ADAPTATION IN *RHODOPSEUDOMONAS SPHEROIDES*

Venetia A. SAUNDERS and O. T. G. JONES

Department of Biochemistry, University of Bristol, Bristol, BS8 1TD, England

1. Introduction

The purple non-sulphur bacterium *Rhodopseudomonas spheroides* may grow anaerobically in the light and aerobically in darkness. Growth in the light involves the elaboration of many membrane-bound vesicles, the chromatophores, bearing the light-harvesting and photoreactive pigments [1]. Photosynthetically-grown cells also possess a well-developed respiratory system and both photosynthetic and respiratory enzyme assemblies are membrane borne and may have components in common. Under aerobic conditions the complex internal membrane system is lost, there is suppression of photopigment (bacteriochlorophyll and carotenoid) synthesis and acquisition of an *a*-type cytochrome which probably functions as a terminal oxidase in respiration [2–4].

In order to elucidate the processes involved in the adaptive formation of electron transport systems in *Rps. spheroides*, electron transport properties of the haem proteins and photopigments have been investigated. There appears to be little difference between the membrane-bound *b*- and *c*-type cytochromes in both aerobically- and photosynthetically-grown cells, while the *a*-type cytochrome, apparent only in aerobically-grown cells, has some of the properties of eukaryotic cytochrome oxidase. Even when cells are grown aerobically they still retain a minimal photosynthetic apparatus and light-induced cytochrome, carotenoid and chlorophyll changes can be demonstrated.

2. Materials and methods

Mutants G-VP and V-2 were prepared by treatment of cells of *Rps. spheroides* strain 2.4.1. with *N*-methyl-*N*-nitrose-*N'*-nitroguanidine [5]. A green facultatively anaerobic strain, designated G-VP, was selected from colonies on agar plates incubated anaerobically in the light. The photosynthetically-incompetent mutant, strain V-2, was selected using the penicillin screening technique [6].

Cultures were grown either aerobically in a vigorously aerated continuous culture apparatus [7] or anaerobically in the light in the medium of Sistrom [8]. Particles were prepared from cells disrupted in a French pressure cell as previously described [7].

Difference spectra were measured using a split-beam spectrophotometer which has been described elsewhere [9]. The dual wavelength spectrophotometer used in this work was essentially based on the design of Chance [10].

The method employed for determination of oxidation–reduction midpoint potentials was that of Dutton et al. [11] using a stirred, gassed cuvette, fitted with platinum and calomel electrodes.

3. Results and discussion

In particulate fractions from aerobically- and photosynthetically-grown cells of the *Rps. spheroides* similar *b*- and *c*-type components were identified both spectroscopically (fig. 1) and potentiometrically (table 1). The emergence of *a*-type cytochrome as terminal oxidase in the aerobically-grown cell appears to be triggered by a variation in oxygen tension. Potentiometric titration at 607 nm *minus* 630 nm using the bacteriochlorophyll-less mutant V-2 revealed the presence of two components with oxidation–reduction mid-point potentials (+375 mV and +200 mV) corresponding well with those of *a* and a_3 of mito-
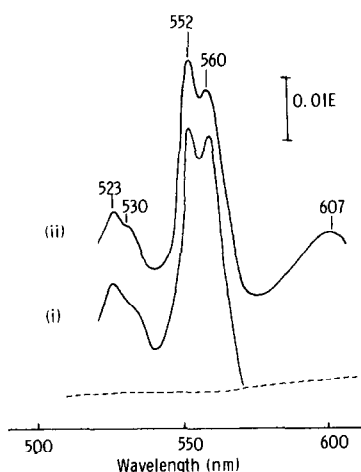
Fig. 1. Dithionite-reduced *minus* oxidized difference spectra of particles prepared from (i) photosynthetically-grown; (ii) aerobically-grown *Rps. spheroides* strain G-VP. Particles were suspended in 10 mM Tris–HCl (pH 7.5) at a concentration of approximately (i) 2.0 mg protein/ml or (ii) 3.5 mg protein/ml; dithionite was added to the test cuvette and spectra recorded at room temperature.

chondria [11]. These components were present in a ratio close to unity.

