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O_2 reduction by photosystem I involves phylloquinone under steady-state illumination

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ABSTRACT

O_2 reduction was investigated in photosystem I (PS I) complexes isolated from cyanobacteria *Synechocystis* sp. PCC 6803 wild type (WT) and *menB* mutant strain, which is unable to synthesize phylloquinone and contains plastoquinone at the quinone-binding site A_1. PS I complexes from WT and *menB* mutant exhibited different dependencies of O_2 reduction on light intensity, namely, the values of O_2 reduction rate in WT did not reach saturation at high intensities, in contrast to the values in *menB* mutant. The obtained results suggest the immediate phylloquinone involvement in the light-induced O_2 reduction by PS I.

1. Introduction

Molecular oxygen, O_2, is involved in numerous biochemical reactions in aerobic cells. In photosynthetic organisms, O_2 can interact with the photosynthetic apparatus, decreasing the quantum yield of CO_2 fixation. Moreover, as a result of such reactions, reactive oxygen species (ROS) are produced. During evolution, the photosynthetic apparatus has been optimized to minimize reactions with O_2 [1,2], although they still occur and may have a positive impact on cellular metabolism [3]. One such reaction, the so-called Mehler reaction, represents O_2 reduction by components of the photosynthetic electron transfer chain (ETC) under illumination [4]. Electron flow to O_2 was shown to occur along with electron transfer (ET) to $NADP^+$ [5,6]. Under conditions of limited NADPH utilization and excessive light, the Mehler reaction prevents over-reduction of the ETC and photoinhibition [7,8]. Moreover, a stable product of the Mehler reaction, H_2O_2, might be a signal messenger indicating ETC functional state [9,10].

The exact components of the ETC involved in O_2 reduction in vivo have not been yet clearly identified. Ferredoxin (Fd), an acceptor of electrons from Photosystem I (PS I), used to be considered as the main O_2 reducing agent [11], although this assumption was challenged [12,13]. Recently, it was shown that reduced Fd was only capable of low rates of O_2 reduction in the presence of $NADP^+$ and its contribution to the total O_2 reduction was 5–10% [14]. This implies that thylakoid membrane-bound components play an essential role in O_2 reduction. Redox-cofactors of PS I are believed to be significant O_2 reducing agents [3,15]. The ETC of PS I consists of the primary electron donor (P_{700}), the primary (A_0), and secondary (A_1, F_X, F_A, and F_B) electron acceptors (Fig. 1A). Under single-flash illumination, the terminal electron cofactors of PS I, the [4Fe–4S] clusters $(F_A/F_B)^-$, are oxidized by O_2 in the absence of Fd [16]. This reaction results in production of the superoxide anion-radical ($O_2^{\cdot-}$) outside the thylakoid membrane. Using the EPR-detector 1-hydroxy-4-isobutyramido-

Abbreviations: DMF, dimethylformamide; ET, electron transfer; ETC, electron transfer chain; Fd, ferredoxin; MV, methyl viologen; PS I, photosystem I; PhQ and $PhQ^{\cdot-}$, phylloquinone and phyllosemiquinone; PQ and $PQ^{\cdot-}$, plastoquinone and plastosemiquinone; ROS, reactive oxygen species; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; TMT-H, 1-hydroxy-4-isobutyramido-2,2,6,6-tetramethylpiperidinium; V_{ET}, $V_{ET}^{O_2}$ and V_{ET}^{MV}, rates of electron transfer as such and to O_2 and MV, respectively; WT, wild type

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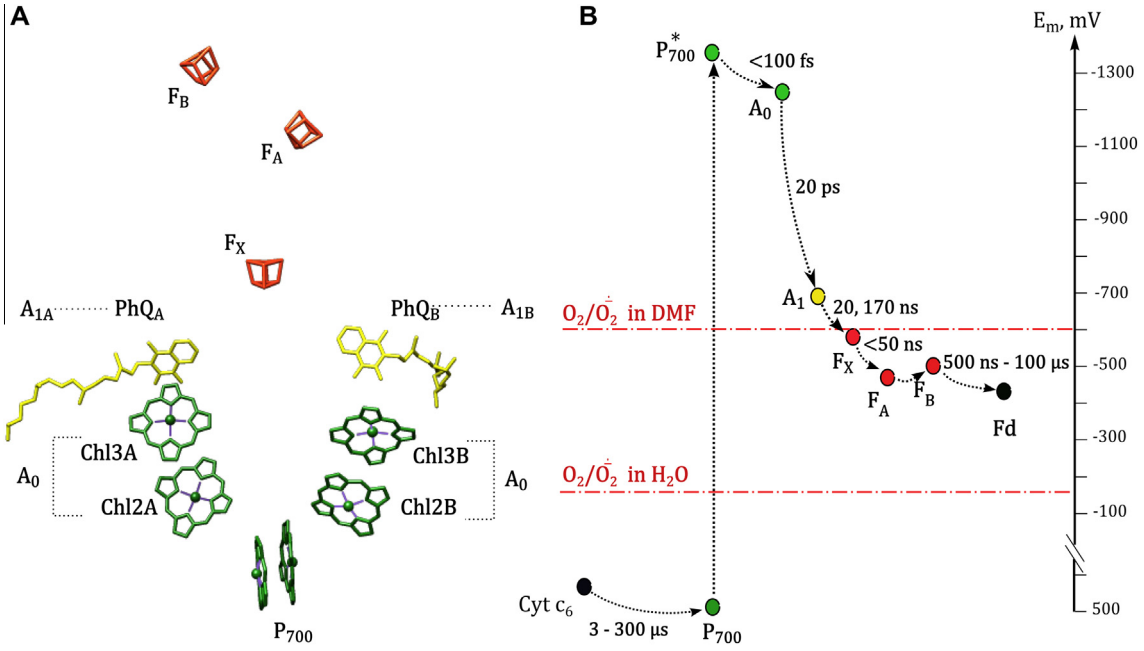

Fig. 1. (A) Arrangement of electron transfer cofactors in PS I. (B) Diagram of forward electron transfer in cyanobacterial PS I with lifetimes and E_m values of the cofactors. E_m values of ($O_2/O_2^{\cdot-}$) in water and dimethylformamide (DMF) are also shown.

