THE IRREVERSIBLE PHOTOREDUCTION OF A LOW POTENTIAL COMPONENT AT LOW TEMPERATURES IN A PREPARATION OF THE GREEN PHOTOSYNTHETIC BACTERIUM *CHLOROBIUM THIOSULPHATOPHILUM*

J. V. JENNINGS and M. C. W. EVANS

Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT, England

Received 21 January 1977

1. Introduction

The reaction centre complex of the purple photosynthetic bacteria is probably the best characterized photosynthetic system at the present time [1]. In this complex the reaction centre bacteriochlorophyll *P*-890 can be photooxidized at either room temperature or at cryogenic temperatures. The photooxidation is coupled to the reduction of an electron-acceptor which may be a quinone–iron complex with $E_m = -150$ mV [1] and this photochemical reaction is reversible even at 4°K.

Relatively little is known about either the electron-transport system or the reaction centre of the green bacteria. The isolation of a reaction centre with properties similar to those of the purple photosynthetic bacteria has been reported [2]. However, there is evidence that membrane preparations from the green photosynthetic bacterium *Chlorobium thiosulphatophilum* can photoreduce electron-acceptors such as ferredoxin with E_m below −400 mV by a noncyclic electron-transport system [3,4] and it has been reported recently that the photooxidation of the cytochrome which is the electron-donor to the reaction centre can be observed at potentials as low as −450 mV [5]. These results suggest that the green photosynthetic bacteria may have a reaction centre more like that of Photosystem I in oxygen-evolving organisms than that of the purple photosynthetic bacteria. This observation is supported by the identification of a number of iron–sulphur proteins in the photosynthetic membrane system of *C. thiosulphatophilum*, including one with $E_m \simeq -550$ mV [6,7].

We have used low temperature EPR spectroscopy to show that *C. thiosulphatophilum* has a reaction centre bacteriochlorophyll which will undergo irreversible photooxidation at 15°K and that this photooxidation is coupled to the reduction of a component with E_m below −500 mV.

Abbreviations: E_m midpoint oxidation–reduction potential, EPR electron paramagnetic resonance

Address correspondence to: Dr M. C. W. Evans, Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT, England

2. Materials and methods

C. thiosulphatophilum (strain Tassajara) was grown as previously described [3]. The cells were suspended in 0.1 M Tris–HCl buffer, pH 7.5, containing 0.01 M $MgCl_2$ and 0.01 M ascorbate and broken by passage through a French Pressure cell at 20 000 psi. The broken-cell suspension was centrifuged at 15 000 × *g* for 15 min to remove whole-cells and large particles. The supernatant was centrifuged at 26 000 × *g* for 15 min. The pellet from this centrifugation containing the photosynthetic membranes was washed once with buffer and resuspended in the same buffer.

EPR samples were prepared in the dark and spectra were recorded with a Varian E4 spectrometer with a Helium Flow System as described previously [8]. Difference spectra were obtained using a Nicolet

1020A digital oscilloscope. Oxidation–reduction potentiometry was carried out, essentially as described by Dutton [8,9].

3. Results and discussion

Figure 1 shows the EPR spectrum of a *C. thiosulphatophilum* membrane preparation at 15°K and microwave power 1 mW in the dark (A), during illumination (B) and in the dark after illumination (C), together with the expanded light minus dark difference spectrum (D). The changes on illumination are more clearly seen in the difference spectrum. This shows the appearance of a free radical signal at $g = 2.00$, which by comparison with other photosynthetic systems we attribute to the photooxidized reaction centre bacteriochlorophyll. The photooxidation of the reaction centre is irreversible under these conditions and the spectrum does not change when the light is turned off. This signal is saturated at low temperatures appearing rather small by comparison with other signals in the sample. At higher microwave powers other components of the preparation can be observed. Figure 2 shows the EPR spectrum of a similar sample in the $g = 1.95–1.85$ region. Illumination at low temperatures of a sample prepared in the dark results in the appearance of a signal at $g = 1.90$. This signal appears in parallel with the oxidation of the reaction centre bacteriochlorophyll and is a result of the reduction of a component of the electron-transport system. The low temperature photoreduction of this component is irreversible. We suggest that this component is part of the primary electron-acceptor complex of the reaction centre and that it accepts an electron from the reaction centre bacteriochlorophyll. The EPR spectrum of this component is similar to that of a Rieski iron–sulphur protein but it has a very low redox potential. The sample shown in fig.2 was prepared at +75 mV. At this potential the iron–sulphur centres in the membrane are fully oxidized (fig.2A) and the appearance of the $g = 1.90$ signal can be clearly seen (fig.2B). The membranes contain three different iron–sulphur centres with signals in the $g = 1.85–1.96$ region and with potentials ranging from 0 mV to as low as −550 mV. The presence of the relatively large signals from these centres make it impossible to accurately

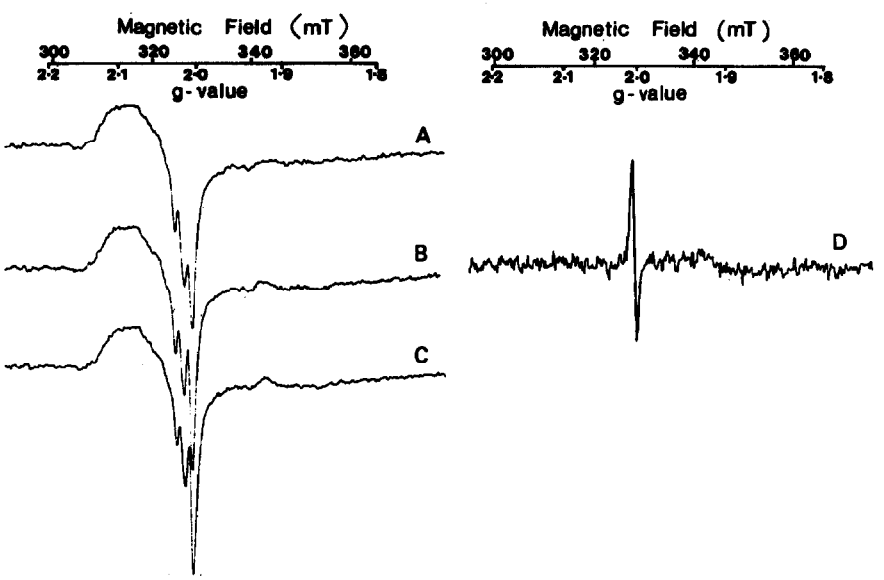

Fig.1. EPR spectra of *C. thiosulphatophilum* membrane particles showing the irreversible photooxidation of the reaction centre bacteriochlorophyll. Sample poised at +50 mV and frozen in the dark (A), during illumination at 15°K (B), in the dark after illumination (C), and in the dark after illumination minus dark difference spectrum expanded 4 × (D). The spectra were obtained under the following EPR conditions: frequency 9.25 GHz, modulation amplitude 10 G, microwave power 1 mW, amplifier gain 500, temperature 15°K.

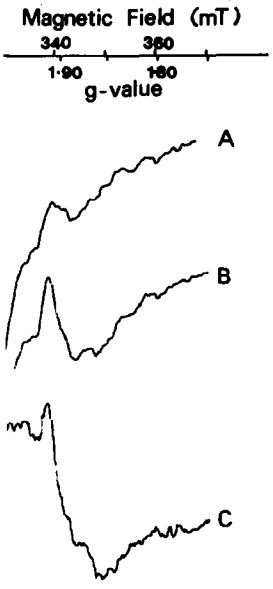

Fig.2. EPR spectra in the $g = 1.95-1.70$ region of *C. thiosulphatophilum* membrane particles showing the irreversible photoreduction of a component at $g = 1.90$. Sample poised at +75 mV and frozen in the dark (A), in the dark after illumination at 15°K (B), and in the dark after illumination minus dark difference spectrum expanded 2 × (C). The spectra were obtained under the following EPR conditions: frequency 9.065 GHz, modulation amplitude 10 G, microwave power 20 mW, amplifier gain 2000, temperature 15°K.

determine the redox potential of the $g = 1.90$ component. We have, however, found that the photoreduction of the $g = 1.90$ centre can be observed at potentials below −500 mV and this ability is lost with $E_m \simeq -550$ mV. The ability to photoreduce the $g = 1.90$ component is also lost if the particles are chemically oxidized with $E_m \simeq +220$ mV, at pH 7.30. Using 'chromatophores' of the same strain of *C. thiosulphatophilum*, Knaff et al. have reported that the reaction centre bacteriochlorophyll has $E_m = +330$ mV [10]. However, the E_m-value of +220 mV corresponds more closely to that reported for the reaction centre bacteriochlorophyll by Prince and Olson [5]. They also reported the loss of ability to photooxidize the reaction centre at below −450 mV. Our results, together with those of Prince and Olson, suggest that the reaction centre of the green photosynthetic bacteria is similar to Photosystem I in plants. Prince and Olson, using purified reaction centres, observed a reversible photooxidation of the reaction centre bacteriochlorophyll, whereas in the more intact membrane fractions used in our experiments this oxidation is irreversibly coupled to the reduction of a low potential component which is most probably an iron–sulphur centre. This suggests that the 1.90 centre is a secondary electron-acceptor as are the iron–sulphur centres in Photosystem I.

Knaff and Malkin [6] reported the identification of a Rieski protein with an EPR signal at $g = 1.90$ and an $E_m = +160$ mV, at pH 7.0, in 'chromatophores' of *C. thiosulphatophilum*. We have not been able to detect a component of this type. However, if Knaff and Malkin prepared their samples in the light, the apparent potential of the $g = 1.90$ component might in fact appear to be in the +200 mV region, as illumination of the samples after freezing (even by normal room-light) would result in the photoreduction of this electron-acceptor. The apparent potential of the $g = 1.90$ component would then, in fact, be the potential of the reaction centre bacteriochlorophyll, i.e., +220 mV.

These results confirm the ability of this reaction centre to generate a reductant with a potential of −550 mV by a direct photochemical reaction and show that the reduction of ferredoxin in this organism can proceed by a noncyclic electron-transport system. This is very different from the system in purple photosynthetic bacteria and supports the suggestion that the green photosynthetic bacteria represent an important stage in the evolution of photosynthesis.

Acknowledgements

This work was supported in part by grants from the UK Science Research Council and the Royal Society.

References

[1] Parsons, W. W. (1974) Ann. Rev. Microbiol. 28, 41–49.
[2] Fowler, C. F., Nugent, N. A. and Fuller, R. C. (1971) Proc. Natl. Acad. Sci. USA 68, 2278–2282.
[3] Evans, M. C. W. and Buchanan, B. B. (1965) Proc. Natl. Acad. Sci. USA 53, 1420–1425.
[4] Buchanan, B. B. and Evans, M. C. W. (1969) Biochim. Biophys. Acta 180, 123–129.

[5] Prince, R. C. and Olson, J. M. (1976) Biochim. Biophys. Acta 423, 357–362.
[6] Knaff, D. B. and Malkin, R. (1976) Biochim. Biophys. Acta 430, 244–252.
[7] Authors' unpublished results.
[8] Evans, M. C. W., Lord, A. V. and Reeves, S. G. (1974) Biochem. J. 138, 177–183.
[9] Dutton, P. L. (1971) Biochim. Biophys. Acta 226, 63–80.
[10] Knaff, D. B., Buchanan, B. B. and Malkin, R. (1973) Biochim. Biophys. Acta 325, 94–101.