Effects of Vinyl Substitutions on Resonance Raman Spectra of (Bacterio)chlorophylls

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Soret resonance and Q,y preresonance Raman spectra are reported and compared for a series of (bacterio)chlorophylls. Chlorophyll a, 2-acetylchlorophyll a, bacteriochlorophyll a and 2-vinylbacteriochlorophyll a were studied in the non-protic solvent tetrahydrofuran. These experiments were designed to identify Raman bands corresponding to the stretching mode(s) of the vinyl group at the C-2 position of ring I of chlorophyll a and 2-vinylbacteriochlorophyll a, and to ascertain whether additional bands corresponding to C=C and/or C=C vibrations could be observed in the 1615-1660 cm\(^{-1}\) region. Raman spectra of chlorophyll a and 2-vinylbacteriochlorophyll a exhibit a 1625 cm\(^{-1}\) band, which is absent from the Raman spectra of 2-acetylchlorophyll a and bacteriochlorophyll a. It is assigned to the vC=Cv mode of the vinyl group. No other band can be definitively assigned to any mode predominantly arising from vinyl motions. The acetyl-containing molecules 2-acetylchlorophyll a and bacteriochlorophyll a give rise to a ca. 1070 cm\(^{-1}\) band, which appears to be related to the presence of the acetyl substituent. The 1615-1660 cm\(^{-1}\) region of the Raman spectra of all four derivatives did not contain any additional band which could be ascribed to modes involving the vCvC and/or vCvCvC coordinates.

INTRODUCTION

In photosynthetic bacteria and plants, bacteriochlorophylls (BChl) and chlorophylls (Chl), respectively, ensure the capture of light energy and its conversion into chemical potential energy. These molecules are generally found as cofactors bound to proteins. Most of the (bacterio)chlorophyll-binding proteins are membrane proteins and, although x-ray and electron crystallography have met with remarkable success in solving the structure of the reaction centre of purple bacteria and two antenna complexes, our knowledge of the structure of the different binding sites of the chlorophylls is still too little to understand the mechanisms by which the proteic moiety influences the pigments properties. Most of the information on chlorophyll structure and environments in vivo is obtained by spectroscopic methods, coupled with chemical and biochemical methods. Resonance Raman (RR) spectroscopy has proved to be one of the most effective methods for obtaining very detailed information on chlorophyll states in their native environment (for reviews see Refs 4 and 5).

Raman spectra of (B)Chl molecules obtained at resonance all contain more than 50 distinct bands and extracting their structural information content requires precise assignment of these bands. Because of the large size, complexity and low symmetry of the chlorophylls, these assignments are still far from comprehensive. Experimental data are still lacking, and only a few normal-mode calculations have been performed, using the QCFF/PI semi-empirical method. Only a few spectral regions have been used so far for characterizing the (B)Chl binding sites in proteins, i.e. a low-wavenumber region (100-350 cm\(^{-1}\)), which contains bands sensitive to the Mg isotopes and a high-wavenumber region (1600-1750 cm\(^{-1}\)). The latter region contains bands arising from the stretching modes of the conjugated carboxyl groups, which are sensitive to intermolecular interactions. It also contains a band at ca. 1610 cm\(^{-1}\), which arises from the stretching modes of methine bridges, and which is sensitive to the number of axial ligands on the central Mg atom of the molecule. Calculations performed by Donohoe et al. and by Boldt et al. predict, in addition to the 1610 cm\(^{-1}\) and a band in the 1620-1650 cm\(^{-1}\) range, of modes involving vCvC and vCvCvCvC internal coordinates in the RR spectra of both BChl a and Chl a. Resonance Raman experiments conducted on BChl a at various excitations did not reveal any bands at these wavenumbers. However, prereesonant Raman spectra of Chl a excited at 1064 nm contained a 1625 cm\(^{-1}\) band, the assignment of which is not obvious. Chl a possesses a conjugated vinyl substituent on ring I at the C-2 position, the C=C stretching mode of which could account for this 1625 cm\(^{-1}\) band. Early Raman studies of Chl a and of vinyl-containing chlorophylls at resonance with the Soret transition did
not reveal any intense band which could primarily arise, 
in the 1600–1650 cm⁻¹ region, from the stretching 
mode of the vinyl C–C bond.¹⁵ Similarly, comparisons 
of Raman spectra obtained at Soret resonance from 
nickel mepyropheophorbide a and from nickel mepyropheophorbide a did not reveal any sizeable activity 
of the vinyl group.⁶ These observations did not agree 
with interpretations of the RR spectra of iron protoporphyrin IX, which ascribed a band at ca. 1620 cm⁻¹ 
to the vinyl C–C stretch.¹⁶ This discrepancy was 
ascribed to a markedly out-of-plane conformation 
of the vinyl group.⁹,¹⁷,¹⁸

