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Calcium depletion modifies the structure of the photosystem II O_2-evolving complex

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Introduction

Although Ca^{2+} is an essential cofactor for O_2 evolution (Ghanotakis, et al., 1984a) and has been shown to play a role in stabilizing the protein structure that ligates Mn (Mei and Yocum, 1991), it is probable that the metal plays additional role(s) in H_2O oxidation. In many Ca^{2+} cofactor proteins, H_2O is present as a ligand. In the case of PSII, the Lewis acidity of Ca^{2+} may be crucial to binding of either H_2O (Riggs-Gelasco, et al., 1996; Pecoraro, et al. 1998) or ^{-}OH (Vrettos et al. 2001b) as substrate intermediates in the S-state cycle. The topological disposition of Ca^{2+} with respect to the Mn cluster has not been explored in detail. In this communication we present results showing that Ca^{2+} can impede access of small reducing agents (NH_2OH, N-methyl NH_2OH) to site(s) of Mn ligation in the O_2-evolving complex. We further show that Ca^{2+} extraction/restoration is a reversible process. Our results are consistent with the existence of a substrate access channel between the solvent and the PSII Mn cluster, which is partially occluded by bound Ca^{2+}.

Materials and Methods

Photosystem II was isolated according to Ghanotakis, et al. (1984b) and small extrinsic proteins were removed by NaCl washing (Ghanotakis, et al. (1984a). Alternatively, Ca^{2+} was released from PSII using the pH 3 citrate method of Ono and Inoue (1988); the metal is extracted but the extrinsic polypeptides remain bound to the intrinsic core of the enzyme system. Reductant access to the Mn cluster was monitored by assaying inhibition of steady-state O_2 evolution rates with DCBQ as the electron acceptor, using samples exposed to hydroxylamines for varying periods of time in the dark. Maximal rates were obtained only when samples were incubated in Ca^{2+} before illumination.

Results

Table 1 presents the results of a series of assays to determine the K_M values for Ca^{2+} and Cl^- in salt-washed and citrate-treated PSII. The single Ca^{2+} K_M exhibited by the polypeptide-depleted sample reflects loss of the diffusion barrier between Ca^{2+} in solution and its PSII binding site, which correlates with extraction of the 23 and 17 kDa polypeptides (Ghanotakis, et al., 1984a). The two K_M values for Ca^{2+} that are detected in the citrate sample are consistent with the presence of structural heterogeneity created by low pH extraction of Ca^{2+}; about 20% of the centers in this sample have lost Ca^{2+} and the 23 and 17 kDa polypeptides, and are therefore partially (ca. 20%) sensitive to inhibition with hydroquinone (data not shown). These centers exhibit a lower K_M than do the remaining, intact centers that retain bound extrinsic polypeptides and are insensitive to hydroquinone. The higher K_M in these

centers must be due to the presence of the 23 and 17 kDa polypeptides that form part of the shield around the OEC. The Cl^- K_M for both PSII samples indicates that Ca^{2+} extraction, regardless of the method, increases the K_M above the level observed in intact PSII.

Table 1: K_M and Vmax values for Ca^{2+} and Cl^- in polypeptide-depleted and citrate-treated PSII preparations.

Photosystem II Preparation

	23,17 kDa Depleted		Citrate-treated	
Cofactor	K_M	Vmax	K_M	Vmax
	(μM)	(μmol/hr/mg Chl)	(μM)	(μmol/hr/mg Chl)
Ca^{2+}	500	170	800	60
Ca^{2+}			6,500	140
Cl^-	1,900	208	1,800	125

The data shown in Fig. 1 below illustrate the contrasting responses of intact PSII and citrate-treated samples to inhibition by hydroxylamines. The most prominent feature of the citrate-treated sample, relative to the intact enzyme, is its enhanced sensitivity to NH_2OH, relative to that of intact PSII, which retains both Ca^{2+} and the small extrinsic polypeptides. As the data show, N-methyl hydroxylamine, but not the dimethyl derivative, is able to reduce and destroy the PSII Mn complex in citrate-treated PSII, but with less efficiency than NH_2OH. Intact PSII is unaffected by the N-methyl NH_2OH derivatives under the conditions used here.

