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Trehalose stimulation of photoinduced electron transfer and oxygen photoconsumption in Mn-depleted photosystem 2 membrane fragments

D.V. Yanykin [a,*,1], A.A. Khorobrykh [a,1], M.D. Mamedov [b], V.V. Klimov [a]

[a] Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino 142290, Moscow Region, Russia
[b] M. V. Lomonosov Moscow State University, A. N. Belozersky Institute of Physical–Chemical Biology, Moscow 119991, Russia

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ABSTRACT

It is known that the removal of manganese from the water-oxidizing complex (WOC) of photosystem 2 (PS2) leads to activation of oxygen photoconsumption (OPC) [Khorobrykh et al., 2002; Yanykin et al., 2010] that is accompanied by the formation of organic hydroperoxides on the electron-donor side of PS2 [Khorobrykh et al., 2011]. In the present work the effect of trehalose on the OPC in Mn-depleted PS2 preparations (apo-WOC-PS2) was investigated. A more than two-fold increase of the OPC is revealed upon the addition of 1 M trehalose. Drastic (30%–70%) inhibition of the OPC upon the addition of either electron acceptor or electron donor indicates that the trehalose-induced activation of the OPC occurs on both donor and acceptor sides of PS2. A two-fold increase in the rate of superoxide-anion radical photoproduction on the electron-acceptor side of PS2 was also shown. Applying the "variable" chlorophyll fluorescence (ΔF) it was shown that the addition of trehalose induces: (i) a significant increase in the ability of exogenous Mn^{2+} to donate electrons to the reaction center of PS2, (ii) slowing down the photoaccumulation of the primary quinone electron acceptor of PS2 (Q_A^-) under aerobic conditions, (iii) acceleration of the reoxidation of Q_A^- by Q_B (and by Q_B^-) as well as the replacement of Q_B^{2-} by a fully oxidized plastoquinone, and (iv) restoration of the electron transfer between the quinone electron carriers in the so-called "closed reaction centers of PS2" (their content in the apo-WOC-PS2 is 41%). It is suggested that the trehalose-induced increase in efficiency of the O_2 interaction with the electron-donor and electron-acceptor sides of apo-WOC-PS2 is due to structural changes leading to both a decrease in the proportion of the "closed PS2 reaction centers" and an increase in the electron transfer rate in PS2.

1. Introduction

Photosystem 2 (PS2) is the pigment–protein complex within a thylakoid membrane of chloroplasts and cyanobacteria where the photoinduced water oxidation and reduction of plastoquinone occur. X-ray crystallography of cyanobacterial PS2 core complexes [1–4] shows that each PS2 monomer consists of 20 protein subunits, 35 chlorophyll molecules, 12 carotenoid molecules and about 20 lipid molecules. In the photochemical reaction center (RC) of PS2, the light energy absorbed by chlorophyll is transformed into the energy of separated charges, and the strongest biological oxidant, $P_{680}^{+\bullet}$, the oxidized primary electron donor of PSII (with the redox potential of 1.1–1.27 V [5–7]) is formed. The $P_{680}^{+\bullet}$ oxidizes the redox-active TyrZ (amino acid residue 161 of D1 protein) which in turn oxidizes the Mn_4CaO_5-cluster (the enzymatic center of the water-oxidizing complex that accumulates the oxidative equivalents required for the oxidation of two water molecules to O_2). The removal of manganese cluster inhibits the electron transfer from water to $TyrZ^{\bullet}$ and $P_{680}^{+\bullet}$ that leads to an increase of the lifetime of the radicals formed as a result of photoinduced charge separation in PS2. Due to the high oxidation potential, $TyrZ^{\bullet}$ and $P_{680}^{+\bullet}$ are capable of oxidation of surrounding molecules [8–10] that, ultimately, leads to damage to the PS2 reaction centers.

In the presence of oxygen, photoreactions taking place in PS2 initiate the formation of reactive oxygen species (ROS). There are a few basic ways of ROS photoproduction in PS2. The generation of singlet oxygen in PS2 occurs mainly through the interaction of triplet-state chlorophyll, ^{3}Chl*, with O_2 [11]. The formation of ^{3}Chl* occurs in PS2 reaction centers when the system lacks photochemically active electron acceptors that promotes charge recombination in the ion-radical pair of PS2 [$P_{680}^{+\bullet}$ $Pheo^{-\bullet}$] with the formation of $^3P_{680}$* [12,13]. Superoxide anion radical ($O_2^{-\bullet}$) production in PS2 occurs in the reaction of one-electron reduction of O_2 by the reduced electron acceptors of PS2 having low redox potentials [14,15]. It is believed that the reduced electron acceptors $Pheo^{-\bullet}$, Q_A^- and Q_B^- are the main sites for superoxide anion radical

