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**STRUCTURAL ORGANIZATION OF THE OXIDIZING SIDE OF PHOTOSYSTEM II****EXOGENOUS REDUCTANTS REDUCE AND DESTROY THE Mn-COMPLEX IN PHOTOSYSTEMS II MEMBRANES DEPLETED OF THE 17 AND 23 kDa POLYPEPTIDES**

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Removal of 23 and 17 kDa water-soluble polypeptides from PS II membranes causes a marked decrease in oxygen-evolution activity, exposes the oxidizing side of PS II to exogenous reductants (Ghanotakis, D.F., Babcock, G.T. and Yocum, C.F. (1984) *Biochim. Biophys. Acta* 765, 388–398) and alters a high-affinity binding site for  $\text{Ca}^{2+}$  in the oxygen-evolving complex (Ghanotakis, D.F., Topper, J.N., Babcock, G.T. and Yocum, C.F. (1984) *FEBS Lett.* 170, 169–173). We have examined further the state of the functional Mn complex in PS II membranes from which the 17 and 23 kDa species have been removed by high-salt treatment. These membranes contain a structurally altered Mn complex which is sensitive to destruction by low concentrations of  $\text{NH}_2\text{OH}$  which cannot, in native PS II membranes, cause extraction of functional Mn. In addition to  $\text{NH}_2\text{OH}$ , a wide range of other small ( $\text{H}_2\text{O}_2$ ,  $\text{NH}_2\text{NH}_2$ ,  $\text{Fe}^{2+}$ ) and bulky (benzidine, hydroquinone) electron donors extract Mn (up to 80%) from the polypeptide-depleted PS II preparations. This extraction is due to reduction of the functional Mn complex since light, which would generate higher oxidation states within the Mn complex, prevents Mn release by reductants. Release of Mn by reductants does not extract the 33 kDa water-soluble protein implicated in Mn binding to the oxidizing side of PS II, although the protein can be partially or totally extracted from Mn-depleted preparations by exposure to high ionic strength or to high (0.8 M) concentrations of Tris. We view our results as evidence for a shield around the Mn complex of the oxygen-evolving complex comprised of the 33 kDa polypeptide along with the 23 and 17 kDa proteins and tightly bound  $\text{Ca}^{2+}$ .

**Introduction**

The oxygen-evolving complex of PS II is a highly ordered structure in which a number of polypeptides interact with one another to provide the appropriate environment for efficient photo-

oxidation of water to molecular oxygen in a linear four-step sequence. The primary events of PS II are associated with the reaction center Chl a species, P-680, and its immediate donor Z. Oxidation of Z (to  $\text{Z}^+$ ) creates the strong oxidant necessary to advance the S-state sequence of the oxygen-evolving complex [1]. The catalytically active members of the oxygen-evolving complex include manganese [2–4] and chloride [5–7]; deletion of either species results in a loss of oxygen-evolution activity. The photocatalysts of PS II

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Abbreviations: Chl, chlorophyll; DCBQ, 2,5-dichloro-*p*-benzoquinone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; PS, photosystem.

activity are associated with hydrophobic proteins (47 and 43 kDa) found in the PS II complex as described by Satoh [8] and Satoh et al. [9]. This complex, although photochemically active, does not possess the capacity to evolve oxygen. Studies by Åkerlund et al. [10] first established a relationship between oxygen-evolution activity and certain water-soluble polypeptides (17 and 23 kDa) which could be removed from inside-out thylakoid vesicles by dilute salt washing. The 23 kDa species possessed a strong reconstitution activity when added to the depleted vesicles, but very little reconstitution activity was associated with the purified 17 kDa polypeptide. The role of water-soluble polypeptides in the oxygen-evolving complex has also been explored by a number of investigators working with the oxygen-evolving PS II membrane preparations. Yamamoto et al. [11] first showed that Tris, in addition to releasing manganese would also solubilize three polypeptides (33, 23 and 17 kDa) from the PS II complex, and Murata et al. [12] used salt-washing procedures to release the 23 and 17 kDa species, also from a PS II preparation. Murata et al. found, however, that release of the two polypeptides did not correlate with a strong inactivation of oxygen evolution, and Toyoshima et al. [13] have suggested that the 17 kDa polypeptide is required for oxygen-evolution activity, in contrast to the report of Åkerlund et al. [10].

