Evidence for two tightly bound iron-quinones in the electron acceptor complex of photosystem II

M.C.W. Evans and R.C. Ford

Department of Botany & Microbiology, University College London, London WC1E 6BT, England

Received 11 November 1985

The EPR signal of the iron-quinone electron acceptor of photosystem II in higher plant chloroplasts is normally difficult to observe. If the preparation is washed with formate to remove bound CO_2 the signal becomes larger. Using formate-washed photosystem II particles from peas the redox potential of the iron-quinone complex has been determined. Two waves are observed in the titrations at $E_m \sim 0$ and ~ -250 mV. The ability to reduce the pheophytin intermediary electron acceptor by illumination at 200 K is largely associated with the −250 mV step. It is suggested that there are two tightly bound quinones in the reaction centre and that the low-potential component is Qa. It is unlikely that the high-potential component is the gating quinone (Qb) as it is not displaced by DCMU and is present in *Chlamydomonas reinhardtii* preparations which lack the gating quinone.

Photosystem II *Electron acceptor* *Iron-quinone* *Plastoquinone* *Photosynthesis*

1. INTRODUCTION

The electron acceptor complex of PS II in plants and algae is thought to contain a pheophytin, which functions as a short-lived intermediary electron carrier, and plastoquinone which functions as the stable electron acceptor [1]. The plastoquinone which is bound to the reaction centre forms a stable semiquinone on reduction. This semiquinone has an unusual EPR spectrum at $g = 1.82$. This spectrum is very similar to that of the iron-quinone complex which forms the stable electron acceptor in the purple bacterial reaction centre [2]. We first observed this spectrum in a purified reaction centre preparation from *Chlamydomonas reinhardtii* [3] and it has subsequently been observed in higher plant preparations [4].

Illumination at 200 K of PS II preparations, and some purple bacterial preparations which have been frozen with the iron-quinone in the semiquinone state results in the reduction of the pheophytin intermediary acceptor. This can be observed as the appearance of a large split radical signal in the low-temperature EPR spectrum. In purple bacteria the ability to photoreduce the pheophytin appears to parallel the reduction of the primary quinone Qa in redox titrations, although the results are complicated by magnetic interactions with the secondary quinone acceptor Qb [5,6]. We have determined the redox potential of the iron-quinone complex in the *C. reinhardtii* preparation [7]. We obtained a midpoint potential $E_m \sim -10$ mV. This is the same as the Qh potential observed in titrations of fluorescence yield of PS II [8–10] and of the C550 absorption change thought to reflect Qa [11]. However, although we initially found that the ability to photoreduce the pheophytin was induced at the same potential it subsequently became apparent that only about 10% of the pheophytin was reducible at this potential [12]. Full reduction of the pheophytin was only possible in samples reduced to about −300 mV. The EPR signal of the iron-quinone in the titra-

Abbreviations: Mes, 2-(*N*-morpholino)ethanesulphonic acid; E_m, midpoint oxidation reduction potential; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS, photosystem

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

tions was smaller than in equivalent samples in which it was reduced by illumination.

Vermaas and Rutherford [13] have recently shown that the EPR signal of the iron-quinone complex, which is normally very small, can be readily observed in higher plant preparations which have been formate-washed to remove CO_2. We have now used pea PS II preparations treated in this way to redetermine the redox properties of the iron-quinone acceptors of PS II. We have obtained evidence for the presence of 2 components. One with $E_m \sim 0$ mV, the other with $E_m \sim -250$ mV. The ability to photoreduce the majority of the pheophytin by 200 K illumination parallels the reduction of the low-potential component.

2. MATERIALS AND METHODS

PS II-enriched preparations from pea chloroplasts were made by the procedure of Ford and Evans [14]. The particles were then washed essentially according to Vermaas and Rutherford [13] to remove CO_2. They were suspended in 50 mM Mes, 25 mM sodium formate, 10 mM NaCl, 5 mM MgCl at pH 6.0 for 1 h at room temperature under N_2. They were then centrifuged at 20000 × *g* for 10 min and the pellet resuspended in 50 mM K Hepes, 25 mM Na formate, 10 mM NaCl, 5 mM $MgCl_2$, pH 7.0 at 1–1.5 mg chlorophyll/ml.

Redox titrations were carried out as in [15]; poised samples were stored in liquid nitrogen in the dark until measured. The following compounds were used as redox mediators, dimethylbenzoquinone, *p*-phenylquinone, dichlorophenolindophenol, thionine, methylene blue, indigotetrasulphonate, anthroquinone-1,5-disulphonate, phenosafranine, safranine-t, neutral red, benzyl viologen and methyl viologen. Titrations were done with varying combinations of mediators to ensure that the observed effects were not due to specific mediator interactions.

