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# Influence of the disaccharide trehalose on the oxidizing side of photosystem II

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## Abstract

The steady-state oxygen evolution rate was previously shown to be stimulated by the disaccharide trehalose in PSII suspension. Here we showed a similar increase in the rate of oxygen evolution in PSII core complexes from spinach in solution and in proteoliposomes in the presence of trehalose. Using direct electrometrical technique, we also revealed that trehalose had no effect on the kinetics of electron transfer from Mn to redox-active-tyrosyl radical, $Y_Z^{\bullet}$ ($S_1 \rightarrow S_2$ transition), while it accelerated the kinetics of electrogenic proton transport during $S_2 \rightarrow S_3$ and $S_4 \rightarrow S_0$ transitions of the water-oxidizing complex (WOC) induced by the first, second, and third laser flashes in dark-adapted PSII samples. These observations imply that the effect of trehalose occurrs due to its interaction with the WOC.

*Additional key words*: effective functioning; osmolyte; photoelectric response; vectorial transfer.

## Introduction

Photosystem II of oxygenic photosynthesis is a thylakoid membrane-embedded protein complex that catalyzes the light-induced transfer of electrons from water to plastoquinone accompanied by the production of molecular oxygen and the release of plastoquinol into the membrane phase. PSII is a large protein complex composed of 17 transmembrane and 3 peripheral subunits and ~100 cofactors (Ferreira *et al*. 2004, Guskov *et al.* 2009, Umena *et al.* 2011). PSII turnover involves a donor [the water oxidizing complex (WOC), redox-active tyrosine $Y_Z$ of the D1 subunit of reaction center (RC), chlorophylls (Chl) of the RC] and an acceptor [pheophytin, a bound plastoquinone ($Q_A$), and an exchangeable plastoquinone ($Q_B$)] side components (Wydrzynski and Satoh 2005, Renger and Kühn 2007, Shevela *et al.* 2012). An electron leaving a specific RC Chl *a* molecules designated as $P_{680}$ is transferred *via* the pheophytin to the primary quinone acceptor, $Q_A$, and subsequently to the terminal plastoquinone $Q_B$. Electron transfer to $Q_B$ produces a semiquinone anion; it is stabilized at the $Q_B$ site and acts as the acceptor in the second electron transfer that reduces the semiquinone to quinol ($Q_BH_2$), which then diffuses out of the $Q_B$ pocket into the quinol pool (Shinkarev and Wraight 1993, Shinkarev 2004).

Water oxidation chemistry occurs in the WOC, which consists of an inorganic $Mn_4CaO_5$ cluster and its surrounding protein matrix. During each catalytic turnover, the Mn cluster cycles *via* five kinetically characterized intermediate states labeled $S_i$ (i = 0–4) (Kok *et al.* 1970, Shinkarev 2004, Wydrzynski and Satoh 2005, Dau and Haumann 2007, Renger and Kühn 2007, Shimada *et al*. 2011). Since the $S_1$ state (basic state) is the most stable in the dark, the first four flashes cause transitions $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, $S_3 \rightarrow S_0$, and $S_0 \rightarrow S_1$ and as a consequence, two water molecules are split into four electrons, four protons, and dioxygen at the catalytic center – the manganese cluster (Kawakami *et al.* 2011, Muh and Zouni 2011, Najafpour *et al*. 2016, Vinyard and Brudvig 2017). The molecular oxygen is released during the $S_3 \rightarrow S_0$ transition *via* intermediate state $S_4$. The WOC is the most fragile site within PSII and is easily susceptible to oxidative damage. In this regard, the study of influence of the protectors

