ON DIFFERENCES IN ACCESSIBILITY AND REDOX ACTIVITY OF D1-Y161 AND D2-Y160 OF PSII AS PROBED BY HALOGENATED ACETATES

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Key words: D2 protein, electron transport, EPR, Hydrophobicity, photosystem 2, polypeptide

1. Introduction

Photosystem II (PSII) includes at least seven intrinsic polypeptides (D1, D2, the α and β subunits of cytochrome b-559, CP47, CP43, and the psbI gene product) and three extrinsic polypeptides with molecular masses of 33, 23, 17 kDa as the key components of this photosystem. A new method has been set up to release sequentially PSII extrinsic polypeptides. When PSII particles were incubated with increasing concentrations of trichloroacetate (TCA), first 17, then 23 and finally 33 kDa polypeptide was released (1). In current research, we have studied how glycinebetaine, a compatible solute, selectively stabilizes the extrinsic polypeptides when PSII particles are treated by different methods. EPR signal measurement displayed that after the removal of the 33 kDa extrinsic polypeptide, small hydrophobic molecules, like halogenated acetates, can access and affect the redox activity of D2-Y160 (2). The model for the microenviroment around D1-Y161 and D2-Y160 are also provided (3).

2. Materials and Methods

Oxygen-evolving photosystem II particles were prepared from spinach by a Triton X-100 method and then were treated with halogenated acetates or other methods as described previously (1; C. Hou unpublished). Polypeptide composition was analyzed by SDS-PAGE in the Laemmli buffer system containing 6M urea using a slab gel containing 5% (stacking gel) and 13.75% (resolving gel) acrylamide. The gel was stained by Coomassie brilliant R-250. The abundance of Mn was measured with a Shimadzu atomic absorption spectrometer (AA-6501F). The dark-stable EPR spectra (signal II_{slow}) were measured with samples frozen in the dark after an illumination of 5 min and then a dark-adaptation of 30 min at room temperature. The light-induced EPR signal II spectra were measured with samples frozen during continuous illumination after a 5 min-illumination at room temperature. The hydrophobic contents (π) for halogenated acetates were estimated according to (4): $\pi = \text{Log } P_X - \text{Log } P_H$, where P_X is the partition coefficient of a halogenated derivative of acetic acid and P_H that of the acetic acid. In order to obtain some ideas about the microenvironments of Y_Z and Y_D, we used the computational methods published by Xiong et al. (3) for the modeling of PSII reaction center of C. reinhaidtii. An UNIX Silicon Graphics Power Series Workstation 4D/440 VGXT was used.

G. Garab (ed.), Photosynthesis: Mechanisms and Effects, Vol. II, 1181–1184.

3. Results and Discussion

3.1. The selective stabilization of extrinsic polypeptides in PSII by glycinebetaine

It was discovered (5) that glycinebetaine markedly stabilizes the PSII extrinsic polypeptides when the particles were treated with 0.8 mol/L tris (pH8.0) or high concentrations of NaCl. When the PSII particles were treated with heat shock or trichloroacetate (TCA), the stabilizing effect became less pronounced. Further study shows that the capability of halogenated acetates to release extrinsic polypeptides in PSII particles follows the order of decreasing molecular hydrophobicities: trichloroacetate (TCA, 1.54) > dichloroacetate (DCA, 1.33) > Monoiodoacetate (MIA, 0.84) > monobromoacetate (MBA, 0.64) > monochloroacetate (MCA, 0.32). The effect of stabilization became weaker as the hydrophobicity of molecules used became stronger. All the results imply that glycinebetaine is effective in stabilizing biopolymer structure against the action of electrolytes, while it is ineffective in protecting extrinsic polypeptides from dissociation induced by halogenated acetates or heat shock that might disturb the hydrophobic interaction between extrinsic polypeptides and the intrinsic parts of PSII core.

