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Reconstruction of the water-oxidizing complex in manganese-depleted Photosystem II by using Schiff base manganese complexes

Mehmet Sayým Karacan*, Güler Somer

Department of Chemistry, Gazi University, 06500 Teknikokullar, Ankara, Turkey

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Abstract

The efficiency of Schiff base manganese complexes in reconstituting Photosystem II (PS II) electron flow and oxygen-evolution capacity was analyzed in PS II deprived of their manganese cluster and the extrinsic regulatory subunits. Three Schiff base complexes, mononuclear $Mn(salpn)_2$, binuclear $[Mn(salpn)]_2(ClO_4)_2$ and μ-oxo binuclear $[Mn(salpn)O]_2$, salpn: 1,3-bis(salicylideneamino)propane, were used for reconstruction of electron transport and oxygen evolution. Order of reconstruction effect is as follows: μ-oxo binuclear Mn(III) > binuclear Mn(III) > mononuclear Mn(II).

Keywords: Photosystem II; Schiff base; Water-oxidizing complex; Reconstruction of manganese-depleted

1. Introduction

The biological production of oxygen and subsequent metabolism of this molecule are among the most important classes of the reactions in the biosphere. So, photosynthetic evolution of oxygen has been the object of very intensive studies. Although significant progress has been achieved, the structure of the water oxidase and the nature of its protein matrix are not yet resolved [1]. However, it is clear that a functionally competent water-oxidizing complex (WOC) contains four manganese [2–6], and these four manganese centers are likely not to be functionally equivalent [7].

Recently, various manganese complexes have been synthesized and analyzed as possible models for the manganese cluster in the WOC [8–13]. Many of these complexes contain binuclear or tetranuclear manganese centers. It therefore appeared interesting to use synthetic binuclear manganese complexes for reconstitution experiments on the oxygen evolution capacity of Photosystem II (PS II).

In our previous work [14], the efficiency of synthetic binuclear complexes in reconstituting PSII was investigated. In this study, three salpn (salpn: 1,3-bis(salicylideneamino)-propane) Schiff base manganese complex is used for the reconstitution of the WOC. Order of reconstruction of electron transport and oxygen evolution is found to be as: mononuclear $Mn(II)(salpn)_2$ > binuclear $[Mn(III)(salpn)]_2(ClO_4)_2$ > μ-oxo binuclear $[Mn(III)(salpn)O]_2$.

2. Materials and methods

Chloroplasts were isolated from spinach leaves according to a procedure described by Whatley and Arnon [15]. PS II enriched preparations were obtained by treatment of chloroplasts with 0.4% digitonin and 0.15% Triton X-100 and centrifugation at 20 000 × *g* using a method described recently [16,17]. These PSII preparations, which are called as DT-20, exhibited O_2 evolution rates of 250–300 μmol/((mg of Chl) h) under saturating light and in the presence of 200 μM phenly-*p*-benzoquinone (Ph-*p*-BQ) plus 300 μM $K_3[Fe(CN)_6]$ as electron acceptor. The DT-20 preparations contained 80–100 Chl molecules per PSII or RC [5,17].

To manganese and the three extrinsic regulatory proteins with apparent molecular masses of 33, 24, and 18 kDa (on the basis of their encoding genes these polypeptides are now designated as PS II-O, PS II-P, and PS II-Q proteins, respectively) were extracted from DT-20 preparations, according to following procedure: was used: samples at 200 μg/ml Chl were incubated for 10 min at 2 °C in a suspension containing 20 mM *N*,*N*,*N*′,*N*′-tetra methylethylenediamine (TEMED), 0.5 M $MgCI_2$, and 20 mM MES–NaOH, pH = 6.5 [18]. After centrifugation at 20 000 × *g*, the pellet was washed twice in a buffer solution of 35 mM NaCl and 20 mM Tris–HCI, pH = 8.0. Photoactivation of the DT-20 samples deprived of

* Corresponding author. Tel.: +90-312-2126030-2789; fax: +90-312-2122279.
E-mail address: mkaracan@gazi.edu.tr (M.S. Karacan).

