FT-IR and Near-Infrared FT-Raman Study of Aggregation of Bacteriochlorophyll *c* in Solutions: Evidence for Involvement of the Ester Group in the Aggregation†

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ABSTRACT: Ultraviolet−visible (UV−Vis) absorption, Fourier-transform infrared (FT-IR), and near-infrared (NIR)-excited FT-Raman spectra have been measured for bacteriochlorophyll *c* (BChl-*c*) in acetone, tetrahydrofuran (THF), pyridine-d_5, carbon disulfide (CS_2), and water-saturated carbon tetrachloride (CCl_4) to investigate its aggregation *in vitro*. The UV−Vis absorption spectra can be classified into two groups. Group I (acetone, THF, and pyridine-d_5 solutions) gives a spectrum with a Q_y band around 665 nm while group II (CS_2 and water-saturated CCl_4 solutions) shows a spectrum typical of BChl-*c* aggregates with a broader red-shifted Q_y band. All the NIR-FT-Raman spectra, which are preresonant with the Q_y band, are very close to those of chlorophyll *a* (Chl-*a*) measured in the corresponding solutions. Bands due to a C=O stretching mode of free and strongly hydrogen-bonded 13^1-keto carbonyl groups appear near 1685 and 1645 cm^{-1}, respectively. In contrast to the FT-Raman spectra, FT-IR spectra of the pyridine-d_5 solution and group II are largely different from those of Chl-*a* in the corresponding solutions, suggesting that BChl-*c* forms quite different types of aggregates. It is clear from the IR spectra that the ester carbonyl group plays an important role in the aggregation for the pyridine-d_5 and group II solutions. Of particular note is that bands due to C=O stretching modes of the ester group are observed at 1733, 1719, and 1705 cm^{-1} in the spectrum of BChl-*c* in water-saturated CCl_4. The appearance of the band at 1705 cm^{-1} suggests that the ester carbonyl groups in some BChl-*c* are engaged in very strong hydrogen-bondings. They may hydrogen-bond with a hydroxyl group which coordinates on the Mg atom.

Bacteriochlorophyll *c* (BChl-*c*)[1] is a member of the green pigment family which occurs only in green photosynthetic bacteria (Capel et al., 1978; Olson, 1980; Blankenship et al., 1988). The major function of BChl-*c* is to harvest light energy, which is then transferred to the adjacent photosynthetic membrane. BChl-*c* exists as aggregates in chlorosomes, the major light-harvesting structures in green photosynthetic bacteria; the direct interactions of the pigment molecules without involvement of proteins seem to be very efficient for energy transfer between the pigments (Brune et al., 1987; Holzwarth et al., 1990; Matsuura et al., 1993). It is well-known that BChl-*c* can form aggregates *in vitro* whose spectroscopic properties are very close to those of BChl-*c* in chlorosomes (Krasnovsky & Bystrova, 1980; Smith et al., 1983).

Figure 1 shows the structure of four homologs of farnesyl BChl-*c* in *Chlorobium* (*C.*) *limicola* f. *thiosulfatophilum*. They are distinguished by substituents at C-8 and C-12. The exact proportion of the four components can vary with the

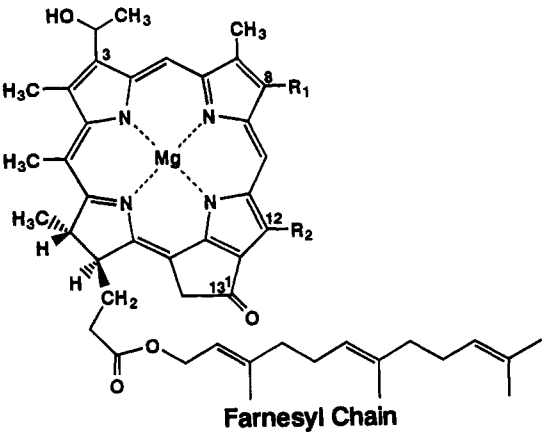

FIGURE 1: Structure of BChl-*c*. R_1 = Et, R_2 = Me, [E,M]BChl-c_F; R_1 = Et, R_2 = Et, [E,E]BChl-c_F; R_1 = *n*-Pr, R_2 = Et, [P,E]-BChl-c_F; R_1 = *i*-Bu, R_2 = Et, [I,E]BChl-c_F.

age of the bacterial culture, but the two main components are usually [E,E] and [P,E]BChl-c_F (Capel et al., 1978; Smith, 1994). The chromophore of BChl-*c* closely resembles that of chlorophyll *a* (Chl-*a*), so that the electronic structures may also be similar to each other. As the structure and properties of Chl-*a* have been studied in detail (Vernon & Seely, 1966; Lutz, 1983; Fujiwara & Tasumi, 1986a,b, 1987; Scheer, 1991; Okada et al., 1993), it may be possible to apply some of the knowledge obtained for Chl-*a* to studies of BChl-*c*. For example, infrared (IR) and Raman marker bands used for Chl-*a* may be useful for BChl-*c* as well (Nozawa et al., 1990). On the other hand, there are some large differences in the formation of aggregates between the two kinds of

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[1] Abbreviations: UV-Vis ultraviolet−visible; FT-IR, Fourier-transform infrared; NIR, near-infrared; BChl, bacteriochlorophyll; THF, tetrahydrofuran; CS_2, carbon disulfide; CCl_4, carbon tetrachloride; *C.*, *Chlorobium*; IR, infrared; HPLC, high-performance liquid chromatography; Chl, chlorophyll.

