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Electrogenic reactions in Mn-depleted photosystem II core particles in the presence of synthetic binuclear Mn complexes

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ABSTRACT

An electrometrical technique was used to investigate electron transfer between synthetic binuclear manganese (Mn) complexes, designated M – 2 and M – 3, and the redox-active neutral tyrosine radical ($Y_Z^{\bullet}$) in proteoliposomes containing Mn-depleted photosystem II (PS II) core particles in response to single laser flashes. In the absence of Mn-containing compounds, the observed flash-induced membrane potential ($\Delta\Psi$) decay was mainly due to charge recombination between the reduced primary quinone acceptor Q_A^- and the oxidized $Y_Z^{\bullet}$. More significant slowing down of the $\Delta\Psi$ decay in the presence of lower concentrations of M – 2 and M – 3 associated with electron donation from Mn in the Mn-binding site to $Y_Z^{\bullet}$ indicates that these synthetic compounds are more effective electron donors than $MnCl_2$. The exponential fitting of the kinetics of additional electrogenic components of $\Delta\Psi$ rise in the presence of Mn-containing compounds revealed the following relative amplitudes (A) and lifetimes (τ): for $MnCl_2$ - A~ 3.5, τ~150 μs, for M – 2 - A~5%, τ~1.4 ms, and for M – 3 - A~5.5%, τ~150 μs. This suggests that the efficiency of the manganese complexes in electron donation depends on the chemical nature of ligands. The experiments with EDTA-treated samples indicated that the ligands for M – 2 and M – 3 are required for their tight binding with the PS II reaction center. The obtained results demonstrate the importance of understanding the molecular mechanism(s) of flash-induced electrogenic reduction of the tyrosine radical $Y_Z^{\bullet}$ by synthetic Mn complexes capable of splitting water into oxygen and reducing equivalents.

1. Introduction

Photosystem II (PS II), a thylakoid membrane water-plastoquinone oxidoreductase, catalyses light-driven oxidation of water to molecular oxygen, protons and electrons. Despite differences in the extrinsic proteins of PS II in plants and cyanobacteria, the central region is highly conserved and have strong similarities in the four core proteins (D1, D2, CP43, CP47) [1–5]. The PS II reaction center (RC) itself, consisting of the D1 and D2 proteins, which harbor all redox-cofactors.

After absorption of light by integral antenna pigments attached to CP43 and CP47 protein subunits, the excitation energy is efficiently transferred to a RC where the following reactions occur: (i) light-induced charge separation into an ion-radical pair between a special pair of chlorophyll *a* (Chl), P_{680}, and the primary plastoquinone acceptor Q_A followed by reduction of the P_{680}^+ by the redox active tyrosine Y_Z (Tyr161 in the D1 subunit of PS II), (ii) oxidative water splitting into molecular oxygen and reducing equivalents *via* a sequence of four redox steps, and (iii) two step reduction of the secondary plastoquinone (PQ) acceptor to plastohydroquinone (PQH_2) under proton uptake [6–10]. The catalytic center of PS II, designated as the water-oxidizing complex (WOC), is the only catalyst used for light-induced water oxidation in Nature and consists of a cluster of four Mn ions and a Ca^{2+} linked by oxo-bonds [1–5].

While investigation of the structures of the native manganese cluster of PS II complex are important, exogenous Mn-complexes

Abbreviations: PS II, Photosystem II; RC, reaction center; WOC, water-oxidizing complex; apo-WOC-PS II, Mn-depleted PS II; $\Delta\Psi$, photovoltage; Y_Z, tyrosine-161 of the D1 subunit; Q_A, primary qunone acceptor; M – 2 and M-3, synthetic binuclear Mn complexes; τ, characteristic time.

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can provide a powerful system to analyze the assembly of the WOC and thus, may lead to a detailed understanding of its function. Various synthetic manganese complexes have been prepared as possible models of the WOC [10–15]. Many of these complexes contain mono-, bi-, tri-, or tetranuclear manganese centers and therefore appeared useful for reconstitution of the Mn-depleted PS II (apo-WOC-PSII), and for examination of their effects on the restoration of the oxygen evolution capacity [16–20]. Certain manganese complexes can reconstitute the WOC, and in some cases higher rates of electron donation and oxygen evolution were observed compared to PS II reconstituted with $MnCl_2$ [16,20].

A great interest to this topic is also caused by the vulnerability of the enzyme catalytic site to various abiotic stresses, especially *in vitro*. In this respect, the D1 subunit of PS II mainly coordinating Mn atoms is particularly susceptible to be inactivated.

Earlier results obtained in the apo-WOC-PS II samples using an electrometrical technique suggest that the electrogenic nature of the electron donation to the oxidized tyrosyl radical $Y_Z^{\bullet}$ is not specific for Mn^{2+}, as an electron donor [21,22].

The present work can be considered as a continuation of the WOC reconstitution studies in the apo-WOC-PS II samples using synthetic binuclear Mn-complexes. Both M – 2 and M – 3 binuclear complexes have been earlier studied in detail by measuring the oxygen evolving activity and electron transfer in PS II membrane fragments [16], although the kinetics of electrogenesis accompanying electron transfer from these compounds to the oxidized $Y_Z^{\bullet}$ was unknown. In this respect, direct detection of the radical pair formation and its decay monitored by photovoltage measurements is an attractive approach that can give complementary information.

2. Materials and methods

Spinach oxygen-evolving PS II core particles were prepared by solubilizing membrane fragments with dodecyl-β-D-maltoside, followed by sucrose gradient centrifugation [23].