2,2,6,6-tetramethylpiperidinium (TMT-H), $O_2^{\cdot-}$ was directly shown to be produced within as well as outside the membrane under steady-state illumination of isolated thylakoids [17,18]. This allowed assuming that at least two cofactors reduce O_2 simultaneously. This assumption is supported by the study of apparent K_m of spinach PS I for O_2 that revealed the existence of at least two sites of O_2 reduction [19]. In the lipid bilayer of the membrane, the midpoint redox-potential (E_m) of ($O_2/O_2^{\cdot-}$) is close to that in dimethylformamide (−500 ÷ −600 mV *vs.* NHE) [20]. Phylloquinones (PhQ), which occupy the quinone-binding sites of PS I (A_1-sites), and the [4Fe–4S] cluster F_X are characterized by rather negative E_m values and are capable of O_2 reduction within the membrane (Fig. 1B). PhQ [21,22] and F_X [23] were proposed to be O_2 reducing cofactors, however there is no direct evidence proving their involvement in O_2 reduction.

In order to investigate the possible role of phyllosemiquinone ($PhQ^{\cdot-}$) at the A_1-site in O_2 reduction, we used PS I isolated from the wild type (WT) and PhQ-lacking mutant strain of *Synechocystis* sp. PCC 6803 with the gene encoding 1,4-hydroxynaphthoyl-CoA-synthase (*MenB*) knocked out [24,25]. In mutant, plastoquinone (PQ) occupies the A_1-sites, with E_m ($PQ/PQ^{\cdot-}$) ~100 mV more positive than E_m ($PhQ/PhQ^{\cdot-}$) in PS I from WT [26]. In this study, it was found that PS I complexes from the WT and *menB* mutant exhibited different dependencies in O_2 reduction on light intensity, and this was interpreted as evidence for direct PhQ involvement in O_2 reduction.

2. Methods

Cells of *Synechocystis* sp. PCC 6803 wild type and mutant strains were grown in BG-11 medium at 30 °C under white fluorescent illumination. PS I complexes were isolated as described in [27]. Reaction medium contained 50 mM HEPES–KOH (pH 7.6), 20 mM NaCl, 5 mM $MgCl_2$, 0.03% β-dodecyl-*n*-maltoside; *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) and sodium ascorbate were added as artificial electron donors to photooxidized P_{700}^+. Redox state of P_{700} was determined by difference in absorption at 810–860 nm with PAM101 equipped with EDP700DW module. A suspension of PS I was placed in a standard chamber (WALZ, Germany) and illuminated by the white light of an incandescent lamp (KL-1500) for 30 s. Light intensity was varied by altering voltage. Light-induced steady-state rate of O_2 reduction was measured as O_2 uptake with Clark type O_2-electrode under stirring at 22 °C. Suspension of PS I was placed in a custom-build thermostatic chamber and illuminated by LED with maximum emission at 660 nm for 1 min. Intensity of the LED was varied by neutral filters or altering electric current. The steady-state rates of ET from PS I to O_2 were proportional to the rates of O_2 uptake because a single PS I turnover resulted in generation of $O_2^{\cdot-}$. In the presence of methyl viologen (MV), a single PS I turnover also resulted in $O_2^{\cdot-}$ generation via the radical form of MV with the rate constant equal to 8×10^8 M^{-1} s^{-1} [28]. Superoxide radicals further produce H_2O_2 in the reaction with ascorbate, present in the suspension at high (5–10 mM) concentration, thereby suppressing spontaneous dismutation of $O_2^{\cdot-}$. The resulting stoichiometry was 1 electron per 1 O_2 molecule consumed [29], and, in our experiments, it was confirmed by addition of superoxide dismutase and catalase. Thus, light-induced O_2 uptake was used as readout of O_2 reduction in the absence and presence of MV. Light intensity as photon flux density µE m^{-2} s^{-1} was determined using a LI-COR model LI-250 quantum meter (Licor, Nebraska, USA).

TMPD-2HCl, sodium ascorbate, MV, β-dodecyl-*n*-maltoside were purchased from Sigma and AppliChem.

3. Results

Steady-state illumination of isolated PS I complexes resulted in O_2 reduction (Fig. 2). In order to accurately study the mechanism of O_2 reduction by PS I under steady-state illumination, this reaction must be the overall ET limiting step. In contrast to O_2, MV is a very efficient acceptor of electrons from the terminal cofactors of PS I, with the rate constant of reduction equal to 10^7 M^{-1} s^{-1} [30]. In the presence of MV, the rate of ET (V_{ET}) is equal to the rate of MV reduction (V_{ET}^{MV}), and this rate is limited by electron donation to P_{700}^+ [31]. The comparison of the rate of ET to O_2 ($V_{ET}^{O_2}$) and V_{ET}^{MV} is a useful approach to reveal the rate-limiting step. The addition