RESONANCE RAMAN SCATTERING OF BACTERIOCHLOROPHYLL, BACTERIOPHEOPHYTIN AND SPHEROIDENE IN REACTION CENTERS OF RHODOPSEUDOMONAS SPHEROIDES

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ABSTRACT : We report and discuss Raman spectra of bacteriochlorophyll a and of bacteriopheophytin a obtained in vitro by resonance effect in their Q_x and Soret electronic bands. Selective excitation of spectra of either of these molecules in reaction centers of Rhodopseudomonas spheroides, strains Y and R 26, was achieved by illumination in their respective Q_x bands. Preliminary interpretation of the spectra yields information about the interactions assumed by these molecules in the reaction centers. Spheroidene bound to reaction centers of strain Y probably affects a conformation different from that assumed by the bulk spheroidene of the chromatophore.

It was recently shown that resonance Raman scattering of the chlorophylls yielded information about their interactions with their environment in chloroplasts of green plants and algae (1,2). In order to extend our observations to photosynthetic bacteria, we studied RR spectra of BChl a and BPheo a in vitro and in chromatophores and reaction centers of R. spheroides, strains Y and R 26. In the following, we propose a preliminary description of these spectra, and preliminary data on the states of BChl a, BPheo a and spheroidene in vivo.

MATERIAL AND METHODS

Chromatophores and LDAO reaction centers of R. spheroides, strain Y (wild type) were prepared as previously described (3). R 26 reaction centers were purified according to ref. 4. No attempt was made to reduce the reaction centers, which were undoubtedly maintained in their oxidized state by the laser beam. BChl a was extracted from R. spheroides Y, and column chromatogra-

Abbreviations : RR : Resonance Raman ; R. : Rhodopseudomonas ; Chl : chlorophyll ; BChl : Bacteriochlorophyll ; BPheo : Bacteriopheophytin.

phed twice, at 10°C and under inert atmosphere, first on polyethylene powder, with water-acetone development, then on cellulose powder, with acetone-petroleum ether development. Samples were tested for purity by absorption spectroscopy and thin layer chromatography. BPheo *a* was prepared by acidification of a BChl solution.

Resonance conditions for Raman scattering of BChl and BPheo were respectively obtained in their Q_x bands using a rhodamine 6G dye laser in the 580-600 nm range and the 528.7 and 514.5 nm lines of an Argon laser. Soret preresonance and resonance conditions were obtained using the 363.8 nm line of Argon and the 441.6 nm emission of a Helium-Cadmium laser. Sample degradation during the experiment was avoided by degassing solutions used at room temperature, and more efficiently, by working at low temperature on solid samples immersed in a flow of gazeous Helium and maintained at ca 35°K outside the illumination site (2).
Spectroscopic methods have been described previously (5).

RESULTS AND DISCUSSION

A RR spectrum of solid BChl *a* excited in the Q_x band, at 35°K, is reproduced in Figure 1A. Vibrational frequencies of the resonance enhanced modes are indicated. Figure 2A displays a RR spectrum obtained from BPheo *a* at 35°K with 528.7 nm excitation. Solution spectra at room temperature presented lower signal to noise ratios, but they differed from the preceding only by details which are accounted for by differences in association states. Stretching modes of ketone and acetyl C = 0 bonds give rise to very weak bands at 1698 and 1665 cm^{-1}, respectively, in the BPheo spectra, which correspond to still weaker bands at ca 1700 and 1670 cm^{-1} for BChl at 35°K. In this latter, partly aggregated, solid sample, a weak 1650 cm^{-1} shoulder may arise from the stretching mode of ketone C = 0 groups linked to Mg (Fig. 1), (6). These modes are more active in Soret resonance conditions, and were observed at 1670 and 1695 cm^{-1} in room temperature spectra of BChl in acetone excited at 363.8 nm. On the contrary, Q_x resonance strongly enhances low frequency modes which are in turn much weaker in Soret resonance. The same observations were made for pheophytins *a* and *b*. BChl yields under Q_x resonance a 295 cm^{-1} complex band absent from the corresponding BPheo spectra. This band correlates with the ca 300 cm^{-1} bands of the chlorophylls, and thus most likely arises in part from a vibration of the $Mg-N_4$ group (5,7). Another contribution of this grouping may be expected in the 352 cm^{-1} band of BChl *a* (7).

