Structure and Protein Binding Interactions of the Primary Donor of the *Chloroflexus aurantiacus* Reaction Center†

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ABSTRACT: Soret resonance, Q_X resonance, and Q_Y near-infrared Fourier transform (FT) (pre)resonance Raman spectroscopies were used to determine pigment−protein interactions of specific bacteriochlorin molecules in the reaction center from *Chloroflexus aurantiacus*. FT Raman spectroscopy, using 1064 nm excitation, was used to selectively obtain preresonance and resonance vibrational Raman spectra of the primary donor (P) of reaction centers (RCs) from *Chloroflexus aurantiacus* in the P° and $P^{\bullet+}$ states, respectively. The FT Raman spectrum of RCs in their neutral P (P°) state exhibits bands at 1605, 1632, 1648, and 1696 cm^{-1} which are attributable to P in its resting neutral state. Specifically, the latter three Raman bands can be assigned to the conjugated C_2 acetyl and C_9 keto carbonyl groups of the bacteriochlorophyll (BChl) molecules constituting P. The observation of at least three such bands is indicative of a non-monomeric nature of P, consistent with the proposal that it is a dimer of BChl molecules. The 1632 cm^{-1} band is consistent only with a hydrogen bonded BChl acetyl carbonyl, while the 1648 cm^{-1} band is assigned to a non-hydrogen bonded acetyl carbonyl. The 1696 cm^{-1} band is consistent only with a non-hydrogen bonded keto carbonyl group; from the unusually high intensity of this latter band compared to the others, we propose that the 1696 cm^{-1} band contains contributions from two keto carbonyl groups, both free of hydrogen bonds. From published protein sequence alignments of the L and M subunits of *Rhodobacter* (*Rb.*) *sphaeroides* and *Chloroflexus aurantiacus* we assign the 1632 cm^{-1} band as arising from the C_2 acetyl carbonyl of the analogous P_M constituent of P, which is hydrogen bonded to tyrosine M187 in the *Chloroflexus* RC, and propose a pigment−protein structural model for the primary donor of *Chloroflexus aurantiacus*. The FT Raman spectrum of RCs in the $P^{\bullet+}$ state indicates that one component of the 1696 cm^{-1} band has upshifted 21 cm^{-1} to 1717 cm^{-1}. Compared to *Rb. sphaeroides* which showed a 26 cm^{-1} upshift for the corresponding band, the 21 cm^{-1} upshift indicates that the + charge is more delocalized over the $P^{\bullet+}$ species of *Chloroflexus*; we estimate that *ca.* 65% of the + charge is localized on one of the two BChl molecules of the *Chloroflexus* primary donor as compared to *ca.* 80% for *Rb. sphaeroides*. The consequences of the proposed structure of the *Chloroflexus* primary donor in terms of its $P^\circ/P^{\bullet+}$ redox midpoint potential are discussed.

Chloroflexus (*Cf.*)[1] *aurantiacus* is a thermophilic, photosynthetic, gliding bacterium which has been placed in the earliest branches of the bacterial phylogenetic tree and is thus proposed to be a photosynthetic common ancestor of all bacteria (Blankenship, 1985, 1992; Nitschke *et al.*, 1996). Although *Cf. aurantiacus* is classified taxonomically as a phototrophic green bacterium (*Chloroflexaceae* family), it shares only partial features with the green sulfur bacteria (*Chlorobiaceae* family). Bacteriochlorophyll (BChl) *c* molecules constitute the bulk of chlorophylls in *Cf. aurantiacus*; these molecules are located and organized in chlorosomes which function solely as light harvesting complexes (Feick & Fuller, 1984). The presence of chlorosomes in both the filamentous and the green sulfur bacteria serves as the main criterium for such a taxonomical classification (Olson, 1980; Feick & Fuller, 1984; Gibson *et al.*, 1985). However, the reaction center (RC) from *Cf. aurantiacus* is most similar to those from purple bacteria (Pierson & Thornber, 1983; Shiozawa *et al.*, 1987 and references therein), since both are bacteriochlorophyll−donor−bacteriopheophytin−quinone acceptor systems (reviewed in Feick *et al.*, 1995). The structural and functional similarities of the *Cf. aurantiacus* RC with those of purple bacteria have been determined from spectroscopic measurements since no crystal structure has been obtained so far.

The three-dimensional X-ray crystallographic structures of RCs from *Rhodopseudomonas* (*Rps.*) *viridis* (Deisenhofer & Michel, 1989; Deisenhofer *et al.*, 1995) and *Rhodobacter* (*Rb.*) *sphaeroides* (Yeates *et al.*, 1988; El-Kabbani *et al.*, 1991; Chirino *et al.*, 1994; Ermler *et al.*, 1994) have revealed the specific spatial arrangements of the cofactors that mediate the electron transfer and the photoinduced stable charge

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[1] Abbreviations: BChl, bacteriochlorophyll; BPhe, bacteriopheophytin; P, primary electron donor; RC(s), reaction center(s); RR, resonance Raman; FT, Fourier transform, FWHM, full width at half-maximum; *Rb., Rhodobacter*; *Cf., Chloroflexus*; *Ro., Roseobacter*; *Rps., Rhodopseudomonas*; *Rvx., Rubrivivax; Rc. Rhodocyclus*; *Rs., Rhodospirillum*; Tris, tris(hydroxymethyl)aminomethane; $C_{12}E_9$, nonaethylene glycol mono-*n*-dodecyl ether.

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separation across the membrane. Specifically, both RCs are composed of three polypeptide subunits (the so-called L, M, and H subunits); the *Rps. viridis* RC, in addition, possesses a fourth tetraheme cytochrome subunit. The L and M subunits noncovalently bind several cofactors including a dimer of BChl molecules constituting the primary donor (P), two monomeric accessory bacteriochlorophyll *a* molecules ($BChl_L$ and $BChl_M$), two bacteriopheophytin molecules ($BPhe_L$ and $BPhe_M$), two quinones, a non-heme iron atom, and a carotenoid molecule. The bacteriochlorins and quinones are spatially distributed in pairs along a pseudo-C_2 symmetry axis which runs across the membrane, thus resulting in two nearly symmetric pigment branches associated with the L and M subunits; only the branch of pigments more closely associated with the L subunit appears to be used for light-driven transmembrane electron transfer. The globular domain of the H subunit makes contact with the cytoplasmic side of the L and M subunits.

The RC from *Cf. aurantiacus* has been well characterized by several biochemical and spectroscopic studies (reviewed in Feick *et al.*, 1995). From these studies, it appears that the general structures of both RCs from *Cf. aurantiacus* and *Rb. sphaeroides* appear to be similar, although the following differences are noteworthy: (i) *Cf. aurantiacus* possesses only two protein subunits with *ca.* 40% identity to the L and M subunits of *Rb. sphaeroides* and *Rps. viridis* (Shiozawa *et al.*, 1987), and it lacks the corresponding H polypeptide subunit which has been proposed to stabilize the complex assembly in the membrane of *Rb. sphaeroides* (Chory *et al.*, 1984); (ii) in the *Cf. aurantiacus* RC, a BPhe *a* molecule replaces one of the accessory (monomeric) BChl *a* molecules (Pierson & Thornber, 1983; Blankenship *et al.*, 1983) found in the purple bacterial RCs, most probably the one located on the M branch; (iii) no carotenoid is found in the isolated RCs from *Cf. aurantiacus* (Pierson *et al.*, 1983); (iv) the type of quinones (menaquinones, Hale *et al.*, 1983) and the transition metal ion (manganese) are different in *Cf. aurantiacus* RC; (v) *Cf. aurantiacus* possesses a RC associated tetraheme cytochrome, cyt c_{554}, (Freeman & Blankenship, 1990; van Vliet *et al.*, 1991), which is neither a tightly-bound tetraheme cytochrome, as in the case of *Rps. viridis*, nor a soluble monoheme cytochrome as for *Rb. sphaeroides*.

