Polymer Vesicles

Supramolecular Self-Assembly of Giant Polymer Vesicles with Controlled Sizes**

*Yongfeng Zhou and Deyue Yan**

Supramolecular self-assembled vesicles have attracted great attention for their potential applications in drug delivery, gene therapy, and model systems of biomembranes.[1] Previous methods to make vesicles include the self-assembly of large copolymers and small amphiphiles such as lipids (liposomes);[2–4] however, compared to liposomes, polymer vesicles possess unique properties such as good stability and permeability. Polymer vesicles have thus become attractive and promising research objects since the first observation of block copolymer vesicles by Eisenberg and co-workers,[2] who called the aggregations of the block copolymer with small hydrophilic fractions ($<20\,\%$) crew-cut micelles. Discher et al. reported another type of polymer vesicle formed from block copolymers having a similar hydrophilic fraction ($35\pm10\,\%$) to liposomes and these were termed polymersomes.[3] Polymer vesicles have also been prepared from other materials, such as, polypeptides, rod-coil polymers, and dendrimers.[5] The vesicle-forming systems based on small amphiphiles, dendrimers, or linear block copolymers have a well-defined molecular structure. The polymer vesicles resulting from the molecular self-assembly of ill-defined polymers such as hyperbranched copolymers have not yet been reported. Furthermore, giant polymer vesicles with diameters of 100 μm have not been observed to date, although Menger et al. have demonstrated in their research on liposomes that "giant vesicles" with diameters of 5–200 μm have many special advantages.[4] We report here the preparation of new kind of polymer vesicle with a controlled size (the diameters of the larger polymer vesicles are above 100 μm) that are generated from the molecular self-assembly of ill-defined hyperbranched copolymers having a high hydrophilic fraction ($>60\,\%$).

Recently, we reported[6] macroscopic molecular self-assembly from a hyperbranched multiarm copolymer (HBPO-star-PEO) in acetone (HBPO = hyperbranched poly(3-ethyl-3-oxetanemethanol), PEO = poly(ethyleneglycol)), which gave rise to multiwalled tubes with diameters of the order of millimeters and lengths of the order of

[*] Dr. Y. Zhou, Prof. Dr. D. Yan
College of Chemistry and Chemical Engineering
State Key Laboratory of Metal Matrix Composites
Shanghai Jiao Tong University
800 Dongchuan Road, Shanghai 200240 (China)
Fax: (+86) 21-54741297
E-mail: dyyan@sjtu.edu.cn

[**] Financial support from the National Natural Science Foundation of China (Nos. 20274024, 50233030, and 20304007) and from the basic research foundation of the Shanghai Science and Technique Committee is acknowledged.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim DOI: 10.1002/ange.200460325

centimeters. It was found that the molecular structure of the HBPO-star-PEO copolymer greatly influenced the macroscopic self-assembly behavior. The work reported herein investigates the self-assembly of HBPO-star-PEO copolymers in water. Three HBPO-star-PEO copolymers with different hydrophilic fractions (PEO volume fraction) denoted HB1, HB2, and HB3 (Figure 1) were synthesized. The overall

