Oxygen-evolving Activity in Photosystem II Core Complex of Photosynthetic Membrane in the Presence of Native Lipid

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The techniques of oxygen electrode polarography and Fourier transform infrared (FT-IR) spectroscopy were employed to explore the involvement of digalactosyl diacylglycerol (DGDG) in functional and structural roles in the photosystem II core complex (PSIICC). It was shown that DGDG exhibited the ability to stimulate the oxygen evolution in PSIICC, which was accompanied by the changes in the structures of PSIICC proteins. The results revealed that there existed hydrogen-bonding interactions between DGDG molecules and PSIICC proteins. It is most likely that the sites of PSIICC interaction with DGDG are in the extrinsic protein of 33 kDa.

Keywords photosystem II core complex, digalactosyl diacylglycerol, oxygen evolution, protein structure

Introduction

Oxygenic photosynthesis is carried out in photosynthetic membranes in which highly organized pigment-protein complexes are embedded in the lipid matrix. It is well established that specific interactions might exist between pigment-protein complexes and lipids. For example,[1] the chlorophyll (Chl) binding protein complexes CP43 and CP47 of photosystem II (PS II) are highly enriched in monogalactosyl diacylglycerol (MGDG). The major Chl *a*/*b* light-harvesting complex of photosystem II (LHC II) is found to be tightly associated with digalactosyl diacylglycerol (DGDG) and phosphatidyl glycerol (PG) predominantly with palmitic acid or 16:1-*trans*-hexadecenoic acid chains. The binding sites for PG molecules in PS II reaction complex (PSIIRC) are suggested to reside in the protein of D1, where PG might sustain the optimal conformation of D1. The coupling factor complex (CF_0-CF_1) has been shown to contain only sulfolipid-sulfoquinovosyl diacylglycerol (SQDG), which seems to be defective in nonphotosynthetic organism.

The fatty acid and glycerolipid compositions of three types of preparations of PS II from spinach thylakoids, namely the PS II membrane, the PS II core complex (PSIICC) and PSIIRC have been well determined.[2] The molecular ratios of lipid to photochemical reaction center II (P680) in these preparations were estimated to be 150:1, 10:1 and 1:1, respectively. It was found that the PSIICC has a specific association with lipid molecules, that is, it contains only three molecules each of MGDG, DGDG and PG per P680. It is noteworthy that the fatty acids of these three lipids are much more saturated (50% of the total fatty acids) than those in the bulk lipids of thylakoid and PS II membrane (10% of the total fatty acids). Since increased saturation of fatty acids results in a more rigid structure of lipid molecules, the occurrence of saturated fatty acids in the lipids suggests that they stabilize the conformation of the polypeptides of this core complex. On the other hand, 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 for the PSIICC to function the

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activities of this complex.

In this regard, however, little information has yet been available on the effects of MGDG, DGDG, PG or their mixtures on the PSIICC activity, and so the above hypothesis needs more experiments to support. This prompted us to investigate the oxygen-evolving characteristics of PSIICC in the presence of the thylakoid membrane lipids. In the present work, the involvement of DGDG in functional and structural roles in PSIICC has been investigated by oxygen electrode polarography and Fourier transform infrared (FT-IR) spectroscopy.

Experimental

Materials

Digalactosyl diacylglycerol (DGDG) was purchased from Sigma Chemical Co. and used without further purification. Spinach (*Spinacia oleraceal L.*) was from local market.

Methods

Preparation of the PSIICC *particles*

The PS II membranes were isolated from chloroplasts of spinach according to Chapman *et al*.[3] with a modification as Yang *et al*.[4] described. And the PSIICC complexes were prepared from the PS II membranes by the method of Yocum *et al*.[5] The final PSIICC pellet was washed 2—3 times in a buffer containing sucrose (0.4 mol/L), NaCl (15 mmol/L), $CaCl_2$ (10 mmol/L), Mes-NaOH (50 mmol/L, pH 6.0) and the resultant PSIICC particles were resuspended in the same buffer. Chlorophyll (Chl) concentrations were determined in 80% (*V*/*V*) acetone solutions using the method of Arnon.[6]

Preparation of PSIICC-DGDG *complex*

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 PSIICC solutions in the same buffer were added to the liposome solution to make a required ratio of lipid to Chl. These preparations, namely PSIICC complexed with DGDG, are denoted hereunder as the PSIICC-DGDG complex.

