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Review

Resonance Raman studies of bacterial reaction centers

Bruno Robert

Service de Biophysique, Département de Biologie, CEN Saclay, Gif-sur-Yvette (France)

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Contents

I. Introduction

Resonance Raman (RR) spectroscopy currently provides detailed information about the structures and conformations of several types of prosthetic groups of proteins, as well as on their molecular interactions with the host protein. In particular, since 1972, when Lutz obtained the first RR spectra of isolated chlorophyll *a* [1], this method has been extensively used for studying (bacterio)chlorophyll–protein interactions in the various complexes involved in the first steps of the photosynthetic process (reviews, [2–4]). The aim of this review is to describe briefly the studies on bacterial reaction centers performed with this technique.

I-A. Bacterial reaction center

Bacterial reaction centers (RCs) are medium-weight membrane proteins in which the transduction of the energy of light into chemical potential energy occurs. Reaction centers of purple bacteria generally consist of three polypeptides, namely L, M and H [5], the primary sequences of which are known for four different bacterial

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Abbreviations: (B)Chl, (bacterio)chlorophyll; (B)Pheo, (bacterio)-pheophytin; *Chr., Chromatium*; PS I, Photosystem I; PS II, Photosystem II; RC, reaction center; *Rb., Rhodobacter; Rps., Rhodopseudomonas;* RR, resonance Raman; *Rsp., Rhodospirillum.*

Correspondence: B. Robert, Service de Biophysique, Département de Biologie, CEN Saclay, 91191 Gif-sur-Yvette Cedex, France.

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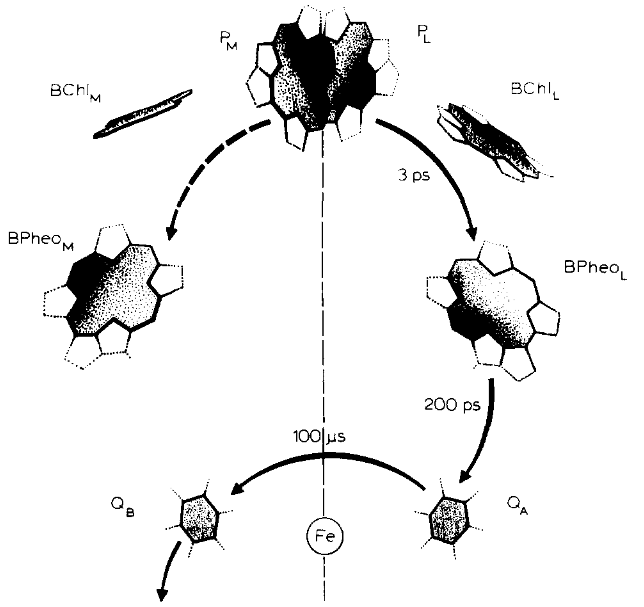


Fig. 1. Relative positions of the prosthetic groups and lifetimes of the primary steps of the electron steps in reaction centers from the *Rb. sphaeroides* bacterium. P, primary electron donor; Q_A, Q_B, quinones; Fe: non-heme iron; arrows: primary steps of electron transfer (lifetime of each step is indicated).

species [6–10] (five, considering the L and M subunits of the RCs of the green bacteria *Chloroflexus aurantiacus* [11,12]). Typically, RCs contain four bacteriochlorophylls (BChl), two bacteriopheophytins (BPheo), two quinones (Q), one (Q_A) of which is more tightly bound to the protein than the other (Q_B), a non-heme iron, and a carotenoid molecule (reviews, [13,14]) (Fig. 1). The excitation of bacterial RC with a photon or singlet exciton triggers the creation of an excited state of a supramolecular structure comprised of two of the four BChls, namely the primary electron donor (P) [15]. The relaxation of this state, which occurs within approx. 3 ps seems to be concomitant with the creation of an electron-hole pair, P^+BChl^- [16]. Only one of the two BChl monomers present in the RC acts as an electron acceptor ($BChl_L$) [16], whilst the role of the second one ($BChl_M$) is limited [17]. From this $BChl_L$ molecule, the electron first jumps to one of the BPheo molecules bound to the protein ($BPheo_L$) in 900 fs [16]. An alternative model, consisting of a single-step transfer of the electron from P to $BPheo_L$ has also been proposed [17a,b,c]. From the $Bpheo_L$ anion so-created the electron is then transferred to Q_A in 240 ps, and, in normal conditions of functioning, from Q_A to Q_B in 100 μs [18]. The most striking property of these protein-pigment complexes is the efficiency with which these electron transfers reactions occur. The yield of formation of the $P^+Q_B^-$ state per incident photon is near 100%. On the other hand, if the electron transfer is blocked between Q_A and Q_B (e.g., at low temperature) or between $BPheo_L$ and Q_A (when RCs are poised in low redox potential conditions), the back-reactions, leading to RC de-excitation, are at least a factor of 10^4 slower than the forward reactions: indeed, the $P^+Q_A^-$ charge recombination lasts approx. 100 ms at room temperature, and the P^+BPheo^- charge recombination, which leads to the formation of a triplet state of P (P^R) occurs within 10 ns [18].

Photochemically active RC from *Rps. viridis* and *Rb. sphaeroides* have been crystallized [19–22] and studied by X-ray diffraction [23–33]. These studies revealed another striking property of the bacterial RCs: a local-two-fold symmetry axis relates the two monomers constituting the special pair, the two accessory BChl molecules, the two BPheo and the two quinones [23] (Fig. 1). RCs thus contain two apparently equivalent branches, only one of which is active for the electron transfer. Similarly, the folding of the L and M polypeptide is similar, each involving five membrane-spanning helices related by the two-fold symmetry [24]. The fact that only one of the two branches appears to be photochemically active for transferring electrons has been the subject of particularly intense investigation. In order to explain this phenomenon, many hypotheses have been put forward, involving local breakages of the overall C_2 symmetry of the RCs at the level of the primary sequence [25], or slight deviations from the symmetry of the relative topology of the pigments that would induce differences in the pigment–pigment electronic couplings between each of the two branches.

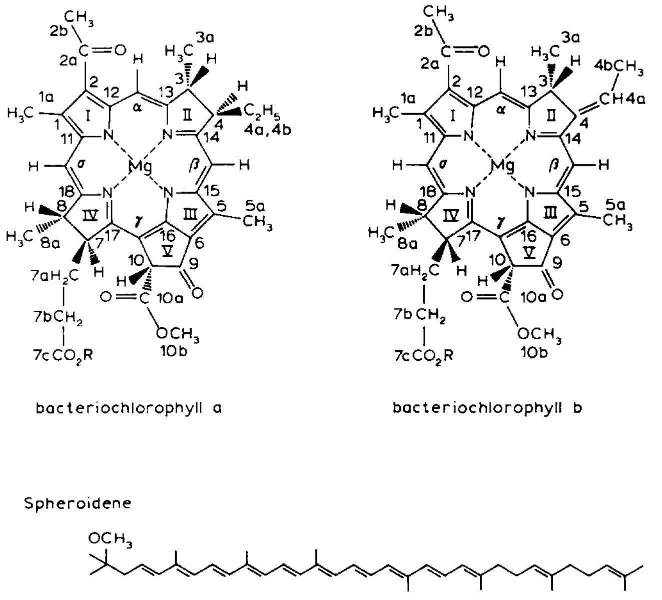
As a vibrational spectroscopic method, RR spectroscopy yields information about ground or lower electronic states of molecules with a submolecular resolution: it thus constitutes a powerful tool for studying the interactions assumed by the different prosthetic groups within the bacterial RCs. From the conclusions drawn from RR spectroscopy, some key problems of the bacterial RCs can be discussed, such as the structure of the primary electron donor [34], the degree of symmetry of the strength of protein–pigment interactions relative to the C_2 axis of the protein [35,36], and also how these interactions change during the first steps of the electron transfer [36].

I-B. Principle of resonance Raman spectroscopy

When polyatomic molecules are irradiated by a monochromatic light, the light scattered by these molecules contains not only the frequency of the incident light (Rayleigh scatter) but also a series of frequency shifts characteristic of some of the vibrational sublevels of the ground-state electronic level of the molecules: this phenomenon constitutes the Raman effect. Practically, for large molecules such as chlorophylls, some of these modes may be considered as arising from particular chemical groupings (such as, for example, $C=O$ groups), which are perturbed by the presence of the other atoms of the molecule. The resolution of Raman spectroscopy is, in this case, submolecular. When the exciting laser beam is tuned to a frequency matching an electronic transition of the irradiated molecule, an enhancement of the Raman effect is observed, that may reach a factor of 10^6: this is the resonance phenomenon. This resonance effect allows the selective observation of resonance Raman spectra of chromophores in media as complex as proteins or even whole membranes or cells [37]. On the other hand, the vibrational modes active in resonance Raman are those coupled with the resonant electronic transition. In particular, for what concerns chlorin derivatives, the vibrational modes observed in RR spectroscopy mainly involve the in-plane modes of the bonds conjugated with the delocalized pi electronic system of the dihydrophorbin ring [2,38].