3.2. Halogenated acetates affects the redox activity of D2-Y160

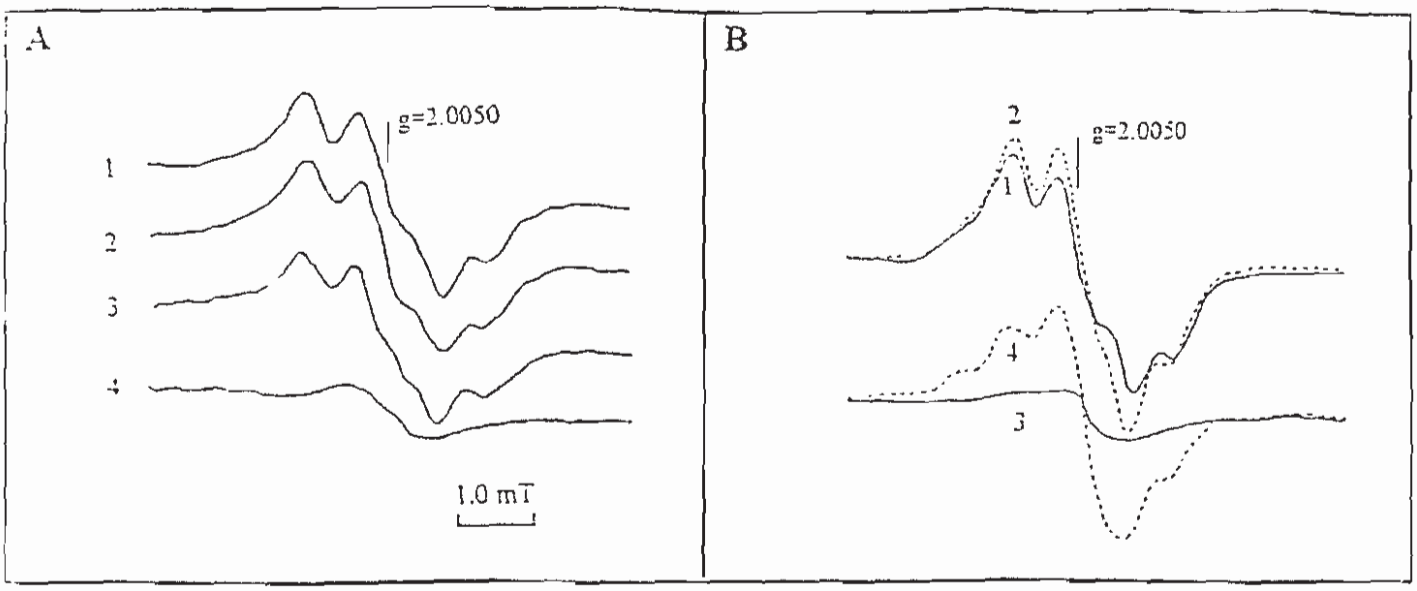

Fig. 1. EPR signals measured in PSII particles depleted of various extrinsic polypeptides with a Varian E-112 spectrometer. Chl concentrations are 3.6, and 3.0 mg/ml respectively in panels A and B. Spectrometer conditions: modulation frequency, 100 kHz; microwave frequency, 9.17 GHz; microwave power, 0.5 mW; response time, 0.25 s; center field set, 3.250×10^{-1} T; sweep width, 2.5×10^{-2} T. Modulation amplitude was 3.2×10^{-4} T. Panel A. Effects of removal of extrinsic polypeptides by TCA-treatment on EPR signal II_{slow}, displaying Y_D^+: (1) control; (2) -17 kDa; (3) -17, -23 kDa; (4) -17, -23, -33 kDa. Panel B. Dark-stable (solid curves) and light-induced (broken curves) EPR signal II spectra of control and TCA-treated PSII particles. Curves 1 and 2, control; curves 3 and 4, 500 mM TCA-treated PSII particles.

