Structural determination of the novel Zn-containing bacteriochlorophyll in *Acidiphilium rubrum*

Masami KOBAYASHI[1*], Machiko AKIYAMA[1], Mayu YAMAMURA[1], Hideo KISE[1], Shinichi TAKAICHI[2], Tadashi WATANABE[3], Keizo SHIMADA[4], Masayo IWAKI[5], Shigeru ITOH[5], Nobuaki ISHIDA[6], Mika KOIZUMI[7], Hiromi KANO[7], Norio WAKAO[8] and Akira HIRAISHI[9]

[1] *Institute of Materials Science, University of Tsukuba, Tsukuba, Ibaraki 305-8573*

[2] *Biological Laboratory, Nippon Medical School, Kawasaki, Kanagawa 211-0063*

[3] *Institute of Industrial Science, University of Tokyo, Minato-ku, Tokyo 106-8558*

[4] *Department of Biology, Tokyo Metropolitan University, Hachioji, Tokyo 192-0397*

[5] *National Institute for Basic Biology, Okazaki, Aichi 444-8585*

[6] *National Food Research Institute, Tsukuba, Ibaraki 305-8642*

[7] *National Institute of Agrobiological Resources, Tsukuba, Ibaraki 305-8602*

[8] *Laboratory of Applied Microbiology, Iwate University, Morioka, Iwate 020-8550*

[9] *Department of Ecological Engineering, Toyohashi University of Technology, Toyohashi, Aichi 441-8580*

JAPAN

ABSTRACT

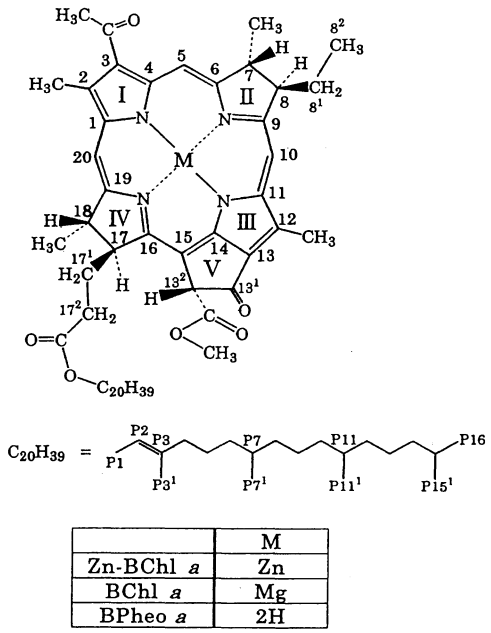
A novel Zn-containing bacteriochlorophyll was found to function as the photosynthetic pigment in an aerobic bacterium *Acidiphilium rubrum* growing under acidic conditions (Wakao *et al.*, *Plant Cell Physiol.*, **37**, 889-893, 1996). In this work, we have unambiguously determined the molecular structure of this pigment by high performance liquid chromatography, absorption, circular dichroism, inductively coupled plasma, fast atom bombardment mass and ^{1}H-nuclear magnetic resonance measurements. The structure of the pigment was identical with that of bacteriochlorophyll *a* esterified with phytol, except that the central metal is zinc in place of magnesium.

Key words: Acidophilic bacteria, *Acidiphilium rubrum*, Bacteriochlorophyll, Bacteriopheophytin, Photosynthesis, Zn-bacteriochlorophyll *a*

INTRODUCTION

The major pigments of both antenna systems and reaction centers (RCs) of photosynthetic organisms are chlorophylls (Chls) in higher plants and algae and bacteriochlorophylls (BChls) in photosynthetic bacteria. Chls and BChls contain magnesium (Mg) as a central metal of substituted porphyrin, chlorin or bacteriochlorin macrocycles. The metal-free forms, namely, pheophytin (Pheo) *a*, bacteriopheophytin (BPheo) *a* (Fig. 1) and BPheo *b*, also function as the primary electron acceptors in the RCs of photosystem 2 of higher plants and algae [1] and the photosynthetic purple bacteria [2,3]. Although some other metal complexes, especially zinc (Zn) complexes, are used in the studies of artificial photosynthesis [4,5], natural occurrence of such a compound had not been reported, till our finding [6].

