Giant Vesicle Formation through Self-Assembly of Complementary Random Copolymers

Faysal Ilhan, Trent H. Galow, Mark Gray, Gilles Clavier, and Vincent M. Rotello*

Department of Chemistry, University of Massachusetts Amherst, Massachusetts 01003

Received April 6, 2000

Molecular self-assembly can be described as the thermodynamically controlled association of molecules into structurally well defined, stable aggregates through noncovalent interactions.[1] Self-assembly is an essential process in biological systems, providing the diverse range of highly ordered structures observed in living organisms. The controlled application of noncovalent interactions also provides a powerful tool for the engineering of man-made systems, allowing the construction of structures spanning the nano- to macroscale range.[2]

Adaptation of self-assembly processes to the controlled aggregation of synthetic polymers provides a useful method for the creation of novel, higher order architectures.[3] We have recently incorporated specific recognition elements into polystyrene-based polymers.[4] Unlike biopolymers such as DNA and RNA,[5] these synthetic polymers are highly flexible and randomly substituted. This combination of randomness and flexibility provides a platform for the creation of highly structured extended systems: in recent studies, we have demonstrated a polymer-mediated self-assembly of gold nanoparticles into highly ordered spherical arrays incorporating up to 5×10^6 individual particles.[6] We report here the extension of this approach to the self-assembly of complementary polymer strands into giant vesicles[7] through specific interchain hydrogen bonding.

Complementarity between polymers was achieved using a diaminopyridine-thymine three-point hydrogen-bonding interaction.[8] The desired random dispersion of functionality in the polymers was obtained via functionalization of a 1:1 random copolymer of styrene and 4-(chloromethyl)styrene.[9] Reaction of this polymer with a diacyldiaminopyridine derivative[10] in the presence of potassium carbonate provided diaminopyridine-functionalized polymer **1** (Figure 1). The complementary thymine-functionalized polymer **2** was synthesized in a similar fashion using thymine-1-acetic acid.

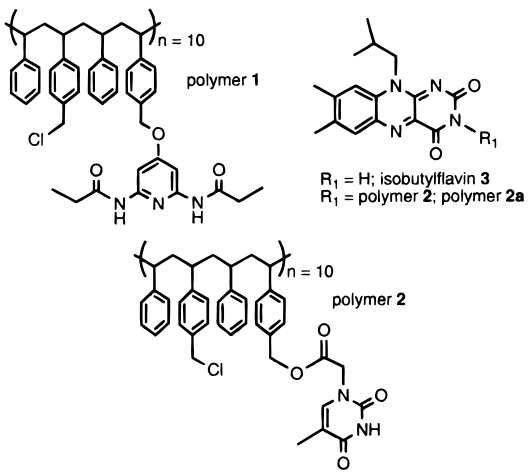

Figure 1. Schematic showing diaminopyridine-based polymer **1**, thymine-based polymer **2**, and partially functionalized polymer **2a** (10% by functional group number). Polymers were prepared from poly-*co*-[4-(chloromethyl)styrene−styrene]; M_n= 5300 g mol^{-1}.

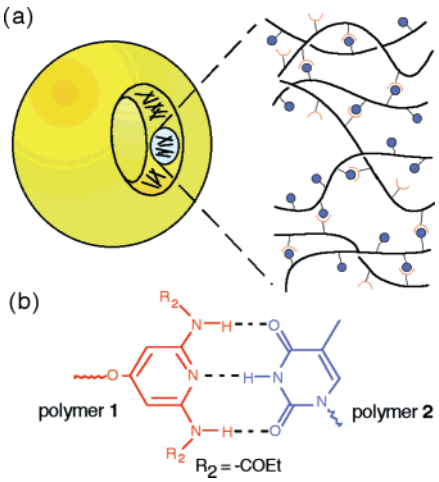

Figure 2. Schematic demonstration of vesicle formation between diaminopyridine-based polymer **1** and thymine-based polymer **2**. (a) Illustration showing molecular recognition within vesicle wall. (b) The corresponding recognition dyads.

Formation of the aggregates was achieved by mixing equal volumes of polymer **1** (3 mg/mL) and polymer **2** (3 mg/mL) in $CHCl_3$ (Figure 2). Light scattering was observed immediately upon mixing of the clear solutions of polymers **1** and **2**, indicating rapid complexation-mediated aggregation. The resulting aggregates were stable indefinitely (>7 days) in solution; however, they dissociated rapidly at elevated temperatures (60 °C).