EPR spectra were recorded using a Jeol FE1X spectrometer fitted with an Oxford instruments ESR 9 cryostat as described [3]. Illumination at 200 K was carried out by immersing the samples in a solid CO_2/ethanol bath in an unsilvered dewar and illuminating with a 1000 W projector for 5 min.

3. RESULTS AND DISCUSSION

The EPR spectra of the iron-quinone complex in formate-washed pea PS II particles reduced at different potentials is shown in fig.1. As in *C. reinhardtii* preparations an iron-quinone complex is reduced in samples at −100 mV. However, exposure to more reducing conditions results in an increase in signal size in these preparations. At −400 mV the signal size is approximately doubled.

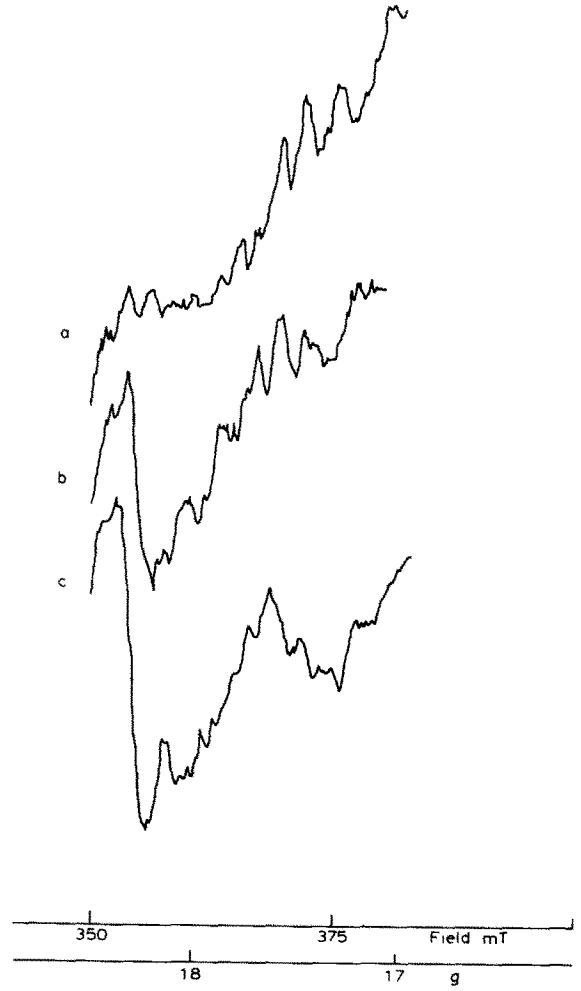

Fig.1. EPR spectra of the iron-quinone complex of formate-washed pea PS II particles. Particles (1.5 mg chlorophyll/ml) were poised at (a) 100 mV, (b) −100 mV, (c) −400 mV in the dark. EPR spectra were recorded at 5 K, microwave power 25 mW, modulation amplitude 1 mT, frequency 9.08 GHz, spectrometer gain 2500.

Redox titrations of these preparations show two waves with $E_m \sim 0$ and ~ -250 mV (fig.2). Although the EPR signals after formate washing are much larger than in untreated preparations the signals are still small and the baseline poor at the concentrations which can be used in titrations. It has proved difficult to obtain accurate E_m values with a range of about 50 mV seen between titrations. The titrations shown were done in the reducing direction, the signals can be reoxidised after the titration but titrations done in the oxidising direction have proved to be difficult because of changes in the baseline during the titration cycle.

