Biosynthesis of δ-Aminolevulinic Acid from Glutamate in *Agmenellum quadruplicatum*[1]

Judith A. Kipe-Nolt and S. Edward Stevens Jr.
Department of Microbiology and Cell Biology, The Pennsylvania State University, University Park, Pennsylvania 16802

ABSTRACT

δ-Aminolevulinic acid accumulated in the culture medium when *Agmenellum quadruplicatum* strain PR-6 was incubated in the presence of levulinic acid, a competitive inhibitor of δ-aminolevulinic acid dehydratase, and specifically labeled glutamate and glycine. The δ-aminolevulinic acid was purified using Dowex 50W-X8 and cleaved by periodate to yield succinic acid and formaldehyde. The distribution of radioactivity in the two fragments suggested that in blue-green algae the carbon skeleton of δ-aminolevulinic acid is derived directly from glutamate. However the possibility of the pathway of δ-aminolevulinic acid synthesis, from glycine and succinyl-coenzyme A also functioning in blue-green algae was not eliminated as uptake of glycine was minimal.

δ-Aminolevulinic acid is the first identified biosynthetic intermediate that is unique to the tetrapyrrole pathway. ALA[2] is the precursor of heme in animals (16) and bacteria (9); of Chl in bacteria (6) and plants (8); and of the phycobilins in red algae (19). In many organisms ALA is formed by the condensation of glycine and succinyl-CoA, catalyzed by ALA synthetase, a pyridoxal-requiring enzyme. The enzymatic activity was first demonstrated in photosynthetic bacteria (12) and chicken erythrocytes (7). ALA synthetase has since been reported in yeast, bacteria, and a number of animal tissues. However, workers have been unable to demonstrate conclusively ALA synthetase activity in green plants and green algae (2).

In studies in which cucumber cotyledons (3), bean and barley leaves (4), maize leaves (15), and the unicellular rhodophyte *Cyanidium caldarium* (10) were incubated in the presence of levulinic acid and labeled compounds; glutamate, α-ketoglutarate, and glutamine were found to donate label to the ALA that accumulated, much more effectively than glycine. Furthermore, from the specific pattern of incorporation of label into ALA, it appears that ALA is formed from the intact carbon skeleton of glutamate in a manner incompatible with the ALA synthetase route (1, 5, 10, 15).

Herein, we report the labeling of ALA from specifically labeled glutamate by a blue-green alga. The evidence suggests that blue-green algae synthesize ALA from glutamate in a manner similar to that observed in green plants and green algae.

MATERIALS AND METHODS

Organism and Culture Conditions. The unicellular marine blue-

green alga, *Agmenellum quadruplicatum* strain PR-6, was isolated by Van Baalen (20). Broth cultures were grown in medium A (18) in a controlled temperature water bath at 39 C. Continuous agitation and CO_2 were provided by bubbling 4% CO_2 in air (v/v) through the cultures. Illumination consisted of four F24T12 CW/HO fluorescent lamps. A large culture of PR-6 was grown to a concentration of 3×10^7 cells/ml. The cells were centrifuged, resuspended in fresh medium A, and divided into four 30-ml cultures. Levulinic acid was added to give a final concentration of 60 mM (13). After 2-h incubation 7 μCi of radiolabeled compounds were added and incubation was continued for 6 h. The specific radioactivity of the labeled compounds was 40–60 μCi/μmol, therefore the concentration of the amino acids in the culture medium was about 5μM.

ALA Determination. After incubation cell suspensions were centrifuged and ALA in the medium was determined using the colorimetric method of Mauzerall and Granick (14), as described in Kipe-Nolt et al. (13).

Isolation of ALA. ALA was isolated using the method described by Beale et al. (5) and Jurgenson et al. (10). Twenty-five-ml aliquots of culture supernatant were added to Dowex 50W-X8 columns (5-ml bed volume in 10-ml plastic syringes) which had been washed with 10 ml 1 N NaOH and 30 ml sodium citrate buffer (pH 3.1). The columns were washed with 30 ml of the same buffer and ALA was eluted with 25 ml of sodium citrate buffer (pH 5.1). The following control experiments were done to check the isolation procedure: [14C]ALA was added to spent medium A and the recovery of label in the pH 5.1 buffer wash was noted. Labeled glutamate and glycine was also added to spent medium A and the recoveries in the pH 5.1 buffer wash noted.

