Volume 210, number 1, 27–30 FEB 04312 January 1987

Light induced calcium binding to mangrove PS II particles

Christopher Preston, Joseph G. Altin+, Fyfe L. Bygrave+ and Christa Critchley

Botany Department and +*Department of Biochemistry, The Faculties, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia*

Received 15 October 1986

A combination calcium selective and oxygen electrode system was used to measure the Ca^{2+} and O_2 concentrations of a solution surrounding mangrove PS II particles. Concomitant uptake of Ca^{2+} and oxygen evolution in the light were observed. The Ca^{2+} bound during the light was released in a subsequent dark period. The Ca^{2+} binding was dependent on both chlorophyll and Ca^{2+} concentrations. Up to 3000 Ca^{2+} were calculated to be bound per reaction centre.

Ca^{2+}; Oxygen evolution; Photosystem II

1. INTRODUCTION

Ca^{2+} has been shown to stimulate the oxygen evolution activity of PS II preparations, particularly those depleted of the 23 and 18 kDa polypeptides associated with the oxygen evolving complex [1–7]. The function of Ca^{2+} in photosynthetic oxygen evolution has yet to be elucidated, although its site of action has been located on the oxidising site of PS II [8] and is suggested to have a role in the oxygen evolving step of the water splitting reaction [9]. It has also been proposed that the 23 kDa polypeptide has a role in providing a high affinity binding site for Ca^{2+} [6,10].

In the present study a calcium selective electrode was used to measure simultaneously Ca^{2+} uptake from solution (and hence Ca^{2+} binding) and oxygen evolution activity by PS II membranes isolated from the mangrove *Avicennia marina*.

Correspondence address: C. Critchley, Botany Department, The Faculties, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia

Abbreviations: AMPD, 2-amino-2-methyl-1,3-propanediol; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LiDS-PAGE, lithium dodecylsulphate-polyacrylamide gel electrophoresis; Mes, 4-morpholineethanesulfonic acid; PBQ, phenyl-*p*-benzoquinone; PS II, photosystem II

2. MATERIALS AND METHODS

Mangrove PS II particles were prepared as described [5], and washed once in 40 mM Mes-AMPD (pH 6.5), 10 mM NaCl, 5 mM $MgCl_2$ and 2 mM EGTA to remove any residual Ca^{2+} bound to the membrane. Ca^{2+} uptake and oxygen evolution were measured simultaneously using a Clark type oxygen electrode with a calcium selective electrode (Radiometer F2112) inserted into the cuvette. The Ca^{2+}-selective electrode system has been described [11,12] except that the flow-through system was not used here. The incubation mixture was coupled to a reference electrode (Radiometer K801) via an agarose-KCl salt-bridge. The Ca^{2+} electrode signal was recorded by a Spectra Physics SP4100 computing integrator.

The assay buffer consisted of 25 mM Mes-AMPD (pH 6.5) with NaCl and $CaCl_2$ added as indicated in the figure legends, Cl^- always being 100 mM. 1 mM $K_3Fe(CN)_6$ and 0.26 mM PBQ were added as electron acceptors. Light was provided by a slide projector and passed through a piece of thin red cellophane with an absorption cut-off below 600 nm. Chlorophyll was deter-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

