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# Effect of alkaline pH on photosynthetic water oxidation and the association of extrinsic proteins with Photosystem Two

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Incubation of a membrane preparation enriched in Photosystem Two (PSII) at alkaline pH inhibited the water-splitting reactions in two distinct steps. Up to pH 8.5 the inhibition was reversible, whereas at higher alkalinities it was irreversible. It was shown that the reversible phase correlated with loss and rebinding of the 23 kDa extrinsic polypeptide. However, after mild alkaline treatments a partial recovery was possible without the binding of the 23 kDa polypeptide when the assay was at the optimal pH of 6.5 and in a medium containing excess $Cl^-$. The irreversible phase was found to be closely linked with the removal of the 33 kDa extrinsic protein of PSII. Treatments with pH values above 8.5 not only caused the 33 kDa protein to be displaced from the PSII-enriched membranes, but also resulted in an irreversible modification of the binding sites such that the extrinsic 33 kDa protein could not reassociate with PSII when the pH was lowered to 6.5. The results obtained with these more extreme alkaline pH treatments support the notion that the 23 kDa protein cannot bind to PSII unless the 33 kDa protein is already bound. The differential effect of pH on the removal of the 23 kDa and 33 kDa proteins contrasted with the data of Kuwabara & Murata [(1983) Plant Cell Physiol. **24**, 741–747], but this discrepancy was accounted for by the use of glycerol in the incubation media.

## INTRODUCTION

Photosystem Two (PSII) is that part of the photosynthetic electron-transport system of plants which is responsible for the oxidation of water. It has been known for some years that its activity is highly sensitive to small changes in pH. With isolated intact thylakoid membranes, inhibition occurs at both acidic and alkaline pH. However, in the case of the latter it is necessary to treat the membranes with appropriate ionophores in order to equilibrate the pH between the suspension medium and the lumenal thylakoid space (Cohn *et al.*, 1975; Reimer & Trebst, 1975; Briantais *et al.*, 1977). From this it can be concluded that alkaline pH inhibits the electron-donation reactions, since these are known to occur on the inner surface of the thylakoid membrane. Understanding the regulation of these electron-donor reactions has been advanced by experiments with PSII-enriched membrane fragments (Ghanotakis & Yocum, 1985; Murata & Miyao, 1985) in which the inner luminal surface is in direct contact with the incubation media (Dunahay *et al.*, 1984). By using this approach it was shown that, above pH 8.0, there was an irreversible loss of activity, whereas milder alkaline treatments caused an inhibition that could be reversed by lowering the pH (Cole *et al.*, 1986). It was suggested that the irreversible loss of activity was probably linked to a removal of extrinsic membrane proteins and a concomitant disruption of the tetrameric manganese cluster thought to play a central role in water oxidation. Indeed, close correlation between the loss of water oxidation and the removal of extrinsic polypeptides has been found for several treatments such as washing PSII-enriched membrane fragments with high salt concentrations, or with alkaline Tris, and by heating them to 55 °C (reviewed in Ghanotakis & Yocum, 1985; Andersson & Akerlund, 1987). The cause of inhibition by mild alkaline treatments is unclear, although, unlike the more extreme treatments, this effect can be reversed easily. Here we report experiments designed to investigate the effect of mild alkaline pH treatments, with particular focus on the relationship between the removal of extrinsic polypeptides and disruption of water oxidation. The study has been aided by the development of a sensitive e.l.i.s.a. for the 23 and 33 kDa extrinsic PSII proteins.

## MATERIALS AND METHODS

### Preparation of PSII-enriched membrane fractions

Chloroplast thylakoids were isolated either from leaves of greenhouse-grown pea (*Pisum sativum* L c.v. Feltham First) or market spinach (*Spinacia oleracea*) by homogenization in 50 mM-$KH_2PO_4$ (pH 7.5, adjusted with NaOH)/0.35 M-KCl/0.5 mM-EDTA, filtration of the leaf brei through ten layers of muslin plus one layer of cottonwool, and centrifugation at 5000 *g* for 10 min at 4 °C. The pellet was resuspended in 6 mM-$MgCl_2$ to rupture any intact plastids by osmotic shock, and buffer was then added to give a final composition of 0.2 M-sucrose/0.1 M-NaCl/3 mM-$MgCl_2$/50 mM-Tricine, pH 8.0, before further centrifugation. A PSII-enriched fraction was prepared essentially as previously described (Berthold *et al.*, 1981) by resuspending the thylakoid

