S1-031

The size of LH1 determined by the ratio of bacteriochlorophyll/ bacterio-pheophytin in purple bacteria containing LH1 only

M Akiyama[1], KVP Nagashima[2], N Wakao[3], H Kise[1], M Hara[4] and M Kobayashi[1*]

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

[2]*Department of Biology, Tokyo Metropolitan University, Hachiohji, 192-0397, Japan*

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

[4]*Tissue Engineering Research Center, AIST, Ikeda, 563-8577, Japan*
([*]FAX: +81-298-55-7440, e-mail: masami@ims.tsukuba.ac.jp)

Key words: antenna size, bacteriochlorophyll, LH1, photosynthetic unit, reaction center

Introduction

The purple photosynthetic bacteria usually contain two types of LH complexes; LH1 and LH2. The amounts of LH2 are modulated by several factors, while the LH1/RC ratio has been thought to be unity (Drews and Golecki 1995). Structural information about LH1 have been obtained from native membranes and RC-LH1 complexes isolated from *Rps. viridis* (Ikeda-Yamasaki *et al*. 1998), *Rsp. molischianum* (Boonstra *et al.* 1994) and *Rsp. rubrum* (Walz and Ghosh 1997), suggesting a single RC inside a closed LH1 ring. Organization of LH1 complexes in a closed ring was also observed by a cryo-electron microscopy study of two-dimensional crystals from *Rsp. rubrum* (Karrasch *et al*. 1995), which showed 16$\alpha\beta$ subunits containing 32 molecules of BChl *a* and was arranged in a ring-like structure that could only accommodate one RC.

However, a perfect cyclic arrangement of LH1 antenna has been questioned, and several recent studies have indicated the imperfect ring (Jungas *et al.* 1999, Akiyama *et al.* 1999). In *Rba. sphaeroides*, the noncircular arrangement was proposed to be related to the presence of a small protein, PufX, which enables the quinone transfer (Francia *et al.* 1999, Frese *et al.* 2000).

In this paper, we reconsidered the models of RC-LH1 complex by determining the BChl/BPhe ratios in six purple bacteria with neither LH2 nor PufX, on the basis of 4 BChls and 2 BPhes in the RC. The BChl/BPhe ratios showed some variation in the range of 12 - 24. We will discuss the possible models of RC-LH1 complex which can interpret the variation.

Materials and Methods

We used six purple bacteria that contain LH1 but neither LH2 nor PufX; *Rps. viridis*, *Rsp. rubrum*, *Rvi. gelatinosus* (LH2-less mutant), *Rbi. marinum*, *Erb. longus* and *A. rubrum*. They were cultured under illumination by a tungsten lamp, pigments were extracted with acetone/methanol (7/3, v/v) mixture, and pigment analyses were performed by normal-phase HPLC, as described elsewhere (Akiyama *et al.* 1999).

Results and Discussion

The molar ratios of BChl/BPhe in six species of purple bacteria were determined by HPLC analyses. If every LH1 ring consisted of 16αβ and surrounded a single RC, the ratio of BChl/BPhe should be unity, namely, 18, because αβ subunit contains 2 BChls and a RC contains 4 BChls and 2 BPhes. However, significant variation was observed (Table 1). In *Erb. longus* the ratio was *ca.* 12, in *A. rubrum* and *Rps. viridis ca.* 14, in *Rsp. rubrum ca.* 18, in *Rbi. marinum* and *Rvi. gelatinosus ca.* 23, suggesting that the size of LH1 is not unity but dependent on the species. The LH1 size in *Erb. longus* was estimated to be 10αβ, 12αβ(γ) in *A. rubrum* and *Rps. viridis*, 16αβ in *Rsp. rubrum*, 21-22αβ in *Rvi. gelatinosus* and *Rbi. marinum* (Table 1).

Table 1. Stoichiometry of BChl/BPhe found in purple bacteria with neither LH2 nor PufX.