We first found a novel bacteriochlorophyll containing Zn as the central metal in an aerobic bacterium *Acidiphilium rubrum* (*A. rubrum*) growing under low pH [6]. The compound is the major pigment in *A. rubrum* and photochemical reaction brings out in the isolated membranes [6]. The Zn-containing bacteriochlorophyll is



	M
Zn-BChl *a*	Zn
BChl *a*	Mg
BPheo *a*	2H

Fig. 1 Molecular structure and carbon numbering of Zn-BChl *a*, BChl *a* (Mg-BChl *a*) and BPheo *a*, according to the IUPAC numbering system.

*Corresponding author: FAX +81-298-55-7440; E-mail:masami@ims.tsukuba.ac.jp

Abbreviations: BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; CD, circular dichroism; FAB-mass, fast atom bombardment mass; ICP, inductively coupled plasma; HPLC, high performance liquid chromatography; LH1, light harvesting complex 1; M-BChl, metallobacteriochlorophyll; NMR, nuclear magnetic resonance; RC, reaction center

present in all established species of *Acidiphilium* [7]. Genes of these bacteria contain the *puf* operon coding the L, M and C subunits of the RC proteins and the α and β subunits of the light harvesting complex with high homology to those in the photosynthetic purple bacteria [8]. The RC complex isolated from *A. rubrum* [9] contains the Zn-containing pigment, but no BChl *a* (Mg-BChl *a*) molecule has been found in the RC [10]. In order to discuss the characteristics of the natural photosystem driven by Zn-containing bacteriochlorophyll in *A. rubrum* in the molecular level, we must know the exact molecular structure of the compound.

In this paper, we determined the molecular structure by high performance liquid chromatography (HPLC), absorption, circular dichroism (CD), inductively coupled plasma (ICP), fast atom bombardment mass (FAB-mass) and ^{1}H-nuclear magnetic resonance (^{1}H-NMR) measurements. The structure of the pigment was identical to that of BChl *a* esterified with phytol, except that the central metal is substituted by Zn in place of Mg (Fig. 1).

MATERIALS AND METHODS

Culturing

Acidiphilium rubrum (ATCC 35905) cells were grown aerobically at 303 K in air bubbled BYG medium at pH 3.5 as described previously [6].

Pigment analysis and preparation

Pigments were extracted from cell suspension (ca. 10 $\mu\ell$) by sonication in a ca. 300-fold volume of acetone/methanol (7/3, v/v) mixture for 2 min in the dark at room temperature. The extract was filtered and dried *in vacuo*. The whole procedure was completed within 5 min.

The pink solid material obtained by the above procedure was immediately dissolved in 10 $\mu\ell$ of chloroform, and injected into a silica HPLC column (Senshupak 1251N, 250×4.6 mm i.d.) cooled to 277 K in an ice-water bath. The pigments were eluted isocratically with degassed hexane/2-propanol/methanol (100/0.7/0.3, v/v) at a flow rate of 1.35 mℓ min^{-1}, and were monitored with a JASCO 875 UV-detector and a JASCO Multiwavelength MD-915 detector.

Sufficiently pure pigments (> 99 %) used for the measurements of the absorption, CD, ICP, FAB-mass and ^{1}H-NMR spectra were extracted from dried cells and purified by preparative-scale HPLC (Senshupak 5251N, 250×20 mm i.d.) at 277 K with hexane/2-propanol (100/3, v/v) at a flow rate of 7 mℓ min^{-1}.

Both BChl *a* of *Rhodobacter (Rba.) sphaeroides* and the Zn-containing pigment of *A. rubrum* were acid treated to yield the metal-free compounds according to the methods described previously [11,12]. The products were purified by preparative-scale HPLC.

Absorption and CD spectrometry

The absorption spectra of the purified pigments were measured in acetone, diethyl ether, benzene and 2-propanol with a JASCO Ubest V-560DS spectrophotometer at room temperature. CD spectra of BChl *a* of *Rba. sphaeroides* and the Zn-containing pigment of *A. rubrum* were measured in benzene with a JASCO J-720W spectropolarimeter at 293 K.

ICP spectrometry

For metal analysis, an aliquot of a diethyl ether solution of the Zn-containing pigment was dried, carbonized with conc. H_2SO_4, and converted to ash at 823 K. Then the sample was dissolved in 10 mℓ of 0.6 M HCℓ and subjected to ICP spectroscopy with a Jarrel-Ash model ICAP-975 ICP-atomic emission spectrometer.