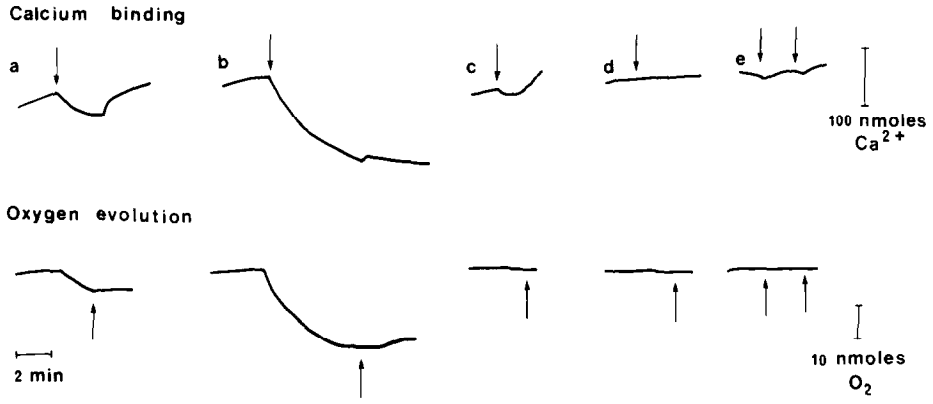

Fig.1. Calcium electrode (upper) and oxygen electrode (lower) traces measured simultaneously. All samples contained 0.5 mM $CaCl_2$ and 99 mM NaCl in the assay buffer except for c which contained 1 mM $CaCl_2$ and 98 mM NaCl. (a) 3.75 μg Chl·ml^{-1}; (b) 7.25 μg Chl·ml^{-1}; (c) 3.75 μg Chl·ml^{-1}, 1.2 μM DCMU; (d) 7.25 μg Chl·ml^{-1} with no electron acceptors; (e) no chlorophyll. ↓, light on; ↑, light off. In trace b oxygen evolution ceased before the light was turned off and the Ca^{2+} binding was irreversible.

mined as in [13] and LiDS-PAGE was performed as described in [14].

3. RESULTS

Light induced Ca^{2+} binding to the mangrove PS II particles was concomitant with the induction of oxygen evolution (fig.1a). In the subsequent dark period Ca^{2+} was released from the membranes. Depletion of electron acceptors led to largely irreversible Ca^{2+} binding and cessation of oxygen evolution in the light (fig.1b). Addition of 1.2 μM DCMU to the assay dramatically reduced Ca^{2+} binding and oxygen evolution activity (fig.1c) as did failure to add any electron acceptors (fig.1d). Buffer plus electron acceptors without membranes did not induce Ca^{2+} uptake signals (fig.1e).

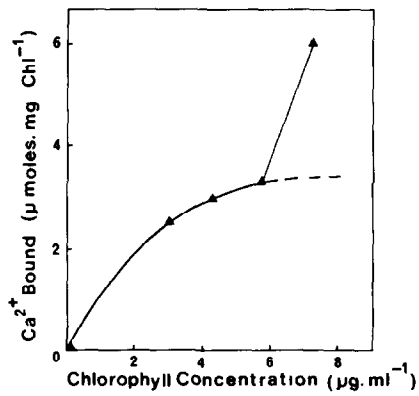

Fig.2. Response of Ca^{2+} binding to chlorophyll concentration. The highest point occurred after depletion of electron acceptors and irreversible Ca^{2+} binding (see also fig.1b). All samples contained 0.5 mM $CaCl_2$, 99 mM NaCl in the assay buffer.

Ca^{2+} binding by the mangrove PS II membranes was dependent on chlorophyll concentration (fig.2). Chlorophyll concentrations above 6 g·ml^{-1} resulted in depletion of electron acceptors and rapidly brought about irreversible Ca^{2+} binding. This also induced more Ca^{2+} to bind, but presumably in a non-specific manner. For this reason most assays were conducted at a lower chlorophyll concentration of 3.75 μg·ml^{-1}.

Ca^{2+} binding was also dependent on Ca^{2+} concentrations with a linear response up to 2 mM Ca^{2+} (fig.3A). This correlated with an increase in oxygen evolution which becomes maximal at this concentration (fig.3B) [5]. At 2 mM external Ca^{2+}, it was calculated that a maximum of 3000 Ca^{2+} bind per reaction centre (assuming 220 Chl per reaction centre [15]).

The mangrove PS II particles used here were depleted of the 23 and 18 kDa polypeptides (fig.4) resulting in a preparation where Ca^{2+} has its maximum effect on oxygen evolution [5].

