

DIRECT DETECTION OF THE ELECTRON ACCEPTOR OF PHOTOSYSTEM II

Evidence that Q is an iron—quinone complex

J. H. A. NUGENT, B. A. DINER⁺ and M. C. W. EVANS

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

⁺Institut de Biologie Physico Chimique, 13 rue Pierre and Marie Curie, 75005 Paris, France

Received 19 December 1980

1. Introduction

The primary photochemistry of the photosystem II reaction centre involves the transfer of an electron from the primary chlorophyll donor (P680) to the primary acceptor, Q. The properties of Q, a specialised plastoquinone [1,2] have been studied in detail using indirect fluorescence measurements [3–6] and absorbance changes [7–12] but no electron paramagnetic resonance (EPR) signal was observed to confirm the suggestion [10,11] that as in bacterial reaction centres, a transition metal ion (probably Fe^{2+}) was complexed to Q.

When Q is chemically reduced, the photoreduction of a pheophytin intermediate electron carrier (I) has been observed [13]. Recent EPR investigations have identified a signal near $g = 2.00$ corresponding to I^- [14–17] which, when present in samples containing Q^- , gives rise to an EPR doublet [14,15] with similar properties to those found in purple photosynthetic bacterial reaction centres (reviewed in [18]). This and further work [19] strongly suggested that the interaction producing the doublet involved a semiquinone—iron complex of the type exhibiting characteristic EPR signals near $g = 1.82$ in purple photosynthetic bacteria [20–23]. Using broken chloroplasts from a mutant of barley (*Hordeum vulgare* viridis zb 63) lacking photosystem I [16,17], the photoreduction of I^- was also observed at 5–77 K indicating the presence of a fast donor to P680 under these conditions.

In [24] a highly active photosystem II particle was prepared from a mutant of the green alga *Chlamydomonas reinhardtii*, originally isolated by P. Bennoun. Fluorescence measurements [24] suggest that the particles lack the secondary quinone acceptor B. Using

these particles we have observed the chemical and photochemical reduction of a component attributed to Q^- which has g -value, lineshape and microwave power characteristics of a semiquinone—iron complex. The photo-induction of the EPR doublet signal of I^- at 5 K when the signal attributed to Q^- is present is also demonstrated.

2. Materials and methods

Intact particles and the further-purified DEAE particles were prepared from *Chlamydomonas reinhardtii* as in [24] and stored at -80°C until used. EPR spectrometry was conducted as in [17] using a Jeol Felix X band EPR spectrometer. Field and g -value scales used in figures are approximate. Illumination of samples was performed using a Barr and Stroud LS II 150 W fibre-optic light source.

3. Results and discussion

EPR investigation of both intact and DEAE-treated photosystem II particles from *C. reinhardtii* revealed EPR spectra of all the components associated with the photosystem II reaction centre observed in chloroplasts from the barley mutant zb 63 [16,17]. These were signal II and the low temperature light-inducible signal II_L [25], a narrow low temperature light-induced signal and at low redox potentials a signal attributed to the pheophytin intermediate. No signals due to photosystem I components were detected in these particles. In addition to the photosystem II components identified, changes were observed in the $g = 1.82$ region of the

spectrum following low-temperature illumination of untreated particles or particles oxidised with 300 μ M ferricyanide before freezing. Fig.1a shows the $g = 1.82$ region of untreated intact particles frozen after dark adaption for 15 min. Fig.1b shows the spectrum of the same sample after illumination for 30 s at 5 K. The irreversible light-induced signal is shown in the difference spectrum, fig.1c. The g -value and lineshape of this signal are very similar to those of the semiquinone-iron signal of the bacterial primary electron acceptor, as are the EPR conditions of very low temperature and high microwave powers required for resolution [20,23].

