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EPR study of charge equilibrium at low temperatures in the S_2 state of oxygen-evolving photosystem II particles

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The stability of EPR Signal II_s in oxygen-evolving PS II particles in the different S-states has been studied as a function of storage time at 77 K. Signal II_s in dark-adapted samples retains the same intensity after 1 month storage at 77 K, while that in the S_2 state produced by illumination at 195 K decays with a half-life time of about 5 days without decay in the manganese multiline intensity observed at 4.5 K. Signal II_s, once decayed in the S_2 state, recovers its original intensity upon dark adaptation of the sample at 210 K accompanied by a decrease in the multiline intensity. A model for the charge recombination process for the S_2 state involving Signal II_s is discussed.

EPR signal II_s; Decay kinetics; Charge recombination; Manganese multiline; PS II particle

1. INTRODUCTION

There has been considerable work on EPR Signal II in chloroplasts and PS II preparations to investigate its origin and the function of the species in the photosynthetic mechanism. Signal II_{vf} has been noted in untreated chloroplasts [1,2], while signal II_f has been observed in Tris-treated chloroplasts [3,4], PS II particles [5], and the purified PS II reaction center [6]. Both signals transiently appear with light illumination and decay in the time range of micro- and milliseconds, respectively. These signals are assigned to Z, the donor to P-680 [7]. Signal II_s has been assigned as the signal observed after dark adaptation and decaying slowly in the time range of a few hours after light illumination [4,8,9]. The signal also has been ascribed to a species situated at the donor side of P-680, although the location of the radical within the polypeptides of the PS II reaction center and its function have not yet been clarified. The radical species of Signal II_s has recently been assigned to a membrane-bound semiquinone cation radical by O'Malley et al. by means of EPR [10] and ENDOR[11] studies.

On the other hand, recent development in the preparation of PS II particles [8,12] and the identification of a multiline EPR found by Dismukes and Siderer [13] have yielded much progress in elucidating the mechanism of oxygen evolution. The multiline signal can be observed only at low temperatures below 20 K and has been assigned to EPR of Mn(III)-Mn(IV) dimer or tetramer originating from Kok's S_2 state [14]. The S_2 state could be created by one short light flash [13,15] or by continuous illumination at about 200 K [16,17]. Here, we have investigated the characteristics of EPR Signal II_s at low temperatures in the S_2 states formed by illumination of a PS II preparation at 195 K.

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Abbreviations: Chl, chlorophyll: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHC, light-harvesting complex; PS II, photosystem II

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2. MATERIALS AND METHODS

PS II particles were prepared from market spinach by the method of Kuwabara and Murata [12] and washed with final suspension buffer which includes 0.2 M sucrose, 20 mM Mops at pH 6.9 and 20 mM NaCl. Oxygen evolution was measured with a Clark type YSI electrode to be about 480 μmol O_2 evolved/mg Chl per h at 25°C in suspension buffer containing PPBQ as an acceptor. The PS II particles were stored in liquid nitrogen with 50 vol.% glycerol added until EPR measurement. The chlorophyll concentration in EPR samples was 5.8 mg/ml.

The EPR sample in a Suprasil quartz tube with its inner diameter of 3 mm was dark-adapted for more than 1 h at 0°C and equilibrated for 5 min at 195 K in a dry ice/methanol mixture. The S_2 state was produced by 5 min illumination at the same temperature by 500 W tungsten-halogen light through a 10 cm thick water layer and Kenko-SR60 filter. With these arrangements only light of wavelengths between 800 and 600 nm was effective in producing the S_2 state. The sample tube was stored in liquid nitrogen in the dark after cessation of illumination.

The EPR spectrum of Signal II_s was observed at X-band with a Varian 109 system at the fixed temperature of 77 K by using a finger-type insertion dewar, or with varying temperature from 87 to 225 K by a home-made nitrogen gas flow system.

