S10-015

Towards understanding the role of calcium in O_2 evolution of PS II

G.B. Stevens[1], P.B. Lukins[2]

[1]*CSIRO Telecommunications and Industrial Physics, Bradfield Rd, West Lindfield, NSW 2070, Australia. gregory.stevens@tip.csiro.au*

[2]*Department of Physical Optics, School of Physics A28, University of Sydney, NSW 2006, Australia. p.lukins@physics.usyd.edu.au*

Keywords: Photosystem II, Ca^{2+}, EGTA, calcium binding site, $P680^+$

Introduction

The importance of Ca^{2+} in PS II was realized when it was observed that O_2 evolution was inhibited in PS II preparations that had been treated by incubation with molar NaCl and chelators to remove bound Ca^{2+}. Although these treatments also resulted in the depletion of the 17 and 23 kDa extrinsic proteins, restoration of O_2 evolution depended on re-addition of Ca^{2+} rather than the extrinsic polypeptides.

It has been reported that the relative extent of $P680^+$ decay with lifetimes of between about 7 ns and 1 μs indicates the proportion of reaction centres in a PS II sample with a functionally intact water-splitting site (Akerlund et al., 1984). Furthermore, the extent of nanosecond $P680^+$ decay was correlated with the rate of O_2 evolution in BBYs that had been inhibited with hydroxylamine (Eckert et al.,1988). These observations encouraged the belief that the extent of the nanosecond $P680^+$ decay can be used to monitor the effects of Ca^{2+} on the electron transfer in O_2-evolving PS II. We investigated the effects of Ca^{2+} on the nanosecond $P680^+$ decay and O_2 evolution of PS II enriched membrane fragments. The Ca^{2+}-selective chelator Ethylene Glycol bis (b-aminoethyl ether)-N,N,N',N'-Tetraacetic Acid (EGTA) and $CaCl_2$ were added to reduce or increase the free-Ca^{2+} concentration.

Materials and methods

PS II enriched thylakoid membrane fragments (BBYs) were isolated from *Spinacia oleracea* according to the method of Berthold et al.(1981) with minor modifications. A low-Ca^{2+} buffer was made in MilliQ water with 400 mM sucrose, 40 mM 2-(N-morpholino)ethanesulfonic acid (MES) and 5 mM $MgCl_2$. Mg^{2+} was chosen as the counter-ion for chloride to avoid interference from ions such as Na^+ which affect the $P680^+$ decay kinetics. The Ca^{2+} contamination in the buffer, as measured by atomic absorption spectroscopy, was less than 1 μM. Typical O_2 evolution rates of BBYs were 500 μmol O_2 (mg Chl)$^{-1}$ h^{-1}. PS II core complexes were prepared using the method of van Leeuwen et al. (1991). Surface-bound calcium was removed from PS II core complexes by serial concentration and dilution in low-Ca^{2+} buffer. Concentration of the cores was achieved by ultrafiltration under pressure using a stirred cell (Amicon model 8050). Several dilutions were required to reduce the free Ca^{2+} concentration to 10 μM. After this treatment, the sample was divided into aliquots and the flash-induced $P680^+$ reduction kinetics measured after addition of varying amounts of $CaCl_2$.

The concentration of free Ca^{2+} was measured with a Ca^{2+} ion-selective electrode. The free Ca^{2+} concentration of the BBY suspension was adjusted by adding the acid form of the Ca^{2+}-

selective chelator EGTA or $CaCl_2$ to the suspension. Transient absorption changes at 830 nm of PS II, which measure the formation and decay of $P680^+$, were made using flash photolysis equipment described by Lukins et al. (1996). Flash-induced 830 nm absorption measurements were made on BBYs and PS II cores suspended in low-Ca^{2+} buffer with 1 mM $K_3Fe(CN)_6$ as an electron acceptor. After the absorption measurements, 30 mM $CaCl_2$ was added and further measurements were made to check whether any changes in the decay kinetics were reversed.

