



## Photochemical and photophysical properties of carotenoid immobilized on a surfactant micellar medium including chlorophyll as an artificial photosynthesis system

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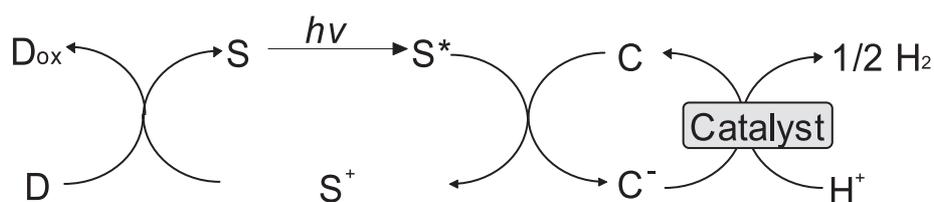
To develop an artificial photosynthesis model, the anionic water-soluble carotenoid dye crocetin was electrostatically immobilized onto the surface of cationic surfactant cetyltrimethylammonium bromide (CTAB) micellar medium including Mg chlorophyll-*a* and *b* (MgChl-*a* and *b*) (Cro/MgChl), and its photophysical properties were studied using UV-vis absorption and fluorescence spectroscopy. The fluorescence of MgChl-*a* and *b* was observed, with the excitation wavelength attributed to the absorption band of crocetin, indicating that photo-induced energy transfer from the photoexcited state of crocetin to MgChl-*a* and *b* occurs. The photostability of MgChl-*a* and *b* in Cro/MgChl was investigated under continuous irradiation. After 60 min irradiation, the absorbance decreases at 660 nm Cro/MgChl and MgChl-*a/b*, without crocetin, were 3.0 and 17%, respectively. These results indicate that the photo-bleaching rate of MgChl-*a/b* in Cro/MgChl on irradiation is suppressed by the crocetin molecule on the surface of micelles.

**Key words:** Artificial photosynthesis, Carotenoid, Crocetin, Light-harvesting Mg chlorophyll-*a*, *b*, Photo-protection

Artificial photosynthesis is attractive as a solar energy conversion system. For example, photoinduced hydrogen

production from water has been studied extensively by means of converting solar energy to chemical energy. Photo-induced hydrogen production systems containing an electron donor (D), a photosensitizer (S), an electron relay (C), and a hydrogen production catalyst have been widely studied, as shown in Scheme 1<sup>1-3</sup>. In this system, colloidal platinum and hydrogenase from *Desulfovibrio vulgaris* (Miyazaki) are widely used as a catalyst<sup>4-7</sup>. We have reported some photoinduced hydrogen production with chlorophyll and colloidal platinum<sup>8,9</sup>. However, chlorophylls purified from green plants are unstable on irradiation. Thus, photosensitizer molecules with a high stability to withstand irradiation are desired for the development of photoinduced hydrogen production. On the other hand, light-harvesting sites in natural photosynthetic proteins consist of Mg chlorophyll-*a* and *b* (MgChl-*a* and *b*) and a carotenoid dye such as  $\beta$ -carotene<sup>10</sup>. These dye molecules play an important role in photosynthesis such as light harvesting, photoinduced electron transfer, and so on. The carotenoid dyes in these proteins absorb light in the blue-green region, in which chlorophyll has a low extinction coefficient, and transfer excitation energy to chlorophyll. Many studies on excitation energy transfer from carotenoids to chlorophylls have been reported<sup>11-14</sup>. On the other hand, carotenoid dyes also have important functions such as in the absorption of UV light, photo-protection of MgChl-*a* and *b*, photosynthetic proteins, and so on<sup>15,16</sup>. Artificial photosynthesis systems based on carotenoid and chlorophyll conjugation via covalent bonds have been reported<sup>17,18</sup>. In photosynthetic proteins, MgChl-*a* and *b* and carotenoid dyes are assembled

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**Scheme 1** Photoinduced hydrogen production system containing an electron donor (D), a photosensitizer (S), an electron relay (C), and a hydrogen production catalyst.

via hydrogen bonds, hydrophobic interactions, and coordination bonds, but not covalently to amino acid residues<sup>19-21</sup>. Moreover, to develop an artificial photosynthesis model, a small separation between energy donors (carotenoids) and acceptors (chlorophylls) is a requirement dictated by the extremely short excited state lifetimes of the carotenoids. Carotenoid dye immobilized onto the surface of a surfactant micellar medium including MgChl-*a* and *b* in the hydrophobic site as an artificial photosynthesis protein is an attractive photosensitized system, because the carotenoid dye molecule on the surface of micelles will act as a UV light cut-off filter, and so the degradation of MgChl-*a/b* will be suppressed.