Saturation of a second pyrrolic C:::C bond in BChl and BPheo with respect to Chl and pheophytin has marked effects on the intensities of the RR bands in the skeletal stretching region (1100-1650 cm^{-1}), while vibrational frequencies remain in close correspondance (5). The 1615 cm^{-1} band increases

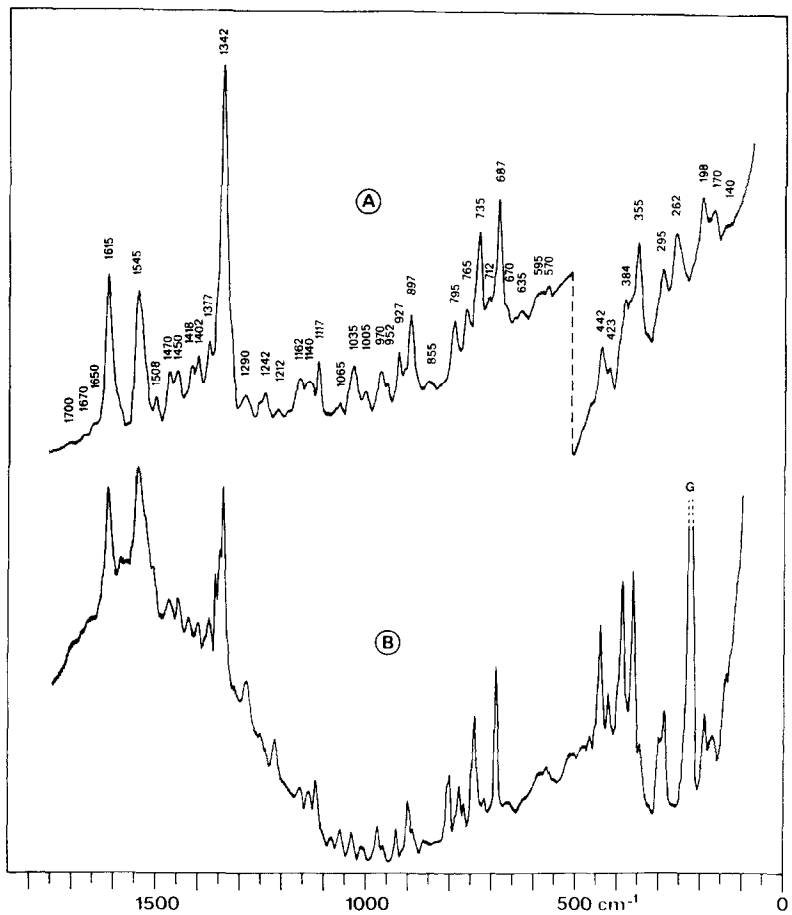

Figure 1 Resonance Raman spectra obtained at 35°K from :
A - Solid bacteriochlorophyll cast from a dry CCl_4 solution. Excitation 579 nm, resolution at 1000 cm^{-1} : 5.5 cm^{-1}.
B - Reaction centers of R. spheroides R 26. Excitation 598 nm. Resolution at 1000 cm^{-1} : 4 cm^{-1}. G : spurious line.

in relative intensity. It must thus preferably be attributed to stretching of methine bridges rather than to stretching of the outer pyrrolic C···C bonds in molecules of both types (5,7). Unexpectedly enough, the RR bands of BChl and BPheo which correlate with Chl bands with high C···N bond stretching participation in the 1050-1200 cm^{-1} region (7), are weak, in both Soret and Q_x resonance, with the only exception of the 1290 cm^{-1} band of BChl, strong in Soret resonance.

Reaction Centers and Chromatophores. Reaction centers of R. spheroides contain four molecules of BChl a and two of BPheo a bound in a still unknown way to a protein moiety (8). By excitation with 600 and 529 nm light, at 35°K, we selectively produced RR spectra of either BChl or BPheo, respectively, in

