Vibrational Spectroscopic Analysis of Self-Aggregates of Synthetic Zinc and Cadmium 13^1-^{18}O-Labeled Bacteriochlorophyll-*d* Analogs

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Zinc and cadmium complexes of methyl 3^1-demethylbacteriopheophorbide-*d* ^{18}O-labeled specifically at the 13^1-oxo moiety were prepared. The FT-IR vibrational band of the 13-carbonyl group was shifted to a much lower wavenumber by their self-aggregation, similarly as in natural light-harvesting antennae.

Most light-harvesting antenna systems of photosynthetic organisms are constructed by a complexation of pigments including chlorophylls, carotenoids, and bilinoids with oligopeptides. Several photosynthetic antennae have been investigated by X-ray crystallographic analyses, and their supramolecular structures were revealed at an atomic level.[1] In contrast, the main peripheral antennae of photosynthetic green bacteria, called chlorosomes, have self-aggregates of specific chlorophyllous molecules, bacteriochlorophyll(BChl)s-*c*, -*d*, and -*e* (Figure 1), without any interaction with oligopeptides.[2] Such large J-aggregates in a chlorosomal core part are investigated to clarify their supramolecules. Bulk (large-scale) components of chlorosomal aggregates are still in debate and proposed to be a rod including single and multiple tubes or an irregular and partially rolled-up lamella based on experimental and calculation results.[2] Moreover, their local structure (short-length bonding motif) has not been determined completely, although the following two bondings are accepted: 1) coordination of the 3^1-hydroxy group of a BChl-*c*/*d*/*e* molecule with the central magnesium of another molecule and 2) stepwise π–π interaction of chlorin moieties along their molecular *y* axes.[3] Vibrational spectroscopic analyses indicated that the coordinated hydroxy group hydrogen-bonds to the 13-keto carbonyl group of the third molecule,[4,5] but very recently other bondings were also speculated: the weak electrostatic interaction of 13^1-oxo group with magnesium to form an asymmetric six-coordinated Mg state[6] or hydrogen-bonding in water-bridged 13-carbonyl groups.[7]

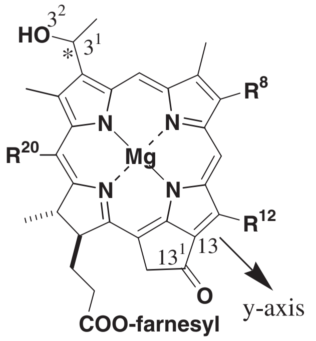
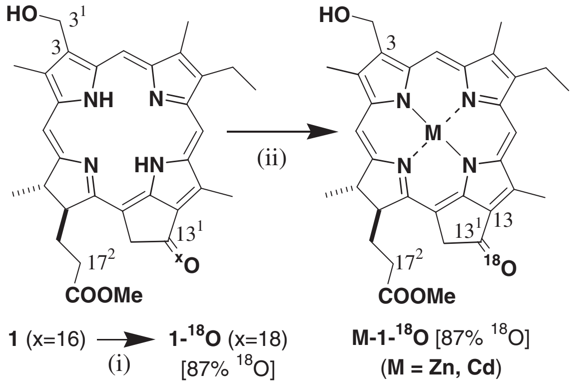


Figure 1. Molecular structures of BChls-*c* (R^{20} = Me) and -*d* (R^{20} = H): R^8: $CH_2CH_{3-n}Me_n$ (n = 0–3) and R^{12}: Me or Et.

Resonance Raman spectra of natural chlorosomes showed a specific shift at around 1640 cm^{-1}.[3,8] The peak was believed to be the vibrational band of 13-carbonyl group in self-aggregates of BChl-*c*, which had dramatically moved from the corresponding band at ca. 1690 cm^{-1} in a monomeric solution. Similar lower shifts in wavenumber were observed for in vitro self-aggregates of BChl-*c* and its models.[3] Such large shifts are explained by an extraordinarily strong hydrogen-bonding of 13-carbonyl group with magnesium-coordinated hydroxy group, $13\text{-C=O}\cdots\text{H–O}(3^2)\cdots\text{Mg}$.[4,5,9] Here, we prepared specifically ^{18}O-labeled models of BChl-*c* and reconfirmed the pronouncedly shifted vibrational band to be the 13-keto carbonyl moieties in chlorosomal self-aggregates using FT-IR spectroscopy. The present models are zinc and cadmium complexes of a synthetic 3-hydroxymethyl-13^1-oxochlorin, which were reported to be useful for preparation of chlorosomal self-aggregates.[10]

