Volume 235, number 1,2, 71–75 FEB 06117 August 1988

Bicarbonate binding and the properties of photosystem II electron acceptors

Jonathan H.A. Nugent, Andrew R. Corrie, Christalla Demetriou, Michael C.W. Evans and Christopher J. Lockett

Department of Biology, University College London, Darwin Building, Gower Street, London WC1E 6BT, England

Received 13 May 1988

We have investigated the quinone-binding region of PS II using exchange reactions to replace key components. Bicarbonate was replaced by formate and Q_B by exogenous quinones or herbicide. The effects of these changes were monitored using the ESR signals from the Q_A iron-semiquinone and Fe^{3+} non-haem iron components. We observed that Q_B binding caused characteristic changes in the Q_A^- ESR signal and confirmed that the characteristics of Q_A^- depend on bicarbonate binding. The non-haem iron was oxidised only under conditions where bicarbonate was bound. The characteristics of bicarbonate binding were observed in *Phormidium laminosum* PS II, showing that the bicarbonate effect occurs in cyanobacteria. The results support a hypothesis which gives a central role to bicarbonate in providing the conditions for electron transfer in both cyanobacteria and higher plant PS II. It is suggested that bicarbonate binds at or close to the non-haem iron and influences the characteristics of Q_A, Q_B and the non-haem iron.

Photosystem II; ESR; Bicarbonate; Herbicide; Iron-quinone

1. INTRODUCTION

In the PS II reaction centre complex, an electron is transferred across the membrane from the reaction centre chlorophyll, P680, to a pheophytin and then to two quinones, Q_A and Q_B. The reaction centre of PS II in higher plants is located on a membrane-protein complex containing two 32 kDa chloroplast-encoded polypeptides termed D_1 and D_2 [1]. Models of PS II [2,3] based on the purple bacterial reaction centre fold the D_1 and D_2 proteins to give five transmembrane helices, with a pocket for the binding of quinone electron acceptors on the stromal side of each polypeptide. The proposed Q_A- and Q_B-binding sites are either side of the non-haem iron atom which is proposed to ligate to both D_1 and D_2. There are a number of PS II characteristics which suggest significant structural differences in the quinone-binding region from that found in the purple bacterial reaction centre. Among these are (i) a requirement for bicarbonate to achieve maximum rates of electron transport to Q_B [4–7] and (ii) oxidation of the non-haem iron Fe^{2+} to Fe^{3+}, which is then able to act as an additional electron acceptor [8–10].

ESR provides a useful probe of the quinone-binding region of PS II as signals are observed from several components including Q_A-Fe [11–13] and the oxidised non-haem iron, Fe^{3+} [8,9,14–17]. Two forms of iron-semiquinone, Q_A-Fe, spectra are observed, a '$g = 1.8$' form [11] seen at low pH and in the presence of formate [18] and a '$g = 1.9$' form at higher pH [12].

Here, we describe experiments which support a simple model for the quinone-binding region linking many of the unusual properties of PS II.

Correspondence address: J.H.A. Nugent, Department of Biology, University College London, Darwin Building, Gower Street, London WC1E 6BT, England

Abbreviations: Chl, chlorophyll; PS II, photosystem II; Mes, 4-morpholineethanesulphonic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DMBQ, 2,6-dimethylbenzoquinone

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

2. EXPERIMENTAL

PS II was prepared by the method of Ford and Evans [19] from market spinach (*Spinacea oleracea*). It was resuspended and stored at 77 K in 20 mM Mes-NaOH, 5 mM $MgCl_2$, 15 mM NaCl and 20% (v/v) glycerol, pH 6.3 (buffer A). Two types of spinach PS II sample were used (i) in 50 mM Hepes, 5 mM $MgCl_2$, 15 mM NaCl and 20% (v/v) glycerol, pH 7.5 (buffer B) and (ii) in buffer A.

