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The primary electron acceptor of green sulfur bacteria is chlorophyll *a* esterified with Δ2,6-phytadienol

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Introduction

The RCs of photosynthetic organisms are classified into two types in regard to the electron acceptors. The first type (type-1) is denoted RC1 which has Fe-S centers in the electron acceptor chain, and another type (type-2) is denoted RC2 having a metal-free chlorophylls, namely, pheophytins, as the primary electron acceptors. The RCs of purple bacteria and filamentous bacteria are classified as type-2, since they contain pheophytins. The RCs of green sulfur bacteria and heliobacteria are type-1, since Fe-S centers are present.

The primary charge separation is brought about by a few specialized chlorophylls in the RCs. The primary electron donor pigments of PS1 and heliobacteria have been identified only recently; a heterodimer of Chl *a*/*a′* constitutes P700 in PS1 (Kobayashi *et al*. 1988, Jordan *et al*. 2001) and a homodimer of BChl *g′* is P798 in heliobacteria (Kobayashi *et al*. 1991). Quite recently, P740 of PS1 in a novel oxygenic alga *Acaryochloris marina* has been found to consist of a Chl *d′* dimer (Akiyama *et al*. 2001). In contrast, it is well-accepted that Chl *a* is A_0 in PS1 (Jordan *et al*. 2001). 8^1-hydroxy Chl *a* has been found to function as A_0 in heliobacteria (Van de Meent *et al.* 1991).

A_0 of green sulfur bacteria was first isolated from *Prosthecochloris aestuarii* by Braumann *et al.* (1986), and was named BChl 663 after its absorption maximum in an organic solvent, which was considered to be a lipophilic form of BChl *c*. The absorption spectra of BChl 663 are very similar to those of Chl *a* (Fig. 1) in organic solvents (Kobayashi *et al*. 1992). The CD spectrum of BChl 663 in benzene shows that BChl 663 is not the prime-type (13^2-epimer) but the normal-type (Kobayashi *et al*. 1992, Kobayashi 1996). The fluorescence quantum yield of BChl 663 *in vitro* is the same as that of Chl *a* (Kobayashi *et al*. 1992, Kobayashi 1996). However, a clear difference is observed on the retention time of BChl 663 on normal-phase HPLC from that of Chl *a* or Chl *a′* (Van de Meent *et al*. 1992, Kobayashi *et al*. 1992, Kobayashi 1996). The above results were rationally explained if BChl 663 was an isomer of Chl *a*.

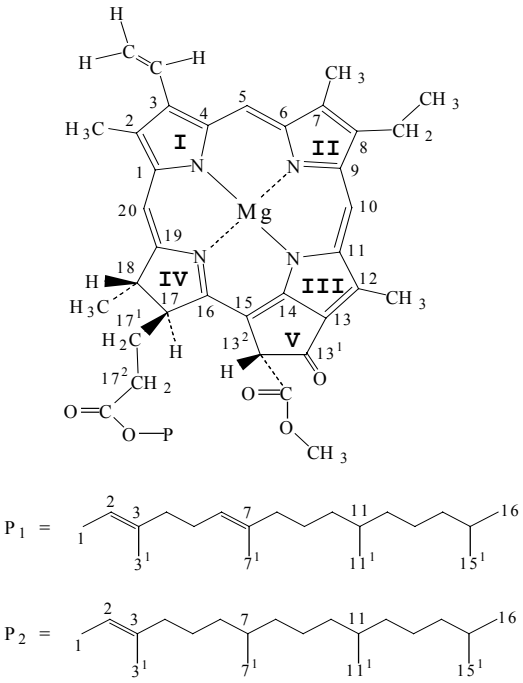

Fig. 1 Molecular structure and carbon numbering of BChl 663 ($P = P_1$) and Chl *a* ($P = P_2$), according to the IUPAC numbering system. The esterifying alcohol of BChl 663 is Δ2,6-phytadienol.

Here, we introduce the structural properties of BChl 663 and BChl *a′*, functioning respectively as A_0 and P840 in the RC of green sulfur bacteria.

Materials and methods

RC complexes were isolated from *Chlorobium tepidum* as described by Permentier *et al*. (2000). BChl 663 was extracted with methanol from RC complexes and then purified as described elsewhere (Kobayashi *et al*. 2000). BChl 663 was analyzed by absorption, FAB-mass and NMR spectrometry. Extinction coefficients of BChl *a* in several organic solvents were reinvestigated to confirm the exact number of BChl *a* in the RC. Plant Chl *a* esterified with phytol is denoted as Chl a_P below.

