm in modified Ehrlich reagent was measured with a Cary model 15 spectrophotometer.

RESULTS

Test for Separation of Glutamate from ALA on Dowex 50. A column of Dowex 50 was prepared as described in the *Materials and Methods* section. A solution consisting of 12.5 ml of saturated $KClO_4$, pH 2, and containing 910,000 cpm of [*1*-^{14}C]glutamic acid and 90 μg of unlabeled ALA, was applied. The column was eluted first with 20 ml of buffer A, then with 15 ml of buffer B. Buffer A eluted more than 90% of the radioactivity and less than 2% of the ALA. The fraction consisting of the final 10 ml of the buffer B effluent contained 98.1% of the applied ALA and only 1800 cpm (0.20% of the applied radioactivity).

Periodate Oxidation of ALA. In a stoppered flask, 50 mg of ALA·HCl and 75 mg of H_5IO_6 were dissolved in 20 ml of water, then 1.25 ml of 0.5 M Na_3PO_4 was added, and the pH was raised to 8.5 with NaOH. After 60 min at 25°, 92.2 mg of dimedone was added and the pH was lowered to 5.6 with HCl. After 60 min more at 25°, the solution was cooled in an ice bath, and methylenebisdimedone was separated by filtration, dried, and weighed. The yield was 77.1 mg (87% yield). The filtrate was acidified to pH 3 with HCl, then extracted with five 20 ml portions of diethyl ether. The combined ether extracts were evaporated and weighed, yielding 19.4 mg of succinic acid (55% yield). If ethyl acetate was used instead of ether, the yield of succinic acid was raised to 87.5%, but ether was chosen in all succeeding experiments, because of its better exclusion of glutamate and ALA. The periodate was found to consume 95% of the original ALA in 60 min at 25°. The scheme for periodate cleavage is shown in Fig. 1 together with the numbering system used.

The above experiment was performed with solutions to which were added 5 μCi of [^{14}C]ALA, labeled either in C_4 or C_5. With labeled [*4*-^{14}C]ALA, only 0.064% of the applied radioactivity was found in the C_5 (i.e., formaldehyde) fragment and 64% was found in the succinic acid fragment. With [5-^{14}C]ALA, 67% of the label was found in the C_5 fragment and only 2% was found in the succinic acid fragment.

The method can be seen to effectively separate C_5 as the dimedone derivative of the formaldehyde, formed on periodate oxidation, from the remainder of the molecule, which is converted to succinic acid by the periodate.

Biosynthesis of [^{14}C]ALA from [^{14}C]Glutamate in Greening Barley. Five gram samples of 7-day-old etiolated barley seedlings consisting of the terminal 80 mm of the leaf blades, approximately 60 per sample, were exposed to dim light for 3 hr, then for 2 more hr while they transpired solutions of [^{14}C]glutamic acid and Lev. Samples 3 and 4 (Table 1) contained [*1*-^{14}C]glutamic acid and 25 mM Lev, while samples 5 and 6 contained [*3,4*-^{14}C]glutamic acid and 25

Table 1. Accumulation of [^{14}C]ALA in greening barley leaves

Solution applied to leaves			Total accumulated ALA			Periodate cleavage products			
						C_5		C_1 through C_4	
Sample no.	Lev (mM)	Source of label	ALA (nmol)	Radioactivity (kcpm)*	% of applied label	Radioactivity (kcpm)	% of total	Radioactivity (kcpm)	% of total
1	0	[*1*-^{14}C]Glu	52	1.8	0.03	—	—	—	—
2	0	[*3,4*-^{14}C]Glu	40	13.8	0.15	—	—	—	—
3	25	[*1*-^{14}C]Glu	720	54.4	0.65	41.3	76	4.1	7.5
4	25	[*1*-^{14}C]Glu	660	47.2	0.57	37.4	79	3.9	8.3
5	25	[*3,4*-^{14}C]Glu	670	65.1	0.82	1.6	2.4	38.6	59
6	25	[*3,4*-^{14}C]Glu	710	75.3	0.95	1.7	2.3	45.5	60