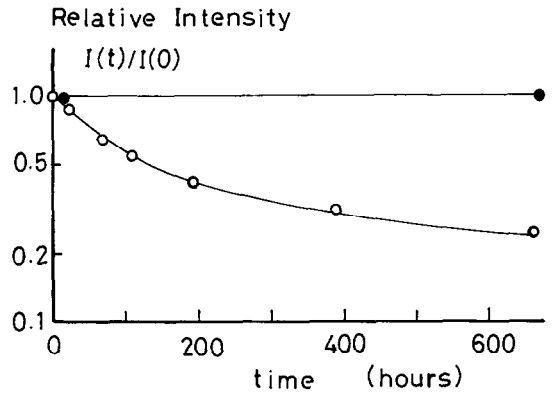

Fig.1. Normalized intensity $I(t)/I(0)$ of EPR Signal II_s at the $g = 2.0117$ position with varying storage time at 77 K for the dark-adapted sample at 0°C (●—●) and the sample illuminated at 195 K (○—○).

The multiline signal due to the S_2 state and Signal II_s were observed at 4.5 K by using an Oxford EPR-900 continuous flow cryostat. The EPR of Q_A^- was detected at about 30 K, where the multiline signal disappears completely. Cr^{3+}-doped MgO attached on the side wall of the TE_{102} cavity was used as an intensity standard over long periods of measurement and the relative error in the Signal II to Cr^{3+} EPR intensity ratio was less than 5% above 77 K. Below 30 K the Cr^{3+} signal was covered by multiline and Q_A^- signals and the error in absolute measurement was estimated to be 15%.

3. RESULTS

In fig.1 we have plotted the relative intensity of EPR Signal II_s measured at the $g = 2.0117$ position in the dark-adapted and illuminated samples after varying storage time at 77 K. The peak height $I(t)$ in the derivative curve of EPR Signal II_s in the dark-adapted sample showed no appreciable decay within 1 month storage at 77 K, while the sample illuminated at 195 K decayed in a biphasic way with its EPR intensity given by the following equation,

$$I(t)/I(0) = A\exp(-t/\tau_1) + (1-A)\exp(-t/\tau_2) \quad (1)$$

where the characteristic time $\tau_1 = 85 \pm 10$ h and is almost constant, while the values $\tau_2 = 1295 \pm 100$ h and $A = 0.6 \pm 0.1$ vary with sample preparation.

In fig.2 the line shapes of Signal II_s observed at 77 K under various conditions are shown; (a) Signal II_s in the dark-adapted sample, (b) that in the S_2 state immediately after illumination at 195 K, and (c) the difference, (b) − (a). The difference spectrum shows only a small signal with $g = 2.0022 \pm 0.0005$ and $H = 9.0$ G. Fig.2d shows Signal II_s in the illuminated sample after 16 days dark storage at 77 K. By subtracting (d) from (b) the decayed part of the signal (e) can be obtained. This signal shape (e) coincided with that shown in fig.2a for the dark-adapted sample by multiplication by 1.6. The decrease in intensity of Signal II_s of the illuminated sample accompanies the decrease in the Q_A^- signal at the $g = 1.84$ position observed at 30 K (not shown).

Even after prolonged storage at 77 K in darkness of the illuminated sample, when the temperature increased to 210 K, Signal II_s shown in fig.2d in-