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their oxygen-evolving complexes was performed in the presence of Schiff base manganese complex and three or four cycles of continuous light ($\lambda > 600$ nm, $I = 55$ W/m^2, illumination of 30–60 s periods separated by 30–40 s of dark). The details of this procedure were described previously [17].

The chlorophyll content of the samples was determined according to [19]. The manganese content was assayed with an Philips PU 9285 flame atomic absorption spectrophotometer.

A single-beam differential spectrophotometer with a phosphoroscope similar to that described previously [16,20] was used for monitoring the light-induced changes of the fluorescence quantum yield at 682 nm. The suspension for fluorescence measurements contained differently treated DT-20 samples at 10 μg/ml Chl, 10 m MM NaCl, 2 mM $MgCI_2$, and 20 mM Tris–HCI, pH = 7.8.

The rate of oxygen evolution was measured with a Clark-type electrode in a 3 ml cell. The sample was illuminated by red light (KC 11 filter) passed through a heat filter consisting of a 5% $CuSO_4$ solution (light intensity at the cell surface ~100 W m^2). The assay mixture contained DT-20 preparations suspended in a buffer solution of 10 mM NaCl, 5 mM $CaCI_2$, 300 mM sucrose, 25 mM MES–NaOH, pH = 6.5, and 200 μM Ph-*p*-BQ/300 μM $K_3[Fe(CN)_6]$ as electron acceptor. Oxygen evolution capacity calculated approximately that recorder calibrated in same conditions as oxygen present and send away.

Manganese Schiff base complexes, $Mn(salpn)_2$ [21], $[Mn(salpn)]_2(ClO_4)_2$ [22] and $[Mn(salpn)O]_2$ [23], H_2salpn =*N*,*N*′-bis(salicylidene)-1,3-diaminopropane were obtained similar to literature procedure.

3. Results and discussion

In order to characterize the function of the samples reconstituted with different manganese Schiff base complexes, comparative measurements were performed with control and TEMED/$MgCl_2$-treated DT-20 preparations using fluorescence measurements and oxygen evolution.

3.1. Fluorescence measurements

Typical traces of the relative fluorescence quantum yield enhancement induced by illumination of different DT-20 preparations with actinic light (is shown in Fig. 1). In control samples (Fig. 1, trace 1), increase of the fluorescence yield is observed due to the light-induced reduction of Q_A. The extent of ΔF amounts to a 4–5-fold increase over the initial value, F_0. ΔF remains virtually invariant after addition of 50 μM EDTA to the control sample (see dotted curve). This