	BChl/Bphe	Size of LH1[a)]
Erb. Longus	11.8 ± 1.8	10αβ
A. rubrum	13.8 ± 1.0	12αβ
Rps. Viridis	14.6 ± 1.6	12αβγ
Rsp. Rubrum	17.5 ± 2.2	16αβ
Rbi. Marinum	23.2 ± 2.6	21αβ
Rvi. gelatinosus[b)]	23.8 ± 2.2	22αβ

a) Antenna size was calculated on the basis of LH1/RC = 1/1.
b) LH2-less mutant

The size of 12αβγ estimated for *Rps. viridis* supports a six-fold symmetry of the ring-like LH1 complex usually observed in the photosynthetic membrane with the electron microscope (Miller 1982, Stark *et al*. 1986), where the LH1 contained 24 BChls *b* and showed a circular structure having a little larger diameter (Ikeda-Yamasaki *et al.* 1998) than 16αβ-LH1 in *Rsp. rubrum* (Karrasch *et al*. 1995). A six-fold symmetry seen in *Rps. viridis* may represent the 12αβγ units. In *Rps. viridis*, γ subunit might play an important role in the quinone transfer (Fig. 1A), although we have no experimental data that elucidate it. In contrast, there is no reports whether γ subunit is present in *Erb. longus* and *A. rubrum*. The 12αβ units without γ protein is too small to surround the RC entirely, because the size of 16αβ ring looks minimum to surround the RC completely (Karrasch *et al*. 1995). Recently, the noncircular arrangement around the RC has been reported by Jungas *et al*. (1999), which shows the C-shaped LH1 antenna enabling the quinone transfer. The LH1 of *Erb. longus* and *A. rubrum* should not be closed but C-shaped antenna (Fig. 1B).

The molar ratio of BChl/BPhe = *ca*. 18 found in *Rsp. rubrum* supports a reported model in which one RC is surrounded by a 16αβ-units (Karrasch *et al.* 1995). However, this arrangement impedes shuttling of quinone (Fig. 1C). In addition, we cannot exclude the dependence of the pigment ratio on culture conditions, *e.g.* light intensities. We are now performing further detailed studies.

Rbi. marinum and *Rvi. gelatinosus* showed remarkably high pigment ratios as shown in Table 1. If all RCs were located in the core of all LH1 rings with a stoichiometry of LH1/RC = 1/1, the size of LH1 should be larger than 20αβ. However, such a large closed ring has no advantage in transferring a quinone (Fig. 1D). It should be noted that smaller antenna size was reported: 12αβ (Francke and Amesz 1995, Kessi *et al.* 1995) and 12-15 αβ (Dawkins *et al*. 1988) for *Rsp. rubrum*; 12αβ (Francke and Amesz 1995) and 16αβ (Qian *et al*. 2000) for *Rbi. marinum*; 12-16αβ (Dawkins *et al*. 1988) for *Rvi. gelatinosus*. Even smaller antenna size of LH1, 10αβ, was estimated for *Rba. sphaeroides* (Fiedor *et al*. 2001). It seems that the antenna size in these bacteria was not constant but sensitive to culture conditions. These

findings gave us the novel idea that there were two types of LH1; one is the C-shaped antenna closely associated with the RC, and another is a small ring antenna located peripherally in a variable ratio to the core complex (Fig. 1E), probably depending on the species and the growth conditions, like LH2. In *Erb. longus*, *A. rubrum* and *Rps. viridis*, it is very likely that they have a core LH1 only (Fig. 1A&B).

Recently, a new theory as to the energy transfer in the purple bacterial photosystems was propounded (Sumi 2000), indicating that symmetrical structure of the RC-LH1 complex is unfavorable for the energy transfer from the LH1 to the RC. Hence, some asymmetrical models presented in Fig. 1 may be likely candidates.