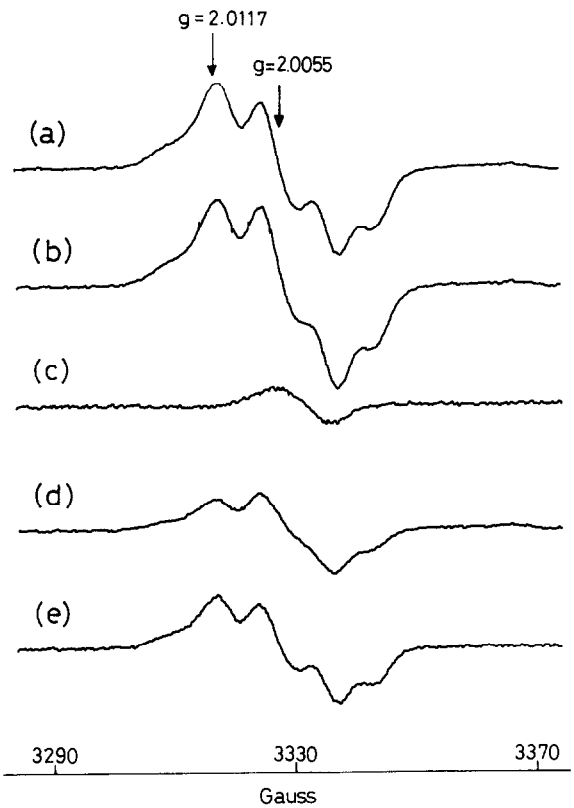

Fig.2. Line shapes of EPR Signal II_s under various conditions; (a) in the sample dark-adapted at 0°C and stored at 77 K; (b) in the sample illuminated for about 5 min at 195 K and stored for about 30 min at 77 K after illumination. (c) Subtraction [(b) − (a)] shows a sharp and small signal with $g = 2.0022$ and $\Delta H = 9$ G. (d) Line shape of EPR Signal II_s in the illuminated sample after 16 days storage at 77 K in the dark. (e) Subtraction [(b) − (d)] shows the line shape of the decayed part of Signal II_s coincides with that in (a) by multiplication by 1.6. EPR conditions: microwave frequency, 9330 MHz; power, 0.04 mW; modulation frequency, 100 kHz; amplitude, 5 G; temperature, 77 K.

creased its intensity again and recovered most of the original intensity within 30 min. Below 190 K we could not observe the recovery in intensity within 1 h, and the critical temperature was about 200 K where 20% recovery was observed within 1 h.

To confirm in which S state Signal II_s recovered its intensity we observed both Signal II_s and the multiline signal at 4.5 K. Fig.3a shows the multiline signal and Signal II_s within 2 h after illumination at 195 K. Fig.3b shows those signals after 76 days storage at 77 K in which Signal II_s decayed to about one-tenth of the original intensity, while the multiline spectrum shows only a slight decrease in intensity. We can also find a decrease in the negative peak between the field strengths 3500 and 3700 G even at 4.5 K in fig.3b compared to fig.3a, which can be ascribable to a decrease in Q_A^- [18] because of the long period of storage. Fig.3c shows the signals after the recovery of Signal II_s by 30 min dark adaptation at 210 K in which the multiline intensity has decreased to less than one-half of the original intensity, indicating that the S_2 state

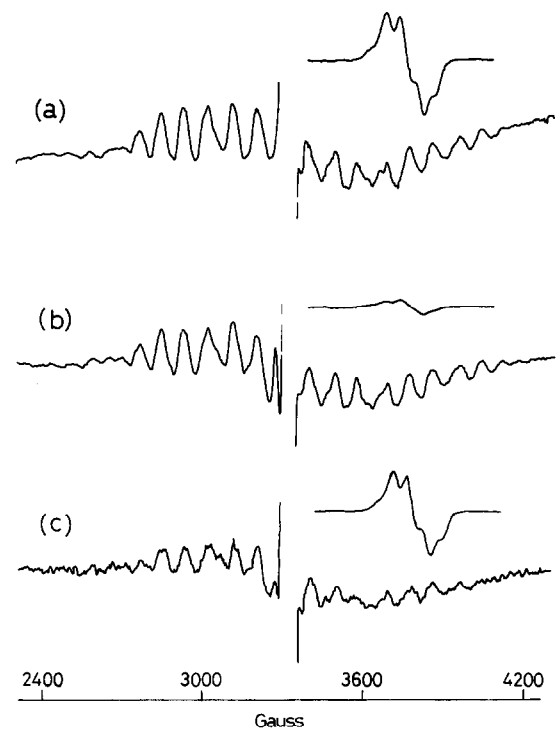

Fig.3. Comparison of multiline EPR and Signal II_s intensities observed at 4.5 K in the illuminated sample (a) immediately after illumination at 195 K, (b) after a prolonged storage time of 76 days at 77 K in the dark and (c) after dark adaptation at an elevated temperature of 210 K. EPR conditions: microwave power, 0.2 mW and 0.8 μW; modulation amplitude, 20 and 5 G for observation of multiline and Signal II_s, respectively. The field sweep range and gain for Signal II_s shown in the right upper part of each figure are one-tenth of those for the multiline. Other conditions are the same as in fig.2.

decayed to some other state accompanying the recovery of Signal II_s intensity. The recovered Signal II_s at 210 K did not decay again upon storage at 77 K in the dark even after long periods.

