Structure and Binding Site of the Primary Electron Acceptor in the Reaction Center of *Chlorobium*†

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ABSTRACT: In isolated, chlorosome-free reaction centers from *Chlorobium limicola* f *thiosulphatophilum*, a chlorin pigment exhibits a Q_y absorption band at 672 nm (Feiler, U., Nitschke, W., & Michel, H. (1992) *Biochemistry 31*, 2608–2614). To characterize the chemical nature of this chlorin pigment and its interactions within the reaction-center protein, selective enhancement of its Raman scattering was achieved by resonant excitation within its Soret band. This is the first time that structural studies of this pigment were performed on the native reaction-center protein. The obtained resonance Raman spectra were consistent with a single population of a chlorophyll *a*(-like) pigment, possessing a vinyl group on ring I, but not with bacteriochlorophyll *c* or bacteriophaeophytin *c*. The stretching frequencies of the C_9-keto carbonyl of this pigment indicates that it is H-bonded to the reaction-center protein. The strength of this H-bond is very close to those of the keto carbonyls of the primary electron acceptors in purple bacterial reaction centers and D1/D2 particles. Since in membranes of *Chlorobiaceae* a transient bleaching at 670 nm is due to the primary acceptor in the reaction center (Nuijs, A. M., Vasmel, H., Joppe, H. L. P., Duysens, L. N. M., & Amesz, J. (1985a) *Biochim. Biophys. Acta 907*, 24–34), we thus conclude that the primary acceptor in *Chlorobium* reaction centers is the characterized chlorophyll *a*(-like) pigment. This pigment might be identical with the so-called BChl-663 molecule which was observed in membranes from *Chlorobiaceae* (van de Meent, E. J., Kobayashi, M., Erkelens, C., van Veelen, P., Otte, S., Inoue, K., Watanabe, T., & Amesz, J. (1992) *Biochim. Biophys. Acta 1102*, 371–378.).

Green sulfur bacteria (*Chlorobiaceae*) are strictly anaerobic, photoautotrophic organisms which grow under strongly reducing conditions (e.g. H_2S-rich environments) in dim light. They are characterized by the presence of a large antenna system, the so-called chlorosomes, reflecting their adaptation to grow under low-light conditions.

A relationship between the reaction centers (RC)[1] of green sulfur bacteria and those of photosystem I (PSI) in plants and cyanobacteria was first postulated in 1968 on the basis of the ability of membrane fragments to perform light-induced NAD^+ reduction (Buchanan & Evans, 1968; Knaff, 1978). This proposed relationship has been supported by the finding that one or more iron sulfur (FeS) centers might participate in light-induced charge separation within the reaction center (Jennings & Evans, 1977; Swarthoff et al., 1981). More recently it has been actually shown that the reaction centers of *Chlorobiaceae* contain the same set of terminal electron acceptors as PSI, i.e. the FeS centers F_A, F_B, and F_X (Nitschke et al., 1990; Feiler et al., 1992; Miller et al., 1992; Kusumoto et al., 1992; Oh-oka et al., 1993). The orientations of both the primary donor and the three FeS centers with respect to the membrane were very similar to those in PSI (Nitschke et al., 1990). Furthermore, indirect evidence was reported for the existence of a quinone-type acceptor, analogous to A_1 in PSI (Nitschke et al., 1990).

Several different variants of (bacterio)chlorin pigments have been proposed to play the role of the primary electron acceptor (i.e. the analogous electron acceptor A_0 in PSI) in the *Chlorobium* reaction center, including bacteriophaeophytin (BPh) *c* (van Bochove et al., 1984; Nuijs et al., 1985a), a bacteriochlorophyll (BChl) *c*-like pigment (Braumann et al., 1986; Shuvalov et al., 1986a) as well as a chlorophyll *a* (Chl) isomer (van de Meent et al., 1992).

However, due to the relatively high amount of BChl*a* in chlorosome-depleted membranes (ca. 100 BChl*a*/P840) optical measurements on nonpurified systems are difficult to interpret and identification of pigments involved in charge separation is often ambiguous. Therefore, preparations of isolated, functional reaction centers were required. Several RC preparations with rather low BChl/P840 ratios have been published (Swarthoff & Amesz, 1979; Vasmel et al., 1983); these, however, show little or no reaction-center photoactivity (Vasmel et al., 1983). A reaction-center preparation of Hurt and Hauska (1984) was still capable of primary charge separation, but the chemical nature of the primary electron acceptor has not yet been studied in this preparation.

