

Excitation Energy Transfer from Self-aggregates of Zinc Chlorins to a Bacteriochlorin in a Silicate Nanocapsule

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Silicate nanocapsules including self-aggregates of zinc methyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (zinc chlorin) and methyl bacteriopyropheorbide-*a* (bacteriochlorin) were prepared by a sol-gel process. Excitation energy transfer from self-aggregates of zinc chlorins to an energy-accepting bacteriochlorin occurred in the silicate nanocapsule, which was similar to a light-harvesting complex (chlorosome) of green photosynthetic bacteria.

Chlorosome is an extramembranous light-harvesting complex of green photosynthetic bacteria. Bacteriochlorophyll (BChl)-*s-c* and *d* (the molecular structure of BChl-*d* is shown in Figure 1A) form self-assemblies in a chlorosome.¹ Harvested light energy by BChl-*d* self-aggregates in a chlorosome is transferred to complexes of BChl-*a* (see Figure 1B) with proteins called baseplates, which are located in a surface membrane of a chlorosome. Chlorosomes, in which no protein participates in the supramolecular structure of BChl-*d* self-aggregates are of much interest in supramolecular photochemistry as well as photosynthesis.

Self-aggregates of BChl-*s-c, d*, and their model compounds in non-polar organic solvents and in aqueous micelles have been studied as structural models of chlorosomal supramolecules.²⁻⁶ However, there has been little report on functional models of chlorosomes,⁷ which mimic the chlorosome-type energy transfer. Photofunctional supramolecules that capture and transfer light-energy efficiently like natural photosynthetic antennas are promising for constructing artificial photosynthetic and high-performance light-energy conversion systems.

Additionally, stabilization of such supramolecules is one of the important points for application of these photoactive supramolecules to highly functional devices. However, self-aggregates of chlorophyllous pigments reported previously were not

stable enough to construct artificial photoactive devices. To overcome this problem, the sol-gel method is useful for preparing organic-inorganic hybrid supramolecules possessing highly mechanical strength.⁸ We preliminarily prepared stable self-aggregates of BChl-*d* model compounds by use of alkoxysilanes as a structural model of chlorosomes.⁹ In this paper, we present a novel functional model of chlorosomes using a sol-gel method, where excitation energy transfer from self-aggregates of zinc chlorins to a bacteriochlorin occurs in a silicate nanocapsule.

Zinc methyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (**1**, Figure 1A), was synthesized from chlorophyll-*a*.¹⁰ Methyl bacteriopyropheorbide-*a* (**2**, Figure 1B) was synthesized from BChl-*a*.¹¹ Metal-free bacteriochlorin **2** was suitable for an energy acceptor in chlorosomal energy transfer, because fluorescent zinc- and magnesium-bacteriochlorins were unstable. Octadecyltriethoxysilane (ODTES) and tetraethoxysilane (TEOS) were purchased from Shin-etsu Chemical Co., Ltd. (Tokyo, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. ODTES (10%, v/v) and TEOS (10%, v/v) were incubated in ethanol containing 1 M HCl (1.5%, v/v) for 30 min. The pH of the ethanol solution of ODTES and TEOS was neutralized, and 10 μ L of the solution was mixed with a 30 μ L ethanol solution of **1** (0.9 mM) and **2** (40 μ M). The mixed solution was dispersed in distilled water (4 mL), and then the aqueous suspension was incubated at 20 °C for 50 h.

Figure 2 shows visible absorption spectra of zinc chlorin **1** and bacteriochlorin **2** in the presence of alkoxysilane molecules (solid curve) and sole **1** with alkoxysilanes (dotted curve) in an aqueous solution, and each pigment in ethanol (**1** and **2** as broken and dashed curve, respectively).¹² In ethanol, **1** had absorption bands at 426 and 652 nm, indicating that **1** was monomeric in the polar organic solution. In the presence of the above partially-hydrolyzed alkoxysilane molecules, Soret and Q_y bands of zinc chlorin **1** in an aqueous solution were shifted to longer

