

RESONANCE RAMAN SPECTRA OF LIGHT-HARVESTING BACTERIOCHLOROPHYLL *a* IN
PIGMENT-PROTEIN COMPLEXES FROM PURPLE PHOTOSYNTHETIC BACTERIA

Hidenori HAYASHI,* Hiro-o HAMAGUCHI, and Mitsuo TASUMI

Department of Chemistry, Faculty of Science, The University of Tokyo,
Hongo, Bunkyo-ku, Tokyo 113

Resonance Raman spectra of light-harvesting bacteriochlorophyll (Bchl)-protein complexes from several species of purple photosynthetic bacteria were measured. Some differences in the Raman spectra were found between the Bchl molecules (in the complexes) which give an absorption band in the 870-890 nm region and those which give absorption bands in the 800-850 nm region.

Elucidation of the state of the chlorophyll molecules *in vivo* is essential for understanding the primary processes of photosynthesis. Many physical and chemical methods have been used for this purpose. Resonance Raman spectroscopy provides a unique technique for probing the state of chlorophyll *in vivo*, because the Raman bands arising from the chlorophyll moiety can be selectively observed using a laser line in resonance with the electronic absorption of chlorophyll.¹⁾

Bacteriochlorophyll *a* (Bchl) is a major photosynthetic pigment of purple photosynthetic bacteria. More than 90% of the Bchl molecules in the photosynthetic bacteria act as light-harvesting antennae. Bchl extracted in an organic solvent exhibits an absorption band at 770 nm due to the Q_y transition, while Bchls in chromatophores show a variety of absorption bands in the 800-900 nm region. The absorption bands of Bchls in chromatophores are typically observed at 800, 850, and 870 nm. These absorption bands are usually called B800, B850, and B870, respectively. This multiplicity of the absorption bands must be related to the inhomogeneity of the Bchl states *in vivo*. Actually a variety of Bchl-protein complexes have been prepared from purple photosynthetic bacteria, and they show the near-infrared absorption bands different from each other.²⁾

Comparison of the resonance Raman spectra of Bchl-protein complexes having different absorption spectra will give new information on the Bchl state *in vivo*. Preliminary data have been reported by Lutz³⁾ on complexes from the R-26 mutant of *Rhodospseudomonas sphaeroides* (mainly containing B870 Bchl) and complexes from the wild type of *R. sphaeroides* (mainly containing B800-850 Bchl). The purpose of the present study is to clarify the relationship between the Bchl state *in vivo* and its absorption type by measuring the resonance Raman spectra of a number of Bchl-protein complexes (and chromatophores) from several strains of purple photosynthetic bacteria. These materials were classified into two groups; one showing the B870 absorption band and the other showing the B800 and/or B850 absorption band(s).

Chromatophores and Bchl-protein complexes were prepared from the carotenoidless mutant of *R. sphaeroides* and the wild types of *R. sphaeroides*, *Rhodospirillum rubrum*, *Rhodopseudomonas palustris*, and *Chromatium vinosum*, according to the procedures reported previously.^{4,5)} The Bchl-protein complexes obtained by these procedures retained the native state of Bchl as judged by their absorption spectra. The Raman spectra were measured at room temperature with the 363.8 nm line of an Ar⁺ laser (NEC GLG3300) which was resonant with the Soret band of Bchl. The absorbance at 370 nm varied between 2-5 from sample to sample. The 351.1 nm laser line and plasma emissions from the laser tube were separated from the 363.8 nm line with a quartz prism. The laser power was less than 2.5 mW at the sample point. To suppress the sample degradation with the laser irradiation, a rotating cell was used. No appreciable change was detected in the absorption spectra recorded before and after each Raman measurement. A Raman spectrometer consisting of a Spex 1401 double monochromator and a photon counting system was used.

