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Assemblies of ionic zinc chlorins assisted by water-soluble polypeptides

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ABSTRACT

Artificial chlorophyll–peptide complexes were prepared by mixing ionic chlorophyll derivatives and oligopeptides in aqueous media containing a small amount of organic solvent. The pigment–peptide complexes provided chlorophyll assemblies showing a sharp red-shifted Qy absorption band concomitant with giant circular dichroism signals. The polypeptide-assisted assemblies of chlorophyllous pigments would afford a good model for photosynthetic apparatuses such as pigment–protein complexes of light-harvesting antennas.

1. Introduction

Chlorophylls are magnesium tetrapyrroles that play important roles of light-harvesting and energy conversion in the primary steps of photosynthesis. Generally, chlorophyll molecules are organized in natural photosynthetic complexes such as light-harvesting (LH) antennas and reaction centers (RCs) to achieve efficient intermolecular energy transfer and electron transfer.[1] Most chlorophyll molecules form complexes with proteins and are arranged in the oligopeptide matrixes. The spatial organization and local environment of chlorophyll molecules in the protein matrix would determine the functionality of the natural pigments.[2] X-ray crystallographic studies on the natural pigment–protein complexes have clarified that the chlorophyll molecules associate with amino acid residues of the proteins, and the biomacromolecules create highly functionalized supramolecular architectures.[3] The specific intermolecular interaction among the chlorophyll molecules and proteins induces distinctive optical properties of the natural photosynthetic pigments, which are investigated by many spectroscopic studies including UV–VIS–NIR absorption, circular dichroism (CD), time resolved fluorescence, transient absorption, etc. For instance, the LH2 antenna complex of purple photosynthetic bacteria has many bacteriochlorophyll (BChl)-*a* molecules that associate with helical protein matrixes and show largely red-shifted absorption bands (800 and 850 nm) compared to its original absorption band of the monomeric state in 7:2 solution of acetone and MeOH (770 nm).[4] The proteins in the LH antenna induce pigment–protein and pigment–pigment interactions, which enable the BChl-*a* molecules to absorb the NIR light. The specific optical property of the bacterial pigment–protein complexes is favorable for the bacteria in adapting to their deep-water habitats. Interestingly, the natural pigment–protein LH antenna were reproducible in vitro.[5] When separately isolated BChl-*a* and proteins from the purple bacteria co-assembled in an aqueous buffer solution, the artificial assembly showed an identical red-shifted absorption band of the naturally occurring LH antennas. The reproduced pigment–protein complexes actually work as LH antennae and can be applied to photoactive devices. Therefore, oligopeptides would be useful materials for making artificial photosynthetic systems.[5–7]

Here, we report artificial complexes of semi-synthetic chlorophyll derivatives with polypeptides. Zinc complexes of chlorins (17,18-dihydroporphyrins) possessing a hydrophilic group

Abbreviations: AN, acetonitrile; AVG, average; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; DMAP, *N*,*N*-dimethyl-4-aminopyridine; TEA, triethylamine; TFA, trifluoroacetic acid; LH, light-harvesting; RC, reaction center; Chl, chlorophyll; BChl, bacteriochlorophyll; THF, tetrahydrofuran; DLS, dynamic light scattering; CD, circular dichroism; HRMS, high resolution mass spectrum; MeOH, methanol; NIR, near-infrared; pAsp, poly-L-aspartic acid; pGlu, poly-L-glutamic acid; pLys, poly-L-lysine; pAla, poly-L-alanine; fwhm, full width at half maximum.

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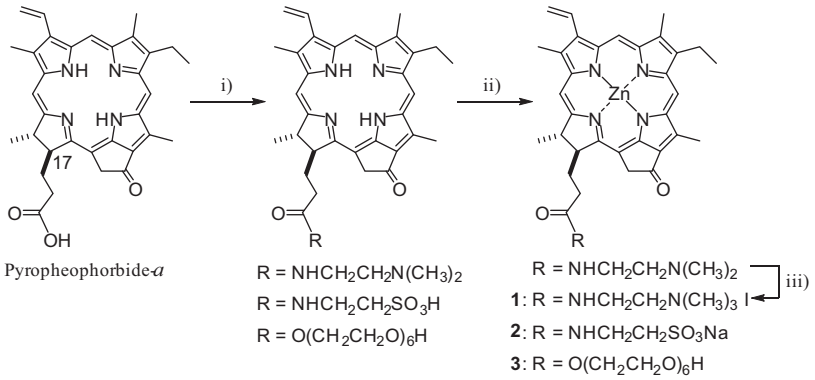

Scheme 1. Synthesis of chlorophyll derivatives possessing a hydrophilic group. (i) Amine or alcohol, EDC·HCl, DMAP/CH_2Cl_2, (ii) $Zn(OAc)_2{\cdot}2H_2O$/MeOH, CH_2Cl_2, (iii) CH_3I/MeOH, CH_2Cl_2.