Accumulation was measured after 2 hr of incubation in the light with Lev plus 8 × 10^6 cpm of specifically labeled [^{14}C]glutamic acid (Glu). The ALA was purified and subjected to alkaline periodate cleavage as described in the *text*.
* Abbreviation: kcpm, cpm × 10^3.

mM Lev. Samples 1 and 2 had [*1*-^{14}C]- and [*3,4*-^{14}C]glutamic acid, respectively, but no Lev. Since samples 1 and 2 did not accumulate ALA in the absence of Lev (Table 1), they served as controls for label incorporation into products other than ALA, which might have co-separated with ALA or its degradation products.

A portion of the accumulated ALA was purified by methods which were earlier reported to yield radiohomogenous ALA-pyrrole (9). As seen in the Table 1, [*1*-^{14}C]- and [*3,4*-^{14}C]glutamic acid contributed approximately equal radioactivity to the ALA, in agreement with previously reported results (9, 10). In the absence of Lev (samples 1 and 2) the ^{14}C incorporation into this fraction was greatly reduced.

Periodate Cleavage of Accumulated ALA. Portions of the accumulated ALA of samples 3 through 6 were treated with periodate. As seen in Table 1, C_5 of ALA (as the dimedone derivative of formaldehyde) was found to be labeled 33 times more effectively by C_1 than by C_3 and C_4 of glutamate. Conversely, C_1 through C_4 of ALA were labeled 7.5 times more effectively by C_3 and C_4 of glutamate than by C_1 of glutamate.

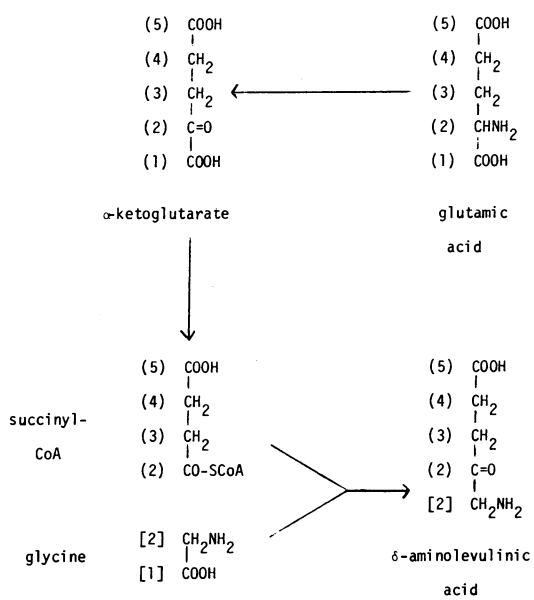

FIG. 2. Illustration of the incorporation of glutamic acid, succinic acid, and glycine into ALA via the ALA synthetase reaction. Note that neither the carboxyl carbon of glycine nor the C_1 carboxyl carbon of glutamic acid are incorporated into the ALA.

DISCUSSION

Conflicting Evidence for the Existence of ALA Synthetase in Plants. Evidence for the existence of ALA synthetase in plants is both fragmentary and contradictory. One preliminary report of the enzyme from spinach leaves was not sufficiently detailed to allow for evaluation of the methods or results (14). [^{14}C]Glycine has been supplied to greening *Chlorella* (15) and wheat seedlings (16), and radioactivity was incorporated into the phorbide moiety of chlorophyll. However, both carbon atoms of glycine contributed to the chlorophyll (15, 16), indicating an indirect route, rather than the succinyl transferase reaction, where the carboxyl carbon of glycine is lost (Fig. 2). Also, the glycine was incorporated less effectively than the more general metabolic intermediate acetate, again indicating an indirect route of incorporation of glycine (15, 16). The succinyl transferase reaction has been reported in extracts of nongreening soybean callus tissues (17). However, the enzyme was not characterized with respect to substrate and cofactor requirements, nor was the possibility excluded that the ALA was formed indirectly from the substrates given. Similarly, the enzyme has been reported in cold-stored potato peel (18). In this case, the reaction seemed to occur to some degree even in a purified enzyme preparation in the absence of the co-factors CoA, ATP, Mg^{++}, and pyridoxal phosphate, all of which are required for the transferase system from other sources. The preferential incorporation of the aminomethyl carbon of glycine compared to the carboxyl carbon was not shown. Finally, the possibility was not excluded that the activity observed was due to contaminating microorganisms in the aged potato peel. Thus, these preliminary claims of ALA synthetase in plant tissue must be accepted with caution until they are confirmed under more rigorously controlled conditions.

