Direct determination of the oxidation reduction potential of the iron–quinone electron acceptor (Q) in photosystem II in *Chlamydomonas reinhardtii*

M.C.W. Evans, J.H.A. Nugent, L.A. Tilling and Y.E. Atkinson

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

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1. INTRODUCTION

The redox properties of the electron acceptors of the photosystem II reaction centre are complex and it has proved difficult to obtain a definitive value for the potential of the quinone electron acceptor Q. Q was originally defined as a component which quenched fluorescence from the light-harvesting chlorophyll associated with photosystem II, reduction of Q chemically or by continuous illumination resulting in increased fluorescence [1]. The extent of this variable fluorescence at different potentials has been used to determine the redox potential of Q. These experiments identify two different quenchers: Q_L with $E_{m7.0} \simeq -250$ mV and Q_H with $E_{m7.0} = 0$ mV (see [2] for review). The precise E_m varies with the organism used and also differences in experimental technique. Direct detection of the electron acceptor is difficult; an absorption change at 550 nm due to an electrochromic absorption shift is an indicator of the redox state of Q. Originally observed at 77 K, it can also be seen as a kinetic change at room temperature. Redox potential determinations for C_{550} vary between +50 mV to −50 mV [2]. In chloroplasts the potential is pH dependent. A recent measurement for a *Chlamydomonas reinhardtii* preparation similar to that used here indicated a potential of 0 mV which was pH independent between pH 5.0 and 8.0 [3]. Q can also be detected optically by the absorption change of the quinone at 320 nm. The change is however small and difficult to measure and its redox properties have not been determined.

We have recently shown that in preparations from a mutant of *C. reinhardtii* lacking photosystem I an EPR signal characteristic of an iron–quinone complex similar to those found in purple bacteria can be detected and identified as an electron acceptor of photosystem II [4]. We have now titrated this EPR signal to obtain a direct measurement of the redox potential of this quinone electron acceptor of photosystem II, and to determine whether it is Q_L or Q_H.

2. MATERIAL AND METHODS

C. reinhardtii Str. F54-14, a mutant lacking the photosystem I reaction centre and the chloroplast ATPase, was a gift from Dr. B. Diner. It was grown and membrane fractions prepared from the cells as described by Diner and Wollman [5]. The EPR signal of the iron–quinone complex is easily lost. We have therefore modified the extraction procedure of Diner and Wollman to provide a partially purified reaction centre preparation in the large quantities required for EPR. The purified membrane fraction was resuspended in 20 mM MES–KOH (pH 5.9) and stored frozen in liquid N_2 until required. After thawing, the concentrated membrane fraction was treated with 1.25% Digitonin, 1% Triton X-100 in 20 mM MES and 0.1 M NaCl at a final chlorophyll concentration of 1.2–1.3 mg/ml. After incubation at 0°C for 2 h without stirring,

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the detergent extract was fractionated with polyethylene glycol 6000. 15 ml of 50% w/v PEG solution was added to each 100 ml of the extract and the preparation centrifuged at 50 000 × *g* for 30 min. The pellet was discarded and another 15 ml/100 ml of original extract volume of 50% PEG added to the supernatant. After centrifugation for 30 min at 50 000 × *g* the pellet contained the photosystem II reaction centre. This pellet was resuspended in 0.1 M MES–KOH (pH 5.9) (or other buffer as required) containing 0.1 M NaCl. The volume of buffer used was such that 1 ml of the final suspension contained the reaction centres from 10–12 mg of chlorophyll in the original membranes. This fraction was either used immediately for the titration or stored in liquid nitrogen. Although it was contaminated with the light-harvesting chlorophyll protein and other membrane fragments including the iron–sulphur centres of succinic dehydrogenase, these contaminants did not interfere with the EPR measurements of Q. Oxidation reduction potential titrations were carried out essentially by the procedure of Dutton [6] as described previously [7]. Titrations were done at 10°C to improve the stability of the preparation. EPR spectra were recorded using a JEOL FEIX spectrometer fitted with an Oxford Instruments ESR 9 liquid helium cryostat [7].