As in the case of purple bacteria, the primary electron donor from *Cf. aurantiacus* is proposed to be a dimer of BChl *a* molecules (Bruce *et al.*, 1982; Blankenship *et al.*, 1983) and its near-infrared absorption maximum is at 865 nm (Pierson & Thornber, 1983). While the $P^{\circ}/P^{\bullet+}$ redox midpoint potential for the primary donor in *Rb. sphaeroides* is about +500 mV (Prince & Dutton, 1978; Moss *et al.*, 1991; Williams *et al.*, 1992; Naragajan *et al.*, 1993; Jia *et al.*, 1994; Beekman *et al.*, 1995), in *Cf. aurantiacus* it is significantly lower (+360 mV; Bruce *et al.*, 1982) and, also, more than 100 mV; higher than that for P_{840} of *Chlorobium* (+240 mV; Fowler *et al.*, 1971; Prince & Olson, 1976). Picosecond spectroscopic studies on *Cf. aurantiacus* indicate that the analogous $BPhe_L$ is an electron acceptor prior to quinone reduction, as is the case of the purple bacterial RC, although the rate of electron transfer to this BPhe *a* molecule is 7 ps at 296 K (Becker *et al.*, 1991) as compared to the *ca.* 3 ps for the purple bacterial RCs (Woodbury *et al.*, 1985; Martin *et al.*, 1986; Holzapfel *et al.*, 1990). As well, the reduction of the first quinone (Q_A) occurs with a time constant of 300 ps (Kirmaier *et al.*, 1983, 1984, 1986; Becker *et al.*, 1991), in contrast to the 200 ps observed in *Rb. sphaeroides* (Kirmaier *et al.*, 1986).

Recently, Cherepy *et al.* (1995) reported the near-infrared Q_Y resonance Raman (RR) spectra of RCs from *Cf. aurantiacus* using shifted-excitation Raman difference spectroscopy (SERDS). The primary donor and the accessory BChl spectra obtained by this technique were compared to the corresponding spectra of *Rb. sphaeroides* RCs. Due to the similarities observed between the SERDS regenerated Raman spectra of both primary donors, Cherepy and co-workers concluded that the nuclear and electronic dynamics are similar for these two bacterial reaction centers.

Near-infrared Fourier transform (FT) Raman spectroscopy has been successfully applied for the characterization of pigment−protein interactions in the primary donor from *Rb. sphaeroides* RCs (Mattioli *et al.*, 1991a, 1994, 1995) as well as those from other bacterial RCs (Mattioli *et al.*, 1992a; Agalidis *et al.*, 1992; Feiler *et al.*, 1995). The 1064 nm excitation wavelength used in those FT Raman experiments provides a selective excitation of the primary donor which exhibits an absorption maximum at 860 nm in the resting (neutral) state and at 1250 nm once it is oxidized. Under these experimental conditions, *ca.* 70% of the intensity observed in the FT Raman spectrum of *Rb. sphaeroides* RCs arises from contributions of preresonantly enhanced neutral P or resonantly enhanced oxidized P (Mattioli *et al.*, 1991a). Consequently, the H-bond pattern of the conjugated carbonyl groups of the primary donor and its protein interactions could be reliably deduced (Mattioli *et al.*, 1994, 1995). Similar selectivity using 1064 nm excitation wavelength can be expected for the primary donor of *Cf. aurantiacus* in its neutral and oxidized states, since the electronic absorption maxima are quite similar to those of *Rb. sphaeroides*. In this work, we have applied near-infrared FT (pre)resonance Raman spectroscopy to obtain specific information on the structure of the *Cf. aurantiacus* primary electron donor as well as its interactions with the protein. Thus, the H-bonding pattern could be deduced for the primary donor from *Cf. aurantiacus*, and from the available primary structure of the RC polypeptide subunits (Ovchinnikov *et al.*, 1988a,b; Shiozawa *et al.*, 1989), possible candidates for the interactions can be proposed. The deduced structure from this analysis and the observed physicochemical properties of the primary donor, such as the $P^{\circ}/P^{\bullet+}$ redox midpoint potential or the delocalization of the unpair electron spin density, can be rationalized in analogy with previous results obtained for *Rb. sphaeroides* mutants where the H-bonding pattern to the primary donor was modified (Mattioli *et al.*, 1994, 1995).

EXPERIMENTAL PROCEDURES

Reaction centers from *Cf. aurantiacus* were prepared and purified as previously described (Shiozawa *et al.*, 1987); these RCs were in a final solution of 20 mM Tris buffer, pH 8, and 0.1% nonaethylene glycol mono-*n*-dodecyl ether ($C_{12}E_9$) detergent. The isolation of RCs from *Rb. sphaeroides* R26 and 2.4.1 was described elsewhere (Robert & Lutz, 1986), except final RCs were in 50 mM Tris-HCl, pH 8, and 0.1% cholate detergent.

Near-infrared Fourier transform (FT) Raman spectra were recorded using a Bruker IFS 66 interferometer coupled to a Bruker FRA 106 Raman module as described elsewhere (Mattioli *et al.*, 1991a). The samples were excited with 180

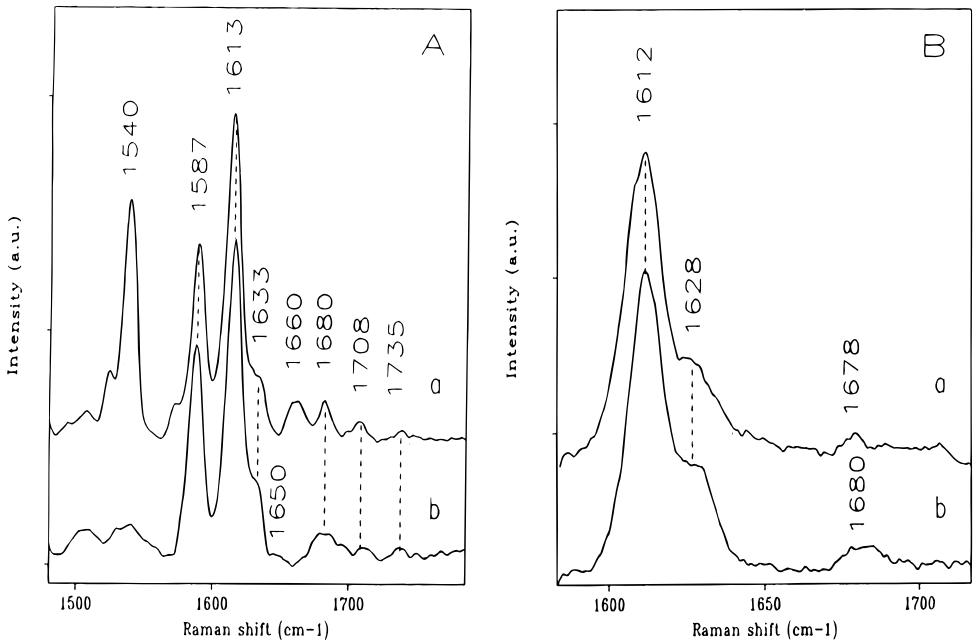

FIGURE 1: Low temperature (10 K) resonance Raman spectra of *Rb. sphaeroides* RCs (a) and *Cf. aurantiacus* RCs (b) excited at 363.8 nm (panel A) and at 530.9 nm (panel B).

and 200 mW of 1064 nm radiation for room and low temperature (10 K) measurements, respectively, which was provided by a diode-pumped Nd:YAG laser; the spectral resolution was 4 cm^{-1}. RC samples either were contained in a sapphire cell for the room temperature measurements or were immersed in a gas-flow cryostat (SMC-TBT, France) regulated by circulation of cold helium gas for the low temperature experiments (10 K). For these experiments, *Cf. aurantiacus* RCs were concentrated using a Centricon microconcentrator system (Amicon) to *ca.* 100 OD at 865 nm. In order to poise the RCs in their reduced (P°) or oxidized ($P^{\bullet +}$) states, sodium dithionite or potassium ferricyanide was used. To obtain FT Raman spectra of fully reduced P, it was necessary to use dithionite since, when poising the RC samples with sodium ascorbate, less than 10% of the RCs were still oxidized as observed by a modest 1−2 cm^{-1} downshift of the 1605 cm^{-1} band.

