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Electron donation by the high-potential haems in *Rhodopseudomonas viridis* reaction centres at low temperatures

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EPR signals due to *c*-type cytochromes in *Rhodopseudomonas viridis* reaction centres are reported. Haems with $E_m = 380$ mV (1), 310 mV (1) and about 0 mV (2) were identified. At redox potentials where the low-potential cytochromes are oxidised, but the high-potential cytochromes are reduced, photooxidation of the high-potential cytochromes is observed as Q_A is photoreduced by low-temperature (15 K) illumination.

Cyt *c*-556→cyt *c*-559→reaction centre→Q_A

However, the signal attributed to Q_A is only 30-40% of the intensity and is narrower than that observed when Q_A is photoreduced with all the haems reduced. It is suggested that reduction of the low-potential haems causes conformational changes in the reaction centre, altering the iron-quinone interaction and the electron-transfer process in the cytochrome chain.

Photosynthetic reaction center; Cytochrome *c*; Iron-quinone; (*Rhodopseudomonas viridis*)

1. INTRODUCTION

The reaction centre of the purple non-sulphur photosynthetic bacterium, *Rhodopseudomonas viridis*, contains four bound *c*-type cytochrome haems: two high-potential haems (cyt *c*-559; E_m 380 mV and cyt *c*-556; E_m 310 mV) and two low-potential haems (cyt *c*-553; E_m 20 mV and cyt *c*-553; E_m −80 mV) [1,2]. Extensive spectroscopic work on *Chromatium* had suggested that the haems were in low-potential–high-potential pairs with the high-potential haem nearest to the membrane surface [3]. However the crystal structure of the reaction centre shows that the four haems are linearly arranged on the polypeptide [4,5]. The relationship of the different potential haems to the physical organisation of the haems is not known and is of some interest in terms of the mechanism of electron transport. All four haems can be photooxidised via P^+ which results in the reduction of the initial, 'stable' electron acceptor Q_A [6]. At room temperature electron donation by the high-potential haems has been investigated kinetically and it has been shown that the highest potential haem is the immediate donor to the reaction centre, electron transfer from the lower potential haem proceeding through this haem [1,2,6]. At low temperature in the solid state it has been reported that only the low-potential (0 mV) haem can function as electron donor to the reaction centre [7]. This was also the case in *Chromatium* [8]. In a recent elegant study Nitschke and Rutherford ([6] and personal communication) have used a mixture of redox poising and orientation studies to show that when the reaction centre haems are fully reduced the 20 mV haem is probably the immediately observed donor on illumination at 4 K. Although their results do not distinguish unequivocally between the 20 mV and 310 mV haems

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Abbreviations: Q_A and Q_B, primary and secondary quinone electron acceptors; cyt, cytochrome; LDAO, lauryldiethylamine *N*-oxide; P, reaction centre chlorophyll

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which have the same orientation. Warming to 80 K resulted in electron transfer from the −80 mV haem to the 20 mV haem. They did not observe cytochrome oxidation at potentials where the low-potential haems were oxidised. However, we have previously observed that in reaction centre preparations part of the Q_A is irreversibly reduced even when the low-potential haems are oxidised [10]. We have now investigated this process and find that the 380 mV haem is the immediate donor to the reaction centre, but that electron transfer from the 320 mV haem to the 380 mV haem occurs even at 5 K.

2. MATERIALS AND METHODS

The growth conditions of *Rps. viridis* and the isolation of chromatophores and reaction centres were as described [7,10,11] except that the samples were concentrated by ultrafiltration. In some preparations the DEAE-cellulose column treatment was omitted and LDAO was removed by treatment of the reaction centres in 0.1 M Tris, 0.5% (v/v) Triton X-100 (pH 8.0) with $Na_2S_2O_4$ (1 mg·ml^{-1}) added by stirring gently at 4°C. Additional $Na_2S_2O_4$ was added, if required, until a drop of the suspension reduced a methyl viologen (50 mg·l^{-1}) solution. The sample was then concentrated by precipitation with 40% ammonium sulphate and dialysis as in [10].