Two tyrosine residues on D1 and D2 polypeptides respectively, denoted asY_Z (D1-Y161) andY_D (D2-Y160), have been identified as redox active components at the

oxidizing side of the PSII electron transport chain. Oxidation of the Y_Z gives rise to characteristic EPR signal $II_{very\ fast}$ (decays in the time.scale of micro- to millisecond) and the appearance of EPR signal II_{solw} (decay in time scale of hours) is due to oxidized Y_D. These two EPR signals have similar spectral characteristics. Although they occur at symmetrical positions in D1 and D2 polypeptides, they are kinetically and functionally different (6).

Fig.1A shows the EPR signal II_{slow} measured in PSII particles in the presence or absence of 17; 17 and 23; 17, 23 and 33 kDa extrinsic polypeptides after TCA-treatment. When the 17 and 23 kDa polypeptides were removed from PSII, the EPR signal II_{slow} remained normal or only slightly modified (curves 2 and 3). However, when the 33 kDa polypeptide was released, the amplitude of the EPR signal II_{slow} was greatly decreased (curve 4), meanwhile 96% of the Mn were also removed. When TCA-treated PSII particles were dialyzed twice against 25 mM Mes-NaOH (pH 6.0) at 4℃ for 6 h in darkness, most of 33 kDa polypeptide had been reconstituted, and the EPR signal II_{slow} was totally restored, but the Mn content was not restored at all. This shows that the presence of 33 kDa polypeptide, not the other polypeptides or Mn is related to the change of the redox characteristic of Y_D.

Fig. 1B shows comparisons of the dark-stable or the light-induced EPR signal II spectra between the control and the 500mM TCA-treated PSII particles. Curves 1 and 3 arise from Y_D^+. The light-induced spectra (broken curves) were recorded for samples illuminated for 5 min at room temperature and then frozen during continuous illumination; therefore both Y_D^+ and Y_Z^+ were observed in the control intact PSII particles (curves 2). It shows that the amplitude of the EPR signal II in the light-induced samples (curve 2) is 20% larger than that in the dark-stable samples (curve 1). In the TCA-treated PSII particles (curve 4), in which the Mn complex had been removed, this caused an accumulation of Y_Z^+. The amplitude of the EPR signal II shows a only 20% decrease after the TCA-treatment in the light-induced PSII particles, not the 85% decrease shown for EPR signal II_{slow} affected by the TCA-treatment, implying that TCA has no obvious decreasing effect on EPR signal II_{fast}, and only a specific effect on Y_D. Further kinetic measurement is specially needed to study the redox property of Y_Z.

When 0.8M Tris (pH8.0) was used to remove the three extrinsic polypeptides, the EPR signal II_{slow} was normal. It was not detected after 100mM TCA treatement. We found that the capability of halogenated acetates to suppress the EPR signal II_{slow} in the Tris-washed PSII particles correlates well with their hydrophobicity(2). We propose that the 33 kDa extrinsic polypeptide might help to keep a special microenvironment around Y_D. When it is removed, the microenvironment around Y_D might become susceptible to attacks by small hydrophobic molecules or other factors.

3.3.The microenvironments of D1-Y161 and D2-Y160

Computational models of D1/D2 polypeptides have been proposed after molecular homology and energy minimization (3, 7). Here we show the microenvironments around D1-Y161 and D2-Y160 (Fig.2). It shows that around D1-Y161, there are 15 amino acids: D1-I163, D1-G164, D1-V160, D1-L159, D1-Q165, D1-P162, D1-A294, D1-G171, D1-D170, D1-M293, D1-T292, D1-S291, D1-A287, D1-L290, D1-F295. Note the absence of D1-H190 within this region. On the other hand, around D2-Y160, there are 18 amino acids: D2-A290, D2-H189, D2-L289, D2-F169, D2-Q164, D2-G163, D2-L162, D2-P161, D2-F185, D2-G285, D2-W191, D2-V287, D2-V286, D2-A170, D2-L158, D2-F157, D2-V156, D2-P171 (see Fig.2 A, B). Note the presence of D2-H189 in the neighborhood. Further experiments will be designed with C. reinhaidtii for the correct analysis of the relationship between their microenvironment and function. Recently, Kim and Barry (8) have suggested that a disparity in the number of accessible proton accepting groups could

influence electron transfer rates and energetics and account for functional differences between Y_Z and Y_D (8). This clearly supports the hypothesis that Y_Z and Y_D differ in their interaction with the environment.

