Charge Equilibrium Reactions S_2 and S_3 States of Photosystem II with Cyt b_{559} and Tyrosine Y_D

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Abstract: Electron transfer from the reduced tyrosine Y_D and cytochrome b_{559} (Cyt b_{559}) to the S_2 and S_3 states of photosystem II was investigated at the temperature of 195 K. Electron transfer reactions were followed by measuring EPR signals of tyrosine $Y_D^{\bullet}$, oxidized Cyt b_{559} and the S_2-state multiline signal. Long term incubation (~90 days) at 195 K causes decay of the majority of S_2 centers up to ~40% of initial value, while in this time scale the intensity of $Y_D^{\bullet}$ radical increases less than 10%. Samples advanced to S_3 state demonstrates an increasing behavior of the S_2-state multiline signal intensity in the beginning of incubation (~20 days) and slow decay up to 40% of maximal amplitude during further incubation of the samples. Similarly to the S_2 sample, small increase in $Y_D^{\bullet}$ radical signal was observed during the S_3 decay. However, in both types of samples prepared in S_2 and S_3 states after 90 days of incubation the signal of oxidized Cyt b_{559} is increased from 45%–50% up to 100% maximal intensity. The results obtained in this study support the conclusion of our early investigations which claimed the reduced Cyt b_{559} as electron source for the S_2 and S_3 states.

Keywords: Photosystem II; S-cycle; Tyrosine; Cytochrome b_{559}; Electron transfer

Introduction

Photosystem II (PSII) of oxygenic species catalyzes water oxidation reactions utilizing power of sunlight in light-induced electron transfer reactions (Barber, 2003). High potential produced in PSII reaction center and required for water oxidation chemistry accumulates through the catalytic S-cycle driven by (Ca-4Mn)-Y_Z-P_{680}-Phe-Q_AQ_B redox sequence. Electron transfer in photosystem II starts with excitation of P_{680} (chlorophyll) resulting in sequential reduction of pheophytin (Phe) and plastoquinone electron acceptors (Q_A, Q_B), and oxidation of tyrosine Y_Z and Ca-4Mn cluster. S-cycle involves five intermediate redox states: S_0-S_4. S_0 is the most reduced state. The S_2 and S_3 states are unstable and decays to the stable S_1 state in the dark. The S_4 state is a transient intermediate between S_3 and S_0 (Debus, 1992; Diner and Babcock, 1996).

In certain circumstances two other inner components of PSII, the redox active tyrosine Y_D and cytochrome b_{559}, may also interfere and support water oxidation reactions by electrons (Buser *et al.*, 1990; Styring and Rutherford, 1988; Vass and Styring, 1991; Stewart and Brudvig, 1998; Hanley *et al.*, 1999; Tompson and Brudvig, 1988; Faller *et al.*, 2001). Electron transfer from tyrosine Y_D and Cyt b_{559} to the S_2 and S_3 states of PSII above 245 K were investigated in previous studies (Styring and Rutherford, 1987, 1988; Feyziyev *et al.*, 2003). The subject of present work is the study of electron transfer reactions between tyrosine Y_D, Cyt b_{559} and S-state cycles (S_2 and S_3 states).

Materials and Methods

BBY-type PSII enriched membrane fragments (Berthold *et al.*, 1984) were prepared from hydroponically grown greenhouse spinach with modifications, described in (Völker *et al.*, 1985). The isolated membranes were resuspended in 50 mmol MES-NaOH buffer pH 6.2, contained 35 mmol NaCl and 300 mmol sucrose at a chlorophyll concentration of 4 mg/ml and stored at liquid nitrogen until use. In the presence of 200 μM PPBQ as an electron acceptor

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the rate of oxygen evolution was ~450 μmol of O_2 (mg Chl)$^{-1}$h^{-1}.

Tyrosine $Y_D^{\bullet}$ and Cyt b_{559} were reduced chemically by an ascorbate and 3.6-diaminodurene (DAD) treatment in complete darkness. The PSII membranes at a chlorophyll concentration of 1 mg/ml were dark adapted for 30 min in complete darkness at room temperature. 5 mmol sodium ascorbate and 1 mmol DAD were added and the suspension was incubated for an additional 30 min. After incubation, the suspension was diluted about 8–10 times and the PSII membranes were precipitated at 30,000 g for 20 min, and thereafter the membranes were washed two times. Last pellet was resuspended in the storage buffer to get the chlorophyll concentration of 4 mg/ml. The treated PSII membranes were transferred into the calibrated EPR tubes. PPBQ (0.5 mmol, dissolved in DMSO, final DMSO concentration of 2% v/v) was added and the samples were illuminated with short (7 ns) light flashes from a Nd:YAG laser (400 mJ/pulse, 532 nm) to advance PSII to the $Y_D^{red}S_2$ (1 flash) or $Y_D^{red}S_3$ (2 flashes, 0.5 Hz) states. After laser flashes, the samples were frozen within 1 s in an ethanol-solid CO_2 bath, and then rapidly transferred into liquid nitrogen where they were stored until used. The dark (non-illuminated) samples were used to study PSII in the S_1 state.