Near-infrared room temperature absorption spectra (800−1300 nm) were recorded with the same FT interferometer described above, operating in absorption mode (Wachtveitl *et al.*, 1993); 1 mL samples at a final concentration of *ca.* 0.2 OD at 812 nm were measured in a 1 cm quartz cuvette. Absorption spectra of P° were obtained from RC samples in the presence of sodium ascorbate, while those of $P^{\bullet +}$ were obtained with no additions and exploiting the actinic effect of the measuring beam as described elsewhere (Mattioli *et al.*, 1995).

Low temperature (10 K) resonance Raman spectra were excited using 363.8 nm radiation from an Ar^+ laser (Coherent Innova 100) or the 530.9 nm line of Kr^+ laser (Coherent Innova 90). The scattered light was collected using a 130° grazing incidence geometry and dispersed through a Jobin-Yvon U-1000 Raman spectrometer equipped with a charged-coupled device detector (Spectraview 2D, Jobin Yvon). Typically, less than 2 mW of power was used for the 363.8 nm excitation, and for the 530.9 nm excitation the power was less than 20 mW. For these experiments, the samples were concentrated to *ca.* 40 OD at 812 nm.

RESULTS AND DISCUSSION

Soret Resonance Raman Spectra of the Cf. aurantiacus RC. The high frequency Soret resonance Raman (RR) spectra of *Cf. aurantiacus* and *Rb. sphaeroides* RCs recorded at 10 K is shown in Figure 1 (panel A). The excitation wavelength used, 363.8 nm, provides a resonance condition under which all the bacteriochlorin pigments should contribute to the RR spectrum. For *Rb. sphaeroides* RCs, the assignments of the RR bands have been discussed elsewhere (Robert & Lutz, 1988; Robert, 1990). The 2.4.1 strain of *Rb. sphaeroides* used in this work contains a carotenoid molecule (sphaeroidene) bound to the RC, as can be observed from the band at 1540 cm^{-1} (Figure 1a, panel A) which corresponds to ν_1 stretching mode of the C═C bonds. In the spectrum of *Cf. aurantiacus* (Figure 1b, panel A), a Raman band in this spectral region is absent, reflecting the lack of carotenoid bound to its RC. Two intense bands in the spectra of both RCs are observed at 1587 and 1613 cm^{-1}. The former band was previously assigned as arising predominantly from the BPhe contributions under these resonance conditions, while the latter was attributed to C_aC_m stretching modes of both BChl *a* and BPhe *a* molecules (Robert & Lutz, 1986). Comparing the RR spectra for both *Cf. aurantiacus* and *Rb. sphaeroides* RCs, it is evident that the ratio of the intensities of 1587 cm^{-1} band to the 1613 cm^{-1} band is higher for *Cf. aurantiacus* (0.67 for *Cf. aurantiacus* and 0.58 for *Rb. sphaeroides*); assuming that the relative resonance enhancements for BPhe and BChl are similar for these two RCs, this ratio reflects the difference in pigment composition of the two RCs (Blankenship *et al.*, 1983). The 1613 cm^{-1} band indicates that the BChl molecules in the RCs are pentacoordinated with only one axial ligand each, for *Cf. aurantiacus* as they are for *Rb. sphaeroides* (Robert & Lutz, 1986).

Raman bands corresponding to the π-conjugated carbonyl group stretching modes of bacteriochlorin molecules are expected in the range 1620−1710 cm^{-1}. For the Soret RR spectra of *Rb. sphaeroides* RCs, the assignments of all the

active carbonyl vibrators of the BChl and BPhe molecules have been previously reported (Robert & Lutz, 1988; see review: Robert, 1990). Comparing the RR spectra of *Rb. sphaeroides* and *Cf. aurantiacus* in this spectral region, we note that, in general, they are similar, yet some significant differences are evident. The most obvious differences in the *Cf. aurantiacus* RC spectrum include (a) the lack of a 1660 cm^{-1} band, (b) the appearance of a weak 1650 cm^{-1} band, and (c) the band centered at 1680 cm^{-1} is broader.

The band at 1660 cm^{-1} in the Soret RR spectrum of *Rb. sphaeroides*, in principle, contains contributions from three different carbonyl groups: (i) the free acetyl carbonyl group of the P_M constituent of the primary donor, (ii) the free acetyl carbonyl group of the accessory $BChl_M$, and (iii) the free acetyl carbonyl group of the accessory $BChl_L$ (Robert, 1990).

(i) The Soret RR spectrum of *Cf. aurantiacus* excited at 363.8 nm does not contain significant contributions of the primary donor. This has been verified by recording RR spectra of RCs poised in their P^o (in the presence of ascorbate) or $P^{\bullet+}$ (in the presence of ferricyanide) states; these spectra were identical, indicating that P does not contribute to the RR spectrum under the experimental conditions used here (data not shown). This situation is comparable to that of the carotenoidless R26 strain of *Rb. sphaeroides* (Lutz & Robert, 1988). Since P is "silent" in the Soret RR spectrum of *Cf. aurantiacus* excited at 363.8 nm, then there should be no contributions from a free acetyl carbonyl (expected to vibrate between *ca.* 1650 and *ca.* 1660 cm^{-1}) of P. However, this alone cannot explain the complete lack of a 1660 cm^{-1} band in its RR spectrum.

(ii) The absence in *Cf. aurantiacus* of the analogous accessory $BChl_M$ found in *Rb. sphaeroides* should have a significant impact on the intensity of a possible 1660 cm^{-1} band in the *Cf. aurantiacus* RR spectrum. Since the analogous molecule is a BPhe and assuming that its acetyl carbonyl is free of H-bonding interactions, then we would expect, from *in vitro* BPhe *a* in the non-H-bonding solvent tetrahydrofuran (Mattioli *et al.*, 1991a), a contribution from this free acetyl carbonyl at *ca.* 1674 cm^{-1}. Indeed, in the *Cf. aurantiacus* RC RR spectrum, the band centered at 1680 cm^{-1} is significantly broader (*ca.* 20 cm^{-1} FWHM) as compared to the similar band in the *Rb. sphaeroides* spectrum (*ca.* 10 cm^{-1} FWHM); this broadening is most likely reflecting the contribution of the free acetyl carbonyl of the extra BPhe molecule in *Cf. aurantiacus*.

(iii) The complete absence of the 1660 cm^{-1} in the *Cf. aurantiacus* RR spectrum, after taking into account the above two points, suggests either (a) that the analogous accessory $BChl_L$ in *Cf. auranticus* is not "active" in this RR spectrum or (b) that the frequency of its acetyl carbonyl stretching mode is different from that of the analogous molecule in *Rb. sphaeroides*. Although point (a) cannot formally be excluded, the weak 1650 cm^{-1} band in the *Cf. aurantiacus* RR spectrum is a candidate for a free acetyl carbonyl arising from this accessory $BChl_L$ molecule. This would indicate that the environment of this BChl molecule is different from that in *Rb. sphaeroides*. The 1650 cm^{-1} band is not present in the *Cf. aurantiacus* Q_X RR spectrum of the BPhe molecules and therefore cannot arise from the BPhe molecules (see discussion below).

