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Photoconsumption of molecular oxygen on both donor and acceptor sides of photosystem II in Mn-depleted subchloroplast membrane fragments

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ABSTRACT

Oxygen consumption in Mn-depleted photosystem II (PSII) preparations under continuous and pulsed illumination is investigated. It is shown that removal of manganese from the water-oxidizing complex (WOC) by high pH treatment leads to a 6-fold increase in the rate of O_2 photoconsumption. The use of exogenous electron acceptors and donors to PSII shows that in Mn-depleted PSII preparations along with the well-known effect of O_2 photoreduction on the acceptor side of PSII, there is light-induced O_2 consumption on the donor side of PSII (nearly 30% and 70%, respectively). It is suggested that the light-induced O_2 uptake on the donor side of PSII is related to interaction of O_2 with radicals produced by photooxidation of organic molecules. The study of flash-induced O_2 uptake finds that removal of Mn from the WOC leads to O_2 photoconsumption with maximum in the first flash, and its yield is comparable with the yield of O_2 evolution on the third flash measured in the PSII samples before Mn removal. The flash-induced O_2 uptake is drastically (by a factor of 1.8) activated by catalytic concentration (5–10 μM, corresponding to 2–4 Mn per RC) of Mn^{2+}, while at higher concentrations (>100 μM) Mn^{2+} inhibits the O_2 photoconsumption (like other electron donors: ferrocyanide and diphenylcarbazide). Inhibitory pre-illumination of the Mn-depleted PSII preparations (resulting in the loss of electron donation from Mn^{2+}) leads to both suppression of flash-induced O_2 uptake and disappearance of the Mn-induced activation of the O_2 photoconsumption. We assume that the light-induced O_2 uptake in Mn-depleted PSII preparations may reflect not only the negative processes leading to photoinhibition but also possible participation of O_2 or its reactive forms in the formation of the inorganic core of the WOC.

1. Introduction

Photosynthetic water oxidation is a fundamental biological process and the main source of both electrons (which are used for fixation of CO_2 at photosynthesis) and molecular oxygen in atmosphere. This process takes place in a pigment–protein complex called photosystem II (PSII). PSII can be divided into two basic functional blocks: (1) the photochemical reaction centre (RC) where light energy absorbed by chlorophyll is transformed into the energy of separated charges, and where the strongest biological oxidant, $P_{680}^{+\cdot}$, the oxidized primary electron donor of PSII (with the redox potential of 1.1–1.27 V [1–3]) is formed, and (2) the water-oxidizing complex (WOC) which contains a Mn_4Ca cluster repeatedly oxidized by $P_{680}^{+\cdot}$. The WOC is oxidized during the sequential absorption of photons and charge separation in PSII. As a result, intermediate S-states (S_0, S_1, S_2, S_3, and S_4) are formed, and the transition from S_4 to S_0 is accompanied by the oxidation of two molecules of water with the formation of O_2.

Interaction of molecular oxygen with electron carriers of PSII leads to the formation of reactive oxygen species: singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$). Singlet oxygen in PSII is mainly generated through the interaction of triplet-state chlorophyll, ^{3}Chl*, with O_2. In turn, ^{3}Chl* is formed in the RC of PSII when the system lacks photochemically active electron acceptors, which facilitates charge recombination in the primary ion–radical pair of PSII [$P_{680}^{+\cdot}Pheo^{-\cdot}$] and results in the formation of $^3P_{680}$* [4,5]. Superoxide anion radicals are produced in PSII through the one-electron reduction of O_2 by low-redox-potential reduced electron acceptors of PSII. It is a common assumption that the reduced primary electron acceptor, $Pheo^{-\cdot}$, both primary and secondary quinone electron acceptors, Q_A^{-} and Q_B^{-}, are the main sites for superoxide generation in PSII [6]. There is also evidence that electrons can be transferred to O_2 from the plastoquinone pool and cytochrome b_{559} [7–9]. Spontaneous or SOD-catalyzed dismutation of $O_2^{-\cdot}$ results in the production of H_2O_2. Hydrogen peroxide formation on the acceptor side of PSII was shown by chemiluminescence method with the use of a luminol-peroxidase assay [10]. It was shown that hydrogen peroxide could also be formed

Abbreviations: PSII, photosystem II; RC, reaction centre; WOC, water-oxidizing complex; Cyt b_{559}, cytochrome b_{559}; Pheo, pheophytin–the primary electron acceptor of PSII; P_{680}, the primary electron donor of PSII; Q_A, the primary plastoquinone electron acceptor of PSII; Q_B, the secondary plastoquinone electron acceptor of PSII; TyrZ, redox active tyrosine residue of D1 protein; Diuron, 3-3,4-dichlorophenyl-1,1-dimethylurea; DPC, diphenylcarbazid; DCBQ, 2,6-dichloro-p-benzoquinone; ΔF, photoinduced changes of chlorophyll fluorescence yield of PSII

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on the donor side of PSII (as a result of two-electron oxidation of water) after WOC modification [10–13]. Hydroxyl radical can be the result of the reduction of H_2O_2 by low-valent transition metals (Fenton reaction). Detailed information on the formation of reactive oxygen species in PSII is presented in a recent review [6].

PSII becomes very sensitive to photoinactivation after damage to the WOC when the electron flow from water cannot compete with the electron withdrawal on the acceptor side. In this case, the donor side mechanism of photoinhibition predominates and the damage to PSII is caused by the long-lived states of $P_{680}^{+\cdot}$ and $TyrZ^{\cdot}$ which can oxidize chlorophyll, carotenoids and amino acids [14–16]. This is supported by the fact that the addition of exogenous electron donors considerably suppresses the rate of photoinhibition in Mn-depleted PSII preparations [14,16].