EPR measurements were performed using a Bruker ESR-500 spectrometer and ST4102 standard cavity, equipped with an Oxford-900 cryostat and ITC-503 temperature controller. The S_2 state multiline signal was measured at 7 K with a microwave power of 13.1 mW and field modulation amplitude 20 G. The EPR signal from tyrosine $Y_D^{\bullet}$ and oxidized Cyt b_{559} was measured at 15 K. The $Y_D^{\bullet}$ radical was detected at microwave power of 1 μW and field modulation amplitude 3.2 G, and 5.3 mW microwave power and 16 G field modulation amplitude used for detection of oxidized Cyt b_{559}.

Results and Discussion

In the presence of reduced tyrosine (Y_D^{red}), the flash-induced turnover of S-cycle is rapidly desynchronized due to misses, the presence of Y_D^{red}, and the lack of a synchronized preflash (Styring and Rutherford, 1987; Feyziyev *et al*., 2003). The EPR signal (Fig. 1A) of oxidized tyrosine was absent in the dark sample (spectrum a) which shows that ascorbate-DAD treatment efficiently reduce tyrosine Y_D. The first flash resulted in appearance of small (~10%–14% of Y_D) tyrosine $Y_D^{\bullet}$ radical signal (spectrum b, state $Y_D^{red}S_2$). After the second flash further increase of the tyrosine $Y_D^{\bullet}$ signal (15%–16% of $Y_D^{\bullet}$, spectrum c) is occurred. Oxidation of the tyrosine results from electron transfer between Y_D^{red} and the oxidized species (preferable S_2 and S_3 states) of PSII prior freezing. Complete oxidation of tyrosine was achieved by room light / dark treatment (30 s vs. 5 min) of the reduced sample at room temperature (spectrum "max"). The registered spectrum of $Y_D^{\bullet}$ was identical with the signal of fully oxidized tyrosine Y_D obtained from the non-treated sample.

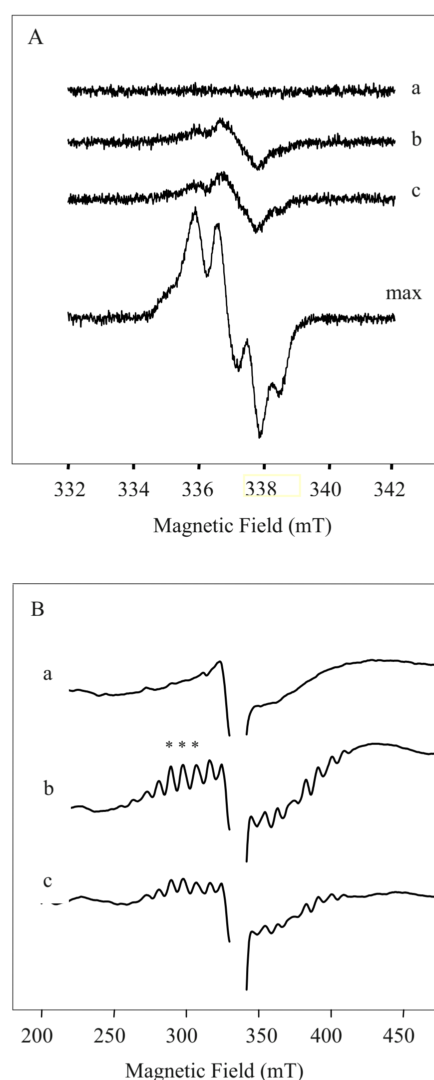

Fig. 1 EPR spectra of tyrosine $Y_D^{\bullet}$ (A) and S_2 state multiline signal (B) from $Y_D^{red}S_1$ (a), $Y_D^{red}S_2$ (b) and $Y_D^{red}S_3$ (c) states. Curve "max" (A) represent the maximal signal from $Y_D^{\bullet}$ registered in chemically reduced samples. The stars indicate the three lines used for the estimation o the multiline signal amplitude. EPR conditions are described in the section of Material and Methods.

Fig.1B demonstrates the EPP spectra of the S_2 state multiline signal. In the dark sample there was no observable multiline signal (spectrum a) indicating that nearly all centers were in the dark stable S_1 state. Illumination of the dark sample at 195 K rises the multiline spectrum (don't shown). The intensity of the signal was taken as maximal and used for estimation of the S_2 and S_3 population in the $Y_D^{red}S_2$ and $Y_D^{red}S_3$ centers. One-flash sample prepared with this reduction and illumination protocol was dominated by the S_2 state (spectrum b), although it also contained a considerable fraction of centers in the S_1 state. The amplitude of S_2 state multiline signal was further increased nearly 10%–20% in such samples. Similarly, a two-flash sample (S_3 state) prepared with our experimental protocol (spectrum c) also contained a large fraction of S_2 centers. Further illumination of the two-flash sample at 195 K hasn't increased the S_2 state of multiline signal indicating that virtually no centers remained in S_1 state. Thus, we can assume that about 80%–90% of PSII was found in the $Y_D^{red}S_2$ state after the first flash and 50%–60% in the $Y_D^{red}S_3$-state after the second flash.