Although differences exist between various laboratories, there is a general consensus that the 17 and 23 kDa polypeptides, either alone or together, are required to obtain high rates of oxygen-evolution activity from the PS II complex, and that the 33 kDa polypeptide is more tightly bound to the oxygen-evolving complex, perhaps as a reflection of its proposed role in the productive binding of manganese [14]. Our recent work with PS II membranes [14–16] showed that preparations depleted of 17 and 23 kDa polypeptides by exposure to 2 M NaCl at pH 6.0, lost 65–80% of their oxygen-evolving capacity; the inhibited rate of oxygen-evolution activity was stimulated by addition of external  $\text{Ca}^{2+}$  at nonphysiological concentrations [15]. Using a dialysis-reconstitution procedure, we subsequently showed that rebinding of the 17 and 23 kDa polypeptides restores oxygen-evolution activity by promoting high-affin-

ity binding of  $\text{Ca}^{2+}$  to the reconstituted membranes [16]. In this communication, we present evidence to show that water-soluble 17 and 23 kDa polypeptides also prevent destruction of the oxygen-evolving complex by a group of exogenous reductants (hydroquinone, benzidine,  $\text{H}_2\text{O}_2$ ,  $\text{NH}_2\text{NH}_2$ ,  $\text{Fe}^{2+}$ ). These reductants, in the absence of the two polypeptides, extract functional Mn from the PS II complex concomitant with irreversible loss of oxygen-evolution activity.

## Materials and Methods

Preparation of the PS II complex was carried out as described in Ref. 16. Release of the 17 and 23 kDa polypeptides was carried out by resuspension of PS II membranes in 2 M NaCl/0.4 M sucrose/50 mM Mes (pH 6.0) followed by incubation for 1 h in the dark (4°C). Salt-treated membranes were recovered by centrifugation at  $40\,000 \times g$  (30 min), washed with a medium comprising 0.4 M sucrose/15 mM NaCl/50 mM Mes (pH 6.0) and finally resuspended in the same medium. Selective reconstitution of high-salt-treated PS II membranes with either polypeptides alone or with polypeptides plus calcium was carried out by the dialysis technique described in Ref. 16.

Manganese release upon treatment of the PS II complex with various chemicals was followed with a Bruker ER-200D EPR spectrometer operated at X-band [4]. Oxygen-evolution activity with  $\text{DCBQ}/\text{Fe}(\text{CN})_6^{3-}$  as a PS II electron acceptor was followed using a Clark-type electrode. The polypeptide content of the PS II membranes was examined by polyacrylamide gel electrophoresis which was carried out as described in Ref. 16.

## Results

Exposure of isolated thylakoid membranes to  $\text{NH}_2\text{OH}$  inactivates oxygen-evolution activity by a mechanism which results in release of functional manganese [17]. In Ref. 18 it was reported that the mechanism of  $\text{NH}_2\text{OH}$  inactivation of the oxygen-evolving complex differs from that of Tris inactivation. Inhibition by Tris is accelerated by low light intensities [17], whereas  $\text{NH}_2\text{OH}$  produces a rapid inactivation in the dark, and it has therefore been proposed that higher S-states are

immune to attack by  $\text{NH}_2\text{OH}$  and that  $\text{NH}_2\text{OH}$ -induced manganese extraction occurs only from the lower S-states [18].