Previously we found increase in of O_2 photoconsumption in PSII preparations at high pH [17]. Based on the study of the effect of diuron, catalase, exogenous electron donors and acceptors of PSII and traps of reactive oxygen species, we suggested that oxygen photoconsumption could be caused by two processes: 1) reduction of O_2 to $O_2^{-\cdot}$ on the acceptor side and 2) the interaction of O_2 with organic radicals as a result of oxidative activity of $P680^{+\cdot}$. In this paper we demonstrate that O_2 photoconsumption does occur on the donor side of PSII; that it is characterized by high quantum yield, and activated by exogenous Mn^{2+} added at catalytic (equivalent to 2–4 Mn per PSII reaction centre) concentrations.

2. Materials and methods

The oxygen-evolving subchloroplast membranes enriched in PSII from spinach leaves were isolated as described previously in [18]. The PS II membranes (2 mg of Chl/mL) were suspended in a medium containing 20 mM Mes–NaOH (pH 6.5), 35 mM NaCl, 0.33 M sucrose and 10% glycerol, and stored at − 76 °C. PS II membranes deprived of Mn were obtained by high pH treatment as described earlier in [19]. The concentration of chlorophyll was assayed in 80% acetone [20].

The rate of the light-induced evolution and consumption of O_2 was measured by monitoring the concentration of O_2 using a Clark-type oxygen electrode for 60 s after the start of continuous saturating actinic illumination ($\lambda > 650$ nm, 1400 μmol photon s^{-1} m^{-2}). The measurements were carried out at 25 °C and 20 μg Chl/mL.

Polarographic detection of O_2 consumption/evolution upon the illumination of PSII preparations by series of saturating light flashes was made using a Clark-type Pt/Ir electrode (diameter 5.5 mm) equipped with a polymer membrane stretched to a thickness of about 1 μm [11,21]. The membrane prevented added electron acceptors and donors from interacting with the polarized electrode. At Chl concentration of 500 μg/mL, the samples were layered into a chamber [22] with 20 μL volume and 0.3 mm thick over the membrane. The electrode was operated at a polarization voltage of 700 mV. Before these measurements, the samples were dark-polarized for 10 min on the covered electrode at 25 °C. The saturating light flashes were made with a xenon Hamamatsu L4633 flash lamp.

The kinetics of photoinduced changes of chlorophyll fluorescence yield (ΔF) were measured in a 10-mm cuvette at room temperature by using a Waltz XE-PAM fluorometer. Actinic light (passed through a BG-39 filter) travelled to the cuvette (10 × 10 mm) in an optical unit through special fiberoptics.

3. Results

3.1. Oxygen consumption under continuous illumination of Mn-depleted PSII preparations

Illumination of untreated PSII preparations in the presence of exogenous electron acceptors (100 μM DCBQ and 1 mM $K_3[Fe(CN)_6]$) resulted in oxygen evolution at the rate of 520 μmol O_2 (mg Chl)$^{-1}$ h^{-1}; meanwhile, oxygen photoconsumption was insignificant (less than 2 μmol O_2 (mg Chl)$^{-1}$ h^{-1}, Fig. 1, Curve 1) in the absence of exogenous electron acceptors.

The removal of Mn and other WOC components (Ca^{2+}, extrinsic proteins) led to a 6-fold increase in the rate of oxygen photoconsumption (up to 12 μmol O_2 (mg Chl)$^{-1}$ h^{-1}, Fig. 1, Curve 2). The inhibition of electron transport between the primary and secondary quinone electron acceptors, Q_A and Q_B, (accompanied by the acceleration of charge recombination within PSII reaction centres) by the addition of 20 μM diuron led to almost complete suppression of O_2 photoconsumption. This demonstrates that O_2 uptake is linked to electron transport in PSII (Fig. 1, Curve 3).

Oxygen uptake caused by electron transfer on the acceptor side of PSII is basically related to the reduction of O_2 to superoxide anion radical which then can be disproportionated to H_2O_2 and O_2. In this case, the reduced electron acceptors of PSII are considered to be a major source of superoxide generation. Fig. 1 demonstrates (Curve 4) that the addition of DCBQ jointly with potassium ferricyanide (a couple that very efficiently takes electrons from the reduced quinone electron carriers in PSII) results in a 30% suppression of the rate of O_2 photoconsumption. A much stronger (70–75%) inhibition of O_2 photoconsumption is induced by the addition of an exogenous electron donor to PSII, 0.5 mM ferrocyanide (Fig. 1, Curve 5), which demonstrates a strong contribution of the donor side of PSII to O_2 photoconsumption. Upon the joint addition of the artificial electron

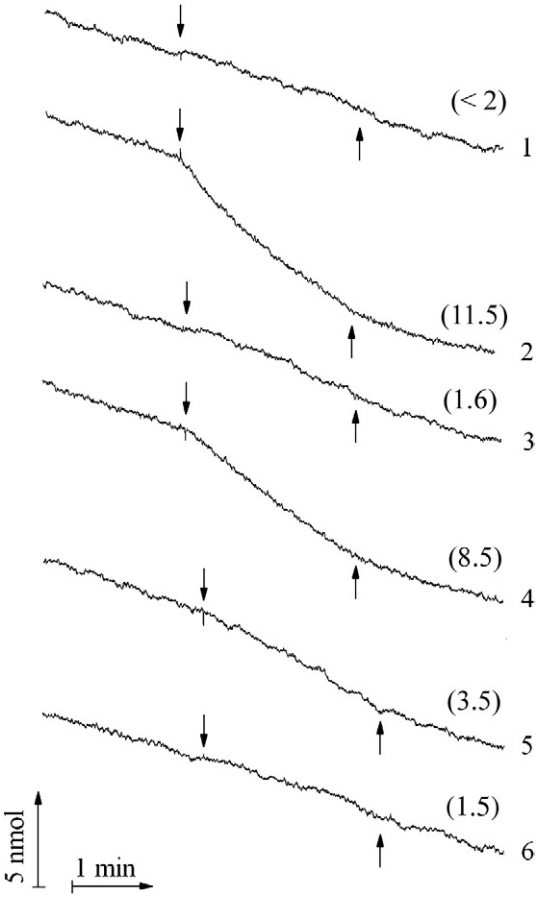

Fig. 1. Kinetics of oxygen photoconsumption in PSII preparations before (1) and after (2–6) removal of Mn. The measurements were made in the medium containing 50 mM MES (pH 6.5) and 35 mM NaCl in the absence (1–2) and in the presence (3–6) of additions: 20 μM DCMU (3), 100 μM DCBQ and 1 mM $K_3[Fe(CN)_6]$ (4), 500 μM $K_4[Fe(CN)_6]$ (5), 100 μM DCBQ, 1 mM $K_3[Fe(CN)_6]$ and 500 μM $K_4[Fe(CN)_6]$ (6). The parenthesized values above the curves show the rate of oxygen consumption (μmol O_2 (mg Chl)$^{-1}$ h^{-1}). ↓ and ↑ — light ($\lambda > 650$ nm, 1400 μmol photon s^{-1} m^{-2}) on and off, respectively.