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*Abbreviations*: Chl – chlorophyll; $P_{680}$ – the primary electron donor of PSII; $Q_A$, $Q_B$ – the primary and the secondary plastoquinone electron acceptors of PSII, respectively; RC – reaction center; $S_0$ – $S_4$ – charge storage states of the WOC; WOC – water-oxidizing complex; $Y_Z$ – redox active tyrosine residue 161 of D1 protein; $\Delta\psi$ – transmembrane electric potential difference.
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on the PSII complexes is of particular interest. Trehalose, a nonreducing disaccharide, which is naturally produced by several species of eubacteria, archaea, some fungi, certain invertebrates, green algae, and plants, has attracted increased attention due to its unique physical-chemical properties. The latter include relative inertness of the glycosidic linkage, the existence of both crystalline and amorphous state, thermostability, high glass transition temperature, high stability over a wide pH range, and high hydrophilicity (Williams *et al.* 1992, Bakaltcheva *et al.* 1994, Hincha *et al.* 1996, Crowe *et al.* 2001, Crowe 2002, Apostolova *et al*. 2005, Jun *et al.* 2008, Palazzo *et al.* 2008, Francia *et al.* 2009, Iturriaga *et al.* 2009, Fernandez *et al*. 2010, Luo *et al*. 2010, Ohtake and Wang 2011, Chang *et al*. 2014, Lunn *et al*. 2014, Malferrari *et al*. 2014, 2016). As for influence of trehalose on isolated PSII complexes, stabilization of PSII-mediated electron transport by the addition of this disaccharide has been demonstrated in oxygen-evolving PSII core preparations (Williams and Gounaris 1992). On the basis of FTIR spectroscopy data (Polander and Barry 2012), it was suggested that trehalose excludes water molecules from the solvation layer of the WOC. Recently it was shown that trehalose in solution significantly stimulates the steady-state rate of $O_2$ evolution in both PSII membrane fragments and PSII core complexes from plants (Mamedov *et al*. 2015). The authors proposed that prevention of the time-dependent degradation of PSII samples in the presence of this disaccharide could be explained by stabilization of the RC. A significant activation of electron transfer on both the acceptor and the donor sides of PSII accompanied by a two-fold increase in the rate of oxygen photo-consumption was revealed upon addition of 1 M trehalose (the latter effect was ten and 40 times lower upon the addition of 1 M sucrose and 1 M glycine-betaine, respectively, in comparison to trehalose) (Yanykin *et al*. 2015). In addition, investigation of the effect of trehalose on photoinhibition in apo-WOC-PSII preparations showed that trehalose increases the capability of manganese (both exogenous and endogenous) to donate electrons to reaction center and strengthens the protective properties of Mn during photoinhibition, probably due to disaccharide-induced structural changes at the oxidizing side (Yanykin *et al*. 2016).

In this work, we investigated the influence of trehalose on the WOC in spinach PSII core particles using polarography and direct electrometrical techniques. The latter allows to detect vectorial transfer of charges by proteins operating as generators of transmembrane electric potential ($\Delta\psi$) (Semenov *et al.* 2006). The data obtained indicate that the effect of trehalose occurs mainly due to its interaction with the WOC.

## Materials and methods

**Purification of PSII samples**: PSII core complexes with an active water-oxidation system were prepared from spinach by solubilizing PSII membrane fragments (Ford and Evans 1983) with dodecyl-*β*-D-maltoside as described in Haag *et al*. (1990). These preparations contain antenna (43, 47 kDa), RC (D1/D2/cytochrome $b_{559}$) proteins, and 33 kDa extrinsic protein. For inactivation of the WOC, a suspension of PSII with 5 μM Chl was titrated to pH 8.3 for 10 min, and then the pH was readjusted to the desired value. $O_2$ evolution in these samples was <10% of the controls [≈1,400 μmol($O_2$) mg(Chl)$^{-1}$ h$^{-1}$].

