Journal of Photochemistry & Photobiology, B: Biology 164 (2016) 236–243

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Trehalose protects Mn-depleted photosystem 2 preparations against the donor-side photoinhibition

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

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

ARTICLE INFO

Article history:
Received 20 July 2016
Received in revised form 15 September 2016
Accepted 21 September 2016
Available online 27 September 2016

Keywords:
Photosystem 2
Oxygen photoconsumption
Photoinhibition
Manganese
Trehalose

ABSTRACT

Recently, it has been shown that the addition of 1 M trehalose leads to the increase of the rate of oxygen photoconsumption associated with activation of electron transport in the reaction center of photosystem 2 (PS2) in Mn-depleted PS2 membranes (apo-WOC-PS2) [37]. In the present work the effect of trehalose on photoinhibition of apo-WOC-PS2 preparations (which are characterized by a high sensitivity to the donor side photoinhibition of PS2) was investigated. The degree of photoinhibition was estimated by the loss of the capability of exogenous electron donor (sodium ascorbate) to reactivate the electron transport (measured by light-induced changes of chlorophyll fluorescence yield (ΔF)) in apo-WOC-PS2. It was found that 1 M trehalose enhanced the Mn^{2+}-dependent suppression of photoinhibition of apo-WOC-PS2: in the presence of trehalose the addition of 0.2 μM Mn^{2+} (corresponding to 2 Mn^{2+} per one reaction center) was sufficient for an almost complete suppression of the donor side photoinhibition of the complex. In the absence of trehalose it was necessary to add 100 μM Mn^{2+} to achieve a similar result. The effect of trehalose was observed during photoinhibition of apo-WOC-PS2 at low (15 μmol photon s^{-1} m^{-2}) and high (200 μmol photon s^{-1} m^{-2}) light intensity. When Mn^{2+} was replaced by other PS2 electron donors (ferrocyanide, DPC) as well as by Ca^{2+} the protective effect of trehalose was not observed. It was also found that 1 M trehalose decreased photoinhibition of apo-WOC-PS2 if the samples contained endogenous manganese (1–2 Mn ions per one RC was enough for the maximum protection effect). It is concluded that structural changes in PS2 caused by the addition of trehalose enhance the capability of photochemical reaction centers of apo-WOC-PS2 to accept electrons from manganese (both exogenous and endogenous), which in turn leads to a considerable suppression of the donor side photoinhibition of PS2.

1. Introduction

Oxygenic photosynthetic organisms using sunlight for growth and development are highly sensitive to photoinhibition (PI). PI is manifested in the form of the light-depended impairment of photochemical processes in the thylakoids of chloroplasts which leads to inhibition of the majority of photosynthetic functions [1–3]. The most susceptible to PI site in thylakoid membrane is photosystem 2 (PS2) – the pigment-protein complex which catalyzes light-dependent water oxidation and plastoquinone reduction. X-ray crystallography of cyanobacterial PS2 core complexes [4–7] shows that each PS2 monomer consists of 20 protein subunits, 35 chlorophyll molecules, 12 carotenoid molecules and about 20 lipid molecules. Light absorption leads to formation of the excited state of the PS2 primary electron donor (chlorophyll dimer P_{680} in the photochemical PS2 reaction center). The energy of this electronic excitation is transformed into the energy of separated charges resulting in the formation of the strongest biological oxidant, P_{680}^{+}•, (with redox potential of 1.1–1.27 V [8–10]). P_{680}^{+}• oxidizes the redox-active TyrZ (amino acid residue 161of 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 and O_2 evolution.