In Fig. 1 are shown the Raman spectra in the 1700-1000 cm⁻¹ region and the absorption spectra in the 800-900 nm region observed for various samples which have an absorption band in the region of 870-890 nm. Although the first two samples were chromatophores, they were practically free from the B800-850 complexes and they showed an absorption band at 870-880 nm and a weak band at 800 nm. Accordingly, we classified these two materials into the same category as the B870-reaction center (RC) complexes. The resonance Raman spectra of the B870 complexes which were obtained from the carotenoidless mutant of *R. sphaeroides* and the wild type of *R. palustris* by the removal of reaction centers were practically the same as those of the B870-RC complexes, although a slight intensity decrease of the band at 1580 cm⁻¹ (probably attributable to bacteriopheophytin in the reaction center¹⁾) was observed. Thus, the Raman spectra in Fig. 1 are due to the Bchls *in vivo* which give the absorption band in the 870-890 nm region (B870 Bchl).

In Fig. 2 are shown the resonance Raman and absorption spectra observed for the B800-850 complexes. The absorption spectra of these materials

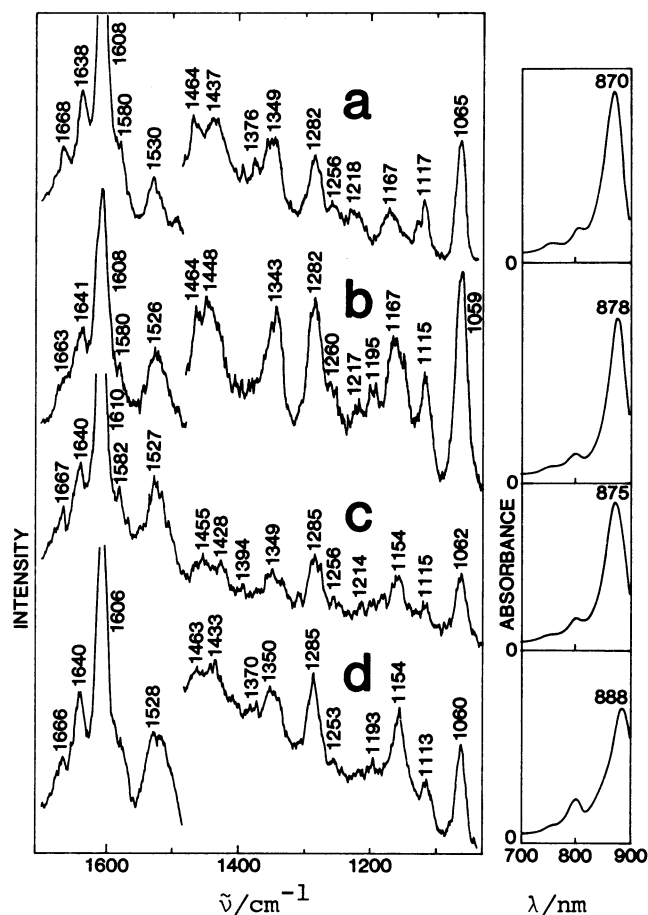


Fig. 1. Resonance Raman spectra (left) and near-infrared absorption spectra (right) of Bchls in a) chromatophores from the carotenoidless mutant of *R. sphaeroides*, b) chromatophores from *R. rubrum*, c) B870-RC complex from *R. palustris*, and B890-RC complex from *C. vinosum*. The resonance Raman spectra were measured with a 363.8 nm excitation light, at room temperature.

are varied from sample to sample with respect to the relative height of the absorption bands at about 850 and 800 nm or the band width of the band at 800 nm. In particular, the complex from *R. palustris* cultured with a low-intensity light shows an absorption spectrum markedly different from the others. However, this complex should be classified into the B800-850 complex, because it has a shoulder at 850 nm in addition to the strong band at 803 nm in the absorption spectrum, and its fluorescence spectrum and protein properties are similar to those of the B800-850 complex from *R. palustris* cultured with a high-intensity light.^{5,6)} Thus, the Raman spectra shown in Fig. 2 are due to the Bchls *in vivo* which give the absorption bands in the 800-850 nm region (B800-850 Bchl).

