Partial Purification of Membrane-bound *b*-Type Cytochrome from *Halobacterium halobium**

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The membranes of *Halobacterium halobium* contain a predominant complex of *b*-type cytochromes, a low level of a *c*-type cytochrome and cytochromes *o* and a_1.[1] We have isolated and partially purified a *b*-type cytochrome fraction.

Halobacterium halobium R_1 was grown aerobically in light for 70 h at 35 °C, in a medium containing KCl, $MgSO_4$, Na_3-citrate, NaCl, $CaCl_2$ and peptone.[2] The cells were harvested by centrifugation (7 000 *g* for 30 min at 4 °C) and were then lysed by suspending them in distilled water and hydrolyzing the DNA with deoxyribonuclease. This gave a clear red lysate [A]. The lysate was centrifuged (114 000 *g* for

Table 1. Enrichment and yield of protoheme during preparation. The heme content was calculated from difference absorbance spectra of pyridine hemochromogens. The protein contents were determined by the Lowry [3] method.

Fraction	Total protein mg	Total protoheme nmol	Protoheme/protein nmol/mg	Purification	Yield %
Lysate of bacteria cells [A]	1 000	74	0.074	1	100
Pellet after centrifugation at 114 000 *g* [B]	154	74	0.48	6.5	100
Supernatant after deoxycholate and phospholipase treatment and centrifugation at 105 000 *g* [C]	124	72	0.58	7.8	97
Fraction after precipitation with 20 % $(NH_4)_2SO_4$ [D]	18	42	2.1	31	60

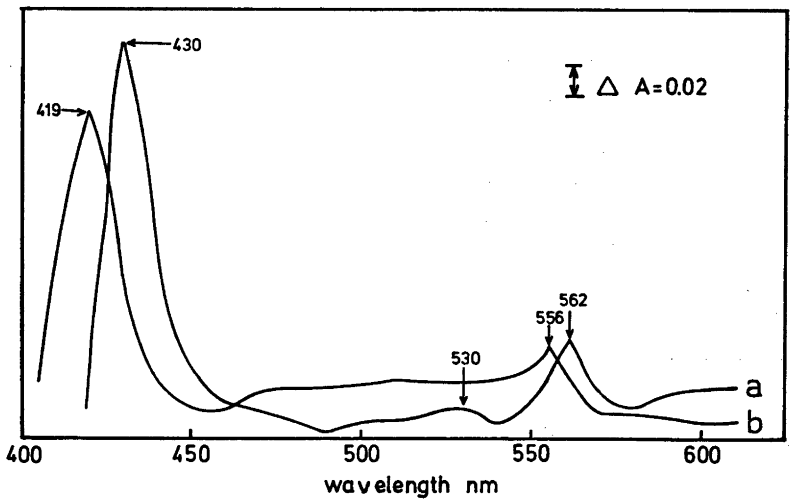

Fig. 1. Spectra at room temperature of fraction [D] after precipitation with 20 % $(NH_4)_2SO_4$ and suspended in Tris-buffer 50 mM (pH 7.8). a, Difference absorbance spectrum (dithionite-reduced minus oxidized). b, Pyridine hemochromogens difference absorbance spectrum (dithionite-reduced minus oxidized).

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2 h at 4 °C) to remove soluble proteins. The pellet was suspended in a Tris-buffer 0.1 M (pH 7.8) [B]. This suspension was incubated with phospholipase A_2 and 1.5 % deoxycholate for 1 h at 35 °C, and then centrifuged (105 000 *g* for 3 h at 4 °C). The supernatant [C] contained 97 % of the *b*-type cytochromes (Table 1). The *b*-type cytochromes were then precipitated with 20 % $(NH_4)_2SO_4$ and the precipitate suspended in a Tris-buffer 50 mM (pH 7.8) [D]. The yield of cytochrome *b* after the precipitation step was 60 % (Table 1), and the degree of purification 30-fold.

The cytochrome absorption spectra have been investigated. Fig. 1a shows a difference spectrum of cytochrome *b* in fraction [D]. There are three maxima: at 562, 530, and 430 nm (α-, β and γ-bands), to be compared with those reported earlier for the membrane-bound cytochrome b_{561} (561, 530 and 430 nm).[1] The heme content in the different fractions was calculated by the pyridine hemochromogen method.[1] Fig. 1b shows a pyridine hemochromogen difference spectrum of fraction [D]. The maximum at 556 nm is characteristic of protoheme,[1] which is the prosthetic group of *b*-type cytochromes.

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Regulation of the Ornithine Decarboxylase Activity by Concomitant Translational and Transcriptional Control during Early Embryonic Development *

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The polyamines putrescine, spermidine and spermine are implicated in the regulation of cell growth and proliferation. A dramatic (80-fold) increase in the activity of the pyridoxal 5′-phosphate-dependent enzyme ornithine decarboxylase (ODC; L-ornithine carboxy lyase; EC 4.1.1.17) precedes gastrulation in the polychete *Ophryotrocha labronica*. ODC catalyzes the initial and rate-limiting step in polyamine biosynthesis, *i.e.*, the conversion of L-ornithine to putrescine. To evaluate the importance of the polyamines in early embryonic development, specific inhibitors of ODC have been used to block their synthesis. DL-α-Methylornithine, a competitive inhibitor of ODC,[1] as well as DL-α-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ODC,[2,3] were found to effectively block pregastrular ODC activity. Experiments in which pregastrular ODC activity was selectively inhibited, strongly suggest that increased ODC activity is required for gastrulation inasmuch as development was blocked at this stage.[4] The block at gastrulation is probably due to interference with nucleolar formation, as indicated by the fact that inhibition of the ODC activity resulted in a marked reduction in the number of nucleoli; all showing an atypical scattered appearance.[5]

It is generally accepted that the embryo does not become genetically autonomous until the stage of gastrulation. Therefore, any pregastrular increase in enzyme activity, like that of ODC, would be expected to depend on stored maternal products, either inactive enzymes or messenger RNAs. However, the possibility remains that embryo genome transcripts are required, at least to some extent, for the initiation of pregastrular events.

Cycloheximide completely prevented development when added at the time of fertilization (0 h). Addition of cycloheximide at 42 h completely eradicated the 48 h ODC activity (Table 1), demonstrating its dependence on protein synthesis and its short biological half-life. Inhibition of protein synthesis completely blocked the increase in pregastrular ODC activity, thus excluding the possibility that the increased ODC

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