4. DISCUSSION

The results in section 3 show that EPR Signal II_s in oxygen-evolving PS II particles in the S_2 state has different decay kinetics at 77 K compared to those in a dark-adapted sample. In the dark-adapted particles at 77 K, where the S-state is expected to be in a mixed state of S_1 and S_0 [19], the intensity of EPR Signal II_s does not change on storage at 77 K. On the other hand, in the sample illuminated at 195 K the S-state exists almost completely in the S_2 state in which the multiline signal due to manganese clusters shows the maximum intensity [16]. Signal II_s in this sample decays on storage at 77 K with its characteristic time τ_1 about 85 h. The characteristic time τ_2 for the slower decay could not be determined exactly because it depends on sample preparations and may be explained by inhomogeneity in integrity of the particles. There was no decay in intensity of the multiline signal during storage of the sample as shown in fig.3b, showing no electron transfer between the water-splitting system (the S-state) and the Signal II_s species at 77 K. The decrease in intensity of Signal II_s, on the other hand, seems to be a consequence of electron transfer from the acceptor side of PS II. In fact, just after illumination we could observe a negative peak in the EPR spectrum at the position of 1.84 at an elevated temperature of 30 K where the multiline signal disappeared. We can ascribe the EPR signal to Q_A^- [18] and electron transfer between Q_A and Signal II species can be considered to occur at 77 K, since the intensity of the signal gradually decreased in proportion to the decrease in intensity of Signal II_s. Nugent et al. [20] also indicated electron donation from the Signal II species to the semiquinone-iron acceptor by freezing under illumination and recombination of both charges by storage at 77 K in the dark of the sample of intact *C. reinhardii* F54-14 particles.

Above 200 K the electron on the reduced Signal II_s species is apparently transferred to the water-splitting system and results in the recovery of EPR Signal II_s intensity and a corresponding decrease in the multiline intensity accompanying the transition from S_2 to the lower valence states. Velthuys and Visser [21] showed that Signal II_s recovered its signal intensity by flash illumination in chloroplasts treated with reduced DCIP and they ascribed the result to the recombination of the S_2 or S_3 state with DCIP-reduced Signal II_s species (D). The recovery in Signal II_s intensity at 210 K resembles this phenomenon and the recombination seems to take place between the S_2 state and the Q_A^--reduced Signal II_s species (D).

The results observed here also seem to have a close relation to the thermoluminescence studied by Rutherford et al. [22] in which recombinations between acceptor quinones and advanced S-states were indicated. We suggest that the Signal II_s species may also take part in this recombination process as an intermediate with a short lifetime.

The small signal which appears in fig.2c may be attributable to some chlorophyll radicals produced by light illumination at low temperature [23]. Its magnitude increases proportionally with deactivation of the water-splitting system, as in the case when the oxygen-evolving system is inactivated by treatment such as with 1 M $CaCl_2$.

Let us summarise our present result of electron transfer and recombination process in the PS II system as follows

$$D^+ + Q_A^- \xrightarrow{77\ K} D + Q_A \qquad (2a)$$

$$S_2 + D \xrightarrow{210\ K} S_1 + D^+, \qquad (2b)$$

where D^+ is the species responsible for Signal II_s and D is the reduced form of D^+. Our study of Signal II_s after prolonged periods of storage at low temperatures has presented a different approach to studying the mechanism of electron transfer in the PS II system by manifesting an intermediate state of charge recombination.

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