FAB-mass spectrometry

The FAB-mass spectrometry of the Zn-containing pigment of *A. rubrum* was performed on a JEOL JMS-MStation in a *m*-nitrobenzyl alcohol matrix to the first decimal place (four significant digits). That of the metal-free compound of the pigment prepared by the acid treatment was determined on a JEOL JMS-SX-102A in the same matrix (to three significant digits). High-resolution mass experiments for the Zn-containing pigment at resolution 5000 were performed on a JEOL JMS-MStation using polyethylene glycol as the standard, with an acceleration potential of 10 keV.

^{1}H-NMR measurements

^{1}H-NMR spectra of the Zn-containing pigment of *A. rubrum*, its metal-free compound, and BPheo *a* prepared from BChl *a* of *Rba. sphaeroides* were measured on a JEOL GSX-270 NMR spectrometer (270 MHz) in acetone-d_6 using TMS (tetramethylsilane) as an internal standard. The pulse angle was 45°, the repetition time was 15 s, and 256 acquisition transients were accumulated. The concentration of the pigments was adjusted to ca. 3 mM, and the measurements were carried out at 263 K. The signals assignment of the Zn-containing pigment of *A. rubrum* and its metal-free compound was conducted referring to the signals of BChl *a* and BPheo *a* of *Rba. sphaeroides*.

RESULTS

HPLC analyses

HPLC was used to analyze the pigments collected in the acetone/methanol extract of *A. rubrum*. The pigments were monitored at 765 nm (Fig. 2). Peaks 1 and 3 (pigments 1 and 3) have exactly the same retention times as peaks of BPheo a_P and BChl a_P, where P denotes phytyl (Fig.1), of *Rba. sphaeroides*, respectively (Fig. 2A and C). The retention time of peak 2 (pigment 2) is obviously different from those of BChl a_P of *Rba. sphaeroides* (Fig. 2C) and BChl a_{GG}, where GG denotes geranyl geranyl, of *Rhodospirillum (Rsp.) rubrum* (Fig. 2D). In our previous paper [6], pigment 2 was assumed to be a Zn-containing bacteriochlorophyll derivative, since the absorption spectrum is similar to BChls, and Zn instead of Mg was detected by ICP measurement.

Acid treatment of pigments 2 and 3 produced a sole compound (Fig. 2B) with the same retention time as those of pigment 1 and

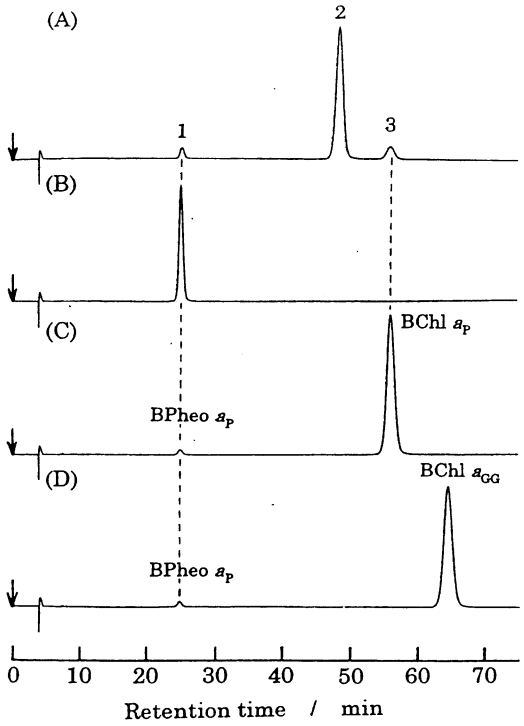


Fig. 2 HPLC elution profiles for (A) extract of *A. rubrum*, (B) acid treated extract of *A. rubrum*, (C) extract of *Rba. sphaeroides*, and (D) extract of *Rsp. rubrum*. Detection wavelength, 765 nm.