I-C. Resonance Raman spectroscopy of bacteriochlorins

The aim of this short section is to review very briefly the information obtained by RR spectroscopy concerning bacteriochlorin pigments which has been used in biological studies, particularly in studies about bacterial RCs. Indeed, this topic has recently been the subject of comprehensive reviews [2,3]. In vitro studies have shown that the higher frequency regions (1550–1750 cm^{-1}) of resonance Raman spectra of BChl *a* and *b* and BPheo *a* and *b* excited in their respective Soret transitions are dominated by a 1610 cm^{-1} band, which partly arises from the methine stretching mode [38] (Fig. 2). The frequency of this mode has been shown to be sensitive to the number of axial ligands coordinated to the central magnesium atom of the BChl molecules, being at 1600 cm^{-1} when this atom binds two axial ligands and at 1615 cm^{-1} when it binds only one [39]. In the same high frequency spectral region one also observes bands that arise from the stretching modes of the conjugated, 9-keto and 2-acetyl carbonyl groups of BChl and BPheo [2,38] (Fig. 2). For BChl *a*, these modes occur near 1700 and 1660 cm^{-1}, respectively, when these groups are free from any intermolecular interactions [38]. In the *b*-type bacteriochlorins, the frequency of the stretching mode of the free-from-interaction 2-acetyl C = O occurs at 1668 cm^{-1}, while that of the 9-keto appears to be unchanged [40,41]. The frequencies of these modes shift down according to the strength of the interaction in which the carbonyl groups are engaged; typically, the frequency of the 2-acetyl carbonyl modes is observed in the 1620–1665 cm^{-1} range, whereas that of the keto groups may be observed between 1660 and 1710 cm^{-1} [2,38].


bacteriochlorophyll a

bacteriochlorophyll b

Spheroidene

Fig. 2. Molecular formulae of pigments present in bacterial RC's: BChl *a* (top left) and BChl *b* (top right), spheroidene (bottom).

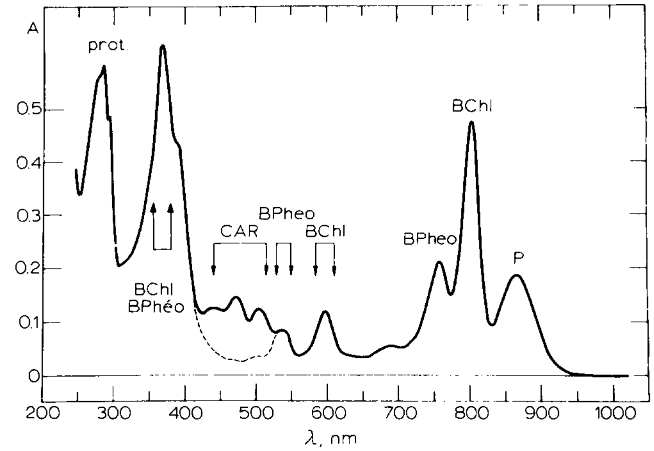


Fig. 3. Electronic absorption spectrum of reaction centers from *Rb. sphaeroides*. Pigments responsible for the different transitions are indicated. Dotted line: carotenoidless RC's.

Both the central Mg and the conjugated carbonyl groups of bacteriochlorin pigments have been shown to play a dominant role in the intermolecular associations in which these molecules are engaged, in vitro as well as in vivo [42]. Moreover, since the 2-acetyl and 9-keto C = O are conjugated with the dihydrophorbin ring of the BChl, their interaction state is expected to play a more important role in running the physicochemical properties of these molecules than the other, non-conjugated, carbonyl groups of the molecule, such as, for example, the ester groups [43]. These conjugated carbonyl modes have thus been extensively studied, in order to determine the interactions of BChls with their host protein.

I-D. Resonance Raman of bacterial reaction centers

An electronic absorption spectrum of the BChl-*a*-containing bacterium *Rb. sphaeroides* is shown in Fig.

3. Selective excitation of the RR spectra of BChls and BPheos within these complexes results when the laser radiation is tuned with the Q_x or Q_y transitions of these pigments. Excitation in the Q_y transitions encounters several difficulties, because of the intrinsic fluorescence of the RCs in this spectral region. Moreover, no RR results have yet been reported on isolated bacteriochlorophyll of bacteriophaeophytin in these resonance conditions. However, by using 755 nm excitation, Bocian et al. obtained RR spectra of the bacteriochlorin pigments bound to RCs [44] (see below). RR experiments conducted with resonance in the 590–610 nm range (i.e., in the Q_x electronic transition of the BChls) have been reported by Lutz et al. [45], but the 9-keto and 2-acetyl $C=O$ stretching modes are inactive under these resonance conditions. Thus, the information that can be extracted from these spectra remains limited. By contrast, these same carbonyl modes appear to be weakly active in the RR spectra of BPheo excited in their Q_x absorption band and experiments conducted on bacterial RCs with resonance excitation in the 530–550 nm range permitted the description of the BPheo–protein interactions as early as in 1980 [35]. On the other hand, selective and intense RR contributions of the carotenoid bound to RCs are easily achieved by using excitations in the 400–514 nm range [45,46]. It thus appears that the only way of obtaining the maximal information concerning the $C=O$ interactions of the BChl bound to bacterial RCs via RR spectroscopy is to perform experiments in Soret resonance. This is one of the reasons why most of the work on bacterial RCs has been conducted with near-ultraviolet excitation. The other major reason is that better quality spectra are obtained under these conditions, because of the strong resonance enhancements and the comparatively small contributions of spurious fluorescence due to impurities (such as oxidation products of BChl) present in the biological preparations.

I-E. Difference resonance Raman of bacterial reaction centers

The drawback in exciting RR spectra of bacterial RC at 363.8 nm, near the maximum of the Soret absorption band, is the loss of the selectivity between the different bacteriochlorin pigments bound to the RCs. In order to alleviate this difficulty, a difference technique for selectively observing the RR spectral contributions of the BChls constituting the primary electron donor (P) was introduced [47]. The basis of this method is that the laser beam used in exciting RR spectra has an actinic effect on the RC preparation, since its wavelength matches an allowed electronic transition of the RC pigments. By varying on the illumination level due to the Raman probing laser, it was shown that transient states, the lifetime of which might be as short as 4 μs [48], could be sizeable populated in bacterial RCs. It was thus possible, by adjusting the irradiance levels on the RC samples during the RR experiments, to build up steady-state population involving different amounts of the 100 ms P^+Q^- and/or of the 100 μs P^R states [48,49,34]. When resonance is ensured with the Soret transition of the neutral pigments, the contributions to the RR spectra of these cation and triplet states are smaller than those of the neutral pigments. This phenomenon is due in part to the smaller extinction coefficients of both of these species in this range [50–52], and also because many porphyrin radicals are weaker scatterers than the corresponding neutral species (see, for example, Refs. 53–55). Hence, differences between RR spectra obtained at low and high irradiance levels arise from the primary donor in its neutral, ground state [34]. Moreover, it has been observed that the primary donor contributes only poorly to the RR spectra obtained with a 363.8 nm excitation of the R 26 carotenoidless mutant of *Rb. sphaeroides* [36]. This allowed Robert and Lutz to observe and study interactions assumed by the accessory BChls and by the BPheos during the first steps of the electron transfer (see below) [36], which would otherwise have been masked by the P contributions in the difference spectrum.

II. Resonance Raman spectroscopy of carotenoids in bacterial reaction centers

Although carotenoids are not essential for the primary electron-transfer reactions which occur in bacterial reaction centers, all reaction centers isolated from wild-type species contain one, specifically bound carotenoid molecule (review, [56]). Carotenoid molecules have been shown to perform two major functions in bacterial RCs: light-harvesting and photoprotection (the role of these molecules in photosynthetic bacteria has been recently reviewed in Ref. 57). Photoprotection is ensured both through the quenching of singlet oxygen and through the direct quenching of chlorophyll triplet states before they can interact with molecular oxygen, thereby preventing single oxygen production [58]. This triplet–triplet transfer has been observed to occur in bacterial reaction centers between the primary electron donor and the bound carotenoid, and the rise-time of the triplet carotenoid (^{T}Car) is approx. 10–20 ns at room temperature [59]. However, the yield of ^{T}Car formation has been found to vary according to the bacterial species from which the reaction centers are extracted, from near 100% in *Rb. sphaeroides* [18] to 0% in *Rps. viridis* [60]. Moreover, Schenck et al. [61] have shown that, in *Rb. sphaeroides* 2.4.1 reaction centers, the yield of the triplet–triplet transfer decreases from 100% to a few percent upon decreasing the temperature from 80 to 30 K.