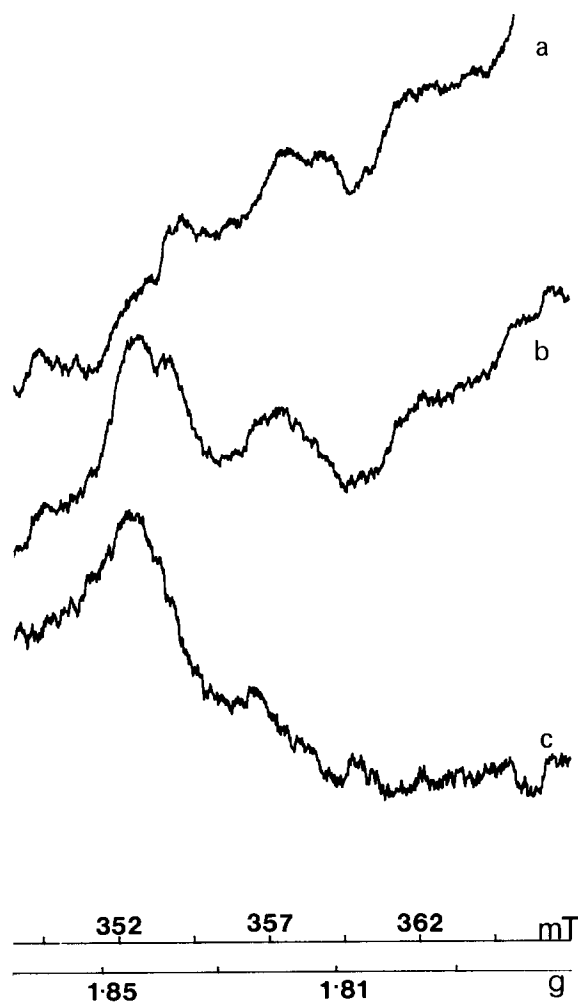


Fig.1. EPR spectra of photosystem II particles frozen after dark adaption for 15 min: (a) dark; (b) after illumination at 5 K; (c) difference spectrum (b - a); microwave power 25 mW; time constant 1 s; scan rate 5 mT/min; modulation amplitude 1 mT; gain 5000; temp. 5 K; frequency 9.1 GHz; 1.1 mg chl/ml.

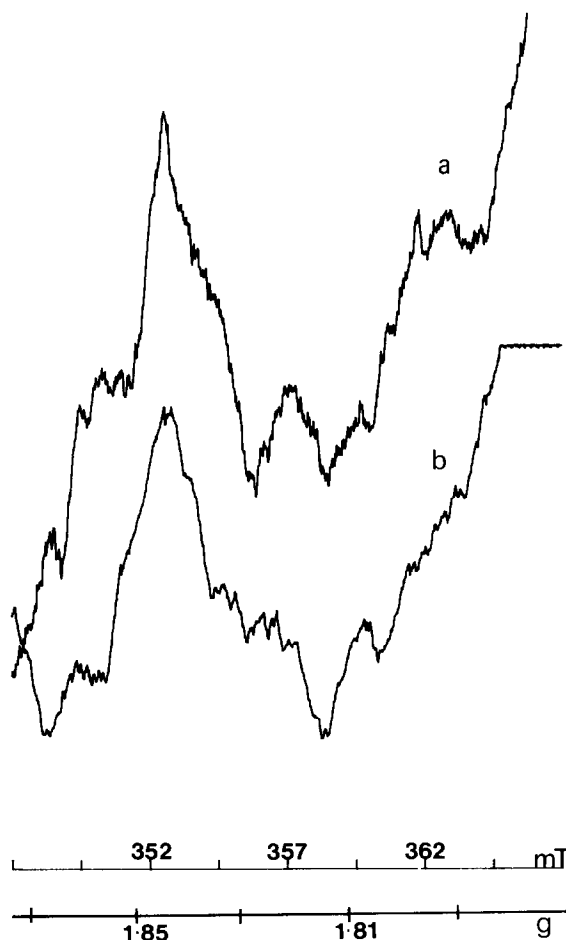


Fig.2. EPR spectra of DEAE-photosystem II particles: (a) frozen in the dark and illuminated at 5 K; (b) chemically reduced by dithionite at pH 6.0 in the dark then frozen. EPR conditions as in fig.1; 1.15 mg chl/ml.

The $g = 1.84$ signal was also observed after illumination of the DEAE-treated photosystem II particles at 5 K (fig.2a) and also after chemical reduction of DEAE particles by dithionite at pH 6 in the dark (fig.2b). This indicates that the signal is due to an acceptor component of photosystem II. A spectrum showing a wider field scan of the $g = 1.82$ region (fig.3c) reveals the broad high-field region of the signal. This spectrum was obtained by subtraction of the spectra of untreated particles frozen in the dark, fig.3a and particles frozen under illumination (fig.3b). When the sample in fig.3 was stored in the dark at 77 K, part of the signal near $g = 1.84$ decayed but this could be restored by further illumination at 5 K. The pattern of behaviour is also followed by signal II_{ir} [25] in these samples suggesting

