Isolation of a calcium-binding protein from an oxygen-evolving photosystem II preparation

Raymond W. Sparrow and Reginald R. England*

Biology Division, Preston Polytechnic, Preston PR1 2TQ., Lancashire, England

Received 15 August 1984

Heat treatment of a highly active photosystem II preparation (600 μmol $O_2 \cdot$ mg $Chl^{-1} \cdot h^{-1}$) caused the release of several polypeptides. One of the polypeptides was found to bind to a fluphenazine-Sepharose affinity column in a calcium-dependent manner. The purified polypeptide was able to stimulate calmodulin-depleted 3',5'-cyclic nucleotide phosphodiesterase, indicating a possible similarity with calmodulin.

Photosystem II *Calcium* *Polypeptide* *Calmodulin*

1. INTRODUCTION

In recent years a considerable amount of evidence has accumulated from studies of cyanobacteria and higher plants, concerning the involvement of calcium in PS II electron transport [1–4]. Authors in [5] have demonstrated that Ca^{2+} was able to restore oxygen evolution in salt-washed PS II particles, depleted of two water-soluble polypeptides (17 and 23 kDa). Authors in [6] showed that Ca^{2+} was required for the retention of certain polypeptides associated with oxygen-evolving PS II particles and that by using the calmodulin antagonist, chlorpromazine, oxygen evolution could be inhibited. A similar inhibition by chlorpromazine and other phenothiazine drugs was also seen in intact chloroplasts [7].

Here, we have investigated the possibility that calmodulin or a similar calcium-binding protein exists in the molecule architecture of PS II.

* To whom correspondence should be addressed

Abbreviations: PS, photosystem; SDS–PAGE, SDS-polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

PS II particles were prepared from lettuce chloroplasts as in [8]. Isolation of the calcium-binding protein was carried out by diluting the PS II particles with buffer containing 1 mM mercaptoethanol, 50 mM Hepes (pH 7.0), to a chlorophyll concentration of 0.2 mg $\cdot$ cm^{-3}, followed by heating at 85°C for 2 min. The preparation was immediately placed on ice, followed by homogenisation with a Ystral blender, set at low speed for 1 min. This suspension was then centrifuged at 30000 × *g* for 45 min and the resultant supernatant was dialysed against 10 vols of 10 mM NH_4HCO_3, followed by 10 vols deionized distilled water. The dialysed supernatant was lyophilised and then resuspended in buffer A (1 mM $CaCl_2$, 10 mM mercaptoethanol, 50 mM Hepes; pH 7.0), prior to loading onto a column containing fluphenazine-Sepharose, previously equilibrated in buffer A. The column was washed with 50 cm^3 column buffer and then exhaustively washed with buffer B (1 mM $CaCl_2$, 0.1 M NaCl, 10 mM mercaptoethanol, 50 mM Hepes; pH 7.0), until the absorbance at 280 nm was <0.01. The calcium-binding protein was eluted with buffer C (0.1 M NaCl, 10 mM mercaptoethanol, 10 mM EGTA, 50 mM Hepes; pH 7.0).

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00 © 1984 Federation of European Biochemical Societies

Fluphenazine-Sepharose 4B was prepared as in [9]. Assay of phosphodiesterase activity was determined as in [10]. SDS–PAGE, oxygen evolution and chlorophyll determinations were performed as in [6]. Absorption spectra were measured on a Perkin-Elmer Lambda 5 spectrophotometer. Fluphenazine·2HCl was purchased from E.R. Squibb and Son; all other chemicals were of the highest grade commercially available.

3. RESULTS AND DISCUSSION

Fig.1 shows a typical elution profile for the calcium-binding protein on fluphenazine-Sepharose. The protein in the presence of Ca^{2+} was strongly bound to the conjugated Sepharose, but could be eluted when 10 mM EGTA replaced Ca^{2+} in the elution buffer.

The UV absorption spectrum for the EGTA-eluted fraction is presented in fig.2. The absorption maximum was at 277 nm and there was no evidence of a peak in the visible region of the spectrum.

The purity of the protein was determined by SDS–PAGE. As shown in fig.3, the protein migrated as a single band, with an estimated molecular mass of 13–15 kDa. This value is slightly lower than published values of 16–18 kDa for the calcium-binding protein calmodulin.

That the protein is similar to calmodulin in activity was confirmed by its ability to stimulate activator-depleted cyclic nucleotide phosphodiesterase, an assay method previously shown to be an indicator for the presence of calcium-dependent regulatory proteins, such as calmodulin [12]. Table 1 shows that a substantial amount of activity is exhibited by the activator-depleted enzyme, without any additions. However, in the presence of commercially available calmodulin or the protein isolated here, the activity of the enzyme was increased by 20%.