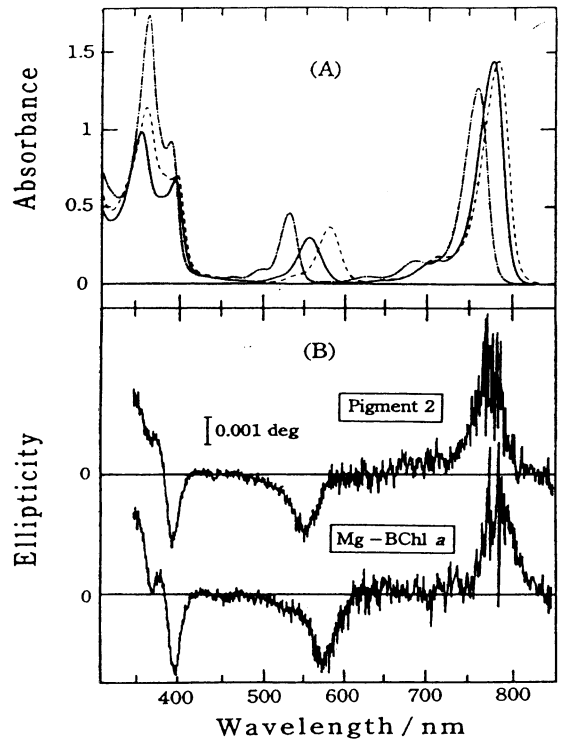


Fig. 3 (A) Absorption spectra of pigments 1 (—·—·), 2 (——) and 3 (---) purified from *A. rubrum*, and (B) CD spectra of pigment 2 purified from *A. rubrum* and BChl *a* purified from *Rba. sphaeroides* in benzene.

the natural BPheo a_P contained in *Rba. sphaeroides* (Fig. 2C) and in *Rsp. rubrum* (Fig. 2D), suggesting that pigment 1 is BPheo a_P (Fig. 1) and that macrocycles of pigments 2 and 3 are the same as that of BPheo a_P. The retention time of BPheo a_P (t = 24.5 min) was obviously different from that of BPheo a_{GG} (t = 29.0 min, not shown in Fig. 2) prepared by the acid treatment of BChl a_{GG} of *Rsp. rubrum*, suggesting that the esterifying alcohol of pigments 1, 2 and 3 is not geranyl geranyol but phytol.

Absorption and CD spectra

The absorption spectra of pigments 1 and 3 are identical to those of BPheo *a* and BChl *a* in the four different organic solvents (Table 1). Minor components, pigments 1 and 3, are hence concluded to be BPheo *a* and BChl *a*, respectively. The absorption spectrum of pigment 2 is similar in shape to that of BChl *a* but different in shape from that of BPheo *a* (Fig. 3A and Table 1). The absorption maxima of pigment 2 were all blue-shifted compared to those of BChl *a* in the four solvents examined here (Table 1). The peak wavelengths in diethyl ether were consistent with those of the artificially prepared Zn-transmetalated BChl *a* (Table 1) reported by Scheer and Hartwich [13]. The absorption maxima of the acid treated pigment 2 were consistent with those of pigment 1 and BPheo *a* (Table 1). The CD spectrum of pigment 2 was similar in shape to that of BChl *a*, although the positions of the peaks differed (Fig. 3B). This indicates that the stereochemistry of C7, C8, C13², C17 and C18 in pigment 2 is the same as in BChl *a*.

Table 1. Absorption maxima (nm) and relative intensities for pigments 1, 2 and 3 of *A. rubrum* and BPheo *a* prepared from pigments 2 and 3 at room temperature.

	Acetone			Diethyl ether			Benzene			2-Propanol		
	B	Q_x	Q_y	B	Q_x	Q_y	B	Q_x	Q_y	B	Q_x	Q_y
Pigment 1	357.0	524.5	746.0	356.5	524.5	748.5	362.0	532.0	758.0	358.5	529.0	750.0
	(100)	(24.9)	(48.2)	(100)	(25.7)	(63.0)	(100)	(26.0)	(71.7)	(100)	(24.5)	(52.1)
BPheo *a*	357.0[a]	524.5[a]	746.0[a]	356.5[a]	524.0[a]	748.5[a]	362.0[a]	532.0[a]	758.0[a]	358.5[a]	529.0[a]	750.0[a]
	357.0[b]	524.5[b]	746.5[b]	357.0[b]	524.5[b]	749.0[b]	362.5[b]	532.0[b]	758.0[b]	358.5[b]	529.0[b]	750.0[b]
	—	—	—	357.0[c]	525.0[c]	749.0[c]	—	—	—	—	—	—
Pigment 2	354.0	562.0	762.0	353.0	559.0	762.5	355.0	556.5	775.5	357.0	571.5	768.0
	(99.6)	(29.4)	(100)	(80.1)	(23.6)	(100)	(68.0)	(20.2)	(100)	(91.4)	(29.3)	(100)
Zn-BChl *a*	—	—	—	353.0[c]	559.0[c]	762.0[c]	—	—	—	—	—	—
Pigment 3	358.5	579.0	769.0	357.0	573.5	770.5	361.0	580.5	781.0	362.5	586.5	775.5
	(94.7)	(27.4)	(100)	(76.7)	(22.3)	(100)	(79.0)	(25.2)	(100)	(84.8)	(21.4)	(100)
Mg-BChl *a*	358.5[d]	579.0[d]	769.0[d]	357.0[c]	573.0[c]	771.0[c]	361.0[d]	580.5[d]	781.0[d]	362.5[d]	586.5[d]	775.5[d]

a) Prepared from pigment 2, b) Prepared from pigment 3, c) From Ref. [13], d) Purified from *Rba. sphaeroides*