The data of Fig. 1 show that incubation of PS II membranes in the dark in the presence of  $\text{NH}_2\text{OH}$  results in inactivation of oxygen-evolution activity of the PS II complex. Incubation with high concentrations of other reductants (hydroquinone, benzidine,  $\text{NH}_2\text{NH}_2$ ,  $\text{H}_2\text{O}_2$ ,  $\text{Fe}^{2+}$ ) failed to inhibit oxygen-evolution activity in intact PS II membranes (see Fig. 1). When the experiments of Fig. 1 were repeated with PS II membranes which had been depleted of the 17 and 23 kDa polypeptides, low concentrations of  $\text{NH}_2\text{OH}$  inhibited oxygen-evolution activity, and in contrast to native PS II membranes, reductants such as those listed in Table I were also inhibitory (see Fig. 2). Fig. 3 shows the time-course of the decay of oxygen-evolution activity in salt-washed PS II membranes after addition of either  $\text{NH}_2\text{OH}$  or hydroquinone (addition of ascorbate along with hydroquinone slightly accelerated the destruction of activity, probably by keeping hydroquinone in its reduced form (data not shown)). Even though a large number of reductants are very effective in destroying the oxygen-evolving complex, compounds like ferrocyanide, ascorbate or a derivative of hydroxyl-

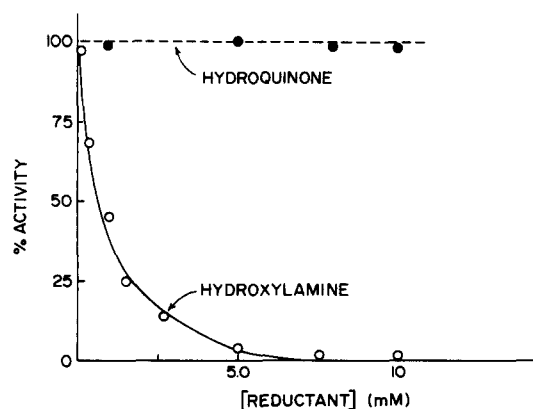


Fig. 1. Effect of reductants on oxygen-evolution activity of the intact PS II complex. PS II membranes in 0.4 M sucrose/15 mM NaCl/50 mM Mes (pH 6.0) (2 mg Chl/ml) were incubated for 1 h in the dark ( $4^\circ\text{C}$ ) with the indicated concentration of either hydroxylamine or hydroquinone, then diluted to 6  $\mu\text{g}$  Chl/ml and examined for oxygen-evolution activity with 0.4 mM DCBQ/3 mM  $\text{Fe}(\text{CN})_6^{3-}$  as an acceptor system. Control activity: 680  $\mu\text{mol O}_2/\text{mg Chl per h}$ .

TABLE I

EFFECT OF LIGHT ON THE DEACTIVATION OF OXYGEN-EVOLUTION ACTIVITY BY EXOGENOUS REDUCTANTS IN HIGH-SALT-TREATED PS II MEMBRANES

High-salt-treated PS II membranes resuspended in a solution comprising 0.4 M sucrose/15 mM NaCl/50 mM Mes (pH 6.0) (6  $\mu\text{g}$  Chl/ml) were treated as shown in the table below ( $25^\circ\text{C}$ ) and then, after addition of 3 mM  $\text{Fe}(\text{CN})_6^{3-}$ , 10 mM  $\text{CaCl}_2$  and 400  $\mu\text{M}$  DCBQ, were assayed for oxygen-evolution activity without any further dilution.

Addition	Incubation	Activity ( $\mu\text{mol O}_2/\text{mg Chl per h}$ )
None	none	520
	5 min in dark	500
	5 min in light	440
Hydroquinone (0.3 mM)+ benzoquinone (0.3 mM)	5 min in dark	40
	5 min in light	420
	5 min in light + 5 min in dark	20
Hydroquinone (0.3 mM)+ benzoquinone (0.3 mM)+ $\text{CaCl}_2$ (10 mM)	5 min in dark	80
Hydroquinone (0.3 mM)	5 min in dark	40
	5 min in light	140
	5 min in light + 5 min in dark	20