Oxygen-evolving PS II particles from the thermophilic cyanobacterium *Phormidium laminosum* were prepared using a method based on that of Stewart and Bendall [20]. Detergent fractionation was performed using membrane fragments at 1 mg/ml Chl with 0.5% (w/v) *N*-dodecyl-*N*,*N*-dimethyl-ammonio-3-propanesulphonate (Serva, Heidelberg) for 40 min at 4°C [21]. The sample was then centrifuged at 100000 × *g* for 1 h and the supernatant containing PS II removed. The PS II was concentrated by precipitation using 10% (w/v) polyethylene glycol 6000 followed by centrifugation at 25000 × *g* for 20 min. The PS II particles were washed twice and finally resuspended in 10 mM Hepes, 10 mM $MgCl_2$, 5 mM disodium hydrogen phosphate, 25% (v/v) glycerol, pH 7.5.

Duplicate samples were placed in ESR tubes and dark-adapted for 4 h unless otherwise stated. Samples were then frozen to 77 K in the dark. Dark-adapted samples were illuminated at 77 K using a 1000 W light source with samples irradiated in liquid N_2 in a silvered dewar for 5 min. Where additions were made involving ethanol as solvent, a maximum of 1% ethanol was added. ESR spectrometry was performed at cryogenic temperatures using a Jeol X-band spectrometer with 100 kHz field modulation and an Oxford Instruments liquid helium cryostat.

3. RESULTS

3.1. *Iron-semiquinone ESR signals*

Only the $g = 1.8$ form of Q_A-Fe had been observed in *P. laminosum* PS II [22], the observation of the signal requiring high levels of DCMU [22] or the ADRY reagent CCCP [23]. This suggested that a magnetic interaction occurred between Q_A-Fe and Q_B which was disturbed on Q_B displacement by DCMU. We have now investigated the conditions required for the $g = 1.9$ form of Q_A-Fe to be observed, if an effect of bicarbonate on the Q_A-Fe signal occurs and whether a signal from bound Q_B can be detected.

A dark-adapted sample of oxygen-evolving *P. laminosum* PS II (fig.1a) had no broad signals from iron-semiquinone components. It did have a complex multiline spectrum not removed by signal averaging and perhaps originating from the manganese complex of the water-oxidation system. Following illumination at 77 K, it was difficult to observe the expected $g = 1.9$ Q_A-Fe spectrum in untreated samples at pH 7.5 (fig.1b). There was a small resonance near $g = 1.9$ attributed to iron-semiquinone but also another resonance near $g = 1.6$ (arrowed). Addition of DMBQ or CCCP increased the size of the $g = 1.9$ resonance obtained following 77 K illumination (fig.1c,d). DMBQ would oxidise any Q_B^- present and would also compete for the Q_B-binding site whilst CCCP deactivates oxidising equivalents on PS II electron donors probably mediating the oxidation of Q_B^-. The spectral characteristics of untreated samples may therefore be explained by bound Q_B^- in un-

