CHEMISTRY LETTERS, pp. 1763-1766, 1991.

Structures of Bacteriochlorophyll c's in Chlorosomes from a New Thermophilic Bacterium Chlorobium tepidum

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

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

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

A new thermophilic bacterium Chlorobium tepidum contained two major bacteriochlorophyll c components whose periphery substituent at 4-position was either an ethyl or a propyl group. Both components possessed ethyl groups at 5-position and the ester alkyl groups at 7-position were farnesyl groups.

Recently a thermophilic green sulfur bacterium has been found and named "Chlorobium tepidum".[1] Green photosynthetic bacteria are characterized by a unique antenna complex known as a chlorosome.[2-3] Chlorosomes contain bacteriochlorophyll (BChl) c as the major pigment and also have proteins, carotenoids and a small amount of BChl a.[1] BChl c of chlorosomes isolated from the thermophilic green bacterium Chlorobium (C.) tepidum has an absorption maximum at 740 nm, which is greatly red-shifted from 668 nm of BChl c in polar organic solvents. This may be attributable to the aggregation structure of the pigments.[4,5] Recently, varieties in the structures and structural adjustment for environmental conditions, which result in spectral red shifts in the near infrared band, have been reported.[4,5] BChl c's in chlorosomes are known to be a mixture of several homologues.[6-8] We have analyzed the pigment composition and identified structures of the major components of BChl c's in chlorosomes from the thermophilic green sulfur bacterium C. tepidum.

C. tepidum was grown and its chlorosomes were isolated by using methods similar to those previously reported.[1,9] BChl c was extracted with methanol (or chloroform) and purified as previously described.[8,10] HPLC (Toso Bio-LC system) with ODS (TSK_{gel} ODS-80T_M) column was used for fractionation to the components. The elusion solvent was composed of methanol and water with a volume ratio of 96 : 4. ^1H NMR spectra were recorded on a Bruker MSL400 FT NMR spectrometer equipped with a dual probe

for ^{1}H and ^{13}C.

Figure 1 shows an elusion profile of the pigment solution after extraction from freeze-dried cell and purification by hexane precipitation. Two major components exist with numerous minor components. The two major fractions (called HPLC1 and HPLC2 in this letter), and two relatively larger peaks (designated by ① and ②) showed absorption spectra which are characteristic of BChl c in methanol solution (Figure 2 for HPLC1 and data not shown for HPLC2, fractions ① and ②). Thus all four fractions have absorption maxima at 668.5 and 435.0 (± 0.5) nm. These absorption peaks indicate that all four fractions have the fundamental BChl c conjugate systems. Many other minor components also showed similar absorption maxima. Therefore it revealed that the chlorosomes from the thermophilic green sulfur bacterium C. tepidum have two major BChl c components with many minor ones.

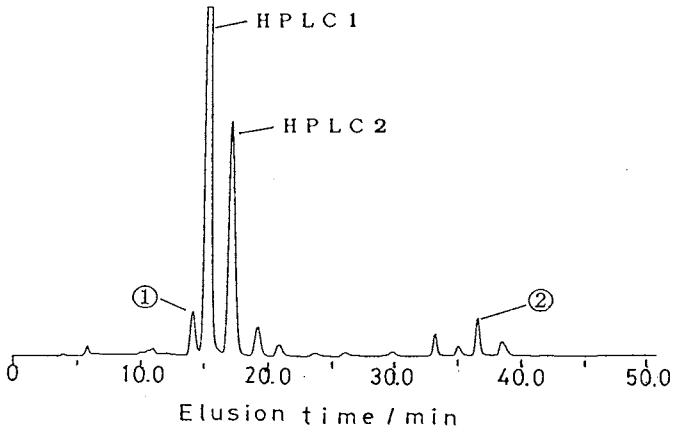

Fig. 1. HPLC elusion profile of methanol extract of the C. tepidum cell.

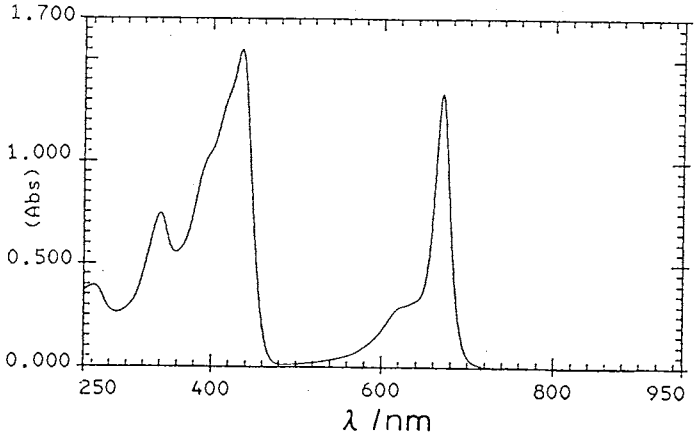

Fig. 2. Absorption spectrum for HPLC1.