Redox titrations were carried out as described previously [7,8,10] in both the oxidative and reductive direction using 25 μM reaction centres based on the content of cytochromes *c*-559 and *c*-556. The following mediators were used at 20 or 50 μM: methylene blue, saffranine O and T, thionine, janus green, indigotetrasulphonate, methyl viologen, benzyl viologen, phenosaffranine and tetramethyl *p*-phenylenediamine. Quinone mediators were avoided because of possible interactions with the quinone-binding sites of the reaction centres. 2,6-Dichlorophenol indophenol which we have used previously was excluded because it was impossible to see the photooxidation of the high-potential cytochromes in its presence, although Q reduction was unaffected. EPR spectra were recorded and signal intensities attributed to Q_A were estimated as described in [10]. Samples were illuminated in the spectrometer with white light from a 150 W halogen lamp focussed through a light guide to the cavity. Illumination was continued for 300 s although Q reduction was virtually instantaneous within the time resolution of the experiment. Theoretical curves were fitted to redox titrations using the Enzfitter curve-fitting programme (Elsevier-Biosoft, Cambridge).

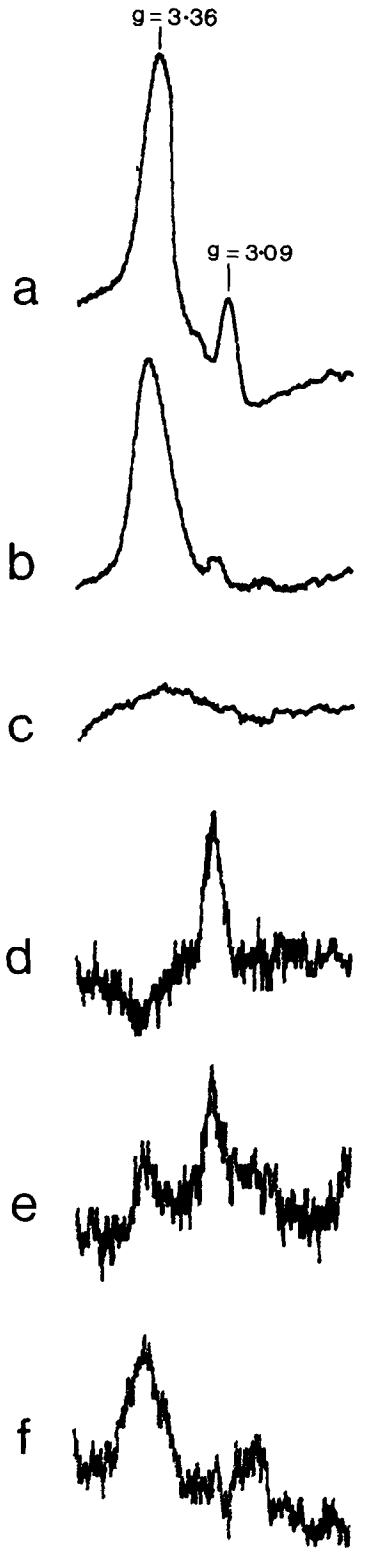
3. RESULTS AND DISCUSSION

Two major EPR signals from *c*-type cytochromes were observed in *Rps. viridis* reaction centres (fig.1). On the basis of redox titrations (fig.2), the $g = 3.09$ signal was attributed to high-potential cyt *c*-559 (E_m 380 mV). The $g = 3.36$ signal is complex as the high-potential cyt *c*-556 (E_m 310 mV) and both the low-potential cytochromes (cyt *c*-553) give rise to signals here. It is not easy to distinguish between these components on the basis of EPR, Nitschke and Rutherford [9] demonstrating minor differences in *g* value of these haems, but we were unable to use these small changes to identify haems in photochemical experiments. Our redox titrations did not allow separation of the two low-potential haems, Nitschke and Rutherford were also unable to distinguish fully these two haems by titration.

Illumination at 5 or 15 K, of a sample poised between 430 and 380 mV, resulted in photoreduction of Q_A and oxidation of the P, but the reaction was rapidly reversed in the dark. However, at potentials more reduced than 380 mV when the high-potential cytochromes become reduced, stable photoreduction of Q_A occurs at these temperatures and photooxidation of cyt *c*-559 occurs (fig.1). As the potential is lowered, so that cyt *c*-556 also becomes reduced, illumination causes oxidation of both *c*-559 and *c*-556. On subsequent scanning, i.e. after a delay of 5 min or less, the signal due to cyt *c*-559 decreases while the signal at $g = 3.36$ increases indicating that electron transfer between the two haems (or electron sharing) has occurred. This is equivalent to the electron transfer sequence observed in optical experiments carried out at room temperature. The initial oxidation of the haems occurs rapidly as does the reduction of Q and is not resolved by these experiments. Although the results indicate electron transfer between the haems part of the cyt *c*-556 is oxidised rapidly within the scan time of the experiment (30 s). Most (80%) of these light-induced changes are reversible in the dark at 77 K in 5 min.

The oxidation of the high-potential haem is coupled to the reduction of a component with a typical iron-quinone complex EPR spectrum in the

Fig.1. EPR spectra of the cytochromes of *Rps. viridis* reaction centres. (a–c) Samples were taken from an oxidation-reduction potential titration as described in section 2. (a) 390 mV; (b) 355 mV; (c) −95 mV. (d–e) Light minus dark difference spectra; the light spectra were recorded after 5 min illumination at 15 K. (d) 355 mV; (e) 275 mV; (f) −95 mV. EPR conditions: temperature, 15 K; frequency, 9.05 GHz; microwave power, 5 mW; modulation amplitude, 10 mT; gain, 2000; light-dark spectra ×5.



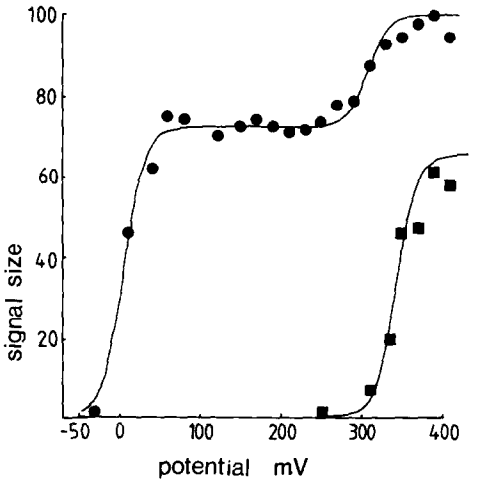


Fig.2. Oxidation-reduction potential titration of the cytochromes of the *Rps. viridis* reaction centre. Signal intensities at $g = 3.09$ (■) and $g = 3.36$ (●) before illumination. The curves drawn are the theoretical curves for one-electron transitions at 350, 305 and 0 mV. EPR conditions as in fig.1.

g = 1.8 region. The spectrum is however slightly different from those previously reported in *Rps. viridis*. Q_B has a peak-to-trough linewidth of 18 mT and the classical Q_A linewidth of 28.5 mT [10,12]. The Q spectrum seen on oxidation of the high-potential haem has a linewidth of 26.0 mT. The difference is very small and would probably not previously have been considered significant; it is however reproducible.

When the low-potential cytochromes are also reduced, Q_A photoreduction occurs but the signal observed is larger (fig.3) and has the classical Q_A linewidth. An increase in the g = 3.36 signal occurs and the potential dependence of the increased intensity indicates that either cyt *c*-553 is now also acting as the donor, or that reduction of these cytochromes has resulted in a change in the properties of Q_A. Photooxidation of the cyt *c*-559 g = 3.09 component is not observed at these low potentials. Although the Q_A signal observed following illumination when the low-potential haems are reduced is much more intense than that induced when the high-potential haems are donors, the extent of cytochrome oxidation is apparently similar. At all potentials between one third and one half of one haem equivalent seems to be photooxidised at 15 K. This suggests that both Q_A signals actually reflect the transfer of a single electron through the

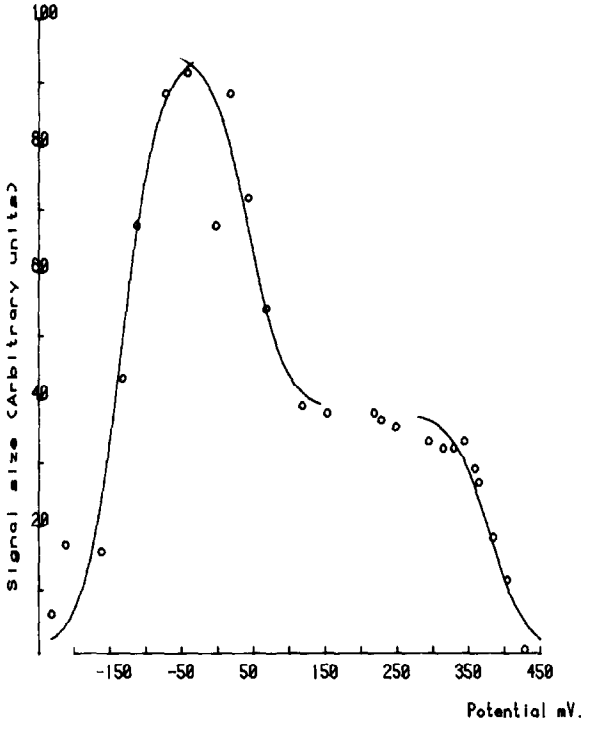


Fig.3. Oxidation-reduction potential of the extent of photoreduction (light-dark) of the iron-quinone (Q_A) complex in reaction centres of *Rps. viridis* after illumination for 5 min at 5 K. The lines drawn are the theoretical curves for a one-electron transition at 380, 45 and −135 mV. EPR conditions: temperature, 5 K; frequency, 9.05 GHz; microwave power, 25 mW; modulation amplitude, 10 mT; gain 1000.

reaction centre. The iron-quinone signal arises as the result of an interaction between the ferrous iron atom and the semiquinone; it is extremely sensitive to the environment and cannot be used as an absolute measure of the extent of Q reduction [13,14].

Prolonged illumination of low-potential samples results in a slow increase in the $g = 3.36$ signal; this probably reflects electron transfer to the pheophytin, reduction of which can be observed at this temperature. Failure to oxidise fully even one haem equivalent, although all of the chemically reducible Q_A is photoreduced, suggests that a proportion of the reaction centres may have lost Q_A during preparation.

The reason for the differences observed in the signal due to Q_A when the low-potential cytochromes are either oxidised or reduced is unclear. It may reflect reaction centre heterogeneity, or the presence as we have suggested previously of two Q_A components, or perhaps the most likely explanation is that reduction of the low-potential haems causes a conformational change in the reaction centre changing the interaction of the quinone and iron. Attempts to separate reaction centres which show only one of these signals have been unsuccessful.

Although the properties of the quinone acceptor cannot yet be fully explained the main result of these experiments is clear. At low temperature as at room temperature, the high-potential haem cyt *c*-559 $g = 3.09$ can be the initial donor to the reaction centre chlorophyll. This suggests that this haem is closest to the reaction centre. We have also found that the *c*-556 $g = 3.36$ haem is oxidised at low temperature, partly rather slowly as a result of transfer of electrons to the *c*-559 haem. This suggests that these haems are adjacent in the peptide chain. When the 0 mV haems are reduced we only observe oxidation of the $g = 3.36$ haem. Nitschke and Rutherford, who were unable to observe oxidation of the high-potential haems, identified the haem oxidised under these circumstances as the 20 mV haem on the basis of potential and orientation, and suggested that it must be next to the 380 mV haem. They were probably unable to see oxidation of the high-potential haems because they used long (10 min) illumination times and a large range of mediators, particularly 2,6-dichlorophenolindophenol, which we have found to conceal the cytochrome oxidation.

However, reduction of the 0 mV haems clearly alters the electron-transfer process. Our results would most easily be explained if the haems were arranged in potential order, reduction of the low-potential haems causing conformational changes which alter the electron transfer process. The observation of an altered Q_A spectrum suggests that reduction of the 0 mV haems does indeed alter the reaction centre structure. Both Rutherford and Nitschke [6] and Dracheva et al. [1,2] prefer a model in which the high- and low-potential haems alternate on the polypeptide because of the magnetic interaction between the low- and high-potential haems. It is difficult in such a model to explain the efficient electron transfer from the 310 mV haem to the 380 mV haem. It is not therefore possible to make an unequivocal model

reconciling the spectroscopic and crystallographic results at present.

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