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Digalactosyl diacylglycerol-mediated Stability toward Thermal Denaturation of Oxygen Evolution in Photosystem II Core Complex

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Introduction

Photosystem II core complex (PS II CC) is a pigment-protein complex that is involved in the electron transport of the photosynthetic process and responsible for the light-induced water oxidation to produce molecular oxygen (Hankamer et al., 1997). It contains at least 10 polypeptides, including hydrophilic and integral membrane polypeptides. In other words, integral polypeptides of PS II CC contain D1 and D2 proteins, two subunits α and β of cytochrome b559, two core antenna proteins or Chl a-binding proteins CP43 and CP47, whereas extrinsic protein consists of a 33-kDa polypeptide that protects the binding site of water molecules involved in the oxidation process (Seidler, 1996). PS II CC also binds different cofactors, pigments and lipids.

The PS II CC contains only three molecules each of monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and phosphatidylglycerol (PG) per P680 (Murata et al., 1990). The molecular ratio of lipid to photochemical reaction center II (P680) in PS II CC was estimated to be 10:1. Since this complex is highly active in photochemical charge separation and oxygen evolution, one can expect that either some or all of these lipid classes are necessary to regulate PS II CC activity and to maintain the structural organization of PS II CC. However, the functional and structural roles of these lipids in PS II CC still remain elusive and ambiguous, and so the foregoing hypothesis needs more evidence to be supported.

In this respect, we attempt to explore the roles of photosynthetic membrane lipids on the protection of the PS II CC against temperature so as to expound how specific lipids take part in the function of the PS II CC involved in the photosynthesis process. We show hereunder that DGDG has a tendency to stabilize the oxygen-evolving activity in PS II CC against the detrimental effects of heat stress.

Materials and Methods

The PS II CC preparations were isolated from chloroplasts of spinach according to Ghanotakis and Yocum (1986). The final PS II CC pellet was washed 2~3 times in a buffer containing 0.4 mol/L sucrose, 15 mmol/L NaCl, 10 mmol/L $CaCl_2$, 50 mmol/L Mes-NaOH (pH 6.0) and the resultant PS II CC particles were resuspended in the same buffer. Chlorophyll (Chl) concentrations were determined in 80% (v/v) acetone solutions using the method of Arnon (1949).

The DGDG liposomes were prepared as the procedure that lipid dissolved in chloroform was dried under a stream of N_2, then dispersed in the above buffer, and followed by sonication for over 30 min. After that, aliquots of PS II CC solutions in the same buffer were added to the liposome solution to make a required ratio of lipid to Chl. These preparations, namely PS II CC complexed with DGDG, are denoted hereunder as the PS II CC ~ DGDG complex.

Heat treatment was carried out by placing samples at a concentration of about 1.0 mg/Chl for 5 min at various temperatures ranging from 25 to 75°C in the dark, and then samples were immediately subject to oxygen evolution measurement and infrared spectroscopy assay.

The determinations of oxygen evolution of samples suspended in buffer containing 500 μmol/L 2,5-dichloro-benzoquione (DCBQ) as electron acceptor were performed using Clark-type electrode (Hansatech D.W. Oxygen electrode Unit, King's Lynn, HK). The samples were irradiated with saturated light for a period of about 1 min.

The samples for infrared measurements at a concentration of 1.0 mg/ml Chl were dropped on a ZnSe window, then dried in a vacuum to be a layer of semihydrated film in darkness. Fourier transform infrared spectra were recorded on a Nicolet Avatar 360 spectrometer (Nicolet Instrument Corporation, USA). 100 interferograms per spectrum, background and sample, were collected and co-added. The analysis of spectra was performed by the methods of De Las Rivas et al. (1997) and Segui et al. (2000).

Results and Discussion

The PS II CC, the minimal and basic functional subunit of PS II for oxygenic photosynthesis process, is highly active in oxygen evolution. In this work, the oxygen-evolving activity in PS II CC was 600 ~ 800 μmol O_2 · mg Chl^{-1}· h^{-1}. It was found that this activity was increased by as high as about 30% when PS II CC interacted with DGDG vesicles, indicating a stimulatory effect of DGDG on the PS II CC activity.