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pigments (Bystrova et al., 1979; Smith et al., 1983; Worcester et al., 1986; Brune et al., 1988; Lutz & van Brakel, 1988; Holzwarth et al., 1990; Nozawa et al., 1991; Olson & Pedersen, 1990; Uehara & Olson, 1992; Uehara et al., 1994, 1995). Those differences arise mainly from the differences in their substituents; Chl-*a* has two ester carbonyl groups at C-13[3] and C-17[3], but BChl-*c* has only one at C-17[3] and a 3-vinyl gruop in Chl-*a* is substituted by a hydroxyl group in BChl-*c*. It has been considered that BChl-*c* can form aggregates by the hydroxyl group (Bystrova et al., 1979; Smith et al., 1983; Lutz & van Brakel, 1988; Uehara et al., 1994). Therefore, knowledge about Chl-*a* aggregation is not always applicable to the studies of BChl-*c*.

Much effort has been made to investigate BChl-*c* aggregation in the chlorosome and solutions (Capel, 1978; Bystrova et al., 1979; Olson, 1980; Smith et al., 1983; Worcester et al., 1986; Brune et al., 1987, 1988; Blankenship et al., 1988; Nozawa et al., 1990; Olson & Pedersen, 1990; Uehara et al., 1991, 1994, 1995; Uehara & Olson, 1992). Bystrova et al. (1979) proposed a model structure for the first time for ground-state interactions of BChl-*c* from measurements of UV−Vis and IR spectra. A different model for the aggregation of BChl-*c* was addressed by Smith et al. (1983). Both models stressed the importance of the hydroxyl group in the aggregation, although the former also considered the involvement of the keto carbonyl group while the latter ignored it. Lutz et al. (1988) discussed a mechanism of polymerization of BChl-*c* based upon resonance Raman measurements. Olson and Pedersen (1990) and Uehara and Olson (1992) investigated the aggregation for each homolog of BChl-*c* in several solutions using UV−Vis absorption and CD spectroscopy and found that there are some differences among the homologs in the mechanism of polymerization. Nozawa et al. (1990) compared resonance Raman spectra of carotenoid-depleted chlorosomes of *Chloroflexus aurantiacus* with those of BChl-*c* in some solvents; they suggested that BChl-*c* exists as five-coordinate species in the chlorosomes and that the keto carbonyl group hydrogen-bonds to the hydroxyl group.

A model structure for the dimer and higher aggregates has been proposed by several groups. Although they have all pointed out that the hydroxyl group plays a key role in the formation of the aggregates, there is still dispute as to the detailed structure.

The purpose of the present study is to provide new insight into the structure of the aggregates of BChl-*c* in solutions. We have employed UV−Vis, IR, and Raman spectroscopy. IR spectroscopy if particularly useful for the studies of the aggregation because not only C=O stretching bands of the keto carbonyl group but also those of the ester carbonyl group appear strongly in IR spectra of BChl-*c* (Uehara et al., 1991). The frequencies of these C=O stretching bands are very sensitive to the strength of the hydrogen-bondings of the keto and ester cabonyl groups, so that one can monitor the states of the keto and ester groups in the aggregates by measuring for IR spectra (Lutz, 1983, 1988; Brune et al., 1988; Uehara et al., 1991, 1995). We already reported the IR spectra of BChl-*c* fragmentary for [E,E], [P,E], [E,M], and [I,E]BChl-c_F in the films and solutions (Uehara et al., 1991). This time we have undertaken a more systematic IR study of BChl-*c* to establish spectra−structure relationships. The most important conclusion here is that the ester carbonyl group of BChl-*c* also plays a key role in the aggregation.

MATERIALS AND METHODS

Sample Preparation. *C. limicola* f. *thiosulfatophilum* was grown and BChl-*c* was isolated and purified according to the method which had been originally proposed by Olson and Pedersen (1990) and improved by Uehara et al. (in preparation): precolumn chromatography was carried out before high-performance liquid chromatography (HPLC) to remove carotenoids and pheophytins by a sugar column using petroleum ether−2-propanol as the mobile phase. The sample employed contained [E,M]BChl-c_F, [E,E]BChl-c_F, [P,E]BChl-c_F, and [I,E]BChl-c_F. It was more than 99% pure as estimated by HPLC equipped with a UV detector.

The concentrations of BChl-*c* were 1.9, 1.5, 1.3, 1.5, and 1.5×10^{-2} M in acetone, THF, pyridine-d_5, CS_2, and water-saturated CCl_4, respectively. Spectrograde solvents were purchased fresh from Dojin Chemical Industries, Ltd. (Kumamoto, Japan), and used without further purification. The water content of acetone, THF, and CS_2 determined by gas chromatography was less than 0.3, 0.3, and 0.02%, respectively. Pyridine-d_5 (99 atom % D, water content less than 0.05%) was furnished from Aldrich Chemical Co., Inc. (Milwaukee, WI).

