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Dimer Structures of Bacteriochlorophyll c from Chlorobium tepidum in $CDCl_3$

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As an aggregate structure in $CDCl_3$ a piggyback type dimer was suggested for bacteriochlorophyll (BChl) c from Chlorobium tepidum on the basis of variations of ^1H NMR chemical shifts accompanied with the aggregate formation and of values of calculated ring current shifts.

Recently a new thermophilic photosynthetic bacterium named Chlorobium (C.) tepidum has been isolated.[1] This is a family of Chlorobiaceae, a green sulfur photosynthetic bacterium. Green bacteria are characterized by a special light harvesting organ called a chlorosome which does not exist in purple photosynthetic bacteria.[2,3] Chlorosomes contain bacteriochlorophyll (BChl) c, more than 50% (by weight) as the major component, and also contain carotenoids and a small amount of BChl a. BChl c of chlorosomes isolated from C. tepidum has an absorption maximum at 740 nm, which is greatly red-shifted from 668 nm of BChl c in polar organic solvents. The red-shifted absorption maximum is characteristic of aggregated BChl c in nonpolar solvents. Recently it has been disclosed that an addition of hexanol to chlorosomes converts aggregated BChl c to its monomeric state, and a dilution or an elimination of hexanol brings the monomer back to an aggregate, reversibly.[3,4] An aggregation property of BChl c is fundamental for elucidation of native chlorosome structures and functions. In this paper we wish to report the structural study of aggregate BChl c in $CDCl_3$ for elucidation of aggregate structures of BChl c in chlorosomes.

Chlorobium tepidum was grown[1] and its BChl c was extracted with methanol (or chloroform) and purified as previously described,[3,5] and the structures of two major components have been determined recently.[5] The major BChl c has either an ethyl or an n-propyl group as a substituent at the 4-position of the chlorin ring as shown in Fig. 1. We designated these

two components as BChl c(Et), and BChl c(n-Pr) in this letter.

^{1}H NMR spectra were recorded on a Bruker MSL400 FT NMR spectrometer equipped with a dual type probe for ^{1}H and ^{13}C.[5] Chemical shifts were referred to TMS. Purified BChl c was dissolved in $CDCl_3$ treated with Na_2CO_3.[6,7]

^{1}H NMR spectra were obtained for one component (BChl c(Et)) in $CDCl_3$, and in $CDCl_3$ with a small amount of CD_3OD (Fig. 2). Homonuclear correlated two dimensional ^{1}H NMR (COSY) spectrum was also observed (data not shown) to aid the signal assignment. The ^{1}H NMR resonances were assigned on the basis of these information together with a previous data in a literature for a similar dimer made of BChl d.[8] It is revealed from Fig. 2, and a number of COSY peaks (data not shown) that in $CDCl_3$ there exist a pair of resonances with an equal intensity for respective protons of BChl c(Et). This is apparent especially for α-H, β-H, δ-C$\underline{H}_3$, 2b-C$\underline{H}_3$, and 3a-C$\underline{H}_3$ protons. These facts indicate that BChl c exists in two different environments in $CDCl_3$. To explain this fact, we need to presume the presence of an asymmetric dimer (a) or (d), or of the mixtures of two symmetric dimers (b) and (c) in Fig. 3. The models (a) to (c) are head to head models where both 2a-hydroxyl groups coordinate to the magnesium ion of the other molecule. The model (a) is a piggy-

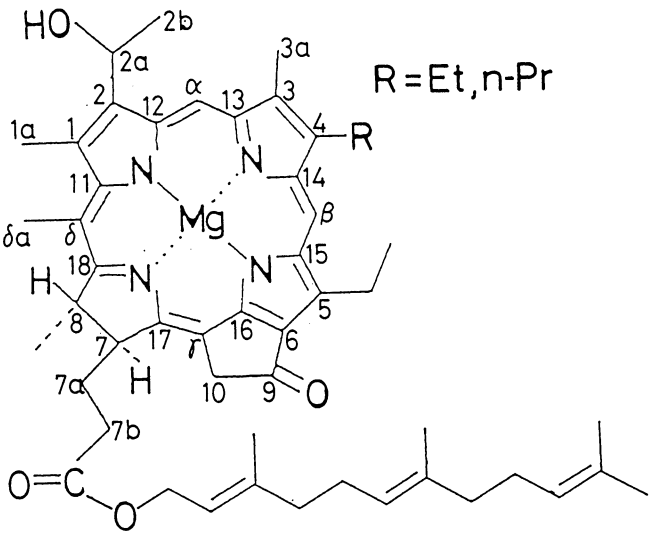

Fig. 1. Structures of BChl c from Chlorobium tepidum.

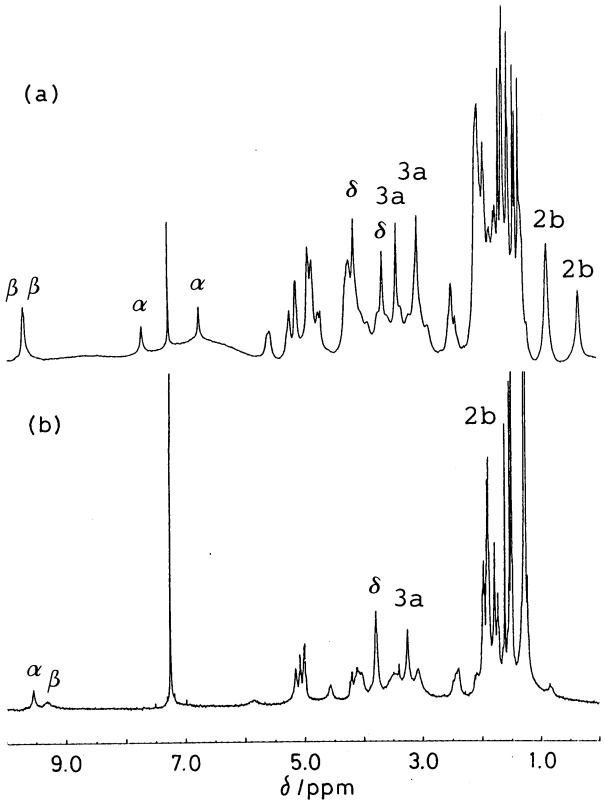

Fig. 2. ^{1}H NMR spectrum of the BChl c(Et) (see text) in $CDCl_3$ (a) and in $CDCl_3$ with a small amount of CD_3OD (b).