At present, there is no commonly accepted view on the precise mechanism of PS2 photoinhibition in vivo [11–14]. However, it is obvious that the D1 protein of the PS2 reaction center is the main target for damage under high light so that the rate of degradation and resynthesis of D1 is significantly higher compared to other PS2 proteins [15,16]. The removal of manganese cluster inhibits the electron transfer from water to TyrZ• and P_{680}^{+}• leading to an increase in the lifetime of the radicals formed as a result of photoinduced charge separation in PS2. Due to

Abbreviations: PS2, photosystem 2; RC, reaction center; WOC, water oxidizing complex; apo-WOC-PS2, photosystem 2 membrane fragments deprived of manganese; Pheo, pheophytin – the primary electron acceptor of PS2; P_{680}, the primary electron donor of PS2; Q_A, the primary plastoquinone electron acceptor of PS2; Q_B, the secondary plastoquinone electron acceptor of PS2; TyrZ, redox active tyrosine residue 161 of D1 protein; DPC, diphenylcarbazide; ΔF, photoinduced changes of chlorophyll fluorescence yield of PS2; Fo, the level of fluorescence induced by the measuring light; Fm, maximal level of fluorescence; PI, photoinhibition; E_m, the midpoint redox potential value.

* Corresponding author.
E-mail address: ya-d-ozh@rambler.ru (D.V. Yanykin).

http://dx.doi.org/10.1016/j.jphotobiol.2016.09.027
1011-1344/

the high oxidation potential, TyrZ• and P_{680}^{+}• are capable of oxidation of surrounding molecules [17–19] that, eventually, leads to the damage of the PS2 reaction centers.

In the presence of oxygen PS2 photoreactions initiate formation of reactive oxygen species. There are several major ways of reactive oxygen species photoproduction in PS2. Generation of singlet oxygen in PS2 occurs mainly through the interaction of triplet-state chlorophyll, ^{3}Chl*, with O_2 [20]. The formation of ^{3}Chl* happens in PS2 RCs when the system lacks photochemically active electron acceptors, which promotes charge recombination in the primary ion-radical pair of PS2 [P_{680}^{+}• $Pheo^{-}$•] with the formation of $^3P_{680}$* [21,22]. Superoxide anion radicals production in PS2 occurs in the reaction of one-electron reduction of O_2 by the reduced electron acceptors of PS2 possessing low redox potentials. It is believed that the reduced primary electron acceptor, $Pheo^{-}$•, both the primary and secondary quinone electron acceptors, Q_A^{-} and Q_B^{-}, are the main sites of superoxide anion radical generation in PS2. There are also proofs that electrons can be transferred to O_2 from the plastoquinone pool and cytochrome b_{559} [23–25]. Spontaneous or superoxide dismutase -catalyzed dismutation of O_2^{-}• results in the production of H_2O_2. Hydrogen peroxide photoformation on the acceptor side of PS2 has been shown by chemiluminescence method based on the use of luminol-peroxidase assay [26]. It was shown that hydrogen peroxide could be also formed on the donor side of PS2 as a result of two-electron oxidation of water after modification of the WOC [26–29]. Hydroxyl radical can be formed 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 the reviews [30,31].

It has been previously found [32,33] that in Mn-depleted PS2 preparations (apo-WOC-PS2) the rate of oxygen photoconsumption induced by redox reactions occurring at the donor side of PS2 significantly increases. It was shown that oxygen photoconsumption on the donor side is probably associated with the formation of organic hydroperoxides via the radical chain mechanism ([34]; see also [35]). Later on it was shown that the oxygen photoconsumption and organic hydroperoxides photoformation are accompanied by photoinhibition of PS2 donor side [36]. A significant activation of electron transfer on both the acceptor side and the donor side of PS2 which is accompanied by a two-fold increase in the rate of oxygen photoconsumption was revealed upon the addition of 1 M trehalose (this effect was 10 and 40 times lower upon the addition of 1 M sucrose and 1 M glycine-betaine, respectively, in comparison to trehalose) [37]. On the one hand, the acceleration in interaction of apo-WOC-PS2 with oxygen should lead to an increase in the degree of apo-WOC-PS2 photoinhibition; on the other hand, it is known that addition of 1 M trehalose induces an increase of the efficiency of the electron donation from Mn^{2+} to PS2 RCs [37]. Furthermore, plants that are capable of trehalose accumulation, better withstand the stress of high light conditions [38].