Spectroscopic Measurements. The NIR-FT-Raman spectra of BChl-*c* in solutions were measured at a spectral resolution of 4 cm^{-1} with a JEOL JRS-FT6500N FT-Raman spectrometer equipped with an InGaAs detector. The 1064 nm line from a Nd:YAG laser (Spectron SL301) was employed for the excitation, and the power at the sample position was typically 100 mW. Generally, several hundred scans were accumulated to ensure an acceptable signal-to-noise ratio. For the Raman measurements, the BChl-*c* solutions were put into a small glass cell and closed with a Teflon cap. The Raman-scattered light from the sample was collected in a back-scattered configuration.

The FT-IR spectra were measured with a Nicolet Magna 550 spectrometer with an MCT detector. All the data were collected at a spectral resolution of 4 cm^{-1}, and generally several hundred scans were accumulated to ensure an acceptable signal-to-noise ratio. The IR spectra presented here are all difference spectra between the spectra of BChl-*c* solutions and those of solvents. For the IR measurements, the sample solutions were put between two CaF_2 windows separated by a 15 μm Teflon spacer.

The UV−Vis absorption spectra were recorded on a Shimadzu UV-3101PC spectrophotometer. The same sample solutions put between the two CaF_2 windows were subjected to the UV−Vis measurements, just before and after the IR measurements.

In order to confirm that sample degradation did not occur during the Raman and IR measurements, UV−Vis absorption spectral measurement and HPLC analysis were performed before and after each spectral measurement. No sample decomposition was detected.

RESULTS

Figure 2a−c shows absorption spectra of BChl-*c* (mixture of the four homologs) in acetone (1.9×10^{-2} M), THF (1.5×10^{-2} M), and pyridine-d_5 (1.3×10^{-2} M), respectively. The spectra of the acetone and THF solutions resemble those of Chl-*a* in the corresponding solvents in terms of not only spectral features but also the locations of Q_y and Soret bands (Sato et al., 1995). Chl-*a* assumes a five- and six-coordinate

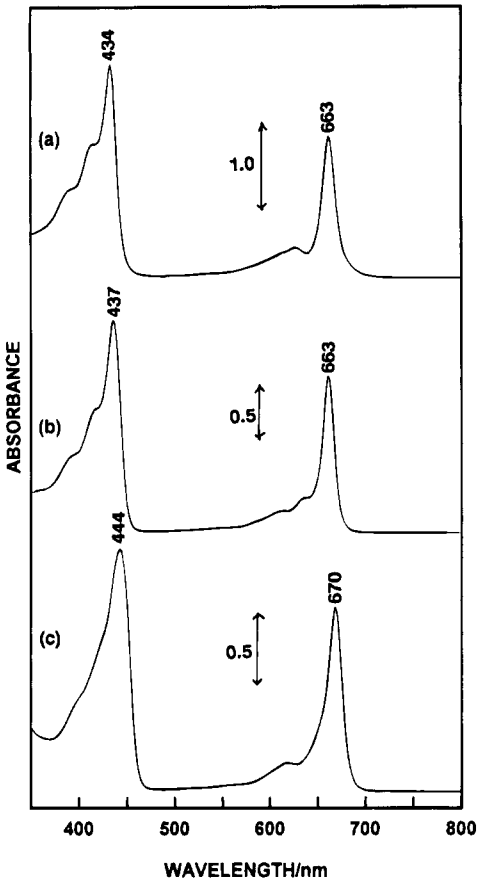

FIGURE 2: Absorption spectra of BChl-*c* in acetone (a; 1.9×10^{-2} M), THF (b; 1.5×10^{-2} M), and pyridine-d_5 (c; 1.3×10^{-2} M).

monomer in acetone and THF, respectively (Tasumi & Fujiwara, 1987; Sato et al., 1995). As will be discussed later based on IR and Raman data, it is found that BChl-*c* also takes a five- and six-coordinated monomer in these solutions. Spectrum c shows a slight band broadening, indicating that BChl-*c* forms aggregates in the pyridine-d_5 solution. This type of absorption spectrum of BChl-*c* aggregation has not been reported yet, and such broadening has never been seen for Chl-*a* in the same solvent (Sato et al., 1995). The red-shift of the Soret and Q_y band in spectrum c may be due to the polarity of solvent, and similar shifts are observed in the absorption spectrum of Chl-*a* in a pyridine-d_5 solution. Hereafter, we refer to the acetone, THF, and pyridine-d_5 solutions as group I.

Figure 3a,b depicts absorption spectra of BChl-*c* in CS_2 (1.5×10^{-2} M) and water-saturated CCl_4 (1.5×10^{-2} M), respectively. Band broadening and shifts are seen in those spectra. Spectra a and b reveal the existence of BChl-*c* aggregation in CS_2 and water-saturated CCl_4. According to the previous paper by Uehara and Olson (1992), it seems that a tetramer of BChl-*c* contributes largely to the spectrum of BChl-*c* in the CS_2 solution and that the contributions of both the tetramer and polymer are obvious only in the spectrum of the water-saturated CCl_4 solution. A shoulder near 745 nm in spectrum b is assignable to the polymer species. Hereafter, we refer to the CS_2 and water-saturated CCl_4 solutions as group II.