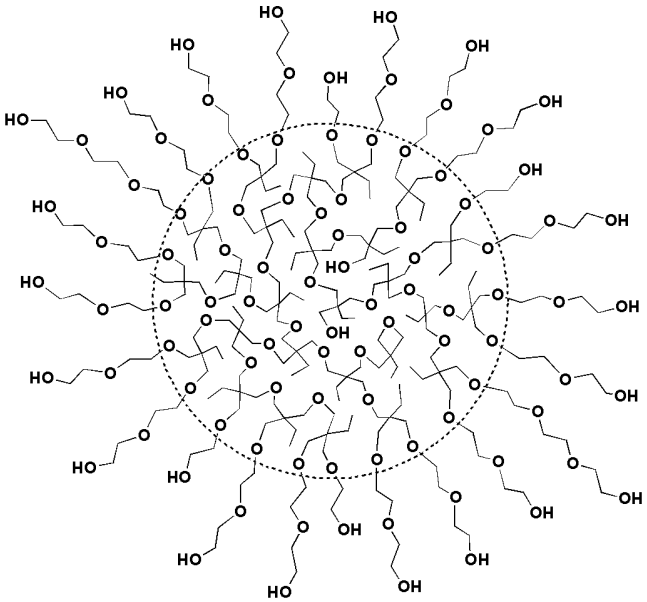

Figure 1. Schematic representation of the HBPO-star-PEO multiarm copolymer. The dashed circle shows the boundary between the HBPO core (inside) and the PEO arms.

structures of HB1–3 are different from those used in the macroscopic molecular self-assembly.[6] The molecular self-assembly of HB1–3 involved directly placing the polymer into deionized water (polymer concentration: 10 mg mL^{-1}) under stirring at room temperature. The properties of HB1–3 and the aggregations are summarized in Table 1, while the synthesis, characterization, and self-assembly of HB1–3 are described in the Supporting Information.

Table 1: Details of vesicle-forming multiarm copolymers.[a]

Sample	$\bar{M}_n$ [g mol^{-1}]	Polydispersity index	f_{EO}	Diameter [μm]	d [nm]
HB1	12200	1.5	0.69	112.8 ± 30	10 ± 2
HB2	16800	1.7	0.79	22.6 ± 4.2	5 ± 2
HB3	20800	1.9	0.86	4.0 ± 1.6	5 ± 2

[a] $\bar{M}_n$: the number-average molecular weight; Polydispersity index: weight-to-number average molecular weight; f_{EO}: the hydrophilic PEO volume fraction; d: the vesicle wall thickness. The HB1–3 samples have the same HBPO cores with a molecular weight of 6400 g mol^{-1}.

The optical and transmission electron microscopy (TEM) images of the resulting self-assembled objects are shown in Figure 2. It appears that HB1–3 directly self-assembled into a well-defined vesicular structure in water, and the vesicles increased in size from 1 to 200 μm as the hydrophilic fraction decreased. The HB2 and HB3 vesicles (polymer concentration less than 100 mg mL^{-1}, other morphologies were found for polymer solutions with concentrations higher than 100 mg mL^{-1}) could be stored for at least two months in water at around 20 °C, but the HB1 vesicles settled under gravity at the bottom of the container underneath the medium. Compared with the polymer vesicles previously reported, the aggregation observed in this work has several unique characters: 1) the vesicles originate from an ill-defined hyperbranched amphiphilic multiarm copolymer; 2) the HB1–3 copolymers that can directly form vesicles in water have a higher hydrophilic fraction (> 60 %) than those forming polymersomes and crew-cut micelles (block copolymers with a hydrophilic fraction higher than 40 % often self-assemble into wormlike and spherical micelles); 3) the HB1–3 vesicles have giant sizes similar to those of giant liposomes,[4] and to the best of our knowledge HB1 provides the largest known polymer vesicles, with diameters larger than 100 μm. In short, the aggregation of the giant vesicles reported herein is different from crew-cut micelles, polymersomes and other polymer vesicles, and results in a new sort of polymer vesicle. A new terminology, branched polymersome, is used to describe this new type of aggregation.

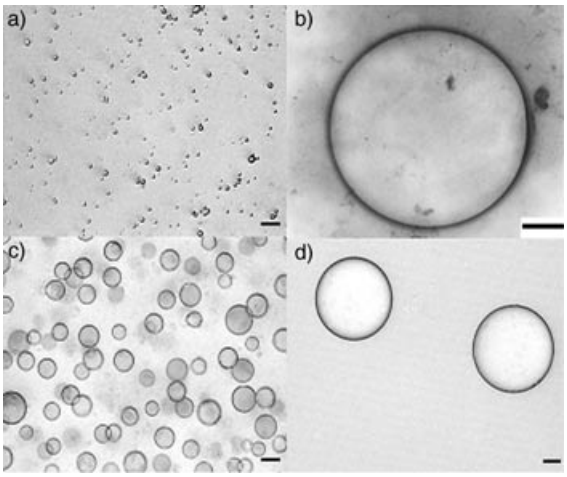

Figure 2. Optical micrographs (a, c, d) and TEM image (b) of HB1–3 vesicles. a), b) HB3 vesicles. c) HB2 vesicles. d) HB1 vesicles. The samples for TEM measurement were negatively stained with 2% aqueous uranyl acetate solution. The scale bars represent 25 μm in (a), (c), and (d), and 250 nm in (b).

