Q_y-Excitation Resonance Raman Spectra of Bacteriochlorophyll Observed under Fluorescence-Free Conditions. Implications for Cofactor Structure in Photosynthetic Proteins

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Resonance Raman (RR) spectroscopy is an attractive technique for probing photosynthetic proteins because the frequencies and intensities of the resonance-enhanced vibrational bands provide information on both the ground and excited states of the photophysically active chromophores.[1] Early RR studies of photosynthetic proteins were conducted exclusively with UV−vis excitation, in resonance with the B or Q_x absorptions of the (bacterio)chlorin pigments.[1a] More recently, RR studies have been extended to the near-infrared region where the photophysically important Q_y states absorb.[2,3] Q_y-excitation RR studies on bacterial reaction centers have allowed selective interrogation of the special pair of bacteriochlorophylls (BChs),[2b−e,3] which serves as the primary electron donor, the two monomeric accessory BChs,[2c−e,3] and the two bacteriopheophytins (BPhs),[2a,d,e] one of which serves as the primary electron acceptor. The acquisition of Q_y-excitation RR data for reaction centers is possible because the high level of fluorescence, which is intrinsic to free BCh or BPh,[4] is strongly quenched in the protein[5] by ultrafast energy[6] and electron-transfer processes.[7] Q_y-excitation RR experiments on reaction centers are still difficult, however, owing to fluorescence from small amounts of adventitious pigments or intact, dysfunctional proteins. The RR studies on reaction centers have shown that the Q_y-excitation scattering characteristics of both the special pair and the accessory BChs[2b−e,3] are distinctly different from those observed with either B or Q_x excitation.[1,2e] In particular, Q_y excitation results in strong scattering from low-frequency modes (<500 cm^{-1}) accompanied by much weaker scattering from high-frequency vibrations (1300−1750 cm^{-1}).[2] The opposite is generally the case for B and Q_x excitation.[1] The Q_y-excitation RR studies of reaction centers also reveal that the frequencies and relative intensities of the low-frequency modes of the special pair are different from those of the accessory BChs.[2c,3] It remains an open question whether the Q_y-excitation RR scattering characteristics of the special pair and the accessory BChs reflect structural and electronic properties unique to reaction centers and whether the low-frequency modes of the special pair are coupled to the initial electron-transfer process.

The first step in assessing the effects of the protein environment on the properties the different cofactors in reaction centers is the comparison of the RR scattering characteristics of these BChs with those of the pigment external to the protein matrix. This approach is feasible for spectra obtained with either B or Q_x excitation.[1] In contrast, Q_y-excitation RR spectra have not been reported for free BCh (or any other photosynthetic pigments) due to the large fluorescence intrinsic to the lowest-energy singlet excited state.[4] Consequently, various alternative approaches have been taken to gain information on the Q_y scattering characteristics of photosynthetic pigments. Several groups have examined pigments in which the naturally occurring Mg(II) ion has been replaced with open shell metals such as Ni(II), Cu(II), or Fe(III).[8] The open shell metals quench the fluorescence; consequently, Q_y-excitation RR spectra can be acquired. Cotton and co-workers have been successful in obtaining high-frequency (600−1750 cm^{-1}) Q_y-excitation RR spectra of the natural plant pigment chlorophyll using surface-enhanced Raman techniques.[9] Here, association with the surface quenches the emission. Others have used FT-Raman spectroscopy to examine natural photosynthetic pigments.[10] These latter experiments avoid interference from fluorescence because the excitation wavelength (1064 nm) is far to the red of the emission. Although useful, all of the above strategies have their limitations. Replacement of the Mg(II) ion with other metals alters the conformation of the macrocycle and hence perturbs its vibrational frequencies and RR scattering characteristics.[8] Such perturbations may also result upon absorbing BCh on a surface. In the FT-Raman experiments, the excitation wavelength is far to the red of the Q_y absorption maximum and, for reaction centers, the RR enhancement pattern is quite different from that observed with resonant excitation.[3,10]

The importance of obtaining Q_y-excitation RR spectra of free photosynthetic pigments prompted us to renew the search for conditions under which interference from fluorescence can be mitigated. In this communication, we show that rigorously dried solid films of BCh exhibit sufficiently attenuated emission that Q_y-excitation RR spectra can be acquired. These spectra represent the first Q_y-excitation RR spectra of BCh external to a protein matrix. The preliminary studies show that certain RR scattering characteristics of BCh in the film are similar to those of the BChs in reaction centers whereas others are very different.