In summary, comparing the Soret-excited RR spectra of RCs from *Rb. sphaeroides* and of *Cf. aurantiacus*, the lack of the 1660 cm^{-1} in the *Cf. aurantiacus* spectrum is consistent with the presence of a BPhe molecule instead of the accessory $BChl_M$ molecule and that the acetyl carbonyl group of this latter molecule is not H-bonded as it is not for $BChl_M$ in *Rb. sphaeroides*. Furthermore, the acetyl carbonyl group of $BChl_L$ molecule in the *Cf. aurantiacus* RC probably vibrates at 1650 cm^{-1} and not at 1660 cm^{-1} as for the case of *Rb. sphaeroides*, reflecting different protein environments in each RC at the level of these carbonyl groups. The frequency 1650 cm^{-1} is too high to reflect an H-bond to this group.

Q_X Resonance Raman Spectra of the Bacteriopheophytin Molecules. To selectively obtain the contributions from the bacteriopheophytins over those from the other pigment molecules, an excitation of 530.9 nm, which is in resonance with the Q_X absorption band of the BPhe molecules, was used to record the spectra of Figure 1 (panel B). For *Rb. sphaeroides*, at cryogenic temperatures this Q_X absorption band splits and resolves into two components at *ca.* 535 and 545 nm arising from $BPhe_M$ and $BPhe_L$, respectively. For the case of *Rb. sphaeroides* RCs at 10 K, 530.9 nm excitation is known to enhance the Raman contributions of $BPhe_M$ preferentially over those of $BPhe_L$ (Lutz, 1980; Robert, 1990). This selectivity with 530.9 nm excitation is observed by the presence of a 1628 cm^{-1} band in the RR spectrum which arises from $BPhe_M$. However, this excitation wavelength does not completely exclude weaker Raman contribution of the $BPhe_L$ contributions (Lutz, 1980).

The Q_X BPhe absorption bands in *Cf. aurantiacus* are modestly resolved into two distinct bands (533 and 540 nm) at low temperature although the relative amplitudes of the bands appear to be *ca.* 2:1 in contrast to the *ca.* 1:1 ratio for *Rb. sphaeroides* (Vasmel *et al.*, 1983; Parot *et al*, 1985). For *Cf. aurantiacus*, the 533 nm component is relatively more intense than the 540 nm component. Assuming that the 540 nm component in the *Cf. aurantiacus* RC absorption spectrum corresponds to the 545 nm component in the *Rb. sphaeroides* absorption spectrum, then the extra intensity under the 533 nm component in *Cf. aurantiacus* could be reflecting the contributions of the extra BPhe molecule.

Figure 1 (panel B) shows a comparison of the 530.9 nm excited RR spectra of RCs from *Cf. aurantiacus* and *Rb. sphaeroides*. As stated above, this wavelength of excitation does not preferentially enhance the $BPhe_L$ molecule in *Rb. sphaeroides* and most likely not the analogous molecule in *Cf. aurantiacus*. Thus, definite conclusions concerning the specific binding interactions of the three BPhe molecules in the *Cf. aurantiacus* RC are not possible at present. However, some observations are noteworthy for the purposes of this paper. These two spectra are essentially identical except for the fact that the band in the *Cf. aurantiacus* spectrum centered at 1680 cm^{-1} is broader (20 cm^{-1} FWHM) than that in the *Rb. sphaeroides* spectrum (9 cm^{-1} FWHM). This broadening most likely reflects the contribution of the extra BPhe molecule in the *Cf. aurantiacus* RC; this observation and conclusion is consistent with the interpretation of the same observed broadening of the 1680 cm^{-1} band seen in the Soret RR spectrum (Figure 1, panel A).

Near-IR FT Raman Spectra of the Cf. aurantiacus RCs in their Reduced P° State: The 600−1600 cm^{-1} Region. The low temperature FT Raman spectra of reduced RC from *Cf. aurantiacus* (B) and *Rb. sphaeroides* (A) excited at 1064 nm are shown in Figure 2. These spectra are from samples held in a cryostat and thus exhibit no Raman contributions

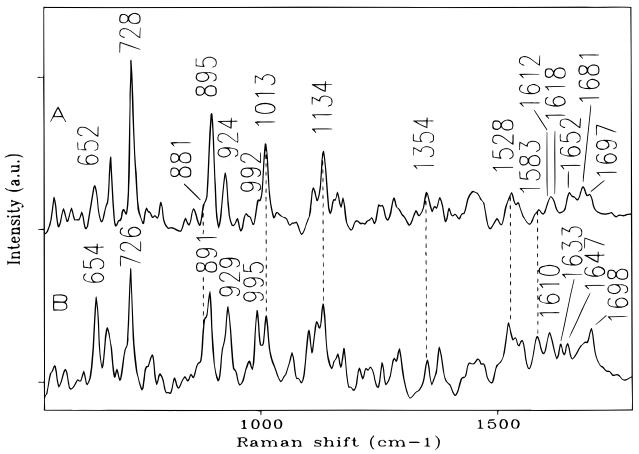

FIGURE 2: Low temperature (10 K) Fourier transform preresonance Raman spectra of *Rb. sphaeroides* RCs (A) and *Cf. aurantiacus* RCs (B) in the presence of ascorbate. Base line subtraction was done with a polynomial fit. Experimental conditions: coaddition of 6000 interferrograms, 1064 nm excitation, 200 mW laser power, spectral resolution of 4 cm^{-1}. The 1610 cm^{-1} band arises from the C_aC_m stretching mode which has upshifted in frequency upon cooling (Mattioli *et al.*, 1992b); this mode is seen to vibrate at 1605 cm^{-1} at room temperature.

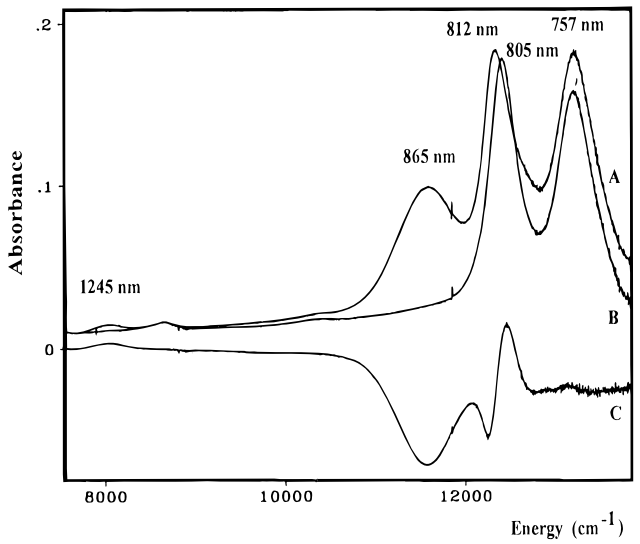

FIGURE 3: Room temperature near-infrared absorption spectra of reduced (A) and oxidized (B) *Cf. aurantiacus* RCs. The computed difference oxidized-minus-reduced spectrum is also shown (C). The band at *ca.* 1150 nm in both reduced and oxidized spectra does not arise from the primary donor since it clearly cancels out in the difference spetrum (C). Spectral resolution is 2 cm^{-1}.

at *ca.* 650 cm^{-1} from the sapphire cell normally used in the room temperature measurements which masks the characteristic BPhe band at *ca.* 645 cm^{-1} (Mattioli *et al.*, 1991a).

In the FT Raman spectra of Figure 2, the relative intensity of the BPhe *a* band at 654 cm^{-1} to that of BChl *a* at 726 cm^{-1} is greater in the case of *Cf. aurantiacus* reaction centers (Figure 2B) as compared to that of *Rb. sphaeroides* (Figure 2A). The same observation is valid for other BPhe bands vibrating at 929 and 995 cm^{-1} as compared to those of BChl *a* vibrating at 891 and 1013 cm^{-1}, respectively. The observed small 2−3 cm^{-1} shifts of most of the above-mentioned bands from *Cf. aurantiacus* RCs as compared to those from *Rb. sphaeroides* could be indicative of environmental or small conformational differences in the bacteriochlorins between both reaction centers. Finally, the 1583 cm^{-1} band observed in the low temperature FT Raman spectrum of *Cf. aurantiacus*, which is also due to BPhe contributions (Mattioli *et al.*, 1991a), is relatively more intense than the corresponding band in the FT Raman spectrum of *Rb. sphaeroides*; although this band has not been explicitly assigned to a particular BPhe mode, it most probably arises from a macrocyclic ring CC mode possessing C_aC_m and/or C_bC_b character (Lutz, 1984; Donohoe *et al.*, 1988; Hu *et al.*, 1993). In summary, these observations in the 600−1600 cm^{-1} region of the FT Raman spectra are all consistent with the higher BPhe to BChl pigment stoichiometry in *Cf. aurantiacus* RC (Blankenship *et al.*, 1983) and could indicate the possibility to observe relatively more intense BPhe contributions in the carbonyl stretching frequency region of the FT Raman spectrum of *Cf. aurantiacus* RCs as compared to that of *Rb. sphaeroides* RCs.