Many Raman bands are commonly observed for all the samples, such as the strong band at 1610 cm^{-1} , and the bands at $1670-40$, $1530-20$, 1350 , 1285 , 1115 , and 1060 cm^{-1} . The Raman spectra in Figs. 1 and 2 are similar to that of the water-soluble Bchl-protein complex obtained from green photosynthetic bacteria,⁷⁾ but do not coincide with those of monomeric or aggregated Bchls *in vitro*.^{1,8)} This implies that the Raman spectra observed in this study are those characteristic to Bchls bound to a protein, namely Bchls in protein complexes.

However, there are new findings regarding the differences between the Raman spectra of B870 Bchl and B800-850 Bchl. All the B870 Bchls in chromatophores and complexes show a distinct band at 1640 cm^{-1} , while the B800-850 complexes exhibit a band at about 1630 cm^{-1} which is observed as a shoulder of the strong band at 1610 cm^{-1} . For the B800-850 complex from the wild type of *R. sphaeroides*, Lutz observed the Raman bands at 1642 and 1635 cm^{-1} and related these bands to the 850 nm and 800 nm absorption bands, respectively.³⁾ However, as shown in Fig. 2, the B800-850 complexes (except for the B800-850 complex from *C. vinosum*) do not show a distinct band at 1640 cm^{-1} , in contrast with the B870 Bchl. The B800-850 complex from *C. vinosum* has a Raman band at 1645 cm^{-1} , but its intensity is relatively low compared with that of the band at 1640 cm^{-1} of the B870 Bchl. According to the infrared studies of Bchl *in vitro*,⁹⁾ and the resonance Raman studies by the Lutz group,^{1,7)}

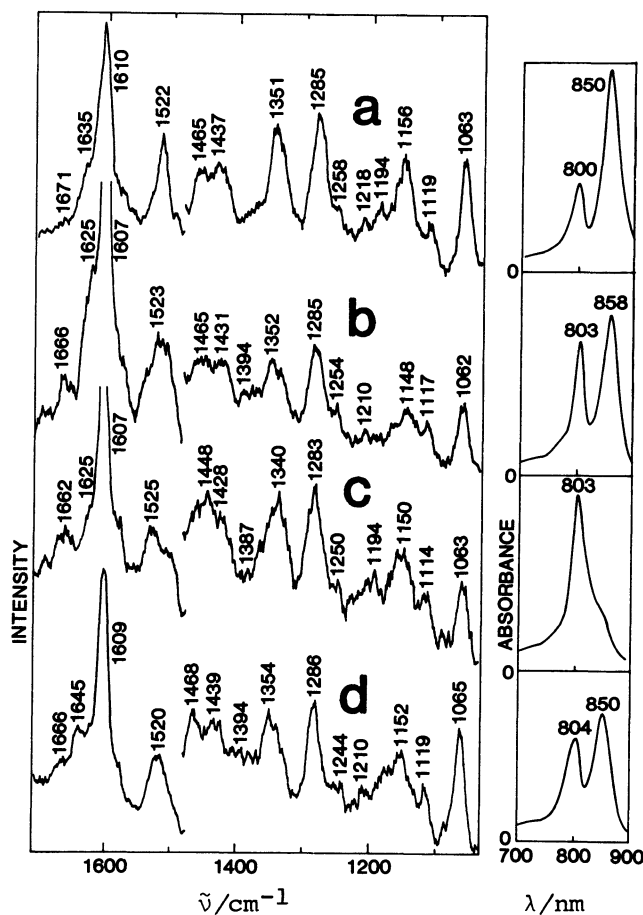


Fig. 2. Resonance Raman spectra (left) and near-infrared absorption spectra (right) of Bchls in B800-850 complexes from a) *R. sphaeroides*, b) *R. palustris* cultured with a high-intensity light (ca. 10,000 lux), c) *R. palustris* cultured with a low-intensity light (ca. 300 lux), and d) *C. vinosum*. The resonance Raman spectra were measured with a 363.8 nm excitation light, at room temperature.