acceptors and donor, the light-induced O_2 uptake was completely inhibited, and the rate of O_2 uptake became comparable to that observed in the PSII preparations before Mn removal (Fig. 1, Curve 6).

Fig. 2 A shows the rate of O_2 photoconsumption versus the concentration of ferricyanide. In the presence of 1 to 10 μM ferricyanide, 30-% inhibition of the O_2 photoconsumption took place. The increase of ferricyanide concentration to 400 μM–1 mM did not lead to further suppression of light-induced O_2 uptake. When we used a more efficient electron acceptor for PSII, DCBQ, O_2 photoconsumption was dramatically suppressed: at a concentration of 2 μM DCBQ, the rate of O_2 uptake decreased by more than 60%. The addition of 1 mM potassium ferricyanide (which supports DCBQ in the oxidized state) markedly reduced the inhibitory effect of DCBQ to the level observed in the presence of a saturating ferricyanide concentration alone (Fig. 2B). The concentration dependence of this effect is presented in Fig. 2C. One of possible explanations of this effect may be that DCBQ taking an electron from the reduced species in PSII may then donate it to $P_{680}^{+\cdot}$ or $TyrZ^{\cdot}$ (and it can induce the inhibition of O_2 photoconsumption like an electron donor), while ferricyanide prevents this process through the oxidation of the reduced DCBQ. Thus, these experiments have shown that the prevention of electron transfer to O_2 by adding artificial electron acceptors does not completely suppress O_2 photoconsumption. The rate of O_2 uptake in the presence of electron acceptors remains rather high (about 70% of the initial level), and it is, in turn, inhibited by electron donors and may be attributed to the donor side of PSII.

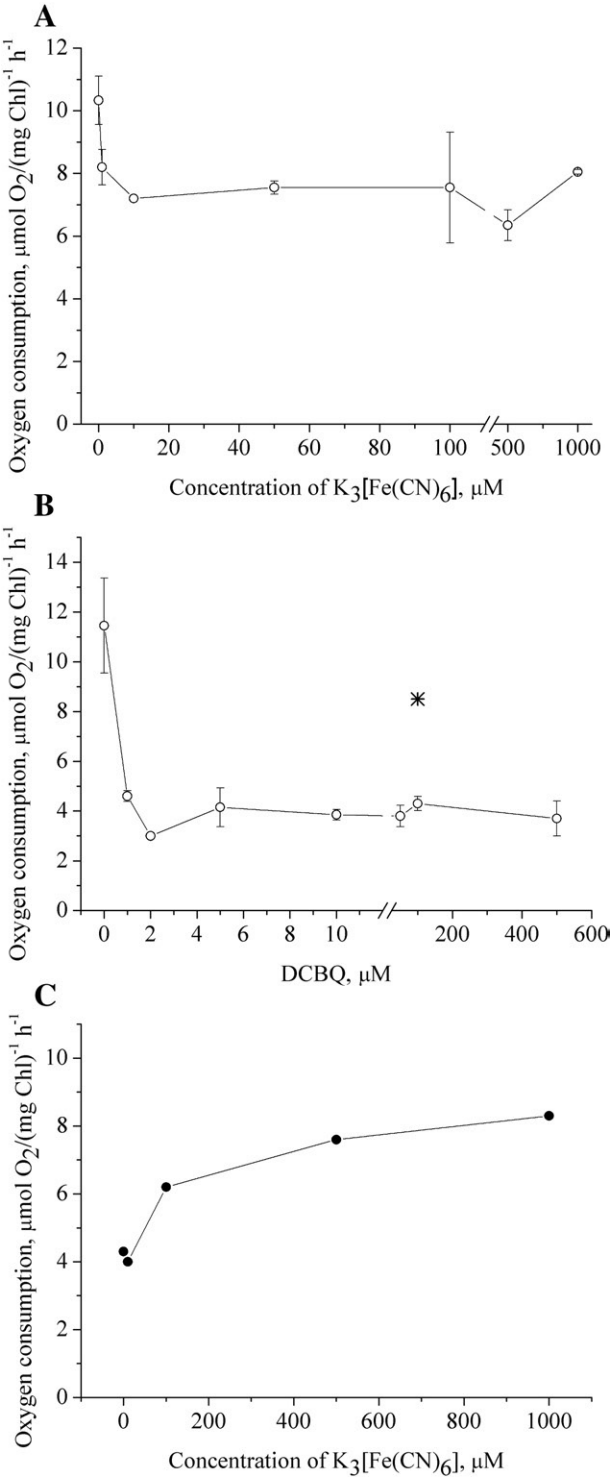

Fig. 2. Dependence of the O_2 photoconsumption rate under the continuous illumination of Mn-depleted PSII preparations on the concentration of exogenous electron acceptors: $K_3[Fe(CN)_6]$ (A), DCBQ (B), $K_3[Fe(CN)_6]$ in the presence of 100 μM DCBQ (C). The measurements were done in a medium containing 50 mM MES (pH 6.5) and 35 mM NaCl at 20 μg Chl/mL. (*) in Fig. 2B shows the O_2 photoconsumption rate in the presence of 100 μM DCBQ and 1 mM $K_3[Fe(CN)_6]$.