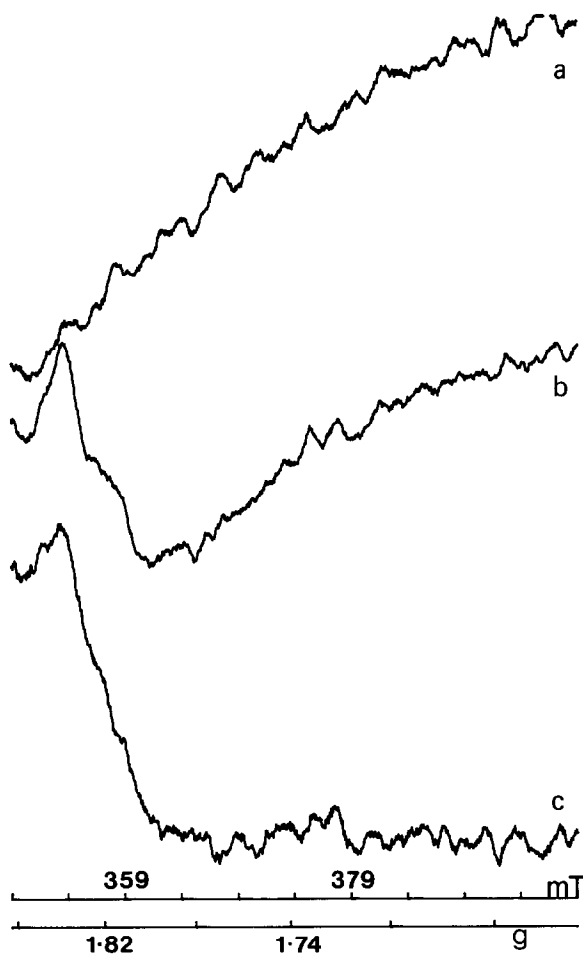


Fig.3. EPR spectra of photosystem II particles: (a) frozen after dark adaption for 15 min; (b) frozen under continuous illumination; (c) difference spectrum (b - a). Conditions as fig.1.

that, at least in this case, signal II_H is the electron donor.

Fig.4 shows the $g = 2.00$ region of particles reduced by dithionite at pH 6.0 in which the $g = 1.84$ was also present (fig.2b). After illumination at 5 K (fig.4b) an increase in signal size is observed with the difference spectrum (fig.4c) showing the appearance of the doublet attributed to the reduced pheophytin intermediate acceptor, I^- [14]. At higher temperature and lower microwave powers the singlet spectrum of I^- is observed. The lineshape of the doublet is not as fully resolved as in [14] but is similar to that shown in [15]. This may be due to the oxidation of a donor to P680 giving rise to a signal near $g = 2.00$ which distorts the central region of the doublet. Fig.4d shows a similar

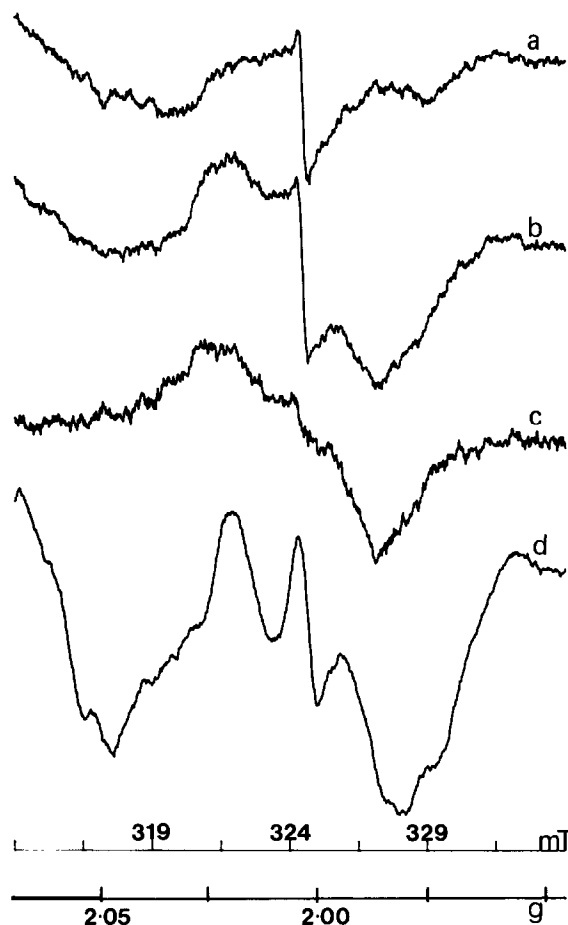


Fig.4. EPR spectra showing light-induced I^- doublet in DEAE-particles: (a) sample dark frozen after reduction with sodium dithionite for 30 min at pH 6.0; (b) same sample after illumination at 5 K; (c) difference spectrum (b - a); microwave power 50 mW; time constant 0.3 s; scan rate 10 mT/min; modulation amplitude 0.2 mT; gain 4000; temp. 5 K; frequency 9.1 GHz; 1.15 mg chl/ml; (d) sample prepared as (b) with modulation amplitude 0.63 mT; time constant 1 s; scan rate 5 mT/min.

sample after illumination but with different EPR conditions which increase in size but may also broaden the doublet.