Determination of oxygen evolution

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

Infrared measurements and analysis of spectra

The samples in D_2O at a concentration of ~1.0 mg Chl/mL were dropped on a ZnSe window, and then dried in a vacuum to be a layer of semihydrated film in the dark. Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet Avatar 360 spectrometer (Nicolet Instrument Corporation, USA) equipped with DGTS KBr detector. The spectra were recorded in the range of 400—4000 cm^{-1} with a spectral resolution of 2 cm^{-1} or 4 cm^{-1} and were apodized with a Happ-Genzel function before Fourier transformation. One hundred interferograms per spectrum, background and sample, were collected and co-added. The removal of the solvent signal was done by subtraction of the IR spectra of buffer from the spectra of each sample within the 1900—1400 cm^{-1} region. An adequate removal of the water band was done by subtracting till a flat baseline in the 1900—1750 cm^{-1} region was achieved. This was equivalent to eliminating the specific band for water at 2125 cm^{-1}.[7]

The different FT-IR spectra difference of between PSIICC-DGDG complex and PSIICC control were obtained by the method of Heimburg and Marsh.[8] In brief, the normalization of the PSIICC-DGDG complex spectrum or the PSIICC spectrum was first performed by defining the areas under the band envelope in the region of study according to the expression $A_0 = kA_n$, where A_n is the integral of the observed surface under the band envelope calculated with the Omnic E. S. P software, version 5.1 (Nicolet Instrument Corporation, USA), k a scale factor that normalizes A_n to 100, and A_0 the normalized area, *i.e.*, 100. Then, the subtraction of the PSIICC control spectrum from that of PSIICC treated by DGDG was done.

Results

DGDG *effect on oxygen-evolving activity of* PSIICC

The PSIICC is highly active in oxygen evolution. In this

work, the oxygen-evolving activity in PSIICC was 600—800 μmol O_2/mg Chl·h at (25.0 ± 0.2) ℃ in darkness. Fig. 1 shows the oxygen evolution activities of PSIICC preparations in the absence of DGDG and in its presence. In this figure, the control samples were PSIICC preparations which were not incubated with DGDG vesicles. The average 100% oxygen evolution in PSIICC is corresponds to 725 μmol O_2/mg Chl·h.

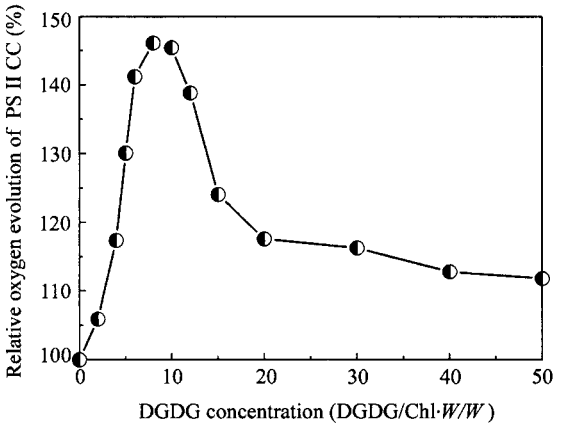

Fig. 1 Dependence of oxygen evolution in PSIICC on the concentration of DGDG. The average 100% oxygen evolution in PSIICC corresponds to 725 μmol O_2/mg Chl·h.

It was first found that this activity of PSIICC particle was enhanced when PSIICC interacted with DGDG, not only at relatively low concentration of DGDG/Chl (mg/mg) (*i.e.*, 2—10 mg DGDG/mg Chl) but also at high values of 12—50 mg DGDG/mg Chl in the region of study.