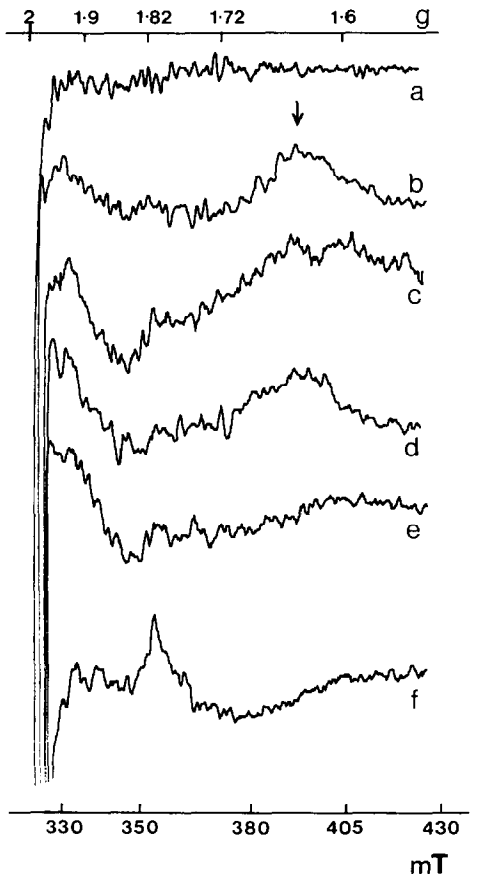

Fig.1. ESR spectra of the iron-semiquinone in cyanobacterial PS II. (a) *P. laminosum* PS II, dark-adapted for 4 h, (b–f) dark-adapted samples following 5 min illumination at 77 K. (b) No additions, (c) + 100 μM DMBQ, (d) + 100 μM CCCP, (e) + 0.5 mM DCMU, (f) + 100 mM sodium formate. Chlorophyll concentration 0.5 mg/ml. ESR conditions: temperature, 4 K; microwave power, 10 mW; modulation width, 1 mT. Spectra shown are the average of three recorded spectra.

treated samples having a magnetic interaction with Q_A^- to decrease the $g = 1.9$ resonance.

Fig.1e shows the effect of addition of the herbicide DCMU to displace Q_B from its binding site. The $g = 1.9$ form of Q_A-Fe was increased in size but the $g = 1.6$ signal was also reduced in size. The requirement for high DCMU concentrations may be to enable Q_B^- as well as Q_B to be displaced. An unexplained effect of DCMU in reducing the size of a small $g = 1.6$ resonance has been observed in Q_A-Fe containing samples of higher plant PS II [12].

By comparison of fig.1b–e, the $g = 1.6$ resonance can be seen to be a marker for Q_B bound to the reaction centre, as it is greatly reduced in size if Q_B is displaced by DCMU and decreased slightly when Q_B is replaced by an artificial Q_B (DMBQ).

Fig.1f shows that the addition of sodium formate to *P. laminosum* PS II to displace bicarbonate caused a change to a $g = 1.8$ form of Q_A. This demonstrates that the effect of bicarbonate displacement in higher plants is also present in cyanobacterial PS II.

3.2. *Non-haem iron* Fe^{3+} g = 6 *ESR signals*

Spinach PS II was used to investigate this signal, as more concentrated samples were needed than were available for *P. laminosum*. Fig.2a shows the $g = 6$ region of untreated spinach PS II at pH 7.5. The peak near $g = 9.7$ from 'rhombic' iron was present in all samples. The peak at $g = 6.7$ was equivalent to that identified in [17] by Diner and Petrouleas as indicating centres with a bound Q_B. The non-haem iron Fe^{3+} was oxidised by ferricyanide at pH 7.5 (fig.2c) but much less was observed at pH 6.3 (fig.2d) as indicated by the appearance of peaks at $g = 8.1$ and $g = 5.7$. The peaks at $g = 8.1$ and $g = 5.7$ were almost completely removed by 77 K illumination confirming that the non-haem iron Fe^{3+} acts as an electron acceptor (not shown).

To investigate the effect of bicarbonate binding we have used samples at pH 7.5 and attempted to oxidise the non-haem iron before and after sodium formate treatment to remove bicarbonate. The formate treatment at this pH caused a change from the $g = 1.9$ to the $g = 1.8$ form of Q_A-Fe in non-oxidised samples (not shown). Formate treatment at pH 7.5 does not change the ESR spectrum in the $g = 6$ region in untreated samples (fig.2b).

