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Photoinduced electron transfer of chlorophyll in lipid bilayer system

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Abstract. Photoinduced electron transfer from chlorophyll-*a* through the interface of dipalmitoylphosphatidylcholine (DPPC) headgroup of the lipid bilayers was studied with electron magnetic resonance (EMR). The photoproduced radicals were identified with electron spin resonance (ESR) and radical yields of chlorophyll-*a* were determined by double integration ESR spectra. The formation of vesicles was identified by changes in measured l_{max} values from diethyl ether solutions to vesicles solutions indirectly, and observed directly with SEM and TEM images. The efficiency of photosynthesis in model system was determined by measuring the amount of chlorophyll-*a* radical yields which were obtained from integration of ESR spectra.

Keywords. Artificial photosynthesis; photoinduced charge separation; electron spin resonance; vesicles.

1. Introduction

Molecular assemblies such as micelles and vesicles are useful in studies concerning the storage of light energy.[1,2] These self-forming molecular assemblies compartmentalize the electron donors and acceptors, relative to the solvent typically water. Although the structures of vesicles and micelles are not as complex as natural membranes, photochemical studies of chlorophyll in such organized assemblies have proved to be relevant to the determination of fundamental properties of chlorophyll in photosynthetic system. Charge separation may be partially controlled by various factors such as the vesicle surface charge, head group variation, and alkyl chain length variation.[3–7] The addition of slightly water soluble, surface-active compounds such as alcohols and cholesterol modify the assembly interface. It has been shown that charge separation may be partially controlled by the addition of such intercalating agents.[8,9] In the current investigation, we have studied systematically the photoionization of chlorophyll-*a* in vesicles differing in charge of the lipid head group without an electron acceptor or scavenger.

2. Experimental procedures

Chlorophyll-*a* was extracted from fresh spinach leaves by the conventional method. Its purity was determined to be 96% from its extinction coefficient in diethyl ether at 660 nm vs the literature value of $8{\cdot}6 \times 10^4\ M^{-1}\ cm^{-1}$.[10] Dipalmitoylphosphatidylcholine (DPPC) was purchased from Sigma Chemical Co. and was used without further purification.

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Buffer solutions were prepared with sodium phosphate, sodium pyrophosphate and sodium ethylene diaminetetraacetate (EDTA) from Aldrich Chemicals.

2.1 *Sample preparation*

DPPC vesicle solutions of chlorophyll-*a* were prepared by the method developed by Huang[11] and modified by Norris *et al*[12]. After the chloroform solutions of DPPC containing chlorophyll-*a* were evaporated, the resulting film was sonicated in aqueous buffer solutions with a Fisher Model 300 sonic dismembrator operated at 30 W with a 4 mm o.d. microtip for 1 h at 55°C (above the liquid crystal gel phase transition temperature). Phosphate buffer solutions contained 0·1 M sodium phosphate, 0·1 M sodium pyrophosphate, and 1 mM EDTA in triply distilled water and were adjusted to *p*H 7·0 with sulfuric acid. *Tris*-HCl buffer solutions were prepared by dissolving 0·5 M tris(hydroxymethyl)aminomethane in triply distilled water and were adjusted to *p*H 7·0 with hydrochloric acid.

The formation of DPPC vesicles containing chlorophyll-*a* was identified by measuring λ_{max} value change from diethyl ether solution to vesicles solutions. Optical absorption spectra were measured in 1 cm path length quartz cell with a Varian CARY 1C UV-Vis spectrophotometer at room temperature. The morphologic analysis of vesicles was carried out by scanning electron microscopy (SEM), with a Hitachi S-4200 FE-SEM, after coating the sample with Pt in vacuum chamber and transmission electron microscopy (TEM), with Jeol JEM-2010, after staining of the sample with uranyl acetate (2%), respectively.

2.2 *Electron spin resonance experiments*

Photoirradiation at 77 K was performed with 300-W Cermax xenon lamp with a power supply form ILC Technology. The light was passed through a 10 cm water filter and a Corning 5030 band pass filter for blue light irradiation ($300\ nm < \lambda_{irr} < 558$). ESR spectra were recorded at X-band on JEOL JEX-FX 2000-300. Mn^{2+} in MgO was used as a magnetic field marker. The efficiency of photosynthesis in model system was determined by measuring the amount of chlorophyll radical yields, which were obtained from integration of spectra.