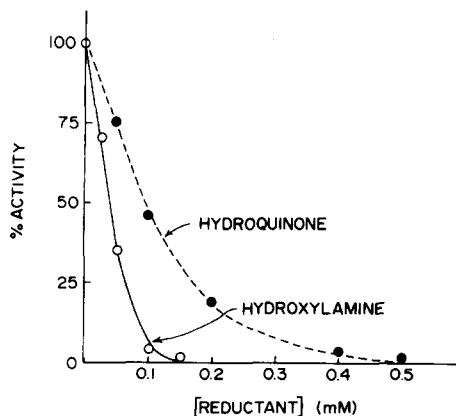


Fig. 2. Effect of reductants on oxygen-evolution activity of 2 M NaCl-treated PS II membranes. Salt-washed membranes in 0.4 M sucrose/15 mM NaCl/50 mM Mes (pH 6.0) (2 mg Chl/ml) were incubated with the indicated concentration of the reductant for 1 h in the dark ( $4^\circ\text{C}$ ), then diluted to 6  $\mu\text{g}$  Chl/ml with the buffer used above and assayed for oxygen-evolution activity in the presence of 10 mM  $\text{CaCl}_2$ /0.4 mM DCBQ/3 mM  $\text{Fe}(\text{CN})_6^{3-}$ . 100% activity: 520  $\mu\text{mol O}_2/\text{mg Chl per h}$ .

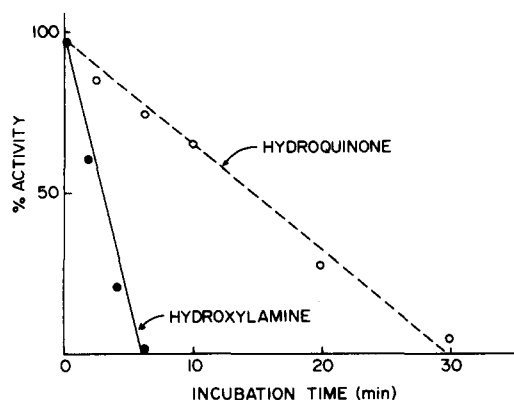


Fig. 3. Time-course of decay of oxygen-evolution activity of salt-washed PS II membranes (2 mg Chl/ml) upon addition of reducing agents. High-salt-treated PS II membranes were incubated with either hydroxylamine (200  $\mu$ M) or hydroquinone (400  $\mu$ M) for the times indicated on the abscissa (4°C, in the dark), then diluted to 6  $\mu$ g Chl/ml and assayed for oxygen-evolution activity (conditions the same as those in Fig. 1).

amine ( $\text{CH}_3\text{NHOCH}_3$ ) have almost no effect on oxygen-evolution activity, which probably reflects their inability to react with higher oxidation states of Mn (see Discussion). When the behavior of the manganese complex of salt-washed PS II membranes was examined after treatment with various reductants, the data of Fig. 4 were obtained. Incubation of the polypeptide-depleted PS II complex with  $\text{NH}_2\text{OH}$  or hydroquinone results in significant release (up to 80%) of functional manganese (the same behavior is observed upon addition of benzidine,  $\text{H}_2\text{O}_2$ ,  $\text{NH}_2\text{NH}_2$  and  $\text{Fe}^{2+}$  (data not shown)). Since it was reported previously [18] that  $\text{NH}_2\text{OH}$  attacks the lower S-states, we examined the effect of reductants on oxygen-evolution activity of salt-washed membranes, under conditions where prolonged exposure of the lower S-states to the reductant was avoided by illumination. As shown in Table I, complete protection of oxygen-evolution activity was possible only when a PS II acceptor (benzoquinone) was present to promote efficient formation of higher oxidation states of the Mn-complex. Since the presence of  $\text{Ca}^{2+}$  during exposure of the salt-washed PS II complex to the reductant does not protect the oxygen-evolving complex against inactivation (Table I), it seems likely that the 17 and 23 kDa polypeptides are the species required to

