CHEMISTRY LETTERS, pp. 1641 - 1644, 1991.

Aggregation Structure of Bacteriochlorophyll c in Chlorosomes from Chlorobium tepidum

Tsunenori NOZAWA,* Manabu SUZUKI, Katsunori OHTOMO, Yumi MORISHITA, Hideo KONAMI,+ and Michael T. MADIGAN++

Department of Molecular Chemistry and Engineering, Faculty of Engineering, Tohoku University, Aoba, Aramaki, Aoba-ku, Sendai 980

+Institute for Chemical Reaction Science, Katahira, Sendai 980

++Department of Micro-biology, Southern Illinois University at Carbondale, Carbondale, Illinois, 62901-6508, U.S.A.

High resolution solid state ^{13}C NMR signals of bacteriochlorophyll (BChl) c were obtained for chlorosomes from Chlorobium tepidum. Observed BChl c signals which resembled those from oligomeric BChl c afforded ring current shift from which an aggregation structure was proposed.

A new thermophilic bacterium Chlorobium (C.) tepidum has chlorosome antenna which are unique light harvesting organs especially for green photosynthetic bacteria.[1] Chlorosomes contain bacteriochlorophyll (BChl) c more than 50% (by weight) as the major component and also carotenoids and a small amount of BChl a. BChl c of chlorosomes isolated from the new thermophilic green sulfur bacterium Chlorobium 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. Since ultrafast excitation energy transfer take place in chlorosomes,[1,2] those pigment organizations have caught attention. Various physicochemical techniques have been applied for their structural elucidation in relation to the functions.[1-6] In a previous paper we have employed CP/MAS ^{13}C NMR spectroscopy to clarify the structure of BChl c in chlorosomes from a green non-sulfur photosynthetic bacterium Chloroflexus (C.) aurantiacus.[6] However the high quantity of carotenoids in chlorosomes from C. aurantiacus hampered to obtain the ^{13}C NMR signals from BChl c in chlorosomes. Presently we have succeeded to obtain clear ^{13}C NMR signals from chlorosomes from C. tepidum. This enabled us to obtain chemical shift values due to ring current shift from which we discussed the aggregation structure of BChl c in chlorosomes in C. tepidum.

Chlorobium tepidum was grown[7] and its chlorosomes were isolated by using methods similar to those previously reported.[1] BChl c was extracted with methanol (or chloroform) and purified as previously described,[1,6] and the structure of major components have been determined recently as shown in Fig. 1.[8] Solid aggregated BChl c was prepared by dissolving BChl c in dichloromethane and precipitating it in large excess of hexane.[6] This sample showed an absorption maximum around 750 nm which indicates the formation of the aggregate, called oligomeric solid BChl c in the text.[6]

CP/MAS ^{13}C NMR spectra were recorded on a Bruker MSL400 FT NMR spectrometer equipped with a double air bearing type CP/MAS probe in a similar manner to that reported.[6] Chemical shift was referred to TMS by setting the carbonyl signal of solid glycine at 176.03 ppm.

CP/MAS ^{13}C NMR spectra for lyophilized chlorosomes which have near infrared absorption spectra identical to those of intact chlorosomes (data not shown) were taken in several measuring conditions (Fig. 1b). CP/MAS ^{13}C NMR spectrum of chlorosomes was compared with those of oligomeric solid BChl c in Fig. 1c. Except for minor resonances possibly due to proteins (around 175, 120, 50, and 30 ppm), lipids (30 ppm) and carotenoids (130, 30 ppm), most of the observed signals of chlorosomes were attributable to