ICP measurements

Zn (15.6 μ g, 238 nmol) was detected with a trace amount (<0.8 μ g) of Fe and Mg in pigment 2 (240 nmol) by ICP spectroscopy. This corresponds to the molar ratio of Zn : pigment 2 = 0.99 : 1.00. No other metals (Ag, Al, Ca, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pd, Se) were detected.

FAB-mass measurements

The FAB-mass spectrum of pigment 2 had a dominant peak at *m/z* 950.5 produced by the molecular ion, M^+ (Fig. 4A). It was 39.9 mass units larger than the peak at *m/z* 910.6 of BChl *a* ($C_{55}H_{74}N_4O_6Mg$) and was the same value of the artificially prepared Zn-BChl *a* ($C_{55}H_{74}N_4O_6{}^{64}Zn$; 950 [14]). The intense peak at *m/z* 672.2 can be explained by the loss of the esterifying phytol ($C_{20}H_{39}OH$) from the pigment. Figure 4B shows the FAB-mass spectrum of the acid treated pigment 2. The dominant peak at *m/z* 888 which coincides with that of the molecular ion of BPheo *a* ($C_{55}H_{74}N_4O_62H$). There is also a peak at *m/z* 610 produced by the fragment $(M\text{-}C_{20}H_{39}+H)^+$, which is produced by eliminating the esterifying phytol. The high-resolution mass measurement of pigment 2 gave a value of *m/z* 950.4935 for the major peak. This can only be explained by one rational formula, $C_{55}H_{74}N_4O_6{}^{64}Zn$ (*Calcd.* 950.4899). The peaks at *m/z* 952.5 and 954.5 in Fig. 4A were produced by M^+ containing isotopes of Zn, $C_{55}H_{74}N_4O_6{}^{66}Zn$ and $C_{55}H_{74}N_4O_6{}^{68}Zn$, respectively.

^{1}H-NMR measurements

Finally, ^{1}H-NMR was used to determine the exact molecular structure of the macrocycle of pigment 2 of *A. rubrum*. The spectrum of the metal-free form prepared from pigment 2 (Fig. 5A) was identical to that of BPheo *a* prepared from BChl *a* of *Rba. sphaeroides* (Fig. 5B). The proton signals linked with nitrogen were observed at -0.82 ppm. The results reveal that pigment 2 has the same macrocycle as BChl *a* and BPheo *a*, and is concluded to be Zn-BChl *a* (Fig. 1). Figure 5C shows the ^{1}H-NMR spectrum of Zn-BChl *a* purified from *A. rubrum* in acetone-d_6. The signal assignments are listed in Table 2.

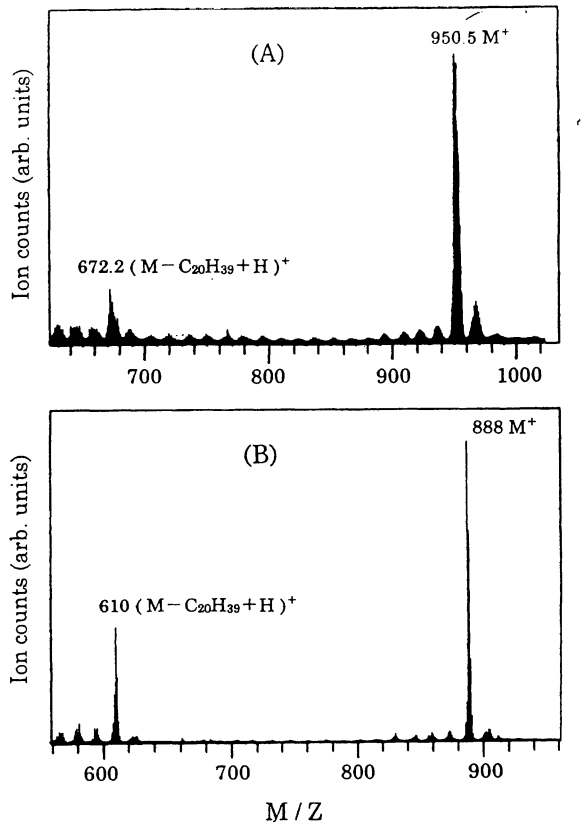


Fig. 4 FAB-mass spectra of (A) pigment 2 purified from *A. rubrum* and (B) acid treated pigment 2.

