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Effect of Phosphatidylcholine on the Steady State Fluorescence of Chlorophyll in Photosystem Ⅱ Particles*

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Abstract Phosphatidylcholine (PC) accounts for less than 1% of the total lipids in plant photosystem II (PSII) particles. In this experiment, PSII particles were reconstituted with PC to construct PSII-PC vesicles. The effect of PC on the steady state fluorescence of chlorophyll (Chl) in PSII particles was studied. The results show that PC significantly affected the fluorescence intensity, but did not obviously affect the fluorescence emission band peak position. PC also did not obviously affect the absorbance at 436 nm or the amide I band peak position in FT-IR spectroscopy of PSII particles. The results suggest that PC may affect the light energy transfer from the antenna chlorophyll molecules to the reaction center chlorophyll molecule (P680).

Key words photosystem II; phosphatidylcholine; chlorophyll; steady state fluorescence

Introduction

The photosynthesis light reaction in plants occurs in the thylakoid membrane of the chloroplasts. The protein complexes in the thylakoid membranes are supermolecular systems consisting of proteins, lipids and pigments which regulate and control the light energy absorption, transfer and conversion[1]. The glycerolipids in the thylakoid membranes play a significant role in maintaining the structure and function of the supermolecular system of the biomembrane. In the past, the study of the role of glycerolipids in biomembrane systems concentrated on animal cell membranes with relatively fewer investigations of plant thylakoid membranes.

The glycerolipid composition of the thylakoid membrane is different from that of general cell membranes. There are five main glycerolipids in thylakoid membranes, monogalactosyl diaylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), sulfoquinovosyl diacylglycerol (SQDG), phosphatidylglycerol (PG) and phosphatidylcholine (PC). Of the total glycerolipids in thylakoid membranes, glycerolglycolipids account for more than 80%, while PC, which is the main glycerolipid in animal cell membranes, accounts for only about 7%[1,2]. Further work is needed to understand the relationship between the special glycerolipid composition of thylakoid membranes and the special function of photosynthetic membranes.

Photosystem II (PSII) is one of the membrane protein complexes in thylakoid membranes. Photosystem II particles (PSII particle) prepared from spinach chloroplast are composed of at least 20 different polypeptides including reaction center proteins, core antenna proteins (CP 43 and CP 47), light-harvesting chlorophyll a/b binding proteins (LHCII), and three extrinsic proteins. The glycerolipid composition of such PSII particle was analyzed by Murata et al[1]. They found that the three glyceroglycolipids accounted for about 90% of

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the total lipids in a PSII particle, while PC accounted for only about 1%. Investigations of the interactions between glycerolipids and membrane proteins of PSII have concentrated on the role of the glyceroglycolipids and PG in the structure and function of PSII[3,4]. However, there have been few investigations of the effects of PC on the structure and function of PSII.

Fluorescence spectroscopy is one of the most extensively used methods in photosynthesis research. It can provide information not only on the structure of the photosynthetic membrane, but also on the photosynthesis process. In our experiment, PSII particles were reconstituted with PC to construct PSII-PC vesicles. The study emphasized the effects of PC on the steady state fluorescence of chlorophyll (Chl) in PSII particles.

1 Materials and Methods

1.1 Materials and sample preparation

PSII particles were isolated from leaves of market spinach as described by Chapman et al[5]. The PSII particles were washed with SMN buffer (0.5 mol/L sucrose, 40 mmol/L Mes-NaOH (pH 6.0), and 10 mmol/L NaCl) by centrifugation and then suspended in the SMN buffer. This preparation will be referred to as PSII suspension. The Mg^{2+}-containing PSII suspension was prepared using Mg^{2+}-containing SMN buffer (0.5 mol/L sucrose, 40 mmol/L Mes-NaOH (pH 6.0), 10 mmol/L NaCl, and 5 mmol/L $MgCl_2$) instead of the SMN buffer in the washing and suspension steps.