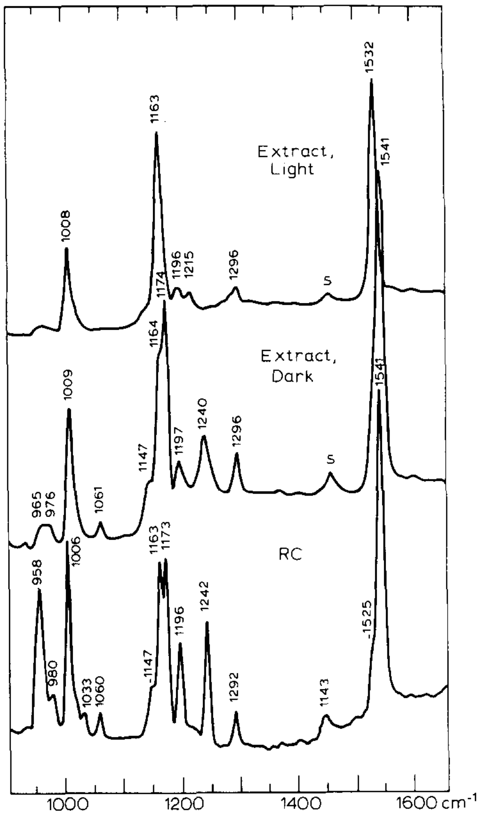


Fig. 4. Resonance Raman spectra (900–1650 cm^{-1} region) of spheroidene, 30 K, 496.5 nm excitation. From bottom to top: RC, spheroidene bound to reaction centers of *Rb. spheroides* 2.4.1. Extract, dark: spheroidene in a total extract from the above RCs. Extract, light: same sample, kept for 1 mn in room light before freezing. S, *iso*-octane solvent bands.

II-A. Carotenoid conformation in bacterial reaction centers

Carotenoids are extremely strong resonant Raman scatterers when illuminated in their lowest allowed electronic transition. These molecules have been studied by RR spectroscopy since 1932, and used in several experimental and theoretical investigations of the resonance Raman effect [62]. RR spectra of C_{40} carotenoids consist essentially of four groups of bands observed at approx. 1530 (ν_1), 1120–1200 (ν_2), 1000 (ν_3) and 960 (ν_4) cm^{-1}.

In 1976, Lutz et al. [45] observed that RR spectra of spheroidene bound to RCs of *Rb. sphaeroides* were quite unusual, and proposed that this molecule assumed a *cis* configuration. Similarly, from absorption and circular dichroism spectra of the carotenoids bound to RCs of *Rsp. rubrum*, Boucher et al. hypothesized that their configurations might be 15-15′-*cis* [64]. Lutz et al. [46] performed RR studies on carotenoids from both the spheroidene and the spirilloxanthin series in reaction centers from *Rb. sphaeroides, Rps. viridis* and *Rsp. rubrum*. They confirmed that all these molecules assumed a common *cis* configuration. Indeed, as shown in Fig. 4, for RC-bound sphaeroidene: - the ν_1 mode is upshifted by 12 cm^{-1} relative to isolated spheroidene - in the ν_2 mode region appears as an intense 1242 cm^{-1} band, which is inactive for all-*trans* carotenoids - the ν_4 modes located at 958 cm^{-1} are as intense as the ν_3 band at 1006 cm^{-1}. These ν_4 modes have been attributed to out-of-plane bending of CH groups of the chain, the resonance Raman activity of which, when exciting in the $^1B_u \rightarrow {}^1A_g$ transition, depends on the planarity of the isoprenic chain. Their high intensity thus was considered as an indication that the carotenoid molecule is not only in a *cis* configuration, but also in a twisted conformation.

In the same study [46], it was further concluded that no isomerization of the bound carotenoid occurred when increasing the temperature from 15 to 250 K in reaction centers of *Rb. sphaeroides* 2.4.1. From this result, and also considering that the carotenoid configuration is the same in both this latter species and in *Rps. viridis* (reaction centers in which no triplet–triplet transfer is observed), it thus appears clear that the carotenoid configuration is not the only parameter that regulates the triplet–triplet transfer.

Agalidis et al. [67] studied the structure of the spheroidene reconstituted in reaction centers of the mutant of *Rb. sphaeroides* deficient in carotenoid synthesis. RR studies of reaction centers reconstituted with spheroidene and spheroidenone showed that the conformations of these molecules were the same as those observed in the wild-type RCs. It thus appears that this conformation is imposed by the protein upon the binding of the molecule.

In the early 1980's, Koyama et al. [68,69] studied 16 different isomers of β-carotene in vitro using RR. At the same period, Saito and Tasumi [65] calculated the normal modes for this molecule in different configurations. From these studies, it was shown that modes ν_1 and ν_2 allow the configuration of the isoprenic chain to be determined. In particular, it was found that the 1240 cm^{-1} mode was active only for molecules in which the 15-15′ C = C bond assumes a *cis* configuration [65]. Koyama et al. [68] thus concluded that the presence of this band in RR spectra of RC-bound carotenoids could be considered as a fingerprint for a central *cis* isomerization. However, this characterization was indirect, considering the chemical differences between β carotene, which has cyclic chain ends, and the RC-bound carotenoids which, as a rule, have open chain ends. Other questions remained open, such as the above-mentioned possibility that the RC-bound carotenoids might assume out-of-plane conformation [66], and the absence of any strong ultraviolet '*cis*' absorption band of the carotenoid in the electronic absorption spectra of RCs, a band which usually characterizes the 15-15′ *cis* carotenoid isomers [46]. From ESR experiments, Chadwick and Frank [70] suggested that the native conformation of the carotenoid in the reaction center involves twists of the molecule at the C6–C7 or higher carbon positions. Lutz et al. [71] recently performed NMR and Raman measurements on spheroidene extracted from bacterial reaction centers in complete darkness: NMR measurements confirmed the the presence of 15-15′ *cis* isomer for the extracted molecules, which also exhibited, as expected, a strong ultraviolet *cis* band. RR measurements lead to the conclusion that, while the configuration of the molecules remained unchanged during extraction (as indicated by comparing the ν_1 to ν_3 frequency modes in Fig. 4) the native out-of-plane conformation relaxed into a planar conformation when the molecule was extracted from its proteic host-site (as indicated by the decrease of the intensity of the ν_4 modes). This out-of-plane conformation must be the origin for the observed weakness of the '*cis*' ultraviolet absorption band for the RC-bound carotenoids. By carefully comparing the relative intensities of the ν_4 bands as well as those of weak out-of-plane modes in the 650–900 cm^{-2} region, in RR spectra of the native and extracted 15-15′ *cis* isomers, and relying on normal mode calculations performed by Saito and Tasumi [65], it was proposed that the twisted parts of the carotenoid bound to the reaction centers were the C8–C12 and/or C8′–C12′ regions [71]. Recently, similar NMR and RR experiments have been conducted with the neurosporene-containing reaction centers of *Rb. sphaeroides* strain G1C [72], and it was concluded that neurosporene adopts the same configuration as spheroidene when bound to reaction centers.

X-ray crystallographic data revealed that the carotenoid in the reaction center of *Rps. viridis* was located in the M subunit, in close proximity to the BChl monomer on the same M subunit (BChl$_M$), approx. 11 Å from the primary donor of electrons [26]. It was further concluded that the configuration of the RC-bound carotenoid was a 13-*cis* with an out-of-plane twist about the C13 position [26]. This conclusion is not in agreement with the RR results, from which it was shown that carotenoids in bacterial reaction centers assume a common 15-*cis* configuration [46,41]. However, the ends of the carotenoid molecule were not resolved in the electron density map, so that there is still a certain degree of uncertainty in the X-ray assignment. Moreover, according to preparation or storage procedures, self-isomerizations have been found to occur in *Rps. viridis* reaction centers (Robert, B., unpublished results). Clearly, work is still needed to establish whether or not, in crystallized *Rps. viridis* reaction centers, the carotenoid retains its native configuration. From the most recent coordinate refinement of the X-ray data of reaction centers from *Rhb. sphaeroides* 2.4.1 it was concluded that the carotenoid was lying near the accessory BChl$_M$ as in *Rps. viridis* [32]. However, because of the resolution of the structure, these authors could only conclude that the 15-*cis* configuration determined by RR spectroscopy is consistent with the electron density, without being able to determine the position of the *cis* bond uniquely [32].

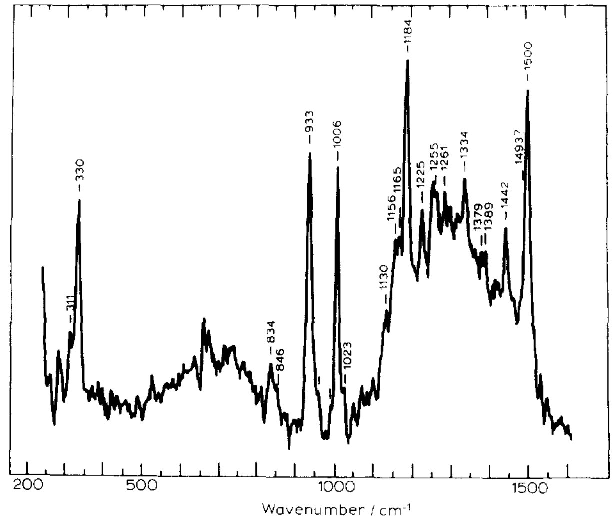


Fig. 5. Resonance Raman spectrum of the triplet state of the spheroidene molecule bound to reaction centers of the *Rb. sphaeroides* bacteria. Difference spectrum obtained by subtracting two RR spectra recorded at different irradiance (high – low irradiance). Excitation wavelength, 545 nm; *T*, 80 K.

II-B. Excited triplet state of RC-bound carotenoids

In 1983, Lutz et al. [73] obtained RR spectra of RC-bound spheroidene, methoxyneurosporene and spheroidenone in their triplet states (^{T}Car), by using laser excitation resonant with the triplet–triplet electronic transition near 540 nm. Their experimental apparatus involved a pulsed actinic laser beam and another cw probe laser beam. RR spectra were recorded using a 5–10 ms gated detection system [73]. More recently, it was shown that it was possible to build-up significant amounts of the ^{T}Car state and to record its RR spectrum with fewer technical constraints, by using a single cw laser excitation at 530–550 nm [48] (Fig. 5). RR spectra of the triplet state of the carotenoid bound to bacterial RCs exhibit marked differences with previously published RR spectra of all-*trans* and *cis* β-carotene in triplet state [74–76]. In particular, the 15 cm^{-1} shift existing between the ν_1 modes in RR spectra of ground-state all-*trans* and RC-bound carotenoids is conserved between RR spectra of the triplet states of these molecules. It was thus concluded that no *cis–trans* isomerization occurs during the triplet to ground-state transition. It is difficult to determine precisely the position of the *cis* bond of the carotenoid molecule in its excited triplet state, since the intense 1242 cm^{-1} band is not present in RR spectra of ^{T}Car. On the other hand, an intense ν_4 band is present in RR spectra of the spheroidene bound to RCs in its excited triplet state, which suggests that the carotenoid molecule keeps its native out-of-plane conformation during the triplet–triplet transition [73].

III. Interactions assumed by bacteriochlorin pigments in bacterial reaction centers

III-A. Resting reaction centers

III-A.1. Interactions assumed by the bacteriopheophytin molecules

In the mid-seventies it was shown that the Q_x electronic transitions of the two BPheo molecules bound to bacterial RCs are resolved at low temperature, and that only one of these transitions is affected by the P^+BPheo^- charge separation [77]. The BPheo molecule that acts as electron acceptor after the first charge separation is located between P and Q_A and is mainly surrounded by the L subunit of the RC [25]. Henceforth this BPheo molecule will be designated as $BPheo_L$, while the second, accessory BPheo, which is related via the C_2 symmetry axis to $BPheo_L$, will be designated as $BPheo_M$.

Resonance Raman spectroscopy was used for determining interaction states of each of the two BPheo molecules present in *Rb. sphaeroides* bacterial reaction centers [35]. it was possible to selectively excite each of these pigments in their Q_x transition at 20 K [35]. From these experiments, it could be concluded that the acceptor $BPheo_L$ had a bound 9-keto C = O group, vibrating at 1678 cm^{-1} [35,36,49], while the homologous chemical grouping of the accessory BPheo was free from interactions [49]. A similar stretching frequency was recently measured from FTIR measurements for the 9-keto carbonyl group of the $BPheo_L$ molecule [78]. Moreover, from RR spectroscopy, it was further concluded that the 2-acetyl C = O groups of both the accessory and acceptor BPheo were H-bonded, vibrating at 1627 and 1633 cm^{-1}, respectively (Fig. 6). More recently, it was reported that these interactions are remarkably conserved in *Rps. viridis* [41] and *Chr. tepidum* reaction centers [79]. A promising approach was employed by Bocian et al. [44], when these authors obtained RR spectra of *Rb. sphaeroides* RCs excited at 752 nm, in the Q_y electronic transition of the bound BPheo molecules. However, because of a lack of ground-state information about the relative contributions of the different bacteriochlorin molecules to these spectra, only tentative conclusions could be drawn from these spectra.

With respect to the keto carbonyl groupings, RR results are in perfect agreement with the conclusions drawn from the refined analysis of the X-ray diffraction data obtained by Michel et al. [25] on *Rps. viridis* reaction centers. Indeed, the latter authors have shown that, in *Rps. viridis* RCs, a glutamic acid residue (Glu L104) assumes the correct orientation and distance to form a hydrogen bond with the keto C = O of $BPheo_L$ [25]. This residue is conserved in *Rb. sphaeroides* [6] and probably constitutes the donor site of the H-bond which is revealed by RR spectroscopy to involve the keto group of $BPheo_L$. On the other hand, the M subunit of the RC proteins of *Rps. viridis* and *Rb. sphaeroides* does not have this glutamic acid residue, but rather valine and threonine residues at the corresponding positions, respectively [9,6,7]. In *Rps. viridis*, the valine M131 side-chain indeed has no suitable H-bond donor group for the 9-keto carbonyl group of $BPheo_M$, in perfect agreement with the RR data. In *Rb. sphaeroides,* the secondary alcohol function group of threonine M133 has been suggested to bind the keto group of $BPheo_M$ [31]. However, RR data indicate that such an interaction does not exist, and thus that this grouping actually should assume a position unfavourable to an H-bond formation. It appears likely that the specific interaction involving the keto carbonyl group of the acceptor molecule, $BPheo_L$, should play a role in the asymmetry of the electron transfer [25,80]. However, Bylina et al. [81] have shown that replacement of the glutamic acid L 104 in *Rhodobacter capsulatus* by a leucine residue had a limited effect on the electron transfer, inducing a decrease in rate for the first charge transfer between P and the acceptor BPheo. This experiment, although directly demonstrating that the H-bonding of the keto carbonyl of $BPheo_L$ indeed has an influence on the electron transfer kinetics, appears to limit its role to a second-order effect. Moënne-Loccoz et al. [82] recently performed RR experiments on PS II particles and demonstrated that the glutamic acid interaction with the

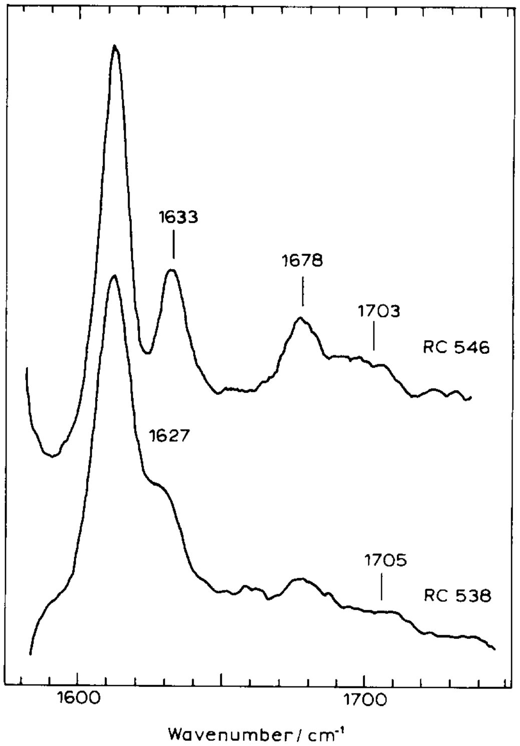


Fig. 6. Resonance Raman spectra of reaction centers from *Rb. sphaeroides* at 30 K excited in the Q_X absorption transition of the bacteriopheophytins at 538 (bottom) and at 546 (top) nm.

acceptor (bacterio)pheophytin is strictly conserved in PS II reaction centers. It is thus tempting to conclude that this interaction should play a crucial role in the first steps of the electron transfer in both bacterial and PS II reaction centers.

The agreement between RR data and the current structural models of RCs derived from X-ray crystallography is not as satisfying when considering the interaction states of the acetyl groups of the BPheo molecules, in RCs of the *Rps. viridis* and *Rb. sphaeroides* species. These carbonyls, which exhibit stretching frequencies downshifted by 33 or 41 cm^{-1} from that of the free vibrator [49], indeed appear to be intermolecularly bound to a significant extent. However, no suitable ligands have yet been found by the crystallographers. In any case, RR spectroscopy indicates that local proteic environments which are remarkably conserved accommodate the two bacteriopheophytin molecules in all the bacterial RCs studied until now [41,79].