To show the correlation between the suppression of O_2 photoconsumption induced by the addition of electron donors and the restoration of electron donation to the RC of PSII, we compared the effect of exogenous electron donors such as ferrocyanide and DPC (as well as Mn^{2+} which is used for the formation of a functionally active manganese cluster during the procedure of photoactivation [21–25]) on these photoprocesses. The restoration of electron donation to PSII reaction centers with exogenous electron donors was demonstrated from the measurements of the photoinduced changes of chlorophyll fluorescence yield (ΔF) of PSII related to the photoreduction of the primary electron acceptor, Q_A. It is known that the removal of manganese from the PSII membranes leads to a drastic decrease in photoinduced ΔF, since these preparations are incapable of Q_A^- photoaccumulation because electrons are no longer donated from the Mn-containing WOC to PSII reaction center. As exogenous electron donors are added, photoinduced ΔF is largely restored as a result of increase in electron flow to PSII reaction center [26]. Fig. 3 shows the restoration of ΔF in Mn-depleted PSII preparations. It increases as ferrocyanide concentration grows; the maximum reactivation of ΔF is observed at 0.5 mM $K_4[Fe(CN)_6]$ (Fig. 3, Curve 6).

Fig. 4 shows a detailed comparison of the effect of exogenous electron donors on the rate of light-induced O_2 uptake (Curves 1) and on the restoration of photoinduced ΔF (Curves 2). As shown in the figure, the inhibition of O_2 photoconsumption in Mn-depleted PSII preparations was observed at concentrations of exogenous electron donors which were efficient for the restoration of ΔF. The maximum decrease in the rate of light-induced O_2 uptake was 70%, 57% and 65% upon the addition of saturating concentrations of electron donors $K_4[Fe(CN)_6]$, DPC and $MnCl_2$, respectively (Fig. 4). One interpretation of these data is that 60% to 70% of oxygen photoconsumption in Mn-depleted preparations occurs on the donor side of PSII.

3.2. Oxygen consumption under illumination of Mn-depleted PSII preparations by a series of saturating microsecond light flashes

The illumination of the untreated O_2-evolving PSII preparations by a series of saturating microsecond light flashes in the presence of exogenous electron acceptor, potassium ferricyanide, led to the flash-induced O_2 evolution with a typical period of four oscillations; maximum of O_2 was observed upon the third flash (while the first flash resulted in neither evolution nor consumption of O_2, Fig. 5, Curve 1). The signal relatively slowly relaxed after a maximum caused by a light flash because the electrode was coated with a 1 μm polymer membrane which, as had been indicated earlier [27,28], slows down the diffusion of O_2 to the electrode. After the removal of Mn from the WOC

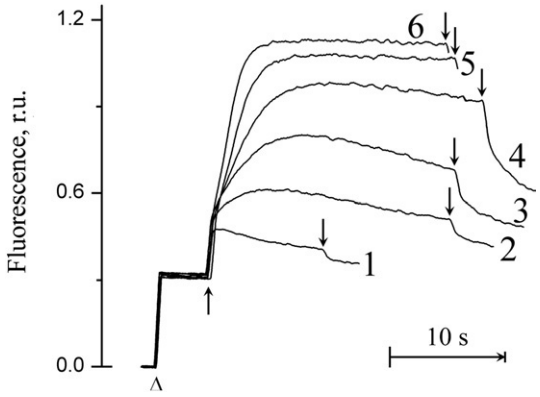

Fig. 3. Kinetics of photoinduced changes of chlorophyll fluorescence yield (ΔF) related to the photoreduction of the primary quinone acceptor, Q_A, in Mn-depleted PSII preparations in the absence (1) and in the presence (2–6) of $K_4[Fe(CN)_6]$: 5 μM (2), 10 μM (3), 50 μM (4), 100 μM (5), 500 μM (6). Δ — switching of the measuring light; ↑ and ↓, actinic light (λ > 600 nm, 450 μmol photon s^{-1} m^{-2}) on and off, respectively.

only O_2 photoconsumption was observed. It was maximal on the first light flash, and the amplitude of the signal was unexpectedly high: it was comparable with the yield of O_2 observed in untreated O_2-evolving PSII preparations on the third light flash in the presence of an electron acceptor (Fig. 5, Curves 1 and 2). The data indicate that O_2 uptake caused by the illumination of Mn-depleted PSII preparations by microsecond saturating light flashes is characterized by a very high efficiency comparable with that of O_2 evolution in untreated PSII preparations. The addition of 20 μM diuron almost completely suppressed the flash-induced consumption of O_2 (Curve 3).

As can be seen (Fig. 5, Curve 2, 4), O_2 photoconsumption is followed by an inversion of the signal after Mn-depleted PSII preparations are illuminated by saturating microsecond light flashes. This phenomenon can be interpreted in two ways. Either it is due to the decomposition of a product (which could be formed as a result of the illumination of the Mn-depleted PSII preparations) and release of O_2, or to slow molecular oxygen diffusion from the medium to the electrode surface. To clarify this point, we measured flash-induced O_2 uptake using chemical trapping techniques in which histidine reacts with $^1O_2$ to form an oxygenation product, $HisO_2$ [29]. Rose bengal (RB) was used as a photosensitizer for the production of $^1O_2$. It was found that flash-induced O_2 uptake in RB-His assay was followed by an inversion of the signal with kinetics similar to that observed in the experiments with Mn-depleted PSII preparations (data not shown). The result can indicate that the apparent O_2 "evolution" observed after the flash-induced O_2 uptake in Mn-depleted PSII preparations is related to O_2 diffusion to the electrode surface from the medium.

