Molecular Assembly of Bachteriochlorophyll *a* and Its Analogues by Synthetic 4α-Helix Polypeptides

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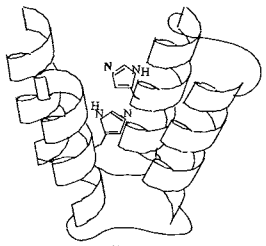
Molecular assemblies of bachteriochlorophyll *a*, BChl *a* and its analogues can be made due to the presence of synthetic 4α-helix polypeptides. UV-vis. and CD spectroscopic properties of BChl *a* derivatives were analogous to those obtained using light-harvesting (LH) polypeptides from photosynthetic bacteria. Thus, appropriate analogous of HAHA are useful in providing an insight into the structural effect of polypeptide on formation the LH / BChl *a* complex as well as an artificial LH complex model.

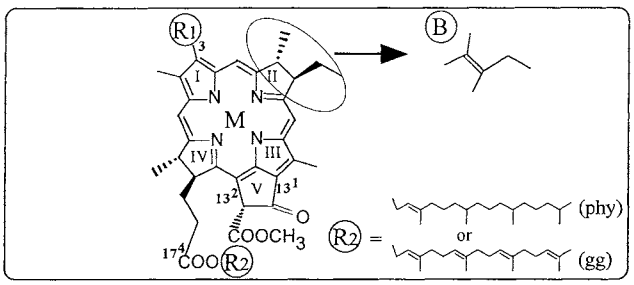
An efficient energy transfer between porphyrin derivatives occurs in the light-harvesting (LH) polypeptides / BChl *a* complex of photosynthetic bacteria.[1,2] It is interesting to note that BChl *a* complex can be organized according to cooperative interactions between the LH polypeptide and BChl *a*, in which Mg atom in BChl *a* is likely to coordinate with the histidine residue in the hydrophobic core of the LH polypeptides and C3 acetyl and C13[1] keto carbonyls of BChl *a* may bind with tryptophane or polar amino acid residues in the C-terminal of LH polypeptides through hydrogen-bondings.[3,4] Several laboratories have demonstrated self-assemblies of porphyrins by using synthetic polypeptides to organize an artificial hemoprotein models.[5,6] However, there has been little study of molecular assembly of BChl *a* and its analogues by using synthetic polypeptides to organize an artificial LH complex. In this paper, we synthesize 4α-helix polypeptides, HAHA and HHHH (Scheme 1) and demonstrate molecular assemblies of BChl *a* and its analogues (Scheme 2) by polypeptides in n-octyl-β-D-glucopyranoside (OG) micelle. The key to the molecular assembly is usefulness in providing an insight into the effect of polypeptide structure on formation of the LH complex as well as in organizing an artificial LH complex. We selected synthetic 4α-helix polypeptides (the 57-peptide) which have histidine residues on the hydrophobic core buried in the hydrophobic domain and also have hydrophilic domain to form 4α-helix bundle structure in aqueous solution. Synthetic 4α-helix polypeptides were prepared and purified by Sephadex LH-60 gel chromatography and then HPLC. These 57-peptide were analyzed by TOF-MS to give expected molecular mass. BChl *a* and its analogues were obtained as described previously.[1,7,8] Zn-BChl *a* was selected because of its stability and similarity to the Mg atom of BChl *a*. The molecular assemblies of BChl *a* and its analogue by using synthetic 4α-helix polypeptides were proceeded as described previously.[7,8]

DAPGELLKA**X**AELLK-
DAPGELLKA**Y**AELLK-
DAPGELLKA**X**AELLK-
DAPGELLKA**Y**AELLK-Naf

	X	Y
HAHA	His	Ala
HHHH	His	His



Scheme 1. Amino acid sequences of synthetic 4α-helix polypeptides.



BChl *a*	: M=Mg	R1= -CHO	R1= gg	
Zn-BChl *a*	: M=Zn	R1= -CHO	R1= phy	
BPheo *a*	: M=H2	R1= -CHO	R1= gg	
Chl *a*	: M=Mg	R1= -CH=CH2	R1= phy	B (Ring II)

Scheme 2. Structure of BChl *a* and its analogues.

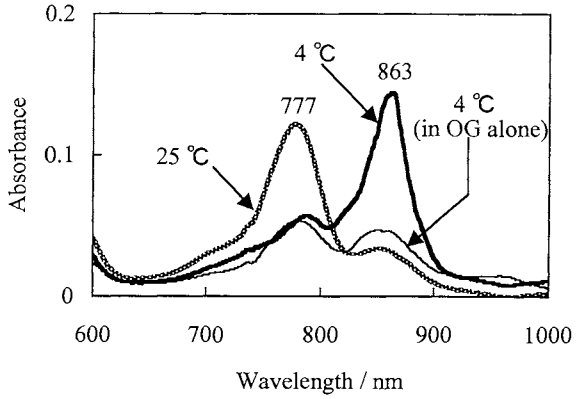


Figure 1. Absorption spectra of BChl *a* in the absence and presence of 4α-helix polypeptide HAHA.
Concentrations : HAHA = 1.73 μM, BChl *a* = 3.45 μM at 0.78% OG solution.