Abbreviations: PS2, photosystem 2; RC, reaction center; WOC, water oxidizing complex; apo-WOC-PS2, photosystem 2 membrane fragments deprived of manganese; P_{680}, the primary electron donor of PS2; Pheo, pheophytin — the primary electron acceptor of PS2; Q_A, the primary plastoquinone electron acceptor of PS2; Q_B, the secondary plastoquinone electron acceptor of PS2; PQ, plastoquinone; TyrZ, redox active tyrosine residue 161 of D1 protein; SOD, superoxide dismutase; Cyt c, cytochrome *c*; ΔF, photoinduced changes of chlorophyll fluorescence yield of PS2; OPC, oxygen photoconsumption.

* Corresponding author.
E-mail address: ya-d-ozh@rambler.ru (D.V. Yanykin).
[1] These authors contributed equally to this work.

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generation in PS2 [16–22]. There are also indications that electrons can be transferred to O_2 from the plastoquinone pool and cytochrome b_{559} [23–25]. Spontaneous or superoxide dismutase (SOD)-catalyzed dismutation of $O_2^{-\bullet}$ results in the production of H_2O_2. Hydrogen peroxide photoformation on the electron-acceptor side of PS2 has been shown by chemiluminescence method based on the use of luminol-peroxidase assay [16]. It was shown that hydrogen peroxide could be also formed on the electron-donor side of PS2 (as a result of two-electron oxidation of water) after modification of the WOC [16,17,26,27]. Hydroxyl radical can arise from the reduction of H_2O_2 by low-valent transition metals via the Fenton reaction. The detailed information on the formation of reactive oxygen species in PS2 is presented in a recent review [28].

It was previously shown [29,30] that in Mn-depleted PS2 preparations (apo-WOC-PS2) a significant increase in the rate of oxygen photoconsumption (OPC) is observed. In doing so, the most part of the OPC (about 70%) is due to redox reactions occurring on the electron-donor side of PS2 and the OPC is probably associated with formation of organic hydroperoxides via a radical chain mechanism. The latter was confirmed using a specific fluorescent probe [31].

The experiments on the OPC in apo-WOC-PS2 were performed in the medium not containing osmolytes [29,30] generally used in the studies of the functioning of membrane fragments of PS2 isolated from various plants. Osmolytes stabilize the PS2 complex against various stress factors (temperature, high light, high salinity) [32–37] as well as stimulate the photochemical activities of PS2 [38]. The use of trehalose is especially interesting due to its unique physicochemical properties such as inertness, thermostability, high vitrification temperature, and stability over a wide pH range [39–42]. It has been shown that trehalose increases plant resistance to drought and salinity [43], has a protective effect on the PS2 during freezing [44] (although, according to other data [37], trehalose does not have a protective effect in the thermal inactivation of isolated reaction centers of PS2 (D1/D2/cyt b559-complex)). It was also shown that trehalose modifies electron transport on the electron-acceptor side of bacterial RC (type 2) [45]. In a recent paper [46] it has been demonstrated that trehalose (0.5–1 M) significantly stimulates the steady-state rate of oxygen evolution in PS2 complexes. The authors suggested that trehalose changes a hydration of PS2 complex that results in the transition of PS2 into conformation more optimal for effective functioning.

In the present work we investigated the effect of trehalose on the oxygen photoconsumption and photoinduced electron transfer in PS2 preparations not containing the water-oxidizing complex.

2. Materials and Methods

Oxygen-evolving PS2 membranes prepared from spinach leaves [47] were suspended (2 mg of Chl/mL) 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. PS2 membranes deprived of Mn were obtained as described earlier [48] with some modifications. PSII membranes at a concentration of 0.25 mg of Chl/mL were incubated in 20 mM CHES/NaOH buffer (pH 9.4) in the presence of 200 mM $MgCl_2$ for 90 s at room temperature followed by dilution with 50 mM MES/NaOH buffer (pH 6.0) to stop the reaction. The membranes were washed with 0.3 M sucrose-MES/NaOH buffer (50 mM, pH 6.0) in the presence of 1 mM EDTA and twice in the EDTA/sucrose-free buffer (50 mM MES/NaOH, pH 6.0, 35 mM NaCl). The Chl concentration was determined in 80% acetone [49].

