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Short Communications

Succinate Dehydrogenase Activity and Succinate-reducible Cytochrome in *Halobacterium halobium* *

CHRISTINA HALLBERG [a] and
LARS HEDERSTEDT [b]

[a] Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden and [b] Department of Bacteriology, Karolinska Institute, S-104 01 Stockholm, Sweden

A close structural and functional association between *b*-type cytochrome and succinate dehydrogenase (E.C. 1.3.99.1, succinate: (acceptor) oxidoreductase) has been demonstrated in several bacterial species.[1,2] Succinate dehydrogenase is tightly membrane-bound and has a similar structure in widely different organisms like mammals,[3] *Neurospora crassa*,[4] and bacteria.[5,6]

Recently two membrane-bound *b*-type cytochromes with absorption maxima at 559 and 562 nm have been separated and partially purified from the aerobic, extremely halophilic bacterium *Halobacterium halobium*.[7] The specific role and position of these cytochromes in the electron transport system of this bacterium is not known. The purpose of this work was to demonstrate the presence of a succinate dehydrogenase in *H. halobium* and to study its possible connection to cytochrome.

Experimental. The *Halobacterium halobium* carotenoid mutant 913-7, originally obtained from Professor J. Lanyi, was grown and harvested as described before.[7] The bacterial cells, washed in basal salt,[7] were disrupted by freezing the bacterial pellet (2.5 g wet weight) in liquid nitrogen and thawing. Subsequently the disrupted cells were homogenized in 5 ml 3 M KCl, 10 mM morpholinopropanesulfonic acid (MOPS), pH 7.2. Deoxyribonuclease (0.2 mg) was added and the suspension was stirred for 30 min at room temperature. This suspension, designated "lysate", was then centrifuged at 48 000 × *g* for 20 min at 4 °C. The supernatant was centrifuged again at the same speed for 2 h at 4 °C and was then called "soluble fraction". The pellet obtained after centrifugation of the "lysate" was washed once in 3 M KCl, 10 mM MOPS, pH 7.2 and finally suspended in the same buffer and was called "membrane fraction".

Succinate dehydrogenase activity was determined in 3 M KCl, 10 mM MOPS, pH 7.2, at 30 °C as the succinate dependent reduction of 2,6-dichlorophenol indophenol (DCIP) at various concentrations (0.01 to 0.20 mg/ml) of phenazine methosulfate (PMS), as described by Singer.[8] Cytochromes were identified by spectrophotometric measurements. Reduced minus oxidized difference absorption spectra were performed at room temperature with a Shimadzu UV-210A double-beam spectrophotometer using a band width of 2 nm and 10 mm light-path cuvettes. Reduced samples were obtained by adding Na-succinate pH 7.7 to a final concentration of 4 mM and to the reference cuvette a compensating volume of buffer was added. Dithionite was added solid. Protein was determined by the method of Lowry *et al.*[9]

Results and discussion. Succinate dehydrogenase activity and cytochrome content were determined in the "soluble fraction" and in the "membrane fraction" from disrupted *H. halobium* cells. Succinate dehydrogenase activity was found only in the "soluble fraction" whereas all the dithionite-reducible cytochromes were found in the "membrane fraction". The finding is in contrast to what has been observed in non-halophilic organisms where the succinate dehydrogenase in buffers of low ionic strength, sediments together with the cytochromes in the membrane fraction. In *Halobacterium salinarium* Aitken and Brown [10] have made a finding similar to ours. They found 82 % of the total succinate dehydrogenase activity in the "cytoplasmic fraction" from cells disrupted by sonication in 4.4 M KCl.

The *H. halobium* succinate dehydrogenase activity is salt dependent. Fig. 1 shows the enzyme activity at different KCl concentrations. Salt dependent succinate dehydrogenase activity has also been reported to be present in *Pseudomonas salinaria (H. salinarium)*.[11]

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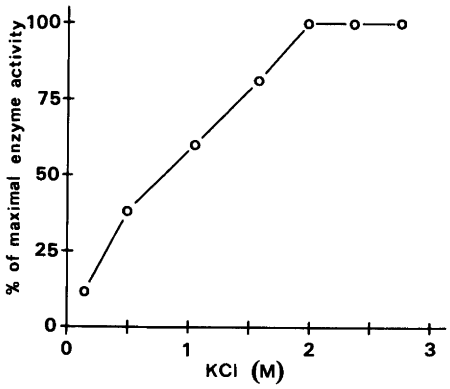

Fig. 1. Succinate dehydrogenase activity in the "soluble fraction" in 10 mM MOPS, pH 7.2 in the presence of different concentrations of KCl. The activity was determined after incubation of the extract for 5 min at 30 °C at each KCl concentration. Maximal enzyme activity was 0,39 nmol of succinate oxidized per minute per mg of protein.