PC (> 99% of purity) was purchased from Sigma Chemical Company. PC vesicle suspension, using the SMN buffer, was prepared according to the method described by Fragata et al[6]. The suspension was mixed with PSII suspension at different PC/Chl mass ratios and then incubated in darkness at 4℃ for 30 min. This preparation will be referred to as PSII-PC vesicles. The Mg^{2+}-containing PSII-PC vesicles were prepared by replacing the SMN buffer with the Mg^{2+}-containing SMN buffer in each preparation step.

The chlorophyll concentration was determined by the method of Arnon[7].

1.2 Instrumentation

The steady state fluorescence spectra of the samples were obtained using a Hitachi 850 fluorescence spectrophotometer at 10℃ with 436 nm as the excitation wavelength. The fluorescence emission spectra were measured between 650 nm and 710 nm. The absorbance of each sample at 436 nm was measured with a Beckman DU-68 spectrophotometer. The infrared measurements of the samples were performed in a Perkin-Elmer FT-IR spectrometer, model Paragon 1000 PC, according to the method described by Shi et al[8].

2 Results and Discussion

2.1 Effect of PC on the steady state fluorescence of Chl in the PSII-PC vesicles

The PSII-PC vesicles with different PC/Chl mass ratios were prepared with the Chl concentration of each sample maintained at 10μg/mL. The fluorescence emission spectra were measured (Fig. 1). Curve 1 is the spectrum of the PSII suspension, which served as a control. The other curves are the spectra of PSII-PC vesicles at different PC/Chl mass ratios. Each spectrum has a peak at 684 nm which indicates that PC did not cause any obvious shift of the maximum band position and suggests that PC reconstitution did not significantly change the hydrophobicity of the micro environment around the chlorophyll molecules. However, the fluorescence emission intensity of each PSII-PC vesicle sample is

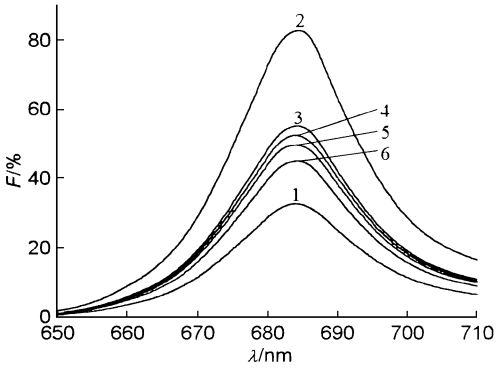

Fig. 1 Fluorescence emission spectra of PSII particles and PSII-PC vesicles for different PC/Chl mass ratios
PC/Chl mass ratios in the samples: (1), 0: 1 (control); (2), 2: 1; (3), 5: 1; (4), 10: 1; (5), 15: 1; (6), 20: 1, λ_{EX} = 436 nm; [Chl] = 10μg/mL

different from that of the control. The emission peak intensity at the 2: 1 of PC/Chl mass ratio is nearly twice the height of the control. As the PC/Chl mass ratio increases further, the emission peak intensity gradually decreases, but still remains higher than that of the control.

The PSII particles and PC vesicles were reconstituted according to the method used for preparing PSII-PG vesicles described by Fragata et al[6]. In their experiment, PSII particles and PG vesicles were mixed at 0℃ for 20 min until the lipid vesicle and membrane protein were reconstituted and almost all the PSII particles have participated in the forming of PSII-PG vesicles. In our experiment, the fluorescence emission spectra of the samples were also measured at the beginning of the incubation of the PC vesicles and PSII particles, but the fluorescence intensity of each sample was not different from that of the control. However, the fluorescence intensity changed obviously after 30 min of incubation as shown in Fig. 1. Our results suggest that the structural reconstitution between PSII particles and PC vesicles occurred within 30 min.