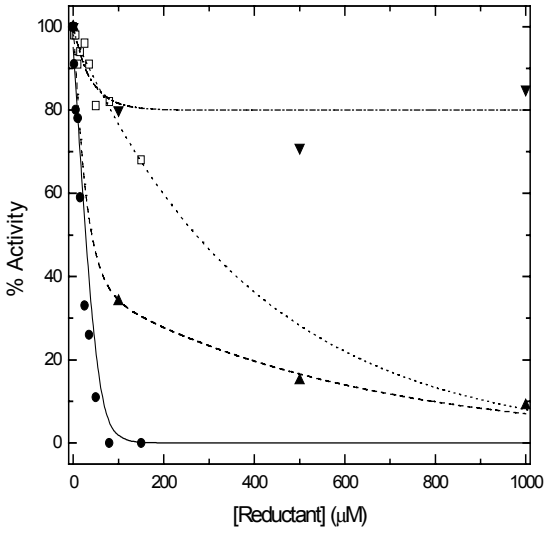

Fig. 1. Effect of hydroxylamines on inhibition of activity in citrate-treated and intact PSII. Symbols are: □, NH_2OH, intact PSII; ●, NH_2OH citrate PSII;-▲,▼,citrate-treated PSII exposed to methyl and di-methyl hydroxylamines, respectively.

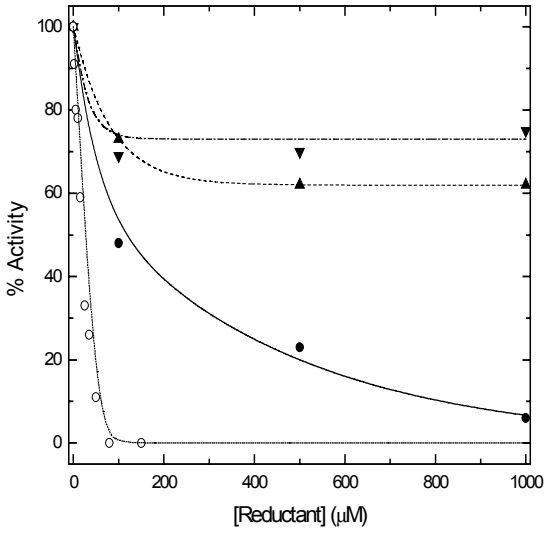

Fig. 2. Ca^{2+} reconstitution of citrate PSII re-stores structural integrity to the OEC. Symbols: ○, NH_2OH, citrate PSII; ●, same after Ca^{2+} re-reconstitution; ▲,▼, same as Fig. 1, but after constitution of citrate-treated PSII.

Figure 2 presents results of similar reductant probing experiments carried out on citrate-treated PSII preparations in which Ca^{2+} had been restored to its binding site. As the figure shows, NH_2OH resistance is partially restored in the Ca^{2+} incubated sample, as is insensitivity to Mn reduction by the methyl derivatives of NH_2OH. Taken together, these data show that removal of Ca^{2+} exposes a sterically constrained pathway between the external medium and the Mn cluster. This shift in the structure of the OEC is reversible, in that rebinding of Ca^{2+} partially occludes the pathway by which NH_2OH and its N-methyl derivative access the Mn cluster.

Calcium depletion of PSII under conditions where the extrinsic polypeptides are retained affects the Cl^- K_M of the enzyme (Table 1); there is little difference in the Cl^- K_M values for salt-washed and for Ca^{2+} extracted samples. The Cl^- affinity of citrate-treated PSII samples was examined after the reconstitution of Ca^{2+} in two ways: first, Ca^{2+}-reconstituted samples were dark incubated with Cl^- for 60 min. and assayed in Ca^{2+}/Cl^- free medium to measure stable reincorporation of Cl^-. The second method used Cl^- addition to the assay medium to assess Cl^- reincorporation in the light, as was done with the samples shown in Table. 1. The results are presented in Table 2. Comparison of these data with those of Table 1 reveal that

Table 2. Effect of Ca^{2+} reconstitution on Cl^- kinetic parameters

Sample Treatment	K_M (μM)	V_{MAX} (μmol O_2/hr/mg Chl)
Dark incubation with Cl^- (no Cl^- in the assay buffer)	350	80
Cl^- added to assay buffer, (no preincubation with Cl^-)	620	120

reincorporation of Ca^{2+} into PSII has decreased the Cl^- K_M (the lower V_{MAX} values are a result of incomplete reconstitution of high affinity Cl^- binding). These samples therefore exhibit the "one site/two affinity" Cl^- binding reported by Lindberg, et al. (1996). It is significant that we cannot observe high affinity Cl^- binding in the dark in Ca^{2+} depleted samples; the Cl^- K_M appears to be about 300 mM (not shown

Discussion

Citrate-induced Ca^{2+} extraction from PSII is not an innocent process; at least 20% of centers are exposed to large reductants (data not shown) and exhibit rapidly exchangeable Ca^{2+} binding, a characteristic of salt-washed PSII preparations lacking the 23 and 17 kDa polypeptides (Table 1). Nevertheless, the remaining 80% of centers in this preparation remain intact, and as we show here, exhibit a poor affinity (high K_M) for Ca^{2+}. The reductant probing approach offers a facile method for assessing both the integrity of the OEC, as well as for examining the steric properties of access channels leading from the external medium to the Mn cluster. We have exploited this methodology to probe the consequences of Ca^{2+} extraction, using the citrate method to produce a sample that is minimally perturbed with respect to binding of the extrinsic polypeptides.

