Substituent Effects on the Aggregation of Bacteriochlorophyll d Homologues Purified from *Chlorobium limicola*

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Synopsis. Six bacteriochlorophyll (BChl) d homologues were isolated from *Chlorobium limicola*. The absorption spectral profiles in hexane–dichloromethane and the solvent effects indicate that the aggregation property of the BChl d's strongly depend on the nature of the peripheral substituents.

Green bacteria are characterized by a special light-harvesting structure called a chlorosome, which is absent from purple photosynthetic bacteria.[1–3] Chlorosomes comprise of pigments (bacteriochlorophyll (BChl) c or d), lipids and proteins, and contain rod-like structures called "rod-elements". Pigment–pigment interactions are predominant in chlorosomes,[4,5] in contrast to other photosynthetic antenna at which pigment–protein interactions dominate in maintaining the structure. It has recently been reported that proteins are present around, but not in, the rod-elements.[6,7] The BChl c's or d's in chlorosomes show absorption maxima at around 740 nm that is far red-shifted from those in polar organic solvents. The variation of the absorption maximum is correlated with the structural organization of the pigments. The varieties observed in the CD spectra are also associated with the structural differences.[8] Chlorosomes from *Chlorobium limicola* contain several homologues of BChl d (Fig. 1);[9] we examined the effect of peripheral substituents on the pigment organization.

Experimental

Materials and Method. *C. limicola* was grown and its chlorosomes were isolated as in a previous study.[10] BChl d was extracted with methanol or chloroform and purified as previously described for BChl c.[11] HPLC (Toso Bio-LC system) with an ODS (TSK_{gel} ODS-$80T_M$) column was used for fractionation of the components with methanol/water (96/4, v/v) used as the eluent. ^1H NMR spectra were recorded on a Bruker MSL400 FT NMR spectrometer equipped with a dual probe for ^1H and ^{13}C. The absorption spectra were observed on a Shimadzu UV-3100 in a 0.2 cm cell.

Results and Discussion

Six major pigment components (designated F1 through F6) were isolated by HPLC. They all showed the same absorption spectrum as that reported for BChl d in methanol.[12] The results of normal ^1H NMR and two-dimensional (COSY) ^1H NMR spectroscopies on each component agreed with the structures for the six BChl d's from *Chlorobium vibrioforme*;[13] the peripheral substituents and relative abundances are given in Fig. 1 and Table 1.

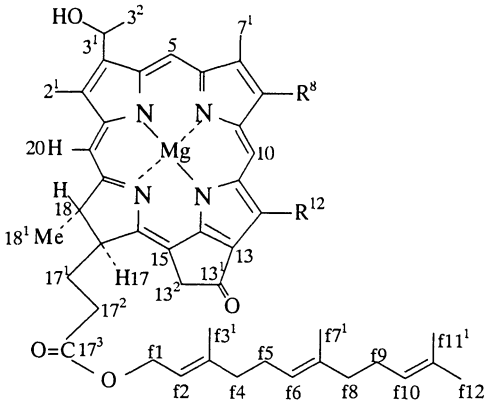

Fig. 1. Structures of BChl d Homologues with IUPAC numbering.

Table 1. R^8 and R^{12} Substituents and Relative Abundance of the BChl d Fractions F1 through F6

	R^8	R^{12}	Relative amount
F1	Et	Me	7.2%
F2	Et	Et	15.6%
F3	*n*-Pr	Me	9.3%
F4	*n*-Pr	Et	33.8%
F5	*i*-Bu	Me	15.5%
F6	*i*-Bu	Et	18.6%

Figure 2 shows the absorption spectra of the six components in hexane. The spectra were taken after the addition of a BChl d dichloromethane solution (20 μl) to hexane (1 ml). The spectral profile did not significantly depend on the concentration when the latter was higher than 5.0×10^{-6} mol dm^{-3}. Comparisons of the spectra between F1 and F2, F3 and F4, and F5 and F6 with a common R^8 substituent disclose that the ethyl group at position 12 generates aggregates, that are characterized by long-wavelength peaks to a greater extent than the methyl group at the same position. Since an aggregate with a longer wavelength peak corresponds to a higher aggregate, the substituent at position 12 is thus found to critically affect the formation of these aggregates. Comparisons among F1, F3, and F5, and among F2, F4, and F6 with a common substituent at position 12 indicate that a larger substituent at posi-