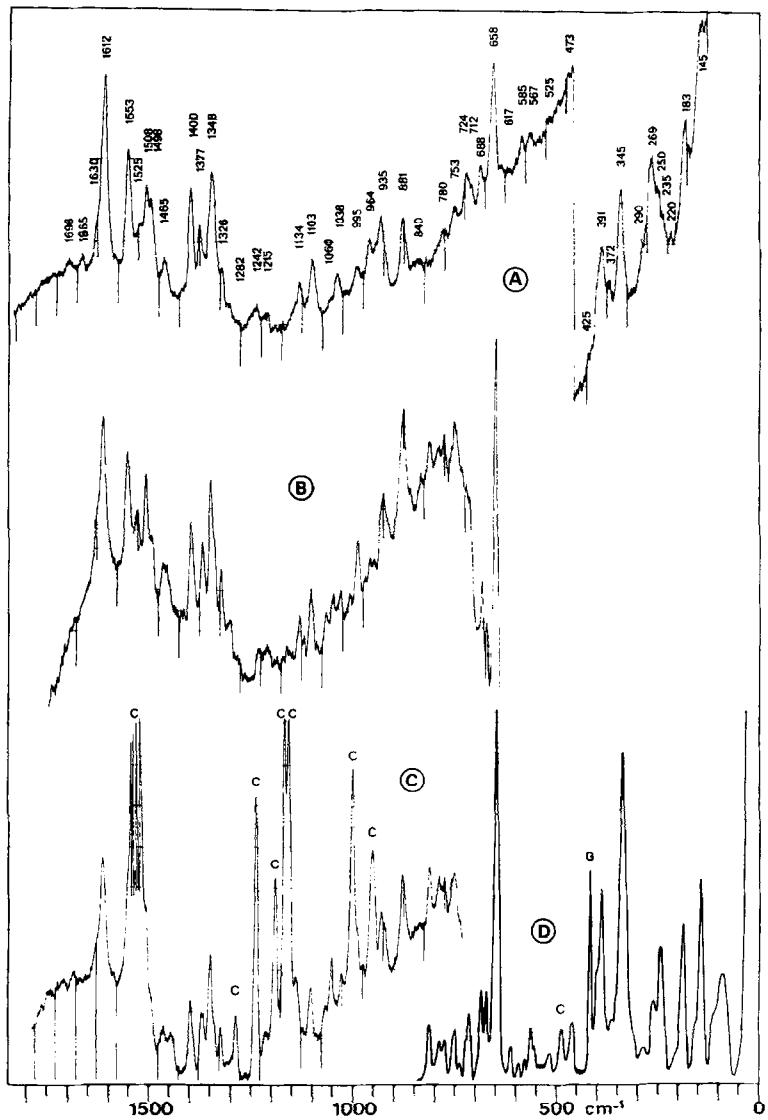

Figure 2 Resonance Raman spectra obtained at 35°K, 528.7 nm excitation, and 5.5 cm^{-1} resolution at 1000 cm^{-1} from :
A- Solid bacteriopheophytin cast from dry CCl_4
B - Reaction centers of R. spheroides R 26, 600-1750 cm^{-1} region.
C - Reaction centers of R. spheroides Y, 700-1750 cm^{-1} region.
C : RR bands of spheroidene.
D - Same sample as in C, 0-850 cm^{-1} region after subtraction of a 539 nm fluorescence band. Relative intensities and half bandwidths of RR bands may have been slightly altered by this operation.
G : spurious line.

reaction centers of R. spheroides Y and R 26, as shown on figures 1 and 2 by comparison with RR spectra of the isolated molecules. This fully confirms previous attributions of the 535 and 600 nm absorption bands of these reaction

centers to Q_x electronic transitions of BPheo and BChl, respectively (8,9).

Bacteriochlorophyll. BChl only contributes to the spectra of oxidized R 26 reaction centers obtained at 600 nm (Fig. 1B), while a very weak contribution of spheroidene occurs for the wild type (see lower). The low temperature spectra are remarkably sharp, as compared to those of solid BChl (Fig. 1A), which is known to assume in these conditions a variety of interaction states (6). As far as all four BChl molecules likely participate significantly to the 600 nm absorption of the oxidized reaction center, each of them should significantly participate to the present RR spectra, whatever, in particular, their oxidation state (9). These four contributions are nevertheless undistinguishable in the present conditions. The 768 and 777 cm^{-1} bands of the reaction centers, however, may result from splitting of the 763 cm^{-1} band of BChl *in vitro*. Such a splitting may correspond to the presence of two categories of BChl differing by their interaction states, as well as to exciton interaction among two identical pairs of interacting BChl molecules. A Fermi resonance involving a combination or harmonic mode (e.g. 2 x 389 cm^{-1}) may also account for this splitting. Interactions assumed by the four BChl are stronger than those occuring for solid BChl *in vitro*, as shown by the large intensity and frequency differences observed between homologous bands in both spectra (Table I). Most of these variations affect the region of planar sketetal deformations and the lower frequencies (150-950 cm^{-1}). The C = O stretching modes could not be studied, however, due to their weakness in Q_x resonance. The frequency shifts reach high values consistent with those observed in Chl-water aggregates (5). Moreover, all the RR bands of BChl in reaction centers which differ markedly in frequency and/or relative intensity from those of BChl *in vitro* correlate with RR bands of Chl which were shown to involve motions of Nitrogen and Magnesium atoms (7) (Table I). This fact also appeared to characterise Chl-water aggregates (5). Whatever the final interpretation of these spectra, they demonstrate that the four BChl molecules of the oxidized reaction center assume rather strong, but nearly identical interaction states.