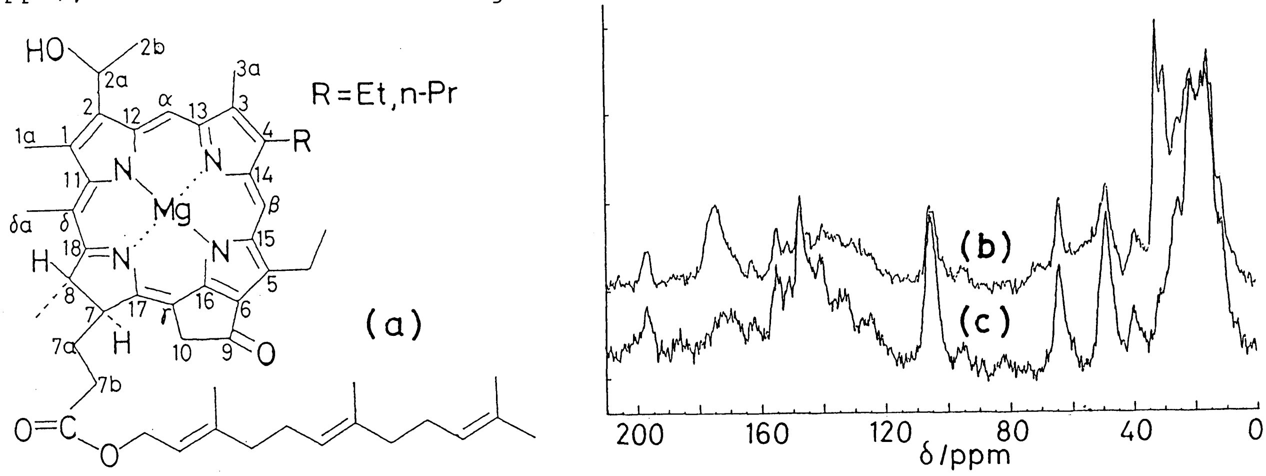

Fig. 1. Structures of BChl c (a), and CP/MAS ^{13}C NMR spectra for chlorosomes (b) and oligomeric solid BChl c (c) from Chlorobium tepidum.

those from BChl c. Comparison of Figs. 1b with 1c indicates that oligomeric solid BChl c has structures similar to those of BChl c in chlorosomes. The assignments of the signals in chlorosomes and oligomeric solid states were tried from the knowledge of the previous solution and solid high resolution NMR data.[1,6] Some of the assignments were shown in Table 1. The differences of the chemical shifts in the

Table 1. ^{13}C NMR Assignment for Chlorosomes and Oligomers

Chemical shift (ppm)	Assignment
196	9
170	18
162.5	16
154.5	2,17
150.5	11
147	12,13
140	5
105	δ
64	2a
48.5	7,8,10
11.7	3a

chlorosomes or the oligomeric solid BChl c from those of the BChl c in solutions can be attributed either to some dynamic structure existed especially in solution or to solid aggregation effects. Since the former is not expected in the present case, the difference can be attributed to the ring current shift of particular BChl c carbons from other BChl c conjugated rings. These values were tabulated in Table 2, along with the calculated values described later.

Several possible models have been considered for aggregates from various investigations.[1-6] In Fig. 2 the model (a) is a basic oligomer model in which each magnesium ion in BChl c has six coordination.[5] At present plausible aggregate structures have been proposed from various physicochemical studies, in which the 2a-hydroxyl group ligates the Mg ion of one BChl c molecule while simultaneously hydrogen bonding to the 9-carbonyl group of another BChl c.[9] The models (b) to (d) were antiparallel aggregation models where rings meet face to face (b), back to back (c) and form piggy bag (d). The model (e) is a parallel aggregation model. The ways of overlap for specific two monomeric BChl c's were shown in Fig. 3.

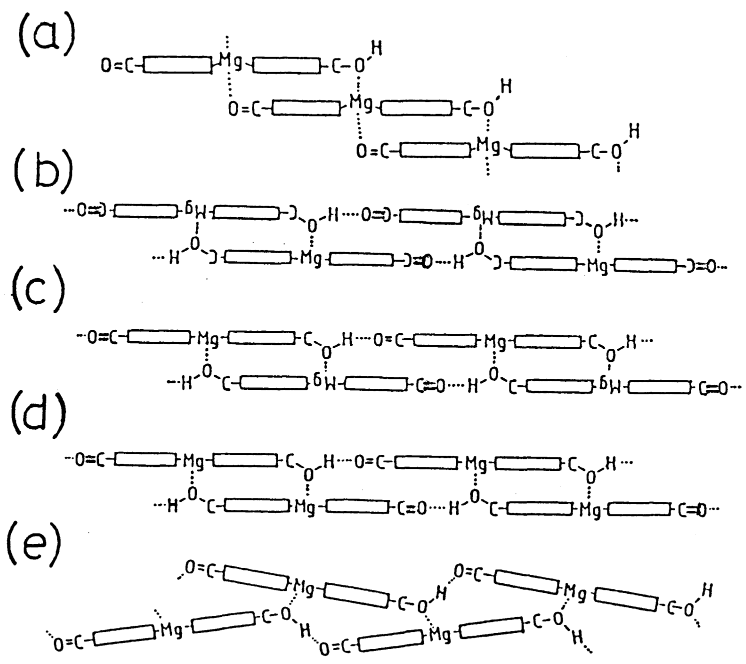

Fig. 2. The model structures of aggregated BChl c.

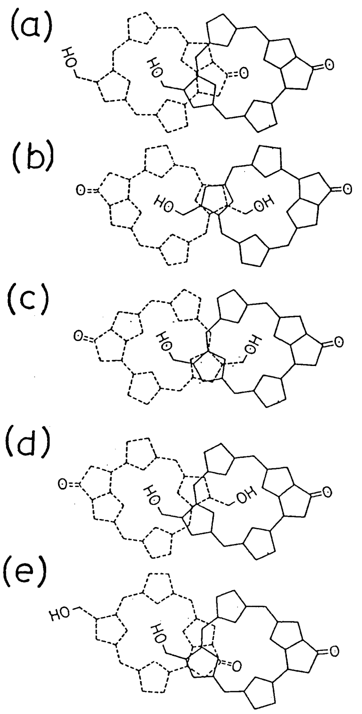

Fig. 3. The overlap of BChl c's in the models in Fig. 2.