Figure 1 shows UV-vis. spectra of BChl *a* in the presence and absence of 4α-helix polypeptide, HAHA in 0.78% OG solution. The Qy absorption band of BChl *a* -monomer at 777 nm decreased, while the absorbance at 863 nm increased with sharpening due to the presence of HAHA especially when cooling from 25 to 4 ℃.

This sharpening and large-red shift of the Qy absorption band of BChl *a* is the first report in the presence of synthetic 4α-helix polypeptide such as HAHA, consistent with the shift of the Qy band in the LH complex of photosynthetic bacteria.It is considered that the red-shift of the Qy band of to 870 nm in the LH polypeptides of photosynthetic bacteria is likely due to exciton coupling interactions between BChl *a*'s and also increasing intensity of the hydrogen bondings between C3 acetyl carbonyl (C3 C=O) group in BChl *a* and the polar amino acid residues of the LH polypeptides such as tryptophane.[3] Thus, the red-shift of the Qy band to 863 nm due to the presence of HAHA may be contributed to the strong interactions of hydrogen-bondings between C3 C=O group in BChl *a* and polar amino acid residues of the polypeptide or between BChl *a*'s. Interestingly, the red shift of the Qy band was not clearly

Table 1. UV-vis. and CD Spectral Data of Bacteriochlorophyll Derivatives in the Presence of Synthetic 4α-helix Polypeptides

		UV-vis.	CD	
BChl derivatives[a]	Polypeptides[b]	Qy/nm	λ_{max}/nm	(10^{-4} θ)
BChl *a*	HAHA	863	868(34)	835(-7.6)
BChl *a*	HHHH	850 780	866(30)	831(-15)
BChl *a*	LH-α[c] + LH-β[c]	870	883(7.1)	856(-5.7)
BChl *a*	none	848 777	868(29)	832(-13)
Zn-BChl *a*	HAHA	858	875(45)	841(-29)
Zn-BChl *a*	none	850 774	872(28)	840(-9.2)

[a][BChl derivatives] = 3.45×10^{-6} mol dm^{-3} in 0.78% OG solution (phosphate buffer pH 7.5) at 4 ℃. [b][Polypeptides] = 3.45×10^{-6} mol dm^{-3} in 0.78% OG solution at 4 ℃. [c]Sepalately isolated from *R. rubrum*.

observed in the presence of HHHH (Table 1). This reason is not clear but is likely to be that α-helicity of HHHH is too poor to form the complex packing together (α-helix content in the complex with BChl *a*, HHHH: 15%, HAHA: 25%). Similar red-shift of the Qy band was observed for Zn-BChl *a* due to the presence of HAHA (Table 1). No red-shift of the Qy band was observed for Chl *a* and BPheo *a*. These results suggested that Mg or Zn atom in BChl *a* and C3 C=O group in BChl *a* played a crucial role to the red-shift of the Qy band, coordinated with histidine and other polar amino acid residues of HAHA. A largely-splitted CD signal at the corresponding the Qy band in the presence of HAHA supported that the strong exciton-coupling between BChl *a*'s (Table 1).[1,8] However magnetic circular dichroism (MCD) data gave us information that BChl *a* may partially aggregates due to the presence of HAHA in OG micelle (data not shown). Furthermore, NIR-FT Raman spectra of Zn-BChl *a* in the presence of HAHA polypeptides in 0.78% OG at 4 ℃ when excited at 1064 nm were measured.[9,10] NIR-FT Raman indicated that bands due to a stretching mode of C13 C=O and C3 C=O groups in Zn-BChl *a* was shifted from 1700 to 1665 cm^{-1} and from 1687 to 1637 cm^{-1}, respectively,[10] according to the presence of HAHA. However, the band at 1603 cm^{-1} which indicates which the 5-coordinated Zn complex was not shifted. These results implied that the stretching modes of C13 C=O and C3 C=O groups in Zn-BChl *a* were shifted by hydrogen bondings between BChls and polar amino acid residues of HAHA. Thus, these UV-vis., CD, and NIR-FT Raman spectra suggested that BChl *a* and its analogue might coordinate with histidine residues as well as polar amino acid residues of HAHA.

Alternatively, to analyze the molecular size of the complex between BChl *a* and HAHA in OG micelle, small angle X-ray scattering was measured.[11] The data indicated that the diameter of the complex between BChl *a* and HAHA in 0.78% OG at 4 ℃ was 9.1 nm, while that of the subunit complex between BChl *a* and LH polypeptide in 0.78% OG at 25 ℃ was 5.5 nm. Thus, the diameter of the former complex is comparable to that of the subunit complex, B820 complex, which may correspond to two BChl *a* and a pair of LH-α and -β polypeptides.[1]

In conclusion, it is demonstrated that molecular assembly of BChl *a* or its analogue can be made due to the presence of synthetic 4α-helix polypeptides especially when HAHA is present at low temperature. Appropriate analogues of the synthetic 4α-helix polypeptides such as HAHA are useful in providing an insight into the effect of polypeptide structure on the molecular assembly as well as in organizing an artificial light-harvesting complex.

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