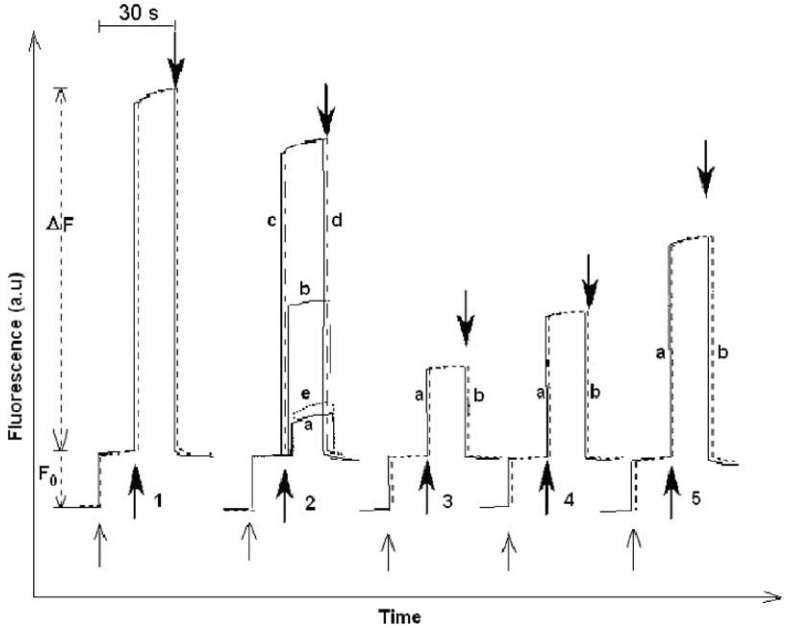

Fig. 1. Fluorescence changes with additions of $MnCl_2$ and Schiff base to Mn-depleted DT-20, ΔF, induced by illumination of DT-20 sample with actinic light. The worked suspension contained PS II preparations (10 μg/ml chlorophyll), 35 mM $MgCI_2$, and 25 mM Tris–HCI, pH = 7.8. The arrows at the bottom indicate the switching on of the modulated measuring light beam ($\lambda \cong 480$ nm, intensity $\cong 0.15$ W/m^2), bold arrows and at the top symbolize the switching on and off, respectively, of the actinic light ($\lambda > 600$ nm, intensity $\cong 100$ W/m^2). 1a: control DT-20 (solid line); 1b: presence of 50 μM EDTA (dashed line); 2a: manganese-depleted DT-20; 2b: the same as curve 2 with addition of 0.2 μM $MnCl_2$ (2 Mn/PS II); 2c: 0.4 μM $MnCl_2$ (4 Mn/PS II); 2d: 0.8 μM $MnCl_2$ (dashed curve) (8 Mn/PS II); 2e: presence of 50 μM EDTA; 3a: 0.2 μM monomeric $Mn(salpn)_2$ complexes addition to 2a (solid line) (2 Mn/PSII); 3b: presence of 50 μM EDTA(dashed line); 4a: 0.1 μM dimeric $[Mn(salpn)_2](ClO_4)_2$ complexes addition to 2a (solid line) (2Mn/PS II); 4b: presence of 50 μM EDTA (dashed line); 5a: 0.1 μM μ-oxo dimeric $[Mn(salpn)_2O](ClO_4)_2$ complexes addition to 2a (solid line) (4 Mn/PS II); 5b: presence of 50 μM EDTA (dashed line).

finding indicates that the chelator at this concentration does not affect the electron transport at the donor side of PS II; i.e., the binding of the functional manganese to the protein is resistant to complexation by external EDTA [24].

Mn-depleted DT-20 sample (Fig. 1, trace 2a) shows markedly different pattern, due to loss of manganese by the TEMED treatment, which deprived of their oxygen evolution capacity. In this case ΔF is largely reduced because of the very limited electron donor capacity. Addition of small amounts of $MnCl_2$ corresponding to about two Mn^{2+} per PS II (trace 2b) caused a significant restoration of ΔF. An almost complete recovery of ΔF can be achieved by addition of more than four Mn^{2+} per PS II (traces 2c and d). However, ΔF is highly susceptible to addition of EDTA (trace 2e, dotted curve), in contrast to the control sample. The EDTA effect shows that Mn^{2+} acts as electron donor of PS II without being tightly bound by the protein matrix. On the other hand, different feature was obtained when manganese Schiff base complexes were used (traces 3–5, Fig. 1) instead of $MnCl_2$. In this case, monomeric Mn(II) complex, dimeric Mn(II) complex and μ-oxo dimeric Mn(III) complex showed approximately 25, 40, 61% recovery of electron transfer, respectively (traces 3–5). Binuclear Mn(III) complex (trace 4) is more efficient than mononuclear Mn(II) complex (trace 3). Addition of EDTA virtually does not affect the extent of ΔF increase due to addition of Schiff base complexes (dotted curves of traces 3–5). Accordingly, manganese Schiff base compounds bind as manganese cluster to Mn-depleted DT-20 preparations thereby permitting an electron transport without getting disrupted by the erogenous chelator EDTA. More additions of the complexes to Mn-depleted DT-20 did not cause an increase extent of ΔF. And it is known that two manganese per PSII is sufficient for photo-induced electron transfer [3,14,20].

