ChemComm

Liposome made of imidazolyl-substituted porphyrin as a single component

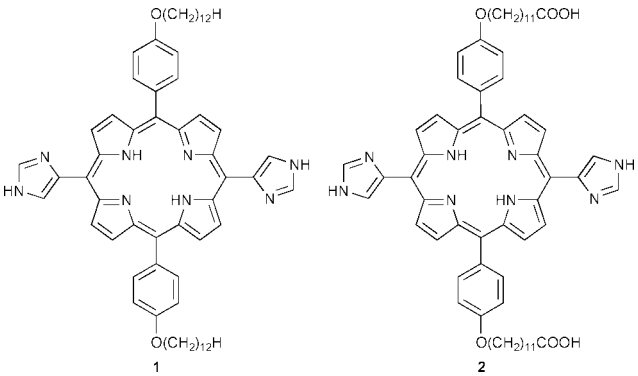
Naoto Nagata, Shin-ichi Kugimiya and Yoshiaki Kobuke*

Graduate School of Materials Science, Nara Institute of Science and Technology, CREST, Japan Science and Technology Corporation (JST), 8916-5 Takayama, Ikoma City, 630-0101, Japan. E-mail: kobuke@ms.aist-nara.ac.jp

Received (in Cambridge, UK) 27th November 2000, Accepted 8th March 2001
First published as an Advance Article on the web 29th March 2001

5,15-Bis(imidazol-4-yl)-10,20-bis(ω-carboxyalkyloxyphenyl)porphyrin 2 was assembled in water to form liposomal dispersions, whose structure were confirmed by DLS, AFM, TEM measurements as well as entrapment of a hydrophilic fluorescent probe.

Solar energy conversion is undertaken efficiently in chloroplasts or photosynthetic bacterial membranes, where chlorophylls or bacteriochlorophylls are arranged in well-organized structures of fixed distances and orientations. Examples are found in photosynthetic bacterial light-harvesting complexes where a large number of bacteriochlorophylls are arranged in a macro-ring form by coordination of imidazolyl side chains from transmembrane α-helices.[1] We have been interested in constructing light harvesting systems from chromophore assemblies without use of peptides.[2] Recently, we found that 5,15-bis(4-dodecyloxyphenyl)-10,20-bis(imidazol-4-yl)porphyrin **1** was assembled through imidazolyl–imidazolyl hydrogen bonds in non-polar media and that the assembly could perform more or less antenna functions.[3] Following on from this, large porphyrin assemblies were planned, to be organized into the form of liposomes by their own combined intermolecular interactions. For this purpose, a bis(imidazolyl)porphyrin **2** having two meso-ω-carboxyalkoxyphenyl substituents was designed and prepared according to Lindsey's procedure[4] from imidazol-4(5)-yl-2,2′-dipyrrylmethane and ethyl 12-(4-formylphenoxy)dodecanoate, followed by hydrolysis.[5]



1 2

Compound **2** was dispersed in water by sonication and subjected to gel filtration column separation to isolate the fraction corresponding to small unilamellar vesicles.[6] Dynamic light scattering (DLS) measurement[7] of the dispersion of **2** elucidated a mean diameter of 27 ± 8 nm. The aqueous sample was then applied on a smooth mica plate and dried in air. Fig. 1(a) shows a top view of atomic force microscope (AFM) measurement and 1(b) the side view along the line in 1(a). The observed full widths at the baseline level were 36, 32 and 38 nm for (A), (B) and (C), respectively. These values are larger than those estimated from DLS measurements because of the geometrical tip/sample convolution effect.[8] Fig. 1(c) shows a TEM image of the liposome dispersions. Many particles with diameters in the range 20–30 nm are observed, along with some aggregates that may have formed through drying under vacuum. These images confirm the size distribution obtained by the DLS measurement.

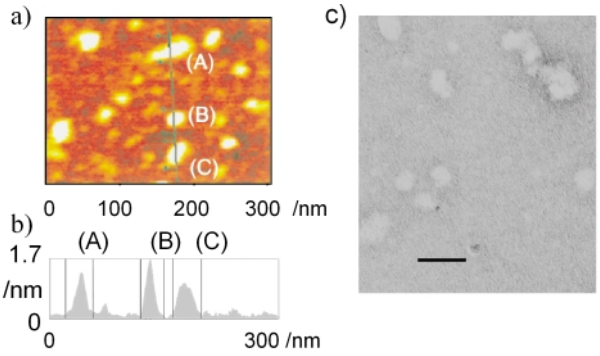


Fig. 1 AFM image of dispersion prepared from **2** developed on mica. a) A top view and b) a cross-section along the line in a). c) TEM image of negatively stained samples of dispersion from **2** with uranyl acetate. (bar = 50 nm).