**Preparation of proteoliposomes**: Reconstitution of PSII complexes into lipid vesicles was carried out by mixing the liposomes resulting from sonication of commercially available soybean phospholipids (*Sigma,* type IV-S) in 50 mM HEPES-NaOH buffer (pH 7.5) containing 1.4% (w/v) octyl-β-D-glucopyranoside with PSII complexes at the lipid/protein ratio of 50:1 (w/w) and incubation for 30 min in the dark. Removal of detergent was performed using a *Sephadex*-50 column (Mamedov *et al*. 1999). The proteoliposome suspension was pelleted at 140,000 × *g* at 4°C for 1 h in a *Coulter Optima L*-90*K* (*Beckman,* USA) ultracentrifuge, and the pellet was resuspended in 25 mM MES-NaOH (pH 6.5) buffer containing 15 mM NaCl, 10 mM $CaCl_2$, 0.3 M sucrose. Potassium ferricyanide (1 mM), as an electron acceptor for $Q_A^-$ , was entrapped within the aqueous inner space of liposomes. All procedures were performed at 4°C.

**Oxygen evolution**: The rates of oxygen evolution under continuous illumination [1,000 μmol(photon) m$^{-2}$ s$^{-1}$] of PSII core particles in solution and in proteoliposomes were measured at 25°C using a Clark-type oxygen electrode at a concentration of 5 μg(Chl) mL$^{-1}$ in the absence and presence of trehalose. The oxygen evolution rates of the samples were 1,400 ± 80 μmol($O_2$) mg(Chl)$^{-1}$ h$^{-1}$. Polarographic detection of $O_2$ evolution upon illumination of samples by series of saturating light flashes was performed at the concentration of 500 μg(Chl) mL$^{-1}$. The light flashes were made with a xenon *L4633* flash lamp (*Hamamatsu*, Japan).

**Voltage transients**: Generation of the voltage transients ($\Delta\psi$) was recorded by direct electrometric technique with resolution of 200 ns as described in Drachev *et al*. (1979) and Semenov *et al.* (2006). The technique includes an arrangement of specimens on the surface of a collodion phospholipid-impregnated film separating two partitions of the measuring cell. The measuring membrane should be very thin to possess large electric capacitance for the recording of fast charge translocation. During the enzyme functioning, PSII creates $\Delta\psi$ on the vesicle membrane, which is then proportionately divided with the measuring

membrane and thus can be detected by Ag/AgCl electrodes situated on its different sides. Typically, the measuring membrane has high resistance of 2–3 GΩ, and the measured ψ decays with a time constant of several seconds. The amplitude of the photoelectric signal is proportional to the dielectrically weighted distances between redox cofactors within protein matrix. A pulsed Nd-*YAG* laser (*YG-481*, *Quantel*, France, λ = 532 nm,pulse half-width 15 ns, flash energy of 40 mJ $cm^{-2}$) was used as a source of light flashes. The kinetic curves were processed and decomposed into exponentials using program packages *Pluk* (Kalaidzidis *et al*. 1997) and *Origin* (*OriginLab Corporation*, USA).

## Results

**Peculiarities of PSII core particles after incorporation into liposomes**: The $S_1$ state of the WOC is the most stable in the dark, and hence in the dark-adapted PSII, maximum oxygen evolution was initially observed in response to the third flash (Fig. 1). The oscillation pattern proved that the PSII core particles in proteoliposomes were similarly synchronized in the dark as in PSII in solution (Haumann *et al.* 1997, Mamedov *et al*. 1999). In contrast to the steady-state conditions, where the rate of oxygen evolution in the presence of trehalose both in solution and in proteoliposomes increased (Table 1), the effect of this disaccharide on the $O_2$ evolution induced by short flashes was not observed (data not shown).