When photosynthetically-grown cells of strain G-VP were transferred to aerobic conditions the preform-

ed bacteriochlorophyll and carotenoid were diluted by cell division but only to a certain level (approximately 5% of that of photosynthetically-grown cells). This level of pigment was then maintained despite subsequent cell divisions. Furthermore the capacity to adapt to the photosynthetic mode was retained irrespective of the number of generations of aerobic growth undergone prior to transfer to anaerobic light conditions.

The small amount of bacteriochlorophyll present in aerobically-grown cells appeared to be photochemically active. In xenon-flash experiments an absorbance decrease occurred at 605 nm not only in photosynthetically-grown but also in aerobically-grown cells, indicating the oxidation of reaction centre components [12]. Furthermore, when the potential in the cuvette, was maintained near to +100 mV the extent

Table 1
Oxidation–reduction mid-point potentials of cytochromes of particles prepared from aerobically-grown and photosynthetically-grown *Rps. spheroides*

Cytochrome type	Growth conditions	E'_0 (pH 7.0) mV
b-type (560 nm minus 570 nm)	Aerobic	(i) + 157
		(ii) + 44
		(iii) −102
	Photosynthetic	(i) + 160
		(ii) + 45
		(iii) −100
c-type (552 nm minus 540 nm)	Aerobic	(i) + 285
		(ii) + 120
	Photosynthetic	(i) + 290
		(ii) *

* Small absorbance changes occurred at potentials below +200 mV but were not characterized accurately.
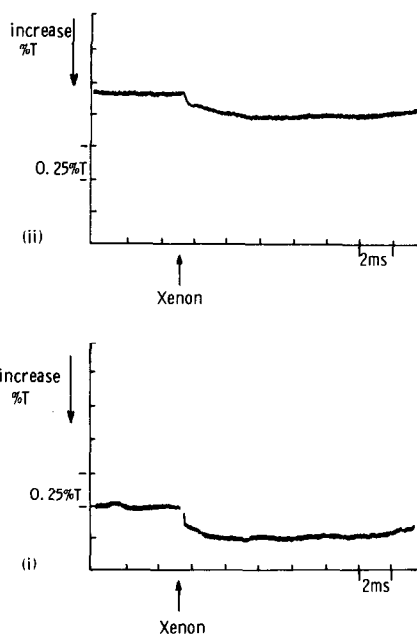

Fig. 2. Xenon-flash-induced cytochrome *c* oxidation in (i) photosynthetically-grown; (ii) aerobically-grown *Rps. spheroides* strain G-VP. Cells were washed and suspended in 10 mM Tris–HCl (pH 7.5) at a concentration of approximately (i) 0.7 mg protein/ml or (ii) 2.7 mg protein/ml. 2.0 mM succinate was added and cells allowed to become anaerobic by respiration in the dark. Absorbance changes were monitored at 552 nm *minus* 540 nm following a 200 μsec xenon flash. The time constant was 100 μsec. A downward deflection represents an oxidation of cytochrome *c*.

of change per mg bacteriochlorophyll at 605 nm was greater (approximately three times greater) for particles from aerobically-grown cells. This suggests that of the small amount of bacteriochlorophyl present in membranes of aerobically-grown cells a much increased proportion is present as reaction centre bacteriochlorophyll.

Oxidation of c-type cytochrome could be activated both by continuous light and xenon flashes (fig. 2) indicating that some operational c-type cytochrome was linked to reaction centre bacteriochlorophyll not only in photosynthetically- but also in aerobically-grown cells.