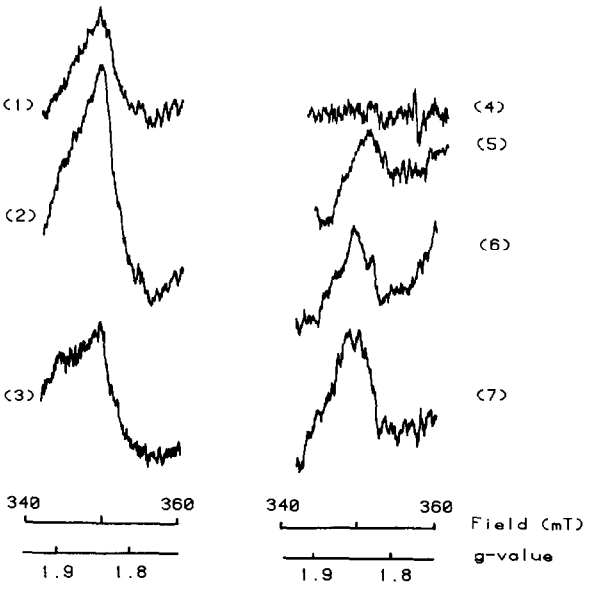

Fig.1. EPR spectra of the iron–quinone electron acceptor of photosystem II. Left: Light induced spectra (1) sample frozen under illumination and stored at 77 K for 2 weeks; (2) sample 1 after illumination at 5 K; (3) Light minus dark difference spectrum (2–1) following 5 K illumination. Right: Spectra of samples poised at different potentials. (4) +100 mV, (5) +35 mV, (6) 0 mV, (7) −80 mV. EPR conditions: microwave power 25 mW; modulation amplitude 1 mT; frequency 9.1 GHz; time constant 0.1 s; scan rate 25 mT/min; instrument gain 2500.

3. RESULTS AND DISCUSSION

Figure 1 shows the EPR spectrum of the iron–quinone complex of the photosystem II reaction centre preparation reduced by illumination, or in samples poised at different potentials in a titration. The line shape of the spectrum is essentially the same in both conditions. However, the maximum signal size observed in titrations is always less than that induced by freezing under illumination, the maximum signal size observed in titrations being about 70% of that in illuminated samples. As we have reported previously, low temperature illumination of samples prepared in the dark causes little reduction of the iron–quinone complex [4]. This is also the case in samples from the titration; it was not possible to increase the signal size in these samples to that of the frozen under illumination sample by low temperature illumination.

Figure 2 shows a typical titration of the iron–quinone complex. The titration curve fits the Nernst curve for $N=1$ with an average midpoint potential $E_m = -10$ mV (Range observed +10 to −30 mV). The titration is reversible using potassium ferricyanide as oxidant and shows no appreciable hysteresis effects. The midpoint potential was pH independent between pH 5.0 and 7.0. We were unable to determine the potential at more alkaline pH as the signal could not be detected in samples at pH 8.0 or above.

These results indicate that the iron–quinone component corresponds to Q_H and also to the component reflected by the C_{550} absorption change. The results differ from those obtained in chloroplasts in which the E_m of C_{550} is pH dependent. A similar effect is seen in purple bacteria where Q_I has a pH dependent midpoint potential in unfractionated membranes but not in isolated

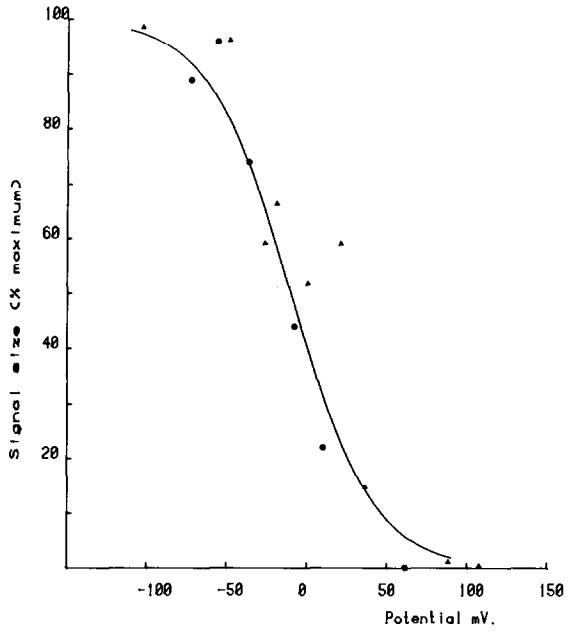

Fig.2. Redox titration at pH 6.0 of the iron–quinone electron acceptor in a preparation from *C. reinhardtii*. The reaction mixture included the following mediators (all at 20 μM): methylene blue, Lauth's violet, pyocyanine, indigo tetrasulphonate, indigo disulphonate and anthroquinone-1,5-disulphonate. The line drawn is the theoretical curve for a one electron acceptor at −10 mV. (•) Oxidative titration. (▲) Reductive titration.

reaction centres. The reason for this difference is unknown [8].

In purple bacteria following reduction of the primary quinone acceptor, a triplet arising from a charge recombination between the pheophytin acceptor and the reaction centre chlorophyll can be observed [8]. A similar triplet can be detected in photosystem II under reducing conditions [9]. This triplet can be observed in this preparation in the presence of excess dithionite [10] but cannot be observed in samples prepared in the titrations with Q_H reduced at potentials between −50 and −100 mV. We have also been unable to induce the signal attributed to a magnetic interaction between Q^-–Fe^{2+} and the pheophytin acceptor in samples at these potentials. These results suggest that another electron acceptor which can function at low temperature is present in these preparations. This acceptor is presumably Q_L but we have not yet detected any EPR signal corresponding to this component.

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