Here, we want to propose another model in which all RCs are located on the outside of the LH1 rings with variety of stoichiometries (Fig. 1F). This model has the advantage in interpreting both the variety of the pigment ratios and the quinone transfer. Unfortunately, however, this unique model contradicts almost all observation ever reported, including the studies by means of electron microscope, which showed the presence of RC in the core of the LH1 ring. We should perform further detailed studies to clarify the architecture of the RC-LH1 complex.

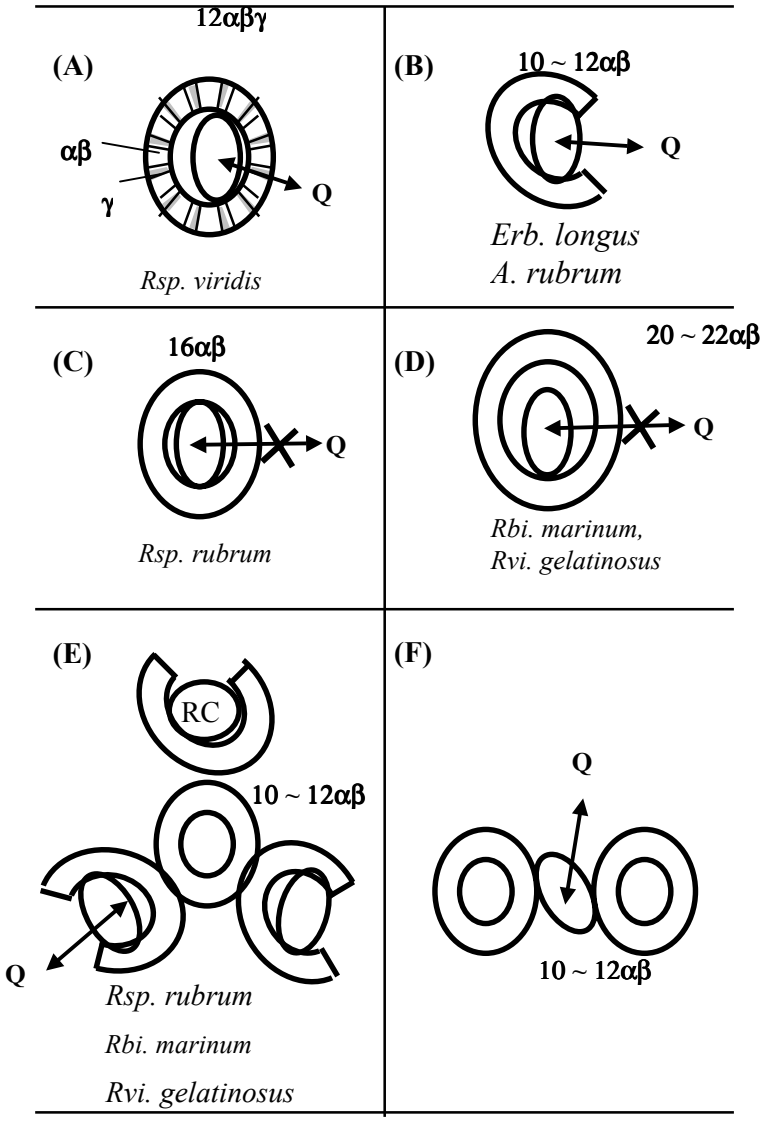

Fig. 1 Proposed models of the RC-LH1 complexes; (A) a 12αβγ LH1 ring surrounding a RC, (B) a C-shaped core LH1, (C) a 16αβ LH1 ring surrounding a RC entirely, (D) a larger than 16αβ LH1 ring surrounding a RC, (E) a combination of C-shaped core LH1 and peripheral LH1 ring, (F) a circumscribed RC. Q : quinone.

Acknowledgements

This work was supported in part by a Research Fellowship of the Japanese Society for the Promotion of Science for Young Scientists.

References

Akiyama M, Nagashima KVP, Hara M, Wakao N, Tominaga K, Kise H and Kobayashi M (1999) Stoichiometries of LH1/RC determined by the molar ratio of BChl/BPhe analyzed

by HPLC in seven species of purple bacteria containing LH1 only. *Photomed Photobiol* **21**, 105-110.

