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Variation in the Size of Light Harvesting 1 of Purple Bacteria

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We examined the bacteriochlorophyll/bacteriopheophytin ratios in several species of purple bacteria containing only LH1. The pigment ratios depended greatly on species. Further, *Rhodospirillum rubrum* showed wide variation when grown under different light intensity, and *Rhodobium marinum* showed significant variation from culture to culture even under the same light conditions. The protein ratios of α/RC and β/RC estimated by SDS-PAGE of chromatophores of *Rsp. rubrum* and *Rbi. marinum* exhibited the ratio of $\beta/\alpha > 1$. These findings gave us the novel idea that there are two types of LH1; one is a C-shaped open antenna composed by αβ units surrounding a RC, and another is a small closed ring antenna composed by αβ units located peripherally in a variable ratio to the core complex like LH2.

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

INTRODUCTION

Purple bacteria have two types of LH complexes, namely, LH1 and LH2. LH1 is closely associated with the RC and the LH1/RC ratio has been thought to be unity, while LH2 is located peripherally and the amount is modulated by the growth conditions. The structures of LH2 antenna complexes have been solved at atomic resolution [1,2], although the structure of LH1 antenna is not clear yet. Organization of LH1 complexes composed of 16αβ subunits as a closed ring was reported in *Rsp. rubrum* [3].

However, a cyclic colsed LH1 antenna in the core complex has been questioned, and a C-shaped LH1 consisting of ca. 12αβ subunits was found to surround a RC in the native tubular membranes of *Rba. Sphaeroides* [4]. Similar small size of LH1 was reported for several purple bacteria [5-8]. In contrast, more enlarged LH1 rings than 16αβ have been suggested on the basis of BChl/BPhe ratios [6,7].

In this paper, we reconsidered the models of the RC-LH1 complexes by determining the BChl/BPhe ratios and the αβ /RC ratios in purple bacteria containing LH1 only. Both ratios had great dependence on the species and growing conditions. We will discuss the possible models of the RC-LH1 complexes which can interpret our results.

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Abbreviations: BChl-bacteriochlorophyll; BPhe-bacteriopheophytin; LH1-light harvesting 1; RC-reaction center

MATERIALS AND METHODS

Four purple bacteria containing neither LH2 nor PufX; *Rhodopseudomonas* (*Rps.*) *viridis*, *Rhodospirillum* (*Rsp.*) *rubrum*, *Rubrivivax* (*Rvi.*) *gelatinosus* (LH2-highly reduced mutant), *Rhodobium* (*Rbi.*) *marinum* were cultured under illumination by a tungsten lamp as previously described [7]. Pigments were analyzed by normal-phase HPLC as described elsewhere [6]. The cells were disrupted by sonication, and then chromatophores were collected by centrifugation. Protein subunits were analysed by SDS-PAGE on a 10-20%(w/v) polyacrylamide gradient gel according to the Laemmli's method [9]. Protein bands were stained with Coomasie Brilliant Blue R-250 and quantitated by densitometry.

RESULTS AND DISCUSSION

The BChl/BPhe ratios obtained by HPLC analyses are shown in Table 1. The BChl/BPhe ratios did not vary with the light intensity in *Rps. viridis* and *Rvi. gelatinosus*, but

Table 1. BChl/BPhe ratios and sizes of LH1 in four purple bacteria grown at different light intensity determined by HPLC.

	BChl/BPhe			Size of LH1[a]		
	Low[i]	Middle[i]	High[i]	Low	Middle	High
Rps. viridis	14.4[c] ± 1.1 (n = 7)	14.4[c] ± 0.8 (n = 8)	14.9[c] ± 0.9 (n = 10)	12α β γ	12α β γ	12α β γ
Rsp. rubrum	14.4[d] ± 2.0 (n = 11)	17.7[d] ± 1.0 (n = 10)	21.6[d] ± 2.0 (n = 10)	12α β	16α β	20α β
Rvi. gelatinosus[b]	19.5 ± 1.9 (n = 25)	21.6 ± 1.9 (n = 18)	21.5 ± 3.3 (n = 18)	18α β	20α β	20α β
Rbi. marinum (A)	16.7[d] ± 1.3 (n = 21)	16.0[d] ± 1.3 (n = 23)	16.3[d] ± 1.5 (n = 17)	15α β	14α β	14α β
(B)	20.9[e,f] ± 1.4 (n = 12)	20.6[e,g] ± 1.6 (n = 12)	21.2[e,h] ± 1.7 (n = 6)	19α β	19α β	19α β

a) Antenna size was calculated on the basis of LH1/RC = 1/1.
b) LH2-highly reduced mutant (LH2/LH1 is less than 0.1).
c) BChl *b*/BPhe *b*, d) BChl = BChl a_{GG},
e) BChl = BChl a_P + BChl a_{THGG} + BChl a_{GG}.
f) BChl a_P/BChl = 0.91, g) BChl a_P/BChl = 0.89, h) BChl a_P/BChl = 0.88.
i) Low: 0.3 klux, Middle: 1 klux, High: 3 klux.

these ratios were very different from each other. In contrast, *Rsp. rubrum* showed a remarkable change. In *Rbi. marinum*, the ratio changed from culture to culture, but seemed to be independent of light intensity.