III-A.2. Interactions assumed by the accessory BChls: resonance Raman studies of borohydride-treated reaction centers

In 1985, it was demonstrated that one of the monomeric BChl of the two present in bacterial RCs could be selectively removed by treating *Rb. sphaeroides* bacterial RCs with sodium borohydride [17]. The removal of this BChl molecule from the reaction centers had little influence on the electron transfer kinetics. It was hence hypothesized that the molecule selectively extracted by the borohydride treatment was the accessory BChl located along the inactive branch of the RCs, i.e., the $BChl_M$ molecule.

From the comparison of non-treated and borohydride-treated *Rb. sphaeroides* RCs it was possible to determine the contributions of the removed BChl in the RR spectra of RCs excited at the maximum of the Soret transition [83,36]. It was concluded that the central Mg atom of this molecule was singly-ligated, and that both of the keto and acetyl C = O groupings of this molecule vibrating at 1685 and 1663 cm^{-1}, respectively, were free from intermolecular interactions or weakly bound [83]. Moreover, these same RR measurements clearly showed that the interactions assumed by the remaining $BChl_L$ monomer and by the acceptor $BPheo_L$ are not perturbed by the borohydride treatment. Indeed, the stretching frequencies in the carbonyl groupings of these molecules are unchanged between the native and borohydride-treated RCs. However, both the acetyl and keto C = O groups of the $BPheo_M$ appear to be shifted by a few wavenumbers after borohydride treatment [83]. These latter results are in good agreement with the hypotheses that the BChl sensitive to borohydride treatment is that one located near the inactive $BPheo_M$, i.e., the accessory $BChl_M$. It is worth noting that, with respect to the interactions assumed by the C = O groupings of this molecule, the conclusions drawn from RR spectroscopy are in complete agreement with those of X-ray diffraction studies: that is, it was concluded from crystallographic data that the central Mg atom of the $BChl_M$ molecule interacts with the M180 His only, and that its 2-acetyl and its 9-keto carbonyl groupings are free from interactions [25,31].

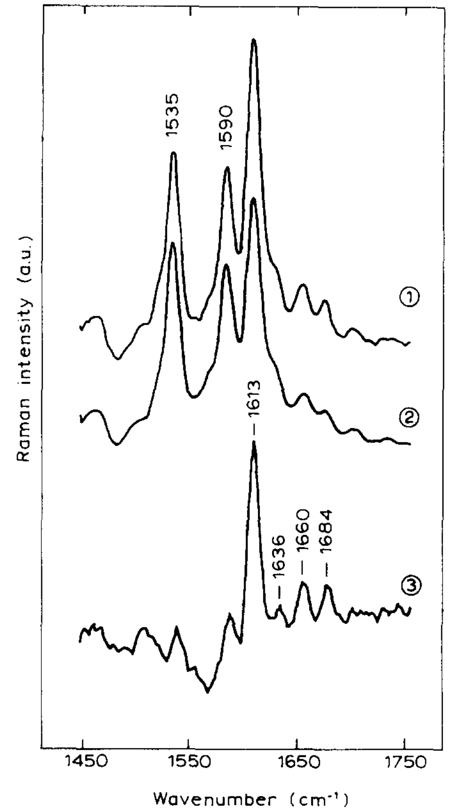


Fig. 7. Resonance Raman spectra (1450–1750 cm^{-1} region) of untreated reaction centers from *Rb. sphaeroides* 2.4.1 at 20 K. Excitation wavelength 363.8 nm. (1) Low irradiance conditions; (2) high irradiance conditions; (3) difference between spectra (a) and (b).

III-A.3. The primary electron donor

As mentioned above, the greatest amount of information from Raman spectra of bacteriochlorophylls excited in the near ultraviolet-visible range is available with Soret resonance. All six bacteriochlorins present in bacterial reaction centers have nearly coincident Soret absorption bands and hence are all more or less contributing in RR spectra excited in this region. Selective vibrational information concerning the primary donor in these resonance conditions was first reported in 1985 [48,49] by using difference RR spectroscopy techniques in studying RC of *Rb. sphaeroides* wild-type (see Section II). Such difference RR spectra contains selective contributions from the two BChls constituting P (namely P_L and P_M) as shown in Fig. 7.

In these difference spectra, the methine bridge stretching modes of the two BChls constituting P were observed at about 1612 cm^{-1} in *Rb. sphaeroides* RCs (see Fig. 7), with no contribution around 1600 cm^{-1} [48,49]. It was thus concluded that both of the BChl molecules constituting P have a single axial ligand coordinated to each of their central Mg atom [48,49,35]. At that time, from preliminary interpretations of the X-ray diffraction patterns of *Rps. viridis* RCs, it was proposed that the central Mg atoms of each of the BChl constituting P had two axial ligands, one being an amino acid side-chain, and the other one being the acetyl carbonyl of the adjacent BChl molecule [23,84]. By contrast, from RR data, it was hypothesized that only the amino acid side-chains were ligating to the central Mg of each of these BChl [35]. In 1986, refinement of the electronic density maps obtained from *Rps. viridis* RC crystals confirmed that the central Mg atoms of both of the BChls constituting P were interacting only with the imidazole side-chains of the L173 and M200 histidine residues, and that the $C = O$ acetyl groups are oriented in positions unfavorable for any binding with the Mg of the adjacent BChl molecule [25]. Since 1986, RR difference spectra of P have been determined from RR spectra of reaction centers from *Rsp. rubrum* [85], *Rps. viridis* [41], *Rb. capsulatus* [79] and *Chr. vinosum* and *tepidum* [79]. From these experiments, it could be concluded that in all of these reaction centers, the BChl molecules constituting P possess a central Mg interacting with a single external ligand, most likely histidines. Recently, it has been proposed from X-ray diffraction studies about *Rb. sphaeroides* RCs that the Mg of P_M could interact with a nearby histidine, whilst the L173 His is too far from the Mg of P_L to coordinate it directly [32]. It was further concluded that the Mg of the P_L molecule would be free from any interaction with axial ligands [32,33]. This hypothesis appears to be unlikely in view of the RR studies previously mentioned.

In the RR spectra of P in RCs of *Rb. sphaeroides,* three distinct bands were present, at 1636, 1660 and 1684 cm^{-1}, respectively, in the carbonyl stretching region (Fig. 7). In order to explain the presence of these three RR bands, it was proposed that the bonding interactions assumed by the conjugated carbonyls of each of these molecules did not obey the two-fold pseudosymmetry of the RCs [35]. The 1636 cm^{-1} and a component of the 1660 cm^{-1} band were assigned to the acetyl $C = O$ stretching modes. It was thus concluded that one of these groups is H-bonded, most likely with a proteic residue, whilst the other one is free from intermolecular interactions. From a comparison of RR spectra of P in *Rb. sphaeroides* and in *Rsp. rubrum* [85] it could be concluded that the 1660 cm^{-1} Raman band was degenerate, also involving the stretching mode of the intermolecularly bound keto carbonyl of one BChl [35]. The 1684 cm^{-1} band was assigned to the keto group of the second BChl. This frequency indicates a weak bonding interaction, or a local polar environment for this group. When the selective contributions of P were extracted from reaction centers of *Rsp. rubrum* [85], *Rps. viridis* [41], *Rb. capsulatus* [79] and *Chr. vinosum* and *tepidum* [79], it surprisingly appeared that the interaction states of at least two of its four conjugated carbonyls were species-dependent. Indeed in *Rps. viridis* reaction centers, the acetyl carbonyls of both of the BChl constituting P appeared to be bound, vibrating at 1627 and 1633 cm^{-1}, respectively [41]. In *Rsp. rubrum*, the keto carbonyl groups were reported to be weakly bound and free from interaction, vibrating at 1684 and 1702 cm^{-1}, respectively [85]. In all other studied reaction centers studied, i.e., *Rb. capsulatus, Chr. vinosum* and *tepidum*, the interaction states of the conjugated carbonyls of P appear to be very similar to those observed in *Rb. sphaeroides* [79].

