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FT-IR Studies on the Aggregation of Bacteriochlorophyll c from Chlorobium limicola

Kaku UEHARA,* Yukihiro OZAKI,+ Kouichi OKADA,+ and John M. OLSON++
Department of Applied Chemistry, College of Engineering,
University of Osaka Prefecture, Sakai, Osaka 591
+Department of Chemistry, School of Science, Kwansei Gakuin University,
Nishinomiya, Hyogo 662
++Institute of Biochemistry, Odense University, Odense M, DK-5230, Denmark

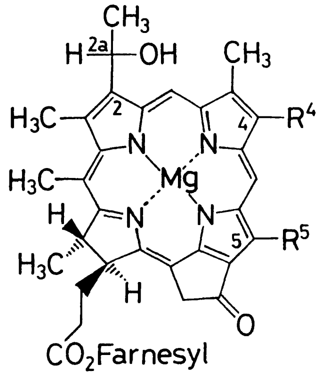
Aggregates of three homologs of bacteriochlorophyll c from Chlorobium limicola in water-saturated carbon tetrachloride and in solid films were studied by means of FT-IR. Four keto-carbonyl stretching peaks were observed at 1703, 1682-4, 1649-51, and 1641-3 cm^{-1} corresponding to free C=O (monomer), free C=O (dimer, tetramer), coordinated C=O (dimer, tetramer), and coordinated C=O (polymer), respectively.

Bacteriochlorophyll c (BChl c) in Chlorobium limicola (green sulfur bacterium) has several homologs including the 2-(R)-(1-hydroxyethyl)-4-ethyl-5-ethyl-homolog (EEF-BChl c), the 2-(R)-(1-hydroxyethyl)-4-n-propyl-5-ethyl-homolog (PEF-BChl c), and the 2-(S)-(1-hydroxyethyl)-4-isobutyl-5-methyl/ethyl-homolog (iBM/EF-BChl c).[1)] Table 1 shows the structural assignments for BChl c. These BChl c homologs occur in the chlorosome as higher aggregates and act as the light harvesting antenna.[2)] Olson and Pedersen[3)] reported that PEF-BChl c (R-configuration) forms dimers absorbing ca. 680 nm and tetramers (Olson and Cox, unpublished) absorbing at ca. 705-710 nm in CH_2Cl_2, $CHCl_3$ and CCl_4. On the other hand, iBM/EF-BChl (S-configuration) in CCl_4 forms aggregates whose absorption (747 nm maximum) and circular dichroism spectra resemble those of the chlorosome. Uehara and Olson (unpublished) identified the forms of BChl c in water-saturated carbon tetrachloride (H_2O-satd CCl_4) as monomer, dimer, tetramer, and polymer from the analysis of visible absorption spectra by the Simplex curve-fitting method (Olson and Cox, unpublished). Ballschmiter and Katz[4,5)] have previously reported the infrared spectra of dimers, oligomers, and water-coordinated aggregates (polymers) of various chlorophylls in solution and in solid films.

In the present paper, we report a new finding that PEF- and iBM/EF-BChl c homologs form polymers in solid films from H_2O-satd CCl_4 solution. Fourier transform-infrared (FT-IR) spectra of H_2O-satd CCl_4 solutions of EEF-, PEF-, and iBM/EF-BChl c homologs are also presented.

Chlorobium limicola f. thiosulfatophilum 6230 (Tassajara) was grown, and BChl c

Table 1. Structural assignments for the BChl c



BChl c	R^4	R^5	Configuration at C-2a
EEF-	Et	Et	R
PEF-	n-Pr	Et	R
iBM/EF-	i-Bu	Me/Et	S

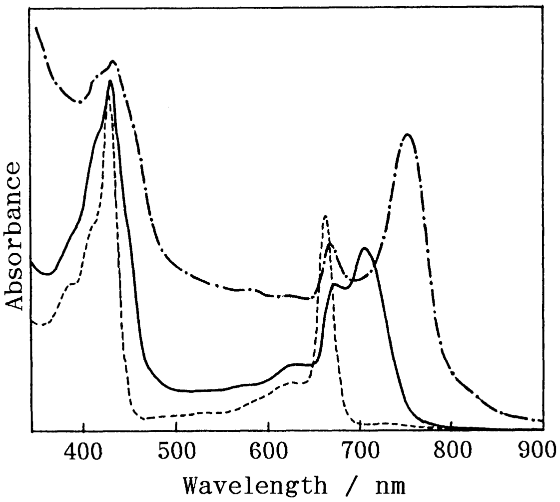


Fig. 1. Absorption spectra of PEF-BChl c in the H_2O-satd CCl_4 (solid), in solid film on CaF_2 from the solution (dot-dash) and in the H_2O-satd CCl_4 solution after addition of 1:200 methanol (dash).