Near-IR FT Raman Spectra of the Reduced and Oxidized Primary Donor: The Carbonyl Stretching Region. It has been previously discussed that FT Raman spectra of reaction centers possessing dimeric BChl *a* primary donors, when excited with 1064 nm radiation, exhibit a selective enhancement of the (pre)resonance spectrum of their reduced and oxidized P (Mattioli *et al.*, 1991a). As seen in Figure 3, the reduced primary donor from *Cf. aurantiacus* exhibits an

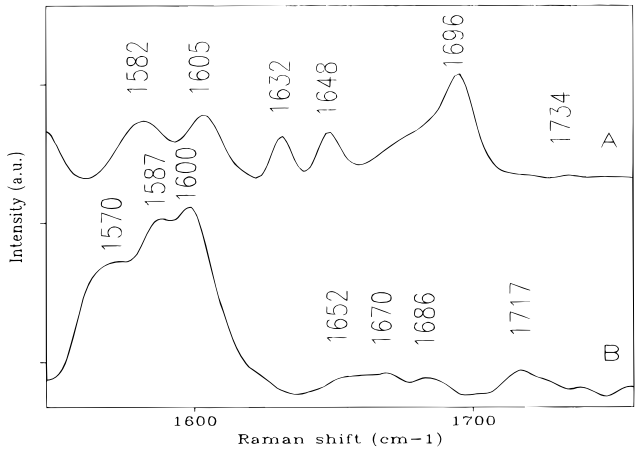

FIGURE 4: Room temperature FT resonance Raman spectra, in the high-frequency region, of *Cf. aurantiacus* RCs chemically reduced (A) and oxidized (B) with sodium dithionite and potasium ferricyanide, respectively. Only the carbonyl region of the spectra is shown. Same experimental conditions as in Figure 2.

optical absorption maximum at 865 nm (Pierson & Thornber, 1983) similar to that of *Rb. sphaeroides*. Moreover, in the oxidized spectrum of *Cf. aurantiacus* RCs this band bleaches while a new band with absorption maximum at 1245 nm (1248 nm for *Rb. sphaeroides* RCs, Wachtveitl *et al.*, 1993) appears. Thus, it is reasonable to expect that the resonance enhancement conditions of P° and $P^{\bullet+}$ in *Cf. aurantiacus* RCs, excited with 1064 nm radiation, would be similar to those of *Rb. sphaeroides* RCs (Mattioli *et al.*, 1991a), i.e., with predominant Raman bands attributable to its reduced and oxidized primary donor.

The room temperature high-frequency FT Raman spectrum of reduced *Cf. aurantiacus* RCs is shown in Figure 4A; it exhibits the following evident bands: 1605, 1632, 1648, and 1696 cm^{-1}, the latter being relatively intense and with a shoulder at *ca.* 1680 cm^{-1}. This shoulder is both weak and broad compared to the other above-mentioned bands, does not appear to bleach upon $P^{\bullet+}$ formation, and thus does not appear to be a candidate for a band corresponding to P (see

below). Finally, there is a weak band at 1734 cm^{-1}. The 1582 cm^{-1} band arises primarily from BPhe contributions (see above).

In the FT Raman spectrum of the oxidized RCs from *Cf. aurantiacus* at room temperature (Figure 4B), the bands at 1632, 1648, and 1696 cm^{-1} clearly disappeared upon $P^{\bullet+}$ formation; thus they were enhanced preresonantly via the 865 nm electronic absorption band (Figure 3) which bleaches upon oxidation; these bands are assigned to P°. Inspection of Figure 4B reveals two obvious new Raman bands at 1600 and 1717 cm^{-1} which appear upon $P^{\bullet+}$ formation; these Raman bands are assigned to $P^{\bullet+}$. The other weaker bands at 1652, 1670, and 1686 cm^{-1} cannot be unambiguously ascribed to $P^{\bullet+}$ since, in principle, they could have been masked underneath the more intense bands of P°. The bands at 1670 and 1686 cm^{-1} appear to correspond to the broad shoulder observed in the FT Raman spectrum for reduced RCs (Figure 4A) and thus were not bleached upon $P^{\bullet+}$ formation. Interestingly, these bands, which are now resolved, appear to correspond to the broad unresolved band centered at 1680 cm^{-1} in the BPhe Q_X RR spectrum (Figure 1, panel B). In the BPhe Q_X resonance Raman spectrum of *Cf. aurantiacus* RCs (Figure 1, panel B), there are significant BPhe *a* contributions in the carbonyl region at *ca.* 1680 cm^{-1}; it was also ascertained that there are relatively more intense BPhe contributions in the 1064 nm excited low temperature FT Raman spectrum of *Cf. aurantiacus* as compared to that of *Rb. sphaeroides* (Figure 2). Thus, we could expect weak contributions from BPhe *a* to be present in the carbonyl region of the FT Raman spectrum (Figure 4A); these BPhe contributions could appear as the shoulder in the low frequency side of the 1696 cm^{-1} band.

The Primary Donor. Of the above-mentioned Raman bands which arise from P°, the 1632, 1648, and 1696 cm^{-1} bands are attributable to C_2 and C_9 π-conjugated carbonyl groups (Lutz, 1984; Mattioli *et al.*, 1991a). The number of these bands (three) is noteworthy and indicates that are at least three conjugated carbonyls belonging to the primary donor. Since a BChl *a* molecule possesses one C_2 acetyl and one C_9 keto carbonyl, giving a total of two conjugated carbonyls, the observation of three conjugated carbonyl Raman bands indicates that more than one BChl *a* molecule is being preresonantly enhanced via the 865 nm transition of P°. This leads to the conclusion that more than one BChl *a* molecule constitutes P of *Cf. aurantiacus*. The simplest model to reconcile this result is, of course, that the number of BChl *a* molecules enhanced via the 865 nm transition is two, thus leading to the proposal that the primary donor of *Cf. aurantiacus* is a dimer of BChl *a* molecules. This proposal has been widely accepted (Bruce *et al.*, 1983; Blankenship *et al.*, 1983) and finds further strong experimental evidence here to support it.