Ground-state interactions assumed by the conjugated carbonyls of the primary donor BChls of *Rps. viridis* as deduced from RR spectroscopy are entirely consistent with the structural model of this RC derived from X-ray crystallography [25]. Indeed, according to this model, the acetyl group of molecule P_L interacts with the side chain of His L168, and that of molecule P_M with the side-chain of Tyr M195. On the other hand, the keto group of P_L may be H-bonded to the hydroxyl group of Thr L248, while that of P_M, being in the vicinity of Ile M282, has no suitable H-bond donor nearby to establish a localized bond (Table I) [25]. The 1660 cm^{-1} frequency was thus attributed to the stretching mode of the 9-keto group of P_L, whilst that of P_M most likely vibrates at 1684 cm^{-1} [41]. On the other hand, the 1635 cm^{-1} frequency, being present in all RR spectra of P, was attributed to the acetyl carbonyl group of P_L, which interacts with L168 His residue, because the latter residue is found to be conserved in the primary sequences of both *Rb. sphaeroides* and *Rps. viridis* [6,7,9]. Consequently, the 1627 cm^{-1} frequency, which is only observed in *Rps. viridis,* has been assigned to the acetyl

TABLE I

Carbonyl stretching frequencies observed in RR spectra of Rb. sphaeroides reaction centers

Frequency (cm^{-1})	$C = O$	Molecule	H-bond donor
1627	acetyl	$Bpheo_M$	?
1633	acetyl	$Bpheo_L$	?
1636	acetyl	P_L	His L168
1660	acetyl	P_M	none
1660	acetyl	$BChl_L$	none
1660	keto	P_L	?
1663	acetyl	$BChl_M$	none
1678	keto	$Bpheo_L$	Glu L104
1684	keto	P_M	none
1685	keto	$BChl_M$	none
1689	keto	$BChl_L$	none
1708	keto	$BPheo_M$	none

C = O of P_M [41]. Indeed, the latter group interacts with the hydroxyl group of Tyr M195, which is replaced by a phenylalanine residue in the M chain of *Rb. sphaeroides* [6,7,9].

Crystallographic studies on *Rb. sphaeroides* RCs led to significantly different conclusions concerning the interactions assumed by the BChls constituting P in this protein. It was first concluded in Ref. 28 that the acetyl carbonyl groups of both the BChl monomers of P were H-bonded, with the His L168 and Tyr M210, respectively. More recently, the same authors reported in Ref. 31 that no hydrogen bonds were observed for these groups, nor for the 9-keto carbonyl groups. Both of these conclusions are at odds with the Raman data, which indicate that one acetyl and one keto group of the BChls of P are H-bonded [35]. More recent results, obtained in Argonne (Tiede, D.M., personal communication), indicate that the His L168 position allows an interaction with the 2-acetyl group of P_L, in accordance with RR conclusions. However, this author does not propose any candidate for the H-bonding of the keto C = O of P_L, which should be H-bonded according to RR spectroscopy. Some questions thus remain open about the partner molecules which interact with the latter carbonyl group.

In the recent sequencing of the L and M polypeptide of *Rsp. rubrum* RC, very few replacements were observed among the amino acids located in the vicinity P between *Rsp. rubrum* and *Rps. sphaeroides* RC [10]. These results are in good agreement with RR spectra of the primary donors in both species, except for the case of the 9-keto group of P_L: indeed, the frequencies of the different carbonyl stretching modes of the BChl constituting P are similar for *Rb. sphaeroides* and *Rsp. rubrum*, except for this group [85]. It vibrates at 1702 cm^{-1} in *Rsp. rubrum*, and is thus clearly free from interactions, whereas its stretching frequency is observed at 1660 cm^{-1} in *Rb. sphaeroides.* As no amino acid replacement is observed between *Rb. sphaeroides* and *Rsp. rubrum* in the local neighborhood of this grouping, this result is all the more surprising. Slight modifications of the C_α backbone and/or of the amino-acid side-chain geometries, which may originates from amino-acid replacements far away from the considered sites, may be invoked for interpreting this apparent discrepancy. However, it is clear that further work is needed to build a coherent structural model of the interactions assumed by this group.

From the similarities between the interactions assumed by the conjugated carbonyls of the BChl of P in the RCs of *Rb. sphaeroides, Chr. tepidum* and *vinosum* [79], it was predicted that most likely the L173 L168 and M200 histidines, as well as the M282 isoleucine, are considered in the primary sequences of these RCs, or at least replaced by equivalent amino acids. On the other hand, the Q_y transition of P is observed at 855 nm and 890 nm at room temperature in *Rb. sphaeroides* and *Chr. vinosum*, respectively (see, for example, Ref. 86). It was thus further concluded that this 460 cm^{-1} energy shift was not due to changes in the intermolecular interactions assumed by the conjugated carbonyls between these RCs [79], but most likely to slight differences affecting the relative geometry of the two BChl constituting P.

III-B. Protein conformational changes occurring during the first steps of electron transfer in bacterial reaction centers

The question of possible protein conformational changes accompanying the first steps of electron transfer has been raised by many authors (see, for example, Refs. 87, 88). The first direct evidence of a local, light-driven, protein conformational change in the bacterial reaction centers were obtained by RR spectroscopy [36]. In RR spectra of reaction centers of the carotenoid-lacking R 26 mutant of *Rb. sphaeroides*, the contributions of the BChl molecules constituting the primary donor were less than a few percent. This result is also verified for other carotenoid-lacking mutants such as that from *Rps. rubrum* [79]. It was thus possible, by using RCs isolated from this strain to study the influence of the primary charge separation on the interaction states of the two monomeric BChls and of the two BPheos: indeed, significantly populating the $P^+Q_A^-$ and/or P^R states in such RCs is expected to result in modifications of the RR spectra primarily arising from these pigments and not, as in the carotenoid-containing strains, from the loss of the contributions of the neutral, ground-state BChls of P [36]. The same methods, involving the control of the dynamic equilibria induced by the actinic effect of the Raman probe laser beam were thus used with bacterial RCs isolated from this strain in order to study the protein–pigment interactions in the $P^+Q_A^-$ and/or P^R states. It was found that $P^+Q_A^-$ formation induces a downshift of the stretching frequency of one keto carbonyl group from 1689 cm^{-1} to 1675 cm^{-1} and a partial bleaching of a 1660 cm^{-1} band [36]. Because these frequencies do not match the frequencies observed for the carbonyl stretching modes of any of the BPheo [35], and because this effect was not sensitive to the removal of the accessory B_M molecule [36], it was concluded that the B_L accessory BChl alone (in fact, the acceptor BChl [16]) was involved in these variations, and thus that the 1660 and 1689 cm^{-1} bands were arising from the stretching modes of its 2-acetyl and 9-keto carbonyls, respectively [36] (Fig. 8). It was further concluded that, in resting RCs, both of these groups are free from intermolecular interactions. As the C = O stretching modes are the only vibrational modes of these molecules observed to be affected by the P^+Q_A formation, it was concluded that these changes in

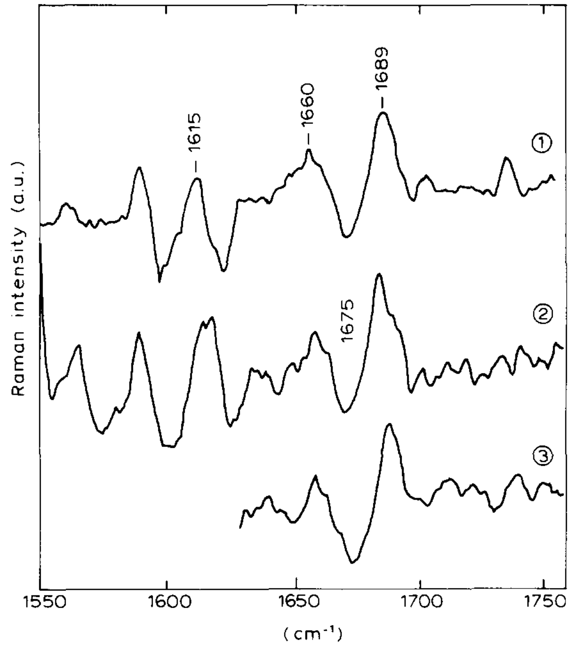


Fig. 8. Difference resonance Raman spectra (1550–1750 cm^{-1} region) obtained from reaction centers of *Rb. sphaeroides* R 26. (1) Untreated, low irradiance minus high irradiance; (2) dithionite-treated, low irradiance minus high irradiance; (3) untreated, low irradiance minus ferricyanide-treated RCs. Experimental conditions excitation wavelength, 363.8 nm; *T*, 20 K.

RR spectra were arising from local proteic events around the B_L molecule [36]. A 15 cm^{-1} shift of a keto carbonyl of a BChl corresponds to an approx. 2.4 kcal/mol variation of the H-bond strength in which it is involved. In order to explain the decrease in intensity of the 1660 cm^{-1} band, a steric repulsion of this grouping out of the plane of the BChl dihydrophorbin cycle was tentatively proposed [36]. It was also shown that these protein conformational changes are associated with the oxidation of the primary donor rather than to the transfer of the electron to the primary quinone Q_A: indeed, chemical oxidation of P by ferricyanide, induces the same RR spectral changes [36] (Fig. 8). More surprising was the fact that similar phenomena must occur in bacterial RCs when the P^R state is formed (Fig. 8) [36]. As it was very surprising that the presence of a charge and of an unpaired electron had the same effect on the local environment of the B_L molecule, two different hypotheses were proposed to explain this result: (i) the protein conformational changes induced by the P^+BPheo^- state may have a proper lifetime which would be longer than that of the triplet state at low temperature (100 μs); or (ii) this change may be triggered by a slight modification of the structure of the primary donor itself, which would be similar in both the P^+ and P^R states [36].