A novel preparation of the reaction-center complex from *Chlorobium limicola* f *thiosulphatophilum* containing the FeS centers F_A and F_B has recently been reported (Feiler et al., 1992). Among several BChl*a* pigments [20–30, some being bound to the remaining FMO light-harvesting protein at the end of the preparation (Feiler et al., 1992; Albouy, D., Feiler, U., Sturgis, J., & Robert, B., manuscript in preparation)], this preparation contains a pigment absorbing at 672 nm. This absorption maximum corresponds roughly to those of any of the three above-mentioned chlorin pigments proposed as primary electron acceptor in the *Chlorobium* reaction center. This preparation is devoid of chlorosome BChl*c* molecules and is thus suited to study the pigment absorbing

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[1] Abbreviations: (B)Chl, (bacterio)chlorophyll; (B)Ph, (bacterio)-phaeophytin; FeS centers F_A, F_B, F_X, iron–sulfur centers F_A, F_B, F_X; FT, Fourier transform; FWHM, full width at half-maximum; PSI/II, photosystem I/II; P840, primary electron donor in green sulfur bacterial reaction centers; RC, reaction center; RR, resonance Raman; PEF, 4-*n*-propyl-5-ethylfarnesyl; THF, tetrahydrofuran.

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at 672 nm using resonance Raman (RR) spectroscopy. This method has previously been successfully used to study (bacterio)chlorin pigments in various photosynthetic systems and yields direct information about the chemical nature and conformation of the respective pigments as well as their interactions with the local environment (Lutz & Robert, 1988). By using this method, both *in vivo* and on pigment extracts obtained from reaction centers, we were able to determine the chemical nature of the pigment giving rise to the 672-nm absorption band and to obtain information concerning the interaction of this pigment with the surrounding protein. Since the gene encoding the reaction-center proteins of *C. limicola* f *thiosulphatophilum* has recently been cloned and sequenced (Büttner et al., 1992), this sequence was used to discuss the binding properties of the primary electron acceptor in the reaction center of *Chlorobium* and to compare them with the situation in purple bacterial reaction centers and those of PSI.

EXPERIMENTAL PROCEDURES

C. limicola f *thiosulphatophilum*, strain tassajara, was obtained from Norbert Pfennig (Konstanz, Germany). The cells were grown under strictly anaerobic conditions in a medium described by Biebl and Pfennig (1978). After 5 days of growth, cells were harvested by low-speed centrifugation and were stored at −80 °C until use.

The reaction-center complex was purified as described by Feiler et al. (1992). An absorption spectrum of the isolated reaction-center complex is shown in Figure 1. For Raman experiments reaction-center preparations were concentrated to 1.2 mM BChl*a* with a Centricon system (Amicon).

Resonance Raman experiments were performed using a Jobin-Yvon spectrometer (Ramanor HG2S-UV). Excitation at 413.1 nm was provided by a krypton laser (Coherent Radiation Inc., Model Innova, 90). Excitation at 441.6 nm was provided by a helium–cadmium laser (Liconix, Model 4050). All RR measurements were carried out at 30 K using a liquid helium cryostat. For details of the recording procedure, see the work of Robert and Lutz (1986).

Fourier-transform (FT) Raman spectra were recorded with a Bruker IFS 66 interferometer coupled to a Bruker FRA 106 Raman module equipped with a continuous, diode-pumped Nd-YAG laser (Mattioli et al., 1991). All FT Raman spectra were recorded at room temperature with a resolution of 4 cm^{-1}.

Total pigment extracts were obtained by adding to ca. 200 μL of concentrated RC samples (OD_{815} = 50) 1 mL of a mixture of acetone and isooctane (3:1, v:v). Once the protein precipitate had settled, the organic phase containing the extracted pigments was transferred to a flask and vacuum dried. For the Raman experiments, the extracted pigments were redissolved in dry THF to ca. OD_{775} = 200. The whole procedure was performed in dim light, with little or no exposure of the extract to oxygen.