It is known that different osmolytes can stabilize the PS2 complex, otherwise quite unstable under various stress factors (temperature, light) [39–44] and stimulate photochemical activity of PS2 [45]. It has been shown that trehalose which differs from other osmolytes by its unique physicochemical properties: inertness, thermostability, high vitrification temperature, stability over a wide pH range [46–49] plays a key role in functional and structural stabilization of membranes and proteins by replacing the water molecules [50] or by formation of a glassy state [51]. Trehalose increases plant resistance to drought and salinity stress [52–54], heavy metals [55], low [56] and high [57] temperatures. It demonstrates a protective effect on PS2 during freeze-thaw [58,59], although according to other data [44] trehalose does not have a protective effect in thermal inactivation of isolated reaction centers of PS2 (D1/D2/cyt *b*559-complex). It was also found that trehalose modifies electron transfer on the acceptor side of the bacterial RCs (type 2) [60] and apo-WOC-PS2 ΦC2 [37]. In a recent work it has been demonstrated that trehalose (1 M) significantly stimulates the steady-state rate of oxygen evolution in PS2 complexes and prevents aggregation and inactivation of PS2 membrane fragments during prolonged storage [61]. The authors suggested that trehalose changed hydration of the PS2 complex that resulted in structural transition of PS2 into conformation more optimal for efficient functioning.

In the present work we investigated the effect of trehalose on the donor-side photoinhibition of PS2 preparations depleted of the water-oxidizing complex.

2. Materials and Methods

Oxygen-evolving PS2 membranes prepared from spinach leaves according to [62] were suspended (at 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 [63] with some modifications [37]. 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 of Mn-depletion. 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). In this case the content of endogenous Mn in the preparations was about 0.5 atoms per one PS2 RC. To prepare apo-WOC-PS2 membranes with different content of endogenous Mn the technique was modified. The increase in the concentration of EDTA from 1 mM to 2 mM or to 10 mM led to the reduction of endogenous Mn content to 0.11 and to 0.06 atoms per one PS2 reaction center, respectively. The decrease in EDTA concentration from 1 mM to 0.1 mM or to 0 mM led to the increase of endogenous Mn content to 1.0–1.1 atoms per one PS2 reaction center. When $MgCl_2$ was not added to the CHES/NaOH buffer (pH 9.4) the content of endogenous Mn in the preparations was about 2.3 atoms per one PS2 reaction center. The main part of this work (Figs. 1–4) was performed using preparations of apo-WOC-PS2 containing 0.06 Mn atoms per one PS2 reaction center.

The Chl concentration was determined in 80% acetone [64]. The content of manganese in PS2 preparations was determined with atomic absorption spectrometer "KVANT-2A" (Cortec, Russia).

The kinetics of photoinduced changes of chlorophyll fluorescence yield (ΔF) were measured in DW2/2 Electrode Chamber (Hansatech Instruments Ltd., UK) connected to the MULTI-COLOR PAM fluorometer (Waltz, Germany) at 25 °C. The measuring light (ML) and the actinic light (AL) traveled from the emitters to the Chamber, and chlorophyll fluorescence from the sample passed to the detector through special perspex rods. The intensity of AL used for the measurements of ΔF after photoinhibition was as low as 15 μmol photon s^{-1} m^{-2} to reduce the inhibitory effect of light at the stage of ΔF measurements.

The photoinhibition of apo-WOC-PS2 was carried out for 5 min in the DW2/2 Electrode Chamber at Chl concentration of 20 μg/ml. As the inhibitory light we used AL of various intensities (λ = 625 nm) from MULTI-COLOR PAM fluorometer. After a five minute photoinhibition the samples were diluted two-fold by the addition of a "compensative" buffer containing sodium ascorbate at concentration that provided the maximum level of ΔF due to efficient electron donation to PS2 reaction centers in the apo-WOC-PS2 [36]. The compensative buffer included 1 M trehalose if photoinhibition was performed in the absence of trehalose, and this buffer did not contain trehalose if photoinhibition was done in the presence of 1 M trehalose, so that all the measurements of photoinhibition with apo-WOC-PS2 were performed in the presence of 0.5 M trehalose and 2 mM ascorbate at Chl concentration of 10 μg/ml.

The degree of photoinhibition of apo-WOC-PS2 was estimated by the loss of the PS2 capability to be reactivated by exogenous electron donors (from the measurements of the photoinduced ΔF in the presence of 2 mM sodium ascorbate) in 10 min after the photoinhibition procedure (as it was described earlier [36]). The amplitude of ΔF