

# 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 ( $600 \mu\text{mol O}_2 \cdot \text{mg Chl}^{-1} \cdot \text{h}^{-1}$ ) 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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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.

## 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  $0.2 \text{ mg} \cdot \text{cm}^{-3}$ , followed by heating at  $85^\circ\text{C}$  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  $30000 \times g$  for 45 min and the resultant supernatant was dialysed against 10 vols of 10 mM  $\text{NH}_4\text{HCO}_3$ , followed by 10 vols deionized distilled water. The dialysed supernatant was lyophilised and then resuspended in buffer A (1 mM  $\text{CaCl}_2$ , 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  $50 \text{ cm}^3$  column buffer and then exhaustively washed with buffer B (1 mM  $\text{CaCl}_2$ , 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).

\* To whom correspondence should be addressed

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

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  $\text{Ca}^{2+}$  was strongly bound to the conjugated Sepharose, but could be eluted when 10 mM EGTA replaced  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ , 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

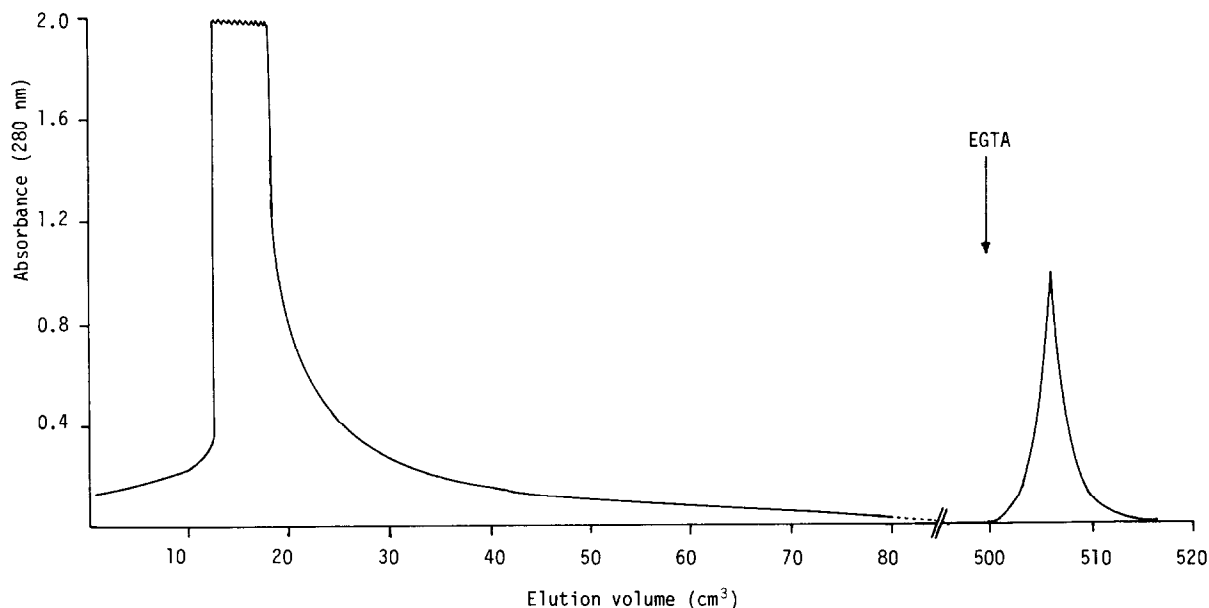


Fig.1. Typical elution profile for the chromatography of a calcium-binding protein on fluphenazine-Sepharose. 10 mM EGTA replaced  $\text{CaCl}_2$  in buffer B as indicated by the arrow.