was extracted from wet cells as described previously.[3,6] FT-IR and absorption spectra were recorded with a JEOL JIR-100 FT-IR spectrometer and a Shimadzu UV-360 spectrophotometer, respectively. H_2O-satd CCl_4 was used as the solvent for liquid film samples (0.995 mM, 1.14 mM, and 1.45 mM for iBM/EF-, EEF-, and PEF-BChl c, respectively). A Spectra Tech Contact Sampler was employed for horizontal FT-IR-ATR measurement (45°, ZnSe) of solid BChl c films from the H_2O-satd CCl_4 solution. A CaF_2 plate was used for visible absorption spectrum measurements of solid BChl c films.

Figure 1 shows absorption spectra of PEF-BChl c in H_2O-satd CCl_4 (108 μM) and of the solid film from solution on CaF_2. In the H_2O-satd CCl_4 the spectral pattern of the Q_y band with two absorption maxima at 673 and 705 nm is similar to that for PEF-BChl c in dry CCl_4 which can be deconvoluted into three components; monomer (662 nm; see Fig. 1, dash line), dimer (680 nm), and tetramer (705 nm)[3] (Olson and Cox, unpublished). On the other hand, the absorption spectrum of the solid film shows a large polymeric band at 750 nm with a small monomeric band at 667 nm. A marked red-shift of the Q_y band from 705 nm to 750 nm upon going from solution to solid film indicates the transformation of dimer and tetramer of PEF-BChl c into polymer.

As shown in Fig. 2 and Table 2, four keto C=O stretching peaks were observed at 1703, 1682-4, 1649-51, and 1641-3 cm^{-1}. The bands at 1703 and 1682-4 cm^{-1} can be assigned to the free keto C=O.[4,7] A marked low-wavenumber shift of the keto C=O peak to lower wavenumber arises from the coordination of the keto C=O to the Mg atom of another BChl c molecule or from the hydrogen bonding with the hydroxy group of another BChl c or water molecule involved in the aggregation[6,8] to form dimer, tetramer, or polymer. The bands at 1649-51 and 1641-3 cm^{-1} might be assigned to coordi-