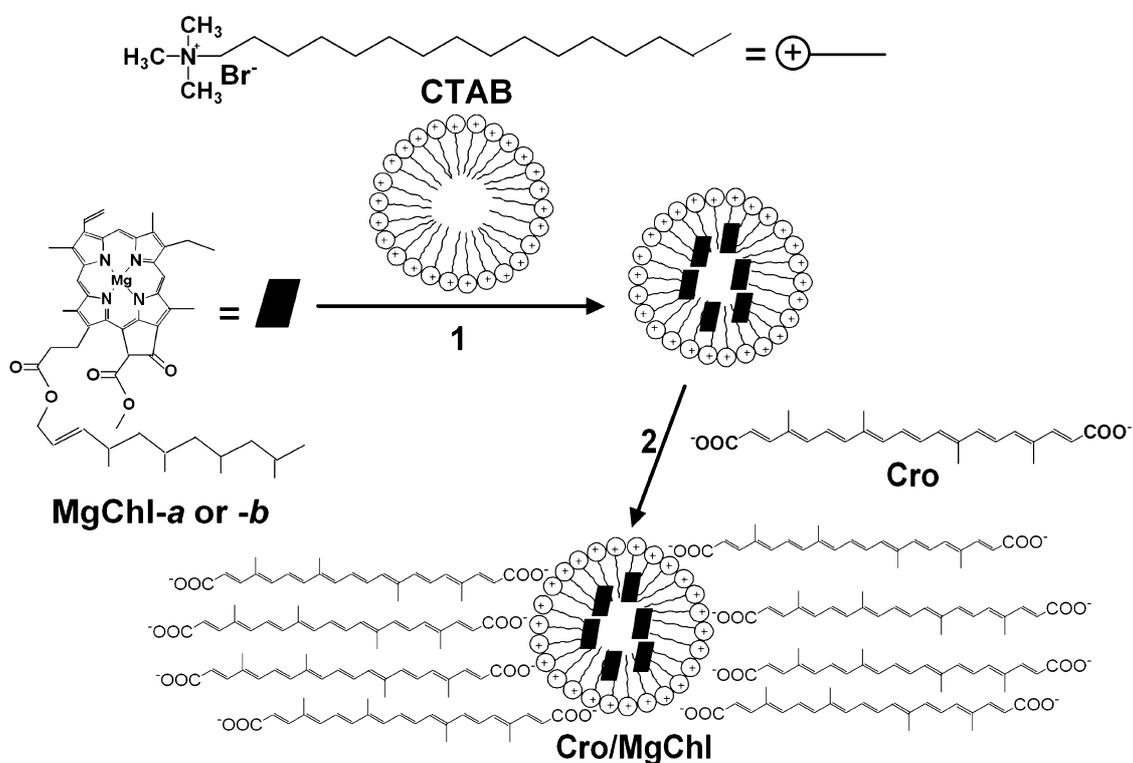
In this work, an artificial photosynthesis system, whereby the anionic water-soluble carotenoid dye crocetin ( $\lambda_{\max} = 536$  nm) was electrostatically immobilized onto the surface of cationic surfactant cetyltrimethylammonium bromide (CTAB) micellar medium including MgChl-*a* and *b*

(Cro/MgChl), was prepared and its photophysical properties such as photo-protection effect of crocetin, and photoinduced energy transfer, were investigated.

### Materials and method

MgChl-*a* and *b* were purchased from Juntech Corporation. The purity of MgChl-*a* and *b* was determined to be 99.0% by HPLC with a UV detector (column: Silica, elutant: *n*-hexane/2-propanol (98.4/1.6) and flow rate: 2.0 ml min<sup>-1</sup>). Crocetin sodium salt was obtained from Kiriya Chemical Co. Ltd., and purification was performed by recrystallization from water-methanol (5 : 1) solution. Cetyltrimethylammonium bromide (CTAB) was purchased from Tokyo Kasei Co., Ltd.