Ring current shifts for respective carbons from 5 surrounding monomeric units were calculated by the method of Abraham,[10] in which two kinds of equivalent dipoles, μ_p and μ_h, were taken 17.3 and 19.0 Å^3, respectively. Structural information was adopted from literatures of X-ray crystal data for BChl a[11] and NMR data for BChl d.[12] The calculated results were summarized in Table 2 and compared with the experimental values. The model (e) shows a good agreement with the experimental values except for the C2a hydroxyl carbon, to which the approximation of the present method will be no good because of too short distance from the ring.

Table 2. Calculated and Observed Ring Current Shifts

Number of atom	Calculated ring current shifts (ppm)					Observed shift (ppm)
	(a)	(b)	(c)	(d)	(e)	(f)
1	-1.71	-1.76	-2.40	-2.24	-1.26	
2	-3.27	-3.32	-3.31	-3.27	-2.48	
3	0.27	-0.30	0.38	0.24	0.12	
4	0.39	0.30	0.44	0.38	0.29	
5	-1.69	0.32	-0.06	0.28	-0.41	-0.5
6	-3.21	0.06	-0.21	0.09	-0.75	
7	0.13	0.50	0.44	0.46	0.38	
8	0.36	0.45	0.41	0.44	0.365	
9	-3.92	-0.78	-0.53	-0.51	-1.33	-2,1
10	-3.08	0.07	0.29	0.27	-0.60	-0.9
α	-1.40	-2.18	-0.56	-1.45	-0.90	-1.0
β	0.24	0.49	0.47	0.45	0.36	
γ	-2.08	0.45	0.43	0.45	0.03	
δ	0.22	0.08	0.01	0.10	0.10	
11	-0.33	-0.89	-0.99	-0.47	-0.33	
12	-2.04	-2.51	-1.65	-1.84	-1.22	
13	-0.06	-0.54	0.23	-0.09	-0.12	
14	0.37	0.40	0.47	0.41	0.34	
15	-0.35	0.49	0.40	0.45	0.23	
16	-2.20	0.43	0.36	0.42	-0.01	0.1
17	-0.41	0.51	0.46	0.48	0.36	
18	0.33	0.38	0.35	0.40	0.31	
7a	0.68	0.78	0.26	0.27	0.14	
7b	0.52	0.66	0.29	0.31	0.19	
a	0.27	0.27	0.17	-0.02	0.17	
1a	-1.06	-0.39	-1.47	-2.70	-0.83	
2a	-3.94	-3.43	-3.69	-3.59	-3.91	-1.4
3a	0.26	-0.89	0.32	0.24	0.12	
4a	0.39	0.39	0.42	0.37	0.34	
2b	-2.52	-4.08	-2.12	-1.98	-1.64	

References

1) D. Brune, T. Nozawa, and R. Blankenship, Biochemistry, **26**, 8644 (1987).
2) K. Smith, L. Kehres, and J. Fajer, J. Am. Chem. Soc., **105**, 1387 (1983).
3) J. M. Olson, D. C. Brune, and P. D. Gerola, "Molecular Biology of Membrane-Bound Complexes in Photrophic Bacteria," ed by G. Drews, and E. A. Dawes, Plenum Press, New York (1990), pp. 227-234.
4) Z. Fetisova, L. Shibaeva, and M. Fok, J. Theor. Biol., **140**, 167 (1989).
5) M. Lutz, and G. van Brakel, "Green Photosynthetic Bacteria," ed by J. M. Olson, Plenum Press, New York (1988), pp. 23-34.
6) T. Nozawa, M. Suzuki, S. Shirai, and S. Kanno, Chem. Lett., **1990**, 1805.
7) M. T. Madigan, Int. J. Syst. Microbiol., **36**, 222 (1986).
8) T. Nozawa, K. Ohtomo, M. Suzuki, Y. Morishita, and M. T. Madigan, Chem. Lett., in press
9) D. C. Brune, G. H. King, and R. E. Blankenship, "Proc. Conf. on Organization and Function of Photosynthetic Antennas," ed by H. Scheer and S. Schneider, Walter de Gruyter, Berlin (1988), pp. 141-151.
10) R. Abraham, S. Fell, and K. Smith, Org. Magn. Reson., **9**, 367 (1977).
11) H. Chow, R. Serlin, and C. Strouse, J. Am. Chem. Soc., **97**, 7230 (1975).
12) R. J. Abraham, and K. M. Smith, J. Am. Chem. Soc., **105**, 1674 (1983).

(Received June 18, 1991)