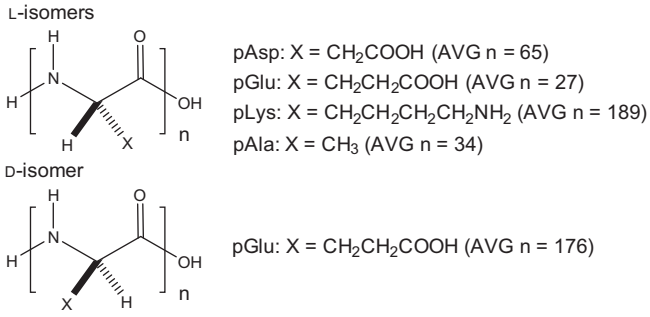

Figure 1. Structure of polypeptides used for complexation of zinc chlorins.

(Scheme 1) and polypeptides (Fig. 1) were used to make models of photosynthetic pigment–protein complexes. A simple mixing of the two components in an aqueous organic solvent provided a pigment–polypeptide complex, in which zinc chlorin molecules self-aggregated to show red-shifted absorption bands and giant CD signals. The artificial complex provided a very simple and readily prepared model of the protein-assisted chlorophyll assemblies.

2. Results and discussion

2.1. Synthesis and properties of amphiphilic zinc chlorins

Semi-synthetic cationic zinc chlorin **1** was prepared according to the reported procedures (Scheme 1).[8,9] A propionic acid side chain at the 17-position of pyropheophorbide-*a* was amidated with *N*,*N*-dimethylethylenediamine followed by zinc metalation and methylation of the tertiary amino group to a cationic quaternary ammonium group. The obtained cationic zinc chlorin was purified by reversed phase HPLC and characterized by ^{1}H NMR and HRMS spectra. Similarly, zinc chlorophyll derivatives possessing an anionic sulfonate group **2** and a nonionic oligooxyethylene group **3** were prepared by the condensation of corresponding amine or alcohol and the zinc metalation. The amphiphilic pigments **1**–**3** showed a Qy absorption band at 658 nm (fwhm: 510 cm^{-1}) in MeOH (Fig. S1), which is a typical absorption band of zinc 3-vinyl-chlorins.[10] Therefore, the introduced hydrophilic groups, ammonium (**1**), sulfonate (**2**) and oligooxyethylene (**3**), did not affect the absorption spectra of the chlorophyll derivatives.

2.2. Complexation of zinc chlorins and polypeptides

Naturally occurring chlorophylls possessing a long esterified alkyl chain are scarcely soluble in aqueous media. In contrast, the modified amphiphilic chlorophyll derivatives **1**–**3** easily dissolved into aqueous buffer solutions containing a small amount of polar organic solvent. A solution of cationic zinc chlorin **1** in MeOH/THF/10 mM Tris–HCl buffer (pH 8.0) = 0.2/15/84.8 (v/v/v) was a clear bluish-green solution and showed a Qy absorption band at 661 nm (fwhm: 550 cm^{-1}) with a tiny negative CD signal. These spectroscopic results are characteristic of monomeric zinc chlorin (dashed line of Fig. 2). When zinc chlorin **1** was mixed with polyaspartic acid (pAsp: Fig. 1) in the same solvent, the Qy absorption band of **1** slightly sharpened (fwhm: 460 cm^{-1}) and shifted bathochromically to 679 nm (solid line of Fig. 2). Additionally, the red-shifted absorption band accompanied giant S-shaped CD signals with positive and negative bands at 684 and 675 nm, respectively. The addition of pAsp to zinc chlorin **1** dramatically changed the optical properties of the chlorophyllous pigment. The solution of **1** and pAsp mixture was homogeneous and showed monodisperse size distribution (polydispersity was 0.36) with an average particle size of 28 nm in dynamic light scattering (DLS) measurement (Fig. S2). Because separately prepared solutions of **1** and pAsp did not show clear DLS signals, the pigment–polypeptide mixture solution contained nano-aggregates of zinc chlorin **1** and

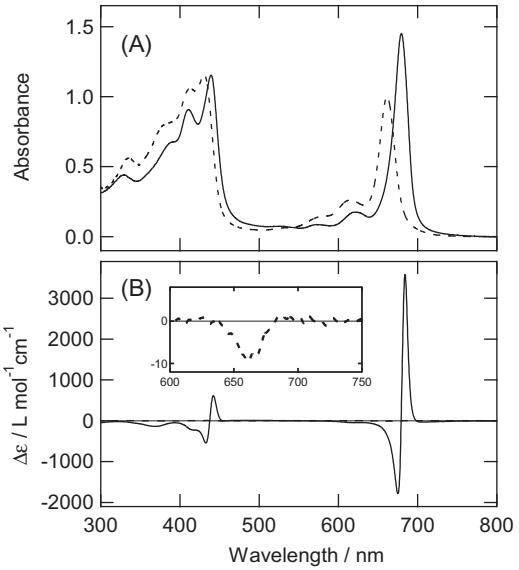

Figure 2. Absorption (A) and CD (B) spectra of zinc chlorin **1** ([**1**] = 20 μM) in 0.2% MeOH, 15% THF and 84.8% 10 mM Tris–HCl buffer (pH 8.0); solid line: with pAsp ([pAsp] = 0.6 μM), dashed line: without pAsp. The inset in (B) shows magnified CD spectra of **1** without pAsp.