The size distributions of HB1–3 vesicles are displayed in Figure 3. The size of the vesicles was measured from the staff gauge in the microscopy studies, and the results were based on an analysis of 200 vesicles for HB2 and HB3 and 100 vesicles for HB1. The average diameters of the vesicles are 4.0 μm for HB3, 22.6 μm for HB2, and 112.8 μm for HB1 (Figure 3 and Table 1). Thus, the size of the generated vesicles increases and the size distribution becomes broader as the hydrophilic fraction of the HBPO-star-PEO molecules decreases from 0.86 to 0.69. However, the distribution of

 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

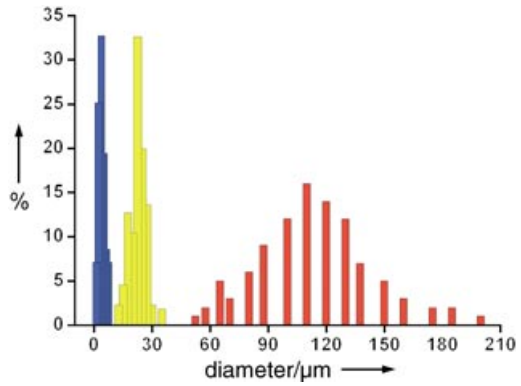

Figure 3. Size distribution of the giant polymer vesicles made from the HBPO-star-PEO multiarm copolymer. The blue, yellow, and red bars represent HB3, HB2, and HB1 vesicles, respectively.

the vesicle size is not directly related to the polydispersity index of the copolymer (Table 1). Discher and Eisenberg[1a] also found that polydispersity is not a strict requirement for vesicle formation. Such a size dependence of the giant vesicles on the copolymer composition as shown in Figure 2 has not been observed before, and it presents an easy approach to the assembly of giant polymer vesicles with well-controlled sizes. In fact, submicroscopic vesicles (not shown) were also obtained through the self-assembly of HBPO-star-PEO with a much higher hydrophilic fraction in water, but they did not fall within the concept of "giant vesicles" discussed in this work. It is necessary to control the vesicle size in technological and scientific applications;[2d] for example, giant vesicles are very good models for the simulation of the fluidization of cell membranes.[4a]

The self-assembly mechanism of HBPO-star-PEO molecules in water includes two aspects: one is the self-assembly interaction, and the other is the molecule packing in the self-assembled vesicle wall. The hydrophobic interaction, which is attributed to the amphiphilic character of HBPO-star-PEO in water, and the formation of hydrogen bonds are responsible for driving the self-assembly. The importance of hydrogen bonds in the self-assembled HB1–3 vesicles has been confirmed by variable-temperature FTIR spectroscopy as well as by temperature- and concentration-dependent ^{1}H NMR spectroscopy. The molecule packing in the vesicle wall was investigated by solution-state ^{1}H NMR data. These studies show that the surface of the vesicle is covered by PEO arms and the HBPO cores are located inside the vesicle wall, thus resulting in the vesicle wall having a sandwich structure. A previous report[6] on the macroscopic molecular self-assembly of HBPO-star-PEO molecules in acetone had shown that the tube walls possessed an alternate PEO and HBPO lamella structure, and thus suggested that the sandwich structure in the HB1–3 vesicle walls consisted of PEO-HBPO-PEO lamellae. In addition, small-angle X-ray scattering (SAXS) experiments further proved the lamellar structure in the HB1–3 vesicle wall. The lamellar thickness measured by SAXS experiments is 5.6 nm for HB3 vesicles, 5.3 nm for HB2 vesicles, and 11.1 nm for HB1 vesicles, which fits well with the wall thickness of the HB1–3 vesicles measured by TEM studies (Table 1). Consideration of the HBPO-star-PEO molecule size, the lamella structure, the lamella thickness, and the vesicle wall thickness leads us suggest that the HB2 and HB3 molecules pack into a monolayer structure in the related vesicle walls in the same way as a triblock copolymer,[7,2g] and the HB1 molecules pack into a bilayer structure similar to that in diblock copolymer vesicles.[2,3] For details of the experiments and analysis of the self-assembly mechanism see the Supporting Information.