Periodate Cleavage of ALA. The ALA in the pH 5.1 buffer wash from the Dowex 50W-X8 columns was cleaved with periodate under alkaline conditions as described by Beale et al. (5). The cleavage products are formaldehyde (C_5) and succinic acid (C_1–C_4) (17). The formaldehyde fragment was precipitated as the dimedone derivative, collected on Millipore filters and assayed for radioactivity (10). The succinic acid fragment was extracted from the resulting filtrate using three 30-ml volumes of diethyl ether. The diethyl ether was evaporated to dryness and the residue was assayed for radioactivity.

The procedure was checked by cleaving [4-14C]ALA and [5-14C]ALA and noting recoveries in the formaldehyde and succinic acid fractions. The recovery of label from glutamate and glycine in the two fractions was also checked.

Uptake of Radiolabeled Compounds. One-ml samples, from cultures of PR-6 containing the specifically labeled glutamate and glycine, were removed at 1-h intervals. The samples were centrifuged and the cells washed with two 8-ml volumes of water. The cells were digested with 1 ml Protosol, decolorized with 0.2 ml 20% benzoyl peroxide in toluene (w/v), and counted in Aquasol-2.

Chemicals. Levulinic acid, periodic acid, and 5,5-dimethyl-1,3-

[2] Abbreviation: ALA: δ-aminolevulinic acid.

cyclohexanedione (dimedone) were purchased from Sigma Chemical Co. Dowex 50W-X8, 100–200 mesh, was from Bio-Rad Labs. DL-[1-^{14}C]Glutamic acid, DL-[5-^{14}C]glutamic acid, [1-^{14}C]glycine, [2-^{14}C]glycine, [4-^{14}C]ALA, [5-^{14}C]ALA, Protosol, and Aquasol-2 were from New England Nuclear.

RESULTS AND DISCUSSION

From four control experiments using [^{14}C]ALA, it was determined that 82% of added ALA was recovered in the pH 5.1 buffer wash of the Dowex 50W-X8 columns. This recovery was not as good as that previously described (5), but enough ALA was isolated for the periodate cleavage experiment. It was also determined that a maximum of 1.3% of applied glutamate and 1.8% of applied glycine was recovered in the Dowex eluate. The lower recovery of ALA and the higher contamination of glutamate, in these experiments as compared to previous work (5), was probably a result of the high salt concentration in medium A which is an artificial seawater medium.

When PR-6 was grown in the presence of levulinic acid and specifically labeled glutamate or glycine, ALA accumulated in the medium. The nmol of accumulated ALA and the amounts of labeled ALA that were recovered in the Dowex pH 5.1 buffer washes are shown in Table I. The radioactivity in the Dowex eluates that could have been accounted for by contaminating glutamate or glycine (using the results from the control experiments given above) was subtracted. None of the ALA that accumulated in the glycine treated cultures was labeled, however this could have been a result of poor uptake of glycine by PR-6. The portion of added glutamate that was recovered in ALA was small. This was not surprising as under these conditions the cells were actively synthesizing their own glutamate from nitrate, which they use preferentially to glutamate as an external nitrogen source. The labeled glutamate would thus be diluted, much of it being used for protein synthesis, and even if ALA was synthesized directly from glutamate one would not expect to recover a large portion of the label in ALA. The recovery of label in ALA, from glutamate but not from glycine, was recovered a number of times using incubation times ranging from 1.5 to 6 h and using nitrate or ammonia as the nitrogen sources.

When 4 μCi of [4-^{14}C]ALA was cleaved with alkaline periodate, 1.7% of the radioactivity was found in the C_5 (formaldehyde) fragment while 24% was recovered in the succinic acid fragment. When 4 μCi of [5-^{14}C]ALA was cleaved with alkaline periodate, 3.6% of the radioactivity was found in the succinic acid fragment and 80% was recovered in the formaldehyde fragment. The above results are averages from three experiments, and they show that the C_5 of ALA was effectively separated from C_1–C_4. Less than 2% of the label from alkaline periodate-treated glutamate and glycine was recovered in the formaldehyde and succinic acid fragments.