The succinate dehydrogenase activity in the "soluble fraction" from *H. halobium* was inhibited by malonate and oxaloacetate, respectively. The enzyme had a K_m for succinate of 3 mM at 30 °C in 3 M KCl buffer. The aerobic metabolism of *H. halobium*, the inhibition data, and the K_m for succinate suggest that the enzyme is a succinate dehydrogenase and not a soluble fumarate reductase of the type commonly found in facultative and anaerobic organisms.[1,2]

Soluble succinate dehydrogenase from beef heart mitochondria is, in contrast to its membrane-bound state, unable to donate the electrons obtained in the oxidation of succinate directly to DCIP.[3] PMS is required as an electron carrier between the enzyme molecule and DCIP. In analogy no succinate dependent reduction of DCIP was detectable in the *H. halobium* "soluble fraction" in the absence of PMS.

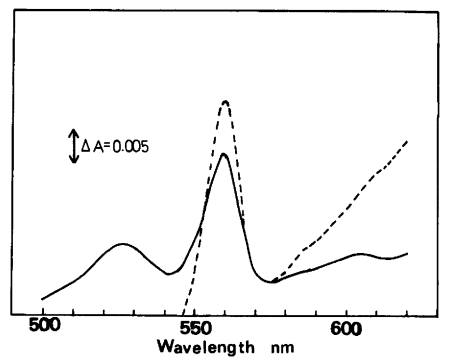

Fig. 2. Difference absorption spectra of "lysate" (12 mg protein/ml). Succinate-reduced *versus* air-oxidized (———). Dithionite-reduced *versus* air-oxidized (– – –).

The reduction of cytochrome(s) by succinate was then measured in different fractions *i.e.* "lysate", "soluble fraction" and "membrane fraction". Fig. 2 shows difference absorption spectra of the "lysate", first reduced by succinate and then by dithionite. About 70% of the dithionite-reducible cytochromes with absorption maxima around 560 nm was reduced by succinate. The reduction of cytochrome(s) by succinate was relatively slow. Maximal reduction was obtained after about 5 min. Possibly this is the time required to get anaerobiosis in the cuvette.

Fig. 3, spectrum b, shows the difference absorption spectrum of the "membrane fraction" reduced by succinate. In this fraction less than 5% of the dithionite-reducible cytochromes with absorption maxima around 560 nm was reduced by succinate. No cytochromes were found in the "soluble fraction". When the "soluble fraction" was added to the "membrane fraction" the reduction of cytochrome(s) by succinate was almost completely recovered to the level of that found in the "lysate" (Fig. 3, spectrum a).

To summarize, a salt dependent succinate dehydrogenase activity, not associated with the fraction that contained the cytochromes, was identified in *H. halobium* cells disrupted and then fractionated by differential centrifugation in high salt. The "soluble fraction" containing the succinate

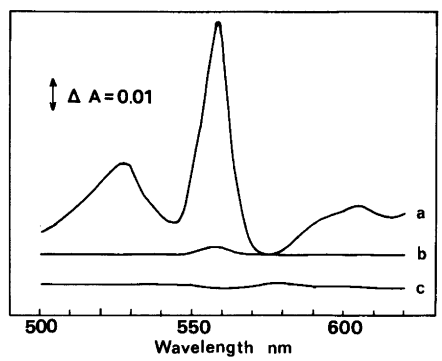

Fig. 3. Difference absorption spectra of succinate-reduced *versus* air-oxidized preparations. a. "Membrane fraction" (final protein concentration 13 mg/ml) mixed with "soluble fraction" (final protein concentration 9 mg/ml) b. "Membrane fraction" (13 mg protein/ml). c. "Soluble fraction" (9 mg protein/ml).

dehydrogenase was found to be required for the reduction of the membrane-bound cytochrome(s) by succinate.

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