The use of Clark-type Pt/Ir electrode equipped with a special polymer membrane allowed us to investigate the effect of exogenous electron acceptors and donors on the flash-induced O_2 uptake. Fig. 6A (Curve 1) shows the effect of DCBQ on flash-induced O_2 uptake. The addition of 10 μM DCBQ led to a 35% inhibition of flash-induced O_2 consumption; the effect was saturated at DCBQ concentrations above 10 μM. The addition of an exogenous electron donor, potassium ferrocyanide, resulted in a 70%–80% suppression of flash-induced O_2 uptake with 75% efficiency at 10 μM $K_4[Fe(CN)_6]$ (Fig. 6A, Curve 2). A similar effect was observed upon the addition of another exogenous electron donor, DPC: at a saturating concentration of DPC (100 μM — 200 μM), the inhibition of flash-induced O_2 uptake reached 65% to 70% (data not shown). Joint addition of an exogenous electron donor (10 μM ferrocyanide) and acceptor (100 μM DCBQ) resulted in a complete inhibition of flash-induced O_2 uptake (data not shown) indicating that O_2 photoconsumption is due to a redox interaction of O_2 with PSII components. Surprisingly, (in contrast to $K_4[Fe(CN)_6]$ and DPC), the addition of Mn^{2+} (from 2 μM to 100 μM) led to a substantial

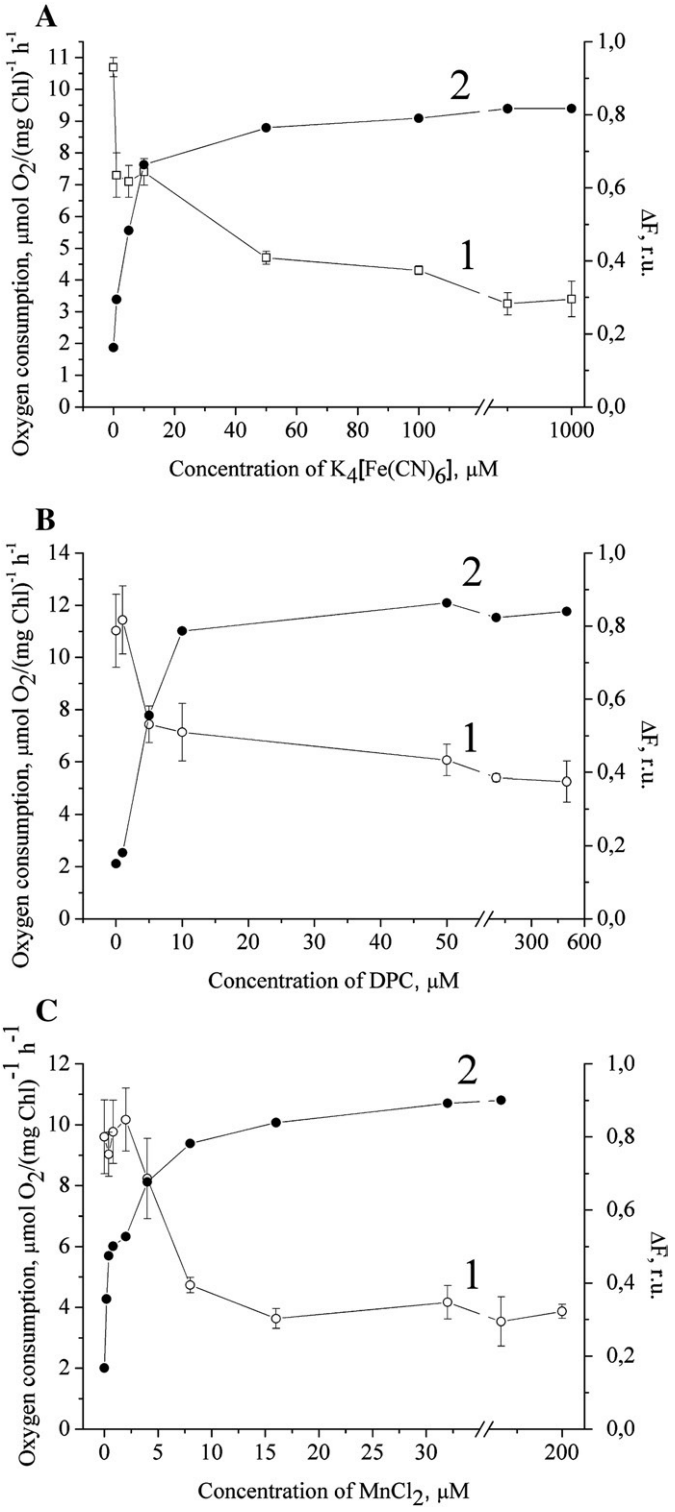

Fig. 4. Comparison of the effect of exogenous electron donors $K_4[Fe(CN)_6]$ (A), DPC (B) and $MnCl_2$ (C) on the O_2 photoconsumption rate (1) and the photoinduced ΔF (2) in Mn-depleted PSII preparations under continuous illumination. The measurements of the O_2 consumption and the photoinduced ΔF were done in a medium containing 50 mM MES (pH 6.5) and 35 mM NaCl at 20 μg Chl/mL and 10 μg Chl/mL, respectively.

activation of flash-induced O_2 uptake, with the maximum effect at 5–10 μM $MnCl_2$ (corresponding to 2–4 Mn per one PSII reaction centre, Fig. 6 B). This effect was most pronounced on the first light flash — O_2 consumption increased by 85% (Fig. 5, Curve 4) upon the addition of 10 μM $MnCl_2$. As we replaced Mn^{2+} by Mg^{2+} (or Ca^{2+}), no activation of