Cro/MgChl was prepared according to the method shown in Figure 1. At first, MgChl-*a* and *b* were solubilized in 20 mM cetyltrimethylammonium bromide (CTAB) micellar



**Figure 1** Schematic representation of the preparation of Cro/MgChl.

medium containing potassium phosphate buffer (pH 7) (procedure 1 in Fig. 1). Then, crocetin solution containing potassium phosphate buffer (pH 7) was added to the CTAB micellar solution containing MgChl-*a* and *b* (procedure 2 in Fig. 1). The concentrations of crocetin, and MgChl-*a* and *b* in Cro/MgChl were 0.43, 0.40, and 0.03 mM, respectively.

UV-vis absorption spectra of MgChl-*a/b*, crocetin, and Cro/MgChl solution were recorded using a spectrophotometer (Multispec-1500 Shimadzu). The fluorescence and excitation properties of MgChl-*a/b*, crocetin, and Cro/MgChl were measured using a spectrofluorophotometer with a 150 W xenon lamp as a visible excitation light source (RF-5300PC Shimadzu). The excitation and emission band-passes were 5.0 nm, respectively.

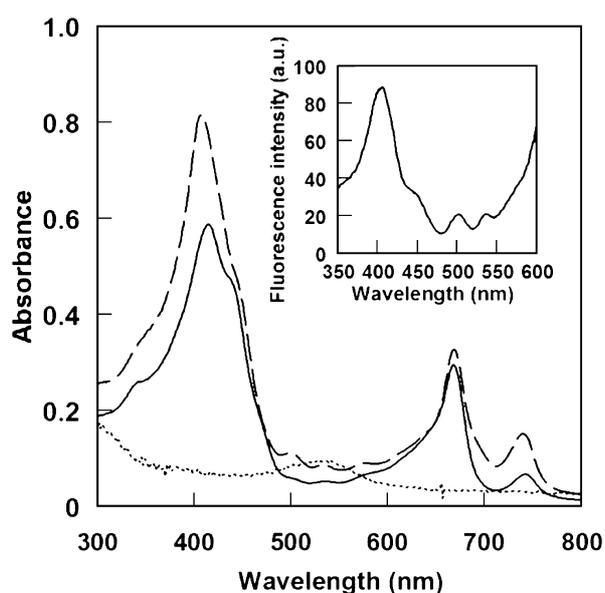
The photostability of MgChl-*a* and *b* in the Cro/MgChl was investigated. The sample solution containing Cro/MgChl in 50 mM potassium phosphate buffer solution (pH 7.0) was deaerated by repeated freeze-pump-thaw cycles 6 times. The reaction volume was 3.0 ml. A Philips KP-8 200W tungsten lamp at a distance of 3.0 cm (light intensity of  $200 \text{ J m}^{-2} \text{ s}^{-1}$ ) was used as a steady state light source. The degradation of MgChl-*a* and *b* was estimated using the absorbance changes of Q bands of chlorophylls at 660 nm.

## Results and Discussion

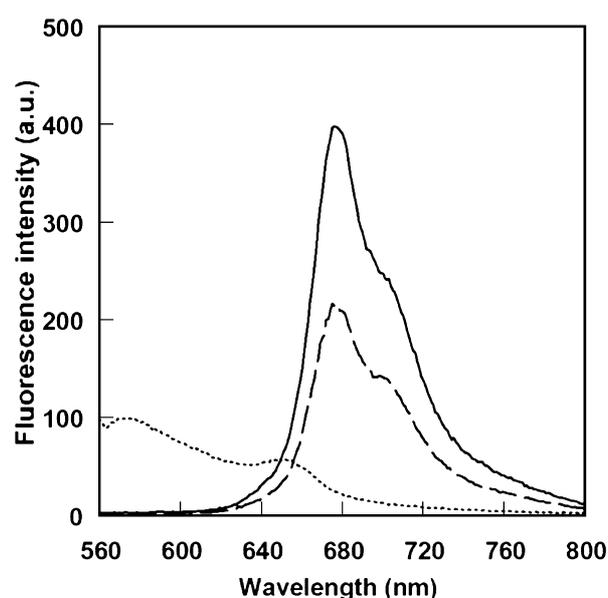
Figure 2 shows the UV-vis absorption spectra of Cro/MgChl (solid line), MgChl-*a/b* (dashed line), and crocetin (dotted line) solution in the presence of CTAB micelles. The inset is the excitation spectrum of Cro/MgChl monitored at 670 nm. From UV-vis absorption spectrum