In the vicinity of $BChl_L$ in bacterial reaction centers from *Rps. viridis*, a likely candidate for binding the keto $C=O$ of B_L is a water molecule [90]. This molecule is bound to the M200 histidine residue, which itself is the axial ligand of the Mg of P_M [90]. Distance between this water molecule and the 9 keto group of B_L is about 2.9 Å, i.e., not far from the optimal distance required for a $C=O \cdots H\text{-}O$ bonding to take place. Conceivably, a slight motion of the M200 histidine could change the strength of this bond and be responsible for the Raman shift observed in both the P^+ and P^R states. According to this hypothesis, P and B_L would be connected through a His $\cdots$ H_2O bridge when P is in the P^+ and/or P^R states. An alternative hypothesis has been proposed which involves the interspecifically conserved M208 Tyr residue. This residue is too far away to bind the keto group of $BChl_L$ in resting reaction centers, but might move enough during charge separation in order to form a hydroxyl $\cdots$ carbonyl H-bond [36]. This hypothesis would involve a larger protein motion than the preceding one. Current RR studies of RCs of *Rb. sphaeroides* in which this tyrosine has been selectively mutated will allow it to be tested (Mattioli, T.A., Robert, B. and Gray, K., unpublished results). In any case, this kind of molecular rearrangement might conceivably play a role during the first steps of the electron transfer, either in reducing the probability of the back reactions or in ensuring the electron transfer on only one of the two possible electron pathways present in the RCs: indeed, this phenomenon appears not to follow to the C_2 symmetry of the protein, being observed even in bacterial RCs in which the $BChl_M$ molecule has been selectively removed [36].

IV. Perspectives

This year, nanosecond time-resolved RR spectroscopy has been successfully applied to RCs of *Rb. sphaeroides* poised in low redox conditions in order to characterize RR spectra of the 10 ns P^+I^- state in these complexes [91], and the influence of the presence of this charge pair on the interactions assumed by the different pigments. In addition, RR spectroscopy and difference methods which have been developed on bacterial RCs have also been recently applied to higher plant photosystems: these permitted the interactions assumed by the primary electron acceptor in PS II reaction centers [82], as well as the structure of the primary donor of PS I [92,93], to be characterized. Similarly, some promising RR experiments have been performed in order to determine the structure of the P680 in PS II RCs [94,95].

Now that the different components present in the carbonyl stretching region have been assigned to the different bacteriochlorin pigments present in the RCs (see Table I), this technique will most likely find a wider range of applications for studying this protein. In particular, it should be extremely useful in characterizing the effect of site-selected mutations in the L and/or M subunits of the RCs in the local environment of the bacteriochlorin pigments, something which is of particular importance for understanding the structure–function relationships in bacterial RCs, and more generally, in electron-transferring proteins.

One would have thought that the interest of such 'structural' spectroscopy would have diminished when the tridimensional structure of bacterial RCs was determined [23–25]. In actual fact, it appears that RR spectroscopy has gained in interest as our structural knowledge of the RCs has improved. RR spectroscopy, indeed, provides information which is complementary to that obtained by crystallographic studies. Moreover, it allows conclusions to be drawn concerning non-crystallized biological photosystems (such as higher plant photosystems or mutant RCs) and about transient states in these photosystems, by using as a reference the crystallographic studies and the RR studies about wild-type and resting bacterial RCs.

References

1 Lutz, M. (1972) C. R. Acad. Sci. 275, 497–502.
2 Lutz, M. (1984) in Advances in Infrared and Raman Spectroscopy (Clark, R.J. and Hester, R.E., eds.), Vol. 11, pp. 211–280, Wiley Heyden, London.
3 Lutz, M. and Robert, B. (1988) in Biological Application of Raman Spectroscopy (Spiro, T.G., ed.), Vol. 3, Ch. 8, Wiley, New York.
4 Robert, B., Nabedryk, E. and Lutz, M. (1989) in Time-Resolved Spectroscopy (Clark, R.J. and Hester, R.E., eds.), pp. 301–335, Wiley Heyden, London.
5 Feher, G. and Okamura, M.Y. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 119–131, Plenum, New York.
6 Williams, J.C., Steiner, L.A. and Feher, G. (1986) Proteins Struct. Funct. Genet. 1, 312–325.
7 Donohue, T.J., Hoger, J.H. and Kaplan, S. (1986) J. Bacteriol. 168, 953–961.
8 Youvan, D.C., Bylina, E.J., Alberti, M., Begusch, H. and Hearst, J.E. (1984) Cell 37, 949–957.
9 Michel, H., Weyer, K.A., Gruenberg, H. and Lottspeich, F. (1985) EMBO J. 4, 1667–1672.
10 Belanger, G., Berard, J., Corriveau, P. and Gingras, G. (1988) J. Biol. Chem. 263, 7632–7638.
11 Ovchinnikov, Y.A., Abdulaev, N.G., Zolotarev, A.S., Shmukler, B.E., Zargarov, A.A., Kutuzov, M.A., Telezhinskaya, N. and Levina, N.B. (1988) FEBS Lett. 231, 237–242.
12 Ovchinnikov, Y.A., Abdulaev, N.G., Shmukler, B.E., Zargarov, A.A., Kutuzov, M.A., Telezhinskaya, I.N., Levina, N.B. and Zolotarev, A.S. (1988) FEBS Lett. 232, 364–368.
13 Parson, W.W. (1987) in Photosynthesis (Amesz, J., ed.), pp. 43–61, Elsevier, Amsterdam.
14 Kirmaier, C. and Holten, D. (1987) Photosynth. Res. 13, 225–260.
15 Norris, J.R., Uphaus, R.A., Crespi, H.L. and Katz, J.J. (1971) Proc. Natl. Acad. Sci. USA 68, 625–628.
16 Holzapfel, W., Finkele, U., Kaiser, W., Oesterhelt, D., Scheer, H., Stilz, H.U. and Zinth, W. (1989) Chem. Phys. Lett. 160, 1–7.
17 Maroti, P., Kirmaier, C., Wraight, C., Holten, D. and Pearlstein, R.M. (1985) Biochim. Biophys. Acta 810, 132–139.
17a Woodbury, N.W., Becker, M., Middendorf, D. and Parson, W.W. (1985) Biochemistry 24, 7516–7521.
17b Martin, J.L., Breton, J., Hoff, A.J., Migus, A. and Antonetti, A. (1986) Proc. Natl. Acad. Sci. USA 83, 957–961.
17c Fleming, G.R., Martin, J.L. and Breton, J. (1988) Nature 333, 190–192.
18 Parson, W.W. (1982) Annu. Rev. Biophys. Bioeng. 11, 57–80.
19 Michel, H. (1982) J. Mol. Biol. 158, 567–572.
20 Chang, C.H., Schiffer, M., Tiede, D.M., Smith, U. and Norris, J.R. (1985) J. Mol. Biol. 186, 201–203.
21 Allen, J.P. and Feher, G. (1984) Proc. Natl. Acad. Sci. USA 81, 4795–1799.
22 Ducruix, B. and Reiss-Husson, F. (1987) J. Mol. Biol. 195, 419–421.
23 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) J. Mol. Biol. 180, 385–398.
24 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature 318, 618–624.
25 Michel, H., Epp, O. and Deisenhofer, J. (1986) EMBO J. 5, 2445–2451.
26 Deisenhofer, J. and Michel, H. (1988) in The Photosynthetic Bacterial Reaction Center (Breton, J. and Vermeglio, A., eds.), pp. 1–3, Plenum Publishing Corp., New York.
27 Chang, C.H., Tiede, D., Tang, J., Smith, U., Norris, J. and Schiffer, M. (1986) FEBS Lett. 205, 82–86.
28 Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1987) Proc. Natl. Acad. Sci. USA 84, 5730–5734.
29 Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1987) Proc. Natl. Acad. Sci. USA 84, 6162–6166.
30 Komiya, H., Yeates, T.O., Rees, D.C., Allen, J.P. and Feher, G. (1988) Proc. Natl. Acad. Sci. USA 85, 9012–9016.
31 Allen, J.P. and Feher, G. (1988) Proc. Natl. Acad. Sci. USA 85, 9012–9016.
32 Yeates, T.O., Komiya, H., Chirino, A., Rees, D.C., Allen, J.P. and Feher, G. (1988) Proc. Natl. Acad. Sci. USA 85, 7993–7997.
33 Feher, G., Allen, J.P., Okamura, M.Y. and Rees, D.C. (1989) Nature 339, 111–116.
34 Robert, B. and Lutz, M. (1986) Biochemistry 5, 2303–2309.
35 Lutz, M. (1980) in Proceedings of the 7th International Conference on Raman Spectroscopy (Murphy, W.F., ed.), pp. 520–523, North Holland, Amsterdam.
36 Robert, B. and Lutz, M. (1988) Biochemistry 27, 5108–5114.
37 Lutz, M. and Breton, J. (1973) Biochem. Biophys. Res. Commun. 53, 413–420.
38 Lutz, M. (1979) Thesis, Université Pierre et Marie Curie, Paris.
39 Cotton, T.M. and Van Duyne, R.P. (1981) J. Am. Chem. Soc. 103, 6020–6024.
40 Robert, B., Steiner, R., Zhou, Q., Scheer, H. and Lutz, M. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. 1, pp. 411–414, Martinus Nijhoff, Dordrecht.
41 Zhou, Q., Robert, B. and Lutz, M. (1989) Biochim. Biophys. Acta 977, 10–18.
42 Katz, J.J. (1973) in Inorganic Biochemistry (Eickorn, G.L., ed.), Ch. 29, Elsevier, Amsterdam.
43 Hanson, L.K., Thompson, M.A., Zerner, M.C. and Fajer, J. (1988) in The Photosynthetic Bacterial Reaction Center (Breton, J. and Vermeglio, A., eds.), pp. 355–367, Plenum, New York.
44 Bocian, D.F., Boldt, N.J., Chadwick, B.W. and Frank, H.A. (1987) FEBS Lett. 214, 92–96.
45 Lutz, M., Kleo, J. and Reiss-Husson, F. (1976) Biochem. Biophys. Res. Commun. 69, 711–717.
46 Lutz, M., Agalidis, I., Hervo, G., Cogdell, R.J. and Reiss-Husson, F. (1978) Biochim. Biophys. Acta 503, 287–303.
47 Robert, B. (1987) Thesis, Université Pierre et Marie Curie, Paris.
48 Robert, B., Szponarski, W. and Lutz, M. (1985) Springer Proc. Phys. 4, 220–224.
49 Lutz, M. and Robert, B. (1985) in Antenna Complexes and Reaction Centers of Photosynthetic Bacteria (Michel-Beyerlee, M.E., ed.), pp. 138–145, Springer, Berlin.
50 Parson, W.W., Clayton, R.K. and Cogdell, R.J. (1975) Biochim. Biophys. Acta 387, 265–278.

51 Davis, M.S., Forman, A., Hanson, L.K., Thornber, J. and Fajer, J. (1979) J. Phys. Chem. 83, 3325–3332.

52 Parson, W.W. and Cogdell, R.J. (1975) Biochim. Biophys. Acta 416, 105–149.

53 Kim, D., Miller, L., Rakhit, G. and Spiro, T.G. (1986) J. Phys. Chem. 90, 3320–3325.

54 Kim, D., Terner, J. and Spiro, T.G. (1986) J. Am. Chem. Soc. 108, 2097–2099.

55 Oertling, W.A., Salehi, A., Chung, Y.C., Leroi, G.E., Chang, C.K. and Babcock, G.T. (1987) J. Phys. Chem. 91, 5887–5898.

56 Siefermann-Harms, D. (1985) Biochim. Biophys. Acta 811, 325–355.

57 Cogdell, R.J. and Frank, H.A. (1987) Biochim. Biophys. Acta 895, 63–79.

58 Mathis, P. (1970) Thesis, Université de Paris, Orsay.

59 Cogdell, R.J., Monger, T.G. and Parson, W.W. (1975) Biochim. Biophys. Acta 408, 189–199.

60 Frank, H.A., Bolt, J.D., De B. Costa, S.M. and Sauer, K. (1980) J. Am. Chem. Soc. 102, 4893–4898.

61 Schenck, C.C., Mathis, P. and Lutz, M. (1984) Photochem. Photobiol. 39, 407–417.

62 Euler, H.V. and Hellström, H. (1932) Z. Phys. Chem., Abt. B 15, 342–344.

63 Rimai, R., Heyde, M.E. and Gill, D. (1973) J. Am. Chem. Soc. 95, 4493–4487.

64 Boucher, F., Van der Rest, M. and Gingras, G. (1977) Biochim. Biophys. Acta 461, 339–357.

65 Saito, S. and Tasumi, M. (1983) J. Raman Spectrosc. 14, 310–321.

66 Szponarski, W., Robert, B., Szalontai, B. and Lutz, M. (1984) Abstracts, 7th International Symposium on Carotenoids, Munich, 23–31.08.1984, p. 37.

67 Agalidis, I., Lutz, M. and Reiss-Husson, F. (1980) Biochim. Biophys. Acta 589, 264–274.

68 Koyama, Y., Takii, T., Saiki, K., Tsukida, K. and Yamashita, K.J. (1982) Biochim. Biophys. Acta 680, 109–118.

69 Koyama, Y., Takii, T., Saiki, K. and Tsukida, K. (1983) Photobiochem. Photobiophys. 5, 139–150.

70 Chadwick, B.W. and Frank, H.A. (1986) Biochim. Biophys. Acta 851, 257–266.

71 Lutz, M., Szponarski, W., Berger, G., Robert, B. and Neumann, J.M. (1987) Biochim. Biophys. Acta 894, 423–433.

72 Koyama, Y., Takatsuka, I., Nakata, M. and Tasumi, M. (1988) J. Raman. Spectrosc. 19, 37–49.

73 Lutz, M., Chinsky, L. and Turpin, P.H. (1983) Photochem. Photobiol. 36, 503–513.

74 Dallinger, R.F., Farguharson, S., Woodruff, W.H. and Rodgers, A.J. (1981) J. Am. Chem. Soc. 103, 7433–7437.

75 Wilbrandt, R. and Jensen, N.H. (1983) in Time-resolved Vibrational Spectroscopy (Atkinson, G.H., ed.), p. 273, Academic Press, New York.

76 Teraoka, J., Hashimoto, H., Matsudaira, S. and Koyama, Y. (1985) Chem. Lett. (Japan) 311–314.

77 Parson, W.W., Clayton, R.K. and Cogdell, R.J. (1975) Biochim. Biophys. Acta 387, 265–278.

78 Nabedryk, E., Mäntele, W., Tavitian, B.A. and Breton, J. (1986) Photochem. Photobiol. 43, 461–465.

79 Zhou (1989) Thesis, Université Pierre et Marie Curie, Paris.

80 Hanson, L.K., Fajer, J., Thompson, M.A. and Zerner, R.C. (1987) J. Am. Chem. Soc. 109, 4728–4730.

81 Bylina, E.J., Kirmaier, C., McDowell, L., Holten, D. and Youvan, D.C. (1988) Nature 336, 182–184.

82 Moenne-Loccoz, P., Robert, B. and Lutz, M. (1989) Biochemistry 28, 3641–3647.

83 Beese, D., Steiner, R., Scheer, H., Angerhofer, A., Robert, B. and Lutz, M. (1988) Photochem. Photobiol. 47, 293–304.

84 Zinth, W., Knapp, E.W., Fischer, S.F., Kaiser, W., Deisenhofer, J. and Michel, H. (1985) Chem. Phys. Lett. 119, 1–4.

85 Zhou, Q., Robert, B. and Lutz, M. (1987) Biochim. Biophys. Acta 890, 368–376.

86 Netzel, T.L., Rentzepis, P.M., Tiede, D.M., Prince, R.C. and Dutton, P.L. (1977) Biochim. Biophys. Acta 460, 467–479.

87 Kirmaier, C., Holten, D. and Parson, W.W. (1985) Biochim. Biophys. Acta 810, 33–48.

88 Tiede, D.M., Kellog, E. and Breton, J. (1987) Biochim. Biophys. Acta 892, 294–302.

89 Zadorozhnyi, B.A. and Ishchenko, I.K. (1965) Opt. Spectrosc. (Engl. Transl.) 19, 306–308.

90 Michel, H. and Deisenhofer, J. (1989) EMBO J. 8, 2149–2169.

91 Mattioli, T., Robert, B. and Lutz, M. (1989) in Spectroscopy of Biological Molecules: State of the Art (Bertoluzza, A., Fagnano, C. and Monti, P., eds.), pp. 303–305, Esculapio, Bologna.

92 Moënne-Loccoz, P., Robert, B. and Lutz, M. (1989) in Techniques and New Developments in Photosynthesis (Barber, J. and Malkin, R., eds.), NATO-ASI series, Plenum, New York, in press.

93 Moënne-Loccoz, P., Robert, B., Ikegami, I. and Lutz, M. (1990) Biochemistry, in press.

94 Moënne-Loccoz, P., Robert, B. and Lutz, M. (1988) in Proceedings of the XIth International Congress on Raman Spectroscopy (Clark, R.J.H. and Long, D.A., eds.), pp. 699–700, J. Wiley, London.

95 Moënne-Loccoz, P., Robert, B. and Lutz, M. (1989) in Proceedings of the VIIIth International Congress on Photosynthesis (Baltscheffsky, M., ed.), Martinus Nijhoff, Dordrecht, in the press.