Demonstration of the Formation of ALA in Greening Plant Tissues. Although ALA has been amply demonstrated to be the precursor of both heme (8) and chlorophyll (19, 20), the synthesis of ALA in plants could be shown only when it was discovered that its metabolism was blocked *in vivo* by Lev, a competive inhibitor of ALA dehydratase (21, 22). This enzyme forms the monopyrrole, porphobilinogen, from two molecules of ALA (8). It was determined by sol-

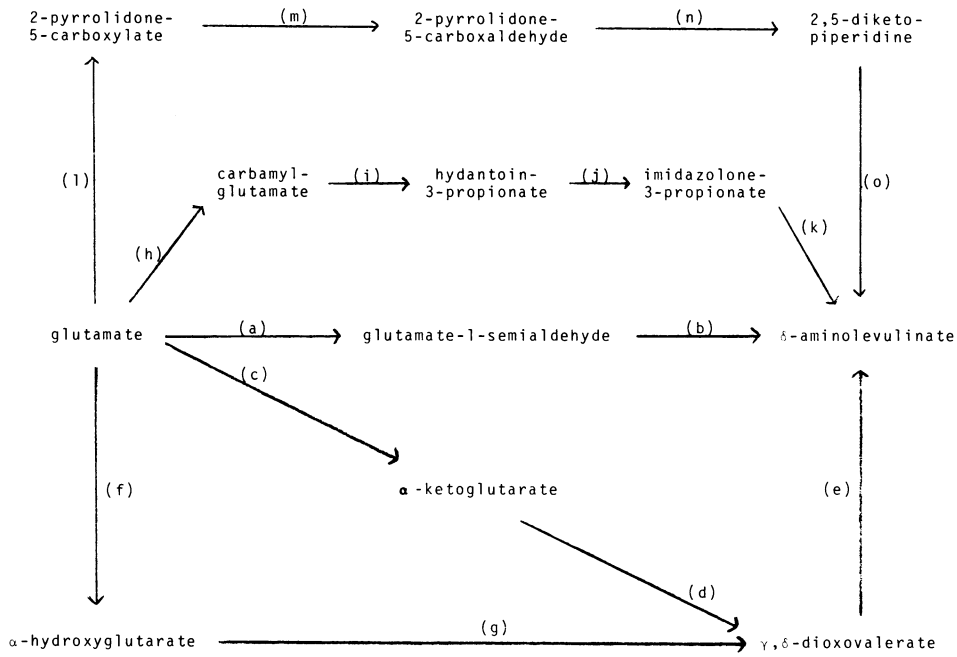

FIG. 3. Possible routes of ALA biosynthesis from glutamic acid. See *text* for discussion.

vent partitioning, paper chromatography, and gas–liquid chromatography that the accumulated material is ALA, and not aminoacetone, a compound present in animal tissues, which often interferes with ALA determinations (9). It was furthermore found that the ALA which accumulates in the presense of Lev in *Chlorella* is not formed from the carbon atoms of the Lev (21).