4-*n*-propyl-5-ethylfarnesyl(PEF)-BChl*c* (Olson & Pedersen, 1990) was a kind gift of John M. Olson (Odense, Denmark); Chl*a* was obtained from Jacques Kléo (Saclay, France).

RESULTS AND DISCUSSION

Figure 1 shows the room-temperature absorption spectrum of the isolated reaction-center complex from *C. limicola* f *thiosulphatophilum*. The arrows indicate both excitation wavelengths used for RR experiments. A detailed description of the pigments present in the RC preparation has been published in Feiler et al. (1992). The absence of a peak at 750 nm demonstrates the removal of chlorosomal BChl*c*. The

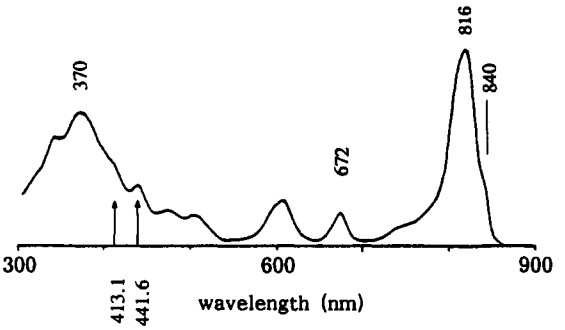

FIGURE 1: Room-temperature absorption spectrum of the reaction-center complex from *C. limicola* f *thiosulphatophilum*. Arrows indicate Raman excitation wavelengths used in this work.

absorption maximum at 816 nm corresponds to BChl*a*, presumably from antenna pigments mostly, and an additional shoulder at ca. 840 nm arises from the primary donor, P840. The Soret absorption band of BChl*a* (a bacteriochlorin pigment) shows a maximum at ca. 370 nm. The Soret transitions of chlorin pigments (i.e. BChl*c*, BPh*c*, Chl*a*, and Ph*a*) are in the range of 410–430 nm. To obtain selective resonance Raman information on chlorin molecules in this preparation, 441.6-nm excitation was chosen. This line is spectrally located on the red side of the chlorin Soret transition and far away (ca. 4000 cm^{-1} lower in energy) from the Soret electronic transition of BChl*a* molecules. Therefore, it is expected to result in a selective enhancement of chlorin contributions relative to contributions of BChl*a*.

In the 1600–1615-cm^{-1} range, RR spectra of (bacterio)-chlorin molecules exhibit a band arising from a stretching mode of the methine bridges. This band has been shown to be sensitive to the coordination state of the central Mg atoms in (bacterio)chlorophyll molecules (Cotton & van Duyne, 1981; Fujiwara & Tasumi, 1986). The 1660–1700-cm^{-1} range of such RR spectra contains bands arising from the C_9-keto carbonyl stretching mode, the frequency of which depends strongly on the interaction state of this group. It is observed at ca. 1700 cm^{-1}, when the keto carbonyl group is free from interaction, and as low as 1660 cm^{-1}, if it is engaged in an H-bonding interaction (Lutz, 1984). Thus, the study of the 1600–1700-cm^{-1} region yields information on the interactions assumed by both the central Mg ion and the keto carbonyl group of (bacterio)chlorophylls.

Resonance Raman Spectra of the Chlorin Pigments of Chlorobium RCs. Figure 2a illustrates the higher frequency region of the RR spectrum of the purified reaction-center complex from *C. limicola* f *thiosuphatophilum* recorded with 441.6-nm excitation. It contains a strong 1550-cm^{-1} band, which dominates this region. This band is characteristic of chlorin pigments under these conditions of excitation (Lutz, 1984). This spectrum also contains two additional bands at 1612 and 1666 cm^{-1}. Together with the 1550-cm^{-1} band, and considering both their frequencies and relative intensities, they constitute a clear fingerprint for a chlorin molecule (Lutz, 1984).

The 1612-cm^{-1} band arises from a methine bridge stretching mode while the 1666-cm^{-1} band must arise from the stretching mode of a conjugated carbonyl group. The fact that it is the only band present in the 1620–1710-cm^{-1} region of the spectrum indicates that the scattering chlorin molecule involves a single conjugated carbonyl group, which hence must be the C_9-keto carbonyl common to all chlorin pigments.