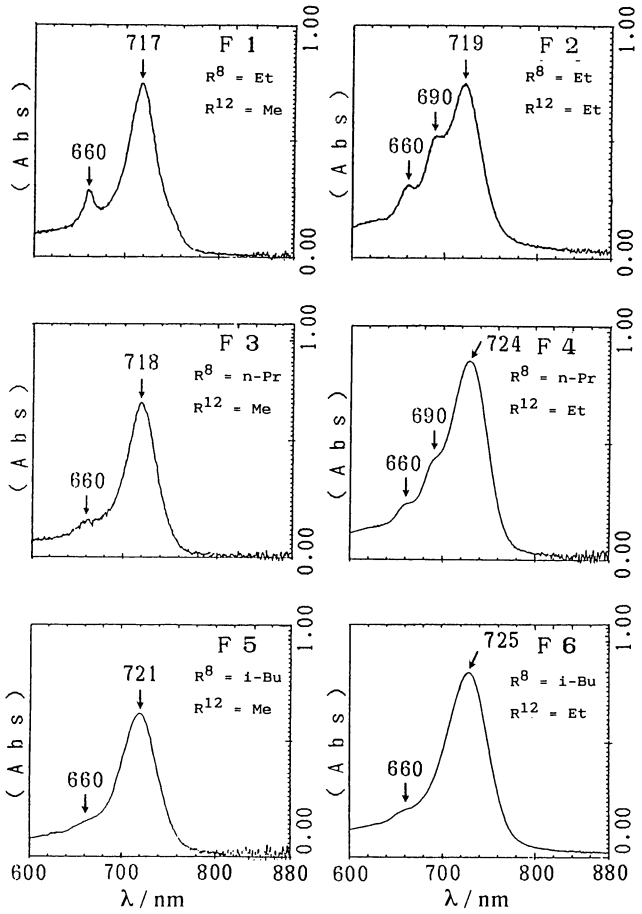

Fig. 2. Absorption spectra of the six BChl d fractions in hexane–dichloromethane (50 : 1, v/v). BChl d concentration, 6.6×10^{-5} mol dm^{-3}; Optical path length, 2 mm.

tion 8 tends to give aggregates with longer wavelength peaks. Hence, the substituents at position 8 also control the formation of higher aggregates.

Table 2 shows the variation of the % fractions of the longest wavelength components with the volume of added dichloromethane. Dichloromethane disintegrates higher aggregates by breaking the hydrophobic interactions in them. A comparison among the pigments with a common R^{12} substituent (among F1, F3, and F5, and among F2, F4, and F6) reveales that the stability of the higher aggregates to this solvent is higher for a pigment with a larger R^8 substituent.

The R^8 and R^{12} substituents thus affect the aggregation number reflected in the absorption peak wavelength. The presence of an inhomogenity in native chlorosomes has been inferred from the absorption and circular dichroism spectra.[8] The various fractions may contribute to this inhomogenity.

Table 2. Variation of the Longest Wavelength Band by Addition of Dichloromethane (V/μl) to 1 ml Hexane Solutions of Pigment Components. The Longest Wavelength Band Fraction was Evaluated by Resolution of Absorption Spectra into Three Components

Fraction	The Longest wavelength component/%				
	V/μl				
	20	40	80	120	160
F1	56.8	55.6	51.9	44.3	30.3
F3	64.9	64.1	60.7	52.2	37.1
F5	67.2	67.0	65.3	61.0	57.7
F2	64.9	60.1	38.5	9.4	7.2
F4	66.0	62.6	53.7	38.5	24.9
F6	65.9	63.0	60.7	56.9	52.5

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