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Recognition-Induced Transformation of Microspheres into Vesicles: Morphology and Size Control

Oktay Uzun, Amitav Sanyal, Hiroshi Nakade, Raymond J. Thibault, and Vincent M. Rotello*

Contribution from the Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003

Received April 23, 2004; E-mail: rotello@chem.umass.edu

Abstract: Polystyrene functionalized with diamidopyridine (DAP) recognition units self-assembles in nonpolar media to form thermally reversible micrometer-scale spherical aggregates. The size and the thermal stability of these microspheres can be controlled by the molecular weight of the polymer. The addition of thymine-functionalized polymer to these self-assembled microspheres converted them into vesicular aggregates with a controlled size. The morphology change was reversible: the addition of DAP-functionalized polymer converted the vesicles back to microspheres.

Introduction

The fabrication of synthetic polymeric vesicles and micelles presents a powerful methodology for the development of structure-driven materials for applications in biosensors,[1] targeting agents,[2] microreactors,[3] and encapsulation/drug delivery.[4] The morphology of these polymeric aggregates can be precisely tuned through presynthesized design of relative hydrophilic/hydrophobic block lengths, ion content, solvent composition, and block arrangement.[5] Recent reports have demonstrated that hydrogen-bonding interactions along the polymer backbone or among the polymer chains can be utilized for the construction of self-assembled polymeric morphologies.[6] The dynamic nature of molecular self-assembly integrates very well with these supramolecular assemblies, as external stimuli can be applied to modulate noncovalent interactions, in turn providing control over the resulting morphology. Amphiphilic molecules such as surfactants and block copolymers in solution self-assemble into highly organized aggregates of various morphologies such as spheres, rods, and vesicles. Morphological transitions are well known to occur from micelle-to-vesicle or vesicle-to-micelle as a function of variations in temperature,[7] shear,[8] concentration,[9] and solvent composition.[10]

In recent studies, we reported that the combination of complementary diamidopyridine (DAP)- and thymine (Thy)-functionalized polymers in nonpolar media results spontaneously in the formation of giant vesicular aggregates or recognition-induced polymersomes (RIPs).[11] These structures are formed through highly specific three-point hydrogen-bonding interactions between the complementary side chains. Progression of this research has led us to explore the potential for further control over size and morphology of these RIPs by probing the effect of chain length and degree of functionalization on the polymer

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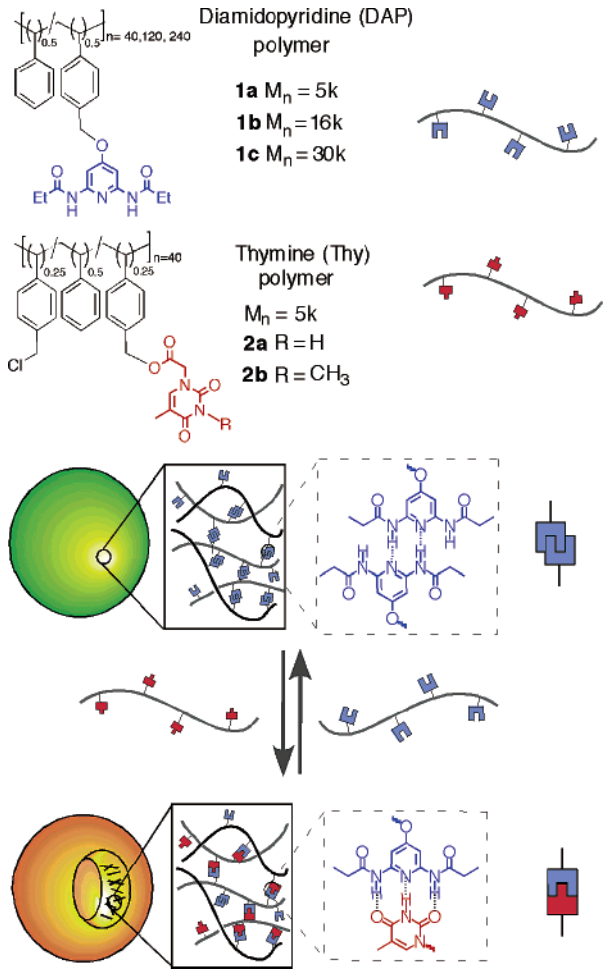

Figure 1. Schematic illustration of the reversible morphology control from microsphere to vesicle using specific noncovalent interactions.

backbone. Herein, we report: (a) the formation of discrete microspheres using self-complementary DAP polymers; and (b) the specific and reversible hydrogen-bonding-induced morphological transition of these polymeric microspheres into vesicular structures via addition of a polymer containing a complementary recognition partner (Figure 1).

Results and Discussion

A series of DAP-functionalized polymers **1** with different molecular weights were obtained by postfunctionalization of 1:1 styrene/4-(chloromethyl)styrene random monoblock copolymers.[12] Aggregate formation was evident by the onset of turbidity in solutions obtained upon dispersing these polymers (3 mg/mL) in $CHCl_3$.[13] The structure of these aggregates was determined by differential interference contrast microscopy (DIC). DIC images showed that well-defined micrometer-sized spherical aggregates were formed (Figure 6a). Further structure analysis was done by laser confocal scanning microscopy (LCSM) using flavin-tagged polymer. The solid fluorescence profile of these spherical assemblies is indicative of a filled sphere rather than the vesicular morphology. The highly localized fluorescence observed in the micrographs indicates

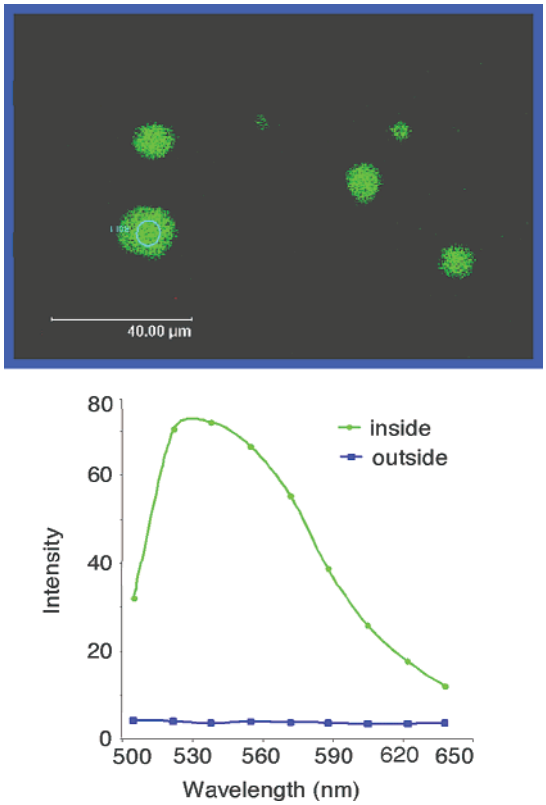

Figure 2. Representative LCSM image of microspheres formed by polymers **1a**−**c**, obtained using polymer **1c** labeled with flavin (E_{max} = 525 nm).

the efficiency of the assembly process (Figure 2). The high degree of DAP-functionalization in polymer **1a**−**c** plays a critical role in the formation of these stable microspheres, enabling dimerization through self-complementary two-point hydrogen bonding.[14,15]

Further evidence for DAP−DAP dimerization in the polymer **1** microspheres was provided using IR spectroscopy. A single, sharp N−H stretching mode at 3424 cm^{-1} was observed with the analogous monomeric 4-benzyloxy-3,5-dipropamidopyridine (benzyl-DAP) in $CHCl_3$ (Figure 3). In contrast, DAP−DAP interactions in polymer **1a** in $CHCl_3$ solution resulted in broad N−H stretching modes at 3290−3300 cm^{-1} with the free N−H stretching at 3424 cm^{-1} still visible. As expected, the IR spectra of solid polymer **1a** exhibited mostly hydrogen-bonded N−H groups with multiple, broad, and red-shifted N−H stretching modes at 3290−3300 cm^{-1}.

Microspheres formed using polymers **1a**−**c** are stable at room temperature. When the microspheres were allowed to remain undisturbed for more than 5 h, a gel-like layer was observed at the air liquid interface. This gelation was presumably due to the aggregation of the polymers; the microspheres could readily be redispersed through gentle agitation (3−5 s). The size of these assemblies was strongly dependent upon the polymer length, with increasing diameters observed with increased polymer molecular weight (Table 1). No noticeable change in size was observed, however, over the concentration range that could be readily studied (from 2 to 5 mg/mL).

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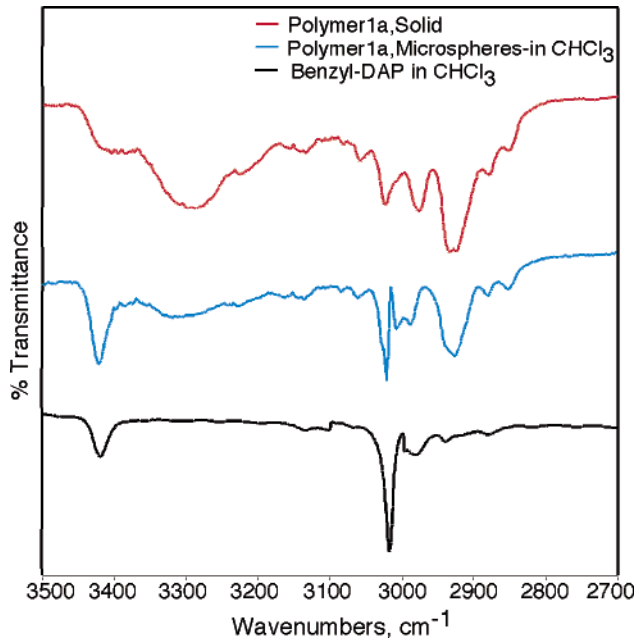

Figure 3. IR spