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BRIEF COMMUNICATION

Reconstitution of the Mn-depleted photosystem 2 by using some manganese complexes

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Abstract

Restoration of electron flow and oxygen-evolution quantity of Mn-depleted photosystem 2 (PS2) was performed with using synthetic manganese complexes $Mn(im)_6Cl_2$, $Mn(im)_2Cl_2$, $Mn(5\text{-}Clsalgy)_2$, and $Mn(salgy)_2$ instead of original manganese cluster for reconstruction of electron transport and oxygen evolution.

Additional key words: chlorophyll fluorescence; oxygen evolution; *Spinacia.*

Biological water oxidation during plant photosynthesis represents a source of electrons for the photon-driven reactions of photosystem 2 (PS2) and leads also to the evolution of molecular di-oxygen. The oxygen-evolving complex (OEC) of the photosynthetic apparatus that catalyses this oxidation contains a cluster of four Mn atoms that acts as the locus of charge accumulation. Calcium ion is essential cofactor of this oxidation (Cheniae and Martin 1970, Yocum *et al.* 1981, Klimov *et al.* 1982a, Allakhverdiev *et al.* 1986, Renger *et al.* 1989, Debus 1992, Renger 1993). Much effort has been concentrated on elucidating the identity of the Mn site (Brudwig and de Paula 1987, Peloquin and Britt 2001, Robblee *et al.* 2001, Carrell *et al.* 2002). Ferreira *et al.* (2004) suggested cube-like Mn_3CaO_4 structure for the cluster.

Up to now, several cluster models containing binuclear or tetranuclear Mn centres have been synthesized as photosynthetic Mn clusters (Christou and Vincent 1987, Vincent and Christou 1989, Wieghardt 1989, Bossek *et al.* 1990, Armstrong 1992, Pecoraro 1992). So it is interesting to use synthetic Mn complexes for the reconstitution studies dealing with the oxygen evolution capacity and electron transport of Mn-depleted PS2.

In our former studies, efficiency of synthetic binuclear manganese complexes: dimeric $L_2Mn(III)Mn(III)L_2(OAc)_2$(M-2), dimeric [LMn(III)-O-Mn(III)$L(Oac)_2(H_2O)_2$](M-3) (Allakhverdiev *et al.* 1994), dimeric $[Mn(salpn)_2](ClO_4)_2$, and dimeric μ-oxo $[Mn(salpn)O]_2$ (Karacan and Somer 2004) were tested for reconstituting Mn-depleted PS2. In the present work, we compared four manganese(II) complexes, monomeric $Mn(im)_6Cl_2$, polymeric $Mn(im)_2Cl_2$, dimeric $Mn(5Clsalgy)_2$, and dimeric $Mn(salgy)_2$ in the reconstitution of the Mn-depleted PS2. The complexes were synthesized in our laboratory according to Chaudhury and Dash (1981) and Dutta and Ray (1977).

Chloroplasts were isolated from fresh and not blossoming spinach leaves according to Whatley and Arnon (1963). PS2-enriched preparations were obtained by treating chloroplasts with 0.4 % digitonin and 0.15 % *Triton X-100* and centrifuging at 20 000×*g* according to the method of Allakhverdiev *et al.* (1992) and Allakhverdiev and Klimov (1992). These PS2 preparations (called DT-20) exhibited O_2 evolution rates of 70–83 mmol kg^{-1}(Chl) s^{-1} under saturating irradiance and in the presence of 200 μM phenyl-*p*-benzoquinone (Ph-*p*-BQ) plus 300 μM $K_3[Fe(CN)_6]$ as electron acceptor. The DT-20 preparations contained 80–100 chlorophyll (Chl) molecules per PS or RC (reaction centre) (Allakhverdiev *et al.* 1986, Allakhverdiev and Klimov 1992). Ten μg Chl per cm^3 contains 1×10^{-7} M RC (Klimov *et al.* 1982a). Manganese and the three extrinsic regulatory proteins with apparent molecular masses of 33, 24, and 18 kDa (based on the encoding genes these polypeptides are now designated as PS2-O, PS2-P, and PS2-Q proteins, respectively) were extracted from DT-20 preparations according to the following procedure: Samples of 200 g(Chl) m^{-3} were incubated for 10 min at 2 °C in a suspension containing 20 mM N,N,N',N'-tetramethyl ethylenediamine (TEMED), 0.5 M $MgCl_2$, and 20 mM