Results

The free Ca^{2+} concentration of the BBYs suspended in low-Ca^{2+} buffer was 350 μM. This is due to weakly bound Ca^{2+} on the surface of the BBY particles. The peak 830 nm absorption change was the same, regardless of the Ca^{2+} concentrations in the buffer. Thus, the extent of formation of the $P680^+$/Pheophytin$^-$ radical pair is unaffected by the presence of the EGTA or free Ca^{2+}. As shown in Fig. 1(a), at Ca^{2+} concentrations above 350 μM, $P680^+$ mostly decayed with time constants in the range 20-350 ns. Below this concentration, the extent of the nanosecond decay diminished as the free Ca^{2+} concentration was lowered and the EGTA concentration was increased. The nanosecond decay is assigned to electron transfer from Y_Z to $P680^+$ in O_2-evolving PS II centres. Therefore the extent of the change in the nanosecond decay suggests that some change in the O_2 evolution under continuous light might occur in BBYs suspended with EGTA. Contrary to our expectations, there was no noticeable reduction in the O_2 evolution of BBYs under continuous illumination even with up to 5 mM EGTA.

The binding of free Ca^{2+} was analysed assuming that the extent of nanosecond $P680^+$ decay was due to changes in the free Ca^{2+} rather than changes in EGTA concentration. A Scatchard plot was fitted with a line with a slope of -0.0523 μM^{-1}, corresponding to k = 19.1 μM. This value is comparable to 13.0 μM for a Ca^{2+} binding site on BBYs observed by Grove and Brudvig (1998). We initially assumed that addition of the EGTA simply extracted free Ca^{2+} and would not interact with the BBYs. Unfortunately, the trend in Fig. 1a may also be due to changes in the extent of bound calcium and/or EGTA. This uncertainty was resolved by varying the Ca^{2+} concentration by serial dilution, which does not introduce unwanted chemical interactions.

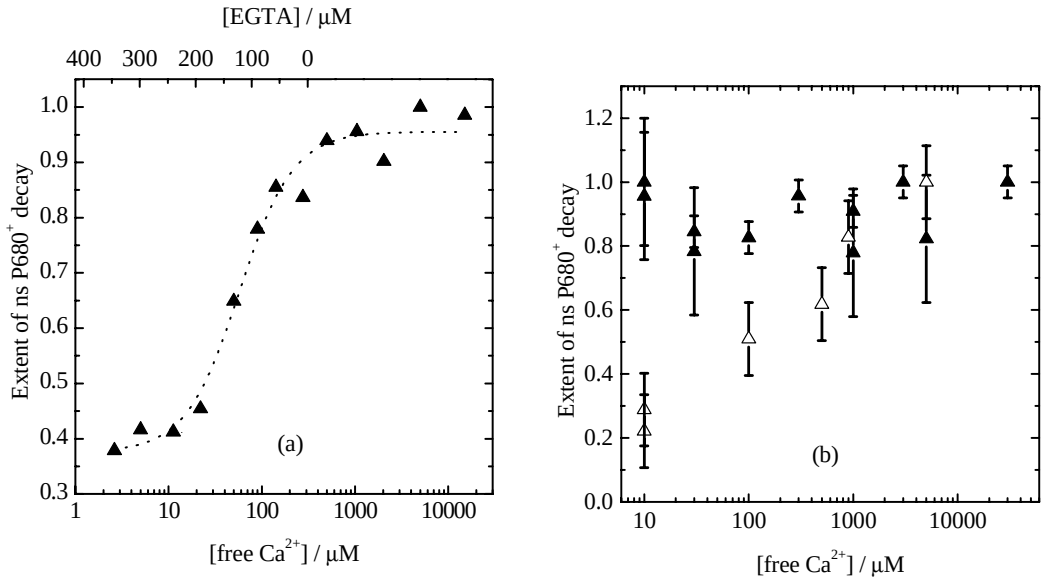

Fig. 1. (a) [Left] Extent of 20–350 ns $P680^+$ decay of BBYs as a function of free Ca^{2+} and EGTA concentration. (b) [Right] Extent of nanosecond $P680^+$ decay of PS II cores in the presence of EGTA (hollow) and after serial dilution and addition of Ca^{2+} (solid).