In Figure 4A, the 1605 cm^{-1} band can be attributed to the C_aC_m methine bridge stretching modes of the two BChl *a* molecules which constitute the primary donor; the observed frequency of this sole and narrow band (16 cm^{-1} FWHM) is indicative of one axial ligand to each BChl molecule (Mattioli *et al.*, 1991a). The 1607 cm^{-1} observed in the FT Raman spectrum of *Rb. sphaeroides* RCs led to similar conclusions (Mattioli *et al.*, 1991a). The X-ray crystal structures of *Rb. sphaeroides* (Ermler *et al.*, 1994) and *Rps. viridis* (Deisenhofer & Michel, 1989; Deisenhofer *et al.*, 1995) revealed that these axial ligands were His L173 and

Table 1: Amino Acid Sequence Alignment of the L and M Subunits in the Vicinity of P for the RCs from *Cf. aurantiacus* and from Other Purple Bacteria

Ro.denitrificans[a]		Y	L	H	F	**H**	Y	N	P	A	**H**	
Rvx. gelatinosus[b]		Y	L	H	F	**H**	Y	N	P	A	**H**	
Rs. rubrum[c]		Y	A	N	F	**H**	Y	N	P	A	**H**	
Rb. capsulatus[d]		Y	G	N	F	**H**	Y	N	P	F	**H**	
Rps.viridis[e]	L164	Y	L	N	W	**H**	Y	N	P	G	**H**	L173
Rb. sphaeroides[f]	L164	Y	G	N	F	**H**	Y	N	P	A	**H**	L173
Cf. aurantiacus[g]	L203	Y	N	N	F	**F**	Y	N	P	F	**H**	L212
Cf. aurantiacus[g]	M183	Y	G	N	F	**Y**	Y	N	P	F	**H**	M192
Rb. sphaeroides[h]	M193	H	G	N	L	**F**	Y	N	P	F	**H**	M202
Rps. viridis[e]	M191	Y	G	N	F	**Y**	Y	C	P	W	**H**	M200
Rb. capsulatus[d]		H	G	N	L	**F**	Y	N	P	F	**H**	
Rs. rubrum[c]		Y	G	N	F	**F**	Y	N	P	F	**H**	
Rvx. gelatinosus[i]		Y	G	N	L	**F**	Y	N	P	F	**H**	
Ro denitrificans[a]		Y	G	N	L	**Y**	Y	N	P	F	**H**	

Note: The amino acid residues designated in underlined boldface correspond to the position of His L173 and His M202 in *Rb. sphaeroides*. Those designated in **boldface** correspond to the position of His L168 and Phe M197 in *Rb. sphaeroides* which are related to the H-bonded P_L C_2 acetyl carbonyl and the non-H-bonded PM C2 acetyl carbonyl groups, respectively. [a] From Liebetanz *et al.* (1991). [b] From Nagashima *et al.* (1994). [c] From Bélanger *et al.* (1988). [d] From Youvan *et al.* (1984). [e] From Michel *et al.* (1986). [f] From Williams *et al.* (1984). [g] From Shiozawa *et al.* (1989). [h] From Williams *et al.* (1983). [i] From Nagashima *et al.* (1993).

M202 (M200 in *Rps. viridis*) for P_L and P_M, respectively. The proposed sequence alignment for both L and M subunits of *Cf. aurantiacus* RC with those of known purple bacteria (Table 1) shows that the His L173 and M202 of *Rb. sphaeroides* are strongly conserved in other species and that His L212 and His M192 are most likely the axial ligands to the two BChl molecules constituting P of *Cf. aurantiacus*. The 2 cm^{-1} downshift of the C_aC_m stretching mode in *Cf. aurantiacus* compared to the that of *Rb. sphaeroides*, *Rb. capsulatus*, *Rsp. rubrum*, and *Rvx. gelatinosus*, which all exhibit a 1607 cm^{-1} C_aC_m band (Mattioli *et al.*, 1992a; Agalidis *et al.*, 1992), might be due to a difference in conformation and planarity of one or both BChl *a* molecules constituting the primary donor (Mattioli *et al.*, 1992b; Ivancich, Lutz, and Mattioli, unpublished results), or could reflect also differences in distances of the histidine axial ligands to the central Mg atoms.

From *in vitro* studies on BChl *a* in solution (Lutz, 1984), it has been shown that the C_2 acetyl carbonyl group may exhibit vibrational frequencies ranging from *ca*. 1660 cm^{-1}, if it is free from protein interactions, down to 1620 cm^{-1} if engaged in an H-bond; the magnitude of the downshift depends on the strength of the H-bond. Similarly, the C_9 keto carbonyl can vibrate from *ca.* 1700 cm^{-1} down to *ca.* 1650 cm^{-1}. By these considerations, the 1632 cm^{-1} band in the *Cf. aurantiacus* P° spectrum (Figure 4A) can be unambiguously assigned to a C_2 acetyl carbonyl engaged in an H-bond. As well, the band at 1696 cm^{-1} can only arise from a C_9 keto carbonyl free of H-bond interactions. In the case of the 1648 cm^{-1} band, it could be due to either a free C_2 acetyl carbonyl or a very strongly H-bonded C_9 keto carbonyl group. Considering that P is a dimer (see above), a band corresponding to the second C_2 acetyl carbonyl group should be identified in the P spectrum; only the 1648 cm^{-1} band satisfies this requirement. Having already assigned the three bands of the spectrum, the second keto carbonyl group should be under the intense 1696 cm^{-1} band. Due to the

Table 2: Assignments of the Observed Carbonyl Stretching Frequencies (cm^{-1}) and H-Bond Donating Amino Acid Residues for P and $P^{\bullet+}$ from *Rb. sphaeroides* and *Cf. aurantiacus*

	$P_L C_2$	H-bond donor	$P_M C_2$	H-bond donor	$P_M C_9$	H-bond donor	$P_L C_9$	H-bond donor	$P_L^{\bullet+} C_9$
Rb. sphaeroides									
WT[a]	1620	His	1653	none	1679	none	1691	none	1715
HF(L168)[a]	**1653**	**none**	1653	none	1679	none	1688	none	1710
FH(M197)[a]	1622	His	**1630**	**His**	1679	none	1691	none	1717
FY(M197)[b]	1620	His	**1636**	**Tyr**	1679	none	1691	none	1720
Cf. aurantiacus[c]	1648	none	1632	Tyr	1696	none	1696	none	1717[d]

[a] Data from Mattioli et al. (1994). [b] Data from Wachtveitl et al. (1993). [c] This work. [d] Tentative assignment to oxidized C_9 keto carbonyl from P_L.

narrow bandwidth of the 1696 cm^{-1} band, and its relatively high intensity, we propose that both C_9 keto carbonyls vibrate with very similar frequencies (1696 cm^{-1}).

From this analysis, we obtain an H-bonding pattern for the primary donor from the *Cf. aurantiacus* RC which is similar to that from *Rb. sphaeroides*, although with the following subtle differences: (i) the H-bond on a C_2 acetyl carbonyl (vibrating at 1632 cm^{-1}) of the *Cf. aurantiacus* primary donor is weaker than that found on the P_L C_2 acetyl carbonyl (vibrating at 1620 cm^{-1}) of *Rb. sphaeroides* (Mattioli *et al.*, 1991a); (ii) the C_9 keto carbonyl groups clearly exhibit two distinct bands in the case of *Rb. sphaeroides* (1679 and 1691 cm^{-1}), but only one band (1696 cm^{-1}) in the case of *Cf. aurantiacus*.

Table 2 lists the carbonyl frequencies of the primary donor from *Rb. sphaeroides* as observed from the near-infrared FT Raman experiments; also, the assignments of these bands with their corresponding H-bond donors are shown (Mattioli *et al.*, 1994; Wachtveitl *et al.*, 1993). Table 1 shows that the histidine residue at position L168 in *Rb. sphaeroides* (which is H-bonded to the P_L acetyl carbonyl group) is conserved in all the known species except in *Cf. aurantiacus*; the latter has a phenylalanine at this position, a residue which cannot serve as an H-bond donor. The symmetry related amino acid residue on the M side of the RC from *Rb. sphaeroides* is a phenylalanine (Phe M197) which is not conserved in *Cf. aurantiacus*, *Rps. viridis*, and *Ro. denitrificans;* instead, a tyrosine residue is at this position. Tyrosine M195 forms an H-bond to the C_2 acetyl carbonyl of P_M in the RC from *Rps. viridis* (Deisenhofer & Michel, 1989; Deisenhofer *et al.*, 1995). FT Raman spectra of a *Rb. sphaeroides* RC mutant in which Phe M197 was genetically replaced by a tyrosine residue (Wachtveitl *et al.*, 1993) also indicated that such a tyrosine forms an H-bond to the P_M acetyl carbonyl.

Considering the above-mentioned differences in the amino acid sequences between *Rb. sphaeroides* and *Cf. aurantiacus*, it is possible to make specific assignments of the carbonyl frequencies of the FT Raman spectra of *Cf. aurantiacus* primary donor. Then, we expect the C_2 acetyl carbonyl band of the analogous P_L constituent of P from *Cf. aurantiacus* to vibrate at a higher frequency than that from *Rb. sphaeroides* (1620 cm^{-1}). This frequency shift is due to the fact that a Phe residue at the corresponding position of His L168 of *Rb. sphaeroides* results in a free P_L acetyl carbonyl. Thus, the observed 1648 cm^{-1} band in Figure 4A can be unambiguously attributed to the free C_2 acetyl carbonyl of P_L. A similar change in the FT Raman spectrum of P° was observed for the HF(L168) mutant of *Rb. sphaeroides* RC (Mattioli *et al.*, 1993); a *ca.* 30 cm^{-1} upshift of the C_2 acetyl carbonyl band of P_L was observed in the FT Raman spectrum of the RC mutant for which the His L168 was genetically replaced by a phenylalanine (see Table 2).

Similarly, it is reasonable to expect that the C_2 acetyl carbonyl band of P_M (which vibrates at 1653 cm^{-1} for the *Rb. sphaeroides* RC) has downshifted in the P° FT Raman spectrum of the *Cf. aurantiacus* RC; the tyrosine residue, in the equivalent position of Phe M197 of *Rb. sphaeroides*, is a good candidate for an H-bond donor to such an acetyl carbonyl group. Assuming a structural analogy with the RCs of *Rb. sphaeroides* and *Rps. viridis*, the 1632 cm^{-1} band should correspond to the C_2 acetyl carbonyl of the analogous P_M constituent in *Cf. aurantiacus* primary donor, which is H-bonded to the above-mentioned tyrosine residue. A comparable frequency of the acetyl carbonyl group was observed in the FT Raman spectra of P° for the mutant FY(M197) of *Rb. sphaeroides* RC (Wachtveitl *et al.*, 1993) where Phe L197 was genetically replaced by a tyrosine residue (see Table 2).

Finally, a summary of our assignments of the C_2 and C_9 keto carbonyls of the primary donor of *Cf. aurantiacus* and a comparison with those of *Rb. sphaeroides* are shown in Table 2. From our proposed structure, the primary donor of *Cf. aurantiacus* RC possesses also only one H-bonded C_2 acetyl carbonyl as does *Rb. sphaeroides* but the donor is a tyrosine instead of a histidine. In addition, the H-bond is with the acetyl carbonyl of P_M and not on P_L as in the case of *Rb. sphaeroides*. A scheme of this model is shown in Figure 5.

The Oxidized Primary Donor. Figure 4B shows the room temperature spectrum of the oxidized RCs from *Cf. aurantiacus*. Bands which are unambiguously attributable to $P^{\bullet+}$ are those at 1600 and 1717 cm^{-1}. The 1600 cm^{-1} band is similar to that of the *Rb. sphaeroides* $P^{\bullet+}$ FT Raman spectrum, attributed to the C_aC_m stretching mode of $P^{\bullet+}$ which had downshifted upon oxidation (Mattioli *et al.*, 1991a). The other new band at 1717 is also attributable to $P^{\bullet+}$, more specifically, to a keto carbonyl group for which the vibrational frequency has shifted to higher frequency upon oxidation (Mattioli *et al.*, 1991a).

The observed upshift of the C_9 keto carbonyl group in the FT Raman spectrum of P upon oxidation provides an estimation of the degree of delocalization of the + charge over P in the cation radical state (Mattioli *et al.*, 1991a). In *Rb. sphaeroides*, the C_9 keto carbonyl vibrating at 1691 cm^{-1} for neutral P shifts to 1717 cm^{-1} upon oxidation. This 26 cm^{-1} upshift, as compared to the 32 cm^{-1} upshift of the keto carbonyl group for (*in vitro*) monomeric BChl a^+ (Mantele *et al.*, 1988; Heald & Cotton, 1990), was interpreted as a *ca.* 80% localization of the + charge on P_L for *Rb.*

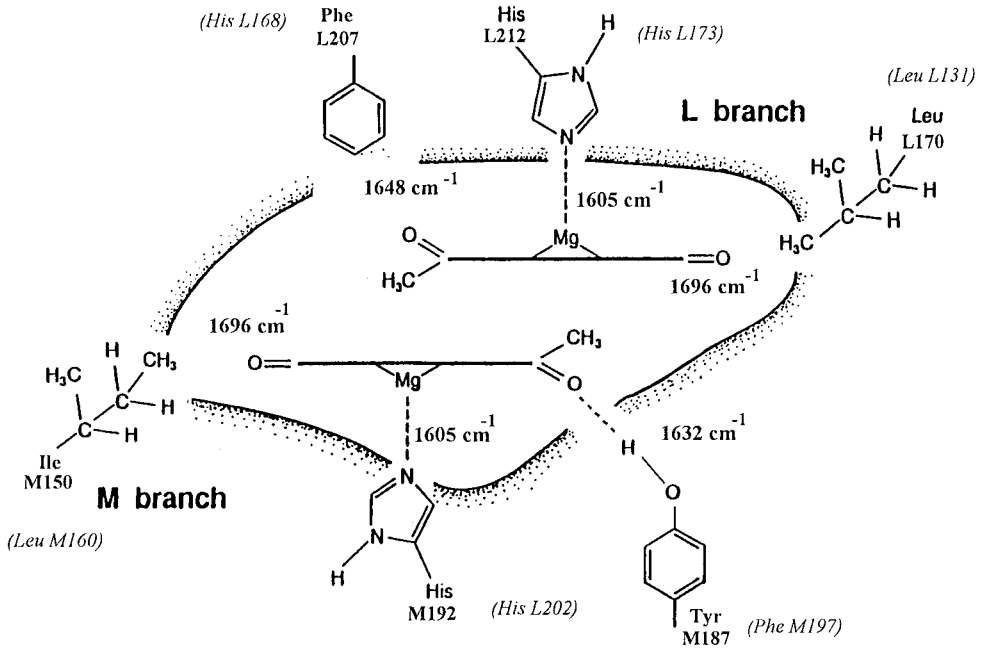

FIGURE 5: Schematic diagram showing the pigment−protein interactions of the proposed structural model for the primary donor microenviroment of the *Cf. aurantiacus* RC, and the assignments of the acetyl and keto carbonyl groups as deduced by FT Raman spectroscopy, the proposed protein sequence alignment (Shiozawa *et al.*, 1989), and assuming structural analogy with the primary donor of *Rb. sphaeroides*. Also shown are possible neighboring residues for the primary donor of *Cf. aurantiacus*, and those in *italics* correspond to those of *Rb. sphaeroides* RC (see Mattioli *et al.*, 1994).

sphaeroides RCs (Mattioli *et al.*, 1991a). In the case of *Cf. aurantiacus* RCs, upon oxidation a 21 cm^{-1} upshift of a keto carbonyl group in the primary donor FT Raman spectrum is observed, and consequently, a 65% localization on one of the BChl *a* molecules of the primary donor is estimated. This indicates that the + charge on $P^{\bullet+}$ of *Cf. aurantiacus* is more delocalized over both BChl molecules, i.e., more symmetric than that of *Rb. sphaeroides*. The degree of localization for *Cf. aurantiacus* does not appear to change at low temperature (10 K) (data not shown).

