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# Heating changed suprastructure of self-assembled zinc methyl bacteriopheophorbide-*c*

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### Introduction

In a chlorosome of green photosynthetic bacteria, a number of bacteriochlorophyll (BChl)-*c*, *d* and -*e* molecules self-aggregate to form rod-like oligomers surrounded with monolayer of galactolipids (Tamiaki 1996 and Olson 1998). The BChl aggregates act as a main lightharvesting antenna and the collected energy is efficiently transferred to BChl-*a* in a baseplate complex. Supramolecular structure of the chlorosomal aggregate is reproduced by selfassembly of isolated BChls-*c*, -*d* and -*e* molecules *in vitro*. Similarly, synthetic zinc analogues of BChl-*c* also form self-aggregates to mimic the structure and function of the supramolecular antenna (Tamiaki et al. 1996 and Miyatake et al. 1999ab, 2001). By the way, some green bacteria are thermophilic and cultured in heated media. For instance, optimum temperature for culturing *Chlorobium tepidum* and *Chloroflexus aurantiacus* is 45 and 55 °C, respectively. However, most of *in vitro* model studies on chlorosomal aggregates have been performed at room temperature. Here we report self-aggregate of zinc methyl bacteriopheophorbide-*c* (zinc chlorin 1 in Fig. 1) in a heated aqueous medium. We found that heating changed supramolecular structure of the artificial aggregate.



Fig.1. Structure of BChl-c (left) and zinc chlorin 1 (right)

#### Materials and methods

Preparation of synthetic zinc chlorins (Tamiaki et al. 1998). Zinc chlorin **1** was prepared from natural BChl-*c* extracted from cultured cells of *Chloroflexus aurantiacus*. The two stereoisomers of  $3^1R$  and  $3^1S$  were separated by a single reversed-phase HPLC run. *Preparation of an artificial aggregate* (Miyatake et al. 1999b). A methanol solution of zinc chlorin **1** and lecithin (phosphatidyl choline from frozen egg yolk, SIGMA USA) was diluted with large volume of 10 mM Tris-HCl buffer (pH=7.5) at room temperature. Final concentration of **1** and lecithin was  $1.0 \cdot 10^{-5}$  and  $3.2 \cdot 10^{-5}$  M, respectively. *Spectroscopic methods*. Absorption spectra were measured with Shimadzu U-3100 spectrophotometer equipped with temperature control unit S-260. Circular dichroism spectra were measured with Jasco J-720W spectropolarimater.

#### **Results and Discussion**

Visible absorption spectra of zinc chlorin 1 were significantly affected by solvent (Table 1). In methanol, both  $3^{1}R$  epimer **1R** and  $3^{1}S$  epimer **1S** showed a Qy band at 662 nm, which correspond to their monomeric forms. When the methanol solution of 1 and lecithin was diluted with a large amount of aqueous buffer at room temperature, it showed a red-shifted Qy absorption band due to the self-aggregation. The aqueous solution of 1R showed a 727-nm absorption peak accompanied by a shoulder at 740 nm. On the other hand, the  $3^{1}S$  epimer 1S gave absorption maxima at 668 and 704 nm, which correspond to remaining monomer and aggregates of **1S**, respectively. Thus, the oligomerization of zinc chlorin **1** was affected by chirality at 3<sup>1</sup> position (Tamiaki et al. 1998 and Yagai et al. 2001). When the aqueous aggregate of **1S** was heated at 55 °C, the absorption spectrum was gradually changed. The Qy absorption band was red-shifted to 731 nm after heating for 1 h. The red-shifted absorption band was unchanged when the solution gradually cooled to room temperature, thus this spectral change was irreversible. This result suggested that the kinetic products of aggregate **1S** converged to thermodynamically favourable structure in a heated medium (Figure 2). Although this spectral change was almost terminated within 1 h at 55 °C, it took more than 5 hours below 50 °C. The aqueous solution was homogeneous throughout measurements without formation of any precipitates, which exhibits that the zinc chlorin 1 was surrounded with the added lecithin molecules.

	in methanol	in an aqueous lecithin solution		
		before heating	after heating at 55°C	
10	(())	727 740(1)	<b>700 740</b> (1)	
IK	662	/2/, /40(sh)	/23, /43(sh)	
<b>1S</b>	662	709	731	

Table 1. Visible absorption maxima (nm) of zinc chlorin 1



Fig.2. Self-assembly of zinc chlorin 1 in an aqueous lecithin solution

CD spectrum of aggregated **1S** was also changed by heating the solution (Table 2). Before heating, CD spectrum of the aggregated **1S** showed small positive bands (699, 717 nm) and a small negative band (666 nm) at the Qy-absorption region. After heating at 55  $^{\circ}$ C, the small CD signals changed to inverse S-shaped signals with a large negative band at 740 nm and a positive band at 727 nm. This large CD of the heated aggregates at the Qy absorption wavelengths suggests that the thermodynamically stable aggregates has well ordered supramolecular structure, in which **1S** molecules aligned along the y-axis (N21–N23 in Fig. 1).

Compared to the remarkable spectral change found in the heated **1S** aggregate, the **1R** aggregates gave no additional band in visible absorption and CD spectra upon heating (Tables 1 and 2). The two Qy absorption bands (727, 740 nm) slightly shifted to 723 and 743 nm. While, the inverse S-shaped CD signals with a positive band at 726 nm and a negative band at 744 nm enlarged about 2.5-fold by heating. These spectra suggested that the basic supramolecular structure of the aggregated **1R** was not changed by heating, i.e., the kinetic product has almost the same supramolecular arrangement with the thermodynamic product. The oligomer of **1R** formed at room temperature was annealed by heating to give well-ordered supramolecular structure with giant CD signals (Miyatake et al. 1999b).

wavelength / nm (ellipticity / mdeg)				
	before heating	after heating		
1R	726 (+476), 743(-517)	726 (+1120), 744 (-1260)		
<b>1S</b>	666 (-9), 699 (+5), 717 (+7)	727 (+188), 740 (-213)		

Table 2. CD peaks of 1 in an aqueous lecithin solution

In summary, self-assembled aggregates of synthetic zinc chlorin was prepared in an aqueous medium. At first, the kinetically favorable structure was appeared in the aggregates prepared at room temperature. The kinetic product of aggregated **1S** might have relatively disordered suprastructure compared to that of the **1R** aggregates. When the aqueous aggregates were heated at 55 °C, both **1R** and **1S** aggregates were annealed to give thermodynamically favorable structure, in which zinc chlorin molecules were well ordered along the y-axis.

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