The frequency of this band at 1666 cm^{-1} shows that the C_9-keto groups of the scattering molecules are involved in intermolecular interactions. The narrow line width of this

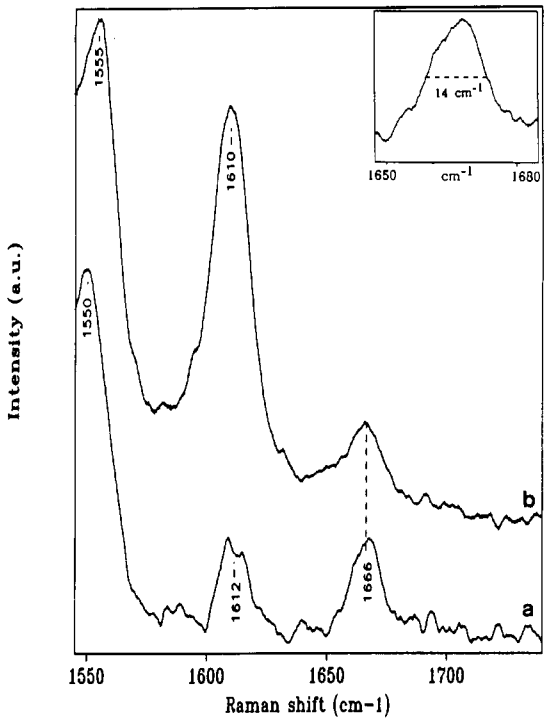

FIGURE 2: RR spectra (1540–1740-cm^{-1} region) of the isolated RC complex from *C. limicola* f *thiosulphatophilum*. Excitation at (a) 441.6 and (b) 413.1 nm. The insert shows the C_9-keto carbonyl band at 441.6-nm excitation. Experimental conditions: temperature, 30 K; laser power, 10 mW.

band (14 cm^{-1} FWHM, see insert of Figure 2) is exactly that of a keto carbonyl stretching mode which is not inhomogeneously broadened (Lutz, 1984). This indicates that the pigments contributing in the RR spectrum of *Chlorobium* RCs excited at 441.6 nm share the same interactions with their environments.

Conceivably, 441.6-nm excitation might have been selectively promoting the Raman scattering of a single population of chlorin pigments amid others which might have been present in the *Chlorobium* RC preparation. In order to check this, we also recorded RR spectra of the same sample, using 413.1-nm excitation (Figure 2b). This excitation is either located on the blue side of, or near, the maximum of the Soret bands of chlorin molecules and is only ca. 2800 cm^{-1} away from the maximum of the Soret of BChl*a*. Excitation (413.1 nm) of *Chlorobium* reaction centers is expected to result in a less selective enhancement of the chlorin molecules and should therefore contain sizeable BChl*a* contributions. However, studies on bacteriochlorins *in vitro* have shown that exciting BChl*a* in the 406–413-nm range resulted in very low activity of the carbonyl-stretching modes, most likely because the dominant contributions to the electronic absorption correspond to the B_X transition. The only observable active mode of BChl*a* in the 1600–1750-cm^{-1} range is the methine bridge stretching mode (Robert, B., unpublished data). The presence of this mode (see in Figure 2) results in the increase of the intensity of the 1610-cm^{-1} mode relative to the 1550-cm^{-1} mode (as already mentioned above, the latter is entirely due to chlorin molecules). The absence of any new band in the 1620–1710-cm^{-1} range (Figure 2b) shows that, indeed, no C=O stretching mode from BChl*a* molecules is observed. With 413.1-nm excitation, only one band in the RR spectrum of the reaction-center complex is observed in the 1660–1710-cm^{-1} range. The frequency and the line width of this band are both identical to what was observed with 441.6-nm excitation. It can therefore be concluded that 441.6-nm excitation results in an