Spectroscopic characteristics of the bacteriochlorophylls of particulate fractions indicated differences between aerobically- and photosynthetically-grown cells (fig. 3) which may reflect differences in the specific arrangements of these molecules within the cellular structure. Since in vivo spectra of bacteriochlorophyll appear to be strongly influenced by reaction centre components [13] the shift to longer wavelength of the spectrum of bacteriochlorophyll of aerobically-grown cells may be due to the apparently high level of reaction centre to bulk bacteriochlorophyll in these cells.
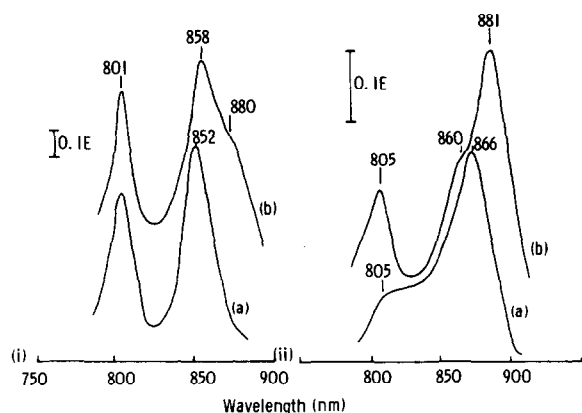

Fig. 4. Light-induced carotenoid changes of particles from (i) photosynthetically-grown; (ii) aerobically-grown Rps. spheroides strain G-VP. Particles were suspended in 10 mM Tris–HCl (pH 7.5) at a concentration of approximately (i) 0.16 mg protein/ml or (ii) 1.3 mg protein/ml. Changes were monitored at 470 nm minus 490 nm at 30°C. Light was switched on and off where indicated.

Fig. 3. Spectra of the bacteriochlorophyll of particles prepared from (i) photosynthetically-grown; (ii) aerobically-grown Rps- spheroides strain G-VP. Particles suspended in 10 mM Tris–HCl (pH 7.5) at a concentration of approximately (i) 0.1 mg protein/ml or (ii) 1.0 mg protein/ml were contained in the test cuvette of the split-beam spectrophotometer 10 mM Tris–HCl (pH 7.5) was in the reference cuvette. Spectra were recorded (a) at room temperature (b) at 77°K.
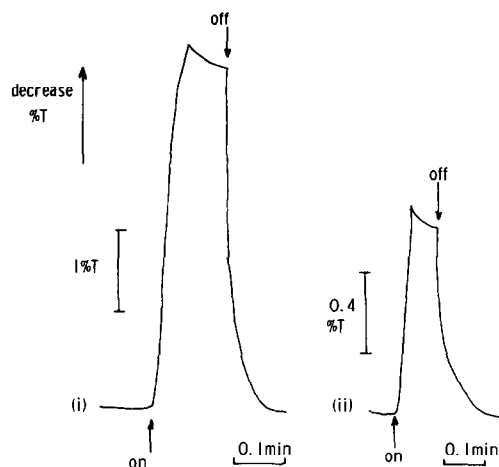
Light-induced absorbance changes in the carotenoid spectra of particles from straing G-VP are shown in fig. 4. Using the ionophorous antibiotic, valinomycin, in the presence of KCl (50 mM) carotenoid changes could be produced which were essentially the same as the light-induced changes except that the magnitude of the changes was less. The presence of such carotenoid changes suggests that photosynthetic electron flow can be used to generate a membrane potential [14] even in aerobically-grown cells.

It would appear therefore that cells grown under aerobic conditions still contain some residual photosynthetic activity. Furthermore, since aerobically-grown cells are virtually devoid of the vesicles characteristic of the photosynthetically-grown cell, it would seem that the photopigments can function in the absence of significant vesicle formation. In addition similar haem protein components of the b- and c-type appear to be involved in electron transport in both types of cell and it seems that the a-type oxidase interacts with them in the aerobically-grown cell.

The capacity of the photosynthetically-grown cell for respiration and the maintenance of a minimal photosynthetic apparatus by the aerobically-grown cell

obviously facilitate reversible adaptation between photosynthetic and aerobic modes of growth for *Rps. spheroides.*

Acknowledgements

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