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Biosynthesis of δ-Aminolevulinic Acid by Blue-Green Algae (Cyanobacteria)

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When levulinic acid, a competitive inhibitor of δ-aminolevulinic acid dehydratase, was added to growing cultures of blue-green algae (cyanobacteria), δ-aminolevulinic acid was excreted into the medium and cell growth was inhibited.

δ-Aminolevulinic acid (ALA) is the first identified biosynthetic intermediate that is unique to the tetrapyrrole pathway. ALA is the precursor of heme in animals (17) and bacteria (10) and of chlorophyll in bacteria (5) and plants (7). Troxler and Brown (19) have shown that radioactively labeled ALA is incorporated into the chromophores (phycocyanobilin and phycoerythrobilin) of phycocyanin and phycoerythrin by red algae. Levulinic acid (LA), which is structurally similar to ALA except for the absence of the amino group, has been shown by Nandi and Shemin (15) to be a competitive inhibitor of ALA dehydratase. ALA dehydratase catalyzes the condensation of two molecules of ALA to form monopyrrole porphobilinogen. Beale (1, 2) demonstrated that LA could be used to induce accumulation of ALA in the medium of a culture of *Chlorella*. LA has subsequently been used for the same purpose in greening higher plant tissues (3, 4, 8), bacteria (9), *Euglena* (16), and *Cyanidium caldarium* (11). The present study reports the use of LA to demonstrate the synthesis of ALA in blue-green algae (cyanobacteria). We believe this to be the first direct demonstration of ALA biosynthesis by the blue-green algae.

Agmenellum quadruplicatum PR-6, *Anacystis marina* 6, and *Coccochloris elabens* Di (20) were grown in medium A (18) at 39°C. *Anacystis nidulans* TX-20 (13) was grown in medium C (18) at 39°C. *Nostoc muscorum*, *Nostoc* sp. strain MAC, and *Anabaena flos-aquae* were grown in medium B (18) at 35°C. Continuous agitation and CO_2 were provided by bubbling 4% (vol/vol) CO_2 in air through all cultures. Illumination consisted of four (two on each side of the bath) F24T12 CW/HO fluorescent lamps. Growth was measured turbidimetrically with a Bausch and Lomb Spectronic 20 spectrometer at 550 nm or by viable cell count (21).

A stock solution of 1 M LA was prepared, adjusted to pH 7.6 with NaOH, filter sterilized (0.45-μm Millipore filter), and added to growing algal cultures. ALA was condensed with acetylacetone and then estimated as the 2-methyl-3-acetyl-4-(3-propionic acid) pyrrole derivative with Ehrlich reagent (14).

ALA produced by *A. quadruplicatum* PR-6 in the presence of 60 mM LA was verified against genuine ALA on silica gel thin-layer plates. ALA was isolated from spent medium (6, 11), converted to the ALA pyrrole (14), developed on thin-layer plates with *n*-butanol-1 N NH_4OH (1:1, vol/vol), and visualized with Ehrlich reagent.

The time course of ALA production by strain PR-6 in the presence of 60 mM LA is shown in Fig. 1. Growth or increase in cell number was completely inhibited when this concentration of LA was added to a culture with an initial cell number of 3.0×10^7 cells per ml. Prior to addition of LA, extracellular ALA was not detectable in the culture fluid. However, upon addition of LA, growth ceased and ALA began to accumulate. Continuous accumulation was observed for up to 48 h, suggesting that ALA does not feedback inhibit its own synthesis.

The amounts of ALA excreted into the medium by strain PR-6 in 20 h when different concentrations of LA were added to different initial cell densities are shown in Fig. 2. Five different concentrations of LA were added to five different culture tubes, all with the same initial cell concentration. After 20 h, the amount of ALA that had accumulated in the medium in each tube was determined. This experiment was repeated four times using four different initial cell concentrations. Cell growth and the amount of ALA accumulated depended on LA concentration and initial cell number.