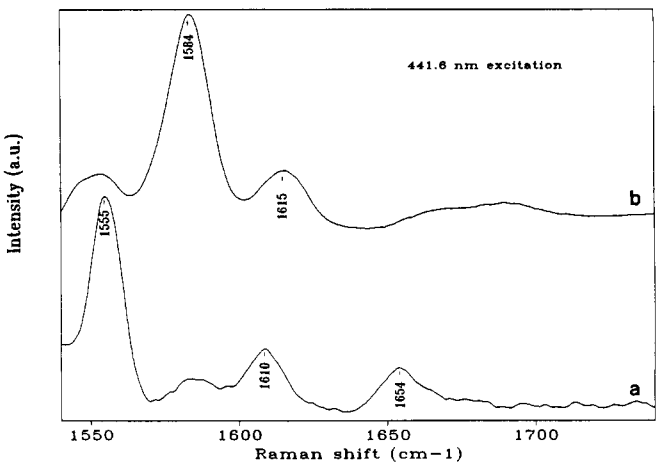

FIGURE 3: RR spectra (1540–1740-cm^{-1} region) of (a) PEF-BChl*c* in 2-propanol and (b) BPh*c* in CCl_4. Both spectra were recorded at 441.6-nm excitation. Experimental conditions: see Figure 2.

essentially selective enhancement of all the chlorin pigments present in *Chlorobium* reaction centers and that all of these molecules share identical interactions with their environmental protein at the level of their keto carbonyl groups.

Chemical Nature of the Chlorin Pigment. In order to obtain information on the chemical nature of the chlorin pigments contained in *Chlorobium* reaction centers, their RR spectra can be compared with those of isolated pigments. Figure 3 displays the RR spectra of BChl*c* in 2-propanol and BPh*c* in CCl_4 with 441.6-nm excitation. In the higher frequency region, BPh*c* spectra exhibit a methine bridge stretching mode, located at 1615 cm^{-1}, as well as an intense 1584-cm^{-1} band, which is characteristic of (bacterio)phaeophytins and is present in their RR spectra under most excitation conditions (Lutz, 1984; Mattioli et al., 1993). In BChl*c* spectra, which are very similar to those of Chl*a* (Lutz & van Brakel, 1988), the most intense band in this region is at 1555 cm^{-1}, while the methine bridge stretching mode occurs at 1610 cm^{-1}. The frequencies of both modes are sensitive to the coordination number of the central Mg ion in BChl*c* and indicate that in this experiment the Mg ion coordinates with one axial ligand only. In BChl*c* spectra, as well as in Chl*a* spectra, a weak band is present at 1584 cm^{-1}, but its intensity is very small relative to those at 1555 cm^{-1}. Comparing the RR spectra of *Chlorobium* reaction centers excited at 441.6 nm (Figure 2a) with those of *c*-type BChl and BPh pigments, respectively (Figure 3), it can be concluded that the *Chlorobium* reaction-center preparation contains no BPh*c*, as indicated by the absence of sizeable contribution at 1584 cm^{-1}.

However, at this stage of analysis it cannot be distinguished by RR spectroscopy whether *Chlorobium* reaction centers contain Chl*a* or BChl*c* since the structure of the chlorin-system macrocycle of both pigments, and therefore their resonance Raman spectra, are similar. However, Chl*a* has a vinyl group in position C_2 of ring I which is conjugated with the chlorin-system macrocycle and which is replaced in BChl*c* by a hydroxyethyl group. In a recent Raman study we attributed a band at ca. 1620 cm^{-1} to the stretching mode of the C=C bond of this group (Feiler et al., 1994). In agreement with this attribution, this band is not observed in the Raman spectra of BChl*c* excited at 1064 nm (Figure 4b), excitation conditions where it is clearly seen in Raman spectra of Chl*a* (Figure 4a). However, as discussed by Feiler et al. (1994), the intensity of this band is smaller in RR spectra excited at 441.6 nm, and it is difficult to observe *in vivo* as most of chlorophyll pigments are 5-coordinated. Under these condi-

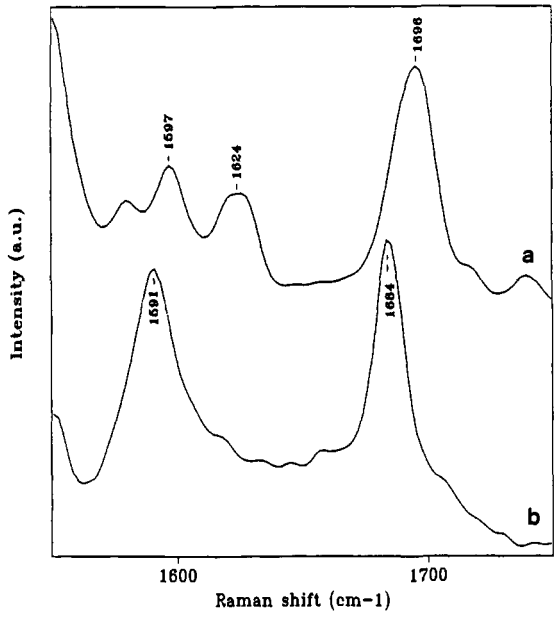

FIGURE 4: FT Raman spectra (1550–1750-cm^{-1} region) of (a) Chl*a* and (b) PEF-BChl*c* both in dry THF. THF contributions are substracted in both spectra. Experimental conditions: temperature, 290 K; excitation wavelength, 1064 nm; laser power, 180 mW.