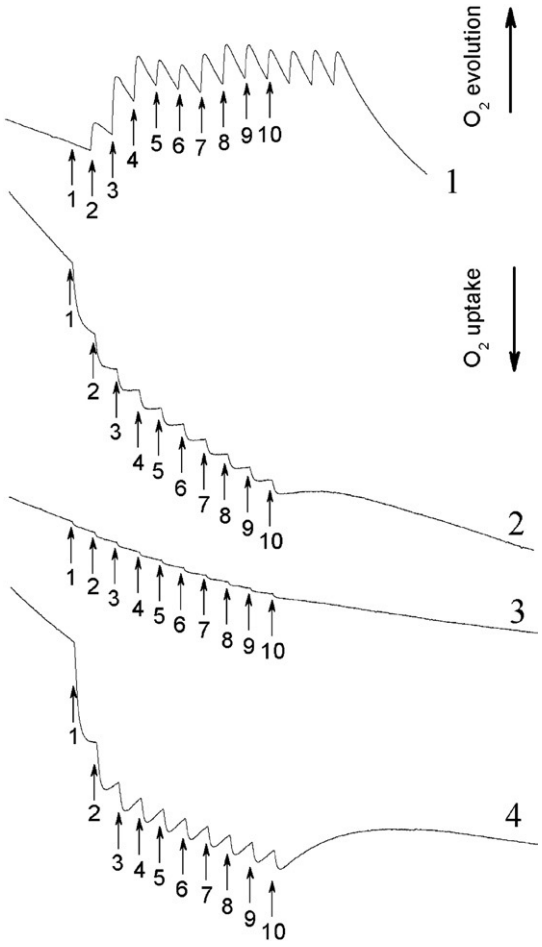

Fig. 5. Original traces of flash-induced oxygen evolution and consumption in PSII preparations before (1) and after (2–4) Mn removal. The measurements were made in a medium containing 50 mM MES (pH 6.5) and 35 mM NaCl in the absence of any additions (Trace 2) and in the presence of 1 mM $K_3[Fe(CN)_6]$ (Trace 1), 20 μM DCMU (Trace 3) and 10 μM $MnCl_2$ (Trace 4) (corresponding to 4 Mn per PSII reaction center) at a Chl concentration of 500 μg/mL. ↑ indicates the light flash (figures under the arrows indicate the flash number).

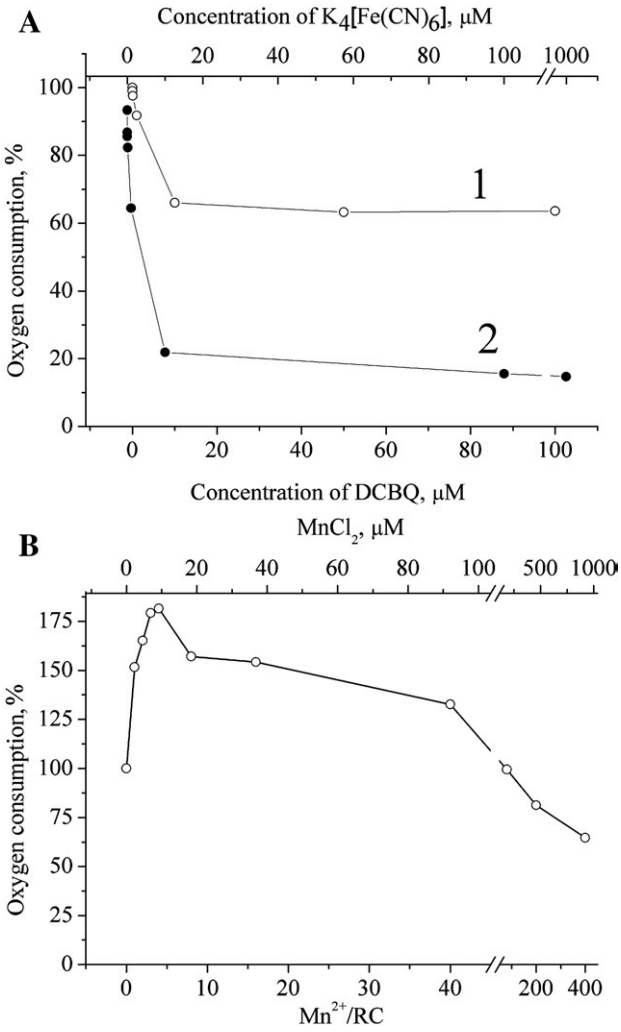

Fig. 6. (A) Dependence of flash-induced oxygen consumption (induced by the first flash) in Mn-depleted PSII preparations (see Fig. 5, Curve 2) on the concentration of DCBQ (1) and $K_4[Fe(CN)_6]$ (2). The measurements were done in a medium containing 50 mM MES (pH 6.5) and 35 mM NaCl at a Chl concentration of 500 μg/mL. (B) Dependence of flash-induced oxygen consumption (the signal was induced by the first flash) in Mn-depleted PSII preparations on the $MnCl_2$ concentration. The measurements were done in a medium containing 50 mM MES (pH 6.5) and 35 mM NaCl at a Chl concentration of 500 μg/mL. 100% is the flash-induced O_2 consumption in Mn-depleted PSII preparations in the absence of additions.

flash-induced O_2 uptake was observed (data not shown). Diuron (20 μM) almost completely inhibited flash-induced O_2 uptake both in the absence and presence of $MnCl_2$. As the $MnCl_2$ concentration grew above 100 μM, the activation was followed by an inhibition of flash-induced O_2 uptake (as in the presence of other regular electron donors).

Fig. 7A shows that pre-illumination of Mn-depleted PSII preparations by continuous light (which is known to lead to the irreversible loss of the capability of PSII donor side to be reactivated by Mn^{2+} [14]) results in the suppression of photoinduced ΔF reactivation by exogenous Mn^{2+}. The pre-treatment also inhibits flash-induced O_2 consumption, leads to a considerable loss of the effect of added Mn^{2+} on flash-induced O_2 consumption, and suppresses both effects of Mn^{2+} (activation at low Mn^{2+} concentrations and inhibition at higher Mn^{2+} concentrations, Fig. 7B).

4. Discussion

The results clearly show that in PSII not containing the WOC, light-induced O_2 uptake on the donor side of PSII takes place along with the well-known effect of O_2 photoconsumption related to the reduction of O_2 on the acceptor side of PSII. This conclusion is based on the fact that light-induced O_2 uptake is suppressed as both exogenous electron acceptors and donors are added to PSII, with the effect of the donors two times higher than that of the acceptors. Whereas O_2 photoconsumption induced by the reduction of O_2 on the acceptor side of PSII is a thoroughly investigated phenomenon, light-induced O_2 uptake related to the donor side of PSII is still largely obscure.

