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Lack of photoactivation capacity in *Scenedesmus obliquus* LF-1 results from loss of half the high-affinity manganese-binding site. Relationship to the unprocessed D1 protein

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The high-affinity binding site for Mn^{2+} is characterized by a decrease in 1,5-diphenylcarbazide (DPC) to 2,6-dichlorophenolindophenol (DCIP) electron transport with NH_2OH-treated spinach Photosystem II (PS II) membrane fragments when micromolar amounts of Mn^{2+} are present in the assay. This site is purported to be the binding site for Mn, functional in O_2 evolution (Hsu, B.-D., Lee, J.-Y. and Pan, R.-L. (1987) Biochim. Biophys. Acta 890, 89–96). We have examined this site in PS II-enriched membranes from *Scenedesmus obliquus* wild-type (WT) and LF-1 mutant cells. LF-1 inserts an unprocessed D1 protein into the photosynthetic membrane, binds approx. 40% of the functional Mn as WT, and does not evolve O_2 (Metz, J.G., Pakrasi, H.B., Seibert, M. and Arntzen, C.J. (1986) FEBS Lett. 205, 269–274). The dissociation constant for added high-affinity Mn^{2+} is about 0.3–0.4 μM in wheat, WT, and LF-1 PS II. However, the relative amount of available high-affinity Mn^{2+}-binding site is about half as much in LF-1 PS II membranes compared to wheat, spinach, and WT PS II membranes. Despite the fact that LF-1 PS II can photoligate Mn, LF-1 cannot be photoactivated as can NH_2OH-treated WT PS II. LF-1 subjected to photoactivating conditions does not reach S_2 as determined by thermoluminescence. This work indicates that the Hsu et al. high-affinity Mn^{2+} site is actually at least two sites, one of which is missing in LF-1, and that successful photoactivation potential requires the presence of all high-affinity Mn^{2+} site. The fact that the full complement of high-affinity Mn^{2+}-binding site is observed in isolated spinach PS II reaction center (D1/D2/cytochrome *b*-559) complex demonstrates that other PS II core proteins do not affect the high-affinity site. Histidine chemical modifier experiments show that one component of the high-affinity site is probably associated with histidine(s) and that this component is missing in LF-1. We conclude that histidine(s) on the D1 protein provides ligand(s) for part of the Mn required for O_2-evolution function and that the balance of the Mn is bound by other amino acids on the proteins composing the PS II reaction center.

Introduction

Substantial progress in understanding the structure and function of Photosystem II (PS II) has been made over the past several years. However, there is still much to be learned about the photosynthetic water-splitting process. It is well known that manganese is associated with the active site of the O_2-evolving complex (OEC). However, the location of the Mn-binding site and precisely how Mn is involved in water-splitting function is not known [1–3].

Recent studies of the *Scenedesmus obliquus* PS II mutant, LF-1, have shown that (1) the alga inserts an unprocessed D1 protein into the photosynthetic membrane [4–6,29], (2) thylakoid membranes isolated from the mutant bind approx. 40% of the Mn bound by wild-type (WT) membranes [4,7], and (3) the mutant does not evolve O_2 [4]. The unprocessed D1 protein has an apparent molecular mass about 2 kDa larger than that of the normal protein and is easily identified by LDS-PAGE. No other large protein alterations detec-

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Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DEPC, diethylpyrocarbonate; DPC, 1,5-diphenylcarbazide; LDS-PAGE, lithium dodecyl sulfate polyacrylamide gel electrophoresis; Mes, 4-morpholineethanesulfonic acid; OEC, oxygen-evolving complex; PS II, Photosystem II; SiMo, silicomolybdate; WT, wild type; SERS, surface-enhanced Raman scattering.

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table by LDS-PAGE between WT and LF-1 have been observed. Furthermore, LF-1 is unaltered functionally on the reducing side, carries out primary charge separation, and retains partial donor-side function [8,9]. Since the mutant does not evolve O_2, it has been assumed that the low Mn content was due to a failure of LF-1 to bind Mn functional in the normal water-splitting process and that this was in some way associated with the altered D1 protein [5]. This was consistent with the work of others who demonstrated that functional Mn was bound to an intrinsic protein, since the extrinsic proteins could be removed from PS II membrane fragments without removing Mn [10,11]. More recent evidence obtained from surface-enhanced Raman scattering (SERS) studies of *Scenedesmus* WT and LF-1 PS II membrane fragments suggest that a binding site for at least part of the functional Mn may reside on the D1 protein itself [7]. This is in agreement with the conclusions of recent ^{125}I-labelling studies [12].