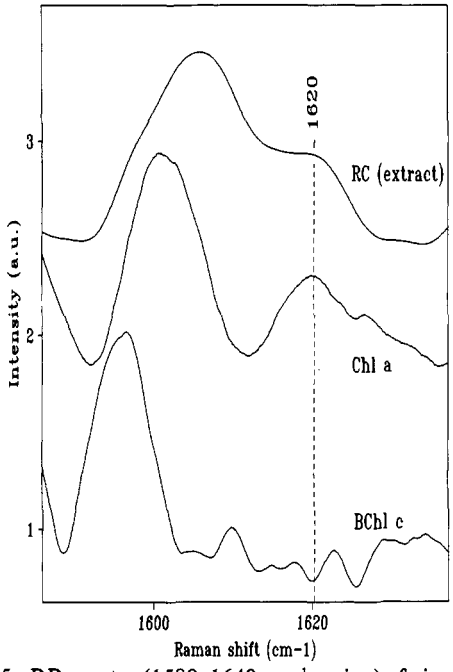

FIGURE 5: RR spectra (1580–1640-cm^{-1} region) of pigment extract from RC from *C. limicola* f *thiosulphatophilum* (top), Chl *a* in THF (middle), and BChl *c* in THF (bottom). Experimental conditions: see Figure 2.

tions, it just appears as a weak shoulder of the ca. 1615-cm^{-1} band arising from the methine bridge stretching modes. In *Chlorobium* reaction centers, we indeed observed that the 1612-cm^{-1} band was slightly asymmetric, thus likely revealing the presence of such a mode (data not shown). In order to observe this mode more clearly, Raman experiments were performed on pigment extracts from *Chlorobium* reaction centers (Figure 5). The goal of this experiment is to change the coordination number of the central Mg ion of the 672-nm absorbing pigment, so that the band arising from its methine stretching modes shifts down to ca. 1600 cm^{-1}. Under these conditions, the 1620-cm^{-1} mode arising from the vinyl stretching mode clearly appears as a band in Chl*a* RR spectra and is missing those of BChl*c*. Figure 5 displays RR spectra (441.6-nm excitation) of Chl*a* and BChl*c* in THF, i.e. conditions where their central Mg ion is expected to be 6-coordinated, as compared to RR spectra of a pigment extract from *Chlorobium* reaction centers. The methine bridge stretching mode, in the latter spectrum, is clearly complex, composed of a main component at 1605 cm^{-1}, with a shoulder at 1600 cm^{-1}. Both of these components indicate that this pigment is now hexacoordinated in the extract. However, presence of a small shoulder indicates some heterogeneity in the sample, which is not surprising in such a pigment extract; this heterogeneity is however limited, as inspection of the carbonyl stretching modes region shows that most of these modes contribute at 1684 cm^{-1}, i.e. that most of these chemical groups are free from intermolecular interactions (data not shown). This spectrum also contains a clear 1620-cm^{-1} shoulder, the intensity and position of which exactly match with the 1620-cm^{-1} mode observed in Chl*a* RR spectra at this excitation and which has been attributed to the vinyl stretching mode of this molecule (Feiler et al. 1994). As a control, the same extraction procedure was applied to chlorosome and BChl*a*-containing samples. Extracts from chlorosomal BChl*c* yielded spectra very similar to that of BChl*c* in Figure 5 (data not shown). When the extraction procedure was applied to BChl*a*-containing samples, no observable signal could be detected in that region under 441.6-nm excitation conditions but carotenoid contributions (data not shown). We thus conclude that this 1620-cm^{-1} band arises from the 672-nm-absorbing pigment in *Chlorobium* RCs, and its existence can be taken as a strong indication that the chlorin molecule present in *Chlorobium* RC does contain a vinyl group as substituent on ring I. Thus this pigment is a Chl*a*-type molecule.