Evidence for the Formation of ALA from Glutamate in Greening Plants. After it became possible to divert ALA away from the chlorophyll pathway through the use of Lev, attempts were made to feed labeled ALA precursors to greening plant tissues in the presence of Lev, and measure the label incorporation into the accumulated ALA (9, 10). It was found that [^{14}C]glycine and [^{14}C]succinic acid were only poorly incorporated into ALA, in contrast to as much as 30-fold greater incorporation of the five-carbon compounds glutamate, α-ketoglutarate and glutamine. Because both glutamic acid and glycine were respired at comparable rates by the tissues studied, the differences in their ability to label ALA could not be attributed to differences in permeability of the cell membranes to these compounds.

Beale and Castelfranco (9, 10) postulated a novel route for ALA biosynthesis in plants, based on the following evidence:

(*1*) In greening bean and barley leaves, and cucumber cotyledons, neither glycine nor succinate are effective ^{14}C contributors to the ALA that accumulates in the presence of Lev. Furthermore, both carbons of glycine are incorporated to the same extent (9, 10). In contrast to these results, in the succinyl transferase found in animal and bacterial systems, glycine and succinate both contribute ^{14}C to the ALA. The C_2 of glycine is incorporated, but C_1 is excluded (8) (Fig. 2).

(*2*) Glutamic acid, α-ketoglutarate, and glutamine are all effective contributors of ^{14}C to ALA in these plant tissues. Furthermore, C_1 of glutamate enters the ALA to the same extent as C_3 and C_4. Therefore, the route is not via α-ketoglutarate dehydrogenase, forming succinyl CoA, for in this reaction the C_1 of α-ketoglutarate is lost (8) (Fig. 2).

A recent report by Castelfranco and Jones provides further support for ALA biosynthesis from glutamate in greening higher plants (23). They found that both heme and chlorophyll are labeled by [^{14}C]glutamate much more effectively than by [^{14}C]glycine in greening barley. It is still possible, however, that the succinyl transferase pathway operates to a small extent in the mitochondria and that the major, 5-carbon pathway may occur in the plastids, where the majority of the heme is found, and all of the chlorophyll is made, in greening plant tissues (23).

In a recent study, Porra and Grimme (24) reported the incorporation of [^{14}C]glycine and [^{14}C]succinate into ALA formed in the presence of Lev in regreening nitrogen-starved *Chlorella fusca*. Neither [^{14}C]glutamate nor α-keto[^{14}C]glutarate were incorporated, but the ability of these compounds to enter the cells was not measured. Nevertheless, we must consider the possibility that higher plants and algae may have different routes of ALA biosynthesis, or that, under different physiological conditions, ALA may arise from different sources.

The experimental results reported here support the conclusion that ALA in greening plant tissues is formed by a route other than that found in animals and bacteria. A rather direct route from glutamate is indicated by the low degree of randomization of the label position from glutamate to ALA during the 2 hr incubation period (Table 1).

The exclusive labeling of C_5 of ALA from C_1 of glutamate, and the remainder of the ALA molecule by C_3 and C_4 of glutamate, indicates that the carbon skeleton of glutamate is converted to ALA intact.

Hypothetical Pathways for ALA Formation from the Intact Carbon Skeleton of Glutamic Acid. In Fig. 3 we have outlined various hypothetical pathways for ALA biosynthesis from glutamate in a manner compatible with our labeling results. Some of these pathways have been previously considered (9, 25). Basically three modifications of the glutamate molecule are required to form ALA: reduction of C_1 from the acid to aldehyde state of oxidation; oxidative removal of the amino group from C_2; and reductive addition of an amino group at C_1. The most direct route would be a two-step process consisting of reduction of C_1 of glutamate

(Fig. 3, step a) followed by internal transamination (step b). The intermediate compound, glutamate-1-semialdehyde, has not been reported in plant extracts.