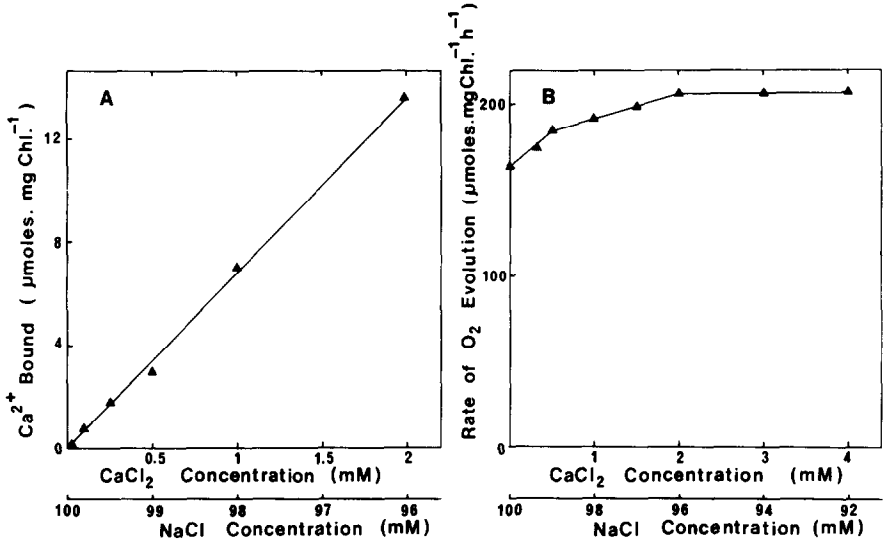

Fig.3. Response of Ca^{2+} binding (A) and oxygen evolution (B) to $CaCl_2$ concentration. Samples contained 3.75 μg Chl·ml^{-1}.

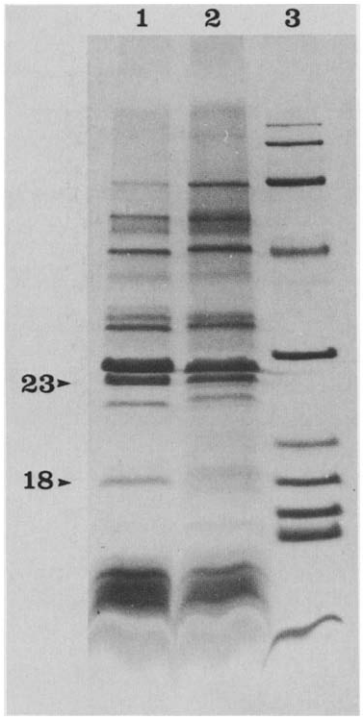

Fig.4. LiDS-polyacrylamide gel of PS II membranes. Lanes: 1, spinach PS II membranes; 2, mangrove PS II membranes; 3, molecular mass markers of 130, 94, 67, 43, 29, 20.1, 17.2, 14.3, 11.7 and 5.7 kDa. The positions of the 23 and 18 kDa polypeptides are indicated. These are absent from the mangrove PS II membranes as shown previously by Western blotting [22].

4. DISCUSSION

Ca^{2+} binding to PS II membranes isolated from mangrove is light induced and reversible in the dark. This pool of reversibly bound Ca^{2+} is not required for oxygen evolution to occur, but maximises the oxygen evolution rate. The binding is dependent on Ca^{2+} in the external medium and up to 3000 Ca^{2+} may be required to bind per reaction centre. This rather large number indicates that our method may not distinguish between specific and non-specific binding. It may also suggest that Ca^{2+} binding is not necessarily confined to a specific protein. These possibilities have to be investigated further, particularly with regard to the role(s) of the 23 and 18 kDa polypeptides which of course are absent from the preparations used in this study.

