

Isolation and Structural Determination of a Complete Set of Intact Bacteriochlorophyll-*d* Homologs and Isomers from Green Sulfur Bacterium *Chlorobium vibrioforme* and Their Aggregation Properties in Hydrophobic Solvents

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Eight intact bacteriochlorophyll (BChl)-*d* homologs and isomers were isolated from a strain of green sulfur bacterium *Chlorobium vibrioforme*. All the molecular structures of the BChl-*d* components were fully determined by a combination of mass spectrometry and ¹H-NMR spectroscopy. The aggregation behavior of the isomerically pure BChls-*d* in hydrophobic organic solvents was examined with respect to the stereoisomeric configuration at the C3¹ position as well as the bulkiness of the C8 and C12 side-chains by using electronic-absorption spectroscopy.

Key words: bacteriochlorophyll-*d*, homologs, photosynthetic antenna, self-assembly, stereoisomers

INTRODUCTION

Green photosynthetic bacteria have a unique antenna complex called chlorosomes [1]. Each chlorosome is assumed to contain 10-30 'rod-elements' having bacteriochlorophyll (BChl)-*c*, *d*, or *e* molecules as the major component [1]. These rod-elements are made of a higher aggregate of those BChl molecules. Chlorosomes are also unique in their content of light-harvesting BChl molecules: the presence of both the stereoisomers of the 1-

hydroxyethyl group at the C3 position and of the stepwise methylated homologs in the C8 and C12 side-chains. It has been systematically examined how the stereoisomeric configuration at the C3¹ position as well as the bulkiness of the side-chains at the C8 and C12 positions affect the supramolecular structures of natural chlorosomes and artificial self-aggregates.

MATERIALS AND METHODS

A strain of *Chlorobium (Chl.) vibrioforme* producing

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exclusively BChl-*d* as a light-harvesting BChl was grown from a single colony [2]. Intact BChl-*d* homologs and isomers were obtained from the cultured cells by similar procedures described previously [3]. Each isomerically pure BChls-*d* was identified by FAB-MS and ¹H-NMR spectra. Sample solutions for electronic-absorption measurements were prepared as described previously [3].

RESULTS AND DISCUSSION

Figure 1 depicts the chemical structure of BChl-*d* possessing a farnesyl ester from the strain of *Chl. vibrioforme*. The R- and S-stereoisomers investigated here are defined for the 3¹-carbon atom. The R-isomers having the ethyl (E) and propyl (P) groups at the C8 position and the methyl (M) and ethyl (E) groups at the C12 position are termed R[E,M], R[P,M], R[E,E] and R[P,E], respectively. The S-isomers are named as S[X,Y].

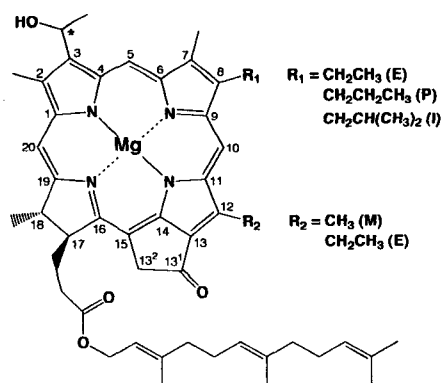


Figure 1. Chemical structure of BChl-*d* from *Chl. vibrioforme*.

Table 1 listed the results of mass spectrometry of the isolated BChls-*d*. Their molecular-ion peaks can be used to identify each BChl-*d* homolog possessing a farnesyl ester. The full assignments of the ¹H-signals by 2D-NMR spectra

establish all the chemical structures of BChls-*d*. The results of mass spectrometry and ¹H-NMR spectroscopy led us to an unequivocal set of assignments of eight BChls-*d* to R[E,M], R[E,E], R[P,M], S[P,M], R[P,E], S[P,E], S[I,M] and S[I,E], respectively.

Table 1. Mass-spectrometry data of BChl-*d* isolated from *Chl. vibrioforme*.