A considerable amount of evidence supports the suggestion that 3 polypeptides of 33, 23 and 16 kDa are components of the oxygen-evolving complex of PS II [13]. It was therefore interesting to determine whether any of these 3 polypeptides were able to bind to fluphenazine-Sepharose. Employing the method in [14] to isolate these polypeptides, it was found that in the presence of Ca^{2+}, none of the proteins were able to be retained by the affinity column (not shown). This implies that the calcium-binding protein we isolated, does

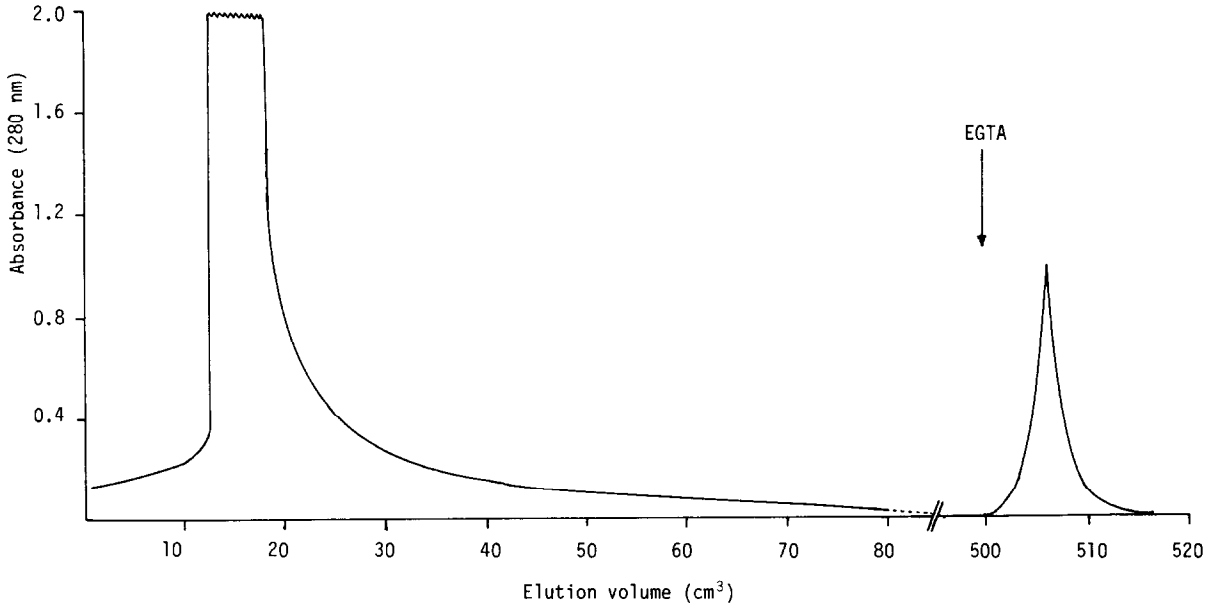

Fig.1. Typical elution profile for the chromatography of a calcium-binding protein on fluphenazine-Sepharose. 10 mM EGTA replaced $CaCl_2$ in buffer B as indicated by the arrow.

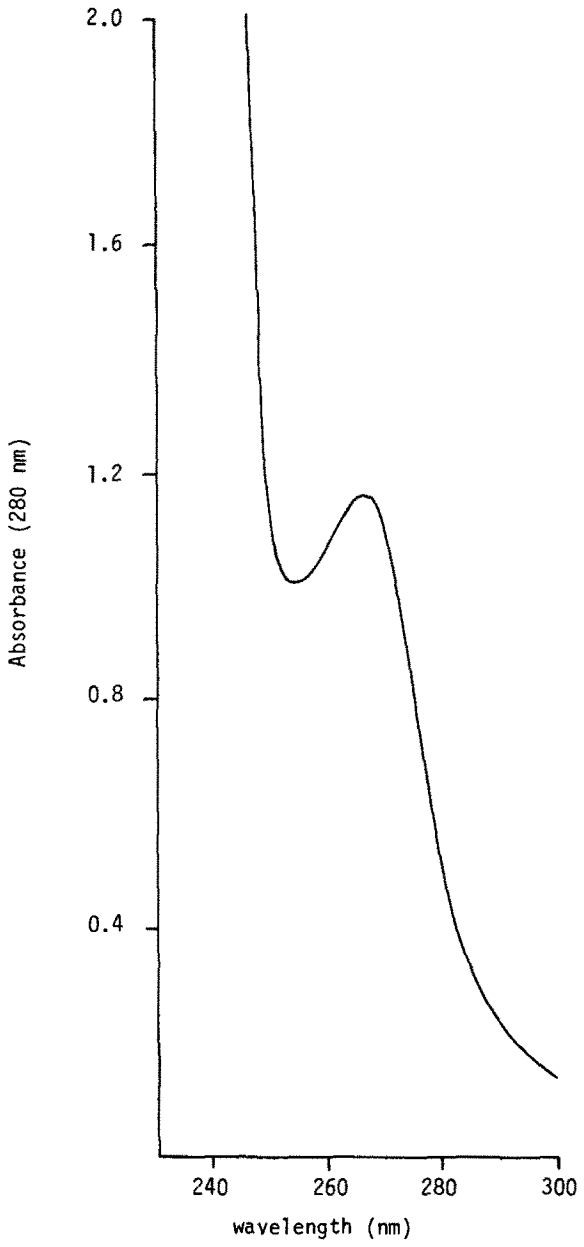

Fig.2. UV absorption spectrum of the calcium-binding protein. Protein concentration was 0.6 mg·cm^{-3} in 50 mM Hepes (pH 7.0).

not correspond to any of the 3 polypeptides removed by Tris washing of PS II particles.

In conclusion, the present results suggest the existence, in an oxygen-evolving PS II preparation, of a heat-stable, low molecular mass calcium-binding protein. Further work is in progress to

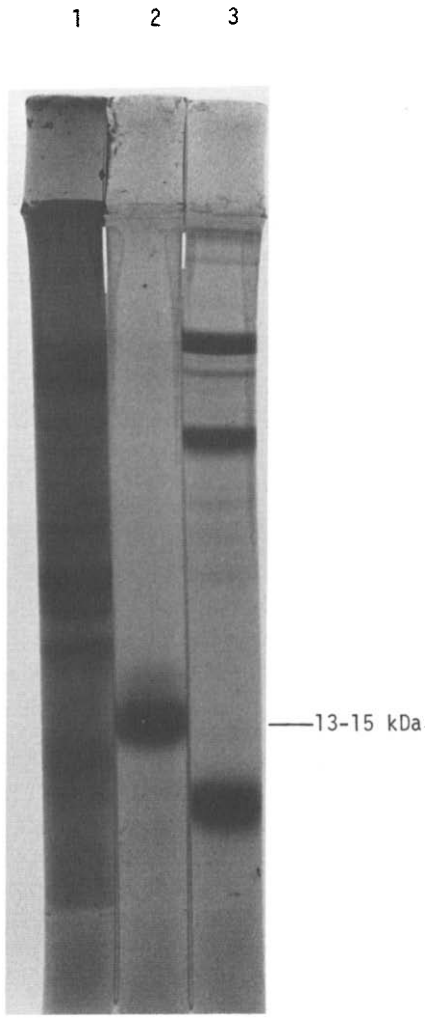

Fig.3. SDS–PAGE of, (1) PS II particles; (2) calcium-binding protein; (3) molecular mass standards: BSA (68 kDa), alcohol dehydrogenase (37 kDa), cytochrome *c* (12.4 kDa).

Table 1

Effect of isolated protein on activity of 3′,5′-cyclic nucleotide phosphodiesterase

Assay mixture	mol PO_4^{2-}·mg protein^{-1}·min^{-1}
Control[a]	33.7
Plus spinach calmodulin	42.0
Plus isolated protein	44.0

[a] As described in [10]

characterise this protein fully and to determine its role within PS II.

ACKNOWLEDGEMENTS

We wish to thank the Science and Engineering Research Council for financial support and Dr E.H. Evans for critical reading of the manuscript.

REFERENCES

[1] Brand, J.J. (1979) FEBS Lett. 103, 114–117.
[2] England, R.R. and Evans, E.H. (1981) FEBS Lett. 134, 175–177.
[3] Yerkes, C.T. and Babcock, G.T. (1981) Biochim. Biophys. Acta 634, 19–29.
[4] Packham, N.K. and Barber, J. (1984) Biochim. Biophys. Acta 764, 17–23.
[5] Ghanotakis, D.S., Babcock, G.T. and Yocum, C.F. (1984) FEBS Lett. 167, 127–130.
[6] England, R.R. and Evans, E.H. (1983) Biochem. J. 210, 473–476.
[7] Barr, R., Troxel, K.S. and Crane, F.L. (1982) Biochem. Biophys. Res. Commun. 104, 1182–1188.
[8] Ford, R. and Evans, M.C.W. (1983) FEBS Lett. 160, 159–164.
[9] Charbonneau, H. and Cormier, M.J. (1979) Biochem. Biophys. Res. Commun. 90, 1039–1047.
[10] Watterson, D.M., Harrelson, W.G. jr, Keller, P.M., Sharief, F. and Vanaman, T.C. (1976) J. Biol. Chem. 251, 4501–4513.
[11] Means, A.R. and Dedman, J.R. (1980) Nature 285, 73–77.
[12] Wolff, D.J. and Brostrom, C.O. (1974) Arch. Biochem. Biophys. 163, 349–358.
[13] Murata, N., Miyao, M. and Kuwabara, T. (1983) in: The Oxygen Evolving System of Photosynthesis (Inoue, Y. et al. eds) pp.213–222, Academic Press, Tokyo.
[14] Kuwabara, T. and Murata, N. (1983) Plant Cell Physiol. 24, 741–747.