Application of the important studies of Hsu et al. [13] to *Scenedesmus* provides a powerful means to examine this possibility in greater detail. After first noting that removal of endogenous Mn increases the DPC donation rate to PS II, these investigators found a high-affinity binding site for exogenous Mn^{2+} on Tris-treated spinach PS II appressed membrane fragments which were depleted of functional Mn and extrinsic proteins. Addition of Mn^{2+} at submicromolar concentrations decreased the rate of DPC-supported DCIP reduction in the treated PS II membranes due to competitive inhibition of DPC electron donation. The anion species present enhances the affinity of the site for Mn^{2+} in the order $NO_3^- > Br^- > Cl^- > CH_3COO^- > SO_4^{2-} > F^-$. This hierarchy is similar to that reported for the expression of O_2-evolution activity [14]. Hsu et al. cited this evidence to mean that the high-affinity Mn^{2+} site is the native site for functional Mn. We report here the use of the above-mentioned assay to identify new properties and a more specific location for at least part of the high-affinity Mn site. This was accomplished by examining WT and mutant PS II membranes from *Scenedesmus* and comparing differences in their Mn-binding properties. A preliminary report of this work has appeared [15].

Materials and Methods

Scenedesmus obliquus WT and LF-1 mutant cells were grown heterotrophically in the dark in 10-l batches and were broken with glass beads. Photosystem-II-enriched fragments were isolated as described previously [16]. Membranes used for Mn assays were exposed to an additional centrifugation step in 1.8 M sucrose to eliminate aberrant material which did not pellet under these conditions. PS II samples were stored in 0.4 M sucrose, 15 mM NaCl, 5 mM $MgCl_2$, and 20 mM Mes (pH 6.5) at $-80\,^\circ$C until use. Spinach and wheat PS II membrane fragments were made by standard procedures [17]. PS II reaction centers (D1/D2/cytochrome *b*-559 protein complex) from spinach were isolated by a modification of the Nanba/Satoh procedure [18] using the polyethylene glycol concentration method as described previously [19].

NH_2OH treatment of the PS II membranes was accomplished by exposing the samples at 1 mg Chl/ml to 5 mM NH_2OH in buffer A (20 mM NaCl, 0.4 M sucrose, and 50 mM Mes (pH 6.5)) for 60 min in the dark at 4 °C. The material was then washed 3 times by centrifugation and resuspended in buffer A at about 2 mg Chl/ml. Photoactivation of PS II membranes was done according to the procedure of Tamura and Cheniae [20]. A suspension containing 100 μM DCIP (5 mM stock), 2 mM $MnCl_2$ (20 mM stock), 50 mM $CaCl_2$ (1 M stock), buffer A, and membranes (200 μg Chl/ml) was incubated for 45 min at 20 °C under cool white fluorescent light (8 W/m^2) in a petri dish to minimize the thickness of the PS II membrane suspension. Photoactivated material for thermoluminescence studies was diluted in buffer A containing 2 mM EDTA, pelleted, and washed in the same EDTA/buffer. The last pellet was resuspended in minimal buffer A. Photoactivated material for Mn assays was treated in a similar manner except that 10 μM A23187 (an ionophore) was included in the second EDTA/buffer centrifugation step and an additional centrifugation step in buffer A was added thereafter.

Samples for histidine chemical-modifier experiments were prepared [28] by incubating 100 μg Chl per ml of PS II membranes with 500 μM diethylpyrocarbonate (from 100 mM stock DEPC) in buffer A for 60 min at 20 °C in the dark. The material (1 ml) was added to 5 ml 10 mM histidine in buffer A and pelleted by centrifugation. The pellet was resuspended in 7 ml of buffer A and washed twice. The final pellet was resuspended in 0.1 ml of buffer A. The untreated controls were incubated under the same conditions but without DEPC.