Electrometrically detected electric responses induced by the first laser flash in dark-adapted liposome-reconstituted intact PSII core complexes are shown in Fig. 2*A*. The asymmetric orientation of PSII in vesicles (WOC is facing the external surface of the proteoliposomal membrane) was previously demonstrated on the basis of negligible influence of sodium dithionite on the amplitude of the photoresponse (Mamedov *et al*. 2006, Gopta *et al.* 2008). $Q_B$ was absent in PSII preparations used in the present work (*see* Haag *et al.* 1990). Under these conditions, a stable charge separation ($S_2Y_ZP_{680}Q_A^-$) in proteoliposomes is observed upon every flash in the presence of ferricyanide (Haumann *et al.* 1997, Mamedov *et al.* 1999). The slow decay of the flash-induced voltage transient suggest that at least ~80% of PSII reaction centers were still active in reducing tyrosyl radical $Y_Z^{\bullet}$ by electrons from the WOC. In Mn-depleted PSII complexes, where the first flash generates $Y_ZP_{680}Q_A$ as a result of $P_{680}^+$ reduction by tyrosine $Y_Z$, the second and subsequent charge separation can only generate $P_{680}^+Q_A^-$ state followed by recombination of this radical pair (Conjeaud and Mathis 1980, Gerken *et al*. 1989, de Wijn and van Gorkom 2002) with characteristic time of 60 μs (Fig. 2*B*).

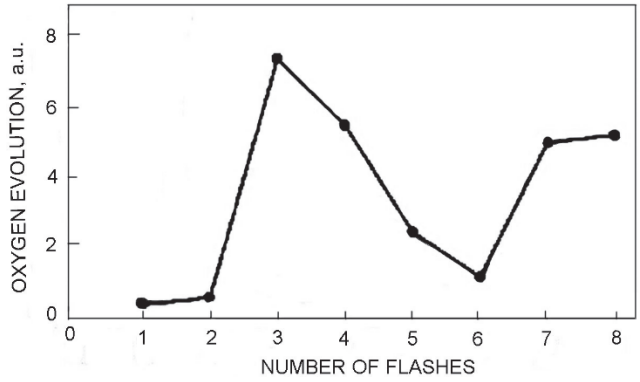


Fig. 1. Oxygen oscillation pattern observed in dark-adapted liposome-reconstituted PSII core particles in a buffer solution containing 25 mM MES (pH 6.5), 15 mM NaCl, 10 mM $CaCl_2$, 0.3 M sucrose, 1 mM potassium ferricyanide. Flash duration: 500 μs, 0.5 Hz repetition.

Table 1. Effect of trehalose (1 M) on the steady-state oxygen evolution in PSII core complexes.

| Sample | [μmol($O_2$) mg(Chl)$^{-1}$ h$^{-1}$] |
|---|---|
| PSII in solution | 1,400 |
| PSII in solution + trehalose | 3,340 |
| Proteoliposomes with PSII | 1,300 |
| Proteoliposomes with PSII + trehalose | 3,000 |

**Electrogenic reactions of the WOC in the presence of trehalose**: The asymmetric incorporation of purified PSII core particles into closed lipid vesicles (Gopta *et al*. 2008) enabled us to investigate the effect of trehalose exclusively on the water-oxidizing side. To resolve electrogenic reactions correlated with the turnover of the WOC, we took the differences of photoelectric responses obtained on consecutive flashes with dark-adapted samples. Using direct electrometrical technique, electrogenic reactions related to $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, and $S_4 \rightarrow S_0$ transitions of the WOC induced by the first, second and third laser flashes were previously observed in dark-adapted PSII cores in the absence of trehalose (Haumann *et al.* 1997, Mamedov *et al*. 1999, 2010).