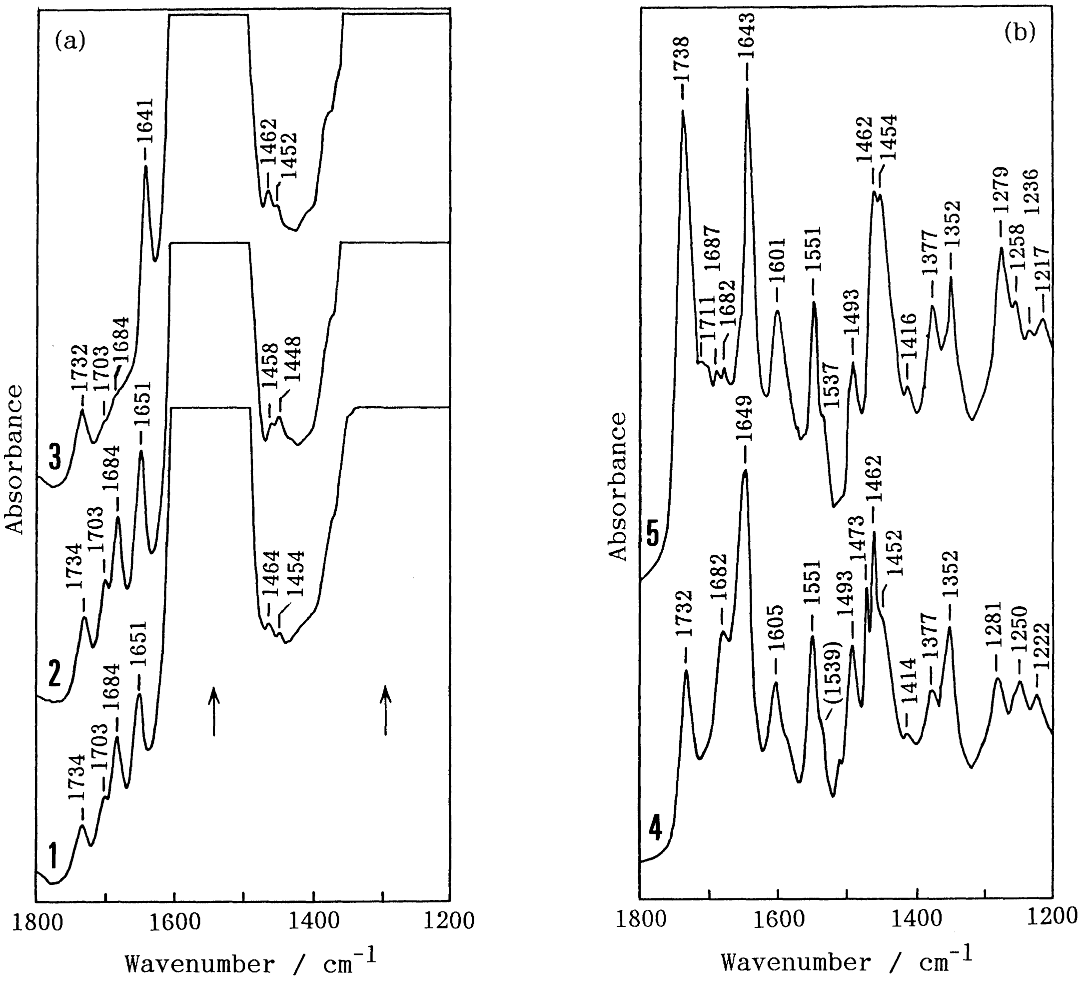


Fig. 2. FT-IR spectra of BChl c homologs in H_2O-satd CCl_4 (a: 1, EEF-; 2, PEF-; 3, iBM/EF-) and in solid films on ZnSe from the solutions (b: 4, EEF-; 5, PEF-). Arrow: absorption band of CCl_4.

Table 2. Comparison of carbonyl peak wavenumbers (cm^{-1}) in FT-IR spectra of BChl c in H_2O-satd CCl_4 and in solid films from solution on ZnSe

Tentative assignment	in H_2O-satd CCl_4 solution EEF-	PEF-	iBM/EF-	in solid film EEF-	PEF-	iBM/EF-
ester C=O	1734 s[e]	1734 s	1732 s	1732 s	1738 s	1734 s
free keto C=O[a]	1703 m	1703 m[f]	1703 sh[g]	-	-	-
free keto C=O[b]	1684 s	1684 s	1684 sh	1682 m	1682 w[h]	-
coordinated keto C=O[c]	1651 s	1651 s	-	1649 s	-	-
coordinated keto C=O[d]	-	-	1641 s	-	1643 s	1641 s

a)(monomer). b)(1/2 from dimer + 1/4 from tetramer). c)(1/2 from dimer + 3/4 from tetramer). d)(polymer). e)s, strong. f)m, medium. g)sh, shoulder. h)w, weak.

nated keto C=O of oligomer4) (dimer, tetramer) and polymer (hydrated aggregates)5), respectively. From the dimer-tetramer ratio in EEF- and PEF-BChl c solutions, the band at 1682-4 cm^{-1} must contain contributions from both dimer and tetramer (1/2 from dimer + 1/4 from tetramer) and the 1649-51 cm^{-1} band must also contain contributions from both dimer and tetramer (1/2 from dimer + 3/4 from tetramer). On the other hand, the iBM/EF-homolog showed a strong polymeric peak at 1641 cm^{-1} with very weak free keto C=O peaks at 1703 and 1684 cm^{-1}. In solid films, however, both the iBM/EF- and the PEF-BChl c homolog showed a strong polymer peak at 1641 and 1643 cm^{-1}, respectively. It is important to note that resonance Raman spectrum of BChl c in the Chlorobium limicola chlorosome shows a single, sharp band at 1639-1640 cm^{-1}.8) The ester C=O stretching peak was observed at 1732-8 cm^{-1} for all three BChl c homologs in solution or solid film, suggesting almost no interaction of the ester C=O with a Mg atom or OH-group of another BChl c molecule. The presence of bands for C=C stretching vibrations at 1551 cm^{-1} (s, strong), 1537-9 cm^{-1} (sh, shoulder) and 1601-5 cm^{-1} (s) do not fall within the 6-coordination ranges and suggest 5-coordination of the Mg atom.8-10) The asymmetric 6-coordinate model11) probably can be excluded. The antiparallel-chain model with 5-coordinate Mg but involving both the 2-hydroxyethyl and 9-keto groups12) is preferred. More detailed studies on the formation and structure of polymeric BChl c aggregates are now in progress.

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