The sequence c–d–e is particularly attractive because step c is the well known glutamate transaminase reaction, and step e, the transamination of γ,δ-dioxovalerate, has been shown to occur in extracts of *Chlorella* (26) and *Rhodopseudomonas* (27). There is presently no evidence for the occurrence of γ,δ-dioxovalerate in plant tissues, and the apparent lability of this compound suggests that it may exist *in vivo* only as an enzyme-bound intermediate.

Another interesting hypothetical three-step sequence is f–g–e. The internal oxidation-reduction of α-hydroxyglutarate to γ,δ-dioxovalerate (step g) is analogous to reversal of the glyoxalase reaction, which catalyzes the rearrangement of methylglyoxal to lactate. The glyoxalase system has been reported to catalyze the reverse of step g (28).

Two different four-step sequences also can be imagined. There is no direct evidence for the occurrence of either of them in greening plants. Sequence h–i–j–k entails carbamylation of glutamate (step h) analogous to the carbamylation of aspartate in the pyrimidine pathway. This is followed by dehydrative cyclization (step i), then reduction of the hydantoin (step j) in a manner similar to reversal of the histidine degradative pathway. Hydrolytic cleavage of the imidazolone propionate (step k) should yield NH_3, CO_2, and ALA. Sequence l-m–n–o involves dehydration of glutamate to 2-pyrrolidone-5-carboxylic acid (step l), reduction to the aldehyde (step m), internal transamination to 2,5-diketopiperidine (step n), then hydrolysis (step o) to ALA.

Other Pathways Now Considered Unlikely. In addition to ALA synthetase, two other previously considered routes for ALA synthesis in greening plants are not compatible with the ^{14}C labeling patterns we report here. One of these involves the cyclization of glutamate to a proline-like intermediate, followed by cleavage of the carbon-nitrogen bond which was originally the α-amino bond of glutamate; the nitrogen then becomes the amino group of ALA (9). In this scheme, C_1 of glutamate would become C_1 of ALA, and C_5 of glutamate would appear as C_5 of ALA. The results of the present paper indicate that this proposed route is not the pathway of ALA synthesis in greening barley. The other previously considered route involves condensation of glyoxalate with propionyl CoA or α-ketoglutarate to yield δ-hydroxylevulinic acid, which might be converted to ALA either by direct amination, or via oxidation to γ,δ-dioxovaleric acid, then transamination (25). Indeed, ALA has been reported to be formed from δ-hydroxylevulinic acid in rat liver mitochondria (29), the δ-hydroxylevulinic acid, in turn, being formed from α-ketoglutarate and glyoxalate. In this pathway the carboxyl carbon of glyoxalate and the α-carboxyl carbon of α-ketoglutarate are lost as CO_2. Therefore, C_1 of glutamate would not be incorporated into ALA, contrary to our present results with plant tissues.

Summary. We have examined the ^{14}C distribution in ALA formed *in vivo* from exogenous specifically labeled [^{14}C]glutamic acid in greening barley leaves. Our results indicate that the carbon skeleton of glutamic acid is incorporated intact into the ALA, in a manner which is incompatible with the ALA synthetase route of formation from succinyl-CoA and glycine. The results presented here allow us to narrow our direction in the present search for an *in vitro* ALA-forming system in greening etiolated higher plants.

Note Added in Proof: We have recently determined that in greening barley the α-keto[*1*-^{14}C]glutarate and α-keto[5-^{14}C]glutarate are both incorporated into ALA two to four times more efficiently than the correspondingly labeled [^{14}C]glutamate isotopes. This result suggests that α-ketoglutarate is an intermediate in the pathway from glutamate to ALA, and lends further support to the Scheme c–d–e of Fig. 3.

We thank Dr. Stanford Moore for suggestions about the chromatographic separations, and Dr. Charlotte S. Russell for information on the periodate cleavage of ALA. We also wish to thank Dr. Alton Meister for his critical reading of the manuscript. This work was supported by National Science Foundation Grant no. GB-39884. S.P.G. was supported in part by a grant from the Carlsberg Foundation.