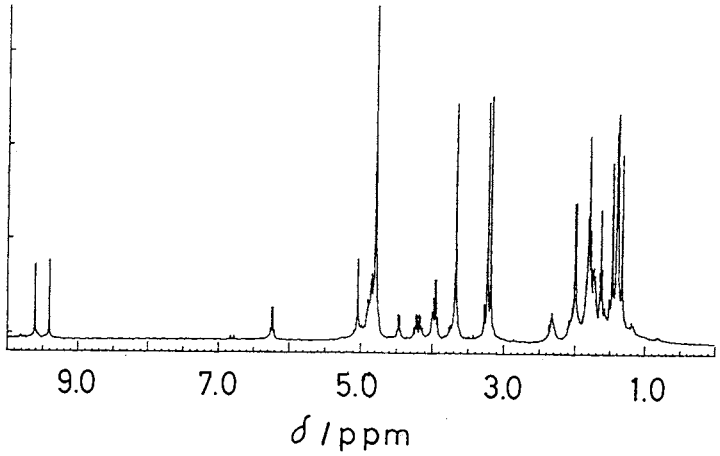

Fig. 3. ^{1}H NMR spectrum of the HPLC1 sample in deuterated methanol.

One dimensional and two dimensional (COSY) ^{1}H NMR spectra were observed for structural determination of HPLC1 and HPLC2, and the results were shown in Figs. 3 and 4 for HPLC1. ^{1}H Homonuclear decoupling NMR spectra were also observed (data not shown). Tables 1 and 2 summarize ^{1}H NMR assignments obtained

from all these data along with those in literature.[8] Figure 5 illustrates the structures derived from them.

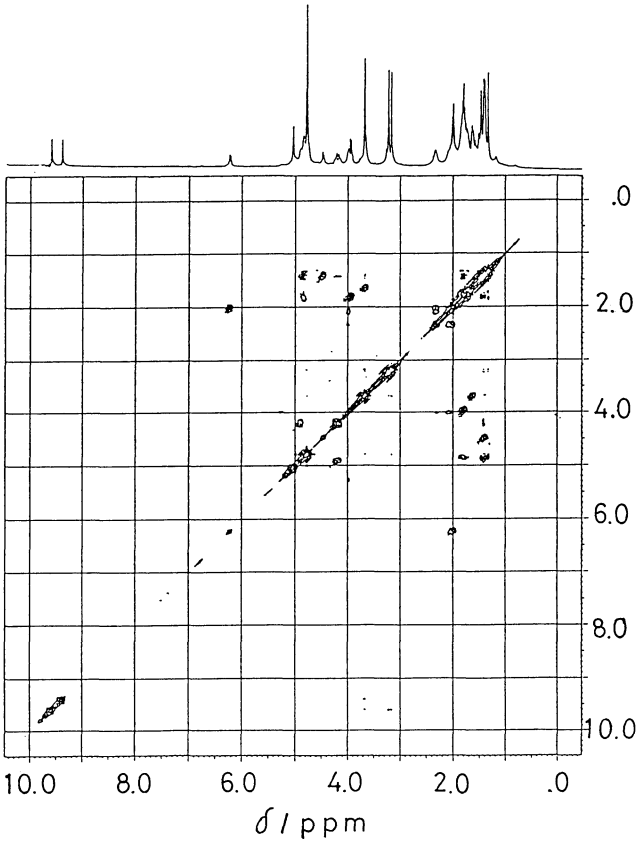

Fig. 4. ^{1}H COSY NMR of the HPLC1 sample in deuterated methanol.

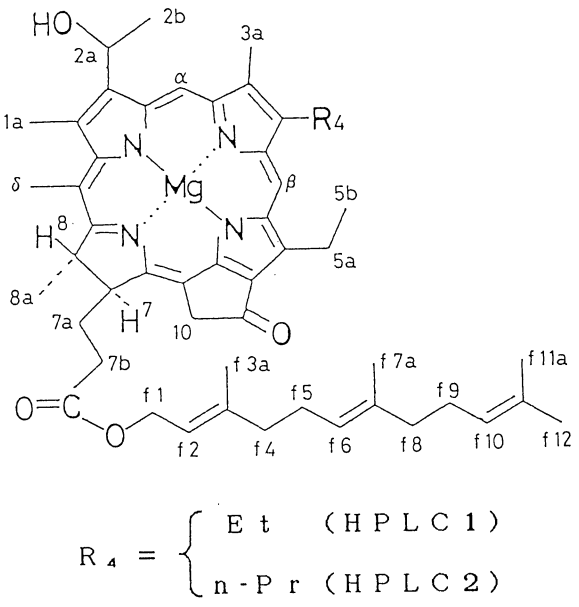

Fig. 5. Structures of BChl c's.

