EPR DETECTION OF THE PRIMARY PHOTOCHEMISTRY OF PHOTOSYSTEM II IN A BARLEY MUTANT LACKING PHOTOSYSTEM I ACTIVITY

J. H. A. NUGENT, Birger Lindberg MØLLER* and M. C. W. EVANS

Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT, England and **Department of Physiology, Carlsberg Laboratory, Gamle Carlsberg Vej 10, 2500 Copenhagen Valby, Denmark*

Received 17 October 1980

1. Introduction

Low temperature electron paramagnetic resonance spectrometry (EPR) allows the study of photosynthetic reactions involving only those components closely linked to the reaction centre. However the study of photosystem II in chloroplasts by this technique is difficult due to the presence of large signals from photosystem I components. The two main approaches to overcome this problem are either the use of detergent subchloroplast particles enriched in photosystem II or the use of chloroplasts from mutants lacking components of photosystem I and the electron-transport chain.

Using the first approach, 4 signals have been observed by EPR at cryogenic temperatures. These are a signal due to cytochrome b_{559} [1,2] which can be photo-oxidised at cryogenic temperatures; a signal II species which also acts as an electron donor to $P680^+$ [3,4] at low temperature; a signal with characteristics of a reduced pheophytin intermediate acceptor, I [5] and a light-induced triplet [6] originating from the recombination of I^- and $P680^+$, the primary donor chlorophyll of photosystem II. Studies using electron spin echo also showed that changes in signal II occurred upon illumination at cryogenic temperatures [7].

Employing the second approach we have now investigated the low temperature EPR characteristics of chloroplasts from a nuclear gene mutant of barley lacking photosystem I. The mutant is one of several identified in barley [8–10]. The study reveals EPR signals pre-masked by signals from photosystem I which confirm the work using subchloroplast particles and provide further information on the photosystem II reaction centre.

2. Materials and methods

Barley (*Hordeum vulgare* c.v. Svalof's Bonus) *viridis* zb^{63} was grown as in [10] and chloroplasts were prepared as in [3] except that centrifugation at 9000 × *g* was required to pellet the broken chloroplasts. The chloroplasts were diluted in a buffer containing 50 mM Hepes (*N*-2-hydroxyethylpiperazine *N'*-2 ethanesulfonic acid) pH 7.6, 5 mM $MgCl_2$ and 0.33 M sorbitol. EPR measurements were performed as in [3] and chlorophyll concentrations calculated as in [11] are given in the legends to the figures. *g*-Values were calculated using a Mn^{2+} standard and the internal standard of signal II, $g = 2.0048$. The field and *g*-scales shown in figures are approximate.

3. Results and discussion

The chloroplasts from *viridis* zb^{63} lack P700 [10] whilst retaining a fully functional photosystem II. We were unable to detect any low temperature photochemistry from photosystem I or any signals from $P700^+$, iron-sulphur centres A, B and X or the Rieske centre using chemical oxidation or reduction. Signals with characteristics of iron–sulphur centres were detected but these did not participate in low temperature photochemistry [12].

The $g = 2.0$ region EPR spectrum of untreated chloroplasts from *viridis* zb^{63} which had been frozen in the dark is shown in fig.1b and has the characteristic lineshape of signal II. After a 30 s illumination at 13 K an increase in signal size is seen (fig.1a). Difference spectra showing the irreversible photo-induced signals after a short <1 s illumination and the 30 s illumination are shown in fig.1c,d. The radical

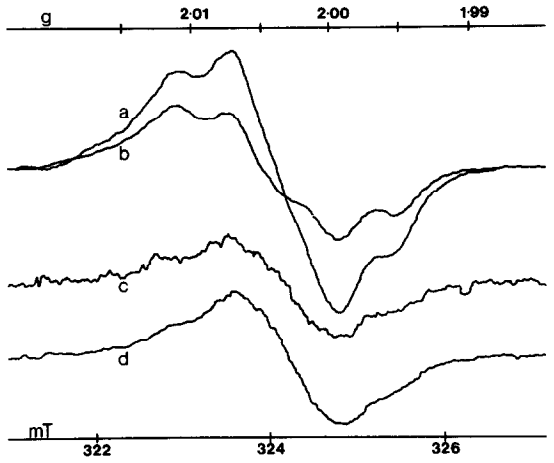

Fig.1. EPR spectra of chloroplasts from *viridis zb*^{63} frozen after dark adaption for 20 min: (a) after 30 s illumination at 13 K; (b) before illumination; (c) difference spectrum (× 2) showing signal induced by <1 s illumination at 13 K; (d) difference spectrum (a) – (b). Microwave power 100 μW; time constant 0.3 s; scan rate 2.5 mT/min; modulation amplitude 0.2 mT; gain 1250; temp. 13 K, frequency 9.104 GHz. Chlorophyll conc. 1.5 mg/ml.