It was therefore considered useful to readdress this 
problem. In this work, we compare resonance and 
preresonance Raman spectra of Chl a and 2-acetyl-Chl 
a on the one hand and of BChl a and 2-vinyl-BChl a on 
the other. From these results we may conclude that (1) 
the 1625 cm⁻¹ feature observed in the RR spectra of 
Chl a is due to the presence of a vinyl group in this molecule, and thus that it should clearly involve the 
vC=C coordinate of the vinyl group, and (2) no addi-
tional vC=C and/or vC=C involving modes other 
than the 1610 cm⁻¹ mode can be observed for either 
Chl a or BChl a in any of the resonance conditions we 
used. Each of these conclusions has consequences for 
the use of resonance Raman data of chlorophylls, i.e. (1) 
the presence of a 1625 cm⁻¹ feature in an RR spectrum 
of a chlorophyll-containing sample may indicate the 
presence of a vinyl group on the chromophore and (2) 
the presence of a 1620–1640 cm⁻¹ feature in RR spectra 
of BChl a-containing samples unambiguously indicate 
the presence of hydrogen-bonded 2-acetyl carbonyl 
groups.

EXPERIMENTAL

Sample preparation

The pigments (Fig. 1) were prepared according to Smith 
and Calvis¹⁵ and Struck et al.²⁰ For Raman experi-
ments, the different pigments were dissolved in dry 
tetrahydrofuran (THF) at concentrations ranging from 
10⁻⁴ to 10⁻³ M. In this polar, non-protic solvent, in this 
concentration range, it was shown that the (B)Chl 
remain monomeric, that the central Mg atom is hexa-
coordinated and that the different conjugated carbonyl 
groups are free from intermolecular interactions.⁹

Raman spectroscopy

Fourier transform (FT) Raman spectroscopy was 
carried out as described by Mattioli et al.¹⁴ using a 
Bruker FRA 106 Raman module coupled with an IFS 
66 interferometer. Excitation radiation of 1064 nm was 
provided by a diode-pumped Nd:YAG laser. FT-
Raman spectra were recorded at room temperature with 
backscattering geometry. Liquid samples were held in a 
quartz tube. Each of the FT-Raman spectra presented is 
the result of the co-addition of 2000–10,000 interfero-
grams. Soret RR spectroscopy was carried out with a 
Jobin-Yvon U 1000 spectrometer equipped with a 
charge-coupled device (CCD) detector. Resonance 
Raman spectra were obtained at low temperature (ca. 
77 K) under conditions similar to those described by 
Robert and Lutz.²¹ Excitation radiation of 441.6 nm 
was provided by a He–Cd continuous laser (Liconix). 
Resonance Raman spectra presented here are the results 
of 1–5 min exposure per spectral element.

RESULTS AND DISCUSSION

Figure 2 displays resonance Raman spectra (441.6 nm 
excitation) and FT-Raman spectra (1064 nm excitation) 
of Chl a (traces 1 and 3) and 2-acetyl-Chl a (traces 2 and 
4), all in the higher wavenumber range (1550–1750 
cm⁻¹). The FT-Raman spectrum of Chl a (trace 3) 
exhibits a distinct band at 1596 cm⁻¹ arising from a 
methine bridge stretching mode;⁴ this low frequency 
indicates that the central Mg atom has two external 
ligands.¹³ Another band is present at above 1684 cm⁻¹ 
in spectra 1 and 3, which arises from the stretching 
mode of the C-9-keto carbonyl group of Chl a. Its high 
frequency indicates that this group is free from any 
bonding interaction. Both of these bands are present in 
the FT-Raman spectrum of 2-acetyl-Chl a (trace 4), as 
expected. A band at 1624 cm⁻¹, which is clearly active 
in the 1064 nm-excited spectrum of Chl a (trace 3), is 
absent from the spectra of 2-acetyl-Chl a, and is 
replaced by a mode at 1660 cm⁻¹ (trace 4). The latter 
band can be safely ascribed to the stretching mode of 
the 2-acetyl carbonyl group considering that the 2-
Figure 2. Resonance Raman spectra (1580–1750 cm⁻¹) of Chl a (1 and 3) and 2-acetyl-Chl a (2 and 4) dissolved in THF, excited at 441.6 (1 and 2) and 1064 nm (3 and 4). Visible-excited RR spectra were recorded at 77 K and Raman spectra excited at 1064 nm were recorded at room temperature.