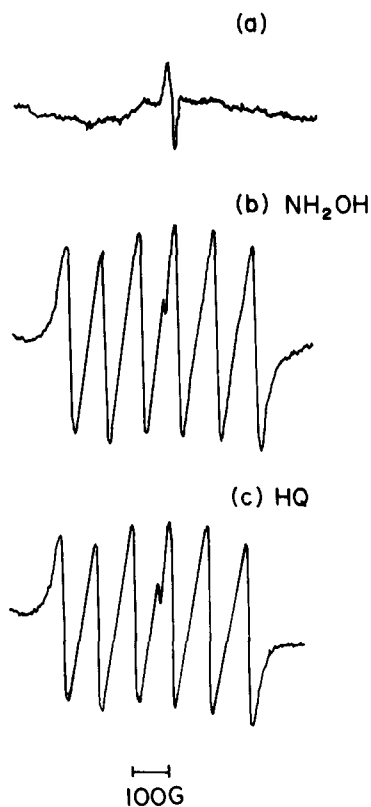


Fig. 4. Effect of reductants on the EPR signal of Mn in high-salt-treated PS II preparations: (a) control salt-washed PS II complex in 0.4 M sucrose/15 mM NaCl/50 mM Mes (pH 6.0) (2 mg/ml) after 1 h incubation on ice in the dark; (b) salt-treated PS II membranes (2 mg Chl/ml, in the buffer used in (a)) incubated with hydroxylamine (200  $\mu$ M) under the conditions described in (a); (c) salt-treated PS II membranes (2 mg Chl/ml) incubated with hydroquinone (HQ) (400  $\mu$ M) under the conditions described above. Instrumental conditions: microwave power, 100 mW; modulation amplitude, 15 Gpp; gain,  $2.5 \cdot 10^5$ ; time constant, 200 ms.

protect the oxidizing side of PS II against destruction by exogenous reductants. To test this hypothesis, we used the technique developed in Ref. 16, which allowed us to first deplete PS II membranes of the 17 and 23 kDa species as well as  $\text{Ca}^{2+}$  and to subsequently reconstitute the extracted PS II complex selectively with either the polypeptides only or with both polypeptides and calcium. As shown in Table II, the presence of the 17 and 23 kDa polypeptides completely protects oxygen-evolution activity, even when readdition of functional calcium has been omitted.

TABLE II

PROTECTIVE EFFECT OF THE 17 and 23 kDa POLYPEPTIDES AGAINST DESTRUCTION OF THE MANGANESE COMPLEX BY HYDROQUINONE (HQ)

PS II membranes (2 mg Chl/ml) depleted of  $\text{Ca}^{2+}$  and the 17 and 23 kDa species were first reconstituted with either the polypeptides or both the polypeptides and  $\text{Ca}^{2+}$  (see Ref. 16), then treated as shown in the table below, and finally examined for oxygen-evolution activity with 0.4 mM DCBQ/3 mM  $\text{Fe}(\text{CN})_6^{3-}$  as an acceptor system in the presence or absence of 10 mM  $\text{CaCl}_2$ .

Reconstituted preparation		Treatment	Activity ( $\mu\text{mol O}_2/\text{mg Chl per h}$ )	
$\text{Ca}^{2+}$	17 + 24 kDa		+ $\text{Ca}^{2+}$	- $\text{Ca}^{2+}$
-	-	none	520	160
-	-	10 mM HQ (30 min in dark)	0	0
-	+	none	530	190
-	+	10 mM HQ (30 min in dark)	500	160
+	+	none	540	510
+	+	10 mM HQ (30 min in dark)	510	490

TABLE III

POLYPEPTIDE AND MANGANESE RELEASE AFTER VARIOUS TREATMENTS OF THE PS II COMPLEX

HQ, hydroquinone.

Treatment	Polypeptide release			Mn release (%)
	33 kDa	23 kDa	17 kDa	
None	-	-	-	0
I 2 M NaCl <sup>a</sup>	-	+	+	4
II 1 mM HQ <sup>a</sup>	-	+	+	75-80
I 2 M NaCl	-	-	-	
II 1 mM HQ	+	+	+	80-85
III 2 M NaCl	-	-	-	
I 2 M NaCl	-	-	-	
II 1 mM HQ	+	+	+	85-90
III 0.8 M Tris <sup>b</sup>	-	-	-	
I 2 M NaCl	-	+	+	5
II 2 M NaCl	-	-	-	

<sup>a</sup> PS II membranes in 0.4 M sucrose/15 mM NaCl/50 mM Mes (pH 6.0) (2 mg/ml) were incubated with the indicated chemical for 1 h in the dark (4°C).

<sup>b</sup> Polypeptide (17, 23 kDa)/Mn-depleted PS II preparations were incubated for 1 h in the dark (4°C) with 0.8 M Tris (pH 8.0).