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MES-NaOH, pH = 6.5 (Ananyev *et al.* 1992). 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 their oxygen-evolving complexes was performed in the presence of manganese complex and using three or four cycles of continuous irradiation (λ>600 nm, 55 W m^{-2}, 30–60 s periods of irradiation separated by 30–40 s of dark). The details of this procedure are described in Allakhverdiev and Klimov (1992). The Chl content of the samples was determined according to Arnon (1949). The Mn content was determined using a *Philips PU 9285* flame atomic absorption spectrophotometer.

A single-beam differential spectrophotometer with a phosphoroscope similar to that described previously (Klimov *et al.* 1982b, Allakhverdiev *et al.* 1992) 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 of 10 mg(Chl) m^{-3}, 10 mM NaCl, 2 mM $MgCl_2$, and 20 mM Tris-HCl, pH = 7.8.

The rate of oxygen evolution was measured in a 3-cm^3 cell with a hand-made Clark-type electrode. The sample was irradiated by red beam (*KC 11* filtre) passing through a heat filter formed by a 5 % (m/v) $CuSO_4$ solution (irradiance at the cell surface ~ 100 W m^{-2}). The assay mixture consisted of DT-20 preparations suspended

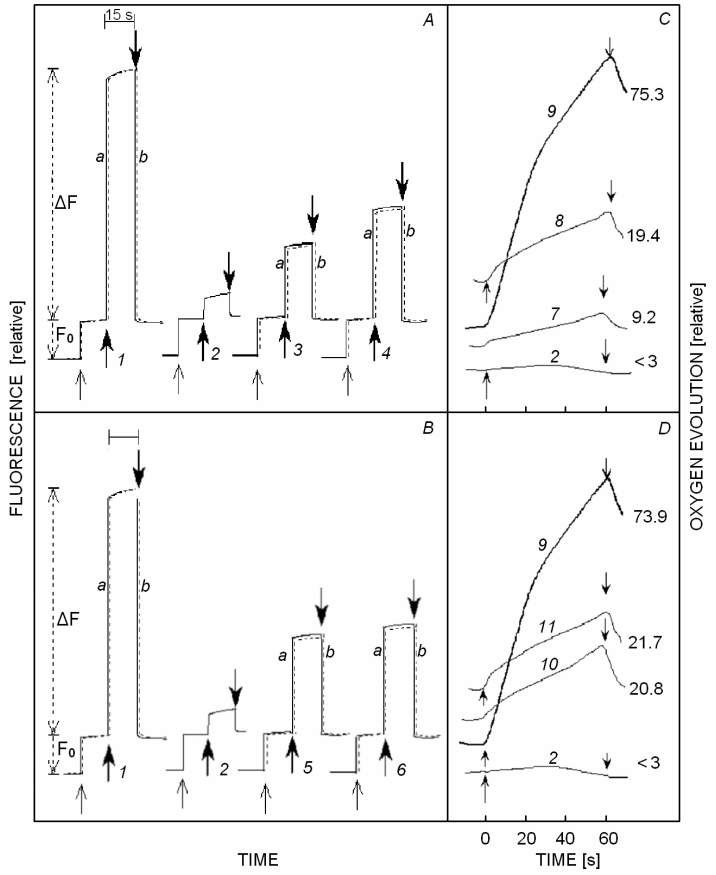

Fig. 1. Changes in fluorescence after "actinic light" irradiation (*A, B*) and oxygen evolution (*C, D*) in DT-20 preparations induced by addition of manganese complexes. The suspension contained PS2 preparations (*A, B*): [10 kg(Chl) m^{-3}], 35 mM $MgCl_2$, and 25 mM Tris-HCI, pH = 7.8; (*C, D*): [20 kg(Chl) m^{-3}], 10 mM NaCl, 5 mM $CaCl_2$, 300 mM sucrose, 50 μM EDTA, 25 mM MES-NaOH, pH = 6.5, 0.2 mM Ph-p-BQ, and 0.3 mM $K_3[Fe(CN)_6]$. (*A, B*): *The arrows at the bottom* indicate the switching on of the modulated measuring beam (λ ≅ 480 nm, irradiance ≅ 0.15 W m^{-2}), *bold arrows* symbolize the switching on and off of the "actinic light", respectively (λ >600 nm, irradiance ≅100 W m^{-2}). *1a*, control DT-20 (*solid line*); *1b*, presence of 50 μM EDTA (*dashed line*); *2*, manganese-depleted DT-20; *3a*, addition of 0.2 μM monomeric $Mn(im)_6Cl_2$ complexes to *2* (*solid line*) (2 