J|A|C|S COMMUNICATIONS

Published on Web 08/18/2010

Polymersome Stomatocytes: Controlled Shape Transformation in Polymer Vesicles

Kyoung Taek Kim,[§,†] Jiahua Zhu,[‡] Silvie A. Meeuwissen,[†] Jeroen J. L. M. Cornelissen,[†] Darrin J. Pochan,[‡] Roeland J. M. Nolte,[†] and Jan C. M. van Hest*[,†]

School of Nano-Biotechnology and Chemical Engineering, Ulsan National Institute of Science and Technology, Ulsan 689-798, Korea, Department of Materials Science and Engineering, University of Delaware, Newark, Delaware 19716, and Institute for Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ, Nijmegen, The Netherlands

Received May 14, 2010; E-mail: j.vanhest@science.ru.nl

Abstract: We report here a controllable shape transformation of polymer vesicles (polymersomes) constructed from block copolymers of which the hydrophobic part is a high-molecular-weight glassy segment. Control over the shape transformation is obtained by kinetic manipulation of the phase behavior of this glassy hydrophobic segment. Kinetic manipulation of the phase behavior of polymer membranes allows for different shapes of polymersomes to be captured at specific times, which directly translates into physically robust nanostructures that are otherwise unobtainable. Combining the morphological diversity of giant liposomes and the physical robustness of polymersomes, our finding can be a general way to realize unusual nanostructures in a predictable manner.

Giant unilamellar vesicles (giant liposomes, 10−50 μm in diameter) readily transform their shapes from spheres to a variety of nonspherical morphologies in correspondence to changes in the environment,[1,2] owing to the fluidity of the phospholipid bilayer membranes. Polymer vesicles (polymersomes) are believed to undergo shape transformations and to show morphological diversity comparable to that of giant liposomes[3] if the polymer membrane possesses enough mobility under ambient conditions.[4] The size regime of polymersomes, often orders of magnitudes smaller than that of giant liposomes, however, makes it difficult to characterize the transient morphologies obtained during the shape transformation. Furthermore, if a glassy hydrophobic block is used,[5] a thermodynamically favored spherical morphology obtained after polymersome formation would be locked due to the glassy polymer membrane; therefore, any further shape transformation of the polymersome would be prevented.

Here we report a controllable shape transformation of polymersomes constructed from block copolymers of which the hydrophobic part is a high-molecular-weight glassy segment. Control over the shape transformation is obtained by kinetic manipulation of the phase behavior of this hydrophobic block.[6] Combining the morphological diversity of giant liposomes and the physical robustness of polymersomes,[7] our finding can be a general way to realize unusual nanostructures in a predictable manner.

In conventional methods for the preparation of polymersomes based on block copolymers possessing a glassy hydrophobic block,[8] organic solvents are used to facilitate their formation process by plasticizing the polymeric aggregates that are formed. We envisaged that this plasticizing effect could also be applied to induce shape transformations in polymersomes by providing enough mobility and permeability to the polymer membrane to allow it to respond to changes in the environment. We took advantage of the fact that the hydrophobic polymer domain displays a phase transition from a solvent-swollen rubbery state to a rigid glassy state, which results in the capture of a transient morphology in a physically robust frozen structure. Kinetic manipulation[6] of this phase behavior of polymer membranes allows for different shapes of polymersomes to be captured at a specific time, which directly translates into physically robust nanostructures that are otherwise unobtainable (Figure 1).

Amphiphilic block copolymers poly(ethylene glycol)-*block*-polystyrene (PEG-*b*-PS), with different molecular weights, were synthesized by atom-transfer radical polymerization (ATRP) starting from PEG-macroinitiators (number-average degree of polymerization (DP_n) = 45 and 115). The DP_n of the PS blocks was adjusted to be in the range of 102−312. All samples showed a narrow size distribution (polydispersity index (PDI) = 1.08−1.18) (Table S1, Supporting Information). At ambient temperature, the glassy nature of the hydrophobic PS block prevents direct hydration of the block copolymer in water. Hence, polymer vesicles from these block copolymers were prepared by a cosolvent method.[8,9]

