J|A|C|S COMMUNICATIONS

Published on Web 01/15/2010

Virus-Templated Assembly of Porphyrins into Light-Harvesting Nanoantennae

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In the natural light-harvesting complexes of cyanobacteria and plants, energy transfer is essential for increasing the exciton flux into the reaction center, where the electron transfer is triggered to drive photosynthesis.[1] Sophisticated self-organization of the natural photosystems serves as a model for artificial photosynthetic systems that require efficient energy and electron transfers.[2] Accordingly, the synthesis and supramolecular self-assembly of a variety of pigments have been widely explored with the aim of constructing photochemical and photoelectronic devices, including photovoltaics, nonlinear optical materials, and photoswitched conductors.[3] Dendritic systems have been widely studied for multipigment arrays; however, it is very challenging and time-consuming to produce such complex molecules in a reasonable yield. Recent studies have shown that biological materials can serve as templates guiding nanoscale organization of pigments via chemical linkage or electrostatic interactions.[4] Viruses are particularly attractive scaffolds because of their highly ordered coat protein structures.[5] M13 viruses have a filamentous structure composed of ~2700 copies of α-helical coat proteins, named pVIII, which are symmetrically arrayed on the viral DNA to compose the ~880 nm long coat that is ~6.5 nm in diameter. The chemical modification of the M13 coat proteins is straightforward because of the exposed N-terminus and Lys residue (both of them are indicated by arrows in Figure 1a) on the viral surface, which can be conjugation sites. The total number of the primary amines available for conjugation is ~5400 per virus. In Figure 1b, the locations of primary amines are indicated as letters, a, b, c, and d for the N-termini and o for the Lys. The calculated average distances between the N-termini and the Lys are oa ≈ 1.0 nm, ob ≈ 1.6 nm, oc ≈ 2.4 nm, and od ≈ 2.4 nm. Thus, it is highly probable that such close distances between the primary amines on the viral surface allow the energy transfer to occur between neighboring pigments attached to the virus, as demonstrated below. Moreover, the flexible N-terminus of pVIII may allow the attached pigments to have considerable freedom in their orientation and thus facilitate interactions between pigments.

Zn(II) deuteroporphyrin IX 2,4-bis(ethylene glycol) (ZnDPEG, Figure 1a) was chosen as a model pigment because of its well-known optical properties, water solubility, and pendant carboxylic groups. The zinc porphyrins were conjugated to pVIII via a carbodiimide coupling reaction (Supporting Information). When collected as pellets, the resulting ZnDPEG-conjugated M13 viruses (denoted ZP-M13) appeared red while unconjugated viruses were white (Figure S1). Two samples, ZP-M13-1 and ZP-M13-2, were prepared using different ratios of ZnDPEG and M13 viruses. Approximately, 1564 and 2900 porphyrins were conjugated per virus for ZP-M13-1 and ZP-M13-2, respectively, as measured from inductively coupled plasma-atomic emission spectrometry.

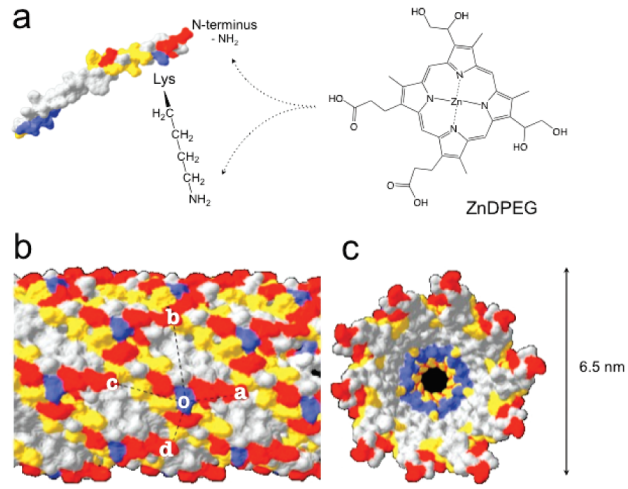

Figure 1. (a). Molecular model of a single pVIII representing negatively charged amino acids in red; positively charged ones in blue; neutral, hydrophilic ones in yellow; and hydrophobic ones in white (software: Swiss-PDB and structure model: 1IFJ). The arrows indicate the sites of primary amines to which ZnDPEG can be conjugated; (b) side view and (c) cross section of the assembled pVIII peptides.

M13 viruses are relatively flexible and thus easily contorted on a flat surface.[6] However, ZP-M13 exhibited a very straight structure (Figure S2). Such a morphological change in ZP-M13 is thought as a result of the interactions of the hydrophobic, π-conjugated macrocycles of the porphyrin with the viral coat protein. Accordingly, the Trp fluorescence of ZP-M13 was considerably lower than that of native M13 viruses (Figure 2a). This change indicated the conformational change of pVIII; that is, the Trp residues were exposed to the outer aqueous environment presumably through their interaction with the attached porphyrins. Protein structures in the natural light harvesting complexes play a critical role in modulating the site energy levels of individual pigments via interactions with protein residues and neighboring pigments, which in turn affect the pathway of excitation energy transfer.[7] Such protein matrix effects could be observed in the absorption spectral changes of ZP-M13. Figure 2b shows the absorption spectrum of monomeric ZnDPEG monomers exhibited a strong Soret band at 406 nm and two weak Q bands at 538 and 574 nm. Compared to free ZnDPEG, the porphyrins assembled on the virus had a considerably broader Soret band. This spectral broadening can be explained by the diverse pigment−protein interactions, which generate different site energies of the excited states. Such heterogeneous microenvironments of the viral surface are illustrated by the various surface functional groups, as shown in Figure 1b, rendering diverse local effects of dipole−dipole interactions and hydrogen bonding to the attached porphyrins.

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10.1021/ja908812b

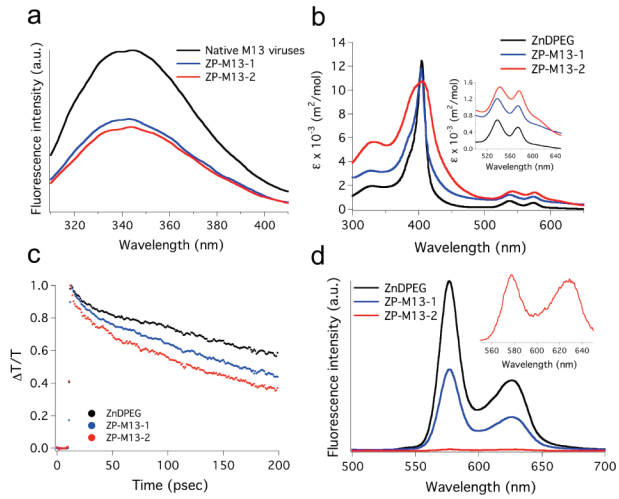

Figure 2. (a) Tryptophan fluorescence emission spectra of native M13 viruses and ZP-M13 with excitation at 295 nm. (b) Absorption, (c) transient absorption at 400 nm, and (d) fluorescence emission with excitation at 400 nm of ZnDPEG, ZP-M13-1, and ZP-M13-2. In (b), ϵ denotes the molar extinction coefficient. The insert in (b) shows a magnified version of the spectra of the Q bands, and the insert in (d) shows a magnified version of the fluorescence emission spectrum of ZP-M13-2.

To verify the exciton migration along the pigments assembled on the virus, pump−probe transient absorption spectroscopy was used (Figure S3). The transient absorption change of the Soret band (Figure 2c) indicated that the excited state of ZP-M13 was relaxed significantly faster than that of ZnDPEG. A two-parameter fit indicated that the dominant relaxation pathway has a lifetime of 273 ± 4 ps for ZP-M13-1 and 223 ± 5 ps for ZP-M13-2, while free ZnDPEG has a lifetime of 415 ± 3 ps (Figure S4 and Table S1). These data imply the delocalization of the excitons through the pigments assembled on the virus. The relatively long distance between the pigments assembled on the virus (~3.5 nm in average for ZP-M13-2) and no significant shift of the Soret band in the absorption spectrum of ZP-M13 suggest that such exciton delocalization is driven via the Förster resonant energy transfer, though the Dexter mechanism seems to contribute to fluorescence quenching process as described below.

The most dramatic optical variation of ZP-M13 was the quenching of the fluorescence emission (Figures 2d and S5). This effect can be explained by a small red shift found in the Q bands of the absorption spectrum of ZP-M13 (538 → 543 nm and 574 → 577 nm, respectively) (Figure 2b). It is known that the red shift of the Q bands reflects the formation of the electronic coupling between pigments. However, such interaction may not be very strong in the ZP-M13 presumably because ZnDPEG lacks *meso*-substituents, which are known to stabilize the *J*-type aggregates.[8] Accordingly, the circular dichroism spectra of the ZP-M13 exhibited no distinctive peaks in the Soret region, indicating the formation of H-type coupling between the conjugated pigments (data not shown). The orbital disordering caused by the pigment−pigment interactions may induce a significant change in the nuclear coordinates of the excited state relative to the ground state. This change can result in the formation of trap sites where the radiative transition becomes much slower than the nonradiative relaxation. This explanation is supported by the result that the fluorescence quenching increases with increasing degree of conjugation, which implies that the Dexter-type electronic interactions between porphyrins are essential for the efficient quenching process.[9]

In summary, we have shown that molecular pigments can be assembled into a light-harvesting antenna using M13 viruses as templates through chemical linkage. The photons absorbed by the pigments assembled on the virus seem to travel through long-range dipole−dipole interactions and be quenched in the trap sites generated by the electron coupling of the pigments. This result implies that the energy transfer of the assembled pigments is controllable by genetically inserting or deleting amino acids on pVIII, which may modify the site energies and the pigment−pigment distances. Through genetic engineering, we are currently making efforts to coassemble catalysts and electron transport mediators with pigments on M13 viruses, aiming to apply such coassembled nanostructures to photochemical systems which often require a large charge flux to catalytic sites through energy transfer.

Acknowledgment. We thank Dr. Thomas Renger and Dr. Rebekah Miller for critical suggestions and CSBi Biophysical Instrumentation Facility for CD spectrophotometer. This work was supported by Eni, S.p.A. through the MIT Energy Initiative Program and NSF Grant No. CHE-0616939.

Supporting Information Available: Experimental details on conjugation procedures and characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Horton, P.; Ruban, A. V.; Walters, R. G. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1996**, *47*, 655–684.
(2) (a) Balzani, V.; Credi, A.; Venturi, M. *ChemSusChem* **2008**, *1*, 26–58. (b) Hasobe, T.; Kamat, P. V.; Troiani, V.; Solladie, N.; Ahn, T. K.; Kim, S. K.; Kim, D.; Kongkanand, A.; Kuwabata, S.; Fukuzumi, S. *J. Phys. Chem.* **2005**, *109*, 19–23. (c) Choi, M. S.; Yamazaki, T.; Yamazaki, I.; Aida, T. *Angew. Chem., Int. Ed.* **2004**, *43*, 150–8.
(3) (a) Steinberg-Yfrach, G.; Liddell, P. A.; Hung, S. C.; Moore, A. L.; Gust, D.; Moore, T. A. *Nature* **1997**, *385*, 239–241. (b) Drain, C. M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5178–82. (c) Kovaric, B. C.; Kokona, B.; Schwab, A. D.; Twomey, M. A.; de Paula, J. C.; Fairman, R. *J. Am. Chem. Soc.* **2006**, *128*, 4166–7. (d) Wang, Z.; Medforth, C. J.; Shelnutt, J. A. *J. Am. Chem. Soc.* **2004**, *126*, 15954–5. (e) Dunetz, J. R.; Sandstrom, C.; Young, E. R.; Baker, P.; Van Name, S. A.; Cathopolous, T.; Fairman, R.; de Paula, J. C.; Akerfeldt, K. S. *Org. Lett.* **2005**, *7*, 2559–61.
(4) (a) Miller, R. A.; Presley, A. D.; Francis, M. B. *J. Am. Chem. Soc.* **2007**, *129*, 3104–9. (b) Fendt, L. A.; Bouamaied, I.; Thoni, S.; Amiot, N.; Stulz, E. *J. Am. Chem. Soc.* **2007**, *129*, 15319–29. (c) Endo, M.; Fujitsuka, M.; Majima, T. *J. Org. Chem.* **2008**, *73*, 1106–12.
(5) (a) Scolaro, L. M.; Castriciano, M. A.; Romeo, A.; Micali, N.; Angelini, N.; Lo Passo, C.; Felici, F. *J. Am. Chem. Soc.* **2006**, *128*, 7446–7. (b) Lee, S. K.; Yun, D. S.; Belcher, A. M. *Biomacromolecules* **2006**, *7*, 14–7. (c) Mao, C.; Solis, D. J.; Reiss, B. D.; Kottmann, S. T.; Sweeney, R. Y.; Hayhurst, A.; Georgiou, G.; Iverson, B.; Belcher, A. M. *Science* **2004**, *303*, 213–7. (d) Nam, K. T.; Kim, D. W.; Yoo, P. J.; Chiang, C. Y.; Meethong, N.; Hammond, P. T.; Chiang, Y. M.; Belcher, A. M. *Science* **2006**, *312*, 885–8.
(6) Khalil, A. S.; Ferrer, J. M.; Brau, R. R.; Kottmann, S. T.; Noren, C. J.; Lang, M. J.; Belcher, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 4892–7.
(7) Raszewski, G.; Saenger, W.; Renger, T. *Biophys. J.* **2005**, *88*, 986–8.
(8) Okada, S.; Segawa, H. *J. Am. Chem. Soc.* **2003**, *125*, 2792–6.
(9) Fudickar, W.; Zimmermann, J.; Ruhlmann, L.; Schneider, J.; Röder, B.; Siggel, U.; Fuhrhop, J.-H. *J. Am. Chem. Soc.* **1999**, *121*, 9539–45.

JA908812B