Preliminary insight into structures of mixed-polymer aggregates was obtained through differential interference contrast (DIC) optical microscopy. Micrographs of mixed solutions of polymers **1** and **2** in $CHCl_3$ clearly show the formation of spherical aggregates (Figure 3). These vesicle-like structures are 3.3 ± 0.9 μm in diameter[11] with a size distribution characteristic of vesicle

(1) Whitesides, G. M.; Mathias, J. P.; Seto, C. T. *Science* **1991**, *254*, 1312.
(2) For examples of self-assembly-based man-made systems, see: Breen, T. L.; Tien, J.; Oliver, S. R. J.; Hadzic, T.; Whitesides, G. M. *Science* **1999**, *284*, 948. Fyfe, M. C. T.; Stoddart, J. F. *Acc. Chem. Res.* **1997**, *30*, 393. Rebek, J. *Acc. Chem. Res.* **1999**, *32*, 278. Stupp S. I.; LeBonheur, V.; Walker, K.; Li, L. S.; Huggins, K. E.; Keser, M.; Amstutz, A. *Science* **1997**, *276*, 384. Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. Sijbesma, R. P.; Beijer, F. H.; Brunsveld, L.; Folmer B. J. B.; Hirschberg, J. H. K. K.; Lange, R. F. M.; Lowe, J. K. L.; Meijer, E. W. *Science* **1997**, *278*, 1601. Gulik-Krzywiche, T.; Fouquey, C.; Lehn, J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 163.
(3) Muthukumar, M.; Ober, C. K.; Thomas, E. L. *Science* **1997**, *277*, 1225. Discher, B. M.; Won, Y.-Y.; Ege, D. S.; Lee, J. C.-M.; Bates, F. S.; Discher, D. E.; Hammer, A. D. *Science* **1999**, *284*, 1143. Kaifer, A. E. *Nature* **1993**, *364*, 484. Frey, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2193. Bassani, D. M.; Lehn, J. M.; Baum, G.; Fenske, D. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1845.
(4) Galow, T. H.; Ilhan, F.; Cooke, G.; Rotello, V. M. *J. Am. Chem. Soc.* **2000**, *122*, 3595. Ilhan, F.; Gray, M.; Blanchette, K.; Rotello, V. *Macromolecules* **1999**, *32*, 6159.
(5) Diederichsen, U. *Angew. Chem., Int. Ed.* **1998**, *37*, 2273. Choi, J. S.; Joo, D. K.; Kim, C. H.; Kim, K.; Park, J. S. *J. Am. Chem. Soc.* **2000**, *122*, 474.
(6) Boal, A. K.; Ilhan, F.; DeRouchey, J. E.; Thurn-Albrecht, T.; Russell, T. P.; Rotello, V. M. *Nature* **2000**, *404*, 746.
(7) Menger, F. M.; Angelova, M. I. *Acc. Chem. Res.* **1998**, *31*, 789. Menger, F. M.; Gabrielson, K. D. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2091. Marchi-Artzner, V.; Lehn, J.-M.; Kunitake, T. *Langmuir* **1998**, *14*, 6470. Ghosh, S.; Lee, S. J.; Ito, K.; Akiyoshi, K.; Sunamoto, J.; Nakatani, Y.; Ourisson, G. *J. Chem. Soc., Chem. Commun.* **2000**, *4*, 267. Wick, R.; Walde, P.; Luisi, P. L. *J. Am. Chem. Soc.* **1995**, *117*, 1435.
(8) For other examples of similar host−guest complexes, see: Niemz, A.; Rotello, V. M. *J. Am. Chem. Soc.* **1997**, *119*, 6833. Muehldorf, A. V.; Van Engen, D.; Warner, J. C.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 6561.
(9) Deans, R.; Ilhan, F.; Rotello, V. M. *Macromolecules* **1999**, *32*, 4956.
(10) Diacyldiaminopyridine derivative was synthesized by modification of a previously published synthetic procedure: Fessmann, T.; Kilburn, J. D. *Angew. Chem., Int. Ed.* **1999**, *38*, 1993.
(11) The average diameter was obtained by sampling 170 individual spheres.

10.1021/ja0011966 CCC: $19.00
Published on Web 06/01/2000

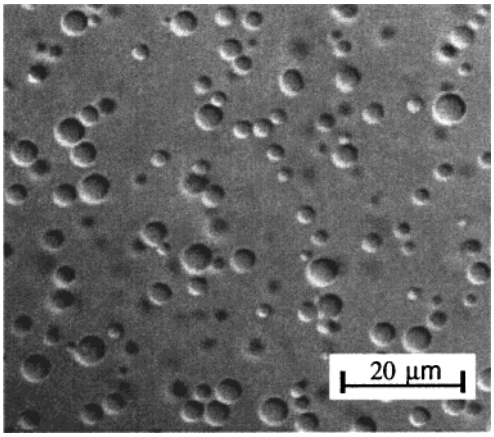

Figure 3. DIC micrograph (×60) clearly showing the spherical aggregates that were obtained in $CHCl_3$. Solutions were prepared from 1 equiv of **1** and 1 equiv of **2**. [**1**] = [**2**] = 3 mg/mL.

systems (Figure 4).[12] As expected from the absence of light scattering, no aggregates were observed for the individual solutions of polymer **1** or **2** in $CHCl_3$.