NIR-FT-Raman spectra of BChl-*c* in acetone, THF, and pyridine-d_5 are shown in spectra a–c, respectively, of Figure 4. An intense band near 1680 cm^{-1} is assignable to a C=O stretching mode of the free keto carbonyl group. It is noted

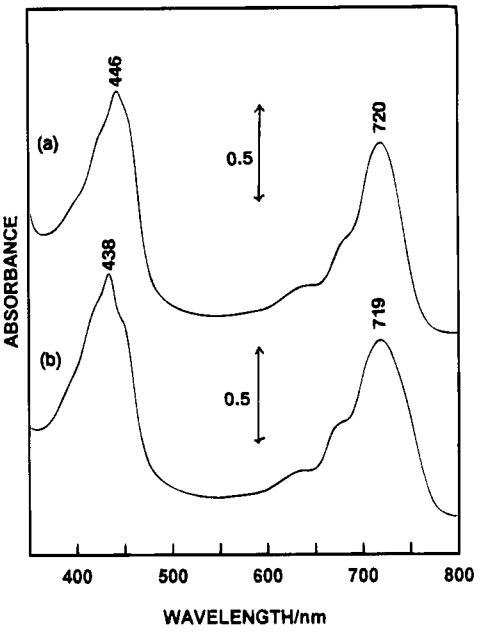

FIGURE 3: Absorption spectra of BChl-*c* in CS_2 (a; 1.5×10^{-3} M) and water-saturated CCl_4 (b; 1.5×10^{-2} M).

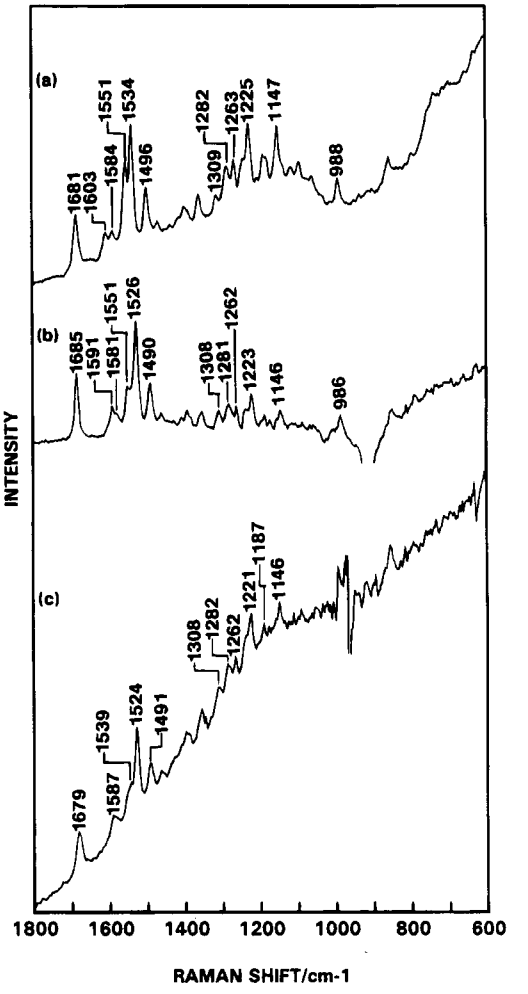

FIGURE 4: NIR-FT-Raman spectra of BChl-*c* in acetone (a; 1.9×10^{-2} M), THF (b; 1.5×10^{-2} M), and pyridine-d_5 (c; 1.3×10^{-2} M).

that the frequency of the band is lower by 10 cm^{-1} than that of the corresponding band due to the free keto carbonyl group of Chl-*a* (Sato et al., 1995). The intense appearnce of the C=O stretching band suggests that the spectra are pre-

resonant *via* the Q_y absorption band because the Q_y excitation induces distortion in the direction of the keto group (*y*-polarized distortion; see Figure 1). There is other evidence for the preresonance effect: very little C−H contribution in the 3100−2800 cm^{-1} region (not shown) of the NIR-FT-Raman spectra. Similar results for the preresonance effect have been observed for Chl-*a* (Sato et al., 1995).

Bands at 1603, 1551, 1534, and 1496 cm^{-1} in spectrum a are assigned to R1, IR4, IR5, and R5 modes based upon comparison of the spectrum with that of Chl-*a* in acetone (Fujiwara & Tasumi, 1986a,b; Tasumi & Fujiwara, 1987). They show very similar behavior to corresponding bands of Chl-*a* upon changing the coordination number; the bands due to the R1 and R5 modes show a significant downward shift upon going from five- to six-coordinated species (compare Figure 4a and Figure 4b). They may be useful marker bands for the coordination number even in the case of BChl-*c*. [Nozawa et al. (1990) already pointed out that some resonance Raman bands can be used as the marker bands commonly for Chl-*a* and BChl-*c*. However, since the spectral pattern in the NIR-FT-Raman spectra is different from that in the resonance Raman spectra (Sato et al., 1995), it is important to confirm which bands can become monitors for the coordination number in the FT-Raman spectra.] The band due to the IR4 mode shifts little because its frequency is much less sensitive to a change in the size of the macrocycle (Tasumi & Fujiwara, 1987).