O_2 evolution from PS II membranes was measured with a Clark-type electrode using 0.4 mM 2,6-dichlorobenzoquinone as the acceptor. PS II electron transport activity was determined spectrophotometrically at 600 nm using a Shimadzu UV-300 spectrophotometer and cross illumination. Xenon light was passed through a $CuSO_4$ solution and a Toshiba R64 filter and then focused on the sample cuvette (625 W/m^2). The spectrophotometer PMT was blocked with a 600 nm narrow band filter, and an extinction coefficient of 13.0 $mM^{-1} \cdot cm^{-1}$ was used for determining rates. The assay solution contained 30 μM DCIP (5 mM stock), 200 μM DPC (25 mM stock) or 1 mM $MnCl_2$ (20 mM stock), buffer A, and 5 μg Chl/ml of membrane material. Small amounts of Mn in the micromolar range were added to the above DPC/DCIP assay solution to de-

termine high-affinity Mn-binding site parameters in the samples [13]. Since DCIP is a very poor electron acceptor when used with isolated spinach PS II reaction complex [18], 200 μg/ml silicomolybdate (SiMo) was substituted for DCIP [21]. The only other changes in the assay procedure for PS II reaction center complex were the replacement of buffer A with 60 mM Tris-HCl (pH 8.5) and the decrease in Chl concentration to 1 μg/ml.

Mn content of the membranes was determined with a Shimadzu flameless atomic absorption spectrometer (AA-640-13), equipped with a graphite furnace (GFA-3). Thermoluminescence glow curves for photoactivated PS II membranes were obtained from 48 μg Chl samples spread onto 2 × 2 cm filter paper squares. The samples were exposed to 5 μs xenon flashes at 20 °C or 45 s of continuous light at −25 °C, frozen immediately in liquid nitrogen, and then allowed to increase in temperature at a uniform rate. Luminescence measurements were then performed as in Ref. 22.

Results

Previous investigators [23] have reported that O_2-evolving capacity and Mn content of *Scenedesmus* LF-1 mutant thylakoid membranes are substantially lower than those found in WT thylakoid membranes, while DCIP photoreduction rates in the presence of an artificial donor are essentially the same in thylakoids from both strains. Table I shows similar results for PS II-enriched membrane fragments isolated from WT and LF-1. Furthermore, the rate of DCIP photoreduction in NH_2OH-treated PS II membranes with 1 mM Mn^{2+} as the donor is about half that observed with DPC as the donor. Table II shows that NH_2OH-treated preparations evolve very little O_2 as expected, since this treatment removes functional Mn (compare Tables I and II). However, after exposure to photoactivating conditions in the presence of Mn^{2+} and Ca^{2+}, WT membranes regain the capacity to evolve O_2 while LF-1 membranes do not. Table II also shows that our inability to photoactivate LF-1 membranes successfully is not due to their lack of Mn-binding capacity. In fact NH_2OH-treated or untreated LF-1 PS II membranes seem to bind more Mn under photoactivating conditions than do NH_2OH-treated WT membranes. This indicates that Mn incorporated into LF-1 is bound differently than that bound to WT membranes.

Fig. 1 shows thermoluminescence profiles for WT and LF-1 membranes subjected to photoactivating conditions. The presence of a B-band representing the

TABLE I

O_2-evolution activity, DCIP photoreduction, and manganese content for Scenedesmus WT and LF-1 PS II membranes

	O_2-evolving activity (μmol O_2 per mg Chl per h)	DCIP photoreduction (μmol per mg Chl per h)		Mn per 200 Chl
		DPC as donor	1 mM $MnCl_2$ as donor	
Wild type	128	123 [a]	64 [a]	5.0
LF-1 mutant	5	128 [a]	61 [a]	1.7

[a] PS II samples for DCIP photoreduction experiments were NH_2OH-treated.

TABLE II

Manganese content and oxygen-evolution capacity of Scenedesmus PS II membranes subjected to photoactivating conditions

Mn/Ca indicate samples that were exposed to ion conditions that support photoactivation in WT membranes. Light is required for successful photoactivation [20].

Sample preparation and treatment	Mn per 200 Chl	O_2 evolution (μmol per mg Chl per h)
NH_2OH-treated WT PS II		
A. None	2.0	29
B. Dark/Mn/Ca	3.4	–
C. Light/Mn/Ca	5.1	132
D. Light/Mn/Ca + NH_2OH	1.6	–
NH_2OH-treated LF-1 PS II		
A. None	1.0	22
B. Dark/Mn/Ca	1.6	–
C. Light/Mn/Ca	6.3	24
D. Light/Mn/Ca + NH_2OH	1.4	–
LF-1 PS II		
A. None	1.7	–
B. Dark/Mn/Ca	2.2	–
C. Light/Mn/Ca	10	–