The data of Fig. 1 show that after Ca^{2+} removal, a pathway for NH_2OH and its N-methyl derivative is present in PSII that permits these reductants to access and destroy the Mn cluster. This pathway imposes steric restrictions on the reductants; N,N-dimethyl hydroxylamine is unable to inhibit H_2O oxidation under the incubation conditions employed for these experiments. This pathway is likely to be identical with that by which small inhibitory ligands like NH_3 as well as substrate H_2O gain access to the Mn cluster. The results

summarized in Fig. 2 and Table 2 show that the "open" conformation of PSII induced by Ca^{2+} extraction can be made to revert partially to a "closed" conformation by the simple expedient of restoring Ca^{2+} to its binding site.

Beyond its role in regulating the structure of the OEC (Mei and Yocum, 1991), Ca^{2+} has been proposed to act as a ligation site for Cl^- (Tommos and Babcock, 1998), for H_2O (Rutherford, 1989; Riggs-Gelasco, et al. 1996; Pecoraro, et al. 1998) or for ^-OH (Vrettos, et al., 2001a). In the absence of Ca^{2+}, Cl^- is unable to restore H_2O oxidation, even at very high concentrations (data not shown). This would not be observed if Ca^{2+} were necessary only to enhance Cl^- binding near the OEC. The alternate proposals view Ca^{2+} as a key component of H_2O oxidation chemistry, i.e., as a site for binding a substrate molecule that attacks a Mn=O species in the S_4 state (Vrettos, et al., 2001a). Our data are consistent with these proposals. As shown in Fig. 3, the topology of Ca^{2+} binding, as revealed by reductant probing, places the metal in a position where it is capable of binding substrate H_2O destined for oxidation by the PSII Mn cluster.

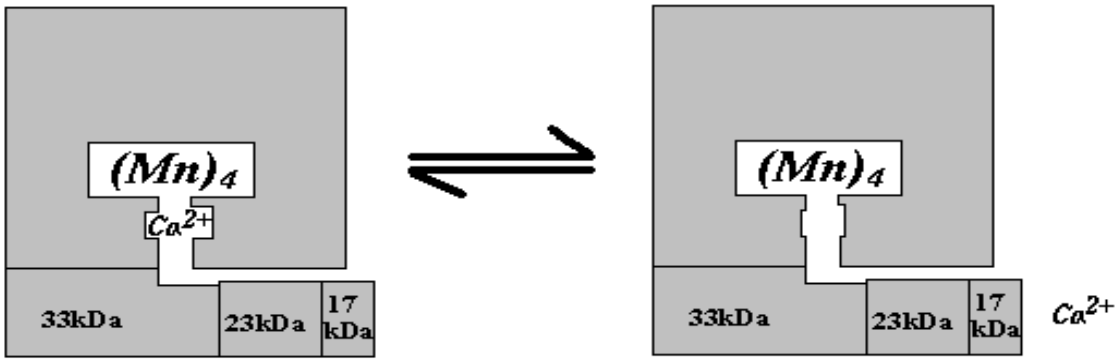

Fig 3. Schematic diagram of an OEC structure illustrating the topological/structural role of Ca^{2+} in blocking rapid access of small reductants to the PSII Mn cluster.

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References

Ghanotakis, D.F., Babcock, G.T. and Yocum, C.F. (1984a) *FEBS Lett.* 167, 127-130.
Ghanotakis, D.F., Babcock, G.T. and Yocum, C.F. (1984b) *Biochim. Biophys. Acta* 765, 388-398.
Lindberg, K., Andreasson, L.-E. (1996) *Biochemistry* **35**, 14259-14267.
Mei, R., Yocum, C.F. (1991) *Biochemistry* **30**, 7836-7842.
Ono, T.-A., Inoue, Y. (1988) *FEBS Lett.* **227,** 147-152.
Pecoraro, V.L., Baldwin, M.J., Caudle, M.T., Hsieh, W.-Y., Law, N.A. (1998) *Pure & Applied Chem.* **70,** 925-929.
Riggs-Gelasco, P., Mei, R., Ghanotakis, D.F., Yocum, C.F., Penner-Hahn, J.E. (1996) *J. Am. Chem. Soc.* **118**, 2400-2410.
Rutherford, A.W. (1989) *Trends Biochem. Sci.* 14, 227-232.
Tommos, C., Babcock, G.T. (1998) *Accts. Chemical Research* **31**, 18-25.
Vrettos, J.S., Limburg, J., and Brudvig, G.W. (2001a) *Biochim. Biophys. Acta* 1503, 229-245.
Vrettos, J.S., Stone, D.A., Brudvig, G.W. (2001b) *Biochemistry* (in press).