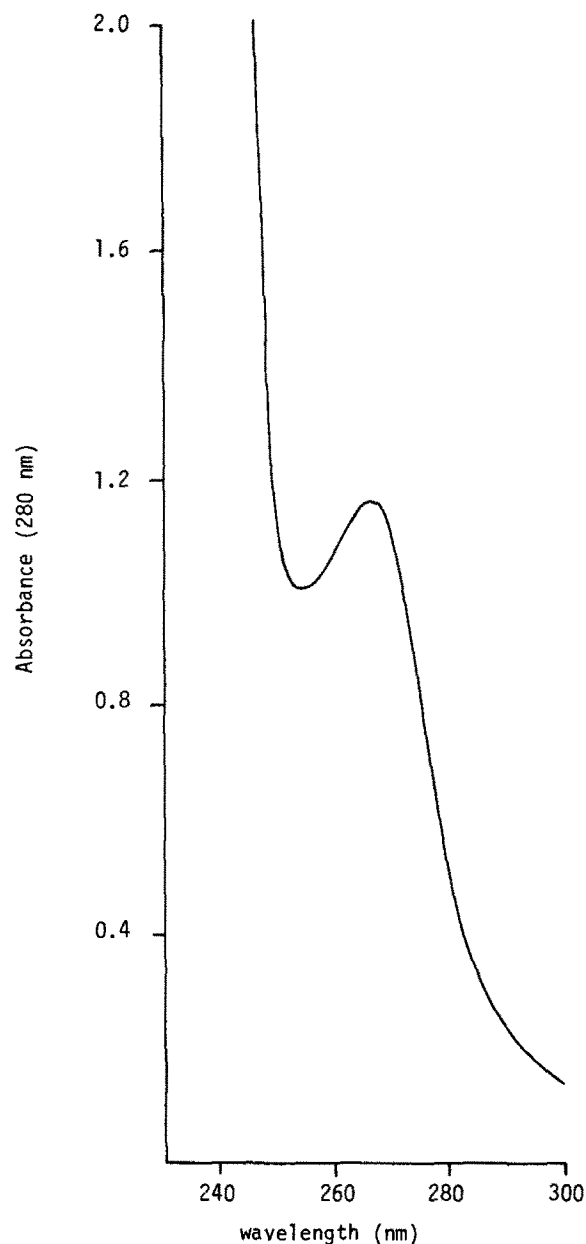


Fig.2. UV absorption spectrum of the calcium-binding protein. Protein concentration was  $0.6 \text{ mg} \cdot \text{cm}^{-3}$  in 50 mM Hepes (pH 7.0).

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

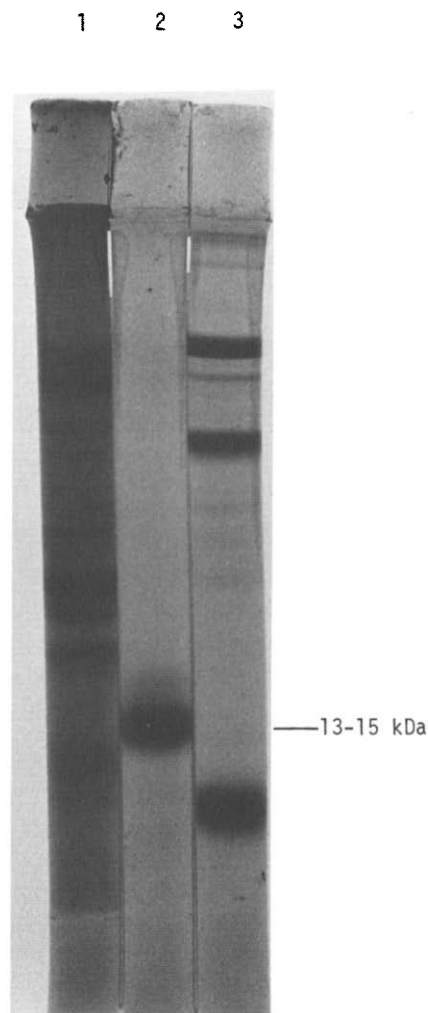


Fig.3. SDS-PAGE of, (1) PS II particles; (2) calcium-binding protein; (3) molecular mass standards: BSA (68 kDa), alcohol dehydrogenase (37 kDa), cytochrome c (12.4 kDa).

Table 1

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

Assay mixture	$\text{mol PO}_4^{2-} \cdot \text{mg protein}^{-1} \cdot \text{min}^{-1}$
Control <sup>a</sup>	33.7
Plus spinach calmodulin	42.0
Plus isolated protein	44.0

<sup>a</sup> 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.