3. Results and discussion

Optical absorption spectra of DPPC vesicle and chlorophyll-*a* in diethyl ether and DPPC vesicles are shown in figure 1. DPPC vesicles do not show absorption in 400–700 nm region. Shift in the absorption bands of chlorophyll in the vesicle solution from the bands in the organic solvents might be caused by different environmental interactions of chlorophyll-*a*.[13] In DPPC vesicle solutions of chlorophyll-*a* an absorption band at 685 nm has been assigned to aggregated chlorophyll-*a*.[14] The formation of hydrated chlorophyll polymer has been reported to give an absorption band at 740 nm.[15] Since there are no absorption bands at 740 or 685 nm in our preparations, we conclude that chlorophyll is solubilized in its monomeric form in our samples. Chlorophyll-*a* solubilized into a phospholipid vesicle, shows absorption band at 670 nm, which is 10 nm red-shifted compared to the absorption band of chlorophyll-*a* at 660 nm in diethylether. This red

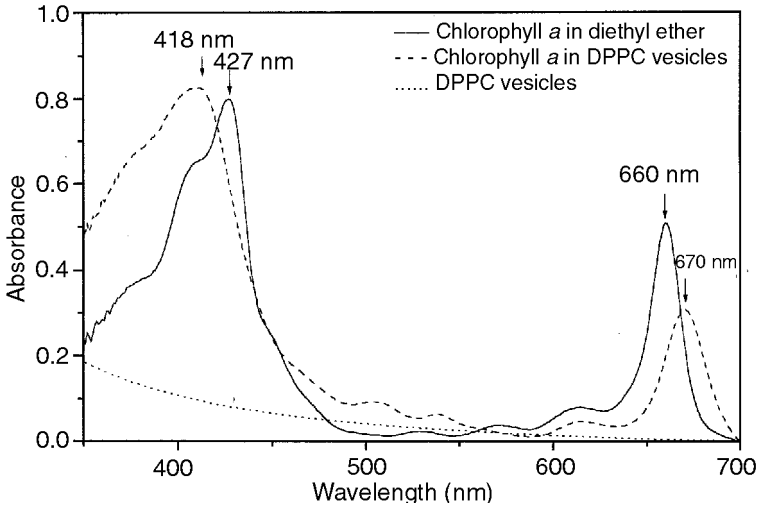

Figure 1. Optical absorption spectra of DPPC vesicle and chlorophyll-*a* in diethyl ether and DPPC vesicles.

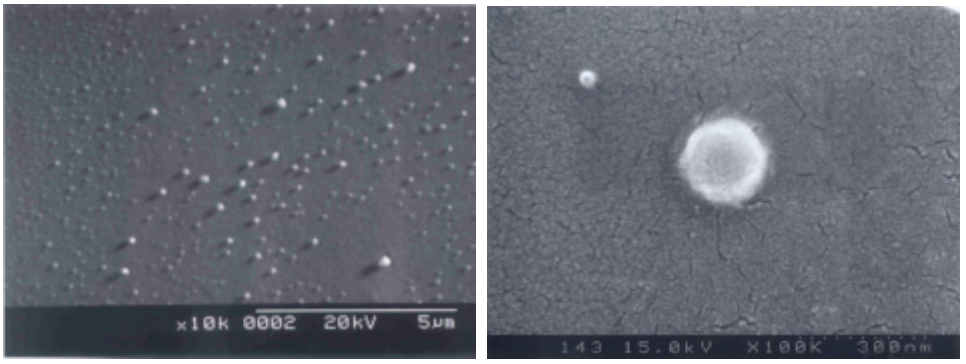

Figure 2. Scanning electron micrographs of DPPC vesicles (left) and DPPC vesicles containing chlorophyll-*a* (right). The size of vesicles is 80–180 nm.

shift is still persisted in DPPC vesicle solution with $\lambda_{max} = 670$ nm.[16] This red shift indicates the chlorin ring being located in a polar environment near the surfactant headgroup region and possibly exposed to the aqueous environment.