An interesting functional difference between *Cf. aurantiacus* and *Rb. sphaeroides* is the initial electron transfer step from P to BPhe, which has been reported to be 7 ps for *Cf. aurantiacus* in contrast to the 3 ps reported for *Rb. sphaeroides* (for a full discussion, see Feick *et al.*, 1995). Despite the observed significant difference in the localization of the + charge over $P^{\bullet+}$ between *Cf. aurantiacus* and *Rb. sphaeroides* RCs (see above), this is probably not a dominant factor which influences the slower initial electron transfer from P to $BPhe_L$ (Wachtveitl *et al.*, 1993) in *Cf. aurantiacus* RCs. This slower electron transfer rate might be largely due to the presence of a leucine residue, at the analogous position of tyrosine M210 of *Rb. sphaeroides* (Shiozawa *et al.*, 1989). Indeed, for *Rb. sphaeroides* mutants in which the Tyr M210 was replaced by a leucine residue, the ET rate significantly changed from 3.5 to 22 ps (Gray *et al.*, 1990; Finkele *et al.*, 1990; Nagarajan *et al.*, 1990). This mutation does not alter any H-bonds on the primary donor (Mattioli *et al.*, 1991b), nor did it affect the + charge localization over $P^{\bullet+}$ (Jones *et al.*, 1994).

H-Bond Effect on the $P^{\circ}/P^{\bullet+}$ Redox Midpoint Potential. A further difference that distinguishes the *Cf. aurantiacus* RC from the *Rb. sphaeroides* RC is its redox properties. For *Cf. aurantiacus*, the $P^{\circ}/P^{\bullet+}$ redox midpoint potential is +360 mV in cytoplasmic membranes (Bruce *et al.*, 1982), and +386 mV (Shuvalov *et al.*, 1986) or +420 mV (Venturoli & Zannoni, 1988) in isolated RCs. In contrast, the following values have been reported for *Rb. sphaeroides*: +440 mV for chromatophores (Prince & Dutton, 1978) and *ca.* +500 mV for isolated RCs (Moss *et al.*, 1991; Williams *et al.*, 1992).

Changes in the $P^{\circ}/P^{\bullet+}$ redox midpoint potential of the primary donor from *Rb. sphaeroides* due to the addition or removal of H-bonds donated by histidine residues have recently been reported for RC mutants of *Rb. sphaeroides* (Lin *et al.*, 1994). These changes in the redox properties were shown to correlate with the number and strength of the H-bonds on the conjugated carbonyls of P (Mattioli *et al.*, 1995). It is noteworthy that the removal of the H-bond on the P_L C_2 acetyl carbonyl, donated by His L168, lowered the redox midpoint potential of P from *Rb. sphaeroides* RC by 95 mV as compared to wild type, while the addition of an H-bond to the P_M acetyl carbonyl, donated by a histidine residue introduced at position M197, raised this midpoint potential by 125 mV (Mattioli *et al.*, 1995). Thus, for the case of mutant RCs of *Rb. sphaeroides*, the addition or removal of H-bonds, donated by histidinyl residues, at the acetyl carbonyl groups of P can modify the $P^{\circ}/P^{\bullet+}$ midpoint potential by ca. 100 mV.

Considering the structural model of the microenvironment of the primary donor of the *Cf. aurantiacus* RC proposed in Figure 5 and assuming that the above-mentioned genetic alterations for the *Rb. sphaeroides* RC would have comparable effects on the $P^{\circ}/P^{\bullet+}$ redox potential in *Cf. aurantiacus*, we would expect that the absence of the H-bond on P_L (donated by His L168 in *Rb. sphaeroides* RCs) is the dominant factor responsible for the significantly lower $P^{\circ}/P^{\bullet+}$ redox potential of *Cf. aurantiacus*. Indeed, the difference in $P^{\circ}/P^{\bullet+}$ redox potential between these two species is 80 mV (taking the chromatophore values) or 80−114 mV (taking isolated RC values, see above), thus being very similar to the difference between the wild type and the

HF(L168) mutant RCs from *Rb. sphaeroides*, i.e., 95 mV. Consequently, in a simplified scheme it seems that only the replacement of one key amino acid (the His at position L168 in *Rb. sphaeroides*) would be enough to modulate the $P^{\circ}/P^{\bullet+}$ redox properties of the primary donor from *Cf. aurantiacus* so that it matches the other cofactors redox properties, i.e., the lower midpoint potentials for the Q_A menaquinone (Bruce *et al.*, 1982; Venturoli & Zannoni, 1988) and the tetraheme cytochrome (+280 mV for the highest potential heme) (Freeman & Blankenship, 1990; van Vliet *et al.*, 1991) as compared to *Rb. sphaeroides* which has a higher potential ubiquinone for Q_A and a higher potential monoheme cyt c_2 (+345 mV; Boyer *et al.*, 1981; Meinhardt & Crofts, 1982) which rereduces $P^{\bullet+}$.

However, the above picture may be oversimplified in light of other relevant *Rb. sphaeroides* mutant RCs. For example, an RC mutant in which a second H-bond was introduced on the primary donor, in particular on the P_M C_2 acetyl carbonyl group via a tyrosine residue (FY(M197)), raised the $P^{\circ}/P^{\bullet+}$ redox potential by only *ca.* 25 mV (Wachtveitl *et al.*, 1993), indicating that the chemical nature of the H-bond donor is also an important factor influencing the redox properties of P. As well, in another *Rb. sphaeroides* RC mutant (YI(M210)) where Tyr M210 was replaced by isoleucine, a residue similar to the leucine residue found at the homologous M210 position in *Cf. aurantiacus* resulted in a 33 mV increase in the $P^{\circ}/P^{\bullet+}$ midpoint potential (Nagarajan *et al.*, 1993). Although the effects of the midpoint potential of these two individual mutations are relatively small as compared to those of adding H-bonds to the acetyl carbonyl groups of P via histidinyl residues, it may be argued that their combined effect (i.e., raising the $P^{\circ}/P^{\bullet+}$ midpoint potential by *ca.* 60 mV) in the RC of *Cf. aurantiacus* could act in off-setting the influence of the absence of the homologous His L168 residue. The fact that the $P^{\circ}/P^{\bullet+}$ midpoint potential of *Cf. aurantiacus* is, in fact, near to 100 mV lower than that of *Rb. sphaeroides* indicates that indeed the absence of the H-bond donated by His L168 is a dominant factor, and that the other effects raising the midpoint potential may be somehow attenuated, or that other unidentified protein factors are acting to further lower the midpoint potential. Clearly, a systematic, comparative study of other native RCs is required to properly answer this question.

CONCLUSION

The primary donor of the reaction center from *Cf. aurantiacus* has been characterized by near-infrared FT (pre)resonance Raman spectroscopy; in particular, the H-bond pattern for the conjugated carbonyl groups and the protein interactions have been determined. In our proposed model, the primary donor of the *Cf. aurantiacus* RC is a BChl *a* dimer with one axial ligand to each BChl molecule, donated by His L212 and His M192; only the C_2 acetyl carbonyl group from P_M is engaged in an H-bond to Tyr M187, in contrast to *Rb. sphaeroides* which has the sole H-bond on the C_2 acetyl carbonyl from P_L and it is donated by a histidine (L168) residue. Taking into account the effects observed in mutant RCs from *Rb. sphaeroides* where the H-bond donated by histidine L168 was removed and where a new H-bond was formed by replacing phenylanine M197 with a tyrosine residue, the proposed structure at the level of the H-bond pattern is consistent with *Cf. aurantiacus* RC having a $P^{\circ}/P^{\bullet+}$ redox midpoint potential *ca.* 100 mV lower than those from *Rb. sphaeroides*. We estimate that the + charge localization in $P^{\bullet+}$ is 65%, favoring one of the two BChl *a* molecules constituting the primary donor.

The results of this work indicate that the $P^{\circ}/P^{\bullet+}$ redox modulations induced by specific amino acid residue interactions present in *Rb. sphaeroides* mutants are also effective in other native RCs, at least in the case of *Cf. aurantiacus*. Other species are currently under investigation. Assuming structural analogies exist, the use of FT Raman spectroscopy for deducing the microenvironmental structure of the primary donor via the analysis of its H-bonding pattern, in combination with known protein sequence alignments of other RCs, suggests that is a useful predictive tool for species with unknown crystal structure.

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