CYTOCHROME c_2 – AN ELECTRON CARRIER SHARED BY THE RESPIRATORY AND PHOTOSYNTHETIC ELECTRON TRANSPORT CHAIN OF *RHODOPSEUDOMONAS CAPSULATA*

A. BACCARINI-MELANDRI, O. T. G. JONES[+] and G. HAUSKA*

Istituto Botanico, Universita di Bologna, Italy, [+]Department of Biochemistry, University of Bristol, England and **FB Biologie, Botanik, Universität Regensburg, FRG*

Received 12 December 1977

1. Introduction

The function of cytochrome c_2 as the primary electron donor to the reaction center in photosynthetic electron transport of Rhodospirillaceae has been clearly established [1,2]. However, its role in the respiratory pathways on which these bacteria depend under heterotrophic growth conditions has been a matter of debate since many years [3–8].

The study of respiration in these organisms is often complicated by the presence of branched electron transport chains [7,9,19] and by possible regulatory phenomena in the activity of the terminal oxidases dependent upon physical parameters of growth, such as oxygen concentration and light intensity [11,12].

The *Rhodopseudomonas capsulata* cytochrome c_2 is thought to function in only one of the branches as electron donor for a cytochrome *b*-type oxidase (cyt. b_{413}) [6,13]. This suggestion rests on experimental evidence, such as the observed redox changes of cytochrome c_2 upon addition of antimycin A or during transition to anaerobiosis, and the lack of re-oxidation of cytochrome c_2 in membranes from a respiration-deficient mutant (M7) in which cytochrome b_{413} is absent [6]. In this communication we present evidence for a role of cytochrome c_2 in respiration of *Rps. capsulata;* definite proof has been obtained by an immunological approach taking into account both the vectorial orientation of the membrane and the complexity of the respiratory chain. These two difficulties have been overcome using spheroplast preparations of a respiratory mutant (M6) endowed with a linear respiratory chain where cytochrome *c* oxidase (cyt. b_{413}) is the only terminal enzyme present [6,10].

The data demonstrate conclusively the role of this cytochrome in the respiratory as well as in the photosynthetic chain of *Rps. capsulata.*

2. Methods

Cells of *Rps. capsulata,* mutant strain M6, were grown under strong aeration in the dark, as in [9]. Cells were harvested at the middle of the logarithmic phase of growth; the preparation of spheroplasts [14] and of the immune gammaglobulin against cytochrome c_2, purified from photosynthetically grown cells, was as in [15].

Succinate oxidation was measured with an oxygen electrode. The reaction mixture is given in the legend to fig.2.

The total amount of gammaglobulin was kept constant at 20 mg/ml, during titration with antibody by adding the required amount of control gammaglobulin.

3. Results and discussion

Antibodies have been used to inhibit the photooxidation of cytochrome c_2 in chromatophores of phototrophically grown *Rps. capsulata* and *sphaeroides* [15]. The presence of cholate was

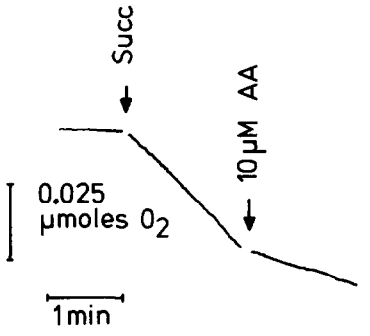

Fig.1. Succinate oxidation by spheroplasts from *Rhodopseudomonas capsulata* M6. The assay mixture (in 1 ml final vol.) consisted of 30 mM Tris–HCl, pH 7.5, 10% sucrose, 5 mM EDTA, 50 mM NaCl. Spheroplasts, equivalent to 1 mg protein, were added in 0.1 ml 50 mM phosphate, pH 7.3, 20% sucrose and 5 mM $MgSO_4$. The reaction was started by adding 5 μmol. succinate. Temp. 30°C. The preparation of spheroplasts used contained 0.4 nmol cytochrome c_2/mg protein. 40% cytochrome c_2 in the original cells was lost during preparation of the spheroplasts. The rate of succinate oxidation calculated from the trace is 0.025 μmol O_2 consumed/mg protein/min. Succ, succinate; AA, antimycin A.

necessary for demonstrating inhibition; this suggests that this cytochrome is trapped within the chromatophores vesicles. In addition cytochrome c_2 could be released, at least partially, by washing the spheroplasts suggesting a location on the periplasmic surface of the plasmalemma, which is thought to be in continuum with the inner surface of the chromatophores [15]. Thus, cytochrome c_2 should be accessible to antibodies in spheroplasts.

Recently spheroplasts have been prepared from cells of *Rps. capsulata* grown aerobically in the dark [14] and it is now possible to use antibodies against cytochrome c_2 for indicating the role of this cytochrome in respiration, Spheroplasts, prepared from the mutant strain M6, can oxidize succinate at a rate comparable to that observed in membranes prepared by French pressure cell breakage of whole cells [6] (fig.1). This activity is observed in spite of the partial loss, during the preparation of spheroplasts, of cytochrome c_2 from the periplasmic space; in these experiments about 40% of the total cytochrome c_2 present in the cells was released, as evaluated by differential spectroscopy. This confirms previous data with photosynthetic [15] and aerobic [14] cells of *Rps. capsulata*, which had indicated that a part of the cytochrome c_2 pool is readily released upon digestion of the cell wall while the remainder is more firmly bound to the membrane. This loss of cytochrome seems however not to impair greatly succinate oxidase activity, which is generally limited kinetically at the flavoprotein level [9]. More detailed studies on this aspect are in progress.

Succinate oxidase in spheroplasts is markedly inhibited by addition of antimycin (80%, see fig.1) and low concentrations of KCN (5.10^{-5} M), in close agreement with results obtained in membrane vesicles of the same mutant strain [6].

This behaviour was taken as evidence that only that branch of the electron transport chain, which channels electrons from succinate to oxygen through cyto-

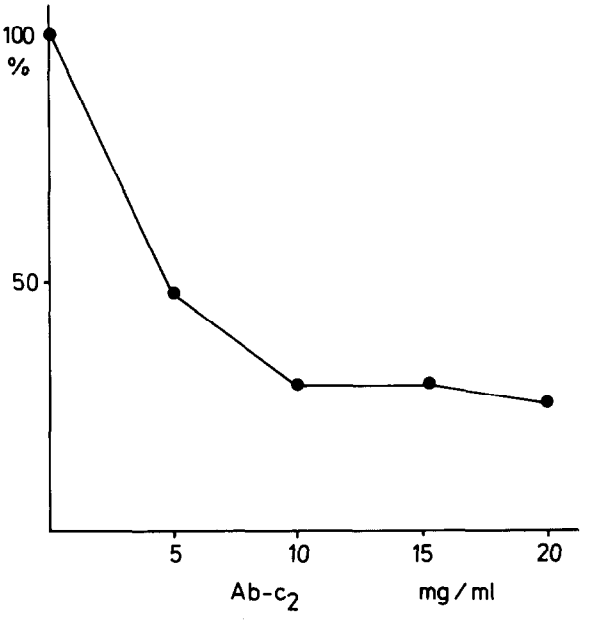

Fig.2. Inhibition of succinate oxidation by antibodies against cytochrome c_2 in spheroplasts from *Rhodopseudomonas capsulata* M6. The assay mixture is described in fig.1 legend. In addition it contained mixtures of gammaglobulin preparations from control and antisera keeping the total amount constant at 20 mg protein. The portion of gammaglobulin stemming from the antiserum is indicated on the abscissa (Ab-c_2). The titer of antibodies in this gammaglobulin preparation was measured as in [15] and was found to be 0.05 nmol/mg protein. The ordinate gives the % oxygen uptake rate after antibody inhibition. The rate of 100% corresponds to 0.017 μmol O_2 consumed/mg protein/min.

chrome *c* oxidase, has significant activity in this mutant [6,16]. Incubation of spheroplasts with the antibody against cytochrome c_2 results in a severe inhibition of succinate oxidase (fig.2). The inhibition seems to level off about 75%, close to the value observed in the presence of antimycin A or cyanide (5.10^{-5} M). The residual rate of oxidation might reflect some minor alternate pathways or, alternatively, a low turnover of the antibody–cytochrome c_2 complex.

If the residual rate is subtracted, half of the cytochrome c_2 present in the spheroplasts (0.2 nmol) seems to be inactivated by about 4 mg antibody preparation. According to the titer (legend fig.2) this corresponds to 0.2 nmol antibody, nicely matching the amount of cytochrome c_2 inhibited.

Analogous experiments with spheroplasts from wild type strains of either *Rps. capsulata* or *sphaeroides* have not yielded clear cut results so far. The action of the alternative oxidase (cytochrome b_{270} [6,13]) might obscure the action of the antibody. The same could hold also for [8] in which an effect on succinate oxidation was not found by replenishing the deficient membrane preparation with cytochrome c_2 in a resolution/reconstitution experiment. Moreover, anaerobic or semiaerobic conditions of growth seem to affect greatly the activity of the alternative oxidases [12]. It should be possible, however, to block the alternative oxidase, in *Rps. capsulata* wild type, with CO, and obtain inhibition by the antibody.

Two lines of evidence suggest the participation of cytochrome c_2 also in respiration of the wild type:

(1) As mentioned above, the cytochrome exhibits the typical redox changes upon addition of electron transport inhibitors or after exhaustion of oxygen [6].

(2) The loss of cytochrome c_2 during preparation of spheroplasts is nicely correlated with a corresponding decrease in NADH-oxidase, when membrane preparations from whole cells and from spheroplasts are compared [14]. It could be established that this decrease reflects a loss in the pathway highly sensitive to KCN, involving cytochrome b_{413}. The KCN-insensitive pathway remains unaffected [14].

It seems therefore clearly established that cytochrome c_2 is indeed functioning as an electron donor for cytochrome b_{413} in respiration as has been anticipated [6,14], in addition to its role in photosynthetic electron transport. Since the antibody used in these studies was prepared against cytochrome c_2 purified from photosynthetically grown cells and was shown to be able to block the photosynthetic oxidation of cytochrome c_2, the observed inhibition of succinate oxidation strongly indicates that the same protein operates both in respiration and photosynthesis. In addition to the coupling factor for phosphorylation [17] this represents a second example of components common to both systems and strengthens the concept of the dual functional role of energy-transducing membranes in facultative photosynthetic bacteria.

Acknowledgements

This work has been supported by a travel grant from NATO. The technical assistance of Mrs Elaine Burd is gratefully acknowledged.

References

[1] Dutton, P. L. and Jackson, J. B. (1972) Eur. J. Biochem. 30, 495–510.
[2] Evans, E. H. and Crofts, A. R. (1974) Biochim. Biophys. Acta 357, 78–88.
[3] Taniguchi, S. and Kamen, M. D. (1965) Biochim. Biophys. Acta 96, 395–428.
[4] Thore, A., Keister, D. L. and San Pietro, A. (1969) Arch. Mikrobiol. 67, 378–396.
[5] Klemme, J. H. and Schlegel, H. G. (1969) Arch. Mikrobiol. 68, 326–354.
[6] Zannoni, D., Melandri, B. A. and Baccarini-Melandri, A. (1976) Biochim. Biophys. Acta 423, 413–430.
[7] King, M. T. and Drews, G. (1975) Arch. Mikrobiol. 102, 219–231.
[8] Hochman, A. and Carmeli, C. (1977) Arch. Biochem. Biophys. 179, 349–357.
[9] Baccarini-Melandri, A., Zannoni, D. and Melandri, B. A. (1973) Biochim. Biophys. Acta 314, 298–311.
[10] Marrs, B. and Gest, H. (1973) J. Bacteriol. 114, 1045–1051.
[11] Sasaki, T., Mokotawa, Y. and Kikichi, G. (1970) Biochim. Biophys. Acta 197, 284–291.
[12] Zannoni, D., Melandri, B. A. and Baccarini-Melandri, A. (1978) Proc. 11th FEBS Meet. Copenhagen, Aug. 1977, in press.
[13] Zannoni, D., Baccarini-Melandri, A., Evans, E. H., Prince, R. C. and Crofts, A. R. (1974) 48, 152–155.

[14] Zannoni, D., Melandri, B. A. and Baccarini-Melandri, A. (1976) Biochim. Biophys. Acta 449, 386–400.
[15] Prince, R. C., Baccarini-Melandri, A., Hauska, G., Melandri, B. A. and Crofts, A. R. (1975) Biochim. Biophys. Acta 387, 212–227.
[16] La Monica, R. F. and Marrs, B. L. (1976) Biochim. Biophys. Acta 423, 431–439.
[17] Baccarini-Melandri, A. and Melandri, B. A. (1972) FEBS Lett. 21, 131–134.