Abbreviations used: DCPIP, 2,6-dichlorophenol-indophenol; DPC, diphenylcarbazide; PSII, Photosystem Two; BTP, Bistris propane; PAGE, polyacrylamide-gel electrophoresis.

pellet to 3 mg of chlorophyll·ml$^{-1}$ in a solubilization buffer (5 mM-$MgCl_2$/15 mM-NaCl/20 mM-Mes, pH 6.3) and incubation on ice, in the dark, for about 45 min before adding 0.5 vol. of 10% (w/v) Triton X-100 (in solubilization buffer). A 30 min incubation on ice in the dark and centrifugation at 40000 *g* for 30 min gave a pellet which was resuspended to 2 mg of chlorophyll·ml$^{-1}$ in a wash medium of 10% (w/v) glycerol in solubilization buffer. The membranes were pelleted once more and resuspended to 4 mg of chlorophyll·ml$^{-1}$ in the glycerol medium for storage in liquid $N_2$.

### pH incubation and reconstitution treatments

Samples of PSII-enriched membranes were washed by dilution to 1 mg of chlorophyll·ml$^{-1}$ in 20 mM-Bistris propane (BTP) buffer (pH 6.5)/5 mM-$MgCl_2$/15 mM-NaCl/10% (w/v) glycerol and centrifugation at 40000 *g* for 20 min at 4 °C. The pellets were resuspended to 1.0 mg of chlorophyll·ml$^{-1}$ in the same medium, but with 10 mM-BTP and pH values between 6.0 and 9.5. After a 10 min incubation on ice in the dark, an equal volume of the same medium, but containing 90 mM-BTP, was added, and a further 10 min incubation carried out before assay of electron-transfer activity at the pH of incubation. For assay at pH 6.5, incubation of the membranes was followed by centrifugation and resuspension in the treatment medium with 50 mM-BTP, pH 6.5. Reconstitution treatments involved an initial 10 min incubation of the preparation in medium with 10 mM-BTP and then addition of medium with 90 mM-BTP at pH 6.5 for a second 10 min incubation, before centrifugation and assay.

### Measurement of electron-transfer activities

Rates of oxygen evolution were determined by using a Clark-type oxygen electrode (Hansatech Ltd., King's Lynn, Norfolk, U.K.) with a quartz/iodine light source at an intensity of 2000 $\mu$E·s$^{-1}$·m$^{-2}$ and samples containing 20 $\mu$g of chlorophyll·ml$^{-1}$ in an assay buffer of 20 mM-BTP (pH 6.5, except where assays were at the treatment pH)/5 mM-$MgCl_2$/15 mM-NaCl/10% (w/v) glycerol/0.5 mM-phenyl-*p*-benzoquinone/2 mM-$K_3Fe(CN)_6$/1 $\mu$M-valinomycin/1 $\mu$M-nigericin maintained at 20 °C. The rate of reduction of 2,6-dichlorophenolindophenol (DCPIP) (50 $\mu$M) was monitored with 0.5 mM-diphenylcarbazide (DPC) as electron donor and 20 $\mu$g of chlorophyll·ml$^{-1}$ in the medium described above, using a Perkin–Elmer 557 dual-beam spectrophotometer (1 cm path length) fitted with side illumination from a quartz/iodine light source with appropriate light guide and transmission filters (Calflex heat filter and 2 mm Schott RG 658 cut-off filter). The intensity of the actinic light at the cuvette surface was 300 $\mu$E·m$^{-2}$·s$^{-1}$. The rate of DCPIP reduction ($A_{560-520}$ = 6.8 mM$^{-1}$·cm$^{-1}$) was measured using a 4 mm Schott B38 broad-band cut-off filter to protect the photomultiplier from scattered actinic light.

### Gel electrophoresis

The protein composition of membrane and supernatant fractions was analysed by SDS/polyacrylamide-gel electrophoresis (PAGE) using Laemmli (1970) buffers and gels with a gradient of 7–17% (w/v) acrylamide run at 4 °C. Proteins were stained with Coomassie Brilliant Blue R-250 for estimation of relative stain intensity in protein bands by scanning the gels at 560 nm in a Shimadzu CS-930 scanning spectrophotometer operated in the transmission mode.

### Enzyme-linked immunoassay

Antibodies were raised to the extrinsic 23 and 33 kDa proteins of PSII-enriched membranes of pea. Membrane samples were washed repeatedly in the Mes pH 6.3 solubilization buffer described above until no chlorophyll could be detected in the supernatant. Washing with 1 M-NaCl added to the buffer gave a supernatant preparation in which only the 23 kDa protein could be detected by gel electrophoresis and Coomassie Blue staining. Further washing with 1 M-$CaCl_2$ added gave just the 33 kDa protein. These proteins provided a source for calibration of the immunoadsorbent assay system (see below). The purities of the proteins were confirmed by liquid column chromatography using Pharmacia FPLC Mono Q ion-exchange and chromatofocusing columns, with eluates monitored at 280 nm. For antibody production using rabbits, final purification of proteins was by preparative SDS/PAGE. The immunoassay was carried out on 2 $\mu$l samples of supernatants, and samples of membranes containing 5 $\mu$g of chlorophyll were prepared in 1 ml of 50 mM-$NaHCO_3$/$Na_2CO_3$ buffer, pH 9.6, before the membrane samples were sonicated briefly in SDS (0.1 mg·ml$^{-1}$). Wells of microtitre plates were filled with 0.2 ml aliquots and a dilution series was prepared. The bound antigen samples were incubated with 1 in 200 dilution of the 23 kDa, or 1 in 500 dilution of the 33 kDa antisera in 0.2 M-NaCl/5 mM-KCl/polyoxyethylene sorbitan monolaurate (Tween 20; 0.5 mg·ml$^{-1}$)/20 mM-phosphate buffer, pH 7.5. The same buffer was used for subsequent attachment of horseradish-peroxidase-conjugated goat anti-rabbit antisera (Sigma: 1 in 1000 dilution). Enzyme activity was assayed at pH 5.0 (0.1 M-citrate buffer) with *o*-phenylenediamine as substrate, and concentrations of samples were determined from the relationship between absorbance (0–0.6 *A*; 490 nm) and dilution series. Quantities of proteins were estimated by reference to a dilution series of the extrinsic proteins in each microtitre plate and assay of these standards by the Lowry *et al.* (1951) method.

## RESULTS

The rates of oxygen evolution for preparations of PSII-enriched membranes which had been incubated at different pH values are given in Fig. 1. When assays were carried out at the same pH as the incubation, there was a severe reduction in activity above pH 7.0, with a pH of 7.7 giving 50% inhibition of the maximum observed rate. A similar profile, but with a shift to slightly higher pH values (pH 8.2 for 50% inhibition), was recorded if incubations were followed by centrifugal precipitation of the membranes and resuspension at pH 6.5 for the assay. Sufficient chloride was included in the assay medium to avoid limitation of activities, since elevated levels of this anion are known to be required for oxygen evolution after removal of extrinsic proteins (Andersson *et al.*, 1984). Inhibition at mild alkaline pH, up to pH 8.5, was overcome if the initial incubation was followed by addition of a low-pH buffer, thus reducing the pH to 6.5 without the need for discarding the original incubation media. In Fig. 1 it can be seen that this method gave almost full recovery after treatments up to about pH 8.5,

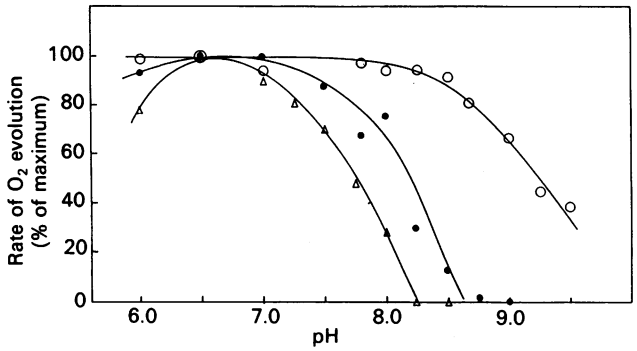


**Fig. 1. Effect of pH on oxygen evolution in PSII-enriched membrane preparations from *Pisum sativum***

All membranes were incubated at pH values indicated on the axis and assayed for activity at the incubation pH (△), after centrifugation and resuspension at pH 6.5 (●) and at pH 6.5 without centrifugation (○). Maximum rates of oxygen evolution were 200 $\mu$mol·h$^{-1}$·mg of chlorophyll$^{-1}$.

and it was only after incubation above pH 9.2 that less than 50% of the activity remained.

