Electron acceptors of photosystem 2 in the cyanobacterium *Phormidium laminosum*

Y.E. Atkinson and M.C.W. Evans

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

Received 16 June 1983

Photosystem 2 preparations with very high rates of oxygen evolution from the thermophilic cyanobacterium *Phormidium laminosum* have been studied by EPR spectrometry. In the presence of DCMU the $g = 1.82$ signal of the iron–quinone electron acceptor (Q) can be observed. It is proposed that DCMU is necessary to disrupt a magnetic interaction, between the semiquinone forms of Q and the secondary acceptor B, which otherwise prevents detection of the Q^-Fe signal. A doublet EPR signal arising from magnetic interaction between Q^-Fe and the reduced intermediary electron acceptor pheophytin (I^-), and a spin-polarized triplet signal assumed to arise from the back reaction between I^- and $P680^+$ can also be seen. Preliminary redox titrations of Q reduction have been carried out, indicating $E_m \simeq 0$ mV.

Phormidium laminosum *Photosystem 2* *Electron acceptor* *Iron–quinone* *Pheophytin* *Triplet*

1. INTRODUCTION

Electron transfer in the photosystem 2 reaction centre involves the photooxidation of the reaction centre chlorophyll P680 and transfer of the electron through a pheophytin intermediary acceptor (I) to a complex containing a primary acceptor (Q) and secondary acceptor (B). The electron acceptors of photosystem 2 seem to be similar to those in purple bacteria.

The primary electron acceptor (Q) of photosystem 2 was thought to be a form of plastoquinone in a special environment from the results of extraction experiments and absorption measurements. Recently Q has been identified directly as an iron–quinone complex by EPR spectrometry in PS2 preparations from a mutant of *Chlamydomonas reinhardtii* lacking photosystem 1 [1] and in chloroplasts of a photosystem 1-less mutant of barley, zb^{63} [2]. In these mutant systems the chemical and photochemical reduction of Q could be observed. The EPR signal of reduced Q showed g-value ($g = 1.82$), lineshape and microwave power saturation characteristics of a semiquinone–iron complex similar to that seen in the purple bacteria [3].

Oxidation–reduction potential titrations of the iron–quinone signal in detergent preparations of *C. reinhardtii* PS2 [4] indicated a pH-independent redox potential (in the range pH 5–7), of $E_m \simeq -10$ mV, similar to that of the higher potential acceptor Q_H observed in fluorescence titrations.

The reduced pheophytin intermediary acceptor can also be detected by EPR. In purple bacterial reaction centres, magnetic interaction between I^- and the Q^-Fe state of the quinone acceptor results in splitting of the pheophytin signal at very low temperatures [4]. A similar splitting of the pheophytin radical signal has been observed in preparations from the *C. reinhardtii* mutant [2] and wild-type higher plants [5,6]. Although the interaction between I^- and Q^-Fe has been detected in preparations from wild-type organisms, it has proved extremely difficult to detect the signal from Q^-Fe itself.

Abbreviations: PS2, photosystem 2; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; EPR, electron paramagnetic resonance; LDAO, lauryldimethylamine oxide; HEPES, *N*-2-hydroxymethylpiperazine-*N*′-2-ethanesulphonic acid

Published by Elsevier Science Publishers B.V.
00145793/83/$ 3.00

We have now used a highly enriched photosystem 2 preparation from the cyanobacterium *Phormidium laminosum* [7] to investigate the iron–quinone in a wild-type organism. We have found that the EPR signal can normally be detected only if the secondary acceptor B is displaced by high concentrations of the inhibitor DCMU. An interaction between the semiquinone –iron complex (Q^-Fe) and reduced pheophytin is observed apparently as in purple bacteria [8] but the reaction centre triplet [9] can only be observed under more reducing conditions.

2. MATERIALS AND METHODS

Oxygen-evolving photosystem 2-enriched fractions were prepared from the thermophilic cyanobacterium *P. laminosum* using the detergent LDAO by an adaptation of the method in [10] and stored at 77 K in (pH 7.5) HEPES–NaOH buffer containing 10 mM $MgCl_2$ and 25% glycerol until used. Oxygen evolution measurements were made at room temperature with a standard Rank oxygen electrode (Rank, Bottisham) in the presence of 25% glycerol and 10 mM Mg^{2+}, and with dimethylbenzoquinone and potassium ferricyanide as electron acceptors. Chlorophyll *a* concentration was measured as in [11]; EPR measurements were performed as in [12]. Difference spectra were obtained and baseline corrections and smoothing of spectra were carried out using a Tektronix 4051 microcomputer. The field and *g*-scales shown in the figures are approximate. Illumination at 200 K was carried out in a dry ice–ethanol bath using a 1000 W projector as light source. The same projector was used for illumination at room temperature, and at low temperature inside the EPR spectrometer cavity. Redox potential titrations were carried out essentially as in [13] as described in [14] except that 500 μM DCMU was included in the titration mixture.