Table 1. ^{1}H NMR Assignment for HPLC1

Chemical shift(ppm)	Multi-plicity	Assignment
9.57 *	s	α-H
9.37 *	s	β-H
6.21 *	q	2a-CH
5.03 *	AB	10-CH_2
4.91	m	f2-CH_2
4.82	m	f6,f10-CH
4.50 *	q	8-CH
4.20	m	f1-CH_2
4.00 *	dd	7-CH_2
3.92	q	5a-CH_2
3.70 *	s	δ-CH_3
3.66 *	q	4a-CH_2
3.21 *	s	1a-CH_3
3.15 *	s	3a-CH_3
2.35 *	m	7b-CH_2
2.07 *	m	7a-CH_2
1.97 *	d	2b-CH_3
1.82	m	f4,f5,f9-CH_3
1.77	t	5b-CH_3
1.70	m	f8-CH_2
1.60 *	t	4b-CH_3
1.44	s	f7a-CH_3
1.42	s	f3a-CH_3
1.40 *	d	8a-CH_3
1.37	s	f11a-CH_3
1.33	s	f12a-CH_3

Table 2. ^{1}H NMR Assignment for HPLC2

Chemical shift(ppm)	Multi-plicity	Assignment
9,57 *	s	α-H
9,35 *	s	β-H
6,22 *	q	2a-CH
5.05 *	AB	10-CH_2
4.90	m	f2-CH_2
4.83	m	f6,f10-CH
4.51 *	q	8-CH
4.22	m	f1-CH_2
4.02 *	dd	7-CH_2
3.93	q	5a-CH_2
3.72 *	s	δ-CH_3
3.62	q	4a-CH_2
3.24 *	s	1a-CH_3
3.15 *	s	3a-CH_3
2.39 *	m	7b-CH_2
2.08 *	m	7a-CH_2
2.08	5	4b-CH_2
1.99 *	d	2b-CH_3
1.83	m	f4,f5,f9-CH_3
1.78	t	5b-CH_3
1.72	m	f8-CH_2
1.48	s	f7a-CH_3
1.42	s	f3a-CH_3
1.40 *	d	8a-CH_3
1.37	s	f11a-CH_3
1.33	s	f12a-CH_3
1.15	t	4c-CH_3

The ^{1}H NMR signals with * signs in Tables 1 and 2 are in agreement with those from BChl c of Chloroflexus (C.) aurantiacus chlorosome fraction 1.[8] Our results are also consistent with the present ^{1}H NMR observation of COSY, homonuclear decoupling and integration values of the signals. Hence these data demonstrate the presence of the basic BChl c conjugate structures in HPLC1 and HPLC2. Differences in structures among BChl c's from C. aurantiacus, and C. tepidum were observed in the substituents at 4-, 5-, and 7-positions. The substituent at the 4-position in HPLC1 was determined as an ethyl group from the signals at 3.66 ppm (quartet) 4a-CH_2 and 1.60 ppm (triplet) 4b-CH_3. While the substituent at the 4-position in HPLC2 was found to be a propyl group on the basis of the ^{1}H NMR signals at 3.62 ppm (triplet) 4a-CH_2, 2.08 ppm (quintet) 4b-CH_2, and 1.15 ppm (triplet) 4c-CH_3. The substituents at 5-position for both HPLC1 and HPLC2 were revealed to be ethyl groups from the signals at 3.92 (3.93) ppm (quartet) 5a-CH_2 and 1.77 (1.78) ppm (triplet) 5b-CH_3. The candidates for the alcohol group which esterified with the propionic acid at the 7-position are farnesyl, phytyl, stearyl. Only a farnesyl group gives the ^{1}H NMR signals which are consistent with the observed signals with assignments given in Tables 1 and 2 like f2-CH (farnesyl number 2 carbon CH) etc.

In conclusion we have shown that the thermophilic green sulfur bacterium C. tepidum has two major BChl c components in its chlorosomes with several minor BChl c fractions. ^{1}H NMR investigation disclosed that HPLC1 and HPLC2 have the basic BChl c conjugate structures with a farnesyl ester at the 7-position, and an ethyl group at the 5-position. At the 4-position HPLC1 possesses an ethyl group and HPLC2 has a propyl group.

This work was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan (#03650638 to T.N.), a Grant from Asahi Glass Foundation and a Grant from Miyagi Industry Promotion Foundation.

References

1) T. M. Wahlund, C. R. Woese, R. W. Castenholz, and M. T. Madigan, Arch. Microbiol., in press.
2) J. M. Olson, Biochim. Biophys. Acta, **594**, 33 (1980).
3) R. E. Blankenship, Photosynthetic Res., **6**, 317 (1985).
4) F. W. Bobe, N. Pfennig, L. L. Swanson, and K. M. Smith, Biochemistry, **29**, 4340 (1990).
5) M. S. Huster and K. M. Smith, Biochemistry, **29**, 4348 (1990).
6) N. Risch, H. Brockmann, Jr., and A. Gloe, Liebigs Ann. Chem., **1979**, 408 (1979).
7) M. B. Caple, H. Chow, and C. E. Strouse, J. Biol. Chem., **253**, 6730 (1978).
8) D. C. Brune, T. Nozawa, and R. E. Blankenship, Biochemistry, **26**, 8644 (1987).
9) M. T. Madigan, Int. J. Syst. Microbiol., **36**, 222 (1986).
10) T. Nozawa, M. Suzuki, S. Shirai, and S. Kanno, Chem. Lett., **1990**, 1805 (1990).

(Received June 3, 1991)