A tentative molecular packing model for the walls of the HB1–3 vesicles is shown in Figure 4. In the monolayer model (Figure 4a) each HBPO-star-PEO molecule behaves as an amphiphilic triblock copolymer (ABA type) and spontaneously segregates into a molecular sandwich with an internal layer consisting of the HBPO core and outer shells of PEO arms as a result of the hydrophobic interaction, while the molecular sandwiches aggregate into giant vesicles. In the bilayer model (Figure 4b) each HBPO-star-PEO molecule segregates into a hydrophobic HBPO part and a hydrophilic PEO part similar to the amphiphilic structure of diblock copolymers, while the hydrophobic parts aggregate together to form the bilayer structure similar to that in vesicles formed by a diblock copolymer. The boundary between the two core layers in Figure 4b does not really exist, and the exaggerated boundary is only shown to illustrate the bilayer structure more clearly. Comparison of the HB1–3 molecules shows that HB1 has shorter hydrophilic PEO arms, which possibly could not stabilize the monolayer structure and resulted in HB1 forming a bilayer structure so as to keep the self-assembled objects stable.

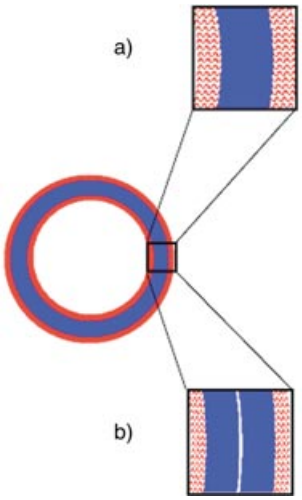

Figure 4. Proposed molecular packing models in the HB1–3 vesicle walls. The blue zones represent the condensed HBPO cores and the red wavy lines denote PEO arms. a) A monolayer structure. b) A bilayer structure. The illustrations are not drawn to scale.

In conclusion, we successfully obtained a new kind of giant polymer vesicle from an ill-defined hyperbranched multiarm copolymer having a high hydrophilic fraction (>60%) through direct self-assembly in water. These polymer vesicles have been termed branched polymersomes. The

 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

size of the branched polymersomes can be easily controlled by adjusting the hydrophilic fraction of the copolymer, with the average diameter of the larger branched polymersomes exceeding 100 μm. Two possible molecular packing models in the vesicle walls are proposed. The large size of the branched polymersomes suggests they will have applications for encapsulation and simulation of the biomembrane process. Further results of the morphology transition and membrane fluidity of these branched polymerases will be reported in the near future.

Received: April 14, 2004
Published Online: August 30, 2004

Keywords: block copolymers · self-assembly · supramolecular chemistry · vesicles

[1] a) D. E. Discher, A. Eisenberg, *Science* **2002**, *297*, 967; b) J.-M. Lehn, *Science* **2002**, *295*, 2400; c) H. Ringsdorf, B. Schlarb, J. Venzmer, *Angew. Chem.* **1988**, *100*, 117; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 113.