produced by illumination appears to be composed of two signals, a small signal II species indicated by the shoulder at $g = 2.01$ plus a narrower 10–12 G species. When the sample was stored in the dark at 77 K overnight the signal decays to that shown in fig.2b. By comparison to fig.1b it can be seen that part of the previously dark-stable signal II has decayed along with most of the signal produced by the first illumination. Reillumination of the sample at 13 K restores the lineshape and size of the signal (fig.2a) to one similar to that after the first illumination (fig.1a). The difference spectrum showing the irreversible light-induced signal, fig.2c reflects the changes noted above as more signal II is generated by the second illumination restoring that lost during dark storage at 77 K. Further dark storage at 77 K followed by re-illumination produces a similar result to that in fig.2. The appearance of signal II represents the donation of an electron to the photosystem II reaction centre whilst the narrow radical may represent the reduction of a quinone acceptor (Q) to the semiquinone or oxidation of another donor. Reduction of the *viridis zb*^{63} chloroplasts with sodium ascorbate prior to freezing in the dark removes the signal II radical. Illumination at cryogenic temperatures then produces mainly the irreversible 11 G wide radical and a signal near $g = 3.0$ attributed to the photo-oxidation of cytochrome b_{559}. The involvement of cytochrome b_{559} as an electron donor may explain the small signal II photo-oxidation after the first low temperature illumination in samples prepared without added reductant. During dark storage at 77 K, cytochrome b_{559} is not re-reduced by electrons from the acceptor side unlike the signal II species, therefore requiring an increased signal II photo-oxidation on re-illumination.

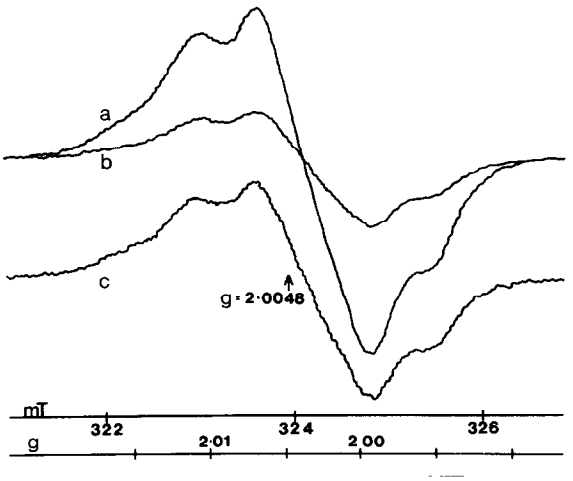

Fig.2. EPR spectrum of sample from fig.1. stored in the dark overnight at 77 K: (a) after 30 s illumination at 13 K; (b) before illumination; (c) difference spectrum (a) – (b); conditions as in fig.1.

Viridis zb^{63} chloroplasts frozen under illumination and then stored at 77 K overnight show a spectrum of signal II (fig.3b) which is slightly larger than that seen in the equivalent frozen dark sample. On illumination at 13 K more signal II appears irreversibly (fig.3a). Fig.3c shows the spectrum of the light-induced signal II_{lt}. This species and the signal II induced in fig.1,2 probably represent the same component. In the sample shown in fig.3, cytochrome b_{559} and possibly other donors are blocked by freezing under illumination.

Reduction of *viridis zb*^{63} chloroplasts by dithionite at pH 7.5 followed by freezing under illumination produces a 13 G radical in the $g = 2.0$ region (fig.4a,b) characteristic of the radical attributed to a reduced pheophytin acceptor of photosystem II [3]. Samples reduced by dithionite but frozen in the dark show only a small radical. However during illumination at 13 K a radical with similar lineshape and g-value ($g = 2.0033$) to the reduced pheophytin signal slowly appears (fig.4c). This indicates the presence of a fast low temperature donor to $P680^+$ which can compete with the back reaction between $P680^+$ and I^- under

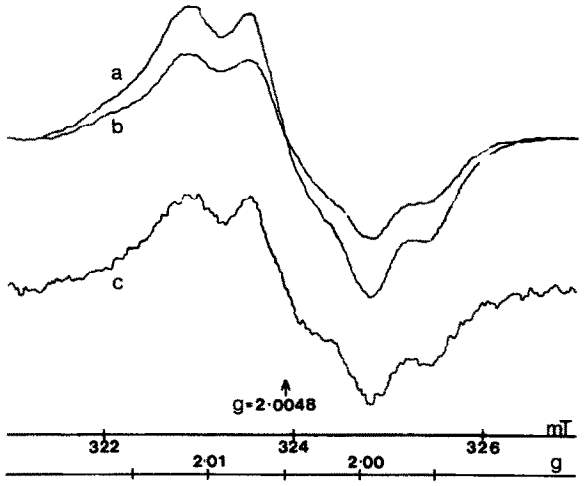

Fig.3. EPR spectra of chloroplasts from *viridis zb*^{63} frozen under illumination and stored in the dark at 77 K: (a) after illumination at 13 K; (b) before illumination; (c) difference (a) – (b) × 2; EPR conditions as in fig.1.