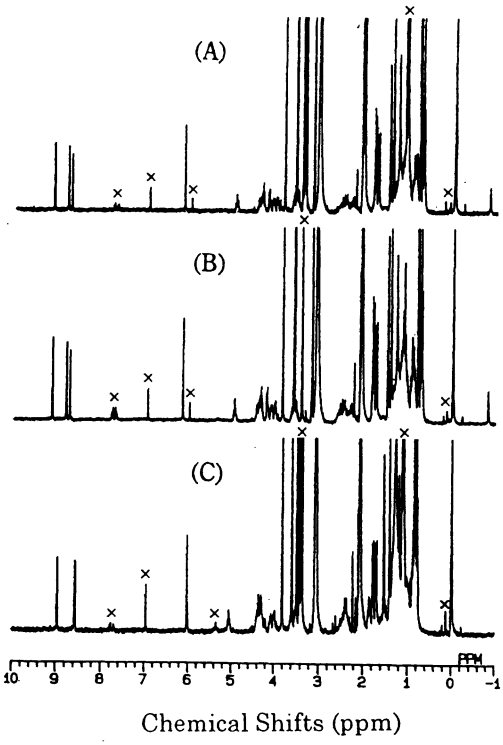


Fig. 5 ^{1}H-NMR spectra of (A) acid treated pigment 2 purified from *A. rubrum*, (B) BPheo *a* prepared by acid treatment of BChl *a* of *Rba. sphaeroides* and (C) pigment 2 of *A. rubrum*, measured in acetone-d_6 at 263 K. The signals at 2.05 ppm and those at 3.62 and 3.4 ppm are due to acetone and water, respectively. Symbol x indicates the signals of impurities.

DISCUSSION

The molecular structure of the Zn-containing pigment in *A. rubrum* was determined to be that of BChl *a* esterified with phytol, except that the central metal is Zn in place of Mg. Thus, we hereafter use an abbreviation, Zn-BChl *a* (Fig. 1), for the Zn-containing bacteriocholorophyll found in *A. rubrum*.

The natural occurrence of BChl with a central metal other than Mg, Zn-BChl *a*, in photosynthetic organisms will provide not only a new tool to study photosystems like as photoreactions and interactions between pigments and proteins, but also rationality to the previous investigations using a series of transmetalated bacteriochlorophyll *a* (M-BChl *a*) [13-20] and chlorophyll *a* (M-Chl *a*) [12,19-24].

Genes of *A. rubrum* contain the *puf* operon coding the L, M and C subunits of the RC proteins and the α and β subunits of

Table 2. ^{1}H-NMR chemical shifts (ppm) for bacteriochlorophylls in acetone-d_6 at 263K

Proton	BPheo *a*[a)]	BPheo *a*[b)]	Zn-BChl *a*[c)]
5	9.12	9.12	8.95
10	8.82	8.81	8.55
20	8.73	8.73	8.57
13^2	6.17	6.17	6.02
P2	4.97	4.97	5.06
18	4.42	4.41	4.39
P1	4.40-4.33	4.40-4.33	4.38,4.29
7	4.22	4.21	4.30
8	4.10	4.10	4.07
17	4.02	4.01	3.99
13^2-OMe	3.84	3.84	3.81
2Me	3.59	3.59	3.49
12Me	3.57	3.57	3.36
3Me	3.06	3.06	3.09
17^1	2.6-2.3	2.6-2.3	2.50,2.37
8^1	2.6-2.3	2.6-2.3	2.40,2.09
17^2	(1.81)	(1.81)	2.02
7Me	1.81	1.81	1.77
18Me	1.74	1.74	1.70
$P3^1$	1.48	1.48	1.54
8^2	~1.1	1.12	1.10
$P15^1$, P16	0.81	0.81	0.85
$P7^1$, $P11^1$	0.74, 0.72	0.74, 0.72	0.81, 0.79
NH	-0.82	-0.82	—

a) Prepared from pigment 2 of *A. rubrum*.
b) Prepared from Mg-BChl *a* of *Rba. sphaeroides*.
c) Pigment 2 purified from *A. rubrum*.