Secondly, the stimulative effect of DGDG on the PSIICC activity is relied on the content of DGDG. For example, one observes a rapid increase of oxygen evolution as the DGDG/Chl increases from 2 to 10 mg DGDG/mg Chl, whereas further increase of DGDG/Chl (12—50 mg DGDG/mg Chl) gives rise to a depressed stimulation of the activity of PSIICC.

From the above results, a direct finding is that there exists the dependence of oxygen evolution in PSIICC on the concentration of DGDG which exhibits a stimulative effect on the oxygen evolution of PSIICC.

DGDG *effect on the infrared absorbance of* PSIICC

The oxygen evolution studies show that the major stimulative effect of DGDG is associated with PSIICC activity. It is therefore reasonable to assume that a specific association of the lipid with the proteins of PSIICC complex may have been affected. It could be shown that the DGDG effect indeed modifies the protein secondary structures in PSIICC characterized by Fourier transform infrared (FT-IR) spectroscopy.

FT-IR spectroscopy has shown its special usefulness in the analysis of protein structure.[7,9-13] It can be successfully applied to detect small changes in the structures of protein complex. The spectral region which is most widely used in infrared investigations is carried out in the band ranging from 1700 cm^{-1} to 1500 cm^{-1}. This is because the region primarily contains the amide I and amide II bands which are very sensitive to conformational changes in the protein secondary structures (*i.e.* α-helix, β-sheet, β-turns, random coil *et al.*). The FT-IR spectroscopic techniques therefore are very useful for detecting minor alterations in the protein structures of PSIICC perturbed, perhaps, by DGDG effect in the present work.

A typical FT-IR spectrum over a range of 1700—1500 cm^{-1} of PSIICC particles and PSIICC-DGDG complex is shown in Fig. 2 [(A) and (B), respectively]. These spectra are characteristic of four main spectral regions in the band, that is, the amide I (1700—1620 cm^{-1}), tyrosine I (1620—1580 cm^{-1}), amide II (1580—1520 cm^{-1}), and tyrosine II (1520—1500 cm^{-1}). It has been well established that the amide I with a strong absorption at about 1654 cm^{-1} arises from the in-plane C = O stretching vibration (80%) with weakly cou-

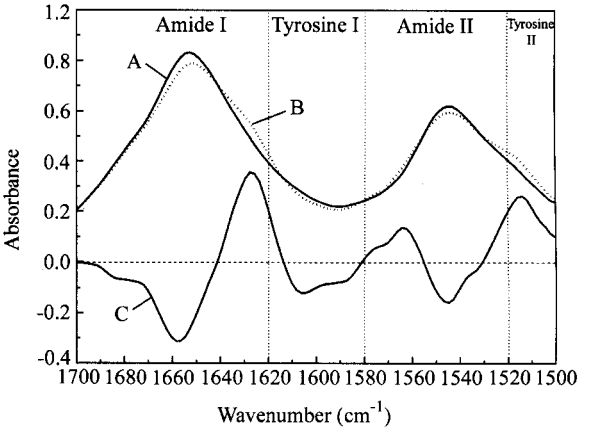

Fig. 2 FT-IR spectrum of PSIICC (A), PSIICC-DGDG particle (B) and the different infrared spectra of (B-A) × 5 (C) in the range of 1700—1500 cm^{-1}. The concentration of DGDG is 10 mg DGDG/mg Chl.

pled with C—N stretching and N—H bending. The amide II of proteins between 1580 cm^{-1} and 1520 cm^{-1} with a maximum of about 1544 cm^{-1} is associated with the in-plane N—H binding (60%) strongly coupled with C—N stretching vibration (40%). The absorbance of tyrosine I is attributed to the vibration of phenol ring skeleton while the tyrosine II arises from the stretching combination with the bending vibration of phenol ring.[9-13]

In the present work, it was noted that DGDG-treated PSIICC exhibits considerable changes in the absorbance compared to the PSIICC particle in the range of 1700—1500 cm^{-1}, namely the four main spectral bands (Fig. 2, curve C). This is discussed below.

Amide I *and amide* II *bands*

The DGDG effect causes significant alterations of the bands of amide I and amide II, which reflects a variation in the polypeptide skeleton of the PSIICC proteins. A downshift of the maximum absorption of the amide I from 1654 cm^{-1} to 1650 cm^{-1} is firstly observed, therefore indicating the DGDG-induced changes in the protein secondary structures of PSIICC. Curve C in Fig. 2 displays clearly a DGDG-induced IR reduction in the amide I region with a minimum at 1657 cm^{-1} (α-helix conformation) and a less pronounced minimum at 1684 cm^{-1} (β-turns). Fig. 2 (C) shows also an IR increase in this region with a maximum at 1626 cm^{-1}. Additionally, in the region of amide II, DGDG-induced changes have experienced an IR absorbance gain with a maximum at 1565 cm^{-1} accompanied by a loss with a minimum at 1545 cm^{-1} which is also assigned to α-helical structure.

From the above results and considerations, a major observation in this work is that DGDG effect on PSIICC gives rise to the modifications in protein conformations. In the amide I region, such a variation in the protein secondary structures exhibits a different tendency. That is to say, a marked positive change in β-strands (~ 1626 cm^{-1}) is accompanied by an opposite change in α-helix content (~ 1658 cm^{-1}) as well as β-turns (~ 1684 cm^{-1}) conformations.

In this respect, it was noted that the β-strands, *i.e.*, extended chains in protein chains are often found as hydrogen-bonding donors and acceptors in amino acid residues which do not participate in intramolecular β-sheets.[7,14] On account of the augmentation of β-strands in protein structures induced by DGDG interaction, one may expect an increase of bound-OH groups in the IR spectrum of PSIICC particles. Fig. 3 displays the FT-IR spectra of PSIICC particles from 3700 cm^{-1} to 3000 cm^{-1} (OH region) (A), PSIICC-DGDG (B), and the different FT-IR spectra (C), *i.e.*, [PSIICC-DGDG]-minus-[PSIICC]. It is obviously seen that the considerable alterations in the OH absorbance spectrum of PSIICC imposed by DGDG interaction also took place. One observes an increase with three maximum at 3430, 3237 and 3139 cm^{-1}. It is remarked that the spectral region around 3430 cm^{-1} is generally assigned to free OH groups, whereas the regions around 3237 and 3139 cm^{-1} are corresponding to bound OH groups.[15] In Fig. 3, the difference in FT-IR spectrum (C) indicates clearly that the treatment of the PSIICC particles with DGDG causes a marked increase in the concentration of bound OH groups concomitantly with an increase in the concentration of free OH. This is mechanistically in accordance with the observation of an increase in the extended chains (β-strands) content in PSIICC accompanied by a decrease in the α-helix content.

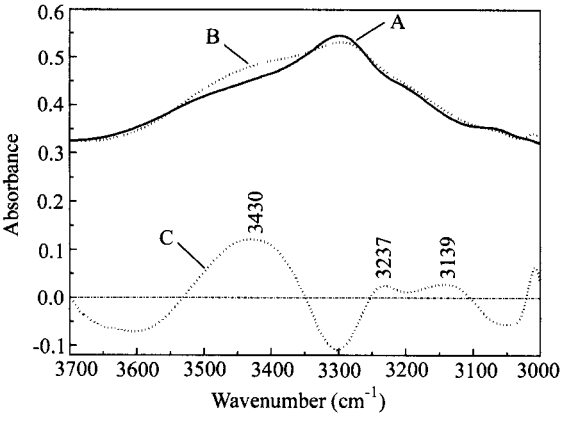

Fig. 3 FT-IR spectra of PSIICC (A), PSIICC-DGDG particle (B) and the different infrared spectra of (B-A) × 6 (C) in the range of 3700—3100 cm^{-1}. The concentration of DGDG is 10 mg DGDG/mg Chl.