these conditions. This donor may be represented by the small linewidth differences seen between the low temperature photo-induced and room temperature photo-induced signals in fig.4. Most of the signal shown in fig.4c decays during overnight storage at 77 K and can be restored by low temperature illumination suggesting that the fast donor is not cytochrome

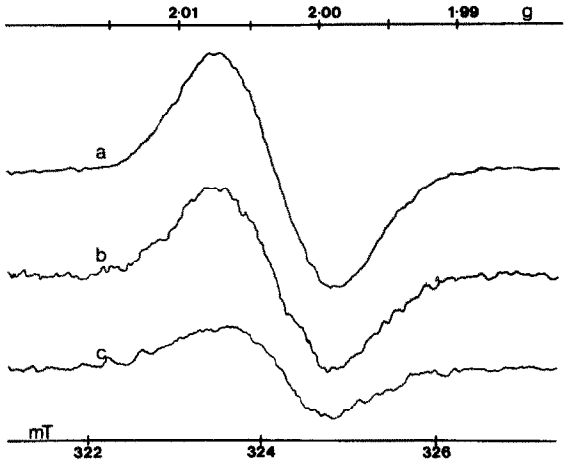

Fig.4. EPR spectra of chloroplasts from *viridis zb*^{63} dark-adapted in the presence of dithionite then: (a) frozen under illumination – EPR conditions as in fig.1 except gain 2500; (b) frozen under illumination – EPR conditions as in fig.1 except gain 5000, microwave power 5 μW; (c) frozen in the dark and illuminated at 13 K; spectrum shows irreversible light-induced signal – EPR conditions as in fig.1 except gain 2500, chlorophyll conc. 4 mg/ml.

b_{559} which donates irreversibly at cryogenic temperatures.

This study confirms the function of cytochrome b_{559} and signal II_{lt} as low temperature electron donors to the photosystem II reaction centre with the latter possibly functioning as the immediate donor to $P680^+$. The signal attributed to I^- now observed in chloroplasts confirms the work on detergent subchloroplast particles [5] and the detection of another radical species with characteristics of an acceptor may be due to the primary quinone acceptor Q. However this latter species may arise from another component, its *g*-value and its absence from samples frozen under illumination (fig.3) or reduced by dithionite in the dark not being consistent with the assignment to Q. Further information from experiments using *viridis zb*^{63} should allow the development of a scheme for electron transport in photosystem II and provide an important link between studies on detergent subchloroplast particles and chloroplasts in their native state.

Acknowledgements

This work was supported by the UK Science Research Council and the EEC Solar Energy Research Programme, contracts 029-76-ES-DK and 019-76-ES-UK. We wish to thank Professor Diter von Wettstein for encouragement and useful discussion during this study.

References

[1] Malkin, R. and Vänngard, T. (1980) FEBS Lett. 111, 228–231.
[2] Nugent, J. H. A. and Evans, M. C. W. (1980) FEBS Lett. 112, 1–4.
[3] Nugent, J. H. A. and Evans, M. C. W. (1979) FEBS Lett. 101, 101–104.
[4] Nugent, J. H. A., Stewart, A. C. and Evans, M. C. W. (1981) submitted.
[5] Klimov, V. V., Dolan, E. and Ke, B. (1980) FEBS Lett. 112, 97–100.
[6] Rutherford, A. W., Paterson, D. R. and Mullet, J. E. (1981) submitted.
[7] Nishi, N. N., Hoff, A. J., Schmidt, J. and van der Waals, J. H. (1978) Chem. Phys. Lett. 58, 164–170.
[8] Møller, B. L., Smillie, R. M. and Høyer-Hansen, G. (1980) Carlsberg Res. Commun. 45, 87–99.
[9] Simpson, D. J. and Von Wettstein, D. (1980) Carlsberg Res. Commun. 45, in press.
[10] Hiller, R. G., Møller, B. L. and Høyer-Hansen, G. (1980) Carlsberg Res. Commun. 45, in press.
[11] Arnon, D. I. (1949) J. Plant Physiol. 24, 1–15.
[12] Nugent, J. H. A., Møller, B. L. and Evans, M. C. W. (1980) Proc. 5 Int. Conf. Photosynth. in press.