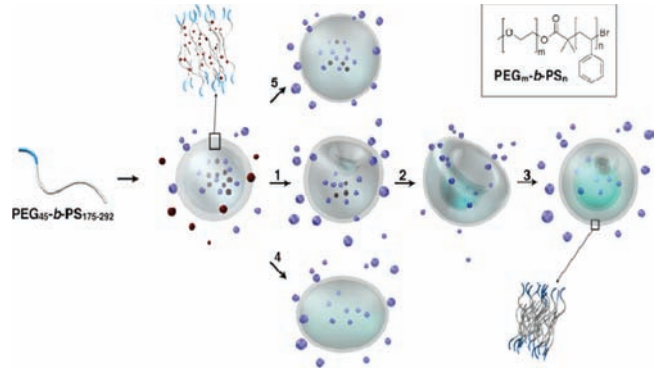

Figure 1. Shape transformation of polymersomes during dialysis of organic solvents (dark red spheres) against water (blue spheres) through a solvent-swollen bilayer membrane (paths 1−3). Shape change also results in prolate formation, depending on the architecture of the block copolymers (path 4). Direct quenching yields spherical polymersomes (path 5).

Following these preparation conditions,[9] a dioxane:THF (1:1 v/v, 1 wt %) solution of PEG_{45}-*b*-PS_{230} yielded a cloudy suspension upon the addition of water. A fraction of this solution (50 μL) was taken from this suspension before dialysis was performed and added at once to a small amount of pure water to rapidly freeze the

[§] Ulsan National Institute of Science and Technology.
[†] Radboud University Nijmegen.
[‡] University of Delaware.

10.1021/ja104154t

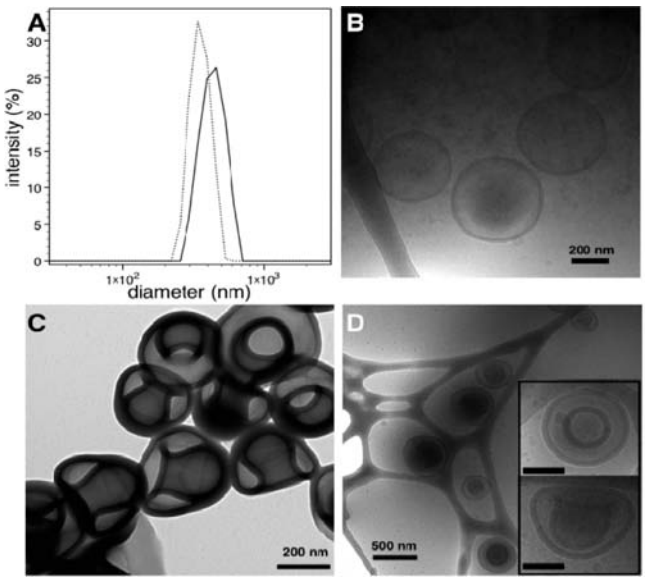

Figure 2. (A) DLS results of the average diameter of polymersomes (D_{av}) before (solid line) and after dialysis of organic solvents from the initial suspension (dotted line). (B) A cryo-TEM image of polymersomes of PEG_{45}-*b*-PS_{230} without shape change obtained by rapid quenching of the suspension in water. Typical dry TEM (C) and cryo-TEM (D) images of stomatocytes after shape transformation of polymersomes of PEG_{45}-*b*-PS_{230}, showing different projections of the structures. Insets in D show top (upper) and side (lower) views of stomatocytes (scale bars: 200 nm).

morphology of the aggregates, which is a common method to prepare spherical polymer vesicles. These rapidly quenched polymersomes showed an average diameter (D_{av}) of 400 nm, with a moderate size distribution (PDI = 0.09), based on dynamic light scattering (DLS) measurements (Figure 2A). The cryo-TEM study of this sample revealed spherical polymer vesicles (Figure 2B), which indicated that a rapid quenching of the PS domain results in vitrification, as commonly observed, due to the high T_g of PS, which provides enough physical strength to the bilayer membrane to preserve the thermodynamically favored spherical morphology. After dialysis of the remaining suspension against pure water, we observed that the D_{av} value of the polymersomes decreased to 350 nm without changing the size distribution (PDI = 0.07) (Figure 2A), which suggests a reduced overall volume of polymersomes without significant vesicle fission.