3.2. Oxygen evolution measurements

In Mn-depleted DT-20 preparations, the oxygen evolution capacity is virtually completely eliminated (Fig. 2, trace 1). A partial restoration of the activity can be achieved by photoactivation with $MnCl_2$ (Fig. 2, trace 2). Restoration of the activity with $Mn(salpn)_2$ complex is for nearly with $MnCl_2$ (Fig. 2, trace 3). Monomeric Mn(II) complex, dimeric Mn(II) complex and μ-oxo dimeric Mn(III) complex showed approximately 20, 37, 52% recovery of O_2 evolution capacity of PSII respectively (traces 3–5). As can be seen a more pronounced effect was obtained in the presence of the dimeric manganese (III) and μ-oxo dimeric manganese (III) complexes (traces 4 and 5, Fig. 2). The initial rate of O_2 evolution of μ-oxo binuclear Mn(III) complex (trace 5) is higher than that of binuclear Mn(III) complex (trace 4). It is shown that these compounds are more efficient than $MnCl_2$ in reconstituting O_2 evolution. All complexes showed maximum effect four manganese per PSII and more additions of complexes did not increase O_2 evolution capacity.

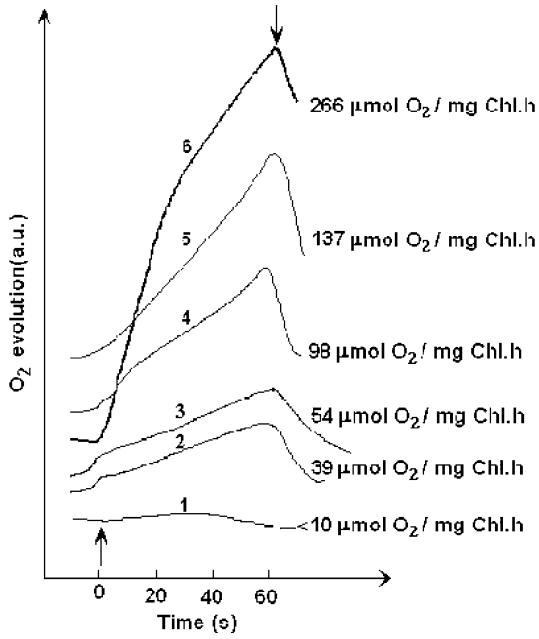

Fig. 2. Polarographic curves of oxygen evolution in DT-20 preparations with addition of Schiff base complexes. The assay suspension contained DT-20 samples (20 μg/ml chlorophyll), 10 mM NaCl, 5 mM $CaCl_2$, 300 mM sucrose, 50 μM EDTA, 25 mM MES–NaOH, pH = 6.5, and 0.2 mM Ph-*p*-BQ/0.3 mM $K_3[Fe(CN)_6]$ as electron acceptor. 1: Mn-depleted DT-20 sample; 2: Mn-depleted DT-20 sample reconstituted with 0.8 μM $MnCl_2$ (4Mn/PS II); 3: Mn-depleted DT-20 sample reconstituted with 0.4 μM Mn(II)$salpn_2$ (4Mn/PS II); 4: Mn-depleted DT-20 sample reconstituted with 0.4 μM Mn(III)$salpn_2$ (4Mn/PS II); 5: Mn-depleted DT-20 sample reconstituted with 0.4 μM μ-O-Mn(II-I)salpn (4Mn/PSII); 6: PS II.