<sup>c</sup> See Fig. 5 and text for extent of extraction.

Another finding of this work concerns the 33 kDa water-soluble polypeptide of the oxygen-evolving complex. Depending on the extraction procedure utilized, one can demonstrate concurrent extraction of Mn and the 33 kDa protein [4] or alternatively, complete release of the 33 kDa species without strong extraction of Mn [19]. When we examined salt-washed PS II membranes from which Mn had been released by hydroquinone incubation, we observed that although as much as 80% of the Mn was extracted from these membranes, little or none of the 33 kDa protein was

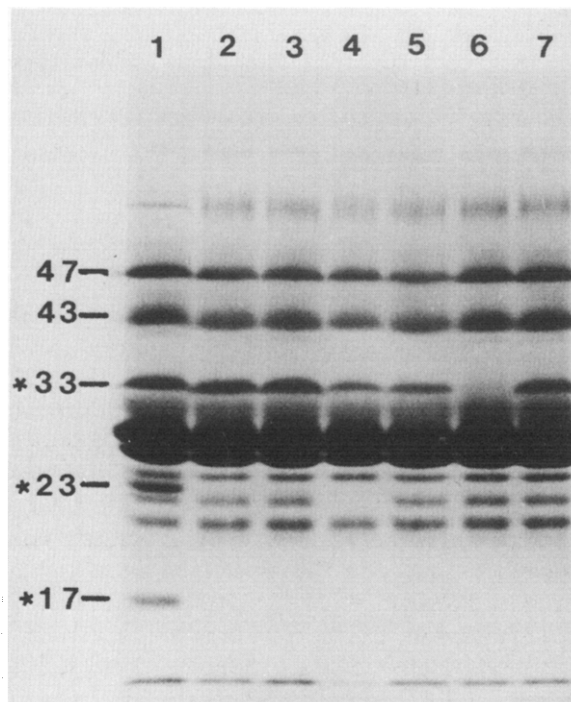


Fig. 5. Gel electrophoresis patterns of PS II membranes: effects of salt washing, treatments with reductants, and subsequent salt, Tris or buffer washing. Electrophoresis was carried out as previously described in Ref. 16; the gel contained 12.5% acrylamide and 2.5 M urea. Samples equivalent to 20  $\mu\text{g}$  Chl were electrophoresed. Numbers on the left-hand side are apparent molecular weights ( $\times 10^3$ ); (\*) denotes water-soluble polypeptides. Lanes: (1) control membranes; (2) 2 M NaCl-washed; (3) 2 M NaCl-washed, then exposed to 1 mM hydroquinone; (4) as 3, then washed with 2 M NaCl (pH 6); (5) as 3, then washed with 2 M NaCl (pH 8); (6) as 3, then washed with 0.8 M Tris, (pH 8); (7) as 3, then washed with 0.4 M sucrose/15 mM NaCl/50 mM Hepes (pH 8). the anomalous extraction of a second polypeptide of about 21 kDa (lane 4) is sometimes observed at pH 6 with high salt, but not with high salt at pH 8.

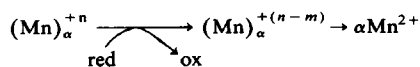
extracted (Fig. 5, lane 3); similar results were obtained for 100  $\mu$ M  $\text{NH}_2\text{OH}$  (data not shown). If, however, the Mn-depleted salt-washed membranes were exposed subsequently to either 2 M NaCl (at pH 6.0 or pH 8.0) or to Tris (0.8 M, pH 8.0) in the dark, extensive (NaCl) or complete (Tris) release of the 33 kDa protein was observed (lanes 4, 5 and 6). We estimate from densitometer tracings that up to 50% of the 33 kDa polypeptide is removed by the high-salt washings (at pH 6 or pH 8) after Mn extraction by hydroquinone exposure (data not shown). This extraction of the protein is due to ionic strength rather than to pH 8.0; a buffer wash of the Mn-depleted membranes at pH 8.0 does not extract the 33 kDa protein (Fig. 5, lane 7). A further treatment of the 17/23 kDa polypeptide-depleted, Mn-containing PS II complex with 2 M NaCl had little effect on the 33 kDa species (Table III). These observations are discussed further in the next section.