2.2 Effect of PC on the A_{436} of the PSII-PC vesicles

The change of the fluorescence emission intensity of each sample could be either due to the change of the fluorescence yield or due to the change of the absorbance at 436 nm which was the excitation wavelength. The absorbance change was evaluated by measuring the absorbance at 436 nm (A_{436}) of different samples of PSII-PC vesicles and PC vesicles (Fig. 2). The results demonstrate that A_{436} of the PSII-PC vesicles is not a function of the PC/Chl mass ratio. Moreover, the PC vesicles had almost no absorption at 436 nm. These results indicate that the change of fluorescence intensity was not caused by variations of the absorption. Since PC did not absorb the light, the absorbance at 436 nm of the PSII-PC vesicles comes from the effect of the PSII particles. Because the fluorescence emission spectrum of PSII particles excited at 436 nm reflects the fluorescence of the chlorophyll in the PSII particles, the results indicate that the fluorescence emission spectrum of PSII-PC vesicles excited at 436 nm is due to the fluorescence of chlorophyll in the PSII-PC vesicles.

2.3 Infrared spectroscopy of the PSII-PC vesicles

The essentially fixed fluorescence peak position in Fig. 1 suggests that PC had not obviously changed the hydrophobicity of the environment around the chlorophyll in PSII. The interaction between lipids and proteins in PSII-PC vesicles was investigated further by measuring the infrared spectra of PSII particles, PC vesicles and PSII-PC vesicles at different PC/Chl mass ratios. Figure 3 shows only the infrared spectrum of PSII-PC vesicles at 15∶1 of PC/Chl mass ratio. The band with a maximum at 1657 cm^{-1} is the amide I band which originates from the carbonyl vibration of the peptide bonds in proteins[9]. The band with a peak at 1228 cm^{-1} originates from the PO_2^- antisymmetric stretch while the peak at 1085 cm^{-1} originates from the PO_2^- symmetric stretch[10]. The amide I band and the phosphate bands do not shift upon PC binding to PSII suggesting that the protein conformation and the PC structure in the PSII-PC vesicles are still maintained in the same state as before reconstitution. PC may insert into the region around the chlorophyll-binding proteins but not into the internal region of them, thus not affecting the micro environment around the chlorophyll molecules in those proteins. This result is consistent with the fixed peak position in the fluorescence spectrum.

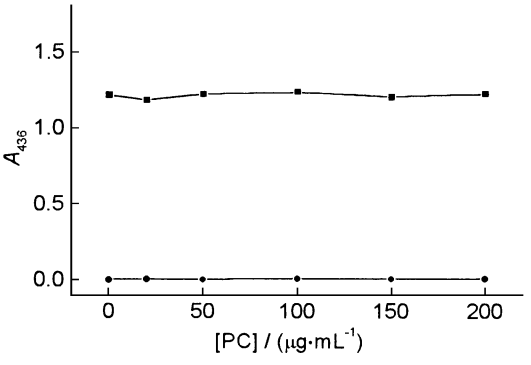

Fig 2 Effect of PC concentration on absorbance of PSII-PC vesicles (■) and PC vesicles (●)
[Chl] in PSII-PC vesicles is 10μg/mL

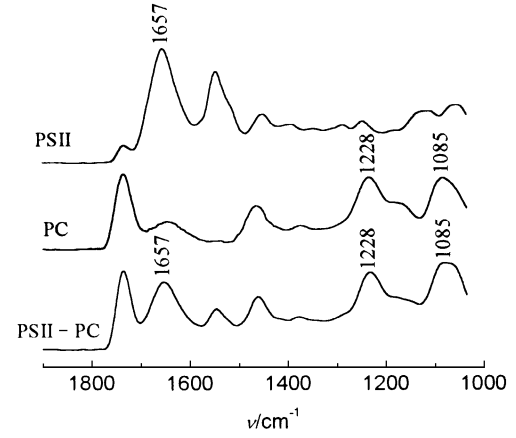

Fig 3 Infrared spectra of PSII particles, PC vesicles and PSII-PC vesicles
PC/Chl mass ratio is 15∶1