4. Conclusion

Binuclear manganese complexes are very efficient PSII electron donors. For complexes used the order of reconstruction and efficiency of PS II electron donor is as follows: μ-oxo dimeric Mn(III) > dimeric Mn(III) > monomeric Mn(II). Donor capacity of manganese Schiff base complexes is not eliminated by complexation with EDTA; however, donor capacity of $MnCl_2$ is eliminated by addition of EDTA as shown by the fluorescence data presented in Fig. 1. This data indicates that binding of Schiff base complexes are rather strong and the site of manganese oxidation is able to interact with complexes containing comparatively bulky ligands, furthermore, oxidation number of the manganese is also important factor, because manganese(III) complexes are more efficient than that of Mn(II) complex. These results supported our previous work.

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References

[1] R.J. Debus, Biochim. Biophys. Acta 1102 (1992) 269–353.
[2] G.M. Cheniae, I.F. Martin, Biochim. Biophys. Acta 197 (1970) 219–239.
[3] V.V. Klimov, S.I. Allakhverdiev, V.A. Shuvalov, A.A. Krasnovsky, FEBS Lett. 148 (1982) 307–312.
[4] C.F. Yocum, C.T. Yerkes, R.E. Blankenship, R.R. Sharp, G.T. Babcock, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 7507–7512.
[5] S.I. Allakhverdiev, M.A. Shafiev, V.V. Klimov, Photobiochem. Photobiophys. 12 (1986) 61–65.
[6] G. Renger, M. Völker, H.J. Eckert, R. Fromme, S. Hohm-Veit, P. Graber, Photochem. Photobiol. 49 (1989) 97–105.
[7] G. Renger, Photosynth. Res. 38 (1993) 229–247.
[8] G. Christou, J.B. Vincent, Biochim. Biophys. Acta 895 (1987) 259–274.
[9] J.B. Vincent, G. Christou, Adv. Inorg. Chem. 33 (1989) 197–257.
[10] K. Wieghardt, Angew. Chem. Int. Ed. Engl. 28 (1989) 1153–1172.
[11] U. Bossek, T. Weyhermüller, K. Wieghardt, B. Nuber, J. Weiss, J. Am. Chem. Soc. 112 (1990) 6387.
[12] V.L. Pecoraro, in: V.L. Pecoraro (Ed.), Manganese Redox Enzymes, VCH Publishers, New York, 1992, pp. 197–231.
[13] W.H. Armstrong, in: V.L. Pecoraro (Ed.), Manganese Redox Enzymes, VCH Publishers, New York, 1992, pp. 261–286.
[14] S.I. Allakhverdiev, M.S. Karacan, G. Somer, N. Karacan, E.M. Khan, S.Y. Rane, S. Padhye, V.V. Klimov, G. Renger, Biochemistry 33 (1994) 12210–12214.
[15] F.R. Whatley, D.I. Arnon, Methods Enzymol. 6 (1963) 308–330.
[16] S.I. Allakhverdiev, Y.N. Kozlov, M.M. El-Sheekh, S. Demeter, V.V. Klimov, Biol. Membr. 9 (1992) 904–910.
[17] S.I. Allakhverdiev, V.V. Klimov, Z. Naturforsch. 47c (1992) 57–62.
[18] G. Ananyev, T. Wydrzynski, G. Renger, V.V. Klimov, Biochim. Biophys. Acta 1100 (1992) 303–311.
[19] D.I. Arnon, Plant Physiol. 24 (1949) 1–15.
[20] V.V. Klimov, S.I. Allakhverdiev, V.A. Shuvalov, A.A. Krasnovsky, Dokl. Akad. Nauk. SSSR 263 (1982) 1001–1007.
[21] C.P. Horwitz, P.J. Winslow, J.T. Warden, C.A. Lisek, Inorg. Chem. 32 (1993) 82–88.
[22] F.M. Ashmawy, J. Chem. Soc., Dalton Trans. (1985) 1391–1397.
[23] J.W. Gohdes, W.H. Armstrong, Inorg. Chem. 31 (1992) 368–373.
[24] T. Wydrzynski, G. Renger, Biochim. Biophys. Acta 851 (1986) 65–74.