In the NIR-FT-Raman spectra of Chl-*a*, a band appears near 1630 cm^{-1} (Sato et al., 1995). The corresponding band is not seen in Figure 4. The band was assigned to the C=C stretching mode of the 3-vinyl group in Chl-*a*. The present result confirms the assignment. It may be used as a good marker for the existence of the vinyl group (Feiler et al., 1994; Sato et al., 1995). Frequencies of bands due to the R1 and R5 modes in spectrum c indicate that the central Mg atom assumes a six-coordinate state in the pyridine-d_5 solution. In contrast to the result of the absorption spectrum described above, no evidence is observed for the aggregation of BChl-*c* in pyridine-d_5 in the Raman spectrum. Clear evidence for the aggregation is seen in the IR spectrum as will be discussed later.

Figure 5 exhibits NIR-FT-Raman spectra of group II. Spectra a and b (the CS_2 and water-saturated CCl_4 solutions, respectively) closely resemble each other. According to the absorption spectra in Figure 3, only the water-saturated CCl_4 solution contains the polymer species. However, major difference cannot be seen between the two Raman spectra (Figure 5a,b).

A band at 1685 cm^{-1} is assignable to the C=O stretching mode of the free keto carbonyl group while that at 1645 cm^{-1} is due to that of the keto carbonyl group strongly hydrogen-bonded to the 3^1-hydroxyl group which coordinates to the Mg atom of another molecule. Bands due to the R1 and R5 modes near 1604 and 1496 cm^{-1}, respectively, suggest that the Mg atom takes a five-coordinate state in the CS_2 and water-saturated CCl_4 solutions.

It should be noted that any contribution from a C=O stretching mode of the ester carbonyl group cannot be observed in Figures 4 and 5. To investigate the behavior of the ester carbonyl group, IR spectroscopy should be applied.

FT-IR spectra of BChl-*c* in THF and pyridine-d_5 are shown in Figure 6a,b, respectively. An IR spectrum of the acetone solution could not be obtained because of the interference

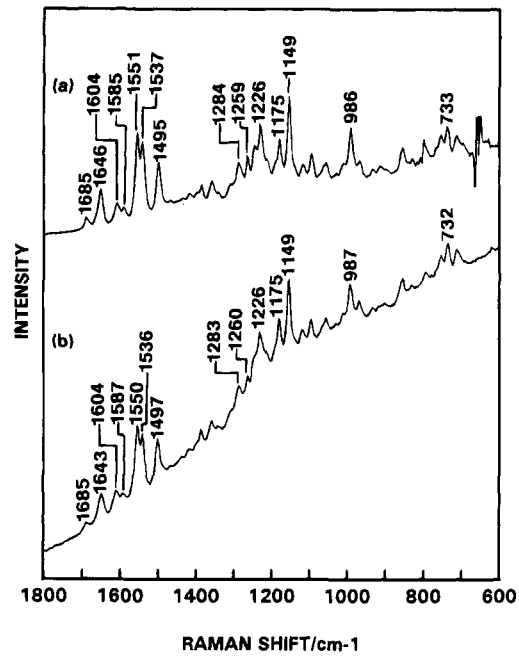

FIGURE 5: NIR-FT-Raman spectra of BChl-*c* in CS_2 (a; 1.5×10^{-2} M) and water-saturated CCl_4 (b; 1.5×10^{-2} M).

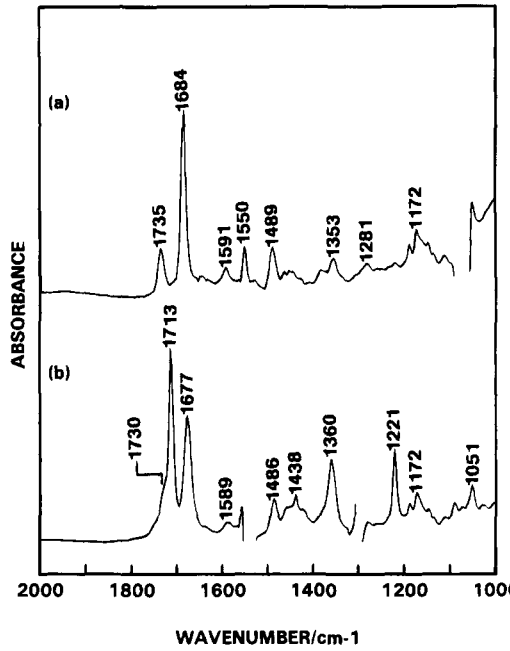

FIGURE 6: FT-IR spectra of BChl-*c* in THF (a; 1.5×10^{-2} M) and pyridine-d_5 (b; 1.3×10^{-2} M).

of strong absorption of the solvent. A band at 1735 cm^{-1} in spectrum a is assignable to a C=O stretching mode of the free ester carbonyl group. This frequency is almost the same as that of the corresponding band of Chl-*a* (the relative intensity of the ester carbonyl band of BChl-*c* is almost half of that of Chl-*a* because Chl-*a* has two ester groups) (Ballschmitter & Katz, 1969; Sato et al., 1995). An intense band at 1684 cm^{-1} arises from the C=O stretching mode of the free keto carbonyl group.