If every LH1 ring consisted of 16αβ and surrounded a single RC, the ratio of BChl/BPhe should be unity, namely 18, because an αβ subunit contains 2 BChls and a RC contains 4 BChls and 2 BPhes, respectively. However, significant variation was seen 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 was calculated to be 12αβγ in *Rps. viridis*, 12-20αβ in *Rsp. rubrum*, 18-20αβ in *Rvi. gelatinosus*, and 14-19αβ in *Rbi. marinum* (Table 1).

A 12αβγ ring is reported to have a little larger diameter [10] than 16αβ-LH1 in *Rsp. rubrum* [3]. In *Rps. viridis*, γ subunit might play an important role in the quinone transfer, while we have no experimental data that elucidate it. The 12-15αβ units without γ protein in *Rsp. rubrum* grown under low light intensity and in *Rbi. marinum* (A) are too small to surround a RC entirely, because the size of 16αβ ring looks minimum to surround a RC completely [3]. We thus surmise that such LH1 antenna may be C-shaped (Fig. 1). The 18-20αβ LH1 rings in *Rsp. rubrum* at high light, *Rvi. gelatinosus* and *Rbi. marinum* (B) are large enough to enclose a RC completely, while such a large closed ring has no advantage in transferring a quinone.

We propose a novel idea that there are two types of LH1; one is a C-shaped antenna surrounding a RC, and another is a small ring antenna located peripherally like LH2 in a variable

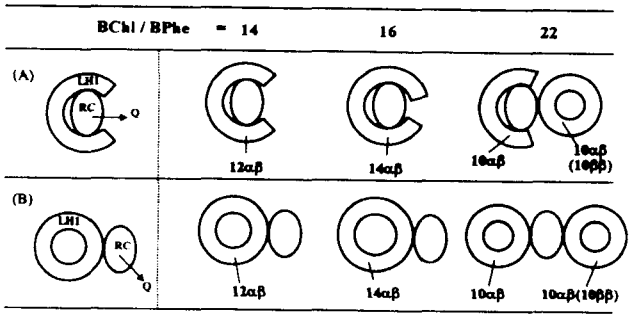

Fig. 1 Proposed models of the RC-LH1 complexes; (A) a combination of C-shaped core LH1 and peripheral LH1 ring, (B) a circumscribed RC. Q: quinone.

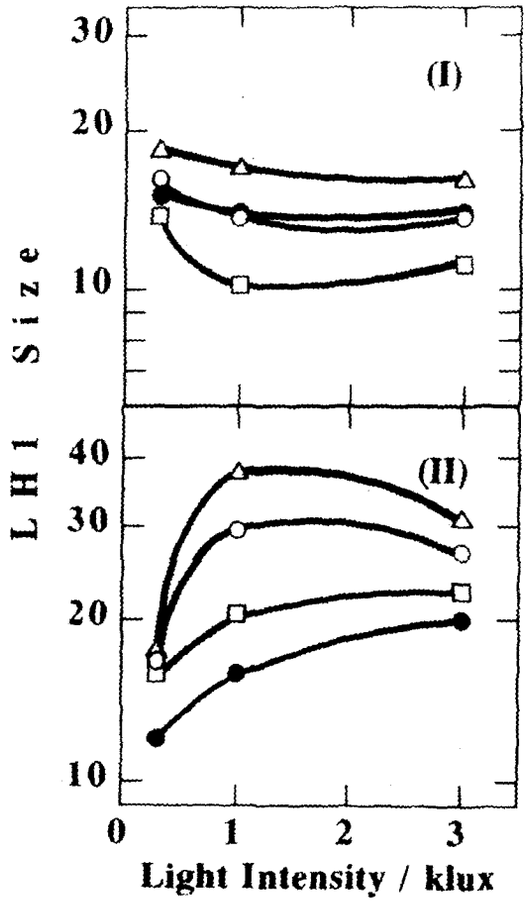

Fig. 2 Dependence of LH1 size of (I) *Rbi. marinum*(A) and (II) *Rsp. rubrum* upon light intensity. □: α/RC, △: β/RC, ○: (α+β)/2RC estimated by SDS-PAGE, and ●: αβ/RC calculated by HPLC analyses.

ratio to the core complex (Fig. 1A). To our surprise, in *Rsp. rubrum* the BChl/BPhe ratio was higher under higher illumination (Table 2), indicating the increase of peripheral LH1 antenna at higher light intensity. This could be regarded as the light adaptation, namely, peripheral LH1 antenna might function as photon-pool to protect the core RC/LH1 complex against strong light by receiving extra photons from the core complex.

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. 1B). This model has the advantage in interpreting both the variety of the pigment ratios and the quinone transfer, although this unique model contradicts almost all observation ever reported.

In order to confirm the variability of LH1 size, we estimated the ratios of αβ/RC in *Rsp. rubrum* and in *Rbi. marinum* (A) by SDS-PAGE. Figure 2 shows the ratios of α/RC, β/RC and their mean values estimated by SDS-PAGE along with the αβ/RC ratios estimated by HPLC. In both bacteria, the β/RC ratios were higher than the α/RC ratios. In *Rbi. marinum*, the mean values, (α+β)/2RC, are in good agreement with the LH1 size calculated by HPLC analyses. However, in *Rsp. rubrum*, the ratios of α/RC, β/RC and their mean values were higher than the LH1 size estimated by HPLC, especially the β/RC ratio was extraordinarily high, suggesting that the data obtained here by SDS-PAGE are not accurate enough. Anyway, the α/β ratio looks not unity but below one, and if this is the case, a peripheral LH1 antenna free of RC inside is likely to consist of not αβ but ββ (Fig. 1).

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