We conclude from these results, and by analogy to bacterial reaction centres, that the component giving rise to the signal near $g = 1.82$ is the primary acceptor Q of photosystem II. The spectrum suggests that the acceptor is a quinone-iron complex with an EPR signal in the semiquinone state. The semiquinone-iron signal can be induced by illumination at room or low temperature and by chemical reduction. When the signal is chemically reduced, the doublet signal of I^-

can be induced by illumination at low temperature. This signal is attributed to interaction between the semiquinone—iron and the pheophytin radical.

Further investigation of the properties of this signal should allow not only a fuller understanding of the electron acceptors of the photosystem II reaction centre but also progress in elucidating the donor chain to P680.

Acknowledgements

We thank the UK Science Research Council, Délégation Général à la Recherche Scientifique et Technique (contract no. 80.7.0157) and the Commissariat à l'Energie Solaire (contract no. 80.75.091) for financial support.

References

- [1] Stiehl, H. H. and Witt, H. T. (1969) *Z. Naturforsch* 246, 1588–1598.
- [2] Van Gorkom, H. J. (1974) *Biochim. Biophys. Acta* 347, 439–442.
- [3] Cramer, W. A. and Butler, W. L. (1969) *Biochim. Biophys. Acta* 172, 503–510.
- [4] Duysens, L. M. N. and Sweers, H. E. (1963) in: *Studies on Microalgae and Photosynthetic Bacteria* (Myachi, S. ed) pp. 353–372, Univ. Tokyo Press.
- [5] Horton, P. and Croze, E. (1979) *Biochim. Biophys. Acta* 545, 188–201.
- [6] Malkin, R. and Barber, J. (1979) *Arch. Biochem. Biophys.* 193, 169–178.
- [7] Okayama, S. and Butler, W. L. (1972) *Plant Physiol.* 49, 769–774.
- [8] Pulles, M. P. J., Kerkhof, P. C. and Ames, J. (1974) *FEBS Lett.* 47, 143–145.
- [9] Cox, R. P. and Bendall, D. S. (1974) *Biochim. Biophys. Acta* 347, 49–59.
- [10] Knaff, D. B. (1975) *Biochim. Biophys. Acta* 376, 583–587.
- [11] Knaff, D. B., Malkin, R., Myron, J. C. and Stoller, M. (1977) *Biochim. Biophys. Acta* 459, 402–411.
- [12] Malkin, R. (1978) *FEBS Lett.* 87, 329–333.
- [13] Klimov, V. V., Klevanik, A. V., Shuvalov, V. A. and Krasnovsky, A. A. (1977) *FEBS Lett.* 82, 183–186.
- [14] Klimov, V. V., Dolan, E. and Ke, B. (1980) *FEBS Lett.* 112, 97–100.
- [15] Rutherford, A. W., Paterson, D. R. and Mullet, J. E. (1981) *Biochim. Biophys. Acta* in press.
- [16] Nugent, J. H. A., Möller, B. L. and Evans, M. C. W. (1981) *Proc. 5th Int. Conf. Photosynth.* in press.
- [17] Nugent, J. H. A., Möller, B. L. and Evans, M. C. W. (1980) *FEBS Lett.* 121, 355–357.
- [18] Sauer, K. (1979) *Ann. Rev. Phys. Chem.* 30, 155–178.
- [19] Klimov, V. V., Dolan, E., Shaw, E. R. and Ke, B. (1981) submitted.
- [20] Tiede, D. M., Prince, R. C., Reed, G. H. and Dutton, P. L. (1976) *FEBS Lett.* 65, 301–304.
- [21] Prince, R. C., Tiede, D. M., Thorner, J. P. and Dutton, P. L. (1977) *Biochim. Biophys. Acta* 462, 467–490.
- [22] Dutton, P. L., Prince, R. C. and Tiede, D. M. (1978) *Photochem. Photobiol.* 28, 939–949.
- [23] Okamura, M. Y., Isaacson, R. A. and Feher, G. (1979) *Biochim. Biophys. Acta* 546, 394–417.
- [24] Diner, B. A. and Wollman, F. A. (1980) *Eur. J. Biochem.* 110, 521–526.
- [25] Nugent, J. H. A. and Evans, M. C. W. (1979) *FEBS Lett.* 101, 101–104.