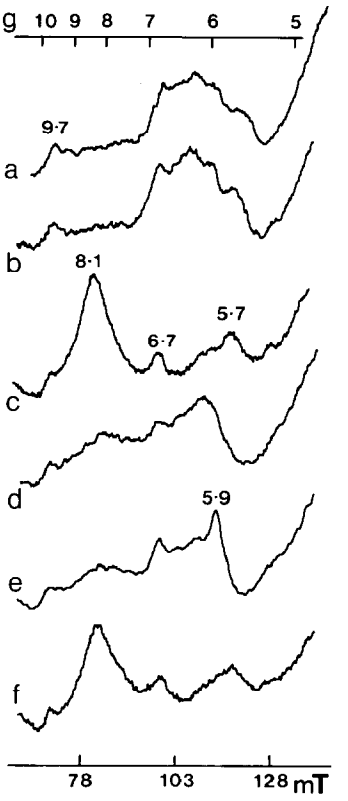

Fig.2. ESR spectra of the $g = 6$ non-haem iron region of spinach PS II. (a) pH 7.5, 1 h dark-adapted at 4°C. (b) pH 7.5, plus 100 mM sodium formate, 1 h dark-adapted. (c) pH 7.5, plus 5 mM potassium ferricyanide, 50 min dark-adapted. (d) pH 6.3, plus 5 mM ferricyanide, 50 min dark-adapted. (e) pH 7.5, plus 100 mM formate, 45 min dark-adapted then 5 mM ferricyanide added and dark-adapted for 45 min. (f) pH 7.5, plus 5 mM ferricyanide, 45 min dark-adapted then 100 mM formate added and dark-adapted for 45 min. Chlorophyll concentration 5 mg/ml. The g values of the major peaks discussed in the text are labelled. ESR conditions as in fig.1 except microwave power, 5 mW.

However, the oxidation by ferricyanide of the iron was prevented in the time shown. A small sharp peak was induced at $g = 5.9$ (fig.2e) but this was unaffected by 77 K illumination. When the non-haem iron was oxidised before addition of formate, the $g = 6$ non-haem iron Fe^{3+} signal was still observed (fig.2f). This indicates that the Fe^{3+} state protects the bicarbonate from removal by formate. The relationships between bicarbonate and the ability to oxidise the non-haem iron therefore suggest a close structural relationship in the quinone-binding region.

4. DISCUSSION

Our results indicate that bicarbonate binding in both cyanobacteria and higher plants affects the properties of Q_A and non-haem iron. We also observed an interaction between Q_A and Q_B in *P. laminosum* PS II. A link between the pH conditions needed to observe the $g = 1.9$ form of Q_A^- and the $g = 6$ ESR signal of oxidised non-haem iron has been established [16,17] suggesting that bicarbonate binding and protonation reactions influence the characteristics of both components. Recently, evidence has been presented that protonation of Q_B may involve the bicarbonate ligand, which was also suggested to be bound to the non-haem iron [24]. The presence of the negatively charged bicarbonate would lower the redox potential of the iron allowing the ferricyanide oxidation in PS II [16,17]. The properties of Q_A, Q_B and non-haem iron are therefore all linked to bicarbonate binding.

The results support a hypothesis which gives a central role to bicarbonate in providing the conditions for electron transfer in both cyanobacteria and higher plant PS II. It is suggested that bicarbonate binds at or close to the non-haem iron, stabilising a proton at the Q_B-binding site and influencing the redox properties of Q_B. The structural changes caused by bicarbonate binding also influence the characteristics of Q_A and stabilise the Fe^{3+} non-haem iron.

We propose that the role of the bidentate ligand, bicarbonate, is to provide negative charge to increase the pK_a of a specific protonated group needed for electron transfer. A protonated group carrying a positive charge may be required at the site of Q_B binding so that the anionic species is stabilised. The positive charge would be either (i) a protonated amino acid residue such as histidine or (ii) a proton carried by the bicarbonate itself. The bicarbonate would be bound to a second positively charged group, another amino acid residue or the non-haem iron. Loss of the positive charge at the Q_B site in the absence of bicarbonate would decrease Q_B binding and impair electron flow. The protonation of Q_B following reduction would also be impaired [24].

Herbicides, especially the phenolic herbicides which carry a negative charge, compete for bicarbonate and Q_B-binding sites [7]. Therefore, some herbicides as well as formate and other anions will interfere with electron flow by influencing Q_B through effects on bicarbonate binding. Bicarbonate affects the shape of the Q_A iron-semiquinone signal through its binding at or near the iron. When bicarbonate is absent the Q_A-Fe signal is similar to that seen in purple bacteria. Allosteric effects caused by the structural changes resulting from bicarbonate binding and charge pairing may be involved in causing the change in ESR characteristics.