3. RESULTS AND DISCUSSION

Typical PS2 preparations from *P. laminosum* showed oxygen evolution activity in the range 1000–2000 μmol O_2 . mg chl^{-1} . h^{-1}. P680 chlorophyll ratios of 1:70 are typical (measured by absorbance change at 820 nm [R.C. Ford, unpublished]) and manganese content is at most 4 Mn/P680. Despite reports of Ca^{2+}-dependence of the oxygen-evolving activity in other cyanobacteria [15], no effect of low concentrations of Ca^{2+} has been observed in this system.

It has proved extremely difficult to observe the signal of the reduced iron–quinone acceptor at $g = 1.82$ in *P. laminosum*. The *C. reinhardtii* preparation in which it was first seen lacks the secondary quinone acceptor B [16]. It has been suggested [17] that the herbicide DCMU which inhibits ferricyanide-dependent oxygen evolution acts by displacing B from its binding site near Q. Thus, if the Q^-Fe signal were being masked by a magnetic interaction between Q and B similar to the one which occurs in purple bacteria, the addition of DCMU to the preparation might remove this interaction and enable the Q^-Fe signal to be detected. Fig. 1 shows the EPR signal of Q^-Fe obtained by the addition of 500 μM DCMU to a *P. laminosum* PS2 preparation. The signal requires very low temperature and high microwave power for resolution [7]; it can be photoinduced by freezing under room temperature illumination (A) or by illumination at 200 K or by chemical reduction

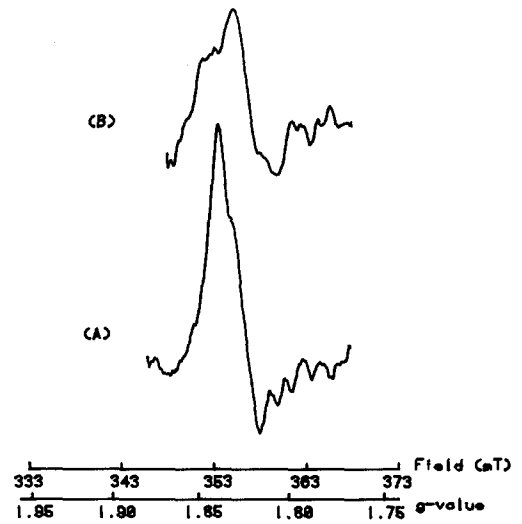

Fig. 1. EPR spectra of the iron–quinone electron acceptor Q^-Fe in *Phormidium laminosum* PS2 preparations: (A) sample frozen under continuous illumination; (B) sample frozen in the dark 10 min after the addition of ~2% (w/v) sodium dithionite at pH 7.5. Both samples contained 500 μM DCMU and ~1 mg chl/ml. EPR conditions: microwave power, 25 mW; temp., 4 K; modulation amplitude, 1 mT; frequency, 9.1 GHz.

with sodium dithionite (B). High concentrations of dithionite are required in order to overcome the effects of the detergent LDAO which is present and which acts as an oxidising agent. Unlike *C. reinhardtii*, where about 30% of the Q^-Fe signal can be induced by low temperature (5 K) illumination, in *P. laminosum* very little signal can normally be photoinduced at 5 K. This difference in low temperature donation is being investigated.

Preliminary oxidation–reduction titrations indicate a midpoint potential for the reduction of Q at pH 6–7.5 around 0 mV, similar to the values of −10 mV obtained for Q in *C. reinhardtii* and 30 mV for the fluorescence titration of Q_H in *P. laminosum* [18]. The presence of LDAO makes the titration difficult, and values obtained for E_m range from +50 to −50 mV in 4 expt.

Fig. 2. shows a doublet signal attributed to the reduced pheophytin acceptor I^- interacting with

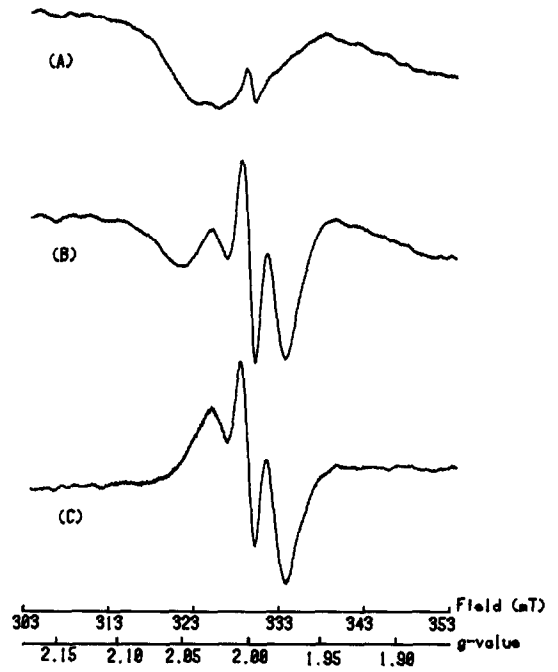

Fig. 2. EPR spectra of the doublet signal resulting from the interaction between reduced pheophytin and semiquinone–iron acceptors in PS2 preparation from *Phormidium laminosum*; ~1 mg chl/ml; (A) sample poised at ~ −200 mV with sodium dithionite and frozen in the dark, (B) after illumination at 200 K for 20 min. (C) Difference spectrum (B)–(A); EPR conditions: microwave power, 50 mW; temp. 6 K; modulation amplitude, 0.5 mT; frequency, 9.1 GHz.

Q^-Fe, after 200 K illumination of dithionite-reduced PS2 from *P. laminosum*. Before (and after) illumination at 200 K the $g = 1.82$ signal of Q^-Fe can be seen if DCMU is present. A small signal showing this interaction can be induced by prolonged illumination at 6 K but the full signal is developed only after 20 min illumination at 200 K. The doublet is centred at $g = 2.003$ and is 7.0–7.5 mT wide. EPR conditions required to observe this split radical signal are high microwave power and low temperature (below 8 K) [5]. At higher temperatures and/or lower power the singlet spectrum of I^- is seen. Redox titrations indicate that the interaction signal can only be induced by 200 K illumination at potentials around that of QFe reduction and at more negative potentials, but the results are not clear since the appearance of the interaction does not exactly parallel that of the Q^-Fe signal.

As in bacterial reaction centres [8], PS2 preparatons from higher plants [19] and *C. reinhardtii* [4], a spin-polarized reaction centre triplet signal can be observed in these preparations. Fig. 3 shows the triplet signal in a *P. laminosum* PS2 sample reduced with excess dithionite at pH 10 and illuminated at 4 K. In *P. laminosum*, like *C.*

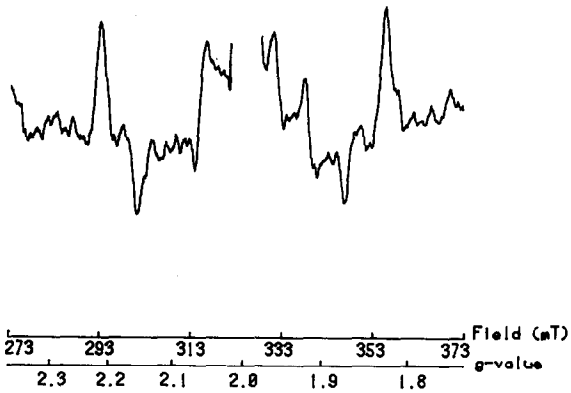

Fig. 3. EPR spectrum of the PS2 reaction centre triplet in a *Phormidium laminosum* PS2 preparation reduced with ~2% sodium dithionite (pH 10) and frozen in the dark. The spectrum shown is a continuously illuminated minus dark difference spectrum at 4 K; ~1 mg chl/ml. The reversible light-induced signal at $g \simeq 1.92$ is probably due to a heating effect on the $g = 1.92$ iron–sulphur signal. EPR conditions: microwave power, 20 μW; temp. 4 K; modulation amplitude, 1.0 mT; frequency, 9.1 GHz.

reinhardtii [4], little or no triplet can be seen at potentials where Q alone is reduced; it appears that an earlier acceptor must also be reduced. According to [18] the *P. laminosum* preparation lacks Q_L, the acceptor observed at around −270 mV in fluorescence titrations of higher plant PS2 preparations, so the nature of this acceptor is as yet unknown.

An iron–sulphur protein with an EPR signal at $g = 2.05$ and $g = 1.92$ and $E_m 7.0 \simeq -270$ mV [20], co-purifies with *P. laminosum* PS2 and can be photoreduced at room temperature. This reduction is only partially inhibited by high concentrations ($\leq 500\ \mu$M) of DCMU. No photoreduction is seen in frozen samples at 15 K or 200 K. There is so far no direct evidence that this protein is a functional acceptor in PS2 electron transport in *P. laminosum*, although work is continuing to further characterize the acceptor side of PSII in this system.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the Commission of the European Communities Solar Energy Programme (contract no. ESD-014-UK) and the UK Science and Engineering Research Council. We would like to thank Miss K. Perks for technical assistance and Mr J Bonnerjea for the use of computer programs.

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