By using DCPIP as the electron acceptor and either water or DPC as electron donor, assays were carried out to determine whether the effect of pH was to inhibit donation of electrons from water to the PSII reaction centre or to impair the function of the reaction centre itself. Fig. 2 gives comparisons of the pH profiles for the two reactions and shows that the water-to-DCPIP activity followed the same pattern of inhibition as for the oxygen-evolution assays given in Fig. 1, and that DPC-to-DCPIP transfer was stimulated rather than inhibited by alkaline pH.

The effect of pH on the protein composition of the PSII enriched membranes was investigated by SDS/

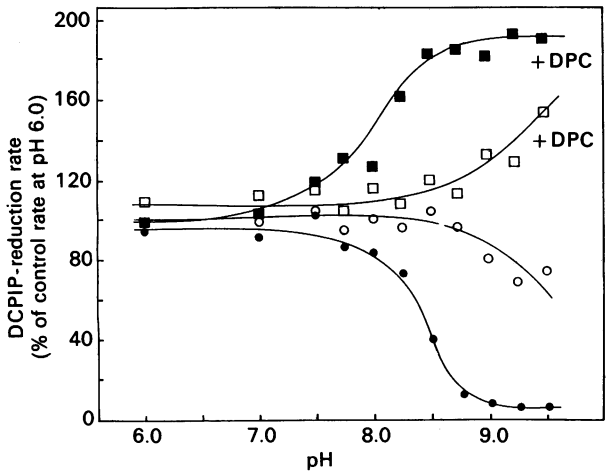


**Fig. 2. Effect of pH on electron-transfer activity of PSII-enriched membrane preparations from *Pisum sativum***

Reduction of DCPIP was assayed with water (●, ○) or with DPC (■, □) as electron donors. Assays were carried out at pH 6.5 and closed and open symbols denote respectively washed and unwashed preparations as described in Fig. 1. At pH 6.0 the rate of DCPIP reduction was 115 $\mu$mol·h$^{-1}$·mg of chlorophyll$^{-1}$.

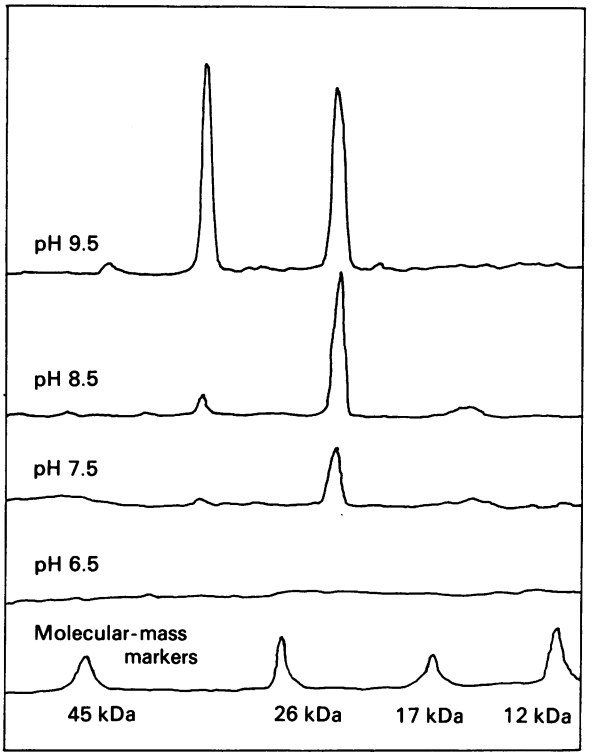


**Fig. 3. Effect of pH on removal of extrinsic proteins from PSII-enriched membranes isolated from *Pisum sativum***

Gels were stained with Coomassie Blue after SDS/PAGE of equal volumes of supernatant fractions from centrifugation of membrane preparations and subjected to scanning densitometry at 560 nm.