The films were prepared by depositing BCh in dry CCl_4 solution onto a quartz window or directly onto the cold tip of a closed-cycle He refrigerator. The solvent was evaporated, and the film was placed under moderate vacuum (10^{-3} Torr) for an extended period (24 h). Films examined before vacuum drying or with drying for only a few hours were found to be highly fluorescent. The fluorescence gradually disappears over the course of a day and reaches a level 4−5 orders of magnitude below its initial value. In contrast, the absorption spectra of the fluorescent and nonfluorescent BCh films are nearly indentical (not shown). The Q_y bands of the BCh film (795

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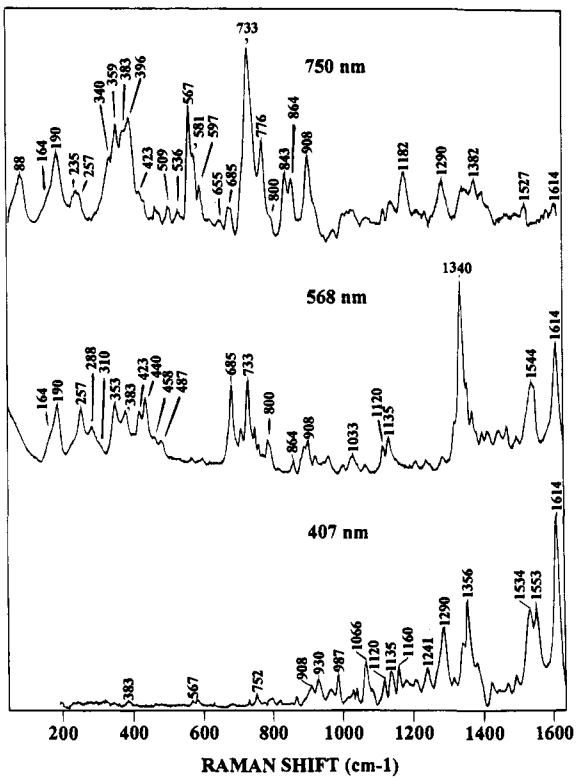

Figure 1. RR spectra of BCh films obtained with Q_y (λ_{ex} = 750 nm), Q_x (λ_{ex} = 568 nm), and B (λ_{ex} = 407 nm) excitation. The RR intensities are corrected for spectrometer and detector response and the λ^{-4} dependence of scattered light. The laser powers were typically 5 mW; the temperature was 15 K.

nm) are slightly broadened and red-shifted compared with those typically observed in solution (770−780 nm).[11] The ~300-cm^{-1} red shift of the Q_y band is suggestive of exciton coupling similar to that which occurs upon dimerization of BCh in dry, noncoordinating solvents.[12] For the solution dimers, the electronic coupling strongly quenches the fluorescence emission.[13] In our experience, however, the solutions can never be made dry enough to reduce the fluorescence to a level which allows the acquisition of Q_y-excitation RR spectra.

The RR spectrum of the BCh film obtained with λ_{ex} = 750 nm is shown in Figure 1 (top trace).[14] This wavelength falls on the blue side of the $Q_y(0,0)$ band where the extinction coefficient is approximately one-half that of λ_{max}. For comparison, Q_x-excitation (λ_{ex} = 568 nm) and B-excitation (λ_{ex} = 407 nm) RR spectra of the film (middle and bottom traces, respectively) are also shown in Figure 1. These latter spectra are similar to those previously reported for BCh films with B and Q_x excitation.[1] Inspection of the figure reveals that the spectral characteristics observed with Q_y excitation are different from those observed with either Q_x or B excitation. The differences include the number, frequency, and relative intensities of the observed RR bands. One striking aspect of the Q_y-excitation RR spectrum of the BCh film is that the low-frequency modes (<500 cm^{-1}) are much more strongly enhanced than the high-frequency vibrations (1300−1750 cm^{-1}). This pattern parallels that observed in the Q_y-excitation RR spectra of the special pair and the accessory BChs in reaction centers.[2] Collectively, these observations indicate that strong enhancement of low-frequency modes is a general characteristic of Q_y excitation of BCh rather than a specific property of BCh in reaction centers.

Although many of the Q_y-excitation RR scattering characteristics observed for the BCh film are similar to those of the cofactors in reaction centers, there are certain notable differences. These differences are most pronounced in the low-frequency region of the RR spectrum. In this region, the frequencies and relative intensities observed for BCh in the film are generally quite different from those observed for either the special pair or the accessory BChs in reaction centers (see refs 2c and 3 for the reaction center data). The fact that the low-frequency Q_y-excitation RR spectrum of the BCh film differs from that of the cofactors in the protein is most likely due to structural variations among the chromophores. Collectively, these observations indicate that the low-frequency RR scattering characteristics of BCh are extremely sensitive to the specific environment in which the chromophore resides. These environmental factors could influence the exact conformation of the macrocycle and/or the specific orientation of the substituent groups. In the case of metalloporphyrins, it is well documented that variations in these structural properties have large effects on the low-frequency RR spectra.[15] The possibility that macrocycle conformation plays a significant role in determining the low-frequency scattering characteristics of BCh is supported by the fact that the RR features of Cu(II)-substituted BCh[8b] are very different from those of BCh in either the film or reaction centers. In particular, the Q_y-excitation spectrum of the Cu(II) complex is characterized by a pattern of weak low- and strong high-frequency modes, opposite the intensity pattern observed for the natural Mg(II) containing pigment (vide supra). The diminished low-frequency RR activity observed for the Cu(II) complex could be due to a more planar macrocycle resulting from four- rather than five-coordination. Regardless of the exact determinants of the low-frequency RR scattering characteristics of BCh, the results reported herein indicate that the differences observed in the Q_y-excitation RR signatures of the special pair versus the accessory BChs in reaction centers are not necessarily due to unique properties arising from the dimeric nature of the special pair. A full description of the low-frequency vibrational characteristics of BCh, both in the film and in reaction centers, will require further studies which include isotopically labeled BCh. Such studies are currently in progress.

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