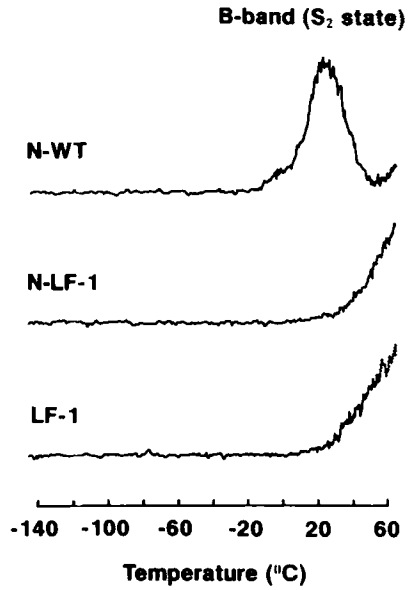

Fig. 1. Thermoluminescence profiles for NH_2OH-treated WT (N-WT), NH_2OH-treated LF-1 (N-LF-1), and untreated LF-1 (LF-1) PS II membranes after exposure to photoactivating conditions. Dark-adapted samples were exposed to one flash at 20 °C prior to freezing. Similar results were obtained when the samples were exposed to continuous light for 45 s at −25 °C (data not shown).

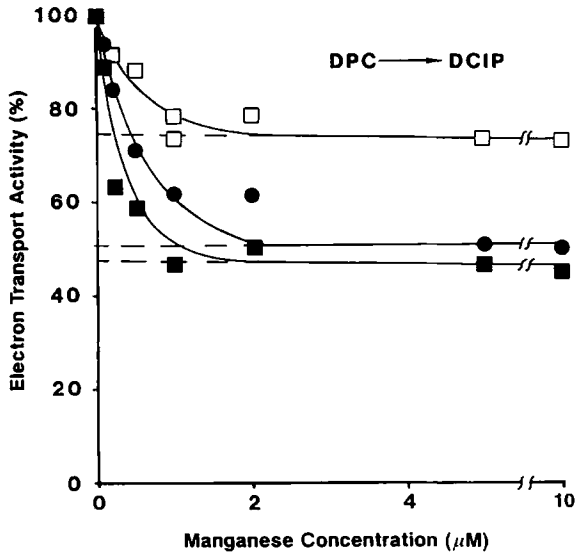

Fig. 2. The high-affinity Mn-binding site in NH_2OH-treated PS II membranes from spinach (●) and *Scenedesmus* WT (■) and in untreated PS II membranes from *Scenedesmus* LF-1 (□). DPC-to-DCIP activity was measured in the presence of different concentrations of added $MnCl_2$. See Materials and Methods for assay conditions.

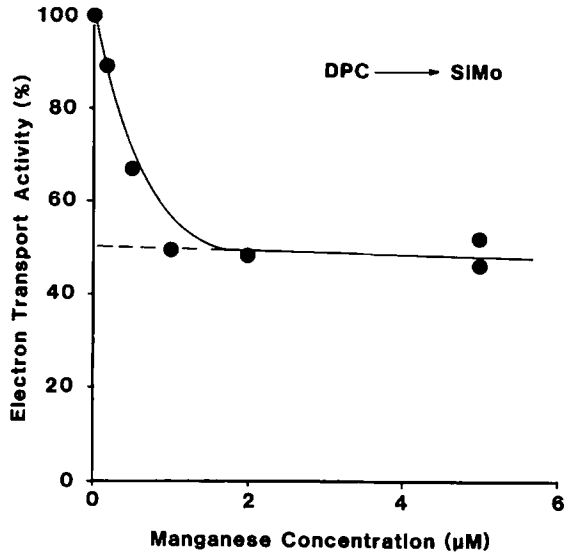

Fig. 3. The high-affinity Mn-binding site in isolated PS II reaction centers (the D1/D2/cytochrome *b*-559 complex) from spinach. Experimental conditions were similar to those in Fig. 2, except silicomolybdate was used as the acceptor (see Materials and Methods).

recombination of the $S_2Q_B^-$ charge pair is expected with WT membranes, since the same samples evolve O_2. The lack of a B-band in the case of the mutant is consistent with the observations in Table II, and demonstrates that LF-1, even after exposure to photoactivating conditions, cannot advance even to the S_2 state. This confirms that the Mn bound to LF-1 under photoactivating conditions (Table II) is not even partially functional.