The saturation-type kinetics of ALA production at different LA concentrations observed in this study were similar to those observed in maize leaves (12). However, they were unlike that observed in a number of other studies in-

cluding bean leaves (12), barley leaves (4), cucumber cotyledons (4), and *C. caldarium* (11); in this last case there was an LA concentration at which maximum ALA was produced, and at higher LA concentrations less ALA was produced.

In cultures with LA concentrations above saturation, cell growth (division) was completely inhibited. However, if the cells were centrifuged and fresh medium without LA was added, the cells recovered after a short lag and growth was normal. In cultures with LA concentrations below saturation, there was an increase in growth rate with a decrease in LA concentration.

The compound that was excreted by strain PR-6 in the presence of LA formed an Ehrlich-positive pyrrole when boiled in acetylacetone. The pyrrole had an R_f of 0.2 in butanol-ammonia, which corresponded to standard ALA-pyrrole. This verified that the compound excreted by strain PR-6 in the presence of LA was ALA.

The usefulness of LA for inhibiting ALA dehydratase was extended to *Nostoc* sp. strain MAC, *A. nidulans* TX-20, *C. elabens* Di, *A. marina* 6, *A. flos-aquae*, and *N. muscorum*. These include unicellular and filamentous algae, marine and freshwater algae, nitrogen-fixing and non-nitrogen-fixing algae, obligate autotrophs, and a facultative heterotroph. Thus, the accumulation of ALA in the presence of LA seems to be a general characteristic among blue-green algae. Gratifyingly unsurprising, our results remove a tacit assumption and exchange it for a fact.

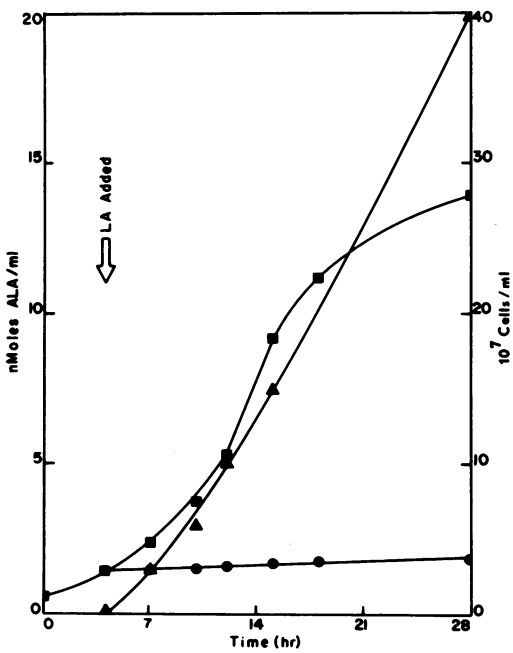

FIG. 1. *Production of ALA and growth of a culture of strain PR-6 in the presence of 60 mM LA.* (▲) *Nanomoles of ALA per milliliter;* (●) *cell number of LA-treated culture;* (■) *cell number of culture without LA.*

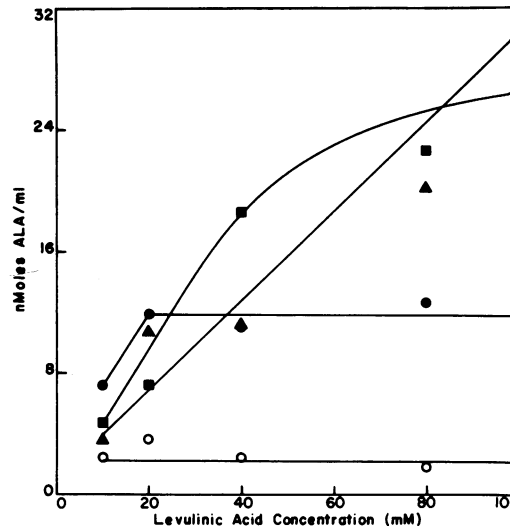

FIG. 2. *Nanomoles of ALA produced per milliliter in 20 h when different concentrations of LA were added to different initial cell densities. Initial cell numbers:* (○) 1.4×10^7 *cells per ml;* (●) 2.7×10^7 *cells per ml;* (■) 5.7×10^7 *cells per ml;* (▲) 8.1×10^7 *cells per ml.*

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