light harvesting complex 1 (LH1) with homologous amino acid sequence to those in the photosynthetic purple bacteria [8]. Comparison of amino acid sequences of the L and M subunits between the *Acidiphilium* species and other purple bacteria shows one characteristic replacement of an amino acid in the region around the special pair, based on the crystal structure of the RC of *Rba. sphaeroides* [2]; His L168, which is highly conserved in other purple bacteria, is replaced by Glu L168 in the *Acidiphilium* species [8]. The RC complex isolated from *A. rubrum* contains four molecules of Zn-BChl *a* and two molecules of BPheo *a* [10]. Since Zn-BChl *a* is shown to have the same macrocycle as that of BChl *a* in the current investigation (Figs. 1 and 5), above findings indicate that special pair and accessary pigments are Zn-BChl *a*, the primary electron acceptor is BPheo *a*, and the pigments are placed with the same geometry in the RC of *A. rubrum* as that in the RC of the purple bacteria. On the other hand, oxidation potential of Zn-BChl *a* measured *in vitro* is more positive than that of BChl *a* [15, 18]. Thus, the characteristic replacement of an amino acid in the region around the special pair from His L168 to Glu L168 seems to be relating to the regulation of the oxidation potential of the special pair composed of Zn-BChl *a* in *A. rubrum*. In *Rba. sphaeroides*, His L168 forms a strong hydrogen bond to the acetyl carbonyl group at ring I of BChl *a* (see Fig. 1) of the special pair [2,25], and contributes to asymmetrical distribution of the positive charge on the special pair. Removal of the hydrogen bond from the His L168 results in an 80 mV decrease of redox potential of the special pair in the RC of *Rba. sphaeroides* [25]. This may be a counter measure of the RC proteins to the elevation of the oxidation potential of Zn-BChl *a* special pair in the RC of *A. rubrum*.

As expected from electronegativity of metals, Zn-BChl *a* exhibits high resistance to acid as compared to BChl *a*; Zn-BChl *a* was pheophytinized roughly 10^6-fold slower than BChl *a* [12,20]. The strong resistance of Zn-BChl *a* to acid seems to be one of the reasons for the use of Zn as the central metal in *A. rubrum*. As is noted in the Pourbaix diagrams for Zn and Mg, Mg^{2+} is the predominant form at neutral pH, while the region where Zn^{2+} is the major form of Zn is limited to low pH [20, 26]. Based on the fact that the macrocycle of Zn-BChl *a* is the same as that of BChl *a*, the macrocycle is considered to be biosynthesized by similar pathways in both *A. rubrum* and the purple bacteria in prior to the metalation. In this context, the low ionization property of Zn is preferable for forming the chelate compounds under low pH conditions using the biosynthesized macrocycle. This may be the primary reason why Zn in place of Mg is the major central metal of the bacteriochlorophyll in *A. rubrum*.

Acknowledgments: We thank Dr. K. Tanaka (JEOL Co., Ltd.), Dr. M. Ogawa (Fuji Photo Film Co., Ltd.) for the measurements of FAB-mass, Dr. K. Inoue (Kanagawa Univ.), Dr. T. Matsunaga (Tokyo Univ. Agr. Tech.) for their kind supply of materials, and Dr. T. Sasaki, Mr. S. Akutsu, Mr. T. Hamano, Mr. Y. Kato and Mr. K. Tominaga (Univ. Tsukuba). This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture, Japan, by a Kurata Research Grant, and by a Research Fellowship of the Japanese Society for the Promotion of Science for Young Scientists.

REFERENCES

[1] Klimov, V. V. and Krasnovskii, A. A., *Photosynthetica*, **15**, 592-609 (1981)

[2] Allen, J. P., Feher, G., Yeates, T. O., Komiya, H. and Ress, D. C., *Proc. Natl. Acad. Sci.* USA, **84**, 6162-6166 (1987)

[3] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H., *Nature*, **318**, 618-624 (1985)

[4] Wasielewski, M. R. and Niemczyk, M. P., *J. Am. Chem. Soc.*, **106**, 5043- 5045 (1984)

[5] Osuka, A., Nakajima, S., Maruyama, K., Mataga, N., Asahi, T., Yamazaki, I., Nishimura, Y., Ohno, T. and Nozaki, K., *J. Am. Chem.Soc.*, **115**, 4577-4589 (1993)