Mn/PS2); *3b*, presence of 50 μM EDTA (*dashed line*); *4a*, 0.2 μM polymeric $Mn(im)_4Cl_2$ complexes added to *2* (*solid line*) (2 Mn/PS2); *4b*, presence of 50 μM EDTA (*dashed line*); *5a*, 0.1 μM dimeric Mn(5-Cl salgy)$_2$ complexes added to *2* (*solid line*) (2 Mn/PS2); *5b*, presence of 50 μM EDTA (*dashed line*); *6a*, 0.1 μM dimeric Mn(salgy)$_2$ complexes added to *2* (*solid line*) (2 Mn/PS2); *6b*, presence of 50 μM EDTA (*dashed line*); *7*, Mn-depleted DT20 sample reconstituted with 0.8 μM monomeric $Mn(im)_6Cl_2$ (4 Mn/PS2); *8*, Mn-depleted DT20 sample reconstituted with 0.8 μM polymeric $Mn(im)_2Cl_2$ (4 Mn/PS2); *9*, PS2; *10*, Mn-depleted DT20 reconstituted with 0.4 μM dimeric Mn(5-Clsalgy)$_2$ (4 Mn/PS2); *11*, Mn-depleted DT20 reconstituted with 0.4 μM dimeric Mn(salgy)$_2$ (4 Mn/PS2). Figures at curves in *C* and *D* give oxygen evolution [mmol(O_2) kg^{-1}(Chl) s^{-1}].

Table 1. Reconstruction of photosystem 2 (PS2) fluorescence ability and oxygen evolution capacity with manganese complexes. Maximum recovery is given for all complexes. [a] taken from Allakhverdiev *et al.* (1994); [b] taken from Karacan and Somer (2004); im: imidazole; salgy: salisilidenglisinato; 5Cl-salgy: 5-chloro salisilidenglisinato, salpn: [N,N-propylenbis(salisilidenaminato]; L: 2-hydroxy-1,4-naphthoquinone monoxime.

Manganese complexes		Approximate reconstruction of PS2 fluorescence [%]	oxygen evolution [%]
dimeric (M-3)[a]	[LMn(III)-O-Mn(III)L$(OAc)_2(H_2O)_2$]	95	78
	$MnCl_2$	86	21
dimeric (M-2)[a]	L_2Mn(III)Mn(III) $L_2(OAc)_2$]	73	47
dimeric	μ-oxo Mn(III) salpn[b], [salpn Mn(III)-O-Mn(III) salpn]	61	52
polymeric	Mn(II)$(im)_2Cl_2$	46	26
dimeric	Mn(II)$(salgy)_2$	45	29
dimeric	Mn(III)salpn [b], [(salpn)Mn(III)-Mn(III)(salpn)]ClO_4	40	37
dimeric	Mn(II)$(5Cl\text{-}salgy)_2$	40	28
monomeric	Mn(II)$(im)_6Cl_2$	30	12
monomeric	Mn(II)salpn[b], Mn(II)(salpn)	25	20

in a buffer solution of 10 mM NaCl, 5 mM $CaCl_2$, 300 mM sucrose, 25 mM MES-NaOH, pH = 6.5, and 200 μM Ph-*p*-BQ and 300 μM $K_3[Fe(CN)_6]$ as electron acceptor. Oxygen evolution capacity was calculated from the recorder calibration which was obtained under the same conditions when oxygen was present or absent.

