J|A|C|S COMMUNICATIONS

Published on Web 12/16/2008

Energy Transfer among Light-Harvesting Macrorings Incorporated into a Bilayer Membrane

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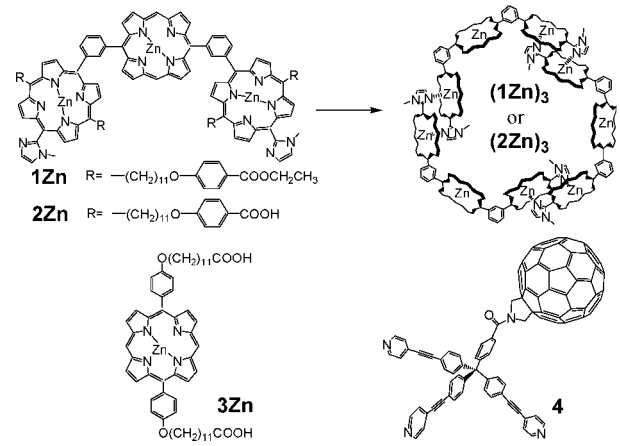
The photosynthetic antenna of purple bacteria is one of the best-characterized membrane proteins. These proteins have two types of light-harvesting complexes, designated as LH1 and LH2. LH1 accommodates the reaction center[1] in its central cavity. LH2 constitutes large peripheral antennas that serve to increase the cross section for light absorption and transfer the excitation energy to the reaction center via LH1.[2] In both complexes, dimeric subunits of bacteriochlorophylls are arranged into macrorings with slipped cofacial structures. Highly efficient excitation energy transfer is performed by the remarkable organization of these pigments in light-harvesting complexes.[3]

In recent years, great efforts in molecular design to mimic antenna structures using covalently linked[4] or self-assembled[5] multiporphyrin arrays have been undertaken. At present, however, only a few studies incorporating porphyrins into a bilayer membrane have been reported.[6] Furthermore, no examples simulating the energy transfer systems between LH1 and LH2 exist in the literature. In a previous paper, we reported the preparation of macroring architectures mimicking the structure of B850 in LH2 by complementary coordination of *m*-phenylene gable porphyrins appended with imidazolyl groups,[7] These cyclic assemblies are regarded as one of the most ideal elements for constructing artificial antenna systems because their fluorescence is not quenched by the assembly into ring structures, even though the chromophores are strongly coupled because of their close proximity. The self-assembled cyclic trisporphyrin trimer provides a cavity to accommodate functional tridentate ligands with large binding constants ($K > 10^7$ dm^3 mol^{-1}).[8]

Here we report on the incorporation of a cyclic assembly made of amphiphilic trisporphyrins into a liposomal membrane to mimic the bacterial antenna system. Four amphiphilic ω-carboxyalkylbenzoyl groups were introduced at two positions of the terminal porphyrins of the trisporphyrin in order to place the dissociated carboxylates near the membrane surface. These chromospheres can be fixed at high concentrations in the bilayer membrane with their planes perpendicular to the membrane surface, similar to LH1 and B850 in LH2. The energy transfer among these cyclic assemblies has been demonstrated by the use of an energy/electron acceptor in the antenna ring.

A trisporphyrin precursor **1Zn** (Scheme 1) was prepared by condensation of 5-(1-methylimidazol-2-yl)-10,15-bis[11-(4-ethoxybenzoyloxy)undecyl]-20-(3-formylphenyl)porphyrin with dipyrromethane, followed by Zn(II) insertion. Carboxyl-terminated trisporphyrin **2Zn** was prepared by hydrolysis of **1Zn**. In order to obtain the cyclic trimers **(1Zn)$_3$** and **(2Zn)$_3$**, **1Zn** and **2Zn** were reorganized according to a published procedure.[8a,b] The cyclic structure was confirmed by ^{1}H NMR and analytical gel permeation chromatography (GPC)−HPLC. The monomeric porphyrin **3Zn** was synthesized as the reference compound.