Spectrum b shows characteristic bands at 1713, 1360, and 1221 cm^{-1} assignable to C=O stretching, C−O−C anti-symmetric stretching , and C−O−C symmetric stretching modes of the ester group, respectively. Comparing the spectrum with that of the THF solution, it is noted that the intensities of these bands are enhanced very much and that

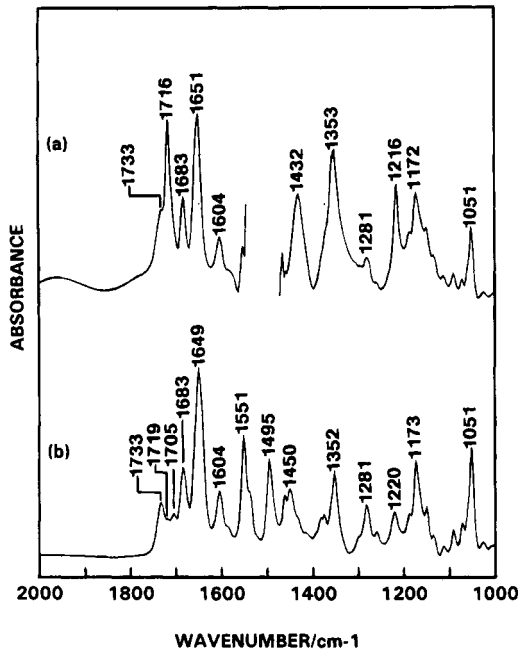

FIGURE 7: FT-IR spectra of BChl-*c* in CS_2 (a; 1.5×10^{-2} M) and water-saturated CCl_4 (b; 1.5×10^{-2} M).

the band due to the C=O stretching mode shows a large downward shift by about 20 cm^{-1}. These observations suggest that the ester C=O group is engaged in strong hydrogen-bonding.

In addition to the band at 1713 cm^{-1}, two bands are observed at 1730 and 1677 cm^{-1} in the C=O stretching band region of Figure 6b. These are assigned to C=O stretching modes of the free ester and keto groups, respectively.

Figure 7 compares FT-IR spectra of the CS_2 (a) and water-saturated CCl_4 (b) solutions. They belong to group II, but we can notice at a glance large differences between the two spectra.

The spectrum of the CS_2 solution (spectrum a) gives four bands in the C=O stretching band region. A strong band at 1651 cm^{-1} is assignable to a C=O stretching mode of the keto group coordinating to the Mg atom of another molecule.

The spectrum of the water-saturated CCl_4 solution (spectrum b) is more complicated. Besides four bands at 1733, 1719, 1683, and 1649 cm^{-1}, there is one more band at 1705 cm^{-1}. Judging from its frequency, the band is assigned to a C=O stretching mode of the strongly hydrogen-bonded ester group or free keto group. The latter possibility is very unlikely because BChl-*c* takes a monomer in THF and acetone and the band due to the free keto group is observed at 1685 cm^{-1} in those solutions. Further support for the assignment to the ester group comes from the following fact. If the band were due to the C=O stretching mode of the free keto group, it should appear also in the NIR-FT-Raman spectrum of BChl-*c* in water-saturated CCl_4, but this is not the case.

DISCUSSION

It is well-known that BChl-*c* can form aggregates *in vitro* as well as *in vivo* easily because of the existence of the hydroxyl group (Bystrova et al., 1979). However, for some solutions such as methanol, it exists in a monomer. A UV−Vis spectrum of the monomeric species is characterized by sharp Soret and Q_y bands at 435 and 666 nm, respectively, and very close to that of the monomeric species of Chl-*a* (Bystrova et al., 1979; Smith et al., 1983; Olson & Pedersen, 1990).

The UV−Vis spectra of BChl-*c* in acetone and THF are almost identical with the spectrum of the monomeric species of BChl-*c* reported by Olson and Pedersen (1990), showing that BChl-*c* assumes a monomer in those solutions. Now, there is little doubt that the IR and Raman bands near 1683 cm^{-1} of BChl-*c* in acetone and THF (Figures 4 and 6) are assigned to a C=O stretching mode of the free keto group and an IR band at 1735 cm^{-1} (Figure 6) is due to a C=O stretching mode of the free ester group. The frequencies of the R1 and R5 bands reveal that BChl-*c* takes five- and six-coordinated species, respectively, in acetone and THF.