Bound Q_B affects the Q_A-Fe signal as demonstrated above. An effect of exogenous quinone at the Q_B site on the $g = 6$ ESR signal of the non-haem iron has also been documented [17], confirming that structural changes in the Q_B pocket are transmitted to Q_A perhaps through movement of the iron. The effects of bicarbonate binding on the redox potential of the iron suggest that both the Q_A and Q_B redox potentials could also be altered by bicarbonate binding. The maintenance of a positively charged Q_B-binding site would raise the midpoint redox potentials of the $Q/Q^{\cdot -}$ and $Q^{\cdot -}/Q^{2-}$ couples and lower the pK_a values providing the conditions for electron transport between Q_A and Q_B. Bicarbonate binding may also influence the redox properties of Q_A. Only the redox potential characteristics of the $g = 1.8$ ESR signal have been measured and some measurements have required the interpretation that there is an electron carrier in addition to Q_A and Q_B [25,26]. Redox titrations of Q_A under conditions where pH, bound bicarbonate and bound Q_B are controlled are now needed.

Acknowledgements: We would like to thank the UK Science and Engineering Research Council for financial support. We would also like to thank Drs Derek Bendall, Julia Hubbard, John Sinclair and Steven Rolfe for useful comments and discussion.

REFERENCES

[1] Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
[2] Trebst, A. (1987) Z. Naturforsch. 42c, 742–750.
[3] Michel, H. and Deisenhofer, J. (1988) Biochemistry 27, 1–77.
[4] Govindjee and Van Rensen, J.J.S. (1978) Biochim. Biophys. Acta 505, 183–213.

[5] Vermaas, W.F.J. and Van Rensen, J.J.S. (1981) Biochim. Biophys. Acta 636, 168–174.
[6] Stemler, A. and Govindjee (1973) Plant Physiol. 52, 119–123.
[7] Van Rensen, J.J.S. (1984) Z. Naturforsch. 39c, 374–377.
[8] Petrouleas, V. and Diner, B.A. (1986) Biochim. Biophys. Acta 849, 265–275.
[9] Nugent, J.H.A. and Evans, M.C.W. (1980) FEBS Lett. 112, 1–4.
[10] Wraight, C.A. (1985) Biochim. Biophys. Acta 809, 320–330.
[11] Nugent, J.H.A., Diner, B.A. and Evans, M.C.W. (1981) FEBS Lett. 124, 241–244.
[12] Rutherford, A.W. and Zimmermann, J.L. (1984) Biochim. Biophys. Acta 767, 168–175.
[13] Nugent, J.H.A., Evans, M.C.W. and Diner, B.A. (1982) Biochim. Biophys. Acta 682, 106–114.
[14] Zimmerman, J.-L. and Rutherford, A.W. (1986) Biochim. Biophys. Acta 851, 416–423.
[15] Itoh, S., Tang, X.-S. and Satoh, K. (1986) FEBS Lett. 205, 275–281.
[16] Petrouleas, V. and Diner, B.A. (1987) Biochim. Biophys. Acta 893, 126–137.
[17] Diner, B.A. and Petrouleas, V. (1987) Biochim. Biophys. Acta 893, 138–148.
[18] Vermaas, W.F.J. and Rutherford, A.W. (1984) FEBS Lett. 175, 243–248.
[19] Ford, R.C. and Evans, M.C.W. (1983) FEBS Lett. 160, 159–163.
[20] Stewart, A.C. and Bendall, D.S. (1979) FEBS Lett. 107, 308–312.
[21] Schatz, G.H. and Witt, H.T. (1984) Photobiochem. Photobiophys. 7, 1–14, 77–89.
[22] Atkinson, Y.E. and Evans, M.C.W. (1983) FEBS Lett. 159, 141–144.
[23] Atkinson, Y.E. and Evans, M.C.W. (1984) in: Advances in Photosynthesis Research (Sybesma, C. ed.) vol.1, pp.139–142, Nijhoff/Junk, Dordrecht.
[24] Van Rensen, J.J.S., Tonk, W.J.M. and De Bruijn, S.M. (1988) FEBS Lett. 226, 347–351.
[25] Evans, M.C.W., Atkinson, Y.E. and Ford, R.C. (1985) Biochim. Biophys. Acta 681, 242–247.
[26] Evans, M.C.W. and Ford, R.C. (1986) FEBS Lett. 195, 290–294.