## Discussion

Previous studies of the mode of action of  $\text{NH}_2\text{OH}$  on the oxygen-evolving complex have revealed that this compound can replace the natural substrate (at low concentrations) [20], extract functional Mn concomitant with loss of oxygen-evolution activity (at higher concentrations) [2–4,18] and also act under certain circumstances to block reduction of  $\text{P-680}^+$  by Z [21,22]. The data of Radmer and Ollinger [20] indicate that the substrate-binding site for  $\text{NH}_2\text{OH}$  resides in a cleft whose dimensions are  $0.45 \times 0.20 \times 0.15$  nm. This cleft is apparently accessible only to a group of small molecules which besides  $\text{NH}_2\text{OH}$  includes  $\text{NH}_2\text{NH}_2$  and  $\text{H}_2\text{O}_2$  [20,23]. In our own investigation of the action of  $\text{NH}_2\text{OH}$  on the oxygen-evolving complex in PS II membranes, we have shown that high concentrations (5–10 mM) of the reagent will produce a strong release of Mn, and the 23 and 17 kDa polypeptides, and produce a release of some, but not all, of the 33 kDa water-soluble polypeptide [14]. Low concentrations (100  $\mu$ M) of  $\text{NH}_2\text{OH}$  cause no significant damage to the oxygen-evolving complex (see Fig. 1), but when used in concert with 2 M NaCl, strong extraction of Mn and all three water-soluble polypeptides was observed [24].

We have previously shown that the 17 and 23 kDa polypeptides are required for a high-affinity  $\text{Ca}^{2+}$ -binding site in the oxidizing side of PS II [16]. As we show in this communication, release of the 17 and 23 kDa polypeptides creates a profound alteration in the structure and stability of the environment of the Mn cluster on the oxidizing side of PS II. In the absence of these two water-soluble polypeptides, oxygen-evolution activity is sensitive to inhibition by low concentrations (approx. 100  $\mu$ M) of  $\text{NH}_2\text{OH}$  (Fig. 2) which, in the presence of the 17 and 23 kDa species, cause no damage to activity (Fig. 1). As we show here, the inactivation phenomenon in salt-washed PS II membranes is not restricted to  $\text{NH}_2\text{OH}$ ; both small reductants ( $\text{H}_2\text{O}_2$ ,  $\text{NH}_2\text{NH}_2$ ,  $\text{Fe}^{2+}$ ) and bulkier ones (benzidine, hydroquinone) are capable of inactivating  $\text{O}_2$ -evolution activity and of releasing Mn from the PS II complex (Fig. 4).

As an explanation for the results presented here, we would propose the following hypothesis. Others have shown that  $\text{NH}_2\text{OH}$  can reduce [20,23] or even 'over-reduce' [18,23] the lower S-states and we have shown that bulky reductants react with higher oxidation states of the Mn-complex in salt-washed PS II preparations [14]. It therefore seems likely that all of the reductants we have examined destroy oxygen-evolution activity by conversion of higher oxidation states of Mn (possibly Mn(III) or Mn(IV)) to Mn(II). Since Mn(II) has no ligand-field stabilization energy in most of its complexes [25], it is possible that the reduced (or 'over-reduced') Mn-complex is unstable and therefore easily destroyed (see Ref. 26 for mechanisms of Mn(III) to Mn(II) reduction by  $\text{NH}_2\text{OH}$ ,  $\text{NH}_2\text{NH}_2$ ,  $\text{H}_2\text{O}_2$ , hydroquinone and  $\text{Fe}^{2+}$  in perchlorate media). The involvement of higher oxidation states of Mn (possibly Mn(III) and Mn(IV)) in the oxygen-evolving complex has been proposed before [27–29]. If  $(\text{Mn})_{\alpha}^{+(n-m)}$  is the unstable form of the Mn-complex, then the reaction which leads to destruction of the oxygen-evolving complex in salt-washed PS II preparations will be:

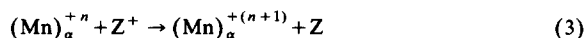
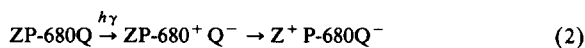


(red =  $\text{NH}_2\text{OH}$ , hydroquinone, benzidine, etc.)