Figure 3. Resonance Raman spectra (1580–1750 cm⁻¹) of BChl a (1 and 3) and 2-vinyl-BChl a (2 and 4) dissolved in THF, excited at 1064 nm (1 and 2) and 363.8 nm (3 and 4). UV-excited RR spectra were recorded at 77 K and Raman spectra excited at 1064 nm were recorded at room temperature.

Figure 3 displays the high-wavenumber regions (1550–1750 cm⁻¹) of the RR spectra of BChl a (traces 1 and 3) and 2-vinyl-BChl a (traces 2 and 4) obtained with 1064 and 363.8 nm excitation. In the BChl a spectra, at both of these excitation wavelengths, the methine stretching band is observed at ca. 1595 cm⁻¹ and, as already mentioned for Chl a, there is no band in the 1600–1660 cm⁻¹ range in these spectra which could be attributed to skeletal modes. As a result of temperature effects (work in progress), this band appears at ca. 1607 cm⁻¹ in spectra 3 and 4. Two bands at 1659 and 1689 cm⁻¹ arise from the stretching modes of the 2-acetyl and 9-keto groups, respectively. In the 2-vinyl-BChl a spectra (traces 2 and 4), the 1660 cm⁻¹ band is not observed, and is replaced by a mode at 1624 cm⁻¹. It should also be noted that the presence of the vinyl group (or the removal of the 2-acetyl group) is accompanied by a 10 cm⁻¹ downshift of the wavenumber of the stretching mode of the 9-keto carbonyl group from ca. 1689 to 1679 cm⁻¹. As for Chl a, the 1624 cm⁻¹ band is stronger in preresonance conditions with the Q₁ electronic transition than in resonance conditions with the Soret electronic transition of 2-vinyl-BChl a. The fact that this band disappears when the vinyl group is removed from the Chl a molecules, and the fact that adding a vinyl group to BChl a results in the presence of such a 1624 cm⁻¹ band, lead to the conclusion that this mode does arise from the stretching frequency of the vinyl substituent at the C-2 position on the chlorin ring. Extensive Raman studies of the vinyl group in various organic compounds²² and in metal porphyrins²²,²³,²⁴ have provided precise wavenumber ranges for the Raman-active modes of this group.
Figures 4 and 5 display the RR spectra of Chl a and 2-acetyl-Chl a with 1064 and 441.6 nm excitation, respectively. These spectra exhibit many relative intensity differences and wavenumber shifts. A weak 1327 cm\(^{-1}\) band is present in Chl a spectra excited at 1064 nm which might arise from the vinyl CH\(_2\) scissoring mode. This band is missing in the spectra of 2-acetyl-Chl a. However, in the RR spectra of nickel methyl-pherophorhide, the band which has been attributed to the vinyl scissoring mode is weak in resonance conditions with the Soret electronic transition (i.e. in resonance with the B state) and is clearly observable only in resonance conditions with the Q\(_{y}\) transition.\(^6\) In the case of Chl a, the 1325 cm\(^{-1}\) band is intense in resonance at 441.6 nm, i.e. in resonance with the B state (Fig. 5). It is worth noting that, on replacing the vinyl group with an acetyl at the C-2 position on the chlorophyll macrocycle, many other perturbations of the RR spectra are observed, which probably result only indirectly from the presence of vinyl and/or acetyl groups, and thus cannot be safely used for diagnosing the chemical structure of a molecule. This is true, in particular, when comparing BChl a and 2-vinyl-BChl a (see Figs 6 and 7): the RR spectra of these two molecules differ in many spectral regions, but no band other than the 1625 cm\(^{-1}\) band can be safely attributed to vinyl contributions.