Fig. 3 depicts the photoelectric responses induced by the first laser flashes in proteoliposomes containing intact (*1*) and Mn-depleted (*2*) PSII particles in the presence of trehalose. The difference between traces 1 and 2 is shown in the inset. The difference revealed additional electrogenic rise component observed in the dark-adapted intact sample. This component was absent in Mn-depleted centers. The relative contribution of this component was ~3% of electrogenic component attributed to formation of the ion-radical pair $Y_Z^{\bullet}Q_A^-$. In the presence of trehalose, the electrogenic rise was well approximated by monoexponential component with lifetime ~40 μs and was attributed to electron transfer from Mn to the tyrosine radical $Y_Z$ ($S_1 \rightarrow S_2$ transition). In doing so, the amplitude and the lifetime of this phase was similar to that of electrogenic

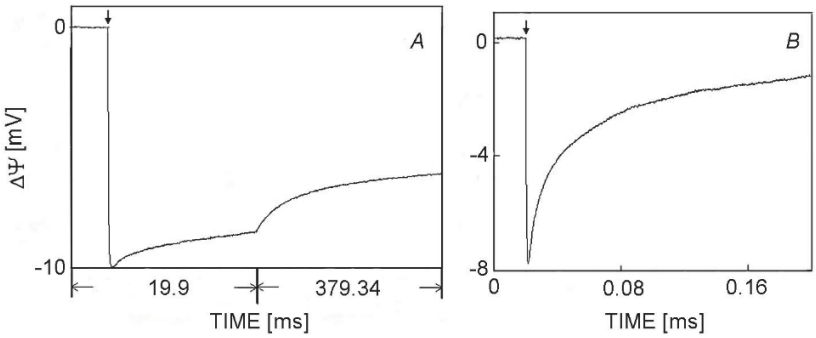


Fig. 2. Photoelectric response in dark-adapted, liposome-reconstituted intact PSII core particles induced by the first laser flash (*A*). Photoelectric response induced by the third flash in the Mn-depleted PSII complexes (*B*). The assay medium containing 25 mM MES (pH 6.5), 15 mM NaCl, 10 mM $CaCl_2$, 1 M trehalose, and 1 mM potassium ferricyanide were used. *Arrows* indicate laser flashes.

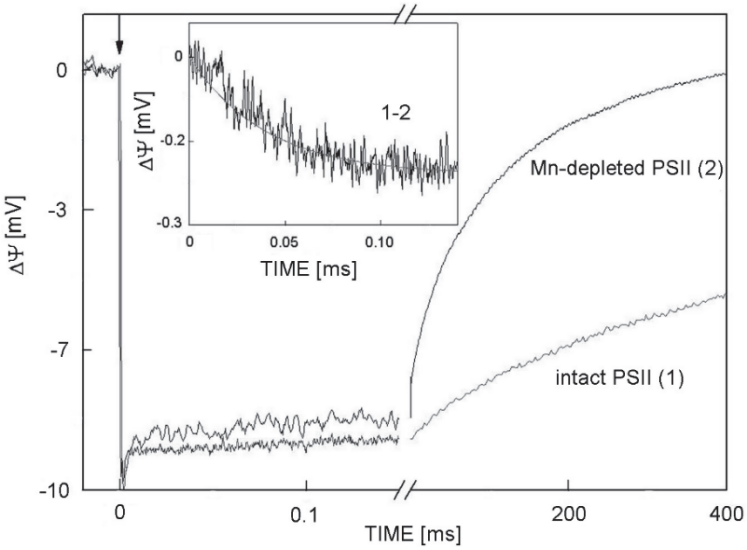


Fig. 3. Photoelectric responses induced by the first laser flashes in dark-adapted, liposome-reconstituted intact (*1*) and Mn-depleted (*2*) PSII core particles in the presence of 1 M trehalose. The difference between traces 1 and 2 is shown in the insert. The assay medium containing 25 mM MES (pH 6.5), 15 mM NaCl, 10 mM $CaCl_2$, 1 M trehalose, and 1 mM potassium ferricyanide were used. Note the different time scale in the insert. *Arrows* indicate laser flashes.

phase observed under the same conditions in the absence of trehalose (*see* Table 2). Note that starting from the dark-stable $S_1$-state, the first flash induces the $S_1 \rightarrow S_2$ transition which involves Mn oxidation without proton release (Haumann *et al*. 2005, Dau and Haumann 2007).