Boonstra AF, Germeroth L and Boekema EJ (1994) Structure of the light harvesting antenna from *Rhodospirillum molischianum* studied by electron microscopy. *Biochim Biophys Acta* **1184**, 227-234.

Dawkins DJ, Ferguson LA and Cogdell R (1988) The structure of the 'core' of the purple bacterial photosynthetic unit. in *Photosynthetic Light-harvesting Systems*, Scheer H and Schneider S (eds), pp. 115-127. Walter de Gruyter, Berlin.

Drews G and Golecki JR (1995) Structure, molecular organization, and biosynthesis of membranes of purple bacteria. in *Anoxygenic Photosynthetic Bacteria*, Blankenship RE, Madigan MT and Bauer CE(eds), pp. 231-257. Kluwer Academic Publishers, Dordrecht.

Fiedor L, Leupold D, Teuchner K, Voigt B, Hunter CN, Scherz A and Scheer H (2001) Excitation trap approach to analyze size and pigment-pigment coupling: Reconstitution of LH1 antenna of *Rhodobacter sphaeroides* with Ni-substituted bacteriochlorophyll. *Biochemistry* **40**, 3737-3747.

Francke C and Amesz J (1995) The size of the photosynthetic unit in purple bacteria. *Photosynth Res* **46**, 347-352.

Francia F, Wang J, Venturoli G, Melandri BA, Barz WP and Oesterhelt D (1999) The reaction center- LH1 antenna complex of *Rhodobacter sphaeroides* contains one PufX molecule which is involved in dimerization of this complex. *Biochemistry* **38**, 6834-6845.

Frese RN, Olsen JD, Branvall RB, Westerhuis WHJ, Hunter CN and Van Grondelle R (2000) The long- range supraorganization of the bacterial photosynthetic unit: A key role for PufX. *Proc Natl Acad Sci USA* **97**, 5197-5202.

Ikeda-Yamasaki I, Odahara T, Mitsuoka K, Fujiyoshi Y and Murata K (1998) Projection map of the reaction center-light harvesting 1 complex from *Rhodopseudomonas viridis* at 10 Å resolution. *FEBS Lett* **425**, 505-508.

Jungas C, Ranck J-L, Rigaud J-L, Joliot P and Verméglio A (1999) Supramolecular organization of the photosynthetic apparatus of *Rhodobacter sphaeroides*. *EMBO J* **18**, 534-542.

Karrasch S, Bullough PA and Ghosh R (1995) The 8.5 Å projection map of the light-harvesting complex I from *Rhodospirillum rubrum* reveals a ring composed of 16 subunits. *EMBO J* **14**, 631-638.

Kessi J, Ghosh R and Bachofen R (1995) Purification of an LHI-RC-complex of *Rhodospirillum rubrum* by solubilization of chromatophores with a short-chain lecithin. *Photosynth Res* **46**, 353-362.

Miller KR (1982) Three-dimensional structure of a photosynthetic membrane. *Nature* **300**, 53-55.

Qian P, Yagura T, Koyama Y and Cogdell RJ (2000) Isolation and purification of the reaction center (RC) and the core (RC-LH1) complex from *Rhodobium marinum*: the LH1 ring of the detergent-solubilized core complex contains 32 bacteriochlorophylls. *Plant Cell Physiol* **41**, 1347-1353.

Stark W, Jay F and Muehlethaler K (1986) Localisation of reaction centre and light harvesting complexes in the photosynthetic unit of *Rhodopseudomonas viridis*. *Arch Microbiol* **146**, 130-133.

Sumi H (2000) Structural strategies in the antenna system of photosynthesis on the basis of quantum- mechanical coherence among pigments. *J Luminescence* **87-9**, 71-76.

Walz T and Ghosh R (1997) Two-dimensional crystallization of the light-harvesting I-reaction centre photounit from *Rhodospirillum rubrum*. *J Mol Biol* **265**, 107-111.