The size of the system is apparently larger than that of spherical micellar aggregates in the range 2–4 nm[9] and coincides with the typical range for small unilamellar vesicles, 20–30 nm. Definitive evidence of vesicle formation, however, would be obtained by proving the presence of an interior aqueous phase and showing it to be capable of keeping polar solutes inside the vesicle. Therefore, a hydrophilic fluorescent probe, pyranine, was co-sonicated with **2** and the dispersion was isolated by Sephadex G-50 gel filtration.[10] Because of the strong absorption of porphyrin **2** and diffraction from the dispersion, no absorption of pyranine was detected in the UV-Vis spectrum. However, the presence of pyranine in the fraction from a gel filtration column was detected by its fluorescence spectra as shown in Fig. 2(a). The fluorescence intensity was low, due to self-quenching at the relatively high initial concentration of 0.3 mM. Fig. 2(b) and (c) show the emission from pyranine after 24 h and 40 h, respectively, from the gel filtration. The intensity gradually increased in a slow time-course and reached a saturation value only after 40 h. In a separate experiment, the fluorescence intensity of pyranine was increased 3× by the addition of 0.1 ml of a 0.1% aqueous solution of Triton X-100. Adsorption of pyranine on the liposomal membrane surface is unfavorable because both components are negatively charged, but in order to safely exclude that possibility the liposome was prepared without the

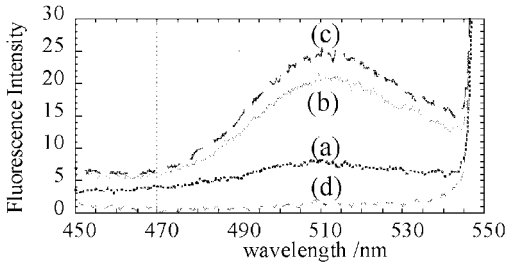


Fig. 2 Fluorescence spectra of porphyrin dispersion co-sonicated with pyranine, λ_{EX} = 280 nm. (a) Just after gel filtration (Sephadex G-50). (b) The same after 24 h and (c) 40 h. (d) Same as (a) without co-sonication, but after treatment with 0.3 mM pyranine, followed by gel filtration.

DOI: 10.1039/b009458g

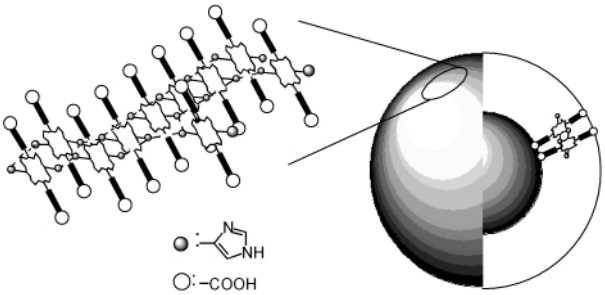


Fig. 3 Schematic representation of the liposome structure of **2** in aqueous solution.

addition of pyranine and than subjected to gel filtration. The resulting liposome fraction was immersed in a 0.3 mM pyranine solution. The liposome fraction, gel-filtered again, gave no fluorescence emission of pyranine (Fig. 2(d)). These results clearly indicate that the membrane can hold the entrapped pyranine and release it slowly to the exterior, when the fluorescence intensity is increased by liberation from the concentration quenching.

Based on these observations, the dispersions are concluded to be liposomes that contain an interior aqueous phase and provide a barrier toward the release of the hydrophilic solute entrapped. Their size corresponds to small unilamellar vesicles. A schematic representation of liposome formation from **2** is shown in Fig. 3. The liposomes we report on here are made only of bis(imidazolyl)porphyrin **2** without any phospholipid such as lecithin. To the best of our knowledge, Fuhrhop and coworkers reported only one example of liposome formation from porphyrins in the literature.[11] In their report, tetrakis[(bixinylamino)-*o*-phenyl]porphyrin was stabilized by side chain polymerization. In our system, the primary driving force for the formation of membrane aggregates comes from hydrophobic and ionic interactions of the amphiphilic compound in water.

The absorption spectrum of **2** dispersed in water (λ_{max} = 426 nm with the bandwidth at the half-height(hbw) of 91 nm resembled that of **1** in cyclohexane (λ_{max} = 432.5 nm and hbw_{Soret} = 86 nm). Both Soret bands were significantly broadened compared to that of **1** in methanol (λ_{max} = 417 nm and hbw_{Soret} = 40 nm). Analysis of ^{1}H-NMR and UV spectral studies shows bisimidazolylporphyrin **1** to assemble into primarily a slipped cofacial orientation through imidazole–imidazole hydrogen bonds in non-polar solvents such as cyclohexane, toluene or $CDCl_3$, but not methanol.[3] Similar structure formation is expected for the dispersion of **2** in water by hydrogen bond networks in the aggregate assembled by hydrophobic interactions. A further stabilization may be provided by hydrogen bond networks by imidazolyl substituents and π–π interactions of porphyrins assembled in the central belt part of the membrane and therefore favoured by an entropy term. The low permeability of entrapped pyranine across the liposomal membrane suggests that a strong hydrogen bond network has been constructed in the central region of the membrane. The liposomal membrane seems rather stiff as judged from AFM and TEM images since the liposomes maintained convex structures even under dry or vacuum conditions, respectively. The symmetrical structure of two carboalkoxyphenyl-substituted porphyrins may not be ideal for the formation of liposomes of small curvatures. However, a membrane-penetrating lipid, glycerol dialkylglycerol tetraether from thermophilic archaebacteria,[12] provides a convincing example of stable liposome formation from such symmetrical transmembrane lipids.