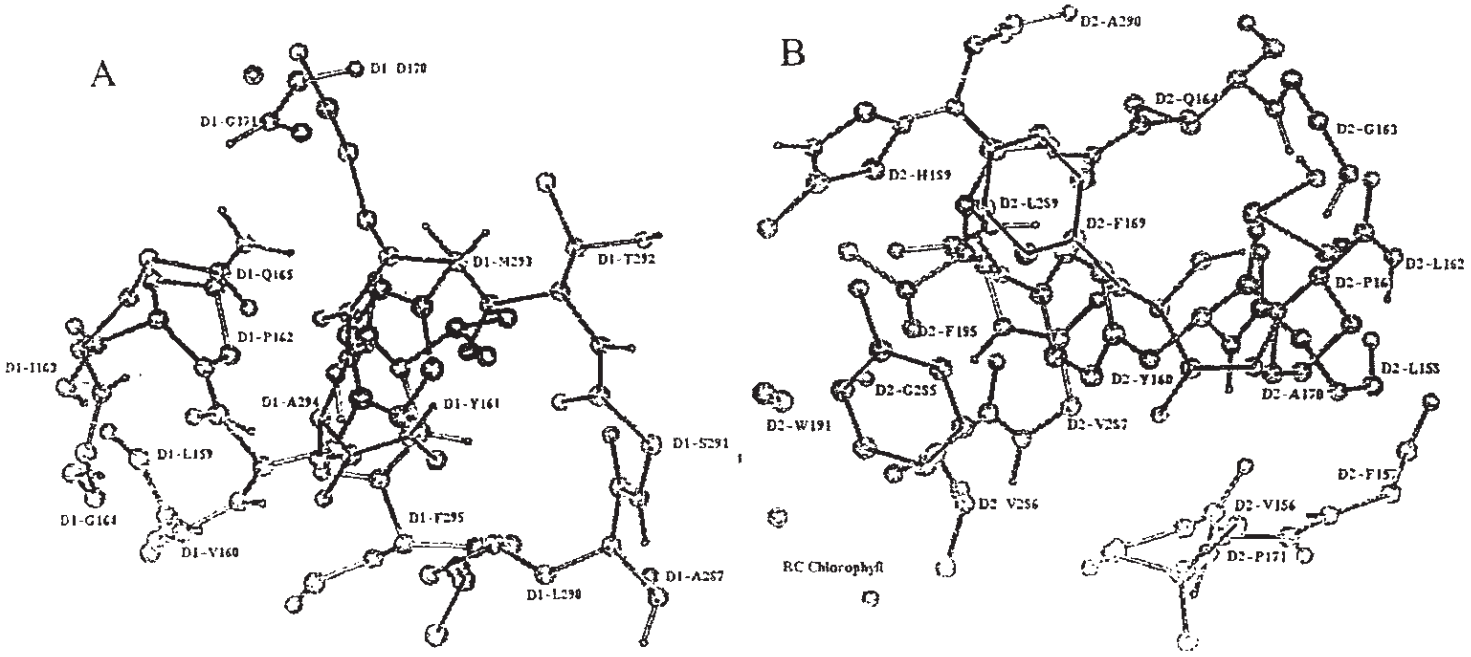

Fig.2. The microenvironment of amino acids, displayed by spheres that have 8A diameters with their origins at the very centers of the D1-Y161 and D2-Y160 molecules of the modeled *C. reinhaidtii.* PSII reaction center, are shown. The knowledge-based 3-dimensional model was established by J. Xiong, S. Subramaniam and Govindjee (1998, Photosyn. Res., in press).

This study was supported by NSF of China (39730040) and the grant for 95 fundamental key items from the Chinese Academy of Sciences.

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