Taking into account the optical kinetic data of Nuijs et al. (1985a) and Shuvalov et al., (1986a), both who observed a transient bleaching at ca. 670 nm due to the reduction of the primary acceptor, we propose that the Chl*a* pigment present in the *Chlorobium* RC is the so-called BChl-663 pigment which has been described, *in vitro*, by Braumann et al. (1986) and by van de Meent et al. (1992) and was proposed to play a role as the primary acceptor. Considering the data on the primary electron acceptor in heliobacteria (Nuijs et al., 1985b), which is also proposed to be a Chl*a*-like molecule (van de Meent et al., 1991), and on A_0 of PSI (Nuijs et al., 1986; Shuvalov et al., 1986b) it appears that, in all PSI-like reaction centers, the primary acceptor is a Chl*a*(-like) molecule.

Binding Properties of the Primary Acceptor of Chlorobium RCs. The RR spectrum of the primary acceptor Chl*a* of *Chlorobium* reaction centers presented in Figure 2a permits discussion of the interaction states of both its Mg atom and of its C_9-keto carbonyl. The frequencies of the 1555- and 1610-cm^{-1} bands are characteristic of a pentacoordinated state of the Mg ion, based on previous RR work on chlorophyll *a* and related molecules (Cotton & van Duyne, 1981; Fujiwara & Tasumi, 1986). The frequencies of both bands would be about 10 cm^{-1} lower for a hexacoordinated species.

As discussed above, the 1666-cm^{-1} frequency of the C_9-keto carbonyl indicates that this group is engaged in intermolecular bonding. This frequency is not low enough to account for the involvement of the keto group in bonding with the Mg ion of another chlorophyll-type molecule (Lutz, 1984). The keto group is thus most likely interacting with the RC protein through H-bonding. Table 1 shows that the stretching mode of this group is downshifted by 29 cm^{-1} from the frequency of the free vibrator in a weakly polar environment.

In Table 1 are also shown the frequencies of the keto carbonyl vibration modes of BPh*a* in the reaction center of *Rhodobacter*

Table 1: Frequencies of the Stretching Modes of C_9-Keto Carbonyl Groups of Primary Acceptors in Various RCs and *in Vitro*

Cofactor	free pigments in THF FT Raman data (cm^{-1})	Primary acceptors RR data (cm^{-1})	Δ (cm^{-1})
Chl*a*	1693[a]	1666[b]	29
Ph*a*	1705[c]	1680[d]	25
BPh*a*	1703[c]	1678[e]	25

[a] This work. [b] RC of *C. limicola* f *thiosulphatophilum*, this work. [c] From the work of Mattioli et al. (1993). [d] D1/D2 particles (Moënne-Loccoz et al. (1989). [e] RC of *Rhodobacter sphaeroides* (Lutz, 1980).

sphaeroides (Lutz, 1980; Robert, 1990), of Ph*a* in D1/D2 particles (Moënne-Loccoz et al., 1989), and both compared with that of Chl*a* in the reaction center of *C. limicola* f *thiosulphatophilum*. In all cases a shift in the position of the keto carbonyl band to lower wavenumbers from "free" to "protein bound", upon H-bonding, is observable.

In the X-ray crystal structure of the reaction center from *Rhodopseudomonas viridis* (Deisenhofer & Michel, 1989), the C_9-keto carbonyl of the primary acceptor BPha_L (H_L) is at H-bonding distance from the presumably protonated glutamic acid L104. No equivalent side chain is present for BPha_M (H_M), which is not involved in electron transfer. These data are in agreement with the RR data on the RC of *R. viridis* and *R. sphaeroides*. The frequency of the C_9-keto group of the acceptor H_L is observed at 1678 cm^{-1}, thus arising from a H-bonded keto carbonyl group, and that of H_M is at ca. 1700 cm^{-1}, arising from a free keto carbonyl group (Lutz, 1980; Zhou et al, 1989; Robert, 1990). RR studies on D1/D2 particles have shown that the H-bond between the acceptor Ph*a* and the protein in D1/D2 particles is similar to that assumed by the H_L in purple bacterial RC (Moënne-Loccoz et al., 1989). The downshifts assumed by the keto stretching modes of the primary acceptors of purple bacterial RCs and of PSII RCs are very close to that observed for the primary electron acceptor of *Chlorobium*. The estimated enthalpies of the H-bonds assumed by these various acceptors are similar and may be roughly calculated by an empirical Badger-type rule to be 10–16 kJ/mol (Zadorozhnyi & Ishchenko, 1965; Tonge & Carey, 1992). Hence, most likely the binding sites of the primary acceptors in these different types of reaction centers share common structural features which might either have been kept from a primitive photosynthetic ancestor or be essential for functionality of these reaction centers.

