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*edited by*

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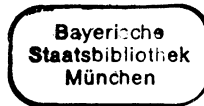
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STRUCTURES OF ANTENNA COMPLEXES AND REACTION CENTERS FROM  
BACTERIOCHLOROPHYLL B-CONTAINING BACTERIA :  
RESONANCE RAMAN STUDIES

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Resonance Raman (RR) spectroscopy yields detailed information about the structure and ground-state environmental interactions assumed by bacteriochlorophyll a (BChl a) and bacteriopheophytin a (Bpheo a) within bacterial pigment-protein complexes (1-3). Recent successes in crystallizing reaction centers (RC) from *Rhodospseudomonas viridis* renewed interest in BChl b- and Bpheo b-containing complexes (4). We here report the first RR spectra of isolated BChl b and Bpheo b, as well as of BChl b-containing antenna and reaction centers. Difficulties due to the high photooxydability of those pigments have been overcome by working at 20 K in anoxic conditions, and by selectively avoiding resonance of decay products (363.8 nm excitation).

RR SPECTRA OF ISOLATED BChL B AND BPHEO B

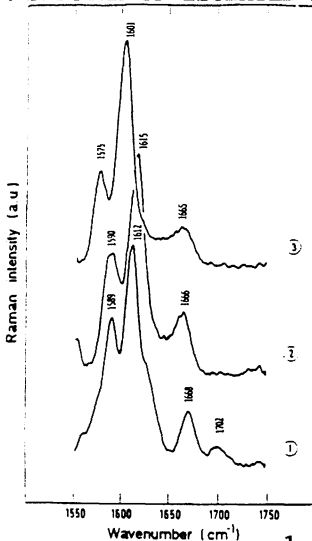


Fig 1 : 1550-1750  $\text{cm}^{-1}$  regions of RR spectra of

- 1) Bpheo b in methanol
  - 2) BChl b in hexane
  - 3) BChl b in methanol
- (T = 20 K ; exc. wv : 364 nm)

Comparison of BChl a and BChl b RR spectra shows that the presence of the ethylidene grouping conjugated to cycle II induces some large ( $> 6 \text{ cm}^{-1}$ ) frequency shifts, principally of bands at 697, 952, 1161, 1218, and 1444  $\text{cm}^{-1}$  (BChl a). Except the 1161  $\text{cm}^{-1}$  one, these modes are weakly sensitive to the  $^{14}\text{N} / ^{15}\text{N}$  substitution and thus should involve motions of the macrocycle periphery (3). BChl b yields two strong bands at 650 and 1351  $\text{cm}^{-1}$  (Bpheo b : 1347  $\text{cm}^{-1}$ ), which are missing in BChl a spectra. These modes do not appear to predominantly arise from the ethylidene grouping.

Differences observable between RR spectra of BChl b and of Bpheo b are very similar to those observed for the a derivatives (3). In particular, characteristic bands of phaeophytins at 270, 777, 1106, 1131 and 1590  $\text{cm}^{-1}$  are present in

Bpheo b RR spectra.

Free carbonyl stretching modes at  $1678$  and  $1700\text{ cm}^{-1}$  (fig 1.3) indicate that the 9-keto carbonyl stretching frequency is not affected by the presence of the additional C=C bond conjugated with cycle II, whereas the stretching mode of the 2-acetyl C=O is upshifted by about  $15\text{ cm}^{-1}$ .

Fig 1.2 and 1.3 compare the higher frequency regions of RR spectra of BChl b in a polar solvent (central Mg 6-coordinated) and self-aggregated in a non polar solvent (central Mg 5-coordinated). This clearly shows that the methine bridge stretching mode of BChl b is sensitive to the coordination state of the central Mg of the molecule, being located, as in BChl a, around  $1614\text{ cm}^{-1}$ , when 5-coordinated, and around  $1600\text{ cm}^{-1}$  when 6-coordinated.

#### INTERACTION STATES OF BChl B IN ANTENNA COMPLEXES

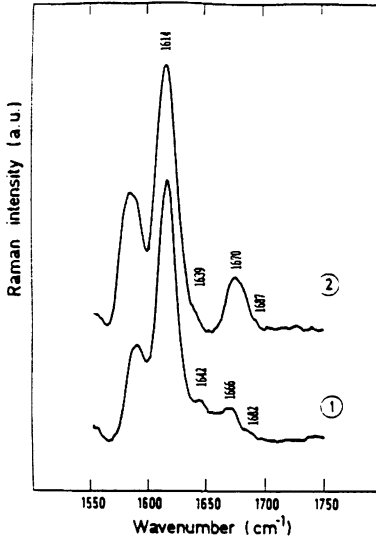


Fig 2 : RR spectra of :  
 1 : B 800-1020 complexes from *Ectothiorhodospira halochloris*  
 2 : Chromatophores from *Rhodospseudomonas viridis*

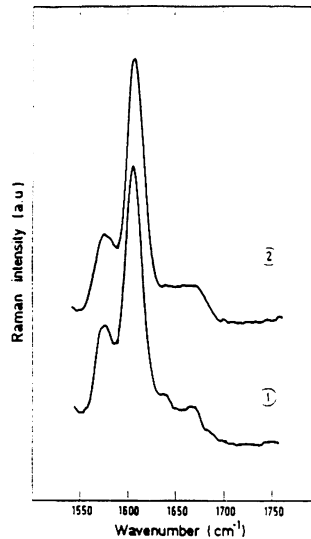


Fig 3 :  
 1 : cf fig. 2.1  
 2 : RR spectra of the same complex after HCl treatment

Fig 2.2 displays the higher frequency region of RR spectra of whole chromatophores of *Rps viridis* : because of the low (0.08) RC : antenna ratio, these spectra essentially arise from B 1015 antenna complexes alone. Fig 2.1 shows the same frequency range for purified B 800-1020 antenna complexes from *Ectothiorhodospira halochloris*. In both of these spectra, the frequency of the methine bridge stretching mode, being located around  $1613\text{ cm}^{-1}$ , clearly demonstrates that, as BChl a, BChl b is preferentially 5-coordinated when bound to protein.