Fig. 2, which examines the properties of the high-affinity Mn-binding site in spinach and *Scenedesmus* PS II membranes, provides a rationale for why LF-1 membranes cannot be successfully photoactivated. NH_2OH-treated spinach and WT PS II membranes lose about half of their DCIP photoreduction activity with DPC as the primary donor when micromolar concentrations of Mn^{2+} are added to the assay medium. However, only about a quarter of the activity is lost when NH_2OH-treated LF-1 (data not shown) or LF-1 PS II membranes are subjected to the same assay. Table III summarizes some of the properties of the high-affinity Mn-binding site obtained from experiments such as in Fig. 2. Despite the difference in the total amount of binding site present in WT and LF-1 membranes, the dissociation constant for Mn^{2+} in both cases is quite similar. Fig. 3 is an experiment similar to those in Fig. 2, except that isolated spinach PS II reaction center (D1/D2/cytochrome *b*-559) complex was examined. As can be seen, the addition of Mn^{2+} reduces the rate of electron transport by about the same amount observed in NH_2OH-treated wheat or WT PS II membrane fragments (Fig. 2). Unfortunately, dissociation constants estimated from the data in Figs. 2 and 3 cannot be directly compared, since the acceptors, pH of the assays, and reaction center concentrations differ in the two cases. Nevertheless, Fig. 3 shows that the entire high-affinity Mn-binding site, as detected by the Hsu et al. method [13] (i.e., about half the activity is lost upon addition of Mn^{2+}), is restricted to those proteins composing the reaction center complex itself. Furthermore, we interpret these data to mean that, compared to WT membranes, LF-1 membranes contain only about half the detectable high-affinity Mn-binding site and that the modified or missing component of the site is related to presence of the unprocessed D1 protein.

In order to examine the molecular environment of the high-affinity Mn-binding site in the photosynthetic

TABLE III

Properties of the high-affinity manganese binding site in wheat and Scenedesmus PS II membranes

Sample preparation	DCIP reduction (μmol per mg Chl per h) with DPC as a donor	Mn dissociation [a] constant (10^{-7} M)	$\frac{V(+)}{V(-)}$ [b]
NH_2OH-treated wheat PS II	379 (181) [c]	4.2	0.46
NH_2OH-treated WT PS II	123 (64)	3.2	0.48
NH_2OH-treated LF-1	128 (61)	3.4	0.75

[a] K_d's were estimated from experiments such as in Fig. 2 and represent the concentration of Mn required to reduce the rate of DPIC reduction half way to the plateau value observed when 2–10 μM Mn^{2+} was present in the assay. Values reported here are quite similar to those obtained by Hsu et al. [13] with spinach PS II membranes at the equivalent salt concentration.

[b] $V(+)/V(-)$ indicates the ratio of DPC-to-DCIP activity in the presence of 2 μM Mn^{2+} to that in the absence of Mn^{2+}.

[c] Values in parentheses are rates with 1 mM Mn as a donor.

membrane, we exposed NH_2OH-treated PS II samples to diethylpyrocarbonate (DEPC), a histidine modifier, prior to performing experiments such as in Fig. 2. The results in Table IV for wheat and WT *Scenedesmus* PS II membranes show that half the high-affinity Mn site is lost when the membranes are pretreated with DEPC. However, LF-1 membranes which have already lost half the high-affinity Mn-binding site (that associated with the unprocessed D1 protein) do not show any additional response to DEPC.

TABLE IV

Effects of the histidine blocker, DEPC, on the high-affinity Mn-binding site

Sample	DEPC	DCIP reduction (μmol per mg Chl per h) with DPC as a donor		$\frac{V(+)}{V(-)}$
		No addition $V(-)$	2 μM Mn^{2+} [a] $V(+)$	
NH_2OH-treated wheat PS II	–	165	85	0.52
	+	148	115	0.78
NH_2OH-treated WT PS II	–	95	49	0.52
	+	92	74	0.80
NH_2OH-treated LF-1 PS II	–	82	62	0.76
	+	81	60	0.75
Untreated LF-1 PS II	–	101	74	0.73
	+	89	64	0.73

[a] Control experiments (Preston and Seibert, unpublished results) demonstrate that DEPC does not affect the shape of the Mn^{2+} inhibition curve (Fig. 2) except for the plateau level and does not affect the Mn^{2+} dissociation constant of any remaining high-affinity Mn-binding site. Thus, 2 μM Mn^{2+} is sufficient to saturate the effect of Mn^{2+} addition in both control and DEPC-treated membranes.