measurement, the absorption maxima of Cro/MgChl were 407, 536, 670, and 740 nm. The absorption bands at 407, 670, and 740 nm were attributed to MgChl-*a* and *b*. The absorption band at 740 nm was due to the aggregation of MgChl-*a* and *b* molecules. The small absorption band at 536 nm due to crocetin was observed in Cro/MgChl. The shape of the absorption spectrum of Cro/MgChl is similar to that on the summation of the spectra of MgChl-*a/b* and crocetin. This result shows no electrostatic interaction among crocetin, and MgChl-*a* and *b* in Cro/MgChl at a ground state.

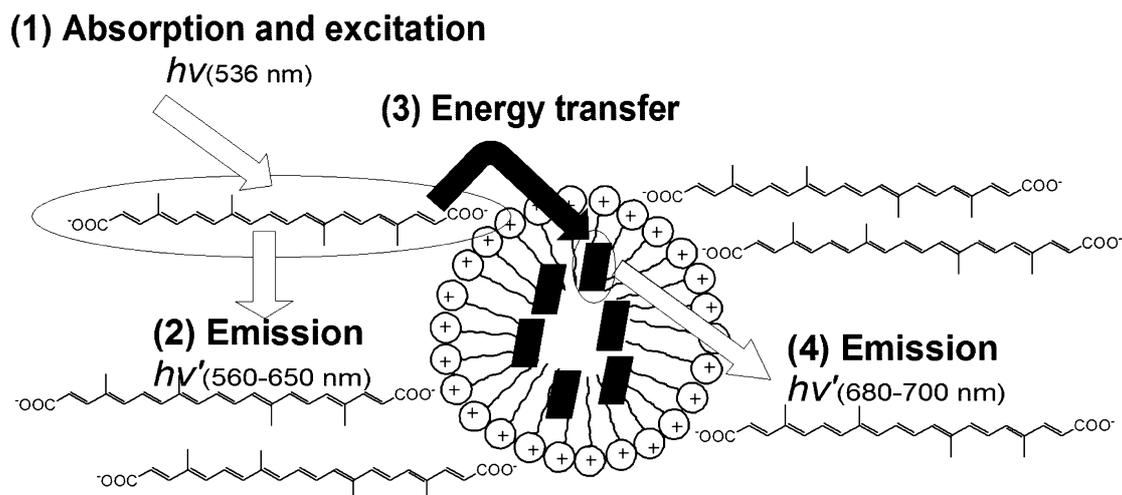
Figure 3 shows the fluorescence emission spectra of Cro/MgChl (solid line) MgChl-*a/b* (dashed line), and crocetin (dotted line) solution. When crocetin is excited with 536 nm due to the absorption band, fluorescence is observed at 572 and 645 nm, as shown in Figure 3 (dotted line). The fluorescence emission spectrum of Cro/MgChl with excitation attributed to the absorption band of crocetin (536 nm) is shown in Figure 3 (solid line). Fluorescence at 680 and 700 nm due to MgChl-*a* and *b* is observed. However, the fluorescence at 572 and 645 nm due to crocetin has disappeared. In contrast, fluorescence is also observed at 680 and 700 nm with excitation due to the absorption band of MgChl-*a* and *b* (660 nm), as shown in Figure 3 (dashed line). Moreover, the weak fluorescence at 680 nm is observed with 536 nm excitation in the CTAB micellar medium including MgChl-*a* and *b* without crocetin (MgChl-*a/b*). The shape of the excitation spectrum of Cro/MgChl is similar to that of the UV-vis absorption spectrum, as shown in Figure 2, and a peak around 536 nm due to crocetin is observed. Thus, the fluorescence of crocetin is quenched by MgChl-*a/b*, and



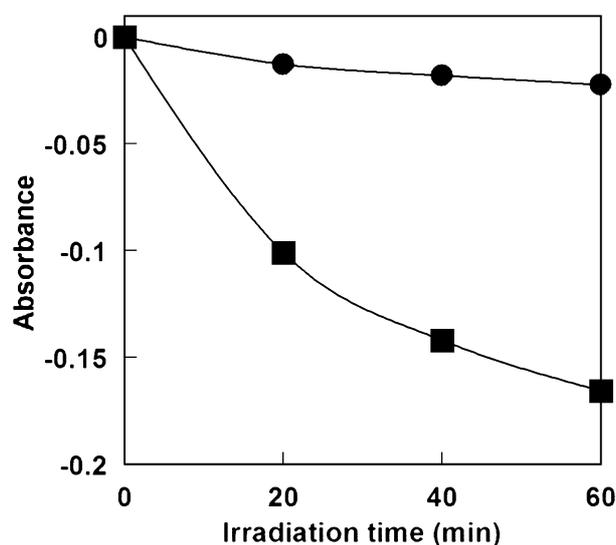
**Figure 2** UV-vis absorption spectra of Cro/MgChl (solid line), MgChl-*a/b* (dashed line), and crocetin (dotted line) solution in the presence of CTAB micelles. The inset is the excitation spectrum of Cro/MgChl monitored at 670 nm.



**Figure 3** Fluorescence emission spectra of Cro/MgChl with excitation at 536 nm (solid line) and 660 nm (dashed line). The dotted line is the emission spectrum of crocetin solution in the presence of CTAB micelles.



**Scheme 2** Schematic representation of the procedure of Cro/MgChl preparation.



**Figure 4** Time dependence of absorbance changes at 660 nm attributed to MgChl-*a* and *b* in Cro/MgChl (circle) and MgChl (square) with irradiation.

photoinduced energy transfer from the photoexcited state of crocetin onto the surface of micelles to the MgChl-*a* and *b* in the hydrophobic site of CTAB micelles occurs, as shown in Scheme 2.

Figure 4 shows the absorbance changes at 660 nm attributed to the absorption band of MgChl-*a/b* in Cro/MgChl (circle) and in MgChl-*a/b* (square) with irradiation time. After 60 min irradiation, the absorbance decreases at 660 nm in Cro/MgChl and MgChl-*a/b* were 3.0 and 17%, respectively. The decrease in the rate of absorbance of MgChl-*a/b* in Cro/MgChl is slower than that of MgChl. These results indicate that the photo-bleaching rate of MgChl-*a/b* in Cro/MgChl on irradiation is suppressed by the crocetin molecule on the surface of micelles. As shown in Figure 2,

crocetin absorbs light at wavelengths less than 400 nm and 536 nm on the surface of CTAB micelles, including MgChl-*a/b*. From the fluorescence of Cro/MgChl, the photoinduced energy transfers from crocetin to the MgChl-*a/b* via the interface of CTAB micelles, as shown in Scheme 2. Since the crocetin molecule on the surface of micelles acts as a cut-off filter, the degradation of MgChl-*a/b* is suppressed.

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#### References

1. Kiwi, J., Kalyanasundaram, K. & Grätzel, M. Visible light induced cleavage of water into hydrogen and oxygen in colloidal and microheterogeneous system. *Struct. Bonding*. **49**, 37–125 (1982).
2. Darwent, J. R., Douglas, P., Harriman, A., Porter, G. & Richoux, M. C. Metal phthalocyanines and porphyrins as photosensitizers for reduction of water to hydrogen. *Coord. Chem. Rev.* **44**, 93–126 (1982).
3. Okura, I., Aono, S. & Yamada, A. Photoinduced electron transfer between Zn(TPPS)<sub>3</sub><sup>3-</sup> and viologens. *J. Phys. Chem.* **89**, 1593–1598 (1985).
4. Okura, I. Hydrogenase and its application for photoinduced hydrogen evolution. *Coord. Chem. Rev.* **68**, 53–101 (1985).
5. Kiwi, J. & Grätzel, M. Protection size factors, and reaction dynamics of colloidal redox catalysis mediating light induced hydrogen evolution from water. *J. Am. Chem. Soc.* **101**, 7214–7217 (1979).
6. Brugger, P. A., Cuendet, P. & Grätzel, M. Ultrafine and specific catalysts affording efficient hydrogen evolution from water under visible light illumination. *J. Am. Chem. Soc.* **103**, 2923–2927 (1981).
7. Okura, I. Application of hydrogenase for photoinduced