PAGE of membrane and supernatant fractions obtained by centrifugation after pH incubation (Fig. 3). Protein analyses were performed in the same experiments in which inhibition of activity was assessed, and it was found that alkaline pH treatment removed polypeptides of molecular mass 23 and 33 kDa. The pH 9.5 supernatant contained both of these proteins, but in the pH 7.5 and 8.5 washes only the 23 kDa protein was detected. Previously Kuwabara & Murata (1983*b*) reported SDS/PAGE analyses of similar pH-wash experiments and demonstrated that the 23 and 33 kDa proteins were removed in equal amounts by different pH incubations. Their experiments differed from ours in the use of PSII-enriched membranes from *Spinacia oleracea* rather than *Pisum sativum*, and they made use of incubation media which did not include glycerol. Using preparations from *S. oleracea* and a buffer without glycerol we confirmed their conclusions (Fig. 4). However, with glycerol (10%, w/v) included in the media, the pH 7.5 and 8.0 treatments caused release of the 23 kDa polypeptide from *S. oleracea* membranes without the loss of the 33 kDa polypeptide. We found no difference between the two species in terms of removal of the 23 and 33 kDa proteins, but one difference between the species was an apparent absence of a 17 kDa extrinsic protein in PSII membrane preparations from *P. sativum* and the existence of a strong 17 kDa band in samples from *S. oleracea* when high-pH washes were analysed by Coomassie Blue staining after SDS/PAGE. Similar differ-

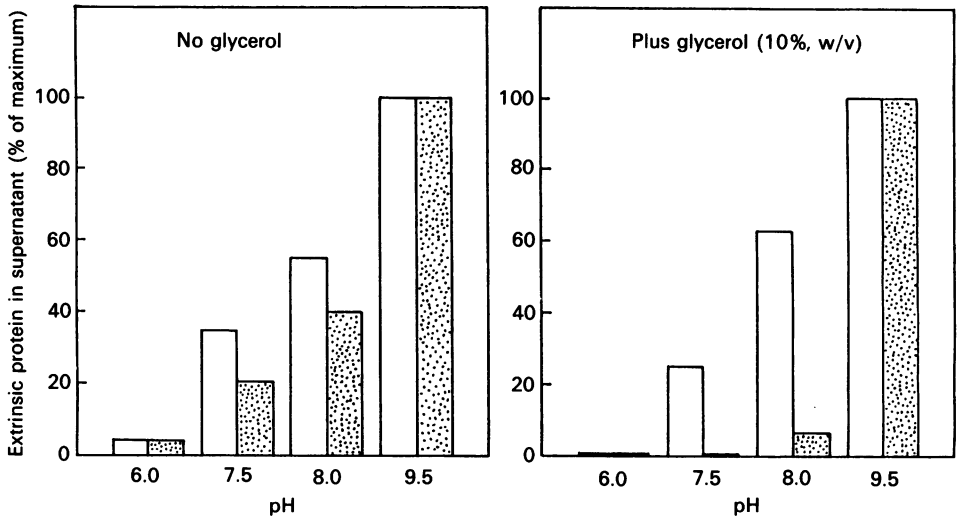


**Fig. 4. Effect of pH on removal of extrinsic proteins from PSII-enriched membranes isolated from *Spinacia oleracea***

Extrinsic 23 and 33 kDa proteins are denoted by open and stippled areas respectively. Incubation media without and with glycerol (10%, w/v) are compared. SDS/PAGE, staining with Coomassie Blue and analysis of gels by scanning densitometry were used to determine relative quantities of proteins. These are expressed as percentages of amounts in the pH 9.5 supernatants.

ences between different genotypes of wheat (*Triticum aestivum* and *T. thaoudar*) have been observed (T. Roscoe, personal communication) and are probably due to differences in the tightness of binding of the protein and consequent differences in its removal during membrane fractionation. In our experiments with *S. oleracea* the effect of alkaline pH on the 17 kDa was essentially the same as for the 23 kDa protein.

The assessment of protein levels in these experiments was by spectrophotometric measurement of absorbance due to Coomassie Blue-stained protein bands using a gel scanner (e.g. Fig. 3). However, for detailed analyses of protein levels as they related to electron-transport activities, an e.l.i.s.a. procedure was adopted. The results given in Fig. 5 demonstrate the linearity of the response to protein quantity and the reproducibility with this assay system.