Discussion

The non-O_2-evolving *Scenedesmus* LF-1 mutant contains an unprocessed D1 protein that seems to affect binding of Mn, functional in the photosynthetic water-splitting process. Although there are some other minor changes in properties recognized in the mutant, as far as we can tell, all other components of PS II associated with normal electron transport are present and appear to function. Consequently, detailed examination of the mutant in comparison with the WT offers us a unique opportunity to ascertain information about the lowered Mn-binding capacity of LF-1 (apparently due to an altered or missing binding site) and the molecular environment that leads to this phenomenon.

Exposure of LF-1 PS II membranes to photoactivating conditions in the presence of Mn^{2+} [20] does not result in the reconstitution of O_2-evolving capacity (Table II) *. On the other hand, LF-1 membranes will bind Mn^{2+} under these same conditions. Although this 'bound' Mn can be removed by NH_2OH treatment, LF-1 membranes exposed to photoactivating conditions do not reach the S_2 state (Fig. 1). Thus, the unprocessed D1 protein in LF-1 membranes affects the conformation of the oxidizing side of PS II to the extent that at least some required Mn cannot be bound in a functional manner. Note that the binding of extrinsic proteins is not required for Mn binding, since Tris-treated PS II membranes can be photoactivated in the absence of the extrinsic proteins [20].

As discussed in the Introduction, Hsu et al. [13] have developed a simple assay to probe for the high-affinity binding site associated with functional Mn of PS II. Such a site is also indicated by analyzing H_2O_2-stimulated Mn^{2+} to DCIP activity [25]. To determine why Mn^{2+} cannot be photoligated onto LF-1 PS II membranes in a manner that leads to photoactivation (note that photoligation and photoactivation are sequential processes; Ref. 20), we examined the properties of the high-affinity Mn site in this material.

Fig. 2 and Table III show that the presence of micromolar concentrations of Mn^{2+} in the DPC to DCIP assay medium decreases electron-transport activity by about 50% in NH_2OH-treated spinach, wheat, and *Scenedesmus* WT PS II membranes, but only by about 25% in *Scenedesmus* LF-1 membranes. The decrease observed in the normal material of all three species is comparable to that observed previously in Tris-treated spinach PS II membranes [13]. Furthermore, the apparent dissociation constant for Mn^{2+} is approximately the same in all preparations (approx. 0.3–0.4 μM, Table III). Since Table I shows for both WT and LF-1 that the rate of DCIP reduction in the presence of 1 mM Mn^{2+} is half that of when DPC is used as a donor, our results suggest that part of the high-affinity Mn^{2+} site observed in WT is converted to a low-affinity site in LF-1. Thus, we conclude that functional Mn requires at least two different high-affinity binding sites.

The question now arises whether the presence of any PS II core protein other than D1, D2, and cytochrome *b*-559 influences the high-affinity Mn-binding site detectable by the Hsu et al. assay [13] in a primary manner. Fig. 3 indicates that this is not the case, since the rate of DPC to SiMo activity in isolated PS II reaction center complex is reduced by about the same amount when Mn^{2+} is present as was the case above for DPC-to-DCIP activity in NH_2OH-treated wheat, spinach, and WT PS II membranes. On the other hand, all attempts to

* Taylor et al. [24] reported the same results but did observe photoactivation (though at a very low rate) after processing the LF-1 D1 protein with WT extract.

photoactivate isolated PS II reaction center complex have failed (data not shown). This suggests that, although the high-affinity Mn-binding site is located on the complex, other proteins and/or cofactors are required for successful photoactivation. Preliminary results show that when exposed to photoactivating conditions, PS II reaction center complex made according to Ref. 19 does bind Mn. Nevertheless, the results in Fig. 2 support the conclusion of Hsu et al. [13] that the high-affinity Mn-binding site is the site to which Mn functional in O_2 evolution binds.