When the dried polymersome solution after dialysis was examined by TEM, we found that the morphology of the polymersomes had completely changed from spheres to stomatocytes,[10] a bowl-shaped vesicle with a "mouth", as shown in Figure 2C (Figures S1 and S2, Supporting Information). The Cryo-TEM images of the solution after dialysis confirmed that the stomatocytes are the consequence of shape transformation of the polymersomes and are not artifacts that result from the sample preparation procedure for TEM (Figure 2D). We further examined the transformations of polymersomes prepared from a series of PEG_{45}-*b*-$PS_{102-292}$ block copolymers by using the same procedure and conditions, which revealed that the formation of stomatocytes occurred when the DP_n of the PS block exceeded 175. All stomatocytes retained their morphology over a long period of time (>4 months) when examined by TEM and did not show any change in diameter or size distribution, as measured by DLS. Even harsh conditions, such as elevated temperatures (70 °C, 1 h) and ultrasonication (45 kHz, 200 W, 5 min at 50 °C), did not alter the morphology of the objects and indicates excellent physical stability, as expected for polymer vesicles with a glassy PS hydrophobic domain.

We reasoned that the shape change of the polymersomes is mainly driven by the decrease in the volume of the inner compartment of the polymersomes during dialysis rather than a drastic change in the surface area of the bilayer membrane (Figure 1). Considering the thickness of the bilayer membrane determined from cryo-TEM images (~26 nm) for polymersomes of PEG_{45}-*b*-PS_{230}, the contour length of the PS_{230} chain (~27 nm), and the molecular weight of the PS block, which exceeds the entanglement length ($DP_n \approx 130$),[11] we may expect that PS chains within the bilayer membrane are most probably in a coiled conformation with chain−chain entanglements.[12] This renders any molecular rearrangements between two membranes difficult during dialysis and suggests that there will be no significant change in the surface area of the polymersomes. The dialysis of the polymersome solution containing organic solvents (50% in volume) against water would, however, cause a rapid expulsion of solvent molecules held within the inner compartment of the polymersomes through the solvent-swollen fluidic PS membrane due to the osmotic pressure. This will continue until the membrane loses its permeability, owing to the loss of solvent molecules, and the PS consequently collapses into the glassy state.[13]

We measured the membrane thickness of polymersomes of PEG_{45}-*b*-PS_{230} in the presence of organic solvents (50% by volume) by cryo-TEM (Figure S3, Supporting Information). The measured thickness (~32 nm) was 20% thicker than those of polymersomes after dialysis against water (~26 nm for spherical vesicles and stomatocytes), which confirms the existence of solvent-swollen membranes. We assumed that the rate of transport of THF and dioxane molecules through the swollen PS membrane would be much higher than that of water molecules on the basis of comparison of the Hildebrand solubility parameters of the solvents (δ = 18.6 $[MPa]^{1/2}$ for THF and 20.5 $[MPa]^{1/2}$ for dioxane) and water (δ = 48 $[MPa]^{1/2}$) with the parameter of homo-PS (δ = 16.6−20.2 $[MPa]^{1/2}$).[14] The unfavorable energy barrier between water and PS would hinder penetration of the swollen PS membrane by the water molecules and replacement of the space occupied by organic solvent molecules, which would consequently decrease the volume of the inner compartment of the polymersomes.

For giant liposomes, shape transformations are reversible due to the fluidity of the phospholipid bilayer membranes, and thus the morphology during shape transformation is only transient.[1,2] In our study, however, the PS domain within the bilayer of polymersomes remains in a solvent-swollen fluidic state during the shape change but subsequently turns into a glassy state as solvent molecules are excluded from the PS domain during dialysis against water. This kinetic freezing of the PS domain provides the basis for the retention of the morphology after shape transformation. From the previous observation that the degree of swelling of the PS domain is higher in THF than in dioxane,[15] we reasoned that the amount of THF in the initial solvent mixture would influence the shape change by affecting the degree of swelling and the rate of vitrification of the PS domain of the membrane. This would consequently lead to a difference in the time allowed for the shape change during dialysis.

For stomatocytes, the diameter of the opening will gradually decrease when the shape change progresses (Figure 1).[1,2] We therefore screened the degree of shape transformation of polymersomes prepared from PEG_{45}-*b*-PS_{292} with a varying amount of THF relative to dioxane in the organic solvent mixtures at a fixed water content (50%) of the suspension. When the THF content in the THF:dioxane mixture was increased from 40 to 65% (by volume), a gradual decrease in the diameter of the opening of stomatocytes after dialysis was observed with both TEM (Figure 3A−D) and cryo-TEM (Figure 3E,F). As shown in Figure 3G, a nearly linear

relationship between the size of the opening of the stomatocytes and the amount of THF used in the initial organic solvent mixture was obtained. The results suggest that the differences in the kinetics of the phase transition of the PS domain in the solvent mixtures can effectively be translated into the final frozen morphologies. A similar effect can also be found by changing the water content of the suspension at a fixed THF:dioxane ratio (1:1 v/v). A reduced water content leads to a slower rate of kinetic vitrification and thus induces a larger shape change, yielding a smaller diameter of the opening of the stomatocytes (Figure S4, Supporting Information).

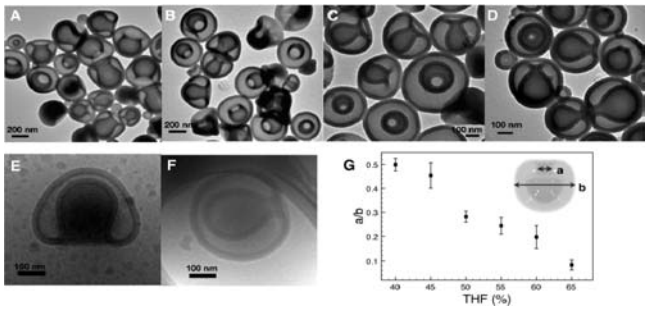

Figure 3. Degree of shape transformation of stomatocytes of PEG-*b*-PS according to the amount of THF to dioxane in the solvent mixture: (a) 50%, (b) 55%, (c) 60%, and (d) 65% THF used by volume). Cryo-TEM images of stomatocytes: (e) 50% THF and (f) 65% THF by volume. (g) Plot of the linear relationship between the amount of THF used in a solvent mixture and the diameter of the opening of the stomatocytes. Each data point was obtained by measuring the inner diameter of the opening and the overall diameter of stomatocytes from dry TEM images of 50 stomatocytes.

According to theoretical studies and experiments with giant liposomes, a higher initial curvature of the bilayer membrane would force the shape transformation to take another route from spheres to prolate/pear-shape vesicles (Figure 1).[1,2] We wondered whether, in our case, the direction of shape transformation could be changed by the introduction of bulkier PEG chains to the hydrophilic domain of the bilayer of the polymersome, while keeping the length of the PS block constant. This modification would increase the initial curvature of the resulting bilayer membrane.[16] Based on the fact that PEG_{115}-*b*-PS_{312} predominantly forms spherical micelles under the same preparation conditions (Figure S5, Supporting Information), we prepared a solution blend of PEG_{45}-*b*-PS_{292}/PEG_{115}-*b*-PS_{312} block copolymers (80:20 by weight) in a THF:dioxane (1:1 by volume) mixture. Under the same experimental conditions that allowed spherical vesicles of pure PEG_{45}-*b*-PS_{292} to become stomatocytes, we observed nearly spherical polymersomes with an indication of shape transformation to prolate/pear-shape vesicles only after dialysis of the suspension solution with 50% water content (Figure S6, Supporting Information). These results indicate that rational control over the shape transformation of polymer vesicles can be obtained by adjusting the architecture of the block copolymer. Our results suggest that the shape transformations of our polymersomes follow the same pathway as those theoretically predicted for the more fluidic giant liposomes made from low-molecular-weight phospholipids.[1,2]

The presence of an inner compartment and an adjustable opening in the polymersome stomatocyte makes it an interesting nanocontainer, which could be accessible by guest molecules and catalytic species. These stomatocyte objects, with controlled morphology and structural robustness, may therefore find various applications, as nano-reaction vessels, for example, which can hold and release guest molecules ranging from small molecules to proteins and further to colloidal particles.

Inspired by the studies on giant liposomes, we have shown that there are interesting possibilities to change the morphologies of polymersomes by the process of shape transformation. Given the potentially unlimited diversity of chemical and physical properties of block copolymers by virtue of the rapidly evolving controlled polymerization methods, the strategy reported here could be a new approach to create polymeric nanostructures with unusual architectures and functions.

Acknowledgment. This work was supported by NWO-ACTS, IBOS, and Encapson. K.T.K. thanks UNIST for support through the 2010 Research Fund.

Supporting Information Available: Experimental details, Table S1, and Figures S1−S6. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Hotani, H. *J. Mol. Biol.* **1984**, *178*, 113. (b) Käs, J.; Sackmann, E. *Biophys. J.* **1991**, *60*, 825. (c) Yanagisawa, M.; Imai, M.; Taniguchi, T. *Phys. Rev. Lett.* **2008**, *100*, 148102.
(2) (a) Seifert, U. *Adv. Phys.* **1997**, *46*, 13. (b) Lipowsky, R. *Nature* **1991**, *349*, 475.
(3) Li, X.; Pivkin, I.; Liang, H.; Karniadakis, G. *Macromolecules* **2009**, *42*, 3195.
(4) Discher, B. M.; Won, Y.-Y.; Ege, D. S.; Lee, J. C.-M.; Bates, F. S.; Discher, D. E.; Hammer, D. A. *Science* **1999**, *284*, 1143.
(5) (a) Yu, K.; Zhang, L.; Eisenberg, A. *Langmuir* **1996**, *12*, 5980. (b) Yu, K.; Eisenberg, A. *Macromolecules* **1998**, *31*, 3509.
(6) (a) Cui, H.; Chen, Z.; Zhong, S.; Wooley, K. L.; Pochan, D. J. *Science* **2007**, *317*, 647. (b) Pochan, D. J.; Chen, Z.; Cui, H.; Hales, K.; Qi, K.; Wooley, K. *Science* **2004**, *306*, 94. (c) Hayward, R. C.; Pochan, D. J. *Macromolecules* **2010**, *43*, 3577.
(7) (a) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967. (b) Discher, D. E.; Ahmed, F. *Ann. Rev. Biomed. Eng.* **2006**, *8*, 323.
(8) (a) Lim, P.; Eisenberg, A. *J. Polym. Sci., Part B: Polym. Phys.* **2004**, *42*, 923. (b) Sanson, C.; Schatz, C.; Le Meins, J.; Brulet, A.; Soun, A.; Lecommandoux, S. *Langmuir* **2010**, *26*, 2751.
(9) PEG-*b*-PS was dissolved in a mixture of THF and 1,4-dioxane, good solvents for both polymer blocks at typical concentration of 1 wt %. With vigorous stirring, water, which is a precipitant for the PS block, was then slowly added to this solution until the water content in the mixture reached 50%. After addition of water, the cloudy suspension was transferred to a dialysis membrane and dialyzed against pure water for 48 h with frequent water changes.
(10) Wong, P. A. *J. Theor. Biol.* **1999**, *196*, 343.
(11) Fetters, J. L.; Lohse, D. J.; Richter, D.; Witten, T. A.; Zirkel, A. *Macromolecules* **1994**, *27*, 4639.
(12) Battaglia, G.; Ryan, A. J. *J. Am. Chem. Soc.* **2005**, *127*, 8757.
(13) A paper discussing fully collapsed polymer vesicles was recently published: Azzam, T.; Eisenberg, A. *Langmuir* **2010**, *26*, 10513.
(14) Brandrup, J.; Immergut, E. H. *Polymer Handbook*, 3rd ed.; Wiley: New York, 1989.
(15) Yu, Y.; Eisenberg, A. *J. Am. Chem. Soc.* **1997**, *119*, 8383.
(16) (a) Israelachivili, J. *Intermolecular and Surface Forces*; Academic Press: London, 1992. (b) van Hest, J. C. M.; Delnoye, D. A. P.; Baars, M. W. P. L.; van Genderen, M. H. P.; Meijer, E. W. *Science* **1995**, *268*, 1592.

JA104154T