It has been suggested that the 23 and 18 kDa proteins provide a high affinity binding site for Ca^{2+} [6,10], although they clearly are not responsible for the light-dependent binding of this particular pool of Ca^{2+}. They may however modify the Ca^{2+} binding sites found here. The Ca^{2+} binding site does not seem to be restricted to the 33 kDa polypeptide either [16,17], but may perhaps involve the 13 kDa Ca^{2+} binding polypeptide of otherwise unknown function isolated by Sparrow and England [18].

The role of Ca^{2+} in photosynthetic oxygen evolution is still to be elucidated. Our observations of light-induced Ca^{2+} binding and release in the dark strongly suggest that Ca^{2+} is required for one or more of the S-states of photosynthetic oxygen evolution [19]. This is unlikely to be the S_1 state as at pH 6.5 all or nearly all of PS II are in this state in the dark [20]. More likely, Ca^{2+} is required for the S_3 to S_0 transition as suggested by Boussac et al. [9]. There is, however, also evidence that in *Synechococcus* PS II preparations Ca^{2+} is essential for electron transport from the primary donor Z to the reaction centre P-680 [21].

ACKNOWLEDGEMENTS

C.P. is the recipient of a Commonwealth Postgraduate Scholarship Award. C.C. acknowledges support from the National Research Fellowship Scheme and a CSIRO Extramural Research Grant. Mrs Neda Plovanic kindly prepared the figures.

REFERENCES

[1] Barr, R., Troxel, K.S. and Crane, F.L. (1980) Biochem. Biophys. Res. Commun. 92, 206–212.
[2] Ghanotakis, D.F., Babcock, G.T. and Yocum, C.F. (1984) FEBS Lett. 167, 127–130.
[3] Nakatani, H.Y. (1984) Biochem. Biophys. Res. Commun. 120, 299–304.
[4] Miyao, M. and Murata, N. (1984) FEBS Lett. 168, 118–120.
[5] Preston, C. and Critchley, C. (1985) FEBS Lett. 184, 318–322.
[6] Ghanotakis, D.F., Topper, J.N., Babcock, G.T. and Yocum, C.F. (1984) FEBS Lett. 170, 169–173.
[7] Boussac, A., Maison-Peteri, B., Etienne, A.L. and Vernotte, C. (1985) Biochim. Biophys. Acta 806, 231–234.
[8] Carpentier, R. and Nakatani, H.Y. (1985) Biochim. Biophys. Acta 808, 288–292.
[9] Boussac, A., Maison-Peteri, B., Vernotte, C. and Etienne, A.L. (1985) Biochim. Biophys. Acta 808, 225–230.
[10] Boussac, A. and Etienne, A.L. (1984) C.R. Acad. Sci. 299, 625–627.
[11] Reinhart, P.H., Taylor, W.M. and Bygrave, F.L. (1982) Biochem. J. 208, 619–630.
[12] Altin, J.G. and Bygrave, F.L. (1985) Biochem. J. 232, 911–917.
[13] Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
[14] Ryrie, I.J. (1983) Eur. J. Biochem. 131, 149–155.
[15] Murata, N., Miyao, M., Omata, T., Matsunami, H. and Kuwabara, T. (1984) Biochim. Biophys. Acta 765, 363–369.
[16] Ono, T. and Inoue, Y. (1984) FEBS Lett. 168, 281–286.
[17] Kuwabara, T., Miyao, M., Murata, T. and Murata, N. (1985) Biochim. Biophys. Acta 806, 283–289.
[18] Sparrow, R.W. and England, P.R. (1984) FEBS Lett. 177, 95–98.
[19] Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475.
[20] Plitjer, J.J., De Groot, A., Van Dijk, M.A. and Van Gorkom, H.J. (1986) FEBS Lett. 195, 313–318.
[21] Satoh, K. and Katoh, S. (1985) FEBS Lett. 190, 199–203.
[22] Andersson, B., Critchley, C., Ryrie, I.J., Jansson, C., Larsson, C. and Anderson, J.M. (1984) FEBS Lett. 168, 113–117.