Structures of aggregates of BChl-*c* in chlorosomes and solutions have been investigated extensively by using various spectroscopic techniques (Capel et al., 1978; Olson, 1980; Smith et al., 1983; Worcester et al., 1986; Brune et al., 1987; Blankenship et al., 1988; Lutz & van Brakel, 1988; Holzwarth, 1990, 1994; Nozawa et al., 1990, 1991; Olson & Pedersen, 1990; Uehara et al., 1991, 194; Uehara & Olson, 1992). Although the proposed structures of the aggregates are somewhat different from each other, almost all the authors have insisted on the importance of the keto carbonyl and hydroxyl groups in the aggregation.

For dimer of BChl-*d*, the involvement of the ester group in aggregation was suggested by Smith et al. (1986), who measured its NMR spectra in solutions. They also inferred a similar role for the ester group in aggregation of BChl-*c*, but they did not give any direct evidence for it. In the following discussion, we demonstrate that the ester carbonyl group does play a key role in some aggregation of BChl-*c*.

The UV−Vis spectrum of BChl-*c* in pyridine-d_5 (Figure 2c) gives a hint of the aggregation of BChl-*c*, but its Raman spectrum is so close to the spectrum of BChl-*c* in THF (Figure 4b,c) that we cannot get any evidence for the aggregation from the Raman spectrum. The IR spectrum, however, provides clear evidence for the aggregation; a band assignable to the C=O stretching mode of the strongly hydrogen-bonded ester carbonyl group is observed at 1713 cm^{-1}. This frequency is very close to that of the C=O stretching mode of the ester carbonyl group of Chl-*a* which is engaged in hydrogen-bonding to the water molecule coordinating on the Mg atom. In the present case, it is likely that the ester carbonyl group is hydrogen-bonded with the hydroxyl group of another BChl-*c*. This is the first time that the involvement of the ester carbonyl group in the aggregation of BChl-*c* has ever been demonstrated.

The IR and Raman frequencies of the C=O stretching mode of the keto carbonyl group show that it is free from a hydrogen-bonding. In addition, the R1 and R5 bands in the IR and Raman spectra reveal that the Mg atom takes a six-coordinate state. For both BChl-*c* and Chl-*a* in pyridine-d_5, the Mg atom assumes a six-coordinate state with two pyridine-d_5 molecules, and the keto carbonyl group does not coordinate on the Mg atom. This happens because pyridine-d_5 is a stronger ligand than the keto carbonyl group. It should also be kept in mind that the BChl-*c* concentration is high (1.3×10^{-2} M) here.

We propose the structure shown in Figure 8a for BChl-*c* in pyridine-d_5. The structure satisfies all the spectroscopic data presented here. In the model, two BChl-*c* planes are not parallel but deviated from parallel to some extent. It is