By using the e.l.i.s.a. technique to analyse supernatant fractions, the results from absorbance measurements of stained gels were confirmed and quantified. For example, it was found that an incubation at about pH 7.7 was sufficient to remove selectively 50% of the 23 kDa

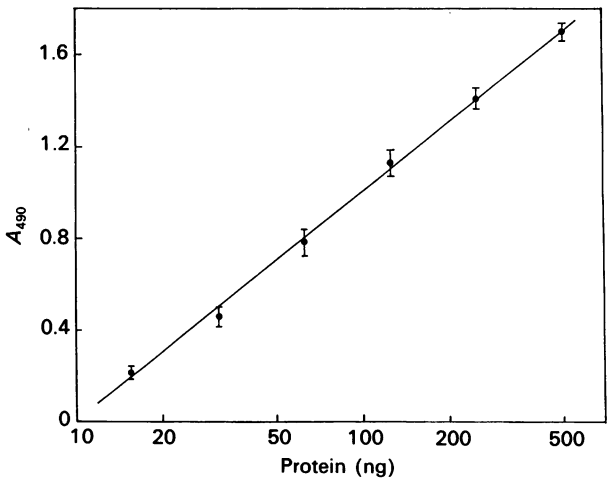


**Fig. 5. E.l.i.s.a of the extrinsic 33 kDa protein of PSII**

The protein standard was obtained by a 1 M-$CaCl_2$ wash of a PSII-enriched membrane preparation previously washed with 1 M-NaCl. The spectrophotometric assay at 490 nm monitored the activity of the antibody–peroxidase conjugate with *o*-phenylenediamine as substrate, and absorbance values with S.E.M. are given.

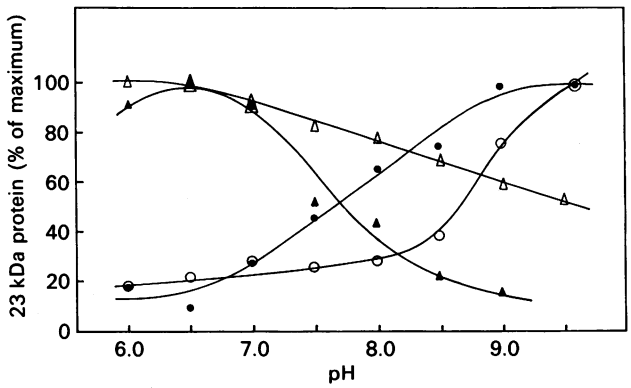


**Fig. 6. Removal of 23 kDa protein from PSII-enriched membranes by pH**

Supernatant fractions (○, ●) and membrane pellets (△, ▲) were assayed after incubation of membranes at different pH values and either immediate centrifugation (closed symbols) or after returning to pH 6.5 (open symbols). Protein was assayed by the e.l.i.s.a. method and expressed as a percentage of the maximum protein level for supernatant or membrane fractions (see the Materials and methods section).

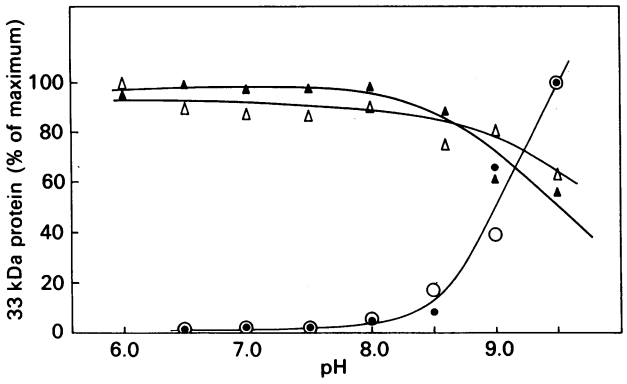


**Fig. 7. Removal of 33 kDa protein from membranes by pH**

Conditions and symbols were as in the legend to Fig. 6.

polypeptide (Fig. 6). This was in the same incubation which gave 50% inhibition of water oxidation assayed at pH 7.7, but only 20% inhibition when assayed at pH 6.5 (after washing) or zero inhibition with no washing but assay at pH 6.5 (see Fig. 1). Fig. 7 shows that around pH 7.7 there was no significant loss of the 33 kDa protein, and only above pH 8.5 was there any noticeable appearance of this protein in the supernatant. Also to be noted in Figs. 6 and 7 is that treatments above pH 8.5 resulted in an inability to rebind both the 23 kDa and 33 kDa proteins, as monitored by their levels in the supernatants after lowering the pH to 6.5. Treatments with pH less than 8.5, when the 33 kDa protein remains bound, allow the 23 kDa protein to rebind in response to lowering the pH. Besides releasing the 33 kDa protein, washing at pH values above 8.5 also resulted in a decrease in manganese associated with the membrane after centrifugation. Assays of membrane preparations by atomic-absorption spectrophotometry gave 2 g-atoms per 250 chlorophyll molecules after a wash at pH 9.0, and less than 0.5 g-atom at pH 9.5.