In a PSII particle, light energy transfer can occur effectively from the chlorophyll molecules in the antenna proteins (LHCII) to the chlorophyll molecules in the core antenna proteins (CP43 and CP47), and then to the reaction center chlorophyll (P680) where the light energy is used to cause a primary chemical reaction[11]. If such energy transfer is inhibited, then more of the light energy absorbed by the chlorophyll molecules will be released in the form of fluorescence. Therefore, if the same amount of light is absorbed by the chlorophyll molecules, then a fluorescence intensity decrease may represent increased energy transfer, and *viœ versa*. According to our results, PC did not affect the A_{436} of PSII particles; therefore the reason that the fluorescence intensity of PSII-PC vesicles is higher than that of PSII particles may be that the PC decreased such energy transfer. Since PC had no obvious effect on the protein conformation and the hydrophobicity of the environment around the chlorophyll molecules in PSII particles, then the increase of fluorescence intensity may be due to that the PC had inserted into the space among LHCII, CP43, CP47 and reaction center proteins, resulting in longer distance between the antenna chlorophyll molecules and P680 thus decreasing the energy transfer to P680.

2.4 Effect of Mg^{2+} on the steady state fluorescence of Chl in the PSII-PC vesicles

Melis et al.[12] had reported that the reaction center may become more tightly coupled to adjacent light harvesting pigment beds under the influence of Mg^{2+}. To further investigate the effect of PC on the steady state fluorescence of PSII particles, we also studied the fluorescence spectra of the Mg^{2+}-containing PSII-PC vesicles. The results showed that the emission peak wavelength shifts from 684 nm to 686 nm indicating that Mg^{2+} affected the hydrophobicity of the environment around the chlorophyll molecules in PSII particles. This result is consistent with the result of Fragata et al.[3] that Mg^{2+} gave rise to the blue shift of the amide I band of PSII particles. Our result also showed that each spectrum of the Mg^{2+}-containing PSII-PC vesicles at different PC/Chl mass ratios has a peak at 686 nm, suggesting that PC did not further alter the hydrophobicity of the environment around the chlorophyll which had been affected by Mg^{2+}. The emission peak intensities of the Mg^{2+}-containing PSII-PC vesicles at 2∶1 or 5∶1 of PC/Chl mass ratios are much higher than that of the Mg^{2+}-containing PSII suspension which served as a control. Increasing the PC/Chl mass ratio caused the emission peak intensity to decrease, but the level was always higher than that of the control. Such changes of the fluorescence intensity are similar to the results for Mg^{2+}-free conditions (Fig. 4). The results infer that the effect of PC on the energy transfer from the antenna chlorophyll molecules to the P680 in PSII-PC vesicles in the absence or presence of Mg^{2+} involves similar mechanisms. However, with the same PC/Chl mass ratio, the fluorescence intensity of the Mg^{2+}-containing PSII-PC vesicles is lower than that of the corresponding Mg^{2+}-free PSII-PC vesicles. Since Mg^{2+} can promote light energy transfer between the reaction center chlorophyll and the chlorophyll molecules in the antenna proteins[12], then Mg^{2+} causes the fluorescence decrease. Therefore, the PC indeed inhibits the light energy transfer from the antenna chlorophyll molecules to P680. This hypothesis needs further study to verify all of the mechanisms.

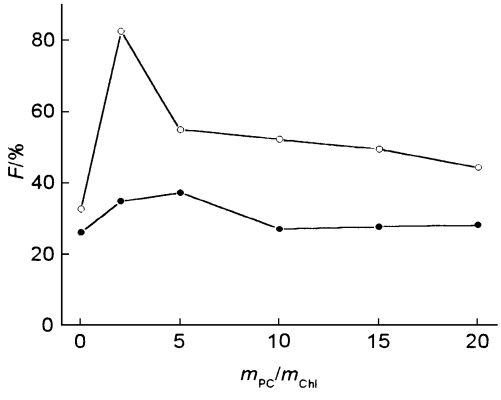

Fig. 4 Effect of PC on the fluorescence emission intensity of PSII-PC vesicles

○, in the absence of Mg^{2+}, maximum emission intensity at 684 nm;

●, in the presence of Mg^{2+}, maximum emission intensity at 686 nm;

[Chl] = 10μg/mL

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