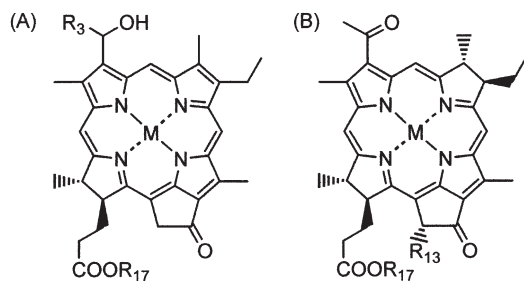


Figure 1. (A) Molecular structures of bacteriochlorophyll (BChl)-*d* and its analog **1**, BChl-*d*: M=Mg, R₃=CH₃, R₁₇=farnesyl, **1**: M=Zn, R₃=H, R₁₇=CH₃. (B) Molecular structures of bacteriochlorophyll-*a* and its analog **2**, BChl-*a*: M=Mg, R₁₃=COOCH₃, R₁₇=phytyl, **2**: M=2H, R₁₃=H, R₁₇=CH₃.

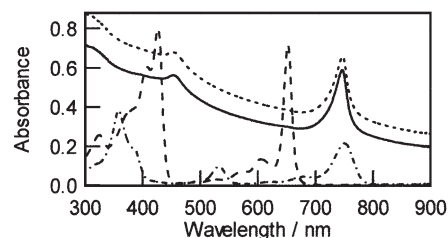


Figure 2. Visible absorption spectra of self-aggregates of zinc chlorin **1** (9 μ M) and bacteriochlorin **2** (0.4 μ M) (solid curve) and self-aggregates of sole **1** (9 μ M, dotted curve) with ODTES (0.025%, v/v) and TEOS (0.025%, v/v) in an aqueous solution, and monomeric **1** (9 μ M, broken curve) and monomeric **2** (4 μ M, dashed curve) in ethanol.

wavelength, and were positioned at 454 and 746 nm, respectively. These red-shifts of both the bands indicate self-aggregation of **1** in the aqueous solution, which is similar to the antenna BChl-*d* organization in natural chlorosomes. In the present preparation, Triton X-100 can hardly decompose the self-aggregates of **1** in the silicate capsule to a monomeric form, whereas self-aggregates in our preliminary silicate capsule were turned to a monomeric form by addition of over ca. 1.0% (v/v) of Triton X-100.⁹ Thus, the partial pre-hydrolysis of alkoxy silane molecules in this report is useful for construction of stable chlorosome-type capsules, and superior to the previous preparation without pre-treatment. Dynamic light scattering measurements showed that the average hydrodynamic diameter of the silicate capsules containing aggregated **1** was 2.7×10^2 nm,¹³ indicating that the capsules had similar sizes to natural chlorosomes. A Q_y band of bacteriochlorin **2** was positioned at 750 nm in ethanol; this band overlapped with the fluorescence emission band from self-aggregates of **1**. This prompted us to study the excitation energy transfer from self-aggregates of zinc chlorin **1** to bacteriochlorin **2** in the silicate capsule, which mimic the energy transfer from antenna BChl-*d* aggregates to BChl-*a* in a chlorosome.

Fluorescence emission spectra of silicate capsules including both **1** and **2** (solid curve) and sole **1** (dotted curve) are shown in Figure 3. Excitation light ($\lambda_{\text{ex}} = 460$ nm) was predominantly absorbed by self-aggregates of **1**, and can hardly excite bacteriochlorin **2** (see Figure 2). The emission band around 750 nm was ascribed to the self-aggregates of zinc chlorin **1**. In the spectra of silicate capsules including both **1** and **2**, the emission around 750 nm was reduced by a factor of 0.67, compared with the capsules containing only aggregated **1**. A new emission band appeared around 815 nm, which originated from bacteriochlorin **2**. The fluorescence from **2** was red-shifted compared to that of monomeric **2** in a polar organic solution. This would be ascribable to electronic interaction between **2** and aggregated **1**. Excitation spectrum of the capsules containing aggregated **1** and **2** (solid curve in Figure 4), which was obtained by 820-nm fluorescence detection, had an intense band around 746 nm attributed to self-aggregates of zinc chlorin **1**. This confirms that the new fluorescence emission of **2** around 815 nm came from excitation energy transfer from self-aggregates of zinc chlorin **1**. Therefore, a novel organic-inorganic hybrid nanocapsule mimicking the energy transfer of chlorosomes was successfully constructed in this study. Energy transfer efficiency in a silicate nanocapsule was 33%, which was estimated from the fluorescence emission intensities in Figure 3. This efficiency was small-