Despite some difficulties in applying the e.l.i.s.a. technique for accurate estimates of quantities of proteins in membrane samples (see Jay, 1987), the data in Figs. 6 and 7 indicate that alkaline pH treatments can totally remove the 23 kDa, but not the 33 kDa, protein. Moreover, the rebinding of the 23 kDa by adjustment to lower pH seems to be related to the level of 33 kDa protein retained on the membranes.

## DISCUSSION

The inhibition of water oxidation by a mild alkaline pH treatment was accompanied by an alteration in the association of the 23 kDa, but not the 33 kDa, extrinsic protein with the thylakoid membrane. There was not only a close correlation between inhibition of activity and the removal of the 23 kDa protein from the membrane, but also between subsequent recovery of activity and rebinding. When rebinding was prevented by removal of the supernatant containing the 23 kDa protein, the partial recovery observed at pH 6.5 must reflect a more direct effect of pH on the function of PSII. These effects were seen in mild alkaline (up to pH 8.5) incubations, and only with higher pH treatments was there a removal of the 33 kDa extrinsic protein from the membrane. This difference in susceptibility to removal of the two proteins has been noted previously in salt-wash experiments in which univalent cations removed the 23 kDa protein but bivalent cations were needed to release both proteins (see Murata & Miyao, 1985; Andersson & Akerlund, 1987). The differential response to pH was not seen in the earlier experiments of Kuwabara & Murata (1983*b*). However, our investigation of the importance of glycerol suggests that the composition of the incubation medium is important for maintaining binding of the 33 kDa protein in mild alkaline conditions. In fact, glycerol and other compounds such as phosphate and citrate are known to promote protein–protein interactions and have been shown to stabilize the association of extrinsic proteins with PSII in cyanobacteria (Katoh & Gantt, 1979; Stewart *et al.*, 1985).

Previous work has shown that the inhibition of water oxidation by incubation of PSII membrane preparations at alkaline pH can be reversed by carrying out assays at pH 6.5 rather than the incubation pH (Cole *et al.*, 1986). However, this work also indicated that there was an irreversible loss of about 50% of maximum activity when incubations were conducted at pH 8.4 and that the activity was almost completely inhibited by treatments above pH 9.0. The irreversible inhibition of oxygen evolution after high-pH treatment is a well-established phenomenon (Ford & Evans, 1985; Plijter *et al.*, 1986; Schlodder & Meyer, 1987) usually attributed to the removal of the extrinsic polypeptides (Kuwabara & Murata, 1983*a*). In our experiments the recovery of activity correlated with rebinding of the 23 kDa protein under conditions when the 33 kDa protein had not been removed, and inability to recover activity correlated with irreversible removal of the 33 kDa protein.

It has been reported that the inhibition of oxygen evolution by the selective removal of the 23 kDa protein can be partially overcome by increasing the concentration of $Ca^{2+}$ (Ghanotakis *et al.*, 1984; Miyao & Murata, 1984; Nakatani, 1984) and $Cl^-$ (Critchley *et al.*, 1984; Imaoka *et al.*, 1984) in the reaction medium. In our assays the $Cl^-$ concentration was 25 mM, and further additions of this anion or $CaCl_2$ did not eliminate the inhibition and recovery of oxygen evolution observed when the 23 kDa protein was removed and rebound. Nevertheless a partial recovery of oxygen evolution after mild alkaline treatments when the 23 kDa had been removed by centrifugation was observed at pH 6.5 (see Fig. 1). This effect may reflect the ability of $Cl^-$ to activate oxygen evolution in a way which is not possible at higher assay pH values, owing to competition with $OH^-$ at an anion-binding site, as suggested by Critchley (1983) and Cole *et al.* (1986). However, this partial recovery was not observed after treatments above pH 8.5. The fact that full recovery was achievable at pH 6.5 under conditions when components of the supernatant were made available clearly indicates that the reversible re-activation of oxygen evolution is not controlled by chloride alone. We also found that the partial reversibility of oxygen evolution after mild pH treatment with the supernatant removed could not be increased by the addition of $Ca^{2+}$. Thus it must be concluded that, despite the fact that $Cl^-$ and $Ca^{2+}$ can substitute to some extent for the removal of the 23 kDa protein, there remains a requirement for the presence of the 23 kDa in order to obtain maximum rates.