The reduced PSII membranes illuminated with one or two flashes were stored at 195 K in total darkness and EPR spectra of the tyrosine $Y_D^{\bullet}$, multiline signal and Cyt b_{559} were registered at different time interval of the incubation.

In 1-flash sample (Fig. 2) which populated by the $Y_D^{red}S_2$ centers, the amplitude of the S_2-state multiline signal demonstrate an exponential decay up to 40% its maximal intensity during long term (~170 days) incubation at 195 K. The decrease of the S_2 multiline signal was concomitant with the oxidation of Cyt b_{559}. During incubation the signal of oxidized Cyt b_{559} rise up to 85% its maximal intensity (determined as a result of 77 K illumination of the sample). Signal of tyrosine $Y_D^{\bullet}$ radical also increased during the incubation; however tyrosine oxidation was significantly slower than oxidation of Cyt b_{559}.

The intensity of S_2 multiline signal registered from 2-flash sample ($Y_D^{red}S_3$ centers) incubated at 195 K shows (Fig. 3) quite different behavior than observed in $Y_D^{red}S_2$ centers: during the first 10–15 days of incubation the signal demonstrates an increasing behavior, and slow decrease followed after more than 200 days of incubation. Signal of oxidized Cyt b_{559} in 2-flash samples rise up to 88%–90% its maximal intensity. Again, the signal of $Y_D^{\bullet}$ radical demonstrate an increasing behavior during the incubation, however similar to the $Y_D^{red}S_2$ samples this process was less manifested than oxidation of Cyt b_{559}. The complex behavior of the S_2 state multiline signal in the $Y_D^{red}S_3$ samples can be explained by the increase of S_2 population in the initial step of the S_3-S_1 decay.

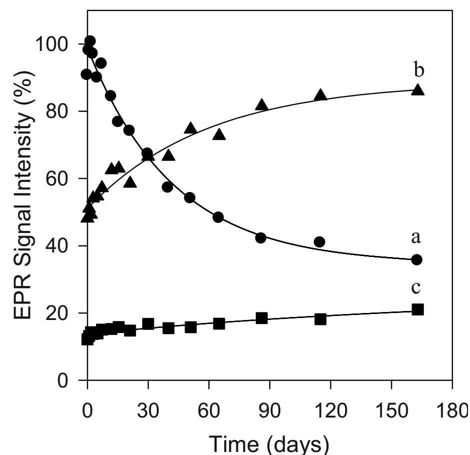

Fig. 2 The amplitudes of S_2 state multiline signal (a), cytochrome b_{559} (b) and tyrosine $Y_D^{\bullet}$ radical (c) registered during incubation of 1-flash sample (initial state was $Y_D^{red}S_2$) at the temperature of 195 K.

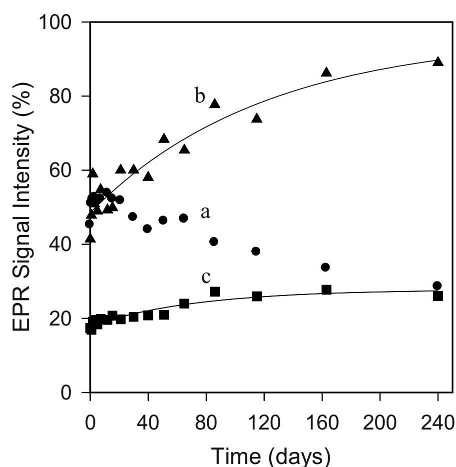

Fig. 3 The amplitudes of S_2 state multiline signal (a), cytochrome b_{559} (b) and tyrosine $Y_D^{\bullet}$ radical (c) registered during incubation of 2-flash sample (initial state was $Y_D^{red}S_3$) at the temperature of 195 K.

In ours previous study (Feyziyev *et al.*, 2003) we show that both reduced tyrosine Y_D and Cyt b_{559} becomes oxidized in significant extent during the dark storage of the S_2 and S_3 samples at 245 K which was nterpreted as a capability of Cyt b_{559} to compete with tyrosine Y_D for electron donation to the highest S-states through the redox equilibrium with other cofactors situated in PSII (for example Car/Chl_Z-P_{680}-Y_Z pathway) or in direct reactions. The results of present investigation demonstrate that during the

decay of both S_2 and S_3 states to S_1 at temperature 195 K significant oxidation of the Cyt b_{559} take place while tyrosine oxidation was negligible. Thus the results of present study may confirm the early interpretation. Apparently very low temperature, as it was shown, blocks the tyrosine Y_D oxidation in both the S_2 and S_3 samples which was manifested by a little increase of $Y_D^{\bullet}$ radical signal. The obtained results show that in the situation when tyrosine Y_D cannot compete for electron donation, is suitable for reduced Cyt b_{559} become a favorable candidate as an electron donor to higher S-states. However either the electron transfer from Cyt b_{559} to the S_2 and S_3 states of PSII is a result of direct reaction or this process occurs through the equilibrium with other components still is not clear.

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