Chlorophyll-*a* was extracted from commercially available dry cells of a cyanobacterial *Spirulina* species,[11] and modified to methyl 3-devinyl-3-hydroxymethylpyropheophorbide-*a* (**1**, methyl 3^1-demethylbacteriopheophorbide-*d*) according to reported procedures[5] (Scheme 1). Treatment of **1** in dichloromethane with 95% ^{18}O-labeled water in the presence of trifluoroacetic acid (TFA) smoothly afforded an 87:13 mixture of 13^1-^{18}O-labeled chlorin **1**-^{18}O and its unlabeled compound **1** after purification.[12] During the biphasic reaction with stirring at room temperature, neither the less reactive 3^1-hydroxy nor 17^2-ester carbonyl oxygen atoms were labeled and **1**-^{18}O with the sole label of the more reactive keto carbonyl oxygen atom was obtained. The resulting free-base **1**-^{18}O in dichloromethane was reacted with metal acetate in methanol to give corresponding



Scheme 1. Synthesis of metal complexes of 13^1-^{18}O-labeled BChl-*d* analog M–**1**-^{18}O (M: Zn and Cd): (i) $H_2^{18}O$ (95% ^{18}O), CF_3COOH, CH_2Cl_2; (ii) $M(OAc)_2 \cdot 2H_2O$, MeOH, CH_2Cl_2.

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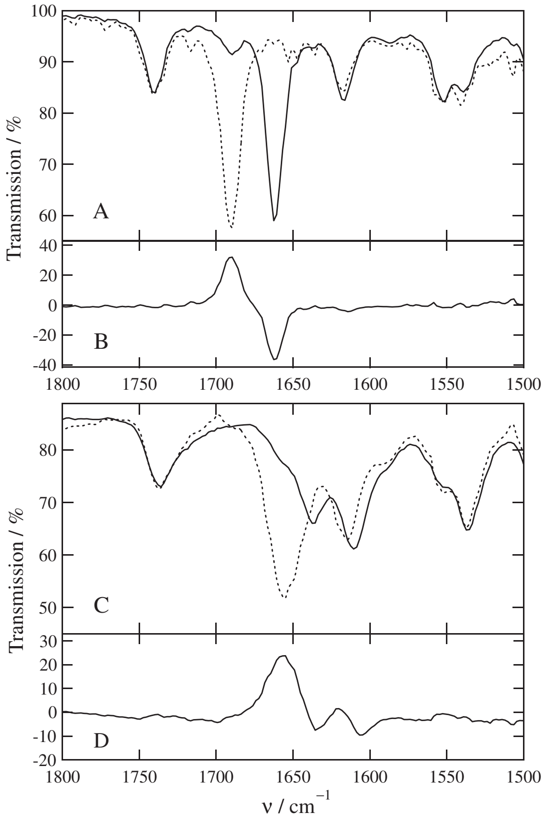

Figure 2. FT-IR spectra of Zn–**1**-^{18}O (87% ^{18}O, solid line) and unlabeled Zn–**1** (broken line) in homogeneous THF solution (A, transmission mode) and in the solid state (C, reflection mode). The difference spectra (B/D) prepared by reduction of solid from broken lines of Figures 2A and 2C: (spectrum of Zn–**1**-^{16}O) − (spectrum of Zn–**1**-^{18}O).

metal complexes without loss of ^{18}O-atom:[13] 30-min stirring at room temperature for zinc[14] and 2-h refluxing for cadmium.[15] Zn– and Cd–**1**-^{18}O (87%-^{18}O) were purified with HPLC and recrystallization, respectively, and fully characterized by their visible, ^{1}H NMR, IR, and MS spectra. Especially, the ^{18}O-labeling degrees were determined by IR and MS spectral analyses reported previously.[12]

In THF, Zn–**1**(-^{18}O) was monomeric with sharp visible absorption maxima ($\lambda_{\max} = 646$ and 423 nm for Q_y and Soret peaks, respectively) to give the 5-coordinated zinc complex possessing a single THF molecule as an axial ligand. From FT-IR absorption spectra in THF (Figures 2A and 2B, transmission mode), the 13-keto-carbonyl vibrational band was exclusively shifted to a 27-cm^{-1} lower wavenumber by its ^{18}O-labeling: ν(13-C=^{16}O) = 1690 and ν(13-C=^{18}O) = 1663 cm^{-1}. The isotopic shift is consistent with the reported values for other compounds (27–30 cm^{-1}).[12]