In summary it has been found that incubation of oxygen-evolving preparations at alkaline pH inhibits the

donor side of PSII by modifying the oxygen-evolving system rather than the reaction centre itself. The inhibition occurs in two distinct phases, one of which is fully reversible (up to pH 8.5) and one of which is not (above pH 8.5). The reversible phase reflects the removal and rebinding of the 23 kDa extrinsic polypeptide without dislodging the 33 kDa. The irreversible phase occurring after high-pH treatments is due to the removal of the 33 kDa protein. It seems that, with pH treatments which remove the 33 kDa protein, there is also a modification to this protein or to the membrane surface that prevents the rebinding of the protein and thus the re-activation of oxygen evolution.

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## REFERENCES

Andersson, B. & Akerlund, H.-E. (1987) Top. Photosynth. **8**, 379–420

Andersson, B., Critchley, C., Ryrie, I. J., Jansson, C., Larsson, C. & Anderson, J. M. (1984) FEBS Lett. **168**, 113–117

Berthold, D. A., Babcock, G. T. & Yocum, C. F. (1981) FEBS Lett. **134**, 231–234

Briantais, J. M., Vernotte, C., Lavergne, J. & Arntzen, C. J. (1977) Biochim. Biophys. Acta **461**, 61–74

Cohn, D. E., Cohen, W. S. & Bertsch, W. (1975) Biochim. Biophys. Acta **376**, 97–104

Cole, J., Boska, M., Blough, N. V. & Sauer, K. (1986) Biochim. Biophys. Acta **848**, 41–47

Critchley, C. (1983) Biochim. Biophys. Acta **724**, 1–5

Critchley, C., Andersson, B., Ryrie, I. J. & Anderson, J. M. (1984) Biochim. Biophys. Acta **767**, 532–539

Dunahay, T. G., Staehelin, L. A., Seibert, M., Ogilvie, P. D. & Berg, S. P. (1984) Biochim. Biophys. Acta **764**, 179–193

Ford, R. C. & Evans, M. C. W. (1985) Biochim. Biophys. Acta **807**, 1–9

Ghanotakis, D. F. & Yocum, C. (1985) Photosynth. Res. **7**, 97–114

Ghanotakis, D. F., Topper, J. N., Babcock, G. T. & Yocum, C. (1984) FEBS Lett. **170**, 169–173

Imaoka, A., Yanaagi, M., Akabori, K. & Toyashima, Y. (1984) FEBS Lett. **176**, 341–345

Jay, F. A. (1987) Top. Photosynth. **8**, 261–303

Katoh, T. & Gantt, E. (1979) Biochim. Biophys. Acta **546**, 383–393

Kuwabara, T. & Murata, N. (1983*a*) Plant Cell Physiol. **23**, 533–539

Kuwabara, T. & Murata, N. (1983*b*) Plant Cell Physiol. **24**, 741–747

Laemmli, U. K. (1970) Nature (London) **227**, 680–685

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275

Miyao, M. & Murata, N. (1984) FEBS Lett. **168**, 118–120

Murata, N. & Miyao, M. (1985) Trends Biochem. Sci. **10**, 122–124

Nakatani, H. Y. (1984) Biochem. Biophys. Res. Commun. **120**, 299–307

Plijter, J. J., de Groot, A., van Dijk, M. A. & van Gorkam, H. J. (1986) FEBS Lett. **195**, 313–318

Reimer, S. & Trebst, A. (1975) Biochem. Physiol. Pflanz, **168**, 225–232

Schlodder, E. & Meyer, B. (1987) Biochim. Biophys. Acta **890**, 23–31

Stewart, A. C., Siczkowski, M. & Ljungberg, U. (1985) FEBS Lett. **193**, 175–179

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