Difference of photoelectric responses induced by the second and the fifth laser flashes (trace 2–5) of dark-adapted PSII core complexes incorporated into liposomes in the presence of 1 M trehalose (after normalization of the fast amplitudes corresponding to the formation $Y_Z^{\bullet}Q_A^-$) is shown in Fig. 4*A*. The second flash induced $S_2 \rightarrow S_3$ transition, while the fifth flash mainly induced $S_1 \rightarrow S_2$ transition, similarly to the first flash, but without contributions from the non-heme iron. The difference presented in Fig. 4*A* revealed component with relative contribution ~5% of $Y_Z^{\bullet}Q_A^-$ formation and a characteristic time of ~150 μs. In the absence of trehalose, the $S_2 \rightarrow S_3$ transition revealed a similar relative amplitude but the kinetics of an additional rising electrogenic component was slower (Table 2); the characteristic time of this component was 240–270 μs. It is believed that during $S_2 \rightarrow S_3$ transition, proton release from the Mn complex and its transfer along a specific proton-conducting pathway towards the water bulk phase is coupled to $\Delta\psi$ generation (Haumann *et al.* 1997, Mamedov *et al.* 1999, Petrova *et al*. 2013).

Table 2. Electrogenic reactions at the water-oxidizing side of PSII. *The figures are taken from (Haumann *et al*. 1997, Mamedov *et al*. 1999, Petrova *et al*. 2013), ** this work.

| Conditions | Relative contributions (% of $Y_Z^{\bullet}Q_A^-$ formation) and lifetimes (in parentheses) of electrogenic reactions during S-state transitions | | |
|---|---|---|---|
| | $S_1 \rightarrow S_2$ | $S_2 \rightarrow S_3$ | $S_4 \rightarrow S_0$ |
| PSII core particles* | ~3 (30–65 μs) | 5–7 (240–270 μs) | 4–6 (~4–6 ms) |
| PSII core particles + trehalose** | ~3 (40–50 μs) | 5 (150–160 μs) | ~5.3 (~2.0–2.2 ms) |

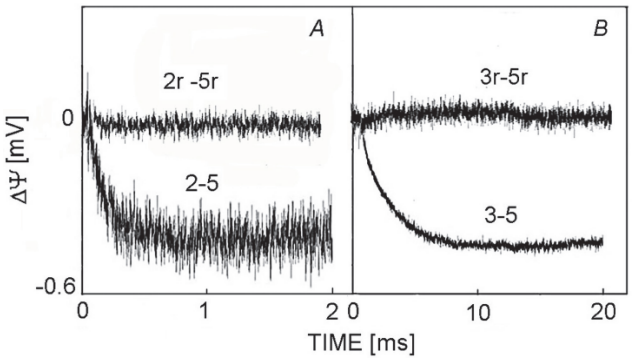


Fig. 4. Differences between photoelectric responses induced by the laser flashes after normalization of the fast amplitude corresponding to the formation $Y_Z^{\bullet}Q_A^-$. 2–5 (*A*) and 3–5 (*B*), flashes 2 (3) minus 5 from dark-adapted material; 2r–5r (insert) and 3r–5r (insert), flashes 2 (3) minus 5 from repetitive excitation. Note the different time scales in panels *A* and *B*.

As a control, the difference of voltage transients induced by the second and fifth flashes upon conditions of repetitive excitation was registered in the presence of 1 M trehalose (Fig. 4*A*, 2r–5r). This difference was close to zero, as expected for an about equal contribution of all S-transitions on both flashes (Haumann *et al*. 1997, Petrova *et al*. 2013).