In resonance with the B transition, the presence of an acetyl group as a substituent at the C-2 position on the (B)Chl macrocycle appears to enhance a ca. 1070 cm\(^{-1}\) band. The RR spectra of 2-acetyl-Chl a contain an intense band at 1068 cm\(^{-1}\) which is very weak in the RR spectra of Chl a molecules (Fig. 5). A similar band is observed at 1072 cm\(^{-1}\) in the RR spectra of BChl a molecules and is absent in those of 2-vinyl-BChl a (Fig. 7). For BChl a molecules, this mode has been reported to be sensitive to \(^{14}\text{N}/^{15}\text{N}\) isotope substitutions in BChl a (it shifts by 7 cm\(^{-1}\) ) and has accordingly been attributed mainly to CN contributions.\(^4\) Similarly, a band was observed at 1071 cm\(^{-1}\) in the RR spectra of nickel deutero-1071 cm\(^{-1}\) it had no counterpart in the RR spectra of formyl nickel deutero-

![Figure 4](image-url)  
**Figure 4.** RR spectra of Chl a and 2-acetyl-Chl a in THF (1000-1750 cm\(^{-1}\) range). Excitation at 1064 nm; samples at room temperature.

![Figure 5](image-url)  
**Figure 5.** RR spectra of Chl a and 2-acetyl-Chl a in THF (1000-1750 cm\(^{-1}\) range). Excitation at 441.6 nm; samples at 77 K.
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porphyrins and of deuteroporphyrin. However, the frequency of these bands does not match with any characteristic frequency expected for in-plane acetyl modes.\textsuperscript{25} Despite this fact, RR data now available on four acetyl-substituted porphyrin derivatives suggest that a 1070 cm\textsuperscript{-1} band in the RR spectra may constitute a reliable marker for this substituent.

In summary, the absence of observable bands in the 1620–1660 cm\textsuperscript{-1} region of the RR spectra of BChl \textit{a} and 2-acetyl-Chl \textit{a}, at resonance either with the \textit{B} states or with the \textit{Q} \textsubscript{2} state, indicates that no mode predominantly involving the \textit{v}C=C\textsubscript{m} coordinates should occur at wavenumbers higher than 1615 cm\textsuperscript{-1}. The 1625 cm\textsuperscript{-1} band of the vinyl-containing compounds should not itself involve these coordinates as it is insensitive to Mg coordination (see below and Ref. \textsuperscript{26}). It therefore appears clear that, in the RR spectra of BChl \textit{a}, the presence of a band in the 1620–1660 wavenumber region is likely to indicate the presence of hydrogen-bonded acetylcarbonyls in the sample considered.

On the other hand, this study has demonstrated that a mode predominantly involving the vinyl C=C stretching coordinate is present in the RR spectra of Chl \textit{a}. This band is more clearly observed in spectra excited in preresonance conditions, at 1064 nm, from Chl \textit{a} samples in which the Mg is hexacoordinated rather than pentacoordinated. Indeed, in the RR spectra of chlorophylls with a pentacoordinated Mg atom, the \textit{v}C=C\textsubscript{m} band occurs around 1610 cm\textsuperscript{-1} and not around 1595 cm\textsuperscript{-1} as in hexacoordinated samples. In these conditions, the vinyl \textit{v}C=C band still occurs close to 1624 cm\textsuperscript{-1}, and hence is only observed as a weak

Figure 6. RR spectra of BChl \textit{a} and 2-vinyl-BChl \textit{a} in THF (1000–1750 cm\textsuperscript{-1} range). Excitation at 1064 nm; samples at room temperature.

Figure 7. RR spectra of BChl \textit{a} and 2-vinyl-BChl \textit{a} in THF (1000–1750 cm\textsuperscript{-1} range). Excitation at 363.8 nm; samples at 77 K.
shoulder on the vC₃C₄ band. This is the reason why this mode may be difficult to see in proteins as the central Mg atom of Chl a is usually pentacoordinated in vivo. Through analysis of the vC₃C₄ bandshape, however, it may be identified in the RR spectra of pentacoordinated samples and in any resonance conditions including the Soret transitions. This mode may therefore safely be used for distinguishing Chl a-type cofactors from other chlorophylls such as BChl c which do not contain vinyl substituents.

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REFERENCES