Tyrosine side chains bands

The absorbance changes resulting from DGDG effect take place not only in the protein secondary structures but also in the tyrosine side chains, as shown Curve C in Fig. 2. In other words, in the region of tyrosine, the dominating modifications are in the absorbance bands at around 1605, 1585 and 1514 cm^{-1}.

The tyrosine residues of PSII proteins are attributed

to aromatic compounds and therefore display phenyl chain absorption in the FTIR spectrum, which is characteristic of the stretching vibration of aromatic ring skeleton between 1620 cm^{-1} and 1500 cm^{-1}.[16-18] As for phenyl ring, it absorbs characteristically around 1600 cm^{-1} and 1500 cm^{-1}. The vibrational mode at ~ 1600 cm^{-1} is representative of the benzene ring stretching fundamental vibration, and the band at ~ 1500 cm^{-1} is assigned to a mixture of ring stretch and deformation. However, these absorbance bands would be affected upon substitution in the phenyl ring. For example, the band at 1600 cm^{-1} splits into two bands at ~ 1600 and ~ 1580 cm^{-1} along with their enhancement intensities when the benzene ring conjugated with the group with lone-pair electrons. Additionally, *para*-substitution in phenyl ring results in an upshift of 1600 cm^{-1} to higher frequency, and when substituting group is electron donor the change in absorbance position from 1500 cm^{-1} to ~ 1510 cm^{-1} will take place. Because tyrosine residues of proteins are rendered with other function groups, characteristic absorption may occur in the region of 1620—1500 cm^{-1}.

According to the considerations as described above, the bands at 1605 and 1585 cm^{-1} appeared in Fig. 2 (C) are assigned to the vibration of phenol ring skeleton while the absorption of 1514 cm^{-1} is associated with the stretching combination with the bending vibration of phenol ring. As Fig. 2 (C) has indicated, the DGDG effect on PSIICC leads to decreases with a minimum at ~ 1605 cm^{-1} and the less pronounced at 1585 cm^{-1}, whereas the major change in the band of tyrosine II is an increase in IR absorbance with a maximum at ~ 1514 cm^{-1}.

In addition, the corresponding absorbance intensities of these bands are considerably changed, as illustrated by the normalized infrared spectra (Curve C) in Fig. 2. This means that the molecular environment of the tyrosine residues of PSIICC proteins has been altered, in as much as the intensity of an infrared absorbance band is proportional to the square of the change in the dipole moment of the vibrational group. It is taken into account that the dipole moment is proportionally related to the local dielectric constant or the polarity of the surrounding molecular environment.[19] Thus, it is concluded that the binding of DGDG to PSIICC affects the microenvironment around the tyrosine residues of PSIICC proteins.

From the above results, a straightforward corollary is that DGDG induces changes in the polypeptide skeleton of the PSIICC proteins. In other words, the interaction of PSIICC with DGDG affects the three-dimensional structures of the PSIICC proteins in the supramolecular complex.

Discussion

PSIICC 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.[20] It contains at least 10 polypeptides, including extrinsic and integral membrane polypeptides. That is to say, integral polypeptides of PSIICC 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.[21] PSIICC also binds different cofactors, pigments and lipids.

This work clearly demonstrates that the DGDG molecules are involved in functional and structural roles in PSIICC. First of all, it has been shown that DGDG is endowed with the ability to enhance the oxygen-evolving activity in PSIICC in the range of lipid concentrations under study. In some instances (*i. e.*, 8—10 mg DGDG/mg Chl) the maximum activation may attain values as high as about 147% of that of control samples without exogenous DGDG vesicles. It means that high efficient oxygen evolution of PSIICC is associated with the presence of DGDG, or, the DGDG molecules play an important role in regulating photosynthetic oxygen evolution activity. The result in this work is in agreement with the suggestion of Murata *et al.*[2] that DGDG molecules are essential to maintain the proper conformation of PSIICC which is a basic functional unit for evolution of oxygen. The motivated effect of DGDG on the oxygen evolution activity of other protein complex of photosynthetic membrane was also observed by several authors.[22-24] This effect of DGDG is interesting since the photosynthetic membrane has 80%—90% of the DGDG molecules distribute in its inner monolayer, that is, in the vicinity of the oxygen evolution complex (OEC) which contains the three extrinsic proteins of 33, 24 and 17 kDa.[1]

