ENERGY TRANSDUCTION IN PHOTOSYNTHETIC BACTERIA. THE NATURE OF CYTOCHROME *C* OXIDASE IN THE RESPIRATORY CHAIN OF *RHODOPSEUDOMONAS CAPSULATA* *

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1. Introduction

In a previous paper [1] it has been demonstrated that in dark grown cells of *Rhodopseudomonas capsulata,* respiration proceeds to oxygen through a branched chain, in which only one arm contains the site of inhibition by antimycin A and the enzymes involved in the oxidation of exogenous cytochrome *c* or of the ascorbate–diaminodurene couple. These conclusions were based mainly on 'in vitro' experiments which showed a lower sensitivity to KCN inhibition of NADH- or succinate-oxidase activities (half inhibition at 10^{-4} M and $5 \cdot 10^{-5}$ M) compared with that of cyt. *c* or ascorbate-DAD oxidase (both showing half inhibition at $2 \cdot 10^{-7}$ M).

The possibility of a branched chain was proposed at the same time also by Marrs and Gest [2] on the basis of the growth characteristics of several respiratory mutants and of the activities present in membranes prepared therefrom. One of these mutant strains, denominated M7, appears to be of particular interest since it lacks completely cyt. *c* oxidase, although it is able to grow heterotrophically on malate.

The results presented in this paper elucidate the nature of the lesion present in this mutant and allow the correlation of cytochrome oxidase activity with the presence of an high potential cytochrome with a spectrum of *b* type.

2. Materials and methods

Aerobic cultures of *Rps. capsulata,* strain St. Louis (American Type Culture Collection No. 23782), were obtained as previously described [1] using a synthetic medium containing malate [3]. Precultures of the M7 mutant, a generous gift from Dr B. Marrs, were grown photosynthetically in the same medium supplemented with ascorbate (0.5 mg/ml) (see [2]) and then transferred for aerobic growth under the same conditions used for the wild type strain.

Membranes were prepared according to standard procedures [1] and washed once with 0.1M glycylglycine buffer, pH 7.2 containing 10 mM $MgCl_2$. The methods used for the measurements of respiratory activities were those outlined in [1]. The protein content was estimated according to Lowry et al. [4].

Redox titrations of cytochromes were performed at pH 7.0 in a medium containing 50 mM KCl and 50 mM 2(n-morpholino) ethane sulphonic acid (MES) using a dual wavelenght spectrophotometer, according to the technique introduced by Dutton et al. [5]. The following redox dyes were used: pyocyanine (16 μM);

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phenazine ethosulfate (16 μM); phenazine methosulfate (16 μM) and diaminodurene (16 μM). Oxidation-reduction potentials were made more positive by adding potassium ferricyanide. Potentials were made more negative by successive additions of ascorbate, succinate, NADH and dithionite.

Absorption spectra at a controlled redox potential were registered with a single beam spectrophotometer and fed into a small computer (Digital Equipment Corp., Maynard, Mass, mod. pdp 11/10), programmed to obtain difference or derivative spectra.

3. Results and discussion

According to Klemme and Schlegel [6] membranes prepared from *Rps. capsulata* strain Kb1, contain only cytochromes of *b* and *c* type, as judged from reduced minus oxidized difference spectra and from pyridine haemochrome spectra; redox differential spectra performed on *Rps. capsulata*, strain St. Louis confirmed that no trace of *a* type cytochrome can be detected in this bacterium (fig. 1).

Redox titrations of *b* type cytochromes, performed at 561–570nm (fig. 2) have indicated the presence of

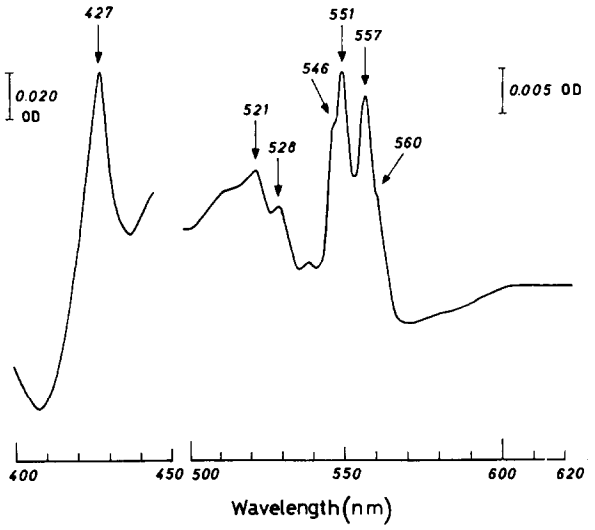

Fig. 1. Dithionitic-reduced minus ferricyanide-oxidized difference spectrum, recorded at −77°K, of the membrane fraction of *Rps. capsulata*, strain St. Louis. Particles were suspended at a concentration of 2.2 mg protein/ml in 50 mM MES (pH 7.0).

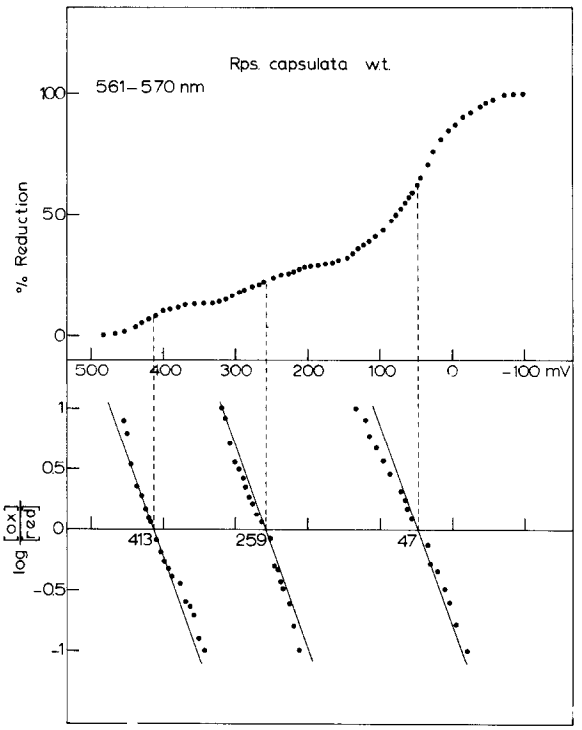

Fig. 2. Potentiometric titration at 561 minus 570 nm in particles prepared from *Rps. capsulata*, strain St. Louis. Particles were suspended in 50 mM MES plus 50 mM KCl (pH 7.0). at a concentration of 2.26 mg protein/ml.

three different components, characterized at pH 7.0 by midpoint potentials at 410 ± 5,260 ± 5 and 47 ± 5 mV and corresponding respectively to 16 ± 5, 12 ± 5 and 88 ± 5% the total *b* type cytochrome present in the membrane preparation. Cytochrome b_{47} had been previously detected in photosynthetic membranes from *Rps. capsulata*, strain St. Louis and from the carotenoid-less mutant AIA pho$^+$ [7]; cytochrome b_{260} and b_{410} appear to be specific for acrobically grown cells. Due to its relatively high midpoint potential, cytochrome b_{410} appeared to us as a good candidate for the role of final oxidase in the respiratory chain. For these reason membranes from the M7 mutant, lacking cytochrome *c* oxidase activity were examined.

M7 is a respiratory mutant, obtained by spontaneous reversion from M5, a mutant completely incapable of aerobic growth and lacking cyt. *c* oxidase activity; in contrast to M5, M7 is again able to grow heterotrophi-

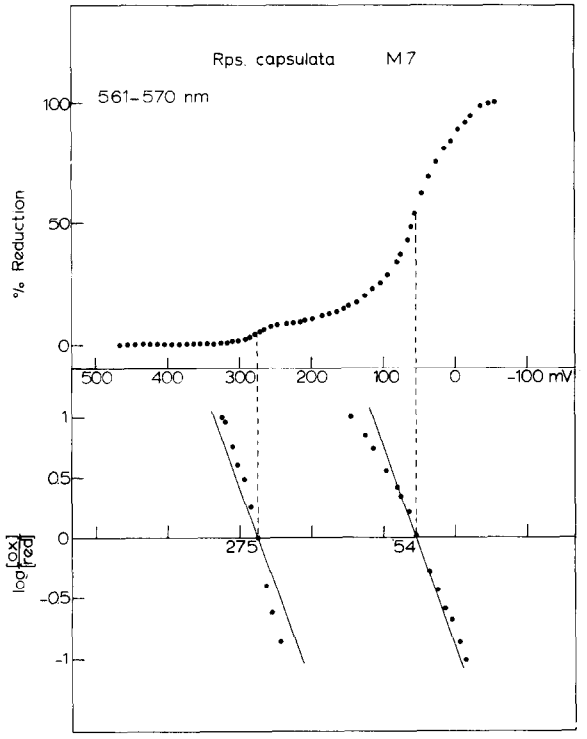

Fig. 3. Potentiometric titration at 561 minus 570 nm in particles prepared from the M7 mutant. Conditions as in fig. 2.

cally, but still lacks cyt. *c* oxidase activity, indicating the presence of an alternative respiratory pathway [2].

The data shown in table 1 compare the respiratory activities of membranes from wild type and M7 cells, grown aerobically. NADH and succinate oxidases appear to be present in both preparations, although

Table 1
Respiratory activities in membranes from *Rhodopseudomonas capsulata*, St. Louis and from the M 7 mutant

Electron donor	Oxidase activity (μeq/hr mg protein)	
	St. Louis strain	M 7 strain
NADH	28	11
Succinate	9	4.6
Cytochrome *c*	20	1.2

considerably reduced in M7; on the other hand cytochrome oxidase appears to be nearly absent in M7 but very active in wild type membranes. In agreement with previously reported data [1,2] this lack of cyt. *c* oxidase activity is correlated with a parallel loss of the ability to oxidize various artificial electron donors as reduced N,N,N′,N′,-tetramethyl-*p*-phenylenediamine or diaminodurene. These results confirm and extend the observations by Marrs and Gest on photosynthetically grown cells [2].

Redox titrations of cytochrome *b* present in membranes from M7 cells show the presence of only two cytochromes, namely cyt. b_{260} and b_{47}, which correspond respectively to about 10 and 90% of the total cytochrome *b*, cyt. b_{410} being completely absent.

This result is a clear cut demonstration that the presence of cyt. b_{410} on the membrane is directly linked to the phenotypic character represented by cytochrome oxidase activity. This conclusion is strengthened by the observation that both cyt. b_{410} and cytochrome oxidase activity are present in membranes from another respiratory mutant, M6 (report in preparation) and if it is considered that both M6 and M7 are spontaneous revertants of M5, a strain affected by a double mutation at the level of terminal oxidases resulting in a complete block of respiration.

The exact nature of the prosthetic group of cyt. b_{410} has not been determined yet; the assignment of this electron carrier to the class of *b* type cytochromes is so far based on the general absence of cytochromes other than *b* and *c* in membranes from *Rps. capsulata* and on its spectral characteristics.

A reduced minus oxidized spectrum of cyt. b_{410} is presented in fig. 4. This spectrum was obtained with the aid of a computarized spectrophotometer as follows: a composite spectrum of cyt. b_{410} plus cyt. c_{342} was derived computing the difference of two absorption spectra, registered at a controlled redox potential of 463 and 358 mV respectively (curve A, compare also fig. 2). A nearly pure spectrum of cyt. c_{342} was then obtained subtracting the spectrum at 358 mV, previously fed into the memory, from a spectrum at 274 mV (curve B). The small contribution of cyt. b_{260}, which becomes apparent between 274 and 358 mV, was calculated to be pratical negligible in curve B.

After normalization of the absorption maximum at 551 nm of cyt. c_{342} this spectrum was subtracted from

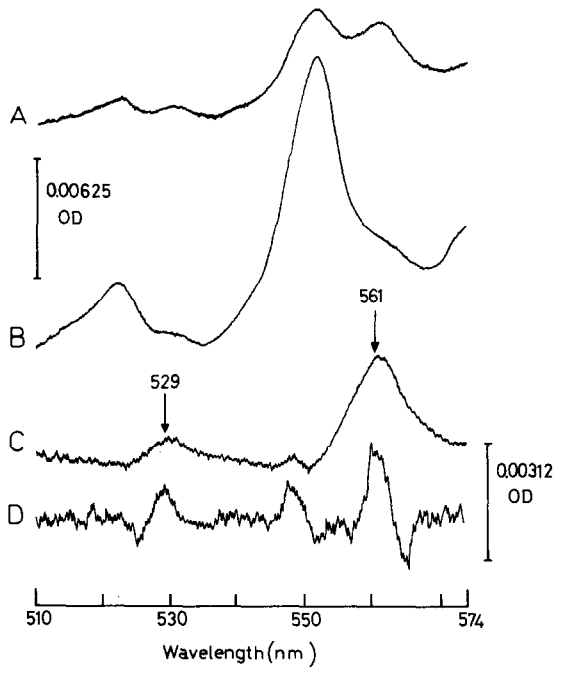

Fig. 4. Reduced minus oxidized difference spectrum of cyt. b_{410}. Particles were suspended at a concentration of 0.75 mg protein/ml in 50 mM morpholino propane sulfonic acid plus 50 mM KCl containing the dyes used in redox titration experiments. Spectra were then recorded at a controlled redox potential. For details see text.

that of curve A and the spectrum of curve C was obtained.

Curve D presents the fourth derivative of spectrum C; two maxima at 561 and 530 nm respectively are clearly related to the α and β bands of a cytochrome of *b* type, namely cyt. b_{410}, while the maximum at 548 nm is due to a second peak in the region of the α band of cytochrome *c* (apparent as small shoulder in curve B) which was not completely eliminated, since the normalization was referred to the maximum at 551 nm.

The only terminal oxidase of *b* type so far described, have been defined as cytochrome *o* [8,9]; the operative definition of this cytochrome rests on its reactivity with CO and on the possibility of detecting a CO-reduced minus reduced difference spectrum.

The terminal oxidase of *Rps. capsulata* clearly has a *b*-type spectrum; however, the lack of sensitivity of cytochrome *c* oxidase activity to CO [6] and the lack of any CO difference spectrum at high redox potential, preclude the identification of the oxidase as an *o*-type cytochrome. The oxidase is an atypical *o*-type cytochrome or perhaps, a new cytochrome oxidase of the *b*-type.

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