When BBYs were treated by serial dilution, the membrane fragments aggregated and became unsuitable for transient absorption measurements which requires good optical transmission. This problem was overcome by using PS II core complexes which did not aggregate. As shown in Fig. 1(b), the cores responded in the same way to EGTA as did BBYs. However, in cores treated by serial dilution, although the extent of the nanosecond $P680^+$ decay varied by up to 20 % over the range of free Ca^{2+} concentrations, it did not decrease to the same extent as it did in the cores with EGTA.

Discussion

Since the extent of nanosecond $P680^+$ decay did not vary significantly when the Ca^{2+} concentration was lowered by serial dilution, we concluded that the decrease in the extent in the flash-induced nanosecond $P680^+$ decay in BBYs and PS II core complexes was mainly due to EGTA binding rather than calcium depletion (Stevens and Lukins, 2001). This conclusion is supported by the observation of van Vliet et al. (1994), in which a range of chelators, including EGTA and MES, modified the dark-stable S2 multiline EPR signal by binding to anion groups on or near the tetranuclear-Mn complex of PS II.

It is curious that the extent of the nanosecond $P680^+$ decay kinetics diminished while the O_2 evolution rate of BBYs remained unaffected in the presence of up to 5 mM EGTA. In these experiments, the O_2 evolution was measured under continuous illumination and the $P680^+$ decay was measured using flash illumination. More importantly, electron transfer from Y_Z to $P680^+$ is not the rate-limiting step in the O_2 evolution reaction. In BBYs with an unoccupied Q_B site, the rate limiting step would be diffusion of PQ to this site and is an order of magnitude larger than other electron transfer rates associated with electron transfer in O_2-

evolving PS II (Renger et al. 1995). A computer simulation, based on the connected units model of PS II (Trissal and Lavergne, 1994) confirmed that when the acceptor side electron transfer was rate-limiting, almost complete abolition of nanosecond $P680^+$ decay kinetics was required before a reduction in steady-state O_2 evolution would be observed.

Acknowledgements

We thank Prof. A.W.D. Larkum and Dr T. Wydrzynski for helpful discussions and gratefully acknowledge financial assistance from the Australian Research Council.

References

Akerlund HE, Brettel K, Witt HT (1984) *Biochim. Biophys. Acta* **765**, 7-11.
Eckert HJ, Wydrzynski T, Renger G (1988) *Biochim. Biophys. Acta* **932**, 240-249.
Berthold DA, Babcock GT, Yocum C (1981) *FEBS Lett.* **134**, 231-234.
Lukins PB, Post SA, Walker PJ, Larkum AWD (1996) *Photosynthesis Research* **49**, 209 - 221.
van Leeuwen PJ, Nieveen MC, van de Meent EJ, Dekker JP, Gorkom HJ (1991) *Photosynthesis Research* **28**, 149-153.
Völker M, Eckert H-J, Renger G (1987) *Biochim. Biophys. Acta* **890**, 66-76.
Grove GN, Brudvig GW (1998) *Biochemistry* **37**, 1532-1539.
van Vliet P, Boussac A, Rutherford AW (1994) *Biochemistry* **33**, 12998-13004.
Stevens GB, Lukins PB (2001) submitted.
Renger G, Eckert H-J, Bergmann A, Bernarding J, Liu B, Napowotzki A, Reifarth F, Eichler HJ (1995) *Aust. J. Plant Physiol.* **22**, 167-181
Trissl H-W, Lavergne J (1994) *Aust. J. Plant Physiol.* **22**, 183-193