1. Granick, S. & Sassa, S. (1971) in *Metabolic Regulation*, ed. Vogel, H. J. (Academic Press, New York), Vol. V, pp. 77–141.
2. Kikuchi, G., Kumar, A., Talmage, P. & Shemin, D. (1958) *J. Biol. Chem.* **233**, 1214–1219.
3. Tait, G. H. (1972) *Biochem. J.* **128**, 32p.
4. Porra, R. J., Irving, E. A. & Tennick, A. M. (1972) *Arch. Biochem. Biophys.* **149**, 563–565.
5. Gibson, K. D., Laver, W. D. & Neuberger, A. (1958) *Biochem. J.* **61**, 618–629.
6. Marver, H. S., Collins, A., Tschudy, D. P. & Rechcigl, M., Jr. (1966) *J. Biol. Chem.* **241**, 4323–4329.
7. Bottomley, S. S. & Smithee, G. A. (1968) *Biochim. Biophys. Acta* **159**, 27–37.
8. Shemin, D., Russell, C. S. & Abramsky, T. (1955) *J. Biol. Chem.* **215**, 613–626.
9. Beale, S. I. & Castelfranco, P. A. (1974) *Plant Physiol.* **53**, 297–303.
10. Beale, S. I. & Castelfranco, P. A. (1973) *Biochem. Biophys. Res. Commun.* **52**, 143–149 (printer's error corrected in Vol. 53).
11. Huffaker, R. C., Obendorf, R. L., Keller, C. J. & Kleinkopf, G. E. (1966) *Plant Physiol.* **41**, 913–918.
12. Mauzerall, D. & Granick, S. (1956) *J. Biol. Chem.* **219**, 435–446.
13. Artom, C. (1957) in *Methods in Enzymology*, eds, Colowick, S. P. & Kaplan, N. O. (Academic Press, New York), Vol. IV, pp. 832–833.
14. Miller, J. W. & Teng, D. (1967) *Proc. Plenary Sess. Int. Congr., Biochem. 7th*, p. 1059.
15. Della Rosa, R. J., Altman, K. I. & Solomon, K. (1953) *J. Biol. Chem.* **202**, 771–779.
16. Roberts, D. W. A. & Perkins, H. J. (1966) *Biochim. Biophys. Acta* **127**, 42–46.
17. Wider de Xifra, E. A., Del C. Battle, A. M. & Tigier, H. (1971) *Biochim. Biophys. Acta* **235**, 511–517.
18. Ramaswamy, N. K. & Nair, P. M. (1973) *Biochim. Biophys. Acta* **293**, 269–277.
19. Nadler, K. & Granick, S. (1970) *Plant Physiol.* **46**, 240–246.
20. Rebeiz, C. A. & Castelfranco, P. A. (1971) *Plant Physiol.* **47**, 33–37.
21. Beale, S. I. (1971) *Plant Physiol.* **48**, 316–319.
22. Beale, S. I. & Castelfranco, P. A. (1974) *Plant Physiol.* **53**, 291–296.
23. Castelfranco, P. A. & Jones. O. T. G. (1975) *Plant Physiol.* **55**, 485–490.
24. Porra, R. J. & Grimme, L. H. (1974) *Arch. Biochem. Biophys.* **164**, 312–321.
25. Tait, G. H. (1968) in *Porphyrins and Related Compounds*, ed. Goodwin, T. W. (Academic Press, New York), pp. 19–34.
26. Gassman, M., Pluscec, J & Bogorad, L. (1968) *Plant Physiol.* **43**, 1411–1414.
27. Neuberger, A. & Turner, J. M. (1963) *Biochim. Biophys. Acta* **67**, 342–345.
28. Jerzykowski, T., Winter, R. & Matuszewski, W. (1973) *Biochem. J.* **135**, 713–719.
29. Wang, F. K., Koch, J. & Stokstad, E. L. R. (1970) *Biochem. Biophys. Res. Commun.* **40**, 576–582.