Scheme 1. Structures of the Cyclic Assembly Formed from the Amphiphilic Trisporphyrins, the Monoporphyrin, and an Internal Energy/Electron Acceptor



Small unilamellar vesicles (SUVs) containing **(2Zn)$_3$** or **3Zn** in their interior were prepared through a sonication procedure.[9] The molar ratios of **(2Zn)$_3$** and **3Zn** to phospholipids were varied in the ranges 1/300 to 1/7500 and 9/300 to 9/1500, respectively. In all cases, no porphyrin remained at the top of the gel filtration column after elution with buffer, indicating that the porphyrins were incorporated quantitatively into the SUVs. The absorption spectrum of **(2Zn)$_3$** incorporated into the vesicles [**(2Zn)$_3$**/lipid molar ratio < 1/300] showed a Soret band split into two peaks at 408 and 440 nm due to exciton coupling between the neighboring porphyrin units, and its maxima and fwhm remained unaltered relative to that in 9/1 $CHCl_3$/MeOH (Figure S4 and Table S1 in the Supporting Information). The absorption spectrum suggests that **(2Zn)$_3$** is incorporated in the membrane while retaining the trimeric macroring structure. In the case of **3Zn**, the Soret band in the bilayer membrane (**3Zn**/lipid = 9/1500) showed a red shift and was broadened (39 nm) compared with that in the homogeneous solution. These differences in the spectra of **(2Zn)$_3$** and **3Zn** may be explained by the fact that the monomeric porphyrin aggregates in the relatively high concentrations in the membrane, while the cyclic trimers are prevented from dense aggregation by their void structures with the reported compound.[6d] Incorporation of **(2Zn)$_3$** into the membrane at molar ratios less than 1/300 reduced the fluorescence due to concentration quenching but kept the intensity higher than 25% of the initial value (Table S1).

The cyclic trimer has three coordination sites of monomeric zinc porphyrins that seem to be the most suitable for cooperative ligation by tridentate ligands. The coordination behavior of the trimer was investigated by using tripodal ligand **4** appended with a C_{60} energy/electron acceptor. The binding constants in $CHCl_3$ were obtained from spectroscopic titrations of **(1Zn)$_3$** at various concentrations

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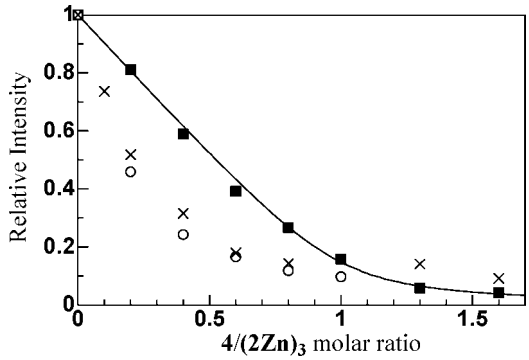

Figure 1. Fluorescence quenching curves at 623 nm upon complexation of macroring **(2Zn)$_3$** with tripodal ligand **4** in SUVs at **(2Zn)$_3$**/lipid molar ratios of 1/1500 (○) and 1/7500 (×) and that of **(1Zn)$_3$** (9.1×10^{-8} M) in $CHCl_3$ solution (■, observed points; solid line, theoretical curve calculated with $K = 3.5 \times 10^8$ M^{-1}).

of **4** by using the characteristic red shift of Soret bands and the fluorescence quenching profile on ligation. The binding constant of the **(1Zn)$_3$**−**4** complex was estimated to be greater than 10^8 M^{-1} (Figure S7 in the Supporting Information), indicating that **4** was selectively and cooperatively incorporated into **(1Zn)$_3$**, as we reported previously for a cyclic trimer with 3-allyloxypropyl groups.[8b] Next, we investigated the spectroscopic properties of **(2Zn)$_3$** toward incorporation of the tripodal guest in the liposomal membrane. **(2Zn)$_3$** and **4** were cosonicated with phospholipids, and the resulting dispersion was isolated by gel filtration as described above. The molar ratios of the host in the phospholipids were examined at 1/1500 and 1/7500, where the host fluorescence was retained. Addition of **4** to **(2Zn)$_3$** resulted in a sharp red shift of the Soret bands at low **4**/**(2Zn)$_3$** ratios, exhibiting behaviors similar to those in homogeneous solution (Figures S6 and S9 in the Supporting Information). Furthermore, a shoulder at 540 nm due to an uncoordinated porphyrin moiety appeared and decreased in intensity with increasing **4** concentration. The 540 nm peak disappeared after the addition of 1 equiv of **4** in a homogeneous solution. These changes can be useful in monitoring coordination on the free porphyrinatozinc (Figure S10 in the Supporting Information). They suggest that the cyclic structure is maintained in SUVs and binds **4** with a large association constant for 1:1 complexation.

The efficiency of excited energy transfer among cyclic trimers was estimated by fluorescence quenching. The relative fluorescence intensities of **(2Zn)$_3$** determined by the addition of various amounts of the guest molecule are illustrated in Figure 1 in comparison with the plot obtained in a homogeneous solution of **(1Zn)$_3$** in $CHCl_3$. When the mixture of **4** and **(2Zn)$_3$** at a molar ratio of 1/5 was put into phospholipid vesicles at a **(2Zn)$_3$**/lipid molar ratio of 1/1500, the quenching efficiency was 54%. This efficiency was about 3 times larger than that of **(1Zn)$_3$**−**4** in a homogeneous solution (19%). The fact that the binding behavior of **(2Zn)$_3$**−**4** remained similar in the bilayer membrane and in a homogeneous solution implies that the three cyclic assemblies in the membrane were quenched by the introduction of one acceptor molecule. Under more dilute conditions at a **(2Zn)$_3$**/lipid molar ratio of 1/7500, the quenching efficiency was slightly decreased. In the case of monomeric porphyrin **3Zn**, the fluorescence quenching remained as low as 28% at a 1/5 molar ratio of **4** to **3Zn** and 30−40% even at a 1/3 molar ratio, corresponding to the equimolar mixture of the pyridyl ligand and the coordination site. Fluorescence quenching data combined with UV−vis spectral changes demonstrate that the

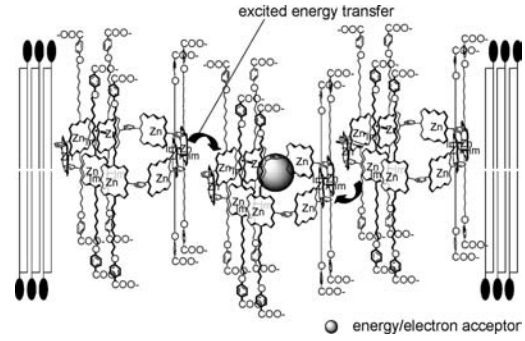

Figure 2. Schematic diagram of excitation energy transfer among macrocyclic antenna complexes in a bilayer membrane.

(2Zn)$_3$ macrorings maintain their binding capacity at the high initial concentrations in the membrane and permit energy transfer among their assemblies, as shown in Figure 2. The cyclic structure is necessary to realize the high affinity and selectivity for binding the tripod ligand **4**.

In this paper, we have examined the incorporation of a cyclic assembly consisting of amphiphilic trisporphyrins into a liposomal membrane. The cyclic assembly **(2Zn)$_3$** was fixed in an orientation similar to bacterial light-harvesting complexes in the phospholipid vesicle. Under the conditions concentrated in the membrane, the fluorescence quenching by incorporation of the energy/electron acceptor to the inner sphere of **(2Zn)$_3$** was enhanced by energy transfer from neighboring **(2Zn)$_3$**. The amphiphilic porphyrin macroring was demonstrated to be a suitable candidate for constructing an artificial photosynthetic antenna or reaction center complex in the membrane.

Supporting Information Available: Synthetic details; GPC, MS, UV−vis, and NMR spectra; UV−vis titration data; and preparation of small unilamellar vesicles. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA8045712