Peak #	Homologs	Molecular-ion peak	
		Observed	Calculated
1	[E,M]	778.5	778.4
2	[E,E]	792.3	792.5
3	[P,M]	792.6	792.5
4	[P,M]	792.5	792.5
5	[P,E]	806.7	806.5
6	[P,E]	806.5	806.5
7	[I,M]	806.5	806.5
8	[I,E]	820.7	820.5

At the high concentration of BChl-*d* in CH₂Cl₂ solution, we examined the initial association state of the identified BChls-*d* to form the core aggregates as a building block for the following higher assembly. For the R-isomers shown in the top panel of Figure 2, the presence of two kinds of aggregates as a major form was resolved in the second derivative spectra of the Q_y absorption. On the contrary, S-isomers shown in the bottom panel of Figure 2 revealed the presence of three kinds of aggregate forms. Therefore, the presence of different structural transformation pathways was observed in terms of the stereoisomeric configuration of the 3-1-hydroxyethyl group.

We then examined the structural transformation of each of the BChl-*d* components starting from the above core aggregates to the higher assembly during the dilution with hexane. First, we examined the dependence of the bulkiness of the C12 side-chain upon the self-assembly of the core aggregates in forming the higher assembly. None

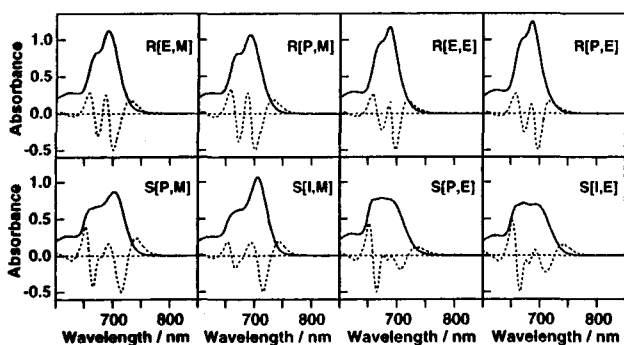


Figure 2. Changes in the Q_y absorption (solid line) in CH_2Cl_2 (2.0×10^{-3} M, optical path length = 0.1 mm) of each (3^1R)-BChl-*d* isomer (upper panel) and each (3^1S)-BChl-*d* isomer (bottom panel). The second derivative spectra of the Q_y absorption are also shown by the dotted lines.

of the contaminant of the core aggregates was observed in either the R- and S-stereoisomeric C12-methyl series homologs ([X,M]) as shown in Figure 3. On the contrary, the presence of the contaminant of the core aggregates was clearly observed in the C12-ethyl series homologs ([X,E]) as shown in Figure 3. Second derivative spectra of the Q_y absorption resolved corresponding wavelengths of the core aggregates. Here, we confirm that the bulkiness of the C12 side-chain strongly affected the self-assembly of the core aggregates in forming the higher assembly. This might be ascribable to introduction of severe steric-hindrance among the core aggregates.

Second, we examined the dependence of the bulkiness of the C8 side-chain upon the self-assembly of the core fragmented aggregates. Changes of [E,E] to [P,E] and [P,E] to [I,E] in [X,E] homologs (shown in Figure 3) exhibit the strongly red-shifted Q_y absorption, although changes in [X,M] homologs (shown in Figure 3) exhibited the slightly red-shifted Q_y absorption. Thus, the bulkiness of the C8 side-chain could affect the self-assembly of the core aggregates in promoting the formation of the higher

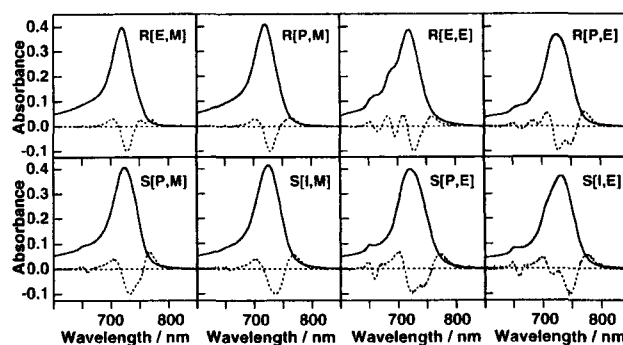


Figure 3. Changes in the Q_y absorption (solid line) upon dilution with 200-fold hexane (1.0×10^{-5} M, optical path length = 10 mm) of each (3^1R)-BChl-*d* isomer (upper panel) and each (3^1S)-BChl-*d* isomer (bottom panel). The second derivative spectra of the Q_y absorption are also shown by the dotted lines.

assembly. This might be ascribable to introduction of the stabilized hydrophobic interaction of the relevant alkyl side-chain at the 8-position.

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