Proof of the vesicular nature of the aggregates formed from polymers **1** and **2** was obtained using confocal laser scanning microscopy (CLSM).[14] For these studies, a flavin-functionalized analogue of polymer **2** (polymer **2a**, Figure 1) was used as a fluorescent probe.[15] CLSM micrographs of the aggregates show that the vesicles consist of bright edges with less fluorescent central regions indicating a hollow interior.[16] While the bulk of the vesicles were unilamellar, a variety of other morphologies were observed, including vesicle-in-vesicle and fused vesicles (Figure 5). Significantly, virtually all of the fluorescence in the solutions studied was contained within the vesicles, indicating highly efficient complexation and concomitant incorporation of the polymer constituents into the vesicles.

In summary, we have synthesized random copolymers with complementary recognition units. The complexation via specific hydrogen bonding between these polymers provides an unprecedented method for the formation of giant vesicles. We are currently investigating the mechanism of formation and the detailed structure of these vesicles. Applications of these vesicles to applied areas of chemistry varying from delivery systems to catalysis are also under investigation and will be reported in due course.

(12) For a theoretical model study of vesicle size distribution, see: Mavelli, F.; Luisi, P. L. *J. Phys. Chem.* **1996**, *100*, 16600.

(13) The density probability function curve was obtained by fitting the data to the following formula from ref 12: $P(r_i) = (2r_i/r_0^2)\exp\{-(r_i/r_0)^2\}$, where $r_0 = (2/\pi^{1/2})r_{av}$. The offset between the experimental and theoretical curves arises from our inability to measure particles smaller than 1.7 μm, and the resultant effect on r_{av}.

(14) Korlach, J.; Schwille, P.; Webb, W. W.; Feigenson, G. W. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8461.

(15) Isobutyl flavin **3** was covalently attached to the starting styrene/(chloromethyl)styrene copolymer, followed by the attachment of thymine-1-acetic acid to provide polymer **2a**. For N(3)-alkylations of N(10)-isobutylflavin, see: Dutra, J. K.; Cuello, A. O.; Rotello, V. M. *Tetrahedron Lett.* **1997**, *38*, 4003 and references therein.

(16) Tsuda, K.; Dol, G. C.; Gensch, T.; Hofkens, J.; Latterini, L.; Weener, J. W.; Meijer, E. W.; De Schryver, F. C. *J. Am. Chem. Soc.* **2000**, *122*, 3445.

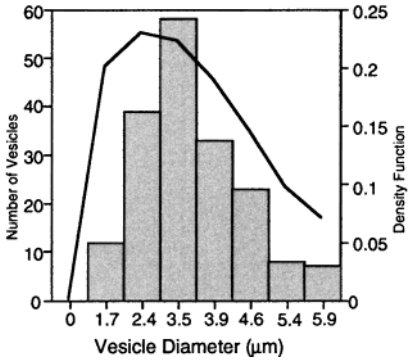

Figure 4. Size distribution histogram and relative density function of the vesicles; a sample of these shown in Figure 3.[13]

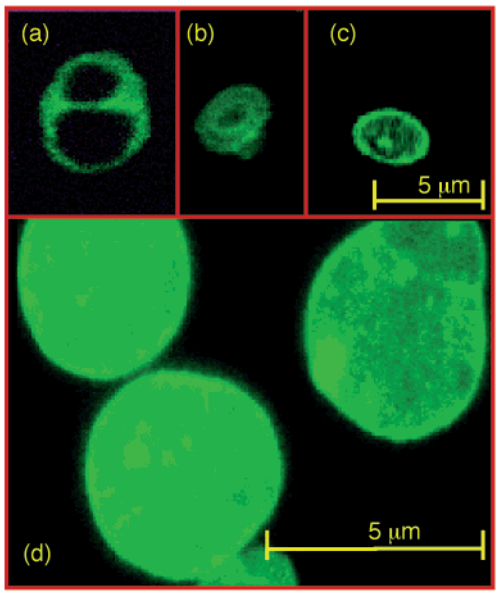

Figure 5. Confocal micrographs of varying architectures in $CHCl_3$, showing the vesicle nature of these mixed-polymer systems. (a) (×60) Fused-vesicle morphology; (b) (×60) vesicle-in-vesicle structure; (c) (×60) regular spherical vesicle morphology; (d) (×120) two spherical vesicles and a fused vesicle. Prepared from 6 equiv of **1**:5 equiv of **2**:1 equiv of **2a**. [**1**] = [**2**] = [**2a**] = 3 mg/mL.

Acknowledgment. This research was supported by the National Science Foundation (DMR-9809365, MERSEC, and CHE-9905492). V.M.R. acknowledges support from the Alfred P. Sloan Foundation, Research Corporation, the Camille and Henry Dreyfus Foundation, The Petroleum Research Fund of the ACS, and the CUMIRP program at the University of Massachusetts. We thank Prof. Thomas P. Russell for helpful discussions, and Dale Callaham from the University of Massachusetts Central Microscopy Facility (NSF BBS 8714235).

Supporting Information Available: Synthesis, ^{1}H NMR, and IR information for polymers **1**, **2**, and **2a** (NMR) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0011966