Scanning electron micrographs of DPPC vesicles (left, × 10,000) and DPPC vesicle containing chlorophyll-*a* (right, × 100,000) are represented in figure 2. Transmission electron micrographs of DPPC vesicles (A, × 100,000) and DPPC vesicle containing chlorophyll-*a* (B, × 100,000) after staining with uranyl acetate are shown in figure 3. The size of vesicles is 80–180 nm. The vesicles were formed almost homogenously in both DPPC and DPPC containing chlorophyll-*a*. The photoionization yields were determined after computation of the double integrals of the observed ESR spectra.

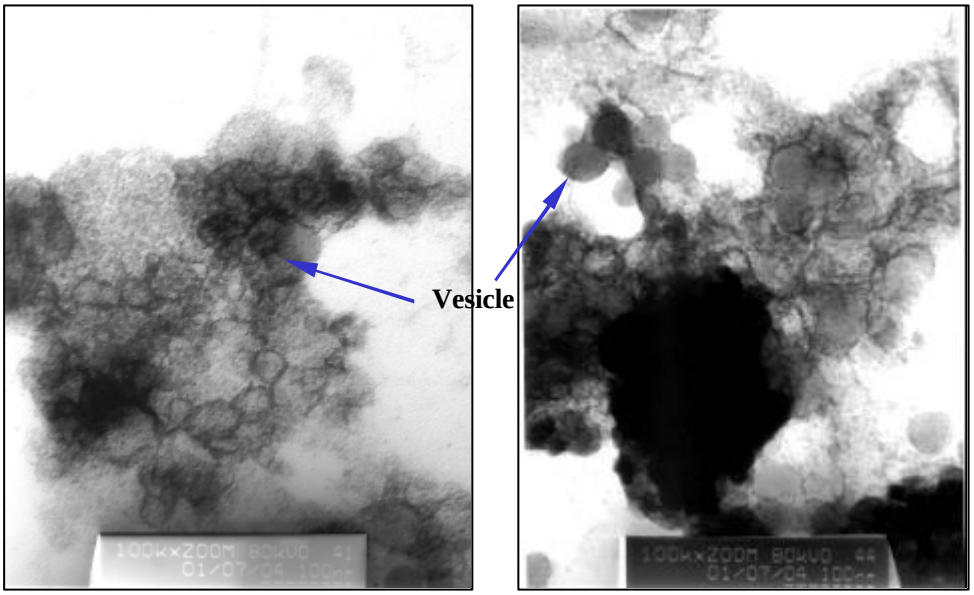

Figure 3. Transmission electron micrographs of DPPC vesicles (A, ×100,000) and DPPC vesicles containing chlorophyll-*a* (B, × 100,000) after staining with uranyl acetate.

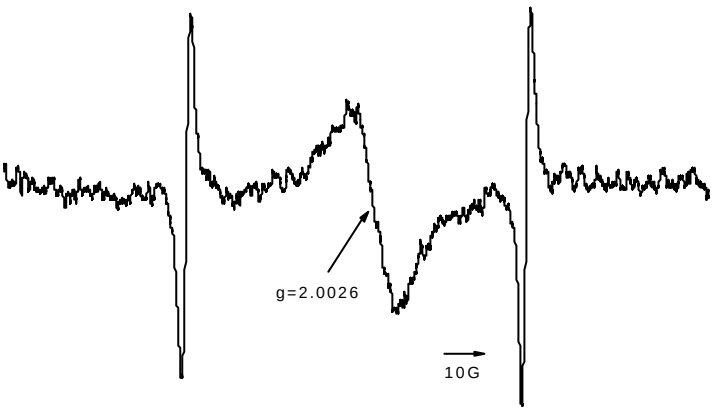

Figure 4. First derivative X-band ESR spectra of chlorophyll-*a* in DPPC vesicles recorded after blue-light irradiation for 90 min at 77 K. The sharp lines on both sides of the spectra are from Mn^{2+} in MgO used as a magnetic field marker.

The photosynthesis of chlorophyll-*a* in vesicles was studied with ESR. No radical formation was observed in DPPC vesicle solutions not containing chlorophyll-*a* after blue-light irradiation at 77 K. After blue-light irradiation of chlorophyll-*a* in DPPC vesicle solutions the ESR singlet was observed. First derivative X-band ESR spectrum of

chlorophyll-*a* in DPPC vesicles after blue-light irradiation for 90 min at 77 K represents a symmetric single ESR line at $g = 2{\cdot}0026$ with a line width of 10·5 G, as shown in figure 4. This ESR singlet did not decay within several days at 77 K. With small magnetic field modulation no extra splitting was observed. The value of *g* and line shape of the singlet did not show any changes at various microwave power levels from 0·2 *m*W to 20 mW. The photoproduced cation radical of chlorophyll-*a* was identified as a broad singlet with $g = 2{\cdot}0026$ in frozen state at 77 K. Dependence of the normalized photoyield of chlorophyll-*a* upon the molar ratio of DPPC in DPPC frozen vesicles is shown in figure 5.