The distribution of radioactivity in accumulated ALA, when PR-6 was grown in the presence of levulinic acid and labeled glutamate or glycine, is shown in Table II. The data have been corrected to 100% yield, using the recoveries given above. Also, the maximum possible label (in the cleavage products) from contaminating glutamate and glycine has been subtracted. The C_1 of glutamate was incorporated into C_5 of ALA about three times

more effectively than it was incorporated into the succinic acid fragment of ALA, whereas the C_5 of glutamate was incorporated into the succinic acid fragment 16 times more effectively than into C_5. The same distribution of radioactivity in ALA as described above was observed in similar experiments in which higher levulinic acid and cell concentrations were used with incubation periods in the presence of labeled compounds ranging from 1.5 to 6 h. When cultures were incubated in the presence of labeled glutamate and glycine but not levulinic acid (no ALA accumulates) the above distribution of radioactivity was not observed. This pattern of incorporation of label into ALA; C_1 of glutamate into C_5 of ALA and C_5 of glutamate into C_1 to C_4 of ALA was the same as that observed in higher plant tissues (5, 15) and red algae (10). It appears that in blue-green algae at least some of the ALA is formed from the intact carbon skeleton of glutamate, and not by the ALA synthetase route observed in bacteria and animal tissues. A number of pathways for the synthesis of ALA from glutamate have been proposed (2, 11).

The possibility of the pathway of ALA synthesis, from glycine and succinyl-CoA, also functioning in blue-green algae was not eliminated as uptake of glycine was minimal. Uptake of specifically labeled compounds is shown in Figure 1. There was a slow, but continuous uptake of [^{14}C]glycine. Over the 5-h time period only 1% of the applied label was taken up by the cells. Even if PR-6 synthesizes ALA from glycine and succinyl-CoA as well as from glutamate, very little label from added glycine would appear in ALA. Varying the pH, the nitrogen source and concentration in the culture medium, and adding cold glycine to the medium,

Table II. *Distribution of Label in Periodate Cleavage Products of ALA*

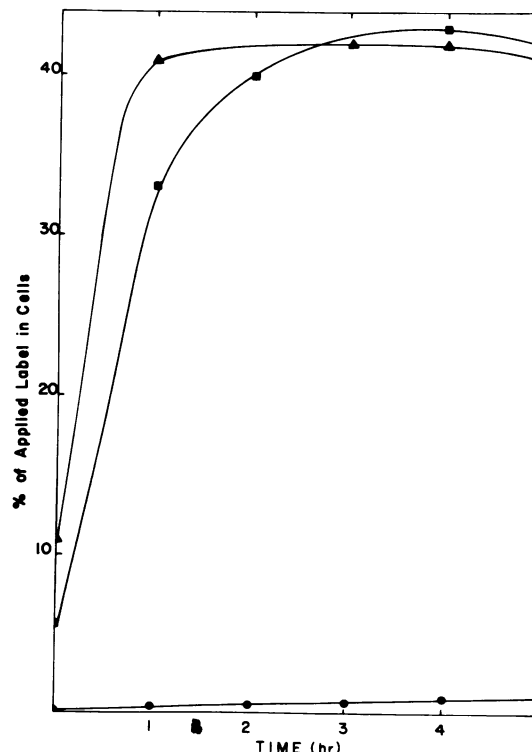| Source of Label	C_5	C_1–C_4
[1-^{14}C]Glutamate	20%	6.3%
[5-^{14}C]Glutamate	1.7%	27.5%
[1-^{14}C]Glycine	0	0
[2-^{14}C]Glycine	0	0

FIG. 1. Uptake of radiolabeled compounds by PR-6. (▲): [1-^{14}C]Glutamate; (■): [5-^{14}C]glutamate; (●): [^{14}C]glycine.

Table I. *Accumulation of ALA*

Source of Label	Labeled Compound Added	ALA Accumulated	Labeled ALA
		nmol	
[1-^{14}C]Glutamate	75	210	0.4
[5-^{14}C]Glutamate	63	218	0.3
[1-^{14}C]Glycine	56	225	0
[2-^{14}C]Glycine	76	210	0

were tested unsuccessfully, to increase the uptake of labeled glycine. Both [1-^{14}C]glutamate and [5-^{14}C]Glutamate were taken up rapidly in the 1st h, then uptake leveled off at around 40% of the applied label. There was a doubling time in the cultures of 6.3 h.

It can be concluded from this study that in the procaryotic blue-green algae, as in eucaryotic plants and algae, a major portion of ALA is probably formed from the intact carbon skeleton of glutamate.

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