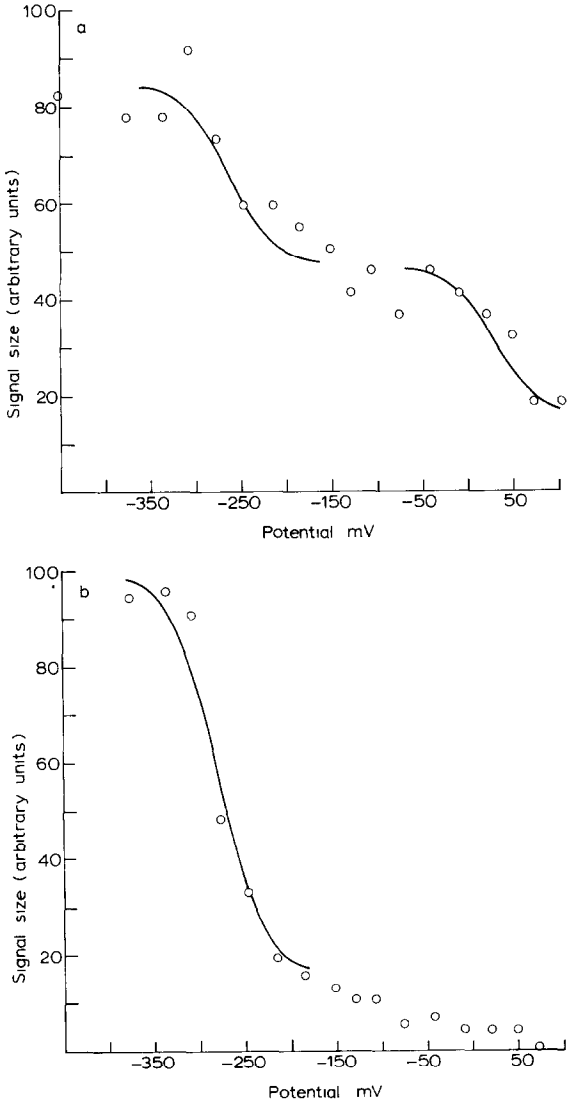

Fig.2. (a) Redox titration of the iron-quinone electron acceptor in formate-washed pea PS II particles (1.5 mg chlorophyll/ml). The full range of mediators was present at 20 μM. The curves drawn are the theoretical curves for i.e. transitions with $E_m = 30$ and -265 mV. (b) Redox titration of the split radical signal induced by 200 K illumination of formate-washed pea PS II particles. The samples were the same as those used in (a). The curve drawn is the theoretical curve for a one-electron transition with $E_m = -280$ mV.

Illumination of the titration samples at 200 K results in the reduction of the pheophytin and appearance of the split radical signal due to interaction between the pheophytin and iron semiquinone. As reported by Vermaas and Rutherford [13] the signal is narrower than in untreated preparations, although we have found considerable variation between preparations. This may reflect varying success in depleting CO_2. The ability to induce the split radical by 200 K illumination always occurred in parallel with the reduction of the iron-quinone complex. As we have reported previously a small fraction of the split signal was observed as the 0 mV component was reduced but most of the signal appeared only when the lower potential component was reduced (fig.2). These results suggest that the lower potential component is in fact Qa.

In purple bacteria 2 iron-quinone components are seen in titrations Qa, and a higher potential component assigned to Qb, the gating quinone. It seems unlikely that the high-potential component seen in these titrations is the gating quinone. The higher potential component is seen in *C. reinhardtii* preparations which do not contain the gating quinone [16]. We have also carried out titrations in the presence of high concentrations of DCMU (100 μM), which might be expected to displace Qb. However, there was apparently no effect of DCMU on the redox properties of the 2 steps in the titration. In these titrations addition of DCMU resulted in the appearance of a signal at $g = 1.82$ which was not removed by oxidation at 350 mV. The 0 mV step in the titration was then difficult to observe, although it could still be seen following 200 K illumination in the titration of the split signal induction (fig.3). The low-potential step in the titration could be observed and the main split signal induction was still associated with it.

As reported previously photoreduction of the iron-quinone could be observed at 5 K in these

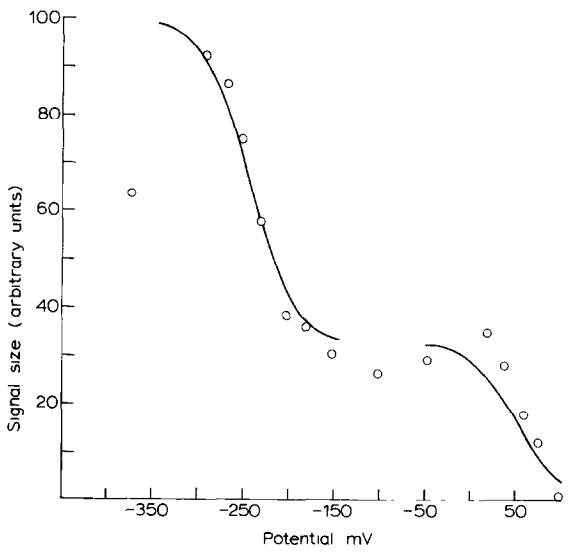

Fig.3. Redox titration of the split radical signal induced by 200 K illumination of formate-washed PS II particles in the presence of 100 μM DCMU. The curves drawn are the theoretical curves for one-electron transitions with E_m = 50 and −245 mV.

preparations. In samples from the titrations photoreduction at 5 K was lost above 0 mV, however 200 K illumination of samples at 0 to −100 mV would then increase the signal size to that observed below −300 mV, suggesting that the loss of measurable photoreduction may reflect changes in the electron donor rather than the reduction of the high potential iron-quinone.