The gene for the reaction centers of *C. limicola* f *thiosulphatophilum* has recently been sequenced (Büttner et al., 1992). Only *one* gene coding for the RC core protein of *Chlorobium* has been determined. This is in contrast to PSI, where two distinguishable genes (psaA, psaB) code for two large, membrane proteins forming a heterodimeric RC core. The existence of only one gene thus strongly implies the presence of a homodimer in the *Chlorobium* RC core (Büttner et al., 1992). Our data are consistent with this proposal. We observed only one population of the Chl*a* pigment (the so-called BChl-663) in the RC preparation. This indicates that there is either only one pigment BChl-663 present or all BChl-663 binding sites are identical. In the case of a homodimer, each of the polypeptides might bind a BChl-663 molecule. This Raman study is the first *in vivo* characterization which supports the proposed homodimeric structure of the *Chlorobium* RC.

As already mentioned above, the primary acceptor, H_L, in the reaction center of *R. viridis* is bound by the protonated glutamic acid L104. The corresponding amino acid in spinach D1/D2 is also a protonated glutamic acid D131 (Michel & Deisenhofer, 1988). According to a hypothetical protein sequence alignment of Robert and Moënne-Loccoz (1990), the aspartic acid Psa A 414 was found to be a possible candidate for binding the primary acceptor in PSI. With inclusion of the corresponding sequence of *C. limicola* f *thiosulphatophilum* in this alignment, glutamine Psc A 397 could be a H-bonding partner for BChl-663.

Chl a as Primary Acceptor: Evolutionary Aspects. In this work we demonstrated that the 672-nm pigment present in the reaction-center preparation of *C. limicola* f *thiosulphatophilum* (Feiler et al., 1992) is a Chl*a*-like pigment. This result is in line with experiments reported by van de Meent et al. (1991, 1992). They proposed that a special chlorin molecule which is present in cells, membranes, and solubilized membrane fractions of green sulfur bacteria (van de Meent et al., 1992) as well as a 8-hydroxychlorophyll present in membranes and antenna–RC complexes of heliobacteria (van de Meent et al., 1991) are likely candidates for being the primary electron acceptors in their respective RCs. Heliobacteria belong to the Gram-positive bacteria which are phylogenetically quite distinct from *Chlorobiaceae* (Woese, 1987). However, in both families, the special pigments seemed to be more closely related to Chl*a* than to BChl*c* or BChl*g*, respectively. It is rather surprising that the phylogenetically distant species *Chlorobium* and *Heliobacterium* both have a chlorophyll as primary acceptor, a pigment which is very similar to the primary acceptor A_0 in PSI reaction centers, whereas all other BChl pigments in these bacterial reaction centers are chemically quite different and are distinguishable from pigments found in PSI from oxygenic organisms with respect to their optical and structural properties. However, this seems less surprising in view of the fact that in all three systems the terminal electron acceptors are low reduction potential FeS centers. To photoreduce the FeS centers, these organisms need to be capable of synthesizing chlorin pigments, which in general have lower reduction potentials than bacteriochlorin pigments and which would then be able to reduce these FeS centers. The fact that the primary acceptor in all PSI-type reaction centers is a chlorin molecule would also be consistent with Granick's hypothesis (Granick, 1957; Pierson & Olson, 1989), which postulates (on the basis of the complexity of the biosynthetic pathways of chlorophyll synthesis) that the photosynthetic ancestor of all photosynthetic organisms which exist today used chlorophyll instead of bacteriochlorophyll. The primary acceptor in *Chlorobium* and *Heliobacterium* would then be an ancestral molecule kept during evolution from a primitive photosynthetic organism.

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