PS II core particles deprived of their manganese and extrinsic subunits (apo-WOC-PS II) were obtained as described in Ref. [19] with some minor modifications. Intact PS II core samples at a concentration of 0.5 mg of Chl/ml were incubated in 0.9 M Tris/HCl buffer (pH 9.0) for 30 min at room temperature followed by dilution with 50 mM MES/NaOH buffer (pH 6.5) to stop the reaction of Mn-depletion. The samples were washed with 0.35 M sucrose-MES/NaOH buffer (50 mM, pH 6.5) in the presence of 1 mM EDTA and twice in the EDTA/sucrose-free buffer (50 mM MES/NaOH, pH 6.5, 15 mM NaCl). In photoactivation experiments, apo-WOC-PS II samples were suspended in a buffer containing 10 mM $CaCl_2$, 15 mM NaCl, 0.35 M sucrose and 50 mM MES (pH 6.5) and in the presence of different manganese-containing compounds. The suspension was illuminated by four cycles of continuous light (>600 nm, I = 55 W m^2, illumination of 30-s periods separated by 30 s of dark) prior to the flash-induced experiments.

Oxygen evolution measurements were done using a Clark type electrode; 1 mM potassium ferricyanide and 100 μM 2,6-dichloro-*p*-benzoquinone were used as electron acceptors for Q_A^-.

Incorporation of PS II particles into proteoliposomes was carried out by sonication with subsequent filtration through a Sephadex G-50 column as described in Ref. [21]. The lipid to protein ratio was about 50 (w/w). The reconstitution of the Mn complexes was performed after formation of proteoliposomes.

The flash-induced membrane potential ($\Delta\Psi$) generation in proteoliposomes with apo-WOC-PS II particles adsorbed on a phospholipid-impregnated collodion film were measured electrometrically using Ag/AgCl electrodes as described previously [22]. Using this system, we can measure electric events accompanying single-turnover flash-induced excitation of PS II. Light flashes were provided by a frequency-doubled Quantel Nd-YAG laser (wavelength, 532 nm; pulse halfwidth, 15 ns; flash energy 50 mJ). All experiments were done at 23 °C. For the analysis of data, the average of 3 independent measurements were taken. The kinetic traces were resolved into individual exponents using Igor Pro v.6.3 (Wavemetrics, Portland, OR).

The present study describes the WOC reconstitution with the binuclear manganese complexes. [Mn(III)Mn(III) $(HNQOX)_4(OAc)_2$] and [Mn(III)-O-Mn(III) $(HNQOX)_2(OAc)_2(H_2O)_2$], symbolized by M – 2 and M – 3, respectively, where HNQOX (2-hydroxy-l.4-naphthoquinone monoxim) and OAC (CH_3COO^-) are the terminal and bridging ligands, respectively [16,17]. These complexes were characterized by spectroscopic and electrochemical methods [24].

3. Results

To date, one of the research directions in the study of photosynthetic energy conversion is to design and synthesize molecular catalysts capable of water splitting reaction linked to a light-driven charge separation system [25]. In this respect, apo-WOC-PS II preparations provide a very promising system to investigate the efficiency of synthetic manganese complexes in reconstituting PS II electron flow and oxygen-evolution capacity.

In this work, direct electrometrical technique has been applied to study the $\Delta\Psi$ generation in apo-WOC-PS II particles in the presence of synthetic binuclear Mn complexes under a single enzyme turnover. Time-resolved electrometrical technique provides a direct approach to obtain information on the kinetics of electrogenic charge transfer reactions and dielectrically weighted transmembrane distances between the redox-cofactors [22].

Fig. 1A shows laser-induced photoelectric responses of the proteoliposomes containing PS II complexes depleted of Mn_4Ca cluster and three extrinsic proteins in the absence of external electron donors or acceptors. The negative sign of the $\Delta\Psi$ generation indicates that the protein donor side is located at the external surface of the membrane and therefore the proteoliposomes might serve as a convenient system for studying the electron donation to oxidized $Y_Z^{\bullet}$ by exogenously added reductants [21,22]. The fast phase (the rise time is faster than the time resolution of the experimental setup (~200 ns)) induced by the first laser flash corresponds to charge separation in PS II RC between the primary electron donor P_{680} and quinone acceptor Q_A, and subsequent P_{680}^+ reduction by transfer of an electron from redox active tyrosine Y_Z with the formation of $Y_Z^{\bullet}Q_A^-$ state [22,26]. Under these conditions, Q_A^- decayed rapidly between flashes (in <0.5 s) and therefore the amplitudes of the $\Delta\Psi$ generation was the same in response to each flash (curve 1B). In contrast to intact samples, where the charge recombination is prevented by WOC reduces $Y_Z^{\bullet}$, the rapid decay of the voltage transient in apo-WOC-PS II particles corresponds to recombination of the electron between Q_A^- and the tyrosyl radical $Y_Z^{\bullet}$ [27–29]. In the absence of additions (Fig. 1, curve 1), $\Delta\Psi$ decay was virtually complete in 400 ms. The kinetics of this decay is approximated reasonably well by a single exponent with τ of ~76 ms, which is related to $Y_Z^{\bullet}Q_A^-$ recombination [19,29]. Under these conditions, the minor slow phase of $\Delta\Psi$ decay with characteristic time (>400 ms) is most probably due to passive discharge of a proteoliposomal membrane which is known to occur in this timescale.

In apo-WOC-PS II complexes, Y_Z reduces P_{680}^+ in the microsecond time scale with pronounced pH dependence [30,31]. The lack of additional electrogenicity in the kinetics of the photoelectric response in this time scale may be due to the acceleration of the forward electron transfer reaction between Y_Z and P_{680}^+ in proteoliposomes. In intact PS II particles, the photoreduced Q_A^- is reoxidised in less than 1 ms by a secondary quinone acceptor Q_B.

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