Results and Discussion

The absorption spectrum of BChl 663 was identical to that of Chl a_P, suggesting a common macrocycle for BChl 663 and Chl a_P. The FAB-mass spectrum of BChl 663 had molecular ion peaks at *m/z* 890.6 M^+ and 891.6 $(M + H)^+$, which were 2.0 mass units smaller than the respective peaks of Chl a_P ($C_{55}H_{72}N_4O_5Mg$, *Calcd.* 892.5353) at *m/z* 892.6 M^+ and 893.6 $(M + H)^+$. Both spectra of BChl 663 and Chl a_P showed an intense peak at the same mass of *m/z* 614.3, due to the loss of the esterifying alcohol. Thus the loss of the 2.0 mass units in BChl 663 was ascribed to a difference in the esterifying alcohol, most probably due to an additional C=C double bond in the phytyl chain of BChl 663.

NMR signals corresponding to all ^1H atoms attached to the macrocycle of Chl a_P were observed in the spectrum of BChl 663 at the same chemical shifts. This confirms that the macrocycle of BChl 663 is identical to that of Chl a_P. However, marked differences were observed in the signals arising from the alcohol chains. A singlet $P7^1$ signal was observed at

the right-hand side of the singlet $P3^1$ signal in BChl 663, while a doublet peak of $P7^1$ overlapped that of $P11^1$ in Chl a_P. A signal of P7-proton, seen in Chl a_P, was lacking, and the P6-proton appeared as a triplet signal near the triplet P2-proton signal in BChl 663. These results indicate the presence of a C=C double bond between P6 and P7 in addition to that between P2 and P3 in BChl 663. The structure of BChl 663 was hence concluded to be Chl *a* esterified with Δ2,6-phytadienol instead of phytol (Fig. 1).

In all RCs prepared from green sulfur bacteria examined so far, small amounts of BChl *a′* have been found to be present, and the BChl 663/BChl *a′* molar ratio was same for the cells and the RC complexes, namely, 4/2 (Kobayashi 1996, Kobayashi *et al*. 2000, Permentier *et al.* 2000). The ratio of BChl *a*/BChl *a′* in the RC complexes was 14/2. The numbers of 16 BChls *a* (of which two should be BChl *a′*) and 4 BChls 663 have been found for several other preparations from green sulfur bacteria (Albouy 1995, Griesbeck *et al*. 1998). We hence concluded that the RC complexes of green sulfur bacteria contain 14 BChls *a*, 2 BChls *a′* and 4 BChls 663. This finding suggests that two molecules of BChl *a′* constitute P840, two molecules of BChl 663 are the primary electron acceptors, and remaining two BChls 663 are the electron transfer accessories, as illustrated in Fig. 2. Quinones were virtually absent in the photochemically active RC complexes examined here, indicating that a quinone is not necessary for electron transfer from P840 to the Fe-S centers, as was also indicated by Frankenberg *et al.* (1996) and Kusumoto *et al.* (1999). It should be natural to assume that there are two parallel electron transfer chain leading from $(BChl\ a')_2$ to Fe-S centers, because the RC complexes consist of the PscA homodimer (Büttner *et al.* 1992).

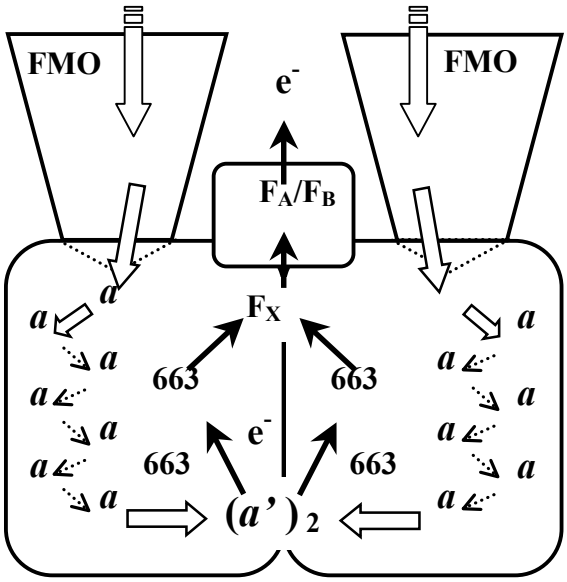

Fig.2 Proposed chlorophyll arrangement in the RC complexes of green sulfur bacteria, where ***a*** is BChl *a*, ***a′*** is BChl *a′*, and **663** is BChl 663. BChls *a* in FMO protein are omitted.

There is a distinct difference between the chlorosomes and the RC of green sulfur bacteria with regard to the esterifying alcohols of chlorophylls: farnesol (C15) is the major alcohol in the chlorosomes and phytol (C20) in the RC. It suggests that the chlorosomes and the RC might be of different evolutionary origin. This absurd but attractive hypothesis might be support in part by the fact that the chlorosomes are attached to the fundamentally different type of RCs, namely, type-1 RC of green sulfur bacteria and type-2 RC of filamentous green bacteria.

Lastly, let us add a few words. May Jan's soul rest in peace !

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