Oxygen measurements were made in a temperature controlled chamber using a Clark type electrode. The rate of the photosynthetic oxygen consumption was measured by monitoring the concentration of oxygen for 60 s after the start of continuous actinic illumination of PS2 membranes.

The kinetics of photoinduced changes of chlorophyll fluorescence yield (ΔF) was measured in a 10-mm cuvette at room temperature by using a MULTI-COLOR PAM fluorometer (Waltz, Germany). Actinic light (AL) traveled to the cuvette (10 × 10 mm) in an optical unit through special fiberoptics. The intensity of AL and saturation 100-ms flash used for the determining of ΔF was 194 μmol photon s^{-1} m^{-2} and 2355 μmol photon s^{-1} m^{-2}, respectively. To determine the effect of trehalose on the Q_A^- photoaccumulation rate the registration of photoinduced ΔF was carried out at AL intensity of 16 μmol photon s^{-1} m^{-2}. The trehalose effect on Q_A^- reoxidation was determined by analysis of the decay kinetics of chlorophyll fluorescence of PS2 induced by a single turnover 10-μs flash (λ = 625 nm, 3055 μmol photon s^{-1} m^{-2}). The analysis of the fluorescence decay kinetics that was performed using a PamWin-3 Software [50] reveals a few kinetic stages of Q_A^- reoxidation. The data presented in the paper is the average of at least three measurements.

To make the measurements under anaerobic conditions the solution with the preparations was purged with argon for 3 min then the argon stream was placed over the surface of the sample.

Light-induced generation of superoxide anion radical in Mn-depleted PS2 membranes was detected by the reduction of cytochrome c (Cyt c) as described earlier [51]. Mn-depleted PS2 membranes were resuspended in a buffer solution containing 50 mM Mes (pH 6.5), 35 mM NaCl and 10 μM Cyt c, with or without 1 M trehalose, to a concentration of 5 μg Chl/ml and divided into two parts placed into the sample and reference cells. After baseline correction, the cuvette with the sample was illuminated with a red light (1200 μmol photon s^{-1} m^{-2}) for 45 s and the differential absorption spectrum (530–570 nm) was recorded. In order to determine Cyt c reduction induced by superoxide anion radicals the same measurement was performed in the presence of superoxide dismutase (SOD). Then the subtraction of differential peak of ΔA at 550 nm obtained in the presence of SOD from the ΔA obtained in the absence of SOD was done ($\Delta A_{550} = \Delta A_{550}^{-SOD} - \Delta A_{550}^{+SOD}$). To estimate the amount of Cyt c reduced by superoxide anion radicals we used the differential extinction coefficient between ferrocytochrome c and ferricytochrome c at 550 nm (21.1 mM^{-1}).

The activity of the SOD (4557 un/mg) was confirmed experimentally by the measurement of suppression of Cyt c reduction. Cyt c was reduced by superoxide anions generated by the xanthine–xanthine oxidase system. Added SOD (1 un/ml) induced a 50% inhibition of the Cyt c reduction. The SOD from bovine erythrocytes, Catalase and Cyt c were purchased from Sigma Aldrich.

3. Results

As shown in Fig. 1 (trace 1) under continuous illumination of apo-WOC-PS2 the oxygen photoconsumption with the rate of

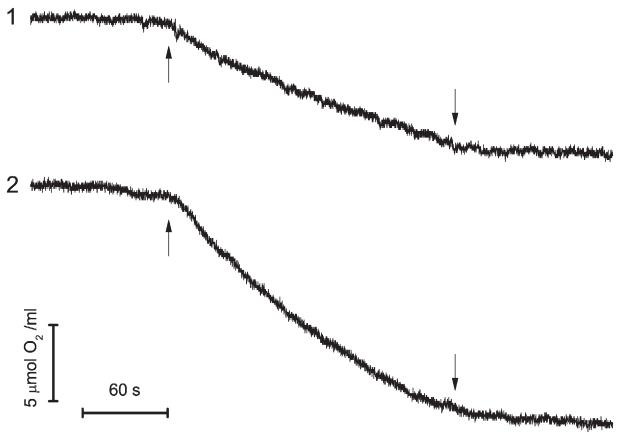

Fig. 1. Kinetics of oxygen photoconsumption (OPC) in Mn-depleted PS2 preparations (apo-WOC-PS2) under continuous illumination (λ = 650 nm, 1200 μmol photon s^{-1} m^{-2}). The measurements were made in the medium containing 50 mM MES (pH 6.5), 35 mM NaCl in the absence (1) and in the presence (2) of 1 M trehalose at a Chl concentration of 20 μg/mL. ↑ and ↓ — light on and light off, respectively.