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Growth and Cytochrome Synthesis in a Hemin-requiring Mutant of *Spirillum itersonii*

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Spirillum itersonii forms considerable amounts of *b*- and *c*-type cytochrome when grown under low aeration, and we have suggested that synthesis of the heme prosthetic group, derived from glycine and succinyl CoA, might be important in regulating the formation of the complete hemoproteins (1). The isolation of a mutant strain requiring hemin (ferric protoporphyrin chloride) has enabled a more direct study of the influence of the prosthetic group on cytochrome synthesis.

The parent strain and microbiological and analytical procedures were as described previously (1). The basal medium was glutamate-succinate-glycine plus 0.1% yeast extract and 0.1% Tween-80 to keep hemin in solution; the stock solution of hemin (2 mM), in 0.01 N NaOH and 50% ethyl alcohol, was added aseptically. The mutant H27/8 was isolated after mutagenesis with *N*-methyl-*N*-nitroso-*N'*-nitroguanidine; treated cells were plated on basal medium containing 0.1 mM δ-aminolevulinate (ALA) and 0.005 mM hemin. Colonies were tested individually for growth with and without these supplements.

Mutant H27/8 responded to hemin or to ALA, although the latter was less effective (Fig. 1); 0.005 mM protoporphyrin and magnesium protoporphyrin were inactive. ALA synthase activity was not detectable in extracts of the mutant, and the block presumably was at this stage.

Hemin was needed for maximum cytochrome synthesis, and only very low levels were found in ALA-grown cells (Table 1). The respiratory rates with glutamate as substrate were, respectively, 4 and 17 μmoles of O_2 per hr per mg of protein for cells grown with 0.1 mM ALA or 0.005 mM hemin.

Both *b*- and *c*-type cytochromes were formed by hemin-grown cells, and the response to aeration was similar to the wild-type (Table 1). Under low aeration, cytochrome synthesis was enhanced, cytochrome *c* being the predominant pigment. High aeration repressed cytochrome synthesis at all concentrations of hemin tested. This does not support the idea that oxygen represses hemoprotein synthesis by favoring succinate utilization via the Krebs cycle and thereby limiting its availability for heme synthesis.

In vitro experiments have suggested that the iron of heme *c* may be incorporated after combination of the protein moiety with protoporphyrinogen; this mechanism would preclude iron protoporphyrin as an intermediate (3). However, experiments with ^{59}Fe-hemin, prepared as described by Falk (2), showed that H27/8 cells formed cytochrome *c* heme directly from exogenous hemin. Cells were grown with ^{59}Fe-hemin (0.004 mM) and the soluble cytochrome *c* purified by chromatography on carboxymethyl-cellulose. The specific activity of the isolated material and of the original hemin were, respectively, 6,220 and 7,300 counts per min per μmole, showing that

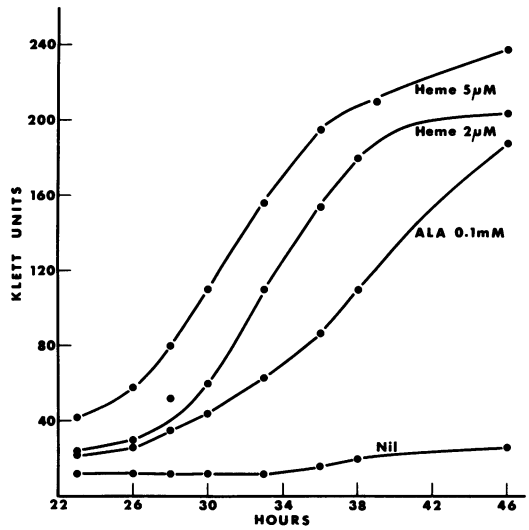

FIG. 1. *Growth of mutant H27/8 in response to hemin and ALA. Nephalo flasks (300-ml) containing 60 ml of medium were inoculated with 3 × 10^8 cells and incubated at 30 C with shaking at 220 rev/min. Growth was followed with the Klett-Summerson colorimeter using filter no. 540. A Klett reading of 100 was equivalent to 50 μg of bacterial protein per ml of culture.*

TABLE 1. *Cytochrome content of Spirillum itersonii mutant H27/8 grown under various conditions*[a]

Growth conditions	Supplements to medium	Cytochrome *b*	Cytochrome *c*
	mM		
High aeration	Hemin, 0.002	0.18	0.18
	Hemin, 0.005	0.27	0.27
	ALA, 0.1	0.05	0.05
Low aeration	Hemin, 0.002	0.23	0.47
	Hemin, 0.005	0.37	0.78
	ALA, 0.1	0.03	0.05
	Hemin, 0.005, plus ALA, 0.1	0.39	0.79

[a] Values expressed as nanomoles per milligram of protein are for cells harvested in the early stationary phase of growth.

at least 85% of the heme *c* had been derived from exogenous hemin without exchange with nonheme iron in the medium.

The mutant apparently did not form apo-cytochrome when grown under conditions which limited the supply of prosthetic group. Respiration of cells grown with 0.1 mM ALA was not stimulated by added hemin, and reconstitution of a cytochrome spectrum did not occur upon addition of hemin to extracts of such cells. Also, cytochrome synthesis, upon addition of hemin to heme-depleted cells, was completely inhibited by puromycin. The behavior of the mutant suggests that formation of the protein moieties of the cytochromes stringently requires the presence of the prosthetic group.

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