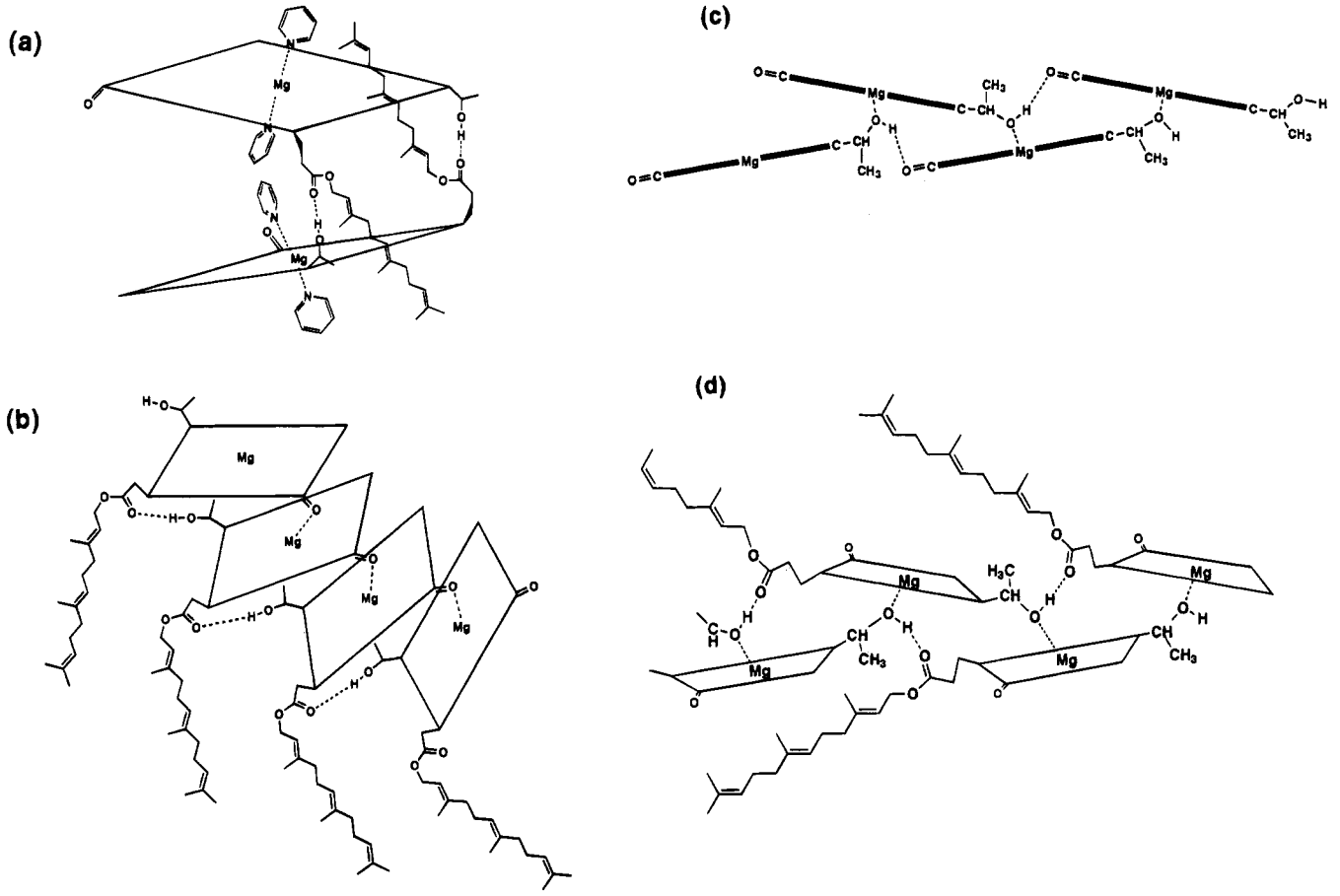

FIGURE 8: Possible structure of aggregation of BChl-*c* in pyridine-d_5 (a), CS_2 (b), and water-saturated CCl_4 (c and d).

very likely that they are closest each other at the side with the farnesyl chain and most distant at the opposite side due to the repulsion between the two pyridine ligands. The estimation based upon the molecular model suggests that the distance between the two planes is at least 10 Å at the closest position. Therefore, it seems that the two planes are far enough away from each other and also not parallel. We think this model can explain the UV−Vis spectrum.

There may be equilibrium between the dimer (structure shown in Figure 8a) and monomer in the pyridine solution of BChl-*c*. The weak shoulder near 1730 cm^{-1} due to the C=O stretching mode of the free ester group is evidence for the coexistence of the monomer.

The absorption spectrum of BChl-*c* in CS_2 has a broad absorption maximum at 720 nm. According to Uehara and Olson (1992), this suggests that the tetramer is the major species in the CS_2 solution. The IR and Raman frequencies of the C=O stretching mode of the keto carbonyl group indicate that some keto groups are free from hydrogen-bonding and some others coordinate on the Mg atom of another BChl-*c*. It is again clear from the IR spectrum that some ester carbonyl groups are engaged in hydrogen-bonding with the hydroxyl group of another BChl-*c*. The Mg atom may be in a five-coordinate state because the R1 band is located at 1604 cm^{-1}. This spectroscopic evidence leads us to propose the structure shown in Figure 8b. It depicts a part of possible oligomers, although from the present IR results, the size of oligomers is not clear. The structure in Figure 8b is close to that of the so-call T-shape model (Chapados, 1988). However, compared to the case of the T-shape model, two chlorin planes in Figure 8b may be less perpendicular to each other because there is a large red-shift in the UV−Vis spectrum.

Uehara and Olson (1992) proposed that BChl-*c* forms monomer, dimer, tetramer, and polymer in water-saturated CCl_4 and that their proportions change with the concentration and homologs. The absorption spectrum of BChl-*c* in water-saturated CCl_4 shown in Figure 5c shows that the oligomers and polymer are the major species in the present case.

Figure 8c shows the structures of the polymer of BChl-*c* in water-saturated CCl_4 previously proposed (Brune et al., 1988). The present spectroscopic data except for the two IR bands at 1719 and 1705 cm^{-1} (Figure 7c) can be explained by the model structures. The two bands at 1719 and 1705 cm^{-1} may be due to C=O stretching modes of the hydrogen-bonded ester carbonyl groups. Probably the former is ascribed to the hydrogen-bonded ester group of the oligomers shown in Figure 8b. Since the band at 1719 cm^{-1} is weak, the population of the oligomers must be small. For the band at 1705 cm^{-1}, we propose the structure shown in Figure 8d. The structure in Figure 8d is similar to that in Figure 8c except that the ester carbonyl group is hydrogen-bonded to the hydroxyl group coordinating on the Mg atom instead of the keto carbonyl group. The band at 1705 cm^{-1} is also weak, so that the structure in Figure 8d should be a minor component.

CONCLUSION

This paper has clearly demonstrated, for the first time, that the ester group plays a key role in some aggregation of BChl-*c*. IR spectroscopy has provided unambiguous evidence for

the involvement of the ester group in the aggregation. Fifteen years ago, Bystrova et al. (1979) already observed a weak band at 1705 cm^{-1} in an IR spectrum of BChl-*c* in CCl_4, but they assigned it to C=O stretching modes of the free keto group, and, therefore, they could not notice the importance of the ester group. Our present systematic study has made the band assignments in the 1750−1640 cm^{-1} region clear. This study has dealt with the mixture of four homologs of BChl-*c*. As the next step, similar study must be done for each homolog, and comparison between the structure of BChl-*c* in solutions and that in chlorosomes is important. Such studies are now in progress in our laboratories.

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