Antenna BChl of Y and R 26 chromatophores yielded RR spectra of low signal to noise ratios, due to spurious fluorescence bands. They were found, however, to have their main features very close in relative intensities and frequencies to those observed for solid BChl. In particular, they exhibited bands at 262 and 295 cm^{-1}.

Bacteriopheophytin. Under 528.7 nm excitation, at 35°K, reaction centers of *R. spheroides* R 26 yield a RR spectrum arising mostly from BPheo *a* scattering (Fig. 2B). A number of supplementary bands and shoulders also arise. Most of them appear to couple with BPheo bands slightly shifted from their *in vitro* positions. These pairs occur at 1457-1467, 1055-1068, 1032-1050, 962-973,

TABLE 1. Differences observed between RR spectra of Bacteriochlorophyll *in vitro* and in reaction centers of *R. spheroides* R 26 at 35°K.

RR bands of BChl in reaction centers (cm^{-1})	Frequency shifts with respect to BChl in vitro (cm^{-1})	Relative intensity variations with respect to BChl in vitro	Relative frequency shifts on $^{15}N+^{26}Mg$ substitution ($10^3 \times \frac{\Delta\nu}{\nu}$)	
			Chl *a* *	Chl *b* *
1510	+8		3	
1425	+7			2
1342		-	2-4	2
1285	-5		6	5
1217	+5	+	7	2
1060	-5	+	7	
1035		-	3	
805	+10		6	
777	+12			4
768	+3			4
742	+7		8	5
692	+5	+	5	6
389	+5	+	10	3
363	+8	+	11	6
287	-8	+	17	13
240?	-20 ?	-		4
190	-8		24	

*: Solids at 35°K, cf reference 7.

925-932, 777-790 and 676-687 cm^{-1}; Moreover, Y reaction centers exhibit two weak RR bands at 1705 and 1685 cm^{-1}, both attributable to the stretching mode of the ketone C = O group of BPheo. This observation may well be related to the splitting of the 535 nm electronic band occuring at 77°K (8). Both phenomena may arise either from markedly different environmental interactions on each of the two BPheo molecules of the reaction center, or from excitonic coupling between these two molecules, if interacting. A number of other bands shift from their *in vitro* positions, e.g. at 1370 (-7 cm^{-1}), 1233 (-9), 1107 (+4) and 653 cm^{-1} (-5 cm^{-1}). Relative intensity variations also occur, particularly below 750 cm^{-1}. Thus the nuclear and π electronic interactions assumed by the two BPheo molecules, whatever their precise origin, are stronger than those occuring in the solid state. Absence of a ca 1665 cm^{-1} band indicates that the acetyl C = O groups of both molecules participate to these interactions.

Spheroidene. RR spectra of reaction centers of the wild type excited in the 515-530 nm region yield bands from a carotenoid (Fig. 2C). Although undoubtedly arising from spheroidene (10,11), this spectrum differs markedly in band positions and relative intensities in the 900-1400 cm^{-1} region from that of bulk spheroidene of the chromatophore. These latter molecules yield RR spectra which differ only in details from those of other natural carotenoids, either *in vitro*

or in vivo, even at low temperature (1, 2, 12). This alteration of the π electronic repartition on the polyene chain may result from an all trans to cis isomerisation, as suggested by both the present Raman data (13), and the 5 to 10 nm blue shift of the visible electronic bands of spheroidene of reaction centers with respect to those of bulk spheroidene. Spheroidene is thus very likely to be an intrinsic component of the reaction centers, as most recently suggested, too, by other experiments (11).

Finally, Y reaction centers yield RR spectra of BChl and BPheo presently undistinguishable from those obtained from the R 26 ones. Thus, any interaction between these molecules and spheroidene must be weak enough to insure this property.

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