- hydrogen evolution. *Biochimie* **68**, 189–199 (1986).
8. Tomonou, Y. & Amao, Y. Visible light induced hydrogen production with Mg chlorophyll-*a* from spirulina and colloidal platinum. *Biometals* **15**, 391–395 (2002).
  9. Tomonou, Y. & Amao, Y. Visible and near-IR light induced biohydrogen production using the system containing Mg chlorophyll-*a* from Spirulina and colloidal platinum. *Biometals* **16**, 419–424 (2004).
  10. Scheer, H. *Chlorophylls* (CRC Press, London, 1991).
  11. Nancy, E. H., Kennis, J. T. M., Dall'Osto, L., Bassi, R. & Graham R. Fleming, G. R. Carotenoid to chlorophyll energy transfer in light harvesting complex II from *Arabidopsis thaliana* probed by femtosecond fluorescence upconversion. *Chem. Phys. Lett.* **379**, 305–313 (2003).
  12. Peterman, E. J. G., Monshouwer, R., van Stokkum, I. H. M., van Grondelle, R. & van Amerongen, H. Ultrafast singlet excitation transfer from carotenoids to chlorophylls via different pathways in light-harvesting complex II of higher plants. *Chem. Phys. Lett.* **264**, 279–284 (1997).
  13. Akimoto, S., Takaichi, S., Ogata, T., Nishimura, Y., Yamazaki I. & Mimuro, M. Excitation energy transfer in carotenoid-chlorophyll protein complexes probed by femtosecond fluorescence decays. *Chem. Phys. Lett.* **260**, 147–152 (1996).
  14. Shreve, A. P., Trautman, J. K., Owens, T. G. & Albrecht, A. C. A femtosecond study of electronic state dynamics of fucoxanthin and implications for photosynthetic carotenoid-to-chlorophyll energy transfer mechanisms. *Chem. Phys. Lett.* **154**, 171–178 (1991).
  15. Matile, P., Hörtensteiner, S. & Thomas, H. Chlorophyll degradation. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 67–95 (1999).
  16. Kim, S. & Jung, J. Inactivation of the respiratory chain in plant mitochondria by visible light: the primary target for damage and endogenous photosensitising chromophores. *J. Photochem. Photobiol. B, Biol.* **29**, 135–139 (1995).
  17. Gould, S. L., Kodis, G., Liddell, P. A., Palacios, R. E., Brune, A., Gust, D., Moore, T. A. & Moore, A. L. Artificial photosynthetic reaction centers with carotenoid antennas. *Tetrahedron* **62**, 2074–2096 (2006).
  18. Gust, D., Moore, T. A., Moore, A. L., Kuciauskas, D., Liddell, P. A. & Halbert, B. D. Mimicry of carotenoid photoprotection in artificial photosynthetic reaction centers: triplet-triplet energy transfer by a relay mechanism. *J. Photochem. Photobiol. B, Biol.* **43**, 209–216 (1998).
  19. Nagata, M., Nango, M., Kashiwada, A., Yamada, S., Ito, S., Sawa, N., Ogawa, M., Iida, K., Kuroono, Y. & Ohtsuka, T. Construction of photosynthetic antenna complex using light-harvesting polypeptide- $\alpha$  from photosynthetic bacteria, *R. rubrum* with zinc substituted bacteriochlorophyll *a*. *Chem. Lett.* **32**, 216–217 (2003).
  20. Iida, K., Kiriya, H., Fukai, A., Konings, W. N. & Nango, M. Two-dimensional self-organization of the light-harvesting polypeptides/BChl *a* complex into a thermostable liposomal membrane. *Langmuir* **17**, 2871–2827 (2001).
  21. Kashiwada, A., Watanabe, H., Tanaka, T. & Nango, M. Molecular assembly of zinc bacteriochlorophyll *a* by synthetic hydrophobic 1 $\alpha$ -helix polypeptides. *Chem. Lett.* **29**, 24–25 (2000).