Secondly, it is evident that the change of oxygen-evolving activity in PSIICC mediated by DGDG has its structural counterpart in modifications in secondary structures of PSIICC proteins, that is, a decrease of α-helical

content with a relatively small reduction in β-turn conformers, whilst β-strands follow an opposite trend. In addition, the binding of DGDG to PSIICC affects the microenvironment around the tyrosine residues of PS II proteins.

In this case, of particular note is the change in β-strands structure, this is because, as discussed above, this kind of substructure of protein is the peptide that is assumed in an extended configuration, and has a hydrogen-bonding pattern formed by peptide residues not taking part in intramolecular structures, even forming intermolecular monomer-monomer interactions.[7,14] Taken into account that the gain in β-strands structures of PSIICC proteins is originated from the interaction of DGDG molecules with PSIICC, therefore, a conclusion can be come at that hydrogen bonds exist between the PSIICC proteins and DGDG molecules. As there are several OH groups per DGDG molecule, it is therefore not difficult to expect the DGDG participation in hydrogen-bonding interactions as a donor or an acceptor with other molecules, for example, amino acid residues of PSIICC proteins. In fact, an increase in bound OH concentration of PSIICC-DGDG complex was indeed observed, as shown in Fig. 2 (C).

From the above considerations, the effect of DGDG is that DGDG molecules attach themselves to the hydrogen-bonding interactions with the PSIICC proteins as the donors or acceptors. This is structurally favored by the three-dimensional characteristics of the DGDG molecule that has a large number of the hydrogen-bond donor and acceptor groups in space.[25] If that is the case, it is expected that the galactolipid intervene in recognition processes at the edges of the PSIICC proteins.

It is interesting since the extrinsic protein in PSIICC is 33 kDa, which is known to bind to PSIICC in the lumenal compartment of photosynthetic membrane.[21] In fact, the evolution of oxygen in PSIICC takes place in the lumen and the protein 33 kDa is indispensable for water-splitting and oxygen liberation process. Therefore, the active sites of PSIICC interaction with DGDG, with great certainty, are in the extrinsic protein of 33 kDa.

33 kDa contains eight tyrosines (Tyr) in a total of 247 amino acid residues.[26] Of these tyrosines, those at 8-, 16-, 110- and 242-position were shown to be largely composed of β-strand structures, *i.e.*, extended chains. It was believed that these four tyrosines situate at or near the aqueous phase of the lumen.[27] Accordingly, the β-strands of those tyrosines locate at or near the outer surface of the 33 kDa protein with close to the aqueous phase of the lumen. Alternatively, these β-strands exist in the proximity of the digalactosyl head group of the DGDG molecules. In the latter case, it is thereby reasonable to expect an increase in the number of hydrogen-bond interactions of the polar group of DGDG with the phenolic ring in the tyrosine residues of the 33 kDa protein. In this context, such a phenomenon was indeed observed in the present work.

Conclusion

A straightforward consequence from the foregoing results and considerations is that DGDG exhibits the ability to stimulate the oxygen evolution in PSIICC, which is accompanied by the changes in the structures of PSIICC proteins. There exist hydrogen-bonding interactions between DGDG molecules and PS II proteins. It is greatly possible that the sites of PSIICC interaction with DGDG are in the extrinsic protein of 33 kDa. It seems that DGDG is indispensable for the maintenance of normal structure for the PSIICC proteins. The maintenance of normal structure for proteins is essential to maintain the physiological activity of the protein complex, as pointed out by several authors.[14,28] In this perspective, the DGDG effect may lead to the molecular rearrangements or local structure order of PSIICC and thus the microenvironments for catalyzing water to oxygen are more appropriate. This effect therefore exhibits an enhancement of oxygen evolution in PSIICC complex.

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