Fig.1 and Fig.2 display respectively the effect of DGDG on the oxygen evolution activity of PS II CC treated by heat at various temperatures for 5 min and incubated with time at 38 °C in darkness. In these two figures, the average 100% oxygen evolution in PS II CC or PS II CC ~ DGDG (10 mg DGDG/mg Chl) is 725 or 943 μmol O_2 · mg Chl^{-1}· h^{-1}, respectively.

As can be seen in Fig.1, the temperature of semi-inactivation of oxygen evolution in PS II CC increased from 40 to about 43 °C in the presence of DGDG. Furthermore, when incubated for 5 min at 45°C, the PS II CC showed no activity any more, whilst DGDG-complexed PS II CC still retained a 16% of activity (100% for 25°C). In addition, from Fig.2, a one-hour incubation at 38°C inactivated absolutely the oxygen evolution for PS II CC. By contrast, there remained about 20% of activity (zero time for 100%) for the complex in the presence of DGDG under the same condition. These results probably reflect the role of DGDG in the protection of PS II CC against the deleterious effects of temperature.

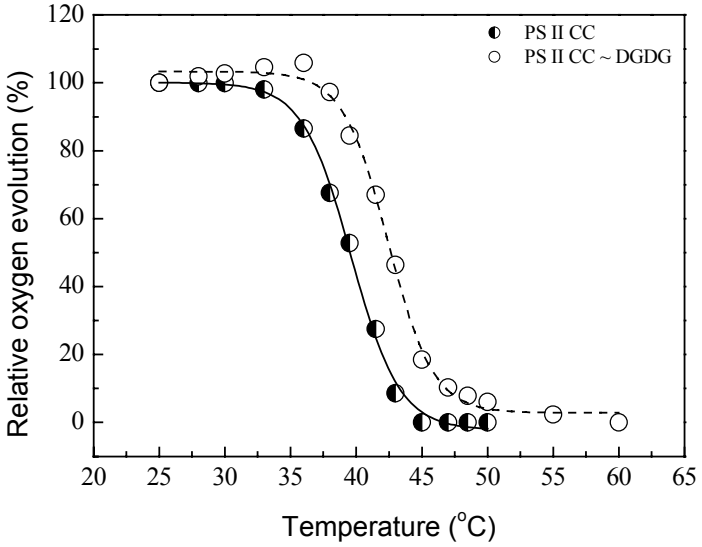

Fig. 1. Effect of heat treatment on the oxygen evolution in PS II CC and PS II CC ~ DGDG complex. The chlorophyll (Chl) concentration is 1.0 mg Chl / ml. The average 100% oxygen evolution in PS II CC or PS II CC ~ DGDG (10 mg DGDG/mg Chl) is 725 or 943 μmol O_2 · mg Chl^{-1}· h^{-1}, respectively.

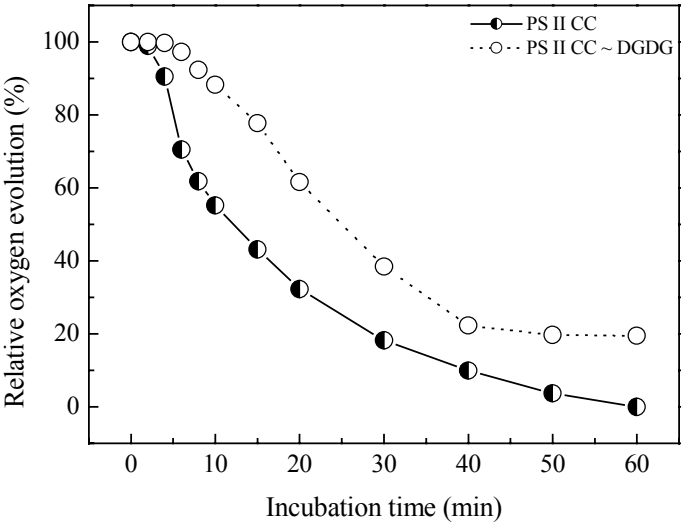

Fig. 2. Oxygen evolution in PS II CC and PS II CC ~ DGDG complex incubated at 38 °C in the dark. The chlorophyll (Chl) concentration is 1.0 mg Chl / ml. The average 100% oxygen evolution in PS II CC or PS II CC ~ DGDG (10 mg DGDG/mg Chl) is 725 or 943 μmol O_2 · mg Chl^{-1}· h^{-1}, respectively.