Porphyrins assembled in liposomal membranes are interesting materials in view of testing functions of light harvesting, light-induced charge separation and electron transfer across the membrane. Research targets along these lines are currently under active investigation.

The authors thank Dr M. Iwano (NAIST) and Mr N. Ohkubo (Seiko Instruments Co.) for the measurement of TEM images and AFM images, respectively.

Notes and references

1 G. McDermott, S. M. Prince, A. A. Free, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell and N. W. Isaacs, *Nature*, 1995, **374**, 517; J. Koepke, X. Hu, C. Muenke, K. Schulten and H. Michel, *Structure*, 1996, **4**, 581.

2 I. V. Rubtsov, Y. Kobuke, H. Miyaji and K. Yoshihara, *Chem. Phys. Lett.*, 1999, **308**, 323.

3 N. Nagata, S. Kugimiya and Y. Kobuke, *Chem. Commun.*, 2000, 1389.

4 C.-H. Lee and J. S. Lindsey, *Tetrahedron*, 1984, **39**, 11 427.

5 5,15-Bis[4-(11-carboxyl)undecyloxyphenyl]-10,20-bis(imidazol-4-yl)-porphyrin **2**: ^{1}H NMR (600 MHz, TFA-D, 40 °C) δ = 9.63 (2H, s, imidazole), 8.91 (2H, s, imidazole), 9.05 (4H, br, pyrrole$^{\beta}$H), 8.85 (4H, br, pyrrole$^{\beta}$H), 8.57 (4H, d, J = 8.1 Hz, phenoxy), 7.79 (4H, d, J = 8.1 Hz, phenoxy), 4.56 (4H, br, O$^{\alpha}$CH$_2$), 2.57 (2H, m, $-^{\beta}$CH$_2-$), 2.16 (4H, br, $-$CH$_2$CO$_2$H), 1.80–1.33 (32H, s, $-$(CH$_2$)$_9-$). UV-vis(CHCl$_3$) λ_{max}/nm: 417, 519, 550, 590, 654. Mass (MALDI-TOF, α-CHCA) m/z 1023.42 (M + H), Calcd ($C_{62}H_{70}N_8O_6$): 1022.54.

6 Preparation of liposomes: bis(imidazolyl)porphyrin **2** (0.5 mg, 0.48 μmol) was dissolved in MeOH (5 mL). After evaporation of the solvent, the sample was dried under vacuum at rt for 6 h. The thin porphyrin film thus obtained was dispersed in a solution of pyranine (0.5 mg) dissolved in 3 mL of distilled water. The dispersion was sonicated in a cold room at 4 °C using a horn-type sonicator (TOMY SEIKO, UR-200) for 5 min (level 4) and left at 4 °C for 10 min cooling. This procedure was repeated 4×. The dispersions free from external pyranine were isolated through a Sephadex G-50 gel filtration column (ø 2 × 20 cm).

7 Dynamic light scattering (DLS) measurement was carried out by DLS-6000 (Photal, Co.). The atomic force microscope (AFM) image of the dispersion of **2** on a mica plate was obtained on a SPI 3800N (Seiko Instruments Co.) according to a dynamic force mode with a Si cantilever (a spring constant of 56 N m^{-1}, tip curvature radius of 10 nm).

8 J. Yang, T. Laurion, T.-C. Jao and J. H. Fendler, *J. Phys. Chem.*, 1994, **98**, 9391.

9 J. H. Fendler, *Membrane Mimetic Chemistry*, John Wiley & Sons, Inc., 1982. Radius of DTAB micelle was reported as 1.67 nm, H. L. Tavernier, A. V. Barzykin, M. Tachiya and M. D. Fayer, *J. Phys. Chem. B*, 1998, **102**, 6078.

10 M. Merritt, M. Lanier, G. Deng and S. L. Regen, *J. Am. Chem. Soc.*, 1998, **120**, 8494.

11 T. Komatsu, E. Tsuchida, C. Böttcher, D. Donner, C. Messerschmidt, U. Siggel, W. Stocker, J. P. Rabe and J.-H. Fuhrhop, *J. Am. Chem. Soc.*, 1997, **119**, 11660.

12 H. Komatsu and P. L.-G. Chong, *Biochemistry*, 1998, **37**, 107.