back type, and the models (b) and (c) are a face to face type and a back to back type, respectively. The model (d) is a parallel head-to-tail model where the 2a-hydroxyl group of one molecule coordinates to the other molecule, and the 9-carbonyl group of one molecule coordinates to the other molecule. It should be noted that protons from 2b-C$\underline{H}_3$ and α-H show high field shifts. Since a ring current makes protons out of the ring plane shift to the high field, and makes ones in the plane shift to the low field, the observed results indicate that the hydroxyethyl group in the 2-position should be out of plane, thus should coordinate to the magnesium ion of the other BChl c. Since the model (d) does not satisfy this qualitative consideration, we only need to consider the models (a) to (c).

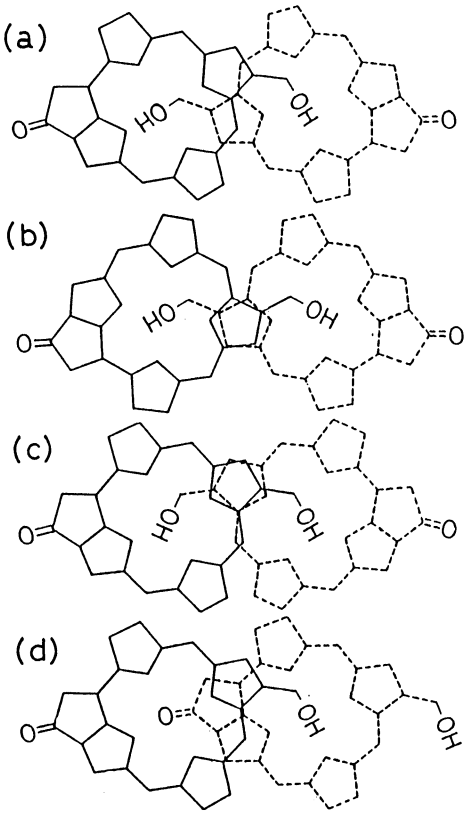

Fig. 3. Possible BChl c dimer models, (a) a head to head model (a piggy back type), (b) a head to head model (a face to face type), (c) a head to head model (a back to back type), and (d) a parallel head to tail model.

The other major component (BChl c(n-Pr)) also gave similar ^{1}H NMR spectra, thus indicating that the two major BChl c components form similar dimeric structures irrespective of the substituents at the 4-position.

From Figs. 2a and 2b the chemical shift changes due to dimer formation were evaluated as the differences of the chemical shifts in $CDCl_3$ from those in $CDCl_3$ with a small amount of CD_3OD, where BChl c exists as a monomer. The results obtained were summarized in Table 1 together with calculated ring current shift data described bellow.

Calculation of a ring current shift for a specific proton from surrounding monomeric unit was done by the method of Abraham,[9] in which two kinds of equivalent dipoles, μ_p and μ_h were taken to be 19.0 and 22.0 A^3, respectively. Structural information was adopted from a literature of X-ray crystal data for BChl a.[10] The calculated results were summarized in Table 1 and compared with the experimentally observed chemical shift changes due to dimer formation. Since experimentally a pair of signals were observed for respective protons, and either one of the symmetric models does not give a pair of the proton signals, and furthermore the chance to form either one of the symmetric dimers may be equal, we should compare the calculation values with the equal mixture of the symmetric

dimers. Thus for the symmetric dimers the calculation values should be combined and compared with the experimental ones. After considering these facts, we can propose from Table 1 that the model (a) offers the values which agree most with experimental values especially for α-H, δ-C$\underline{H}_3$, and 3a-C$\underline{H}_3$ positions, where the ring current shift of these positions should be very sensitive to the aggregation structures of the models. The present calculation method may not be good for the protons where the distance from the ring is too short, because of the dipole approximation. This may explain the larger values calculated for 2a-C$\underline{H}$ as compared to the experimental ones. In conclusion BChl c's form piggyback type dimers in $CDCl_3$ irrespective of the substituent (either an ethyl or an n-propyl) at the 4-position.

Table 1. Observed and calculated ring current shift values

Chemical shift differences (ppm)		Calculated ring current shift (ppm) (a)Asymmetric (Piggy back)	(b)Symmetric (Face to Face)	(Back to Back)
α-H	-1.85	-2.09	-1.40	
	-2.83	-3.38		-4.07
β-H	0.38	0.18	0.17	
	0.36	0.16		0.20
δ-C$\underline{H}_3$	0.33	0.18	0.03	
	-0.15	-0.22		0.12
2a-C$\underline{H}$	-2.54	-5.37		-4.63
	-3.32	-5.39	-5.55	
2b-C$\underline{H}_3$	-1.02	-2.21		-2.17
	-1.56	-2.43	-2.41	
3a-C$\underline{H}_3$	0.15	0.11	0.09	
	-0.21	-0.19		-1.23

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