The difference between photoelectric responses induced by the third and fifth flashes from dark-adapted PSII core samples in the presence of 1 M trehalose (Fig. 4*B*, 3–5) revealed much slower additional kinetic component induced by the third flash. The relative contribution of this component (which is mainly due to $S_4 \rightarrow S_0$ transition) was ~5% of $Y_Z^{\bullet}Q_A^-$ formation with a characteristic time of ~2.2 ms. The difference between voltage transients induced by the third and fifth flashes of the PSII samples in the absence of trehalose revealed a similar relative amplitude (~5% of $Y_Z^{\bullet}Q_A^-$) of this component but with about twofold slower kinetics (Table 2).

The difference between photoelectric responses 3 and 5 from repetitive excitation (Fig. 4*B*, 3r–5r) was negligible in accordance with an about equal contribution of all S-transitions on each flash.

The $S_0 \rightarrow S_1$ transition of the WOC induced by the fourth flash most likely involved oxidation of the Mn complex by radical $Y_Z$ as well as charge-compensating deprotonation of an amino acid residue located in the vicinity of the manganese cluster (Haumann *et al.* 2005, Dau and Haumann 2007). The subtraction of the fifth from the fourth flash-induced photoelectric signal did not reveal any difference (data not shown). Since the fifth flash is considered similar to the first flash, the photoelectric signals in both cases are due to the sum of charge separation steps including electron transfer between $P_{680}$ and $Q_A$, reduction of $P_{680}^+$ by $Y_Z$, and subsequent re-reduction of $Y_Z^{\bullet}$ by Mn. In doing so, the proton released during the $S_0 \rightarrow S_1$ transition is electrically silent (Haumann *et al.* 1997, Semenov *et al.* 2008, Petrova *et al*. 2013).

The relative amplitudes and characteristic times of the individual S-transitions in PSII core complexes in the absence and in the presence of trehalose are summarized in Table 1.

## Discussion

Trehalose stabilizes the structure of proteins and lipid bilayers and serves as a medium for various biological reactions (Crowe *et al*. 1984*,* Harrigan *et al*. 1990, Crowe *et al*. 2001, Villarreal *et al*. 2004, Kan *et al*. 2015). Chemically, trehalose forms hydrogen bonds with polar residues in proteins and phospholipids in cell membranes, and may therefore substitute for water that normally comprises the 'hydration shell' surrounding biomolecules (Carpenter and Crowe 1989, Jain and Roy 2009, Crowe *et al*. 2014). Among the oligosaccharides, trehalose has the highest ability for hydration (Villarreal *et al.* 2004).

We have previously shown that trehalose stimulates the steady-state rate of oxygen evolution in PSII complexes in a buffer solution (Mamedov *et al*. 2015). Here presented results (Table 1) about similarity of the rate of oxygen evolving activities of PSII cores in solution and in proteoliposomes imply that the effect of this disaccharide could be related to its influence on the donor side of PSII. This conclusion has been supported by the following reasons. First, the addition of trehalose had no effect on the overall rate of $Q_A^-$ reoxidation, as assessed by single flash-induced Chl fluorescence decay kinetics in the presence of artificial electron acceptor in PSII core particles (Mamedov *et al*. 2015). An analogous data was earlier obtained for PSII membrane fragments in the case of sucrose and glycerol (Halversson and Barry 2003). On the other hand, since the phospholipid membrane is impermeable for trehalose, added disaccharide can only interact with the water-oxidizing side of PSII. The similarity of the steady-state rates of oxygen evolution in solution and in proteoliposomes (Table 1) indicate in favor of this assumption. The results obtained with PSII membrane fragments by measurements of the steady-state oxygen-evolving activities and $S_2$ state of the WOC by EPR spectroscopy also indicate the effect of cosolvents (sucrose, glycerol) on the donor side (Halversson and Barry 2003).

As it was shown earlier (Haumann *et al*. 1997, Mamedov *et al*. 1999, Petrova *et al*. 2013), in dark-adapted PSII samples, only $S_2 \rightarrow S_3$ and $S_4 \rightarrow S_0$ transitions, induced by the second and third laser flashes, respectively, and attributed to proton transport from immediate environment of the Mn complex to the water bulk phase, are coupled to $\Delta\psi$ generation. The kinetics of these processes is slowed down in a $D_2O$ containing assay medium ($H_2O$–$D_2O$ exchange).