[2] a) L. Zhang, A. Eisenberg, *Science* **1995**, *268*, 1728; b) L. Zhang, A. Eisenberg, *Science* **1996**, *272*, 1777; c) L. Zhang, A. Eisenberg, *Polym. Adv. Technol.* **1998**, *9*, 677; d) H. Shen, A. Eisenberg, *Angew. Chem.* **2000**, *112*, 13448; *Angew. Chem. Int. Ed.* **2000**, *39*, 3310; e) L. Luo, A. Eisenberg, *J. Am. Chem. Soc.* **2001**, *123*, 1012; f) L. Luo, A. Eisenberg, *Angew. Chem.* **2002**, *114*, 1043; *Angew. Chem. Int. Ed.* **2002**, *41*, 1001; g) F. Liu, A. Eisenberg, *J. Am. Chem. Soc.* **2003**, *125*, 15059.

[3] a) B. M. Discher, Y.-Y. Won, D. Ege, J. C.-M. Lee, F. S. Bates, D. E. Discher, D. A. Hammer, *Science* **1999**, *284*, 1143; b) B. M. Discher, D. A. Hammer, F. S. Bates, D. E. Discher, *Curr. Opin. Colloid Interface Sci.* **2000**, *5*, 125; c) J. C.-M. Lee, H. Bermudez, B. M. Discher, M. A. Sheehan, Y.-Y. Won, F. S. Bates, D. E. Discher, *Biotechnol. Bioeng.* **2001**, *73*, 135; d) H. Bermudez, A. K. Brannan, D. A. Hammer, F. S. Bates, D. E. Discher, *Macromolecules* **2002**, *35*, 8203; f) F. Ahmed, A. Hategan, D. E. Discher, B. M. Discher, *Langmuir* **2003**, *19*, 6505.

[4] a) F. M. Menger, K. D. Gabrielson, *Angew. Chem.* **1995**, *107*, 2260; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2091; b) F. M. Menger, J. S. Keiper, *Adv. Mater.* **1998**, *10*, 888; c) F. M. Menger, J. S. Keiper, K. L. Caran, *J. Am. Chem. Soc.* **2002**, *124*, 11842; d) F. M. Menger, V. A. Seredyuk, M. V. Kitaeva, A. A. Yaroslavov, N. S. Melik-Nubarov, *J. Am. Chem. Soc.* **2003**, *125*, 2846; e) W. H. Binder, V. Barragan, F. M. Menger, *Angew. Chem.* **2003**, *115*, 5980; *Angew. Chem. Int. Ed.* **2003**, *42*, 5802.

[5] a) H. Kukula, H. Schlaad, M. Antonietti, S. Förster, *J. Am. Chem. Soc.* **2002**, *124*, 1658; b) M. Antonietti, S. Förster, *Adv. Mater.* **2003**, *15*, 1323; c) F. Chécot, S. Lecommandoux, Y. Gnanou, H.-A. Klok, *Angew. Chem.* **2002**, *114*, 1395; *Angew. Chem. Int. Ed.* **2002**, *41*, 1340; d) S. I. Stupp, V. LeBonheur, K. Walker, L. S. Li, K. E. Huggins, M. Keser, A. Amstutz, *Science* **1997**, *276*, 384; e) J. C. M. Vanhest, D. A. P. Delnoye, M. W. P. L. Baars, M. H. P. Van Genderen, E. W. Meijer, *Science* **1995**, *268*, 1592.

[6] D. Yan, Y. Zhou, J. Hou, *Science* **2004**, *303*, 65.

[7] a) H. Schuch, J. Klingler, P. Rossmanith, T. Frechen, M. Gerst, J. Feldthusen, A. H. E. Müller, *Macromolecules* **2000**, *33*, 1734; b) H. Xu, R. Erhardt, V. Abetz, A. H. E. Müller, W. A. Goedel, *Langmuir* **2001**, *17*, 6787; c) R. Erhardt, M. Zhang, A. Böker, H. Zettl, C. Abetz, P. Frederik, G. Krausch, V. Abetz, A. H. E. Müller, *J. Am. Chem. Soc.* **2003**, *125*, 3260; d) K. Schillén, K. Bryskhe, Y. S. Mel'nikova, *Macromolecules* **1999**, *32*, 6885.

 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim