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Light-harvesting properties of zinc complex of chlorophyll-*a* from *spirulina* in surfactant micellar media

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Abstract

Zn chlorophyll-*a* was prepareted from Mg chlorophyll-*a* from *spirulina* and the optical properties of the ground state and the photoexited state of Zn chlorophyll-*a* in aqueous surfactant micellar media were studied using UV-vis absorption, fluorescence emission spectra, electrochemical and fluorescence lifetime measurements. In comparison of the UV-vis absorption and fluorescence emission spectra of Zn chlorophyll-*a* and Mg chlorophyll-*a*, the blue-shift in the absorption bands and emission peak of Zn chlorophyll-*a* was observed. The energies of the first excited singlet state of Zn chlorophyll-*a* was 1.87 eV. The first oxidation and reduction potentials of the photoexcited singlet state of Zn chlorophyll-*a* were −0.67 and 0.60 V, respectively. Fluorescence lifetime of Zn chlorophyll-*a* was 9.0 ns in CTAB micellar solution. The fluorescence lifetime of Zn chlorophyll-*a* is shorter than that of Mg chlorophyll-*a* (9.8 ns). The photositability of Zn chlorophyll-*a* was superior to that of Mg chlorophyll-*a* in various pH conditions.

Introduction

Chlorophyll-*a* acts as the light-harvesting in photosynthesis of green plant and exhibits physiological functions of the photolysis of water, the reduction of $NADP^+$ and carbon dioxide fixation under visible light irradiation (Scheer 1991). Chlorophyll-*a* consists of chlorine ring like porphyin and phytol. Mg complex with chlorophyll-*a* exists in green plant. Mg chlorophyll-*a* has absorption maximum in 432 and 670 nm and is attractive compound as a visible photosensitizer for the means of converting solar energy to chemical energy or such as a photoinduced hydrogen evolution (Darwent *et al.* 1982, Okura 1985, Tomonou & Amao 2002) to electrochemical energy (Wada *et al.* 1996, Kitamura *et al.* 2001, Komori & Amao 2003). However, Mg chlorophyll-*a* is unstable against the irradiation. On the other hand, Zn bacteriochlorophyll-*a* was founded in an aerobic bacterium *Acidiphilium rubrum* (Wakao *et al.* 1996). Some studies on the preparation and characterization of the Zn chlorophylls and Zn bacteriochlorophylls have been reported (Nagashima *et al.* 1997; Akiyama *et al.* 1998; Kobayashi *et al.* 1998). Water-soluble Zn porphyrins have been widely used as effective photosensitizers (Okura 1985; Okura *et al.* 1985), for these porphyrins have absorption band in the visible light region (380–600 nm). As the Zn porphyrins are stable against the irradiation and effective photosensitizers, Zn chlorophyll-*a* are attractive compound as a stable visible photosensitizer. For using the chlorophyll-*a* to the photoenergy conversion system such as photoinduced hydrogen production from water, the studies on the optical properties of chlorophyll-*a* in the aqueous media are needed. Since chlorophyll-*a* is hydrophobic compound, chlorophyll-*a* is solubilized to the aqueous media using surfactant micellar. We previously reported the photoinduced hydrogen production with the photosensitization of Mg or Zn chlorophyll-*a* in

the surfactant micellar system using cethyltrimethylammonium bromide (CTAB) (Takeuchi & Amao 2003; Tomonou & Amao 2003). However, the optical properties of the photoexcited state of chlorophyll-*a* in the CTAB micellar media have not been clarified.

In this paper we describe the preparation of Zn chlorophyll-*a* from Mg chlorophyll-*a* from *spirulina*, the properties of light-harvesting function of Zn chlorophyll-*a* and the effect of pH on the photostability of Zn chlorophyll-*a* in the CTAB micellar media.

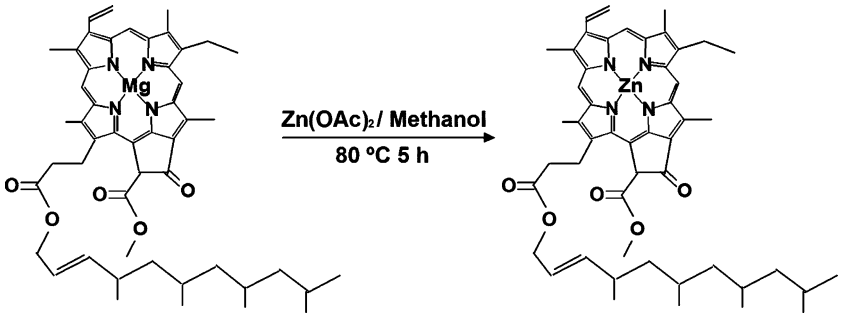
Materials and methods

Reagents

Mg chlorophyll-*a* from *spirulina* and zinc acetate dihydrate was obtained from Wako Chemical Co. Ltd (Osaka, Japan). All the other reagents were higher grade available.

Preparation of Zn chlorophyll-a

Zn chlorophyll-*a* was synthesized by refluxing Mg chlorophyll-*a* (50 mg, 56 μmol) with about 10 times molar equivalent of zinc acetate dihydrate in 100 ml of methanol at 80 °C for 5 h. The reaction scheme is indicated in Scheme 1.



The insert of zinc ion into chlorine ring of chlorophyll-*a* was monitored by visible absorption spectra. During the reaction, the characteristic absorption bands of Zn chlorophyll-*a* at 421 and 662 nm increased and the absorbance bands at 433 and 668 nm of Mg chlorophyll-*a* decreased gradually. After the mixture was cooled to room temperature, the solvent was removed under vacuum and then the reaction mixture was washed with water to remove the unreacted zinc acetate dihydrate. Finally, Zn chlorophyll-*a* was precipitated in water. Zn chlorophyll-*a* was collected by filtration and washed with water and then the sample mixture is passed to silica gel column chromatography. The purification was performed by recrystallization from water–methanol (**5**:1) solution.

Spectroscopic measurements

UV-vis absorption spectra of Zn and Mg chlorophyll-*a* were recorded using spectrophotometer (Multispec-1500 Shimadzu). The molar absorption coefficients at absorption maxima of Zn chlorophyll-*a* were determined by the linear plot of absorbance vs. Zn chlorophyll-*a* concentration in methanol. Fluorescence emission spectra of Zn and Mg chlorophyll-*a* were measured using spectrofluorophotometer with a 150 W xenon lamp as a visible excitation light source (RF-5300PC Shimadzu). The excitation and emission bandpasses were 5.0 nm, respectively. The excitation wavelength was 600 nm. In these experiments the absorbance at the excitation wavelength was kept constant to be 0.2 for all the sample solutions.

Fluorescence lifetime measurement

Measurements of fluorescence lifetime of Zn and Mg chlorophyll-*a* in CTAB micellar solution were carried out using the system consisting of a nanoflash Xe arc lamp with 334 nm (pulse width 1.5 ns) as an excitation light source and Stroboscopic detector (Time Master Series PTI-3000 Photon Technology International) at 25 °C. In these experiments the absorbance at the excitation wavelength was kept constant to be 0.4 for all the sample solutions. The monitored wavelengths for Zn and Mg chlorophyll-*a* were 664 and 678 nm, respectively.

Electrochemical measurements

The redox potentials were determined by cyclic voltammetry (Hokuto denko HAG1510m). All measurements were carried out under nitrogen gas in solutions containing 0.1 mol dm^{-3} of *tert-n*-butylammoniumhexafluorophospate and acetonitrile at a platinum working electrode. A platinum wire was used as a counter electrode. All potentials are relative to the Ag/AgCl electrode used as the reference.

*Photostability of Zn and Mg chlorophyll-*a *in CTAB micellar*

Photostabilities of Zn and Mg chlorophyll-*a* were tested by irradiation with visible light using 200 W tungsten lamp. The reaction system consisted of 15 μmol dm^{-3} of Zn or Mg chlorophyll-*a* in 3.0 ml of 10 mmol dm^{-3} potassium phosphate buffer. Zn and Mg chlorophyll-*a* are solubilized with 10 mmol dm^{-3} of CTAB, since Zn and Mg chlorophyll-*a* are insoluble to aqueous solution. To investigate the effect of pH on the photostabilities of Zn and Mg chlorophyll-*a*, the pH values were changed between 4.0 and 8.0. The sample solution was deaerated by repeated freeze-pump-thaw cycles and irradiated with a 200 W tungsten lamp at a distance of 3.0 cm, with a light intensity of 200 J m^{-2} s^{-1}, at 30 °C. The light of the wavelength less than 390 nm was removed by Toshiba L-39 cut-off filter (Tokyo, Japan). The photoinduced breach of Zn and Mg chlorophyll-*a* were monitored the absorption change using UV-vis spectrophotometer at 662 and 670 nm, respectively.

Results and discussion

UV-vis absorption spectra of Zn and Mg chlorophyll-a in CTAB micelle

Figures 1 (a) and (b) show UV-vis absorption spectra of Zn and Mg chlorophyll-*a*. The solid and dotted lines are spectra in CTAB micellar solution and in methanol solution, respectively. The absorption bands of Zn chlorophyll-*a* are 421 nm due to Soret band and 662 nm due to Q band. In contrast, the absorption bands of Mg chlorophyll-*a* were 433 nm attributed to Soret band and 668 nm attributed to Q band. In both cases of Zn and Mg chlorophyll-*a*, the absorption spectra in CTAB micellar solution are similar to that of methanol solution. In comparison of the absorption spectra of Zn and Mg chlorophyll-*a*, the blue-shift in the absorption bands of Zn chlorophyll-*a* was observed. The reason for the blue-shift in the absorption bands of Zn chlorophyll-*a* is as follows. As the electron negativity of Zn is lower than that

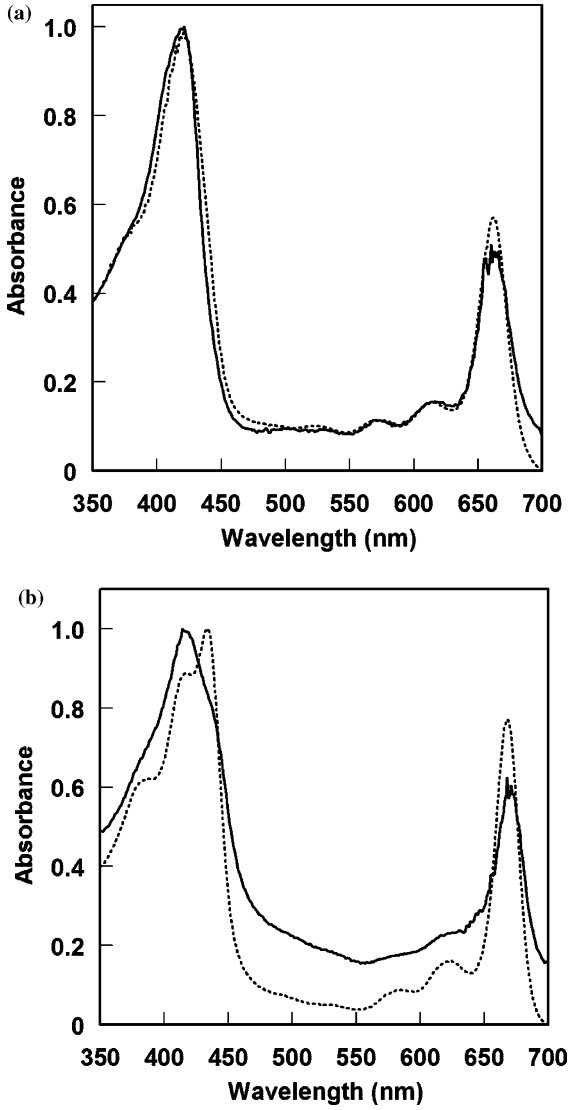


Figure 1. UV-vis absorption spectra of Zn chlorophyll-*a* (a) and Mg chlorophyll-*a* (b). The solid and dotted lines are spectra in CTAB micellar solution and in methanol solution, respectively. The concentrations of Zn and Mg chlorophyll-*a* were 79 and 16 μmol dm^{-3}, respectively.

of Mg, the electron density of chlorophyll ring is reduced and the redox potential of Zn chlorophyll-*a* also is reduced compared with that of Mg chlorophyll-*a*. Thus, the blue-shift in the absorption bands of Zn chlorophyll-*a* is observed. The absorption maxima and molar absorption coefficients of Zn and Mg chlorophyll-*a* in methanol solution were summarized in Table 1. The molar absorption coefficients of Zn chlorophyll-*a* were at Soret and Q bands about six times lower than those of Mg chlorophyll-*a*. As the interaction between Zn chlorophyll-*a* molecules is stronger than that of Mg chlorophyll-*a*, the molar absorption coefficients of Zn chlorophyll-*a* decrease compared with those of Mg chlorophyll-*a*.

*Fluorescence spectra of Zn and Mg chlorophyll-*a *in CTAB micelle*

Figures 2 (a) and (b) show fluorescence emission spectra of Zn and Mg chlorophyll-*a* with 600 nm excitation. The solid and dotted lines are spectra in CTAB micellar solution and in methanol solution, respectively. The fluorescence emission peaks of Zn and Mg chlorophyll-*a* were observed at 664 and 678 nm, respectively. In both cases of Zn and Mg chlorophyll-*a*, the maximum of emission intensity in CTAB micellar solution is larger than that of methanol solution. In comparison of the fluorescence emission spectra of Zn and Mg chlorophyll-*a*, the blue-shift in the emission peak of Zn chlorophyll-*a* was observed as well as that in the absorption spectra. The reason for the blue-shift in the emission peak of Zn chlorophyll-*a* also is as follows. As the electron negativity of Zn is lower than that of Mg, the electron density of chlorophyll ring is reduced and the redox potential of Zn chlorophyll-*a* also is reduced compared with that of Mg chlorophyll-*a*.

Table 1. Wavelengths of absorption maxima and molar absorption coefficients of Zn chlorophyll-*a* and Mg chlorophyll-*a* in methanol solution.

	Absorption maxima	
	Soret band (nm)	Q-band (nm)
Zn chlorophyll-*a*	421	662
	(13,000)[a]	(7100)
Mg chlorophyll-*a*	433	668
	(61,000)	(47,000)

[a]The unit of molar absorption coefficient was mol^{-1} dm^3 cm^{-1}.

*Properties of the photoexcited state of Zn and Mg chlorophyll-*a

The energy levels of Zn and Mg chlorophyll-*a* are studied by the electrochemical measurements. The results are listed in Table 2. The energies of the

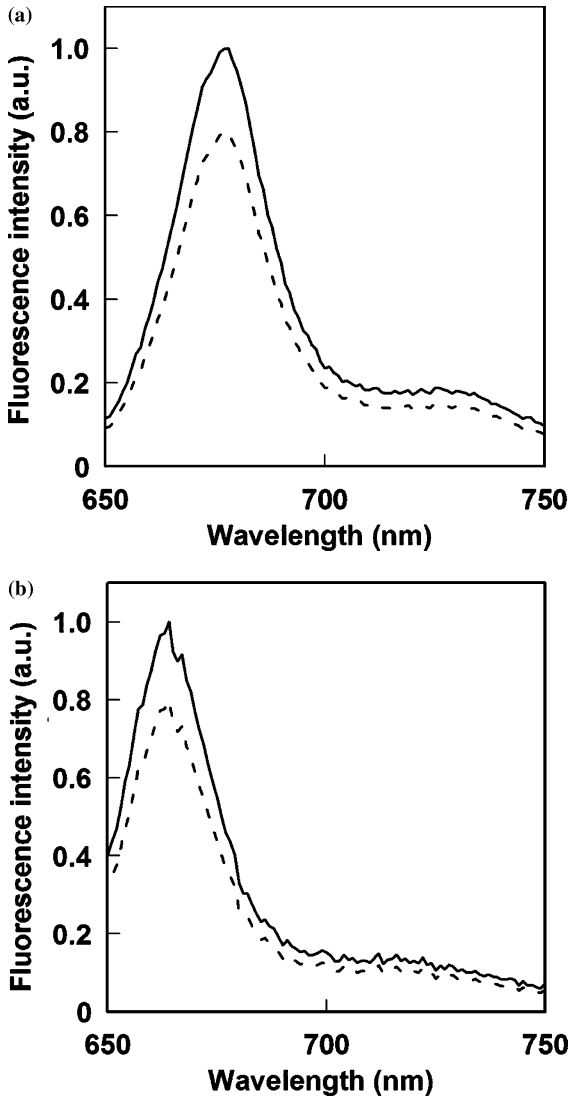


Figure 2. Fluorescence spectra of Zn chlorophyll-*a* (a) and Mg chlorophyll-*a* (b). The solid and dotted lines are spectra in CTAB micellar solution and in methanol solution, respectively. The excitation wavelength was 600 nm. The concentrations of Zn and Mg chlorophyll-*a* were 0.6 and 0.4 μmol dm^{-3}, respectively.

Table 2. The energies of the first excited singlet states, first oxidation and reduction potentials of the excited singlet states of Zn and Mg chlorophyll-*a*.

	First excited singlet state (eV)	First oxidation potential of the excited singlet state (V)	First reduction potential of the excited singlet state (V)
Zn chlorophyll-*a*	1.87	−0.67	0.60
Mg chlorophyll-*a*	1.84	−0.58	0.61

[a]All potentials are relative to the Ag/AgCl electrode used as the reference.

first excited singlet states of the Zn and Mg chlorophyll-*a* were calculated from the average values of the frequencies with the longest wavelength for absorption maxima and the shortest wavelength of fluorescence emission maxima. The first oxidation and reduction potentials of the photoexcited singlet state of Zn and Mg chlorophyll-*a* were calculated from one-electron oxidation and reduction potentials of Zn and Mg chlorophyll-*a*. The one-electron oxidation and reduction potentials of Zn chlorophyll-*a* were 1.26 and −1.23 V, respectively. On the other hand, the one-electron oxidation and reduction potentials of Mg chlorophyll-*a* were 1.20 and −1.27 V, respectively. The energies of the first excited singlet states of Zn and Mg chlorophyll-*a* were 1.87 and 1.84 eV, respectively. The first oxidation and reduction potentials of the photoexcited singlet state of Zn chlorophyll-*a* were −0.67 and 0.60 V, respectively. In contrast, first oxidation and reduction potentials of the photoexcited singlet state of Mg chlorophyll-*a* were −0.58 and 0.61 V, respectively.

*Fluorescence lifetimes of Zn and Mg chlorophyll-*a *in CTAB micelle*

Typical fluorescence decay profiles of Zn (a) and Mg chlorophyll-*a* (b) with 334 nm the excitation are shown in Figure 3. The lamp decay is shown in Figure 3 (c). The monitored wavelengths for Zn and Mg chlorophyll-*a* were 664 and 678 nm, respectively. The fluorescence decays of Zn and Mg chlorophyll-*a* consisted of a single component. Fluorescence lifetimes of Zn and Mg chlorophyll-*a* in CTAB micellar solution were estimated to be 9.0 and 9.8 ns, respectively. In contrast, fluorescence lifetimes of Zn and Mg chlorophyll-*a* in methanol solution were estimated to be 5.2 and 5.9 ns,

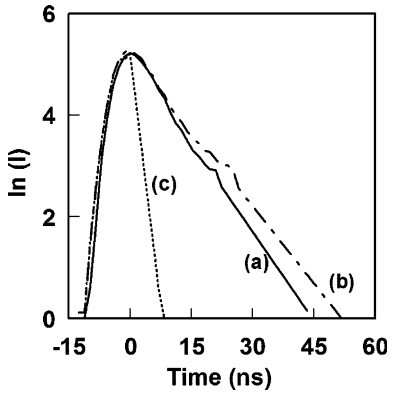


Figure 3. Fluorescence decay curves of Zn chlorophyll-*a* (a) and Mg chlorophyll-*a* (b) in CTAB micellar solution. Lamp decay is curve (c). The excitation wavelength was 334 nm. The concentrations of Zn and Mg chlorophyll-*a* were 0.6 and 0.4 μmol dm^{-3}, respectively.

respectively. Thus, the fluorescence lifetime in CTAB micellar solution is longer than that of methanol solution and the fluorescence lifetime of Zn chlorophyll-*a* is slightly shorter than that of Mg chlorophyll-*a*.

*Photostability of Zn and Mg chlorophyll-*a *in CTAB micelle*

Figure 4 shows the absorbance changes at 662 nm attributed to the absorption maximum of Zn chlorophyll-*a* (closed circle) and at 670 nm attributed to the absorption maximum of Mg chlorophyll-*a* (open circle) with irradiation time in CTAB micellar solution. The absorbance decrease of Zn chlorophyll-*a* was slower than that of Mg chlorophyll-*a* solution as shown in Figure 4, indicating that the decomposition ratio of Zn chlorophyll-*a* was suppressed compared with that of Mg chlorophyll-*a* against irradiation. Thus, Zn chlorophyll-*a* was superior to that of Mg chlorophyll-*a* in the photostability. From the

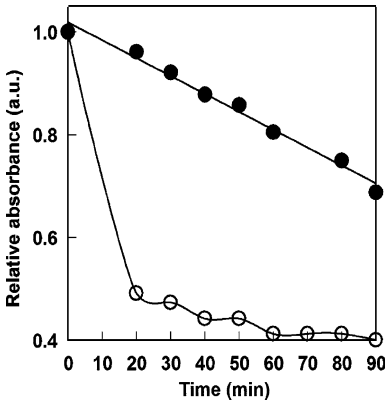


Figure 4. Time dependence of the absorbance changes of Zn (closed circle) and Mg chlorophyll-*a* (open circle) at absorption maxima under steady-state irradiation.

result of time dependence of UV-vis absorption spectrum change of Zn and Mg chlorophyll-*a* with irradiation, the no formation of metal-free chlorophyll-*a* is observed and solution breaching only is observed. Thus, the decomposing Zn and Mg chlorophyll-*a* with irradiation is attributed to the breaking double bond of chlorin ring of chlorophyll-*a*.

Next let us focus on the photostability of Zn and Mg chlorophyll-*a* under various pH conditions. Figure 5 shows the photoinduced decomposition of Zn or Mg chlorophyll-*a* under various pH conditions. The decomposition ratio of Zn or Mg chlorophyll-*a* was determined by the ratio of absorbance at absorption maximum before and after irradiation. The irradiation time was 90 min. For Mg chlorophyll-*a*, the decomposition ratio under various pH conditions was changed between 25 and 40%. At pH 4, the 40% decomposition of Mg chlorophyll-*a* was observed. In contrast to Mg chlorophyll-*a*, the decomposition under various pH conditions was suppressed and the ratio was changed between 15 and 25%. Especially the photoinduced decomposition of Zn chlorophyll-*a* was suppressed under acidic condition of pH 4. These results show that the photositability of Zn chlorophyll-*a* was superior to that of Mg chlorophyll-*a* in various pH conditions. From the result of time dependence of UV-vis absorption spectrum change of Zn and Mg chlorophyll-*a* with irradiation, the no formation of metal-free chlorophyll-*a* also is observed. Thus, the decomposing Zn and Mg chlorophyll-*a* with irradiation under various pH conditions also is attributed to the breaking double bond of chlorin ring of chlorophyll-*a*.

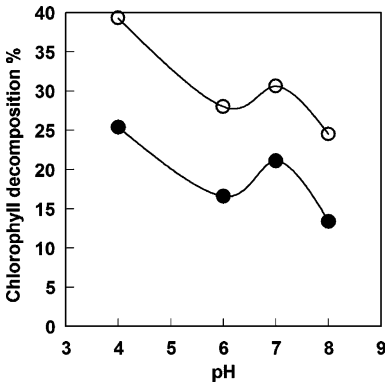


Figure 5. Photoinduced decomposition ratio of Zn (closed circle) and Mg chlorophyll-*a* (open circle) under various pH conditions. The irradiation time was 90 min.

Conclusion

In this work, Zn chlorophyll-*a* was prepareted by refluxing Mg chlorophyll-*a* from *spirulina* with an excess zinc acetate. Light-harvesting function of Zn chlorophyll-*a* in CTAB micellar solution were studied using UV-vis absorption and fluorescence emission spectra. In comparison of the UV-vis absorption and fluorescence emission spectra of Zn and Mg chlorophyll-*a*, the blue-shift in the absorption bands and emission peak of Zn chlorophyll-*a* was observed and the molar absorption coefficients of Zn chlorophyll-*a* were about six times lower than those of Mg chlorophyll-*a*. The properties of the photoexcited singlet state of Zn chlorophyll-*a* were studied using fluorescence emission spectra, electrochemical measurement and fluorescence lifetime measurement. The energies of the first excited singlet state of Zn chlorophyll-*a* was 1.87 eV. The first oxidation and reduction potentials of the photoexcited singlet state of Zn chlorophyll-*a* were −0.67 and 0.60 V, respectively. Fluorescence lifetime of Zn chlorophyll-*a* was 9.0 ns in CTAB micellar solution. The fluorescence lifetime of Zn chlorophyll-*a* is shorter than that of Mg chlorophyll-*a* (9.8 ns). The photostability of Zn chlorophyll-*a* was investigated under various pH conditions. The photositability of Zn chlorophyll-*a*

was superior to that of Mg chlorophyll-*a* in various pH conditions.

Acknowledgements

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