One can assume the following possible mechanism for the O_2 photoconsumption on the donor side of PSII. The loss of electron donation from water to the RC of PSII leads to the oxidation of organic molecules (R) by cation-radical $P_{680}^{+\cdot}$ or $TyrZ^{\cdot}$, and, consequently, to the formation of organic radicals, $R^{\cdot}$. (The illumination of PSII preparations lacking a WOC is known to result in the formation of the long-lived cation-radical $P_{680}^{+\cdot}$ which, in turn, may produce $TyrZ^{\cdot}$ as well as oxidize His [30,31], carotenoids and accessory chlorophyll, Chl_Z, [16,32] (see also Supplementary materials), which would produce corresponding radicals. The photobleaching of carotenoids in the presence of electron acceptor, 3,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, is more pronounced in the presence of molecular oxygen [16]). It is known that organic radicals can interact with O_2 to form corresponding peroxyl radicals, $ROO^{\cdot}$ [33,34], which are then transformed into hydroperoxides, ROOH, as a result of protonation (Reaction (1)). Previously, we attributed

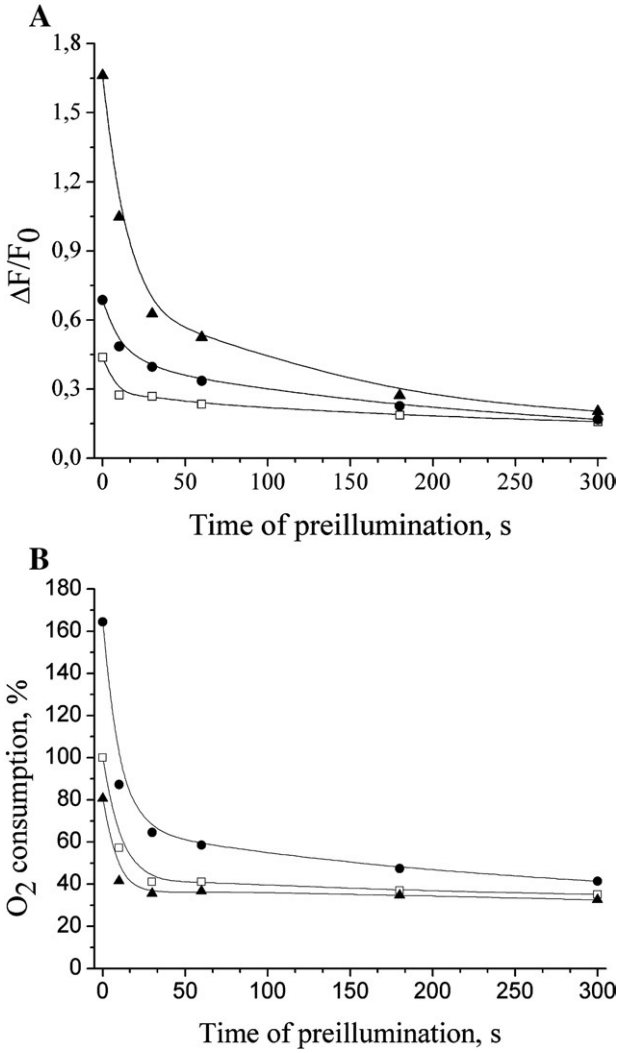

Fig. 7. Effect of inhibitory pre-illumination on the photoinduced ΔF (A) and the flash-induced O_2 uptake (B) in Mn-depleted PSII preparations. Lines in figure (A) represent the value of photoinduced ΔF in the absence (□) and in the presence of 0.2 μM $MnCl_2$ (●) and 20 μM $MnCl_2$ (▲), which corresponds to 4 and 400 Mn per PSII reaction centre, respectively. Lines in figure (B) represent flash-induced O_2 uptake (the signal was induced by the first flash) in the absence (□) and in the presence of 10 μM $MnCl_2$ (●) and 1 mM $MnCl_2$ (▲), which corresponds to 4 and 400 Mn per PSII reaction centre, respectively. The pre-illuminations of Mn-depleted PSII preparations were done by continuous light (λ > 650 nm, 900 μmol photon s^{-1} m^{-2}) in a medium containing 50 mM MES (pH 6.5) and 35 mM NaCl in the absence of additions at a chlorophyll concentration of 500 μg/mL. The measurements of flash-induced O_2 uptake and photo-induced ΔF were done at chlorophyll concentrations of 500 μg/mL and 10 μg/mL, respectively.

this mechanism to the activation of oxygen photoconsumption in PSII preparations at high pH [17].

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot} \xrightarrow{H^+} ROOH \quad (1)$$

Thus, if we assume that O_2 photoconsumption is the sum of two processes – the reduction of O_2 on the acceptor side, leading to the formation of $O_2^{-\cdot}$, and the interaction of O_2 with organic radicals on the donor side of PSII according to Reaction (1), – and if we discount other utilizations of the separated charges within the RC, two O_2 molecules can be consumed per one electron (transferred through the PSII reaction centre): one on the donor side (Reaction (1)) and the other on the acceptor side of PSII (Reaction (2)). Superoxide anion radical may be disproportionated to H_2O_2 and O_2, which ultimately results in a twofold decrease in O_2 photoconsumption related to the acceptor side of PSII (Reaction (3)).

$$O_2 + e \rightarrow O_2^{-\cdot} \quad (2)$$

$$O_2^{-\cdot} + H^+ \rightarrow {}^1/_2 H_2O_2 + {}^1/_2 O_2 \quad (3)$$

In this case, the contributions of the donor and acceptor sides to O_2 photoconsumption will be about 2/3 and 1/3, respectively. This is close to the 70/30 ratio reported above for the effects of the exogenous electron donors and acceptors, respectively, on O_2 photoconsumption. Nevertheless, this is a formal and approximate evaluation of the contribution of the acceptor and donor sides of PSII to O_2 photoconsumption, since it does not take into account the ratio between the rate constants of charge recombination within the RC, electron transfer to O_2, and interaction of other (endogenous) electron acceptors and donors with the separated charge in PSII.

Our experiments on O_2 uptake upon the illumination of an Mn-depleted PSII preparation by a series of microsecond saturating light flashes show that O_2 consumption occurs already on the first flash and is comparable with the yield of O_2 evolution on the third flash in the PSII samples before Mn removal (Fig. 5, Curves 1 and 2). If we assume that the yield of O_2 evolution in the untreated PSII preparations on the third saturating flash is one molecule per RC then the amount of O_2 consumed on the first flash in the Mn-depleted PSII will be 0.8–0.9 molecules of O_2 per RC. These data suggest that reactions leading to O_2 uptake effectively compete with charge recombination and other ways of using the separated charges, and the contribution of the donor side of PSII (as shown by the effect of electron donors on flash-induced O_2 uptake) is two times higher than that of the acceptor side.

The study of Mn^{2+} effect on the flash-induced O_2 consumption in Mn-depleted PSII preparations provided a quite unexpected result: whereas at rather high concentrations (>100 μM) Mn^{2+} inhibits flash-induced O_2 uptake (like other electron donors such as potassium ferrocyanide and DPC), at very low (catalytic) concentrations (corresponding to 2–4 Mn^{2+} per RC) Mn^{2+} activates (by a factor of 1.8) the flash-induced O_2 consumption. The dependence of Mn^{2+} effect on its concentration (Fig. 6B) suggests that there is an overlap of two processes: Mn^{2+}-induced activation and Mn^{2+}-induced inhibition of flash-induced O_2 uptake. The nature of the former process is not quite clear (possible reactions are presented below), while the latter one is evidently due to the decrease in the long-lived states of $P_{680}^{+\cdot}$ or $TyrZ^{\cdot}$. It prevents the formation of organic radicals and, accordingly, prevents O_2 photoconsumption according to Reaction (1).

Mn-depleted PSII preparations are very sensitive to the inhibitory action of light. We have shown that the illumination of Mn-depleted PSII preparations inactivates the donor side of PSII, clearly due to the photoaccumulation of the long-lived state of $P_{680}^{+\cdot}$ (or $TyrZ^{\cdot}$). If Mn-depleted PSII preparations had been pre-illuminated in the absence of Mn^{2+}, the PSII could no more be reactivated by Mn^{2+} [14]. Our results show that the loss of the ability of PSII to be reactivated by Mn^{2+} as a result of inhibitory pre-illumination is accompanied by both the suppression of flash-induced O_2 uptake and the disappearance of the Mn-induced activation of O_2 photoconsumption (Fig. 7). The results reveal that only the donor side of PSII capable of "functional" redox interaction with Mn^{2+} can be involved in these effects. It is important to note that the photoinhibition procedure we used inflicts certain damage upon the donor side of PSII while the photoinduced charge separation in PSII reaction centres remains active (as was shown earlier by the measurement of photoaccumulation of $P_{680}^{+\cdot}$ after such a treatment [14]). One can assume that O_2 photoconsumption (including that in the presence of Mn^{2+}) is related to the photoinhibition of the donor side of PSII. However, according to a previous paper [14], the addition of catalytic concentration of Mn^{2+} (2–4 Mn per RC) prevents, rather than activates, the photoinhibition. Therefore, the O_2 photoconsumption on the donor side of PSII (especially that

observed in the presence of Mn^{2+}) in clearly not due to the known process of the photoinhibition of the PSII donor side.

Consider possible reactions involving Mn^{2+} which could stimulate the O_2 photoconsumption observed in the Mn-depleted PSII preparations.

a) Mn^{2+} binding in Mn-depleted PSII preparations may lead to structural changes in PSII which, in turn, may result in the activation of O_2 consumption both on the acceptor and the donor sides of PSII. However, there is no such effect upon the addition of another divalent cation, Mg^{2+}, which does not support this suggestion.
b) The activation of flash-induced O_2 consumption upon the addition of catalytic Mn^{2+} concentration may be due to the oxidation of organic molecules via their interaction with Mn^{3+} formed as a result of the photooxidation of the added Mn^{2+}.
c) Mn^{2+}-induced activation of flash-induced O_2 uptake may be due to the redox interaction of Mn^{2+} (free or bound with the PSII reaction centres) with reactive oxygen species formed upon the illumination of PSII. For example, Mn^{II} phenanthroline complexes can react with hydroperoxides to generate hydroxyl radical ($HO^{\cdot}$) [35]; Mn^{II} pyrophosphate can interact with $O_2^{\cdot-}$ to form Mn^{III} pyrophosphate and hydroperoxyl anion, HO^{2-} [36]; and Mn^{II} histidine (as well as Mn^{II} bicarbonate) complexes are capable of decomposing H_2O_2 into $HO^{\cdot}$ and $O_2^{\cdot-}$ [37]. Hence the reactions produce radicals which may cause additional O_2 consumption.
d) It is possible that O_2 photoconsumption (characterized by a high quantum efficiency, strongly activated by Mn^{2+}, and requiring the PSII donor side capable of redox interaction with added Mn^{2+}) reflects the involvement of O_2 or its reactive forms in the photoassembly of the inorganic Mn-containing core of the WOC, in particular, in the formation of Mn^{3+}(di-μ-oxo) complex believed to be a key intermediate for the assembly of the Mn cluster [38]. Earlier oxygen photoconsumption during photoreactivation of the WOC was observed in thylakoids (though it required the presence of electron donors and was attributed to the Mehler type electron transport from PSII to molecular oxygen via PSI) [39].

Thus, the O_2 photoconsumption in PSII preparations after the removal of Mn and other components (Ca^{2+}, external proteins) of the WOC is a result of reactions taking place on both acceptor and donor sides of PSII. It is characterized by a high quantum yield (comparable with that of flash-induced oxygen evolution), activated by catalytic concentration of Mn^{2+}, and may reflect the participation of O_2 (or its reactive forms) in the formation of the inorganic core of the WOC as well as the negative processes leading to photoinhibition.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbabio.2010.01.014.

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