A concentrated solution of Zn–**1**(-^{18}O) in THF was diluted with excess hexane to give green precipitates after standing overnight. The resulting solid film showed red-shifted and broadened $\lambda_{\max} = 740$ and 450 nm, compared with monomeric maxima (vide supra). The visible absorption spectral change clearly indicated the formation of chlorosomal self-aggregates in the solid state.[5] The self-aggregation of Zn–**1** shifted the 13-C=^{16}O band from 1690 (monomer in THF) to 1655 cm^{-1} in the dried solid state (measured in reflection mode), while the other peaks moved less: ν(17^{2}-C=O) = 1741 → 1738 and ν(chlorin-π-skeleton-C=C/C=N) = 1617 → 1614 cm^{-1} (see broken lines of Figures 2A and 2C). The 35-cm^{-1} shift is ascribable to a special hydrogen bond as 13-C=O···HO(3^{2})···Zn (see Graphical Abstract).[5]

In contrast, self-aggregates of Zn–**1**-^{18}O (87%-^{18}O) in a solid state gave a complex IR spectrum (see solid lines of Figures 2A and 2C). The ester carbonyl band was shifted slightly by self-aggregation (1741 → 1736 cm^{-1}) and the shoulder at around 1655 cm^{-1} was observed due to the presence of a special hydrogen-bonding 13-C=^{16}O in chlorosomal self-aggregates of 13% unlabeled Zn–**1**. Since the aggregation shift for 13-C=O is determined to be 35 cm^{-1} from the above spectra in unlabeled **1**, a 1628 cm^{-1} peak is estimated for the 13-C=^{18}O in self-aggregates of Zn–**1**-^{18}O, but no apparent peak was seen in the proposed position (see solid lines of Figures 2C and 2D). A main peak was observed at 1611 cm^{-1} and a smaller band was measured at 1637 cm^{-1}. These two peaks must result from the vibrational bands of 13-C=^{18}O and chlorin-π-skeleton-C=C/C=N in chlorosomal self-aggregates of Zn–**1**-^{18}O. The estimated peak position for the former is 1628 cm^{-1} which is near the latter at 1614 cm^{-1} proposed from the value in self-aggregates of unlabeled Zn–**1** (vide supra). The two bands were coupled together to give the shifted bands at 1637 (>1628) and 1611 (<1614) cm^{-1}. Such resonance couplings were previously reported in chlorin molecules, where the nearby situation (<20 cm^{-1}) of the vibrational bands was necessary for their coupling.[13] In the present system with an estimated 14-cm^{-1} (=1628 − 1614) difference, the criterion also holds.

Cadmium chlorophyll derivatives Cd–**1**(-^{18}O) in dichloromethane and pyridine (2:1) gave a bluish green-colored homogeneous solution to produce monomeric species with $\lambda_{\max} = 656$ and 434 nm where the 5-coordinated cadmium complex possessed a single pyridine molecule as an axial ligand. FT-IR difference spectrum in the solution shown in Figure 3B (the solid minus broken lines of Figure 3A) clearly indicates that the sole 13-keto-carbonyl vibrational band was shifted to a 29-cm^{-1} lower wavenumber by its ^{18}O-labeling: ν(13-C=^{16}O) = 1678 and ν(13-C=^{18}O) = 1649 cm^{-1}. The isotopic shift is also consistent with the values mentioned above (27–30 cm^{-1}).

A monomeric solution of Cd–**1**(-^{18}O) in methanol and dichloromethane (1:3) was diluted with cyclohexane to quickly give dark green precipitates possessing red-shifted and broadened $\lambda_{\max} = 744$ and 462 nm, due to chlorosomal self-aggregation in the solid state. A self-aggregation shift observed in the 13-C=^{16}O vibrational band of unlabeled Cd–**1** is 34 cm^{-1} (=1678 − 1644, see broken lines of Figures 3A and 3C), which is almost the same as the value in Zn–**1** (35 cm^{-1}). In the self-aggregates of Cd–**1** in the dried solid state, a special hydrogen bond [13-C=O···HO(3^{2})···Cd] is also present, shown above in zinc complexes. The 13-keto-carbonyl vibrational band in the solid self-aggregates moved from 1644 (C=^{16}O) to 1620 cm^{-1} (C=^{18}O) instead of a small shift of chlorin-π-skeleton-C=C/C=N mode at around 1590 cm^{-1}. The predominant shift of ν(13-C=O) in the self-aggregates of Cd–**1** by the selective ^{18}O-labeling shown in the difference FT-IR spectrum (Figure 3D) indicates that the band shifted dramatically to a lower wavenumber by chlorosomal self-aggregation is assigned

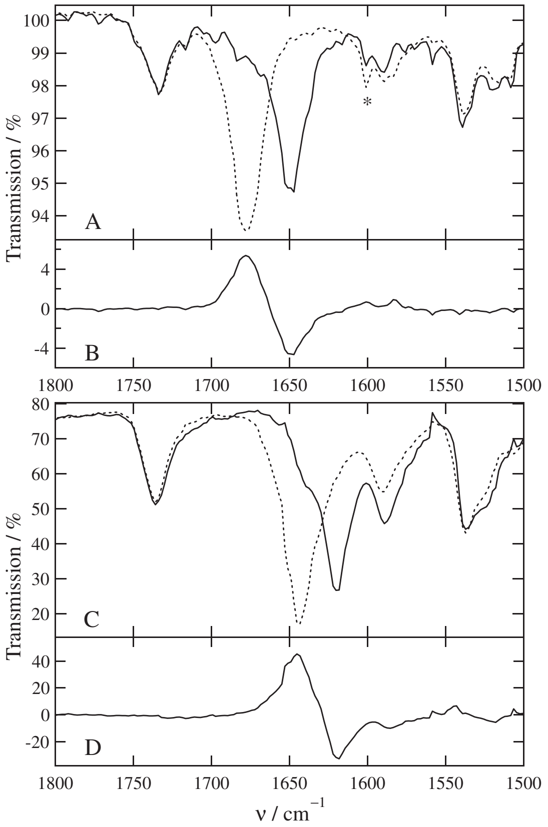

Figure 3. FT-IR spectra of Cd–**1**-^{18}O (87% ^{18}O, solid line) and unlabeled Cd–**1** (broken line) in homogeneous CH_2Cl_2 and C_5H_5N (2:1) solution (A, transmission mode) and in the solid state (C, reflection mode). The difference spectra (B/D) prepared by reduction of solid from broken lines of Figures 3A and 3C: (spectrum of Cd–**1**-^{16}O) − (spectrum of Cd–**1**-^{18}O). The peaks at 1601 cm^{-1} shown by an asterisk * of Figure 3A come from pyridine.

to the specially bonded 13-C=O stretching mode (see Graphical Abstract). The difference in wavenumber between ν(13-C=^{18}O) and ν(chlorin-π-skeleton-C=C/C=N) of $(\text{Cd–}\mathbf{1}\text{-}^{18}\text{O})_n$ is 31 cm^{-1} (=1620 − 1589), which is larger than 20 cm^{-1}, so no resonance coupling could be observed according to the reported criterion[13] (vide supra).

In conclusion, selective ^{18}O-labeling of chlorophyll chromophores has been proven to be useful for elucidation of complex biological (supra)molecular structures. Reconfirmation of significant lower wavenumber shifted 13-keto-carbonyl stretching bands in artificial chlorosomal self-aggregates of chlorophyll derivatives indicates that natural self-aggregates of BChls-*c*/*d*/*e* in a chlorosome should be produced by a special hydrogen-bonding of their 13-carbonyl groups, 13-C=O···HO(3^2)···Mg.

Experimental

General. All apparatus were reported in Ref. 5. ^{18}O-Labeled free-base **1**-^{18}O[12] as well as unlabeled metal complexes Zn–**1**[5] and Cd–**1**[15] are available in the literature.

Synthesis of Zinc Methyl 13^1-^{18}O-Labeled 3^1-Demethylbacteriopheophorbide-*d* (Zn–1-^{18}O). According to reported procedures,[14] **1**-^{18}O (87% ^{18}O) was metallated with zinc acetate after stirring at room temperature for 30 min to give a 13:87 mixture of unlabeled Zn–**1** and titled Zn–**1**-^{18}O: IR (THF): 3437 (3^1-O–H), 1741 (17^2-C=O), 1690 (13-C=^{16}O), 1663 (13-C=^{18}O), 1617 cm^{-1} (C=C); MS (FAB): found, *m*/*z* 616. Calcd for $C_{33}H_{34}N_4O_3{}^{18}OZn$ $[M + H^+]$, 616; its vis and ^{1}H NMR data were the same as those of unlabeled Zn–**1**, see Ref. 5.

Synthesis of Cadmium Methyl 13^1-^{18}O-Labeled 3^1-Demethylbacteriopheophorbide-*d* (Cd–1-^{18}O). According to reported procedures,[15] **1**-^{18}O (87% ^{18}O) was metallated with cadmium acetate after refluxing for 2 h to give a 13:87 mixture of unlabeled Cd–**1** and titled Cd–**1**-^{18}O: IR (CH_2Cl_2/C_5H_5N = 2/1): 1734 (17^2-C=O), 1649 (13-C=^{18}O), 1590 cm^{-1} (C=C); MS (FAB): found, *m*/*z* 666. Calcd for $C_{33}H_{34}N_4O_3{}^{18}OCd$ $[M + H^+]$, 666; its vis and ^{1}H NMR data were the same as those of unlabeled Cd–**1**, see Ref. 15.

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