In control samples (Fig. 1*A,B*, traces *1*), fluorescence yield increased due to the light-induced reduction of Q_A (primary plastoquinone acceptor of PS2). ΔF remained unchanged after addition of 50 μM EDTA to the control sample (see *dotted curve*). This and previous findings confirm that EDTA does not influence the electron transport at the donor side of PS2 as a chelator with manganese (Allakhverdiev *et al.* 1994, Karacan and Somer 2004). Mn-depleted DT-20 sample (Fig. 1*A,B*, trace *2*) showed only a negligible electron transport. When monomeric Mn$(im)_6Cl_2$ complex was used for reconstitution of electron transport in Mn-depleted DT-20 preparation, an almost 1/3 extent of ΔF was found (Fig. 1, trace *3*). Polymeric Mn$(im)_2Cl_2$ complex induced an approximately 46 % recovery of electron transfer (Fig. 1*A*, trace *4*). Other dimeric Mn $(5Clsalgy)_2$ and dimeric Mn$(salgy)_2$ complexes showed a 40 or 45 % recovery of electron transfer, respectively (Fig. 1*B*, traces *8* and *9*). Addition of EDTA did not affect the ΔF increase due to addition of Mn complexes (*dotted curves* in Fig. 1*B*, traces *5* and *6*). Accordingly, Mn compounds bind as Mn cluster to Mn-depleted DT-20 preparations thereby permitting an electron transport without getting disrupted by the exogenous chelator EDTA. More additions of the complexes to Mn depleted DT-20 did not cause an increase in ΔF. Two Mn atoms per PS2 were sufficient for photoinduced electron transfer (Fig. 1*A*, traces *3* and *4*) as also found in previous works (Klimov *et al.* 1982a,b, Allakhverdiev *et al.* 1994, Karacan and Somer 2004).

In Mn-depleted DT-20 preparations, the oxygen evolution capacity was eliminated (Fig. 1*C,D*, trace *2*). Monomeric Mn$(im)_6Cl_2$ and polymeric Mn$(im)_2Cl_2$ complexes induced an approximately 12 or 26 % recovery of O_2 evolution capacity of PS2, respectively (Fig. 1*C*, traces *7* and *8*). The rate of O_2 evolution for polymeric Mn$(im)_2Cl_2$ complex was higher than that for the monomeric complex, but both of them showed lower recovery of PS2. Restoration of the activity with dimeric Mn$(5\text{-}Cl(salgy)_2$ and Mn$(salgy)_2$ complexes showed 28 or 29 % recovery of O_2 evolution, respectively (Fig. 1*D*, traces *10* and *11*). Hence these compounds exhibited a similar recovery of O_2 evolution. All complexes showed maximum effect for four Mn atoms per PS2 and addition of further complexes did not increase the O_2 evolution capacity.

Reconstruction of PS2 fluorescence yield and oxygen evolution capacity of Mn complexes is given in Table 1. The comparison of reconstitution efficiency of the Mn complexes led to following results: (*a*) Bi-nuclear Mn complexes were more efficient than monomeric complexes in electron donation for PS2 and reconstitution of O_2 evolution. (*b*) Manganese(III) complexes were more efficient than Mn(II) complexes and the μ-oxo dimeric Mn(III) complexes were more efficient than the dimeric Mn(II) complexes. (*c*) Manganese complexes forming bulky ligands such as M-2, M-3, dimeric μ-O-Mn(III) salpn, Mn(III)salpn, and polymeric Mn(II)$(im)_4Cl_2$ were more efficient than Mn^{+2} (Mn aqua complex) and the others in electron donation for PS2 and restoring of O_2 evolution.

The OEC is a cube-like Mn_3CaO_4 cluster with each metal ion having three μ-oxo bridges connected to the fourth Mn ion by a mono-μ-oxo bridge in the extended region (Ferreira *et al.* 2004). This model explains the high efficiency of the dimeric μ-oxo Mn(III) complexes for the reconstitution of Mn-depleted PS2. According to this model, μ_3-oxo bridged Mn trimer (Limburg *et al.* 2001, Li *et al.* 2002, Yan 2003) plus Mn monomer complexes will be used in further studies.

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