the Raman band at $1640\text{-}30\text{ cm}^{-1}$ are attributed to the carbonyl group ($\text{C}_2=\text{O}$) of Bchl bound to the protein moiety. The differences in the Raman spectra between the B870 Bchl and B800-850 Bchl seem to reflect the difference in the environment of this carbonyl group.

The Raman band observed at 1640 cm^{-1} commonly for the B870 Bchl may indicate the similarity of the interaction between Bchl and its environment in the materials classified in the B870-RC complex. On the other hand, the Raman bands at $1650\text{-}25\text{ cm}^{-1}$ observed for the B800-850 Bchls are generally weaker in intensity than the 1640 cm^{-1} band of the B870 Bchl. In addition, they are more varied in frequency and in intensity from sample to sample (Fig. 2). These results agree with the observations that the B800-850 complexes exhibit more varieties in absorption spectra, circular dichroism, and protein properties than the B870-RC complexes, depending on their sources (bacterial species) and culture conditions.^{5,6)}

In addition to the Raman bands at the carbonyl stretching region, there are some bands which appear to be different for the B870 Bchl and B800-850 Bchl. The bands at $1530\text{-}25\text{ cm}^{-1}$ observed in the spectra of the B870-RC complexes shift to $1525\text{-}20\text{ cm}^{-1}$ in those of the B800-850 complexes. The relative intensity of the bands at 1060 and 790 cm^{-1} (not shown in figures) appears to be different between the B870-RC and B800-850 complexes.

There are some bands whose frequencies and intensities varied slightly from sample to sample, such as two or three overlapping bands at $1350\text{-}40$ or $1470\text{-}30\text{ cm}^{-1}$, weak bands at $1250\text{-}1180$ and around 1400 cm^{-1} , and the band superimposed with the Raman band of carotenoids at 1150 cm^{-1} . However, at present, it is not possible to find any relationship between these Raman bands and the Bchl absorption types.

In conclusion, it is now clear that a significant difference exists at least in the $1650\text{-}1620\text{ cm}^{-1}$ region between the Raman spectra of the B800-850 and B870 complexes. This probably reflects the environmental differences around the $\text{C}_2=\text{O}$ group of Bchls in these two types of complexes. Whether the differences of the absorption spectra in the $800\text{-}900\text{ nm}$ region can be explained by these environmental differences is to be studied in future.

References

- 1) M. Lutz, J. Kleo, and F. Reiss-Husson, *Biochem. Biophys. Res. Commun.*, **69**, 711 (1976).
- 2) R.J. Cogdell and J.P. Thornber, *FEBS Lett.*, **122**, 1 (1980).
- 3) M. Lutz, *Photosynth., Proc. Int. Congr.*, **3**, 461 (1981).
- 4) K. Sauer and L.A. Austin, *Biochemistry*, **17**, 2011 (1978).
- 5) H. Hayashi, M. Miyao, and S. Morita, *J. Biochem.*, **91**, 1017 (1982).
- 6) H. Hayashi, M. Nakano, and S. Morita, *J. Biochem.*, **92**, 1805 (1982).
- 7) M. Lutz, A.J. Hoff, and L. Brehamet, *Biochim. Biophys. Acta*, **679**, 331 (1982).
- 8) T.M. Cotton and R.P. Van Duyne, *J. Am. Chem. Soc.*, **103**, 6020 (1981).
- 9) J.J. Katz, R.C. Dougherty, and L.J. Boucher, "The chlorophyll," ed by L.P. Vernon and G.R. Seely, Academic Press, New York (1966), p.185.

(Received September 14, 1983)