We have attempted to clarify these results by comparative experiments with *Rhodopseudomonas viridis* chromatophores. In untreated chromatophores Qa and Qb can be detected and very complex titrations are observed [5]. We treated the chromatophores with *o*-phenanthroline, which is thought to displace Qb. We then expected to see a simple titration of Qa. In fact we obtained results very similar to those presented here for PS II. The results will be presented in detail elsewhere (in preparation). In summary, we observed 2 steps on the titration of Q and of the 200 K photoreduction of the pheophytin. The Q spectrum was that of Qa at both steps; no Qb spectrum was observed. Photoreduction of Q was lost only when the low-potential component had been reduced. Although the interpretation of these experiments is difficult in terms of current models of the *R. viridis* reaction centre they increase our confidence in the experimental results obtained in PS II.

The simplest interpretation of the results would be that there are two tightly bound quinones in the acceptor complex of PS II. These might be equivalent to Qa and Qb. However, as discussed above it is unlikely that the 0 mV component, Qh C550, is the gating quinone. The experiments suggest that there are 2 quinones which are tightly bound to the reaction centre, preceding the transiently bound gating quinone. If both of these quinones participate in electron transport current models of the electron transfer pathway may have to be revised. If they function in series the gating mechanism may be as proposed by Bouges-Bouquet [17] and Wraight [18]. However, if they function in parallel quite different models may be required. We have proposed one such model in which the transiently bound quinone is reduced by the concerted action of the 2 tightly bound semiquinones [19]. While there are a number of lines of evidence suggesting that there are multiple acceptors in PS II this is not the case in the bacterial reaction centre. There are also a number of reports which suggest that Q1 is not a quinone, and Witt [20] has recently presented a convincing experiment showing that only one electron can be stabilised between pheophytin and the DCMU block. These experiments cannot therefore be fully explained at present, experiments with purified reaction centres with defined quinone content are required to identify definitively the origin of the 2 redox components in the quinone complex.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the UK SERC. We are grateful to Ms I. Gallagher and Mrs S. McNeill for technical assistance.

REFERENCES

[1] Klimov, V.V., Dolan, E., Shaw, E.R. and Ke, B. (1980) Proc. Natl. Acad. Sci. USA 77, 7227–7231.
[2] Feher, G. and Okamura, M.Y. (1979) in: The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R. eds) pp.349–396, Plenum, New York.

[3] Nugent, J.H.A., Diner, B.A. and Evans, M.C.W. (1981) FEBS Lett. 124, 241–244.
[4] Rutherford, A.W., Zimmerman, J.L. and Mathis, P. (1983) FEBS Lett. 165, 156–162.
[5] Rutherford, A.W., Heathcote, P. and Evans, M.C.W. (1979) Biochem. J. 182, 515–523.
[6] Rutherford, A.W. and Evans, M.C.W. (1980) FEBS Lett. 110, 257–261.
[7] Evans, M.C.W., Nugent, J.H.A., Tilling, L.A. and Atkinson, Y.E. (1982) FEBS Lett. 145, 176–178.
[8] Golbeck, J.H. and Kok, B. (1979) Biochim. Biophys. Acta 547, 347–360.
[9] Horton, P. and Croze, E. (1979) Biochim. Biophys. Acta 546, 93–105.
[10] Karukstis, K.K. and Sauer, K. (1983) Biochim. Biophys. Acta 725, 246–253.
[11] Diner, B.A. and Delsome, R. (1983) Biochim. Biophys. Acta 722, 443–451.
[12] Evans, M.C.W., Atkinson, Y.E. and Ford, R.C. (1985) Biochim. Biophys. Acta 806, 247–254.
[13] Vermaas, W.F.J. and Rutherford, A.W. (1984) FEBS Lett. 175, 243–247.
[14] Ford, R.C. and Evans, M.C.W. (1983) FEBS Lett. 160, 159–164.
[15] Evans, M.C.W., Lord, A.V. and Reeves, S.G. (1974) Biochem. J. 138, 177–183.
[16] Diner, B.A. and Wollman, F.A. (1980) Eur. J. Biochem. 110, 521–526.
[17] Bouges-Bouquet (1980) Biochim. Biophys. Acta 594, 85–103.
[18] Wraight, C.A. (1979) Photochem. Photobiol. 30, 767–778.
[19] Evans, M.C.W. (1985) Physiol. Veg. 23, in press.
[20] Brettel, K., Schlodder, E. and Witt, H.T. (1985) Photobiochem. Photobiophys. 9, 205–213.