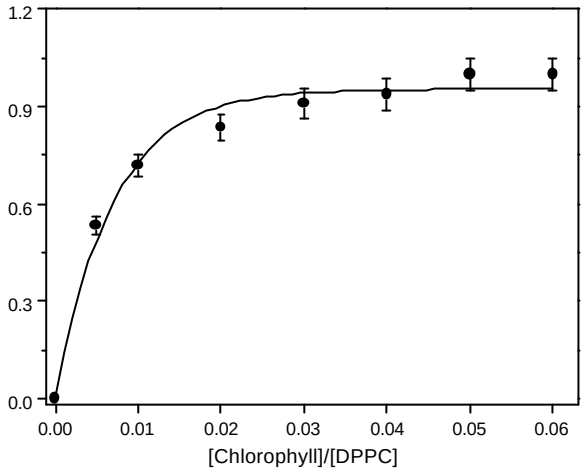

Figure 5. Dependence of the normalized photoyield of chlorophyll-*a* upon the molar ratio of DPPC in DPPC frozen vesicles. Photoirradiation was carried out with blue-light for 10 min at 77 K.

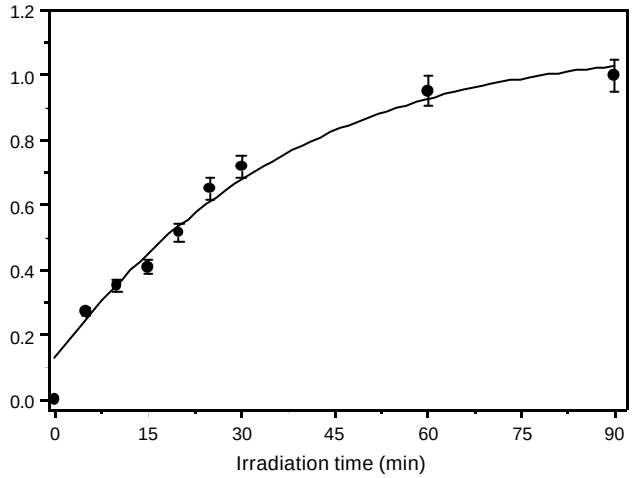

Figure 6. Dependence of the normalized photoyield of chlorophyll-*a* upon the blue-light irradiation in DPPC frozen vesicles at 77 K. [Chlorophyll-*a*]/[DPPC] molar ratio is 0·03.

Photoirradiation was carried out with blue-light for 20 min at 77 K. The normalized photoyield increases linearly with the chlorophyll-*a*/DPPC molar ratio up to 0·03 and thereafter reaches a plateau. The time dependence of the normalized photoyield of chlorophyll-*a* upon the blue-light irradiation in DPPC frozen vesicles at 77 K is shown in figure 6. The normalized photoyield increases rapidly with blue-light irradiation up to 30 min and then increases slowly after 30 min, and finally saturated at 70 min photoirradiation.

4. Conclusions

The formation of DPPC vesicles containing chlorophyll-*a* was identified by a 10 nm shift to the red region compared to diethyl ethyl solution in which $\lambda_{max} = 660$ nm. From SEM images, we could confirm that the vesicles were formed almost homogenously in both DPPC vesicles and DPPC vesicles containing chlorophyll-a with a size of 80–180 nm. After blue-light irradiation of chlorophyll-*a* in DPPC vesicle solutions, the ESR singlet was observed. The photoproduced cation radical of chlorophyll-*a* was identified as a broad singlet with $g = 2{\cdot}0026$ in frozen state at 77 K. From TEM images we could observe round shaped DPPC vesicle. The normalized photoyield increases linearly with the chlorophyll-*a*/DPPC molar ratio up to 0·03 and then is constant. The normalized photoyield increases rapidly with blue-light irradiation up to 30 min and then increases slowly after 30 min, and finally saturated at 70 min photoirradiation.

Acknowledgments

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