In the carbonyl stretching frequency region, RR spectra of B 1015 complexes from *Rps viridis* are quite different from those of the supposedly (5) homologous BChl a-containing B 880 complexes

from Rhodospirillales (1). Indeed, if the number of conjugated C=O vibrators observable in the spectra (three) is consistent with the stoichiometry proposed for these complexes (2 BChl b / complex), the frequencies of these vibrators (1639, 1670 and 1687  $\text{cm}^{-1}$ ) are different from those observed in B 880 complexes (1). Indeed, the present spectra show that one acetyl carbonyl of one of the two BChl b is intermolecularly bound, vibrating at 1639  $\text{cm}^{-1}$ , whereas that of the other is free, vibrating at 1670  $\text{cm}^{-1}$ . Moreover, the latter Raman band is very likely degenerate, and most probably involves one keto carbonyl, then intermolecularly bound. The second keto group vibrates at 1687  $\text{cm}^{-1}$  and is only weakly interacting. One thus has to conclude that ground-state molecular interactions assumed by BChl b in B 1015 complexes differ from those assumed by BChl a in B 880 complexes : in the latter, which form a homogeneous class from a structural point of view (1), both of the acetyl carbonyls of the BChl a molecules are intermolecularly bound, and vibrate around 1643  $\text{cm}^{-1}$ .

RR spectra of B 800-1020 of halochloris show that at least four unequivalent BChl b are present in these complexes (table 1). This result agrees with the stoichiometry deduced from biochemical data (5 BChl b / complex)(6). Acid treatment of this complex induces a shift of the 1020 nm transition to 960 nm (6). This treatment affects the stretching frequencies of no more than two acetyl and two keto carbonyl groups (Fig 3 and Table 1). This confirms the hypothesis according to which (7) two out of the five BChl b molecules participate to the 1020 nm absorption band.

B 880 ( <i>Rsp rubrum</i> )	B 1015	B 800-1020 1630	viridis RC	sphaeroides RC
	1639		1628	1628
1644		1643 ↓	1634	1633
		1651	1654	
		1657		1660
		1664	1664 (?)	
1667	1671	1668 ↓	1671	
1674		1677		1678
	1681	1686 ↓	1684	1684
			1709 (?)	1705

Table 1 : compared frequencies of BChl b- and BChl a-containing complexes. (↓ : intensity variations induced by HCl treatment)

#### BCHL B-CONTAINING REACTION CENTERS

We obtained RR spectra of reaction centers from *Rps viridis* (fig 4). In the lower and medium frequencies regions of these spectra, the main bands predominantly arise from Bpheo b and BChl b appears to poorly contribute. However, in the higher frequency region, the low 1590 : 1615  $\text{cm}^{-1}$  intensity ratio indicates a strong participation of BChl b. Moreover, the carbonyl stretching frequency region does not contain all of the 4 frequencies that are observed when resonance is with the 535-545 nm transition, hence selectively enhancing Bpheo contributions(8). Such a frequency-dependent balance of BChl / Bpheo contributions is also observed in RR spectra of RCs from *Rhb sphaeroides* R 26

excited at 364 nm (Robert, B. unpublished results).

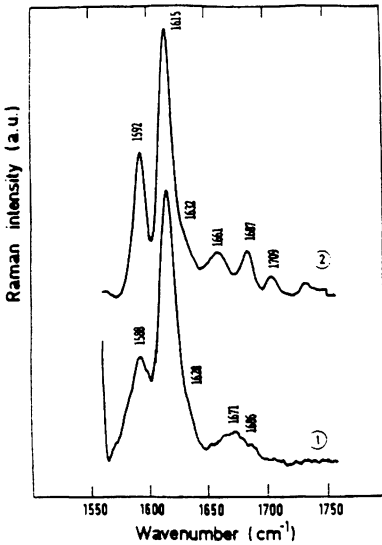


Fig 4 : RR spectra of reaction centers (1550-1750  $\text{cm}^{-1}$  region) from : 1, Rps viridis  
2, Rhb sphaeroides  
T = 20 K  
Excitation wavelength : 364 nm

The frequencies observed in the carbonyl stretching region of the spectra only partially match with those observed for Rhb sphaeroides (table 1). Some of these differences may not indicate differences in interaction states of conjugated carbonyls, but may arise from the above-mentioned difference in stretching frequencies of free acetyl C=O in BChl a and BChl b. For example, the 1670 and 1660  $\text{cm}^{-1}$  features observed for Rps viridis and Rhb sphaeroides RCs, respectively, may both arise from interaction-free acetyl groups. On the other hand, the 1628  $\text{cm}^{-1}$  frequency, which, in RR spectra of Rhb sphaeroides RCs arises from the 535nm-absorbing Bpheo (8), most probably arises, in RR spectra of Rps viridis RCs, from the primary donor (6, 9). A more detailed account of RR spectroscopy of the primary donor of Rps viridis is given in these Proceedings (9).

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