We demonstrate in Table IV that DEPC treatment reduces the apparent amount of high-affinity Mn-binding site in *Scenedesmus* WT PS II membranes by about 50% but does not affect the amount of site apparent in *Scenedesmus* LF-1 membranes. Since untreated WT membranes contain at least two sites and untreated LF-1 membranes contain at least one fewer site, we conclude that the missing site in LF-1 (related to the presence of the unprocessed D1 protein) is the site affected by DEPC treatment. Although DEPC is known to react with a number of amino side groups, Tamura et al. [28] have provided chemical reversal, p*K*a, and inactivation rate constant evidence showing that DEPC-induced loss of Mn^{2+} photoligation and photoactivation potential results from specific modification of histidyl residues in PS II membranes. This does not preclude non-specific modification of PS II core proteins, but histidine modification is specifically implicated in the protein affecting functional Mn-binding properties. Thus, the evidence presented in Table IV suggests that histidine(s) on the D1 protein provides a ligand(s) for binding functional Mn. Tamura et al. [28] make a similar conclusion based on ^{14}C-DEPC radiolabelling studies of unphotoactivated and photoactivated PS II membranes from wheat which, of course, contains the normal processed D1 protein. These two studies taken together indicated that the loss of the high-affinity Mn site associated with the presence of the unprocessed D1 protein in LF-1 is not due to 'shielding' of histidine residues on PS II proteins other than D1 by the unprocessed transit C-terminus peptide.

In light of this evidence, we conclude that of at least two high-affinity Mn-binding sites localized on the D1/D2/cytochrome *b*-559 PS II reaction center complex, one site is associated with histidine(s) on the D1 protein. Furthermore, we suggest that in the LF-1 mutant steric hindrance due to the presence of the unprocessed C-terminus on the D1 protein results in the loss of high-affinity Mn-binding properties (see also Ref. 5) of the histidine site. This is consistent with Diner et al. [6] who argue that the altered Mn-binding properties in LF-1 are due primarily to structural changes in D1. Reducing-side function, including atrazine-binding constants [5,8,23] and low-temperature $Q_A^- Fe^{2+}$ EPR signals [9] both of which should be very sensitive to structural changes on the stromal side of the membrane, is not affected in LF-1. This strongly suggests that the C-terminus extension on the unprocessed D1 protein resides on the lumenal side of the PS II membrane where functional Mn is expected to bind and where partially altered Mn-binding properties are observed (model b of Ref. 6).

Although the current study provides no specific location for histidine(s) implicated in Mn binding, one can speculate using Trebst's D1/D2 folding model [26]. D1 contains ten histidines and D2 contains eight. Five of these histidines (118, 190, 198, 215, and 272) are located in symmetric positions on the two proteins. If the data in Table IV can be taken at face value, then only one Mn-binding site on the PS II reaction center, that on the D1 protein, involves histidine(s). This would suggest that D1 histidine(s) 92, 195, 252, and 332 having no counterpart on D2 should be considered. On the other hand, His-195 is on helix IV of D1 and His-252 is on the stromal side of the membrane and will not be considered further. Since steric effects due to the unprocessed carboxyl end of D1 in LF-1 should have the greatest effect on that end of the protein, His-332 should be a primary candidate for binding Mn, though His-92 cannot be specifically eliminated. His-337 is a special case at this point – it has a near symmetric histidine at position 339 on D2, but it may or may not be on that part of the carboxyl end of D1 that is processed. One must also consider the fact that our chemical modifier experiments do not specifically eliminate the possibility of an Mn-binding histidine site on D2. In this case, the symmetrically placed His-190 can also be considered (His-118 is in the center of helix II and His-198, His-215, and His-272 have been assigned to other PS II electron transport carriers (Ref. 26, and by analogy to the bacterial reaction center)). However, Dismukes [27] suggests that from analogy with the bacterial reaction center, His-190 is likely to bind chlorophyll.

If the other high-affinity Mn-binding site(s) does not involve histidine, as our evidence (taken at face value) suggests, the site might be located on another part of the D1 protein distant from the carboxyl end or on the D2 protein. Dismukes [27] states that besides histidine, lysine, carboxylate donors such as aspartate and glutamate, and primary amides such as asparagine and glutamine are candidates for Mn-binding amino acids. Tamura et al. [28] in preliminary carboxyl modifier experiments suggest Mn^{2+}-specific substrate protection of the high-affinity site. This argues for involvement of aspartate and/or glutamate in that part of the high-affinity Mn-binding site not associated with D1 histidine. Hopefully, future studies of the PS II reaction center will be of use in sorting out current speculations as to the location of the Mn-binding sites identified in this investigation.

Note added in proof:

Since this paper was accepted Takahashi et al. [30] reported that the D1 protein is processed between Ala-344 and Ala-345. Based on this information we also consider His-337 on D1 as a candidate for binding Mn.

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