The present work showed that trehalose accelerated the rate of the electrogenic proton transport during $S_2 \rightarrow S_3$ and $S_4 \rightarrow S_0$ transitions, while the kinetics of transfer of an electron from the manganese complex to the redox-active tyrosyl radical, $Y_Z^{\bullet}$ ($S_1 \rightarrow S_2$ transition) within the protein, was not affected (Table 2).

In addition to reaction center D1 subunit, the 33 kDa extrinsic protein (also referred to as the Mn-stabilizing protein) connecting the WOC active site to the aqueous phase, also participates in a proton-transfer network (Shutova *et al*. 2007, McEvoy and Brudvig 2008*,* Shoji *et al*. 2013, Najafpour *et al.* 2016). Proton transfer requires precise distance and orientation of the chain of hydrogen-bonded water molecules and can conceivably be controlled by protein dynamics. In doing so, the $S_2 \rightarrow S_3$ and $S_4 \rightarrow S_0$ transitions can be more susceptible to structural perturbation induced by dehydration (Noguchi and Sugiura 2002).

Experiments carried out at similar viscosity for trehalose (1 M) and sucrose (1.42 M) (Ohtake and Wang 2011) at 23ºC imply that observed findings are probably due to specific physical and chemical properties of trehalose. The increase in viscosity, due to addition of glycerol or sucrose, has a potential to slow the rate of chemical reactions that are diffusion-limited or involve large scale translational motion (Uribe and Sampedro 2003). There is no electron or proton transfer reactions in PSII that is known to involve such a large-scale translational motion (Halversson and Barry 2003). A candidate reaction, for a diffusion-associated effect, might be protonation of the twice reduced secondary quinone acceptor $Q_B$ induced by the second laser flash, but PSII preparations used did not contain $Q_B$. It seems therefore less likely that an increase in viscosity would increase the steady-state oxygen evolution rate in PSII core particles and this is in harmony with data obtained in the case of sucrose of PSII membrane fragments (Halversson and Barry 2003). In fact, in the presence of osmolytes (sucrose, glycerol), a co-solvent-induced shift in the $pK_\alpha$ of an aspartic or glutamic acid side chain, as well as structural changes at the active site of PSII, are attributable to a change in preferential hydration of the WOC (Halverson and Barry 2003). These carboxylic acid residues are involved in light-induced proton transfer in PSII. Another possible interpretation is that the effects of trehalose are attributed to exclusion of water from the solvation layer of the WOC (Polander and Barry 2012). The latter assumption was based on the FTIR spectroscopy data derived from spinach PSII core particles. This idea is basically consistent with the general view that trehalose competes with water for hydrogen-bonding interactions at protein surfaces (Jain and Roy 2009).

Thus, similar increase of the oxygen evolution rates of PSII core complexes in solution and in proteoliposomes (Table 1), as well as acceleration of vectorial proton transfer during $S_2 \rightarrow S_3$ and $S_4 \rightarrow S_0$ transitions of the WOC in the presence of trehalose (Table 2), imply that this disaccharide mainly interacts with the donor side. We assume that *in vitro* trehalose keeps the water-oxidizing complex in a more optimal conformation for effective functioning, *i.e.*, the catalytic site of PSII is fixed in a conformation that allows more efficient release of the products ($O_2$ and protons) as a result of preferential hydration. However, much remains to be learned about fundamental issues concerning the effect of trehalose and some other osmolytes (sucrose, glycerol) on biostructures. In addition, we conclude that the proteoliposomes are much more convenient for the study of the effects of osmolytes because they do not contain detergent, while the isolated photosynthetic membrane protein complexes in a buffer solution are surrounded by both water molecules and by a detergent belt (Golub *et al*. 2017).

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