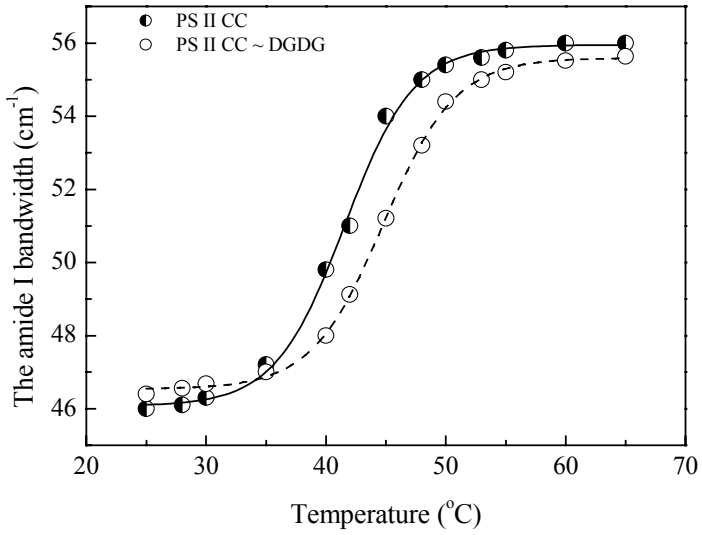

Fig. 3. The changes in the amide I bandwidth of PS II CC or PS II CC ~ DGDG in D_2O at half-height of the maximum peak from 1700 and 1600 cm^{-1} against temperature. The chlorophyll (Chl) concentration is 1.0 mg Chl / ml. The mass ratio of DGDG to PS II CC is 10 mg DGDG/mg Chl.

Fig.3 is a representation of the changes in the width of the amide I band of PS II CC or PS II CC ~ DGDG in D_2O at half-height between 1700 and 1600 cm^{-1} in the temperature range from 25 to 65 °C. The isosbestic points of the sigmoidal curves occur at ~41.3°C for PS II CC and at 44.7°C for PS II CC ~ DGDG, respectively. These transition temperatures in the amide I bandwidth are almost correspond to the inactivation transitions in the curves of Fig.1, although not exactly the same ones. These midpoints can be considered to be the main and distinct thermal transition in infrared spectra (De Las Rivas et al., 1997). Thus, the infrared findings are another indication of the regulatory effect of DGDG on PS II CC against thermal denaturation.

In our other infrared study, we have found that the interaction of DGDG with PS II CC primarily leaded to a decrease in α-helix content parallel to an increase in β-strand of the protein secondary structures of PS II CC. In addition, the conformation and microenvironment of tyrosyl residue of PS II CC proteins were markedly affected. Furthermore, the DGDG effect also gave rise to a considerable increase in the concentration of bound OH. The results demonstrated that the DGDG action on PS II CC is related to the polar and/or fatty acid parts of DGDG, which results in the redistributions of secondary bonds in PS II CC proteins. It was suggested that the active sites of PS II CC with DGDG is in the extrinsic protein of 33 kDa, as pointed out by Gabashvili et al. (1998).

As a conclusion, the above results and considerations indicate that DGDG has the ability to enhance the thermal stability of oxygen evolution in PS II CC. It is likely that DGDG-induced restraint on the inhibitory effect from heat is due to the molecular rearrangements of PS II CC proteins upon interactions with DGDG. We remark that the role of DGDG is probably on the protection of 33 kDa protein from disassociating from PS II CC so as to hinder the Mn release from the oxygen evolution complex induced by heat stress.

Acknowledgements

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References

Arnon D I (1949) *Plant Physiol*. **14**, 1-15.
De Las Rivas J, Barber J (1997) *Biochemistry* **36**, 8897-8903.
Gabashvili I S, Menikh A, Segui J, Fragata M (1998) *J. Mol. Struct.* **444**, 123-133.
Ghanotakis D F, Yocum C F (1986) *FEBS Lett.* **3449**, 244-248.
Hankamer B, Barber J, Boekema E (1997) *Annu. Rev. Plant Physiol.Plant Mol. Biol.* **48**, 641-671.
Murata M, Higashi S, Fujimura Y (1990) *Biochim. Biophys. Acta.***1019**, 261-268.
Segui J A, Maire V, Gabashvili I S, Fragata M (2000) *J.Photochem. Photobiol. B: Biol.* **56**, 39-47.
Seidler A (1996) *Biochim.Biophys. Acta.* **1277**, 35-60.