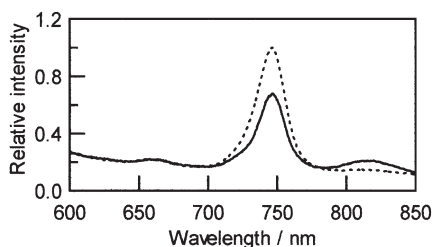


Figure 3. Fluorescence emission spectra of self-aggregates of zinc chlorin **1** and bacteriochlorin **2** (solid curve) and self-aggregates of sole **1** (dotted curve) with ODTES (0.025%, v/v) and TEOS (0.025%, v/v) in an aqueous solution. Excitation wavelength, 460 nm.

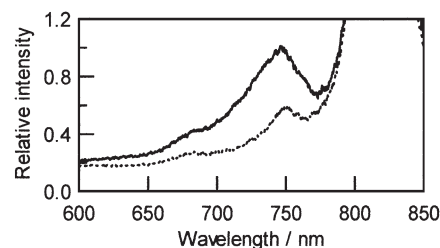


Figure 4. Fluorescence excitation spectra of self-aggregates of zinc chlorin **1** and bacteriochlorin **2** (solid curve) and self-aggregates of sole **1** (dotted curve) with ODTES (0.025%, v/v) and TEOS (0.025%, v/v) in an aqueous solution. Detection wavelength, 820 nm.

er than those observed in the aqueous micelle with α -lecithin (ca. 60%).⁷ The decrease of energy transfer efficiency in the chlorosome model could be attributable to the change in the distance and/or the orientation of energy-donating aggregates of zinc chlorin **1** and an energy accepting bacteriochlorin **2**. Formation of siloxane bonds might cause these changes in the chlorosomal nanocapsule. The molecular design of alkoxy silane analogs and the sol-gel process, therefore, are expected to improve the efficiency of exciton energy transfer in such silicate capsules.

To summarize, the present supramolecule is significantly stable compared with the reported aqueous micellar self-assemblies in which similar chlorosomal energy transfer occurs, because of siloxane network formation on the surface of the self-aggregates. The supramolecular system in this study would lead to a stable and efficient light-energy conversion device mimicking photosynthetic antenna apparatus.

References and Notes

- 1 J. M. Olson, *Photochem. Photobiol.*, **67**, 61 (1998).
- 2 M. Hirota, T. Moriyama, K. Shimada, M. Miller, J. M. Olson, and K. Matsuura, *Biochim. Biophys. Acta*, **1099**, 271 (1992).
- 3 T. Nozawa, K. Ohtomo, N. Takeshita, Y. Morishita, M. Osawa, and M. T. Madigan, *Bull. Chem. Soc. Jpn.*, **65**, 3493 (1992).
- 4 K. Uehara, M. Mimuro, Y. Ozaki, and J. M. Olson, *Photosynth. Res.*, **41**, 235 (1994).
- 5 D. B. Steensgaard, H. Wackerbarth, P. Hildebrandt, and A. R. Holzwarth, *J. Phys. Chem. B*, **104**, 10379 (2000).
- 6 T. Mizoguchi, Y. Saga, and H. Tamiaki, *Photochem. Photobiol. Sci.*, **1**, 780 (2002).
- 7 T. Miyatake, H. Tamiaki, A. R. Holzwarth, and K. Schaffner, *Helv. Chim. Acta*, **82**, 797 (1999).
- 8 H. Schmidt, *J. Sol-Gel Sci. Technol.*, **1**, 217 (1994).
- 9 Y. Saga, T. Miyatake, and H. Tamiaki, *Bioorg. Med. Chem. Lett.*, **12**, 1229 (2002).
- 10 H. Tamiaki, M. Amakawa, Y. Shimono, R. Tanikaga, A. R. Holzwarth, and K. Schaffner, *Photochem. Photobiol.*, **63**, 92 (1996).
- 11 H. Tamiaki, M. Kouraba, K. Takeda, S. Kondo, and R. Tanikaga, *Tetrahedron: Asymmetry*, **9**, 2101 (1998).
- 12 Visible absorption and fluorescence spectra were measured with Hitachi U-3500 and F-4500 spectrophotometers, respectively.
- 13 DLS measurements were performed with a Malvern Zetasizer Nano-ZS particle size analyzer.