Biosynthesis of δ-aminolaevulic acid by extracts of *Rhodopseudomonas spheroides*

There is strong evidence to support the hypothesis that the initial reaction in porphyrin synthesis is the condensation of glycine with an asymmetric derivative of succinate to form ALA*[1]. A recent report from this department described the isolation of a particulate system from anaemic chickens which catalyses a net synthesis of ALA from glycine and α-ketoglutarate or succinate[2]; and it was later found that after suitable treatment the system forms ALA from glycine and succinyl-CoA[3]. It has how been found that extracts of *Rps. spheroides* carry out the same reaction. The work of LASCELLES[4] indicates that this organism forms ALA when grown anaerobically in the light; however, it appears that there is also considerable ALA formation by extracts of organisms grown aerobically in the dark. This report is concerned only with dark-grown organisms.

The strain of *Rps. spheroides* was that used by LASCELLES[4]. Cells were grown aerobically in medium "S" at 34° from a 1% inoculum of light-adapted organisms grown as described by LASCELLES; they were harvested after 20–24 h, by which time the density of organisms was 0.7–0.9 mg dry wt./ml. The cells were suspended in 0.05 *M* triethanolamine buffer, pH 7.4 (about 10 ml/g dry wt.) and disintegrated on a Mullard ultrasonic generator, type E 759 OA (25 kc/sec for 30 min). The suspension was centrifuged for 1 h at 105,000 × *g*; the clear pink solution contained all the activity.

It was found that extracts made in this manner rapidly metabolize ALA aerobically or *in vacuo*. However after storage for 3–5 days at —20° they lose some of their ability to destroy ALA, and it is possible to demonstrate a net synthesis of this compound. Dialysis against triethanolamine buffer also inactivates the system which metabolises ALA. Table I shows the formation of ALA from glycine and succinyl-CoA generated enzymically either from succinate (Expt. 1) or from α-ketoglutarate (Expt. 2). In the former case no enzymes apart from the extract need to be added, but in the latter case it is necessary to add α-ketoglutaric and glutamic dehydrogenases. It can be seen that in both cases the system is almost completely inactive in the absence of glycine or PyP, and that activity is greatly reduced when CoA is omitted. The requirement for added PyP in this system is probably the most conclusive evidence so far that this substance is involved in ALA formation.

TABLE I

Each tube contained 100 μmoles triethanolamine buffer, pH 7.4, 20 μmoles $MgSO_4$, 50 μmoles glycine, 0.06 μmole CoA, 0.1 μmole PyP, and 0.6 ml of *Rps. spheroides* extract. In Expt. 1 the tubes also contained 5 μmoles succinate and 6 μmoles ATP; in Expt. 2 they contained 5 μmoles α-ketoglutarate, 0.1 μmole DPN, 2.5 μmoles cysteine, 20 μmoles $(NH_4)_2SO_4$, 49 μg α-ketoglutaric dehydrogenase[5], and 100 μg crystalline glutamic dehydrogenase (obtained from Boehringer and Soehne, Mannheim). Final volume 1.5 ml; incubated for 1 h *in vacuo* at 37°, deproteinised with 5% trichloroacetic acid, and estimated for ALA by condensation with acetylacetone[6].

	ALA synthesised (μmole/h)	
	Expt. 1	*Expt. 2*
Complete system	0.33	0.12
No glycine	0.00	0.01
No CoA	0.03	0.04
No PyP	0.03	0.01

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Received February 1st, 1958

* The following abbreviations are used: ATP, adenosine triphosphate; ALA, δ-aminolaevulic acid; CoA, coenzyme A; DPN, diphosphopyridine nucleotide; PyP, pyridoxal phosphate.