[6] Wakao, N., Yokoi, N., Isoyama, N., Hiraishi, A., Shimada, K., Kobayashi, M., Kise, H., Iwaki, M., Itoh, S., Takaichi, S. and Sakurai, Y., *Plant Cell Physiol.*, **37**, 889-893 (1996)

[7] Wakao, N., Hiraishi, A., Shimada, K., Kobayashi, M., Takaichi, S., Iwaki, M. and Itoh, S., in *The Phototrophic Prokaryotes*, eds. by Peschek, G. A., Loffelhardt, W. and Schmetterer, G., Plenum Publishing Co., New York, 745-750 (1998)

[8] Nagashima, K. V. P., Matsuura, K., Wakao, N., Hiraishi,

A. and Shimada, K., *Plant Cell Physiol.*, **38**, 1249-1258 (1997)

[9] Shimada, K., Itoh, S., Iwaki, M., Nagashima, K. V. P., Matsuura, K., Kobayashi, M. and Wakao, N., *Proc. 11th Int'l Cong. on Photosynthesis*, 1998, *in press.*

[10] Akiyama, M., Kobayashi, M., Kise, H., Hara, M., Wakao, N. and Shimada, K., *Photomed. Photobiol.*, **20**, 1998, *in press.*

[11] Watanabe, T., Hongu, A., Honda, K., Nakazato, M., Konno, M. and Saitoh, S., *Anal. Chem.*, **56**, 251-256 (1984)

[12] Kobayashi, M., Yamamura, M., Akiyama, M., Kise, H., Inoue, K., Hara, M., Takaichi, S., Wakao, N., Yahara, K. and Watanabe, T., *Anal. Sci.* (1998) *in press.*

[13] Scheer, H. and Hartwich, G., in *Anoxygenic Photosynthetic Bacteria*, eds. by Blankenship, R. E., Madigan, M. T. and Bauer, C. E., Kluwer Academic Publishers, Dordrecht, 649-663 (1995)

[14] Hartwich, G., Fiedor, L., Simonin, I., Cmiel, E., Schäfer, W., Noy, D., Scherz, A. and Scheer, H., *J. Am. Chem. Soc.*, **120**, 3675-3683 (1998)

[15] Geskes, C., Hartwich, G., Scheer, H., Mantele, W. and Heinze, J., *J. Am. Chem. Soc.*, **117**, 7776-7783 (1995)

[16] Hartwich, G., Geskes, C., Scheer, H., Heinze, J. and Mantele, W., *J. Am. Chem. Soc.*, **117**, 7784-7790 (1995)

[17] Näveke, A., Scheer, H., Robert, B. and Sturgis, J., in *Photosynthesis: from Light to Biosphere*, ed. by Mathis, P., Kluwer Academic Publishers, Dordrecht, Vol. **1**, 259-262 (1995).

[18] Noy, D., Fiedor, L., Hartwich, G., Scheer, H. and Scherz, A., *J. Am. Chem. Soc.*, **120**, 3684-3693 (1998)

[19] Kobayashi, M., Akiyama, M., Yamamura, M., Kise, H., Wakao, N., Ishida, N., Koizumi, M., Kano, H. and Watanabe, T., *Zeitschrift Physikalische Chemie* (1998) *in press.*

[20] Yamamura, M., Kobayashi, M., Inoue, K., Hara, M., Wakao, N., Kano, H., Watanabe, T., Akiyama, M. and Kise, H., *Proc. 11th Int'l Cong. on Photosynthesis*, (1998) *in press.*

[21] Baker, B. L. and Hodgson, G. W., *J. Phys. Chem.*, **65**, 1078-1079 (1961)

[22] Berezin, B. D. and Sosnikov, N. I., *Russ. J. Phys. Chem.*, **38**, 663-666 (1964)

[23] Watanabe, T., Machida, K., Suzuki, H., Kobayashi, M. and Honda, K., *Coord. Chem. Rev.*, **64**, 207-224 (1985)

[24] Watanabe, T. and Kobayashi, M., in *Chlorophylls*, ed. by Scheer, H., CRC Press, Boca Raton, 287-315 (1991)

[25] Mattioli, T. A., Williams J. C., Allen, J. P. and Robert B., *Biochemistry*, **33**, 1636-1643 (1994)

[26] Kobayashi, M., Akiyama, M., Kise, H., Takaichi, S., Shimada, K., Itoh, S., Hiraishi, A. and Wakao, N., *J. Mineralog. Soc. Jpn.*, **26**, 117-120 (1997)