(1)

Upon illumination the following reactions will

also take place:



According to the above scheme, the protective effect of light observed in Table I would be the result of a competition between reactions 1 and 3. In such a model, the presence of the 17 and 23 kDa polypeptides would facilitate the formation of a more stable Mn-complex, probably in concert with tightly-bound  $\text{Ca}^{2+}$ , and in addition would allow only molecules of a certain size to penetrate and react with Mn (see Ref. 20). The observations that reductants such as ascorbate, ferrocyanide or *O,N*-dimethylhydroxylamine do not extract Mn from the polypeptide-depleted PS II membranes probably reflect a slow reaction of these reductants with higher oxidation states of manganase. It is known, for example, that methylation of the hydroxyl group in hydroxylamine causes a 500-fold decrease in the rate constant of the reaction between the reductant and Mn(III) in perchlorate media [26]. The fact that of the various reductants tested, only  $\text{NH}_2\text{OH}$  at high concentrations can overcome the barrier posed by the 17/23 kDa polypeptides and destroy the oxygen-evolving complex in the intact PS II complex could actually reflect the complex effects of  $\text{NH}_2\text{OH}$  on the oxidizing side of Photosystem II. For example,  $\text{NH}_2\text{OH}$ , an analog of two molecules of water, is known to bind and react with the oxygen-evolving complex [20]. In addition to its redox properties, the involvement of  $\text{NH}_2\text{OH}$  in the modification of a protein at the oxidizing side of PS II, which results in inhibition of the Z to P-680 electron transfer, has also been suggested [21]. We propose then that in the intact PS II system, destruction of the oxygen-evolving complex by  $\text{NH}_2\text{OH}$  (at higher concentrations) is due to its ability to penetrate easily and react with the Mn-complex (one molecule of  $\text{NH}_2\text{OH}$  can reduce up to six equivalents of Mn(III) [26]) and perhaps also to perturbation of the structural organization of the oxidizing side of Photosystem II produced by  $\text{NH}_2\text{OH}$  itself. In our work with low concentrations of  $\text{NH}_2\text{OH}$  and hydroquinone, it is apparent that substantial (up to 80%) amounts of functional Mn can be released

from salt-washed PS II without loss of the 33 kDa protein. The results of Fig. 5 show that after Mn release, the 33 kDa species can be partially, but not completely, released by high ionic strength treatment at low (6.0) or high (8.0) pH. Since high ionic strength alone does not release the 33 kDa polypeptide from the salt-washed Mn-containing PS II complex, we conclude that the presence of functional Mn stabilizes the binding of the 33 kDa polypeptide to the PS II complex.

In summary, our results present new information on the roles of water-soluble polypeptides of PS II in maintaining the structural integrity of the oxygen-evolving complex and its pool of functional Mn. Although we cannot determine the oxidation states of Mn in the oxygen-evolving complex, it is clear from our data that at least some of the oxidation states are higher than +2, and these higher oxidation states are, by virtue of their associated ligand-field stabilization energies, a necessary element in maintaining the structural integrity of the oxygen-evolving complex. We believe that the 23, 17 and 33 kDa polypeptides are organized so as to provide a structure which stabilizes and also shields the Mn-complex (or at least part of it) from reaction with exogenous reductants. Part of this structure is comprised of the 23 and 17 kDa proteins, which are bound into the structure by ionic interactions which are sensitive to high salt concentrations. The interaction of the 33 kDa polypeptide within the PS II complex is also ionic, but is further stabilized by the presence of higher oxidation states of Mn. Removal of the 17 and 23 kDa proteins modifies the structure of the oxygen-evolving complex so that it is less stable and even larger reductants such as hydroquinone and benzidine can now react with the Mn cluster to reduce and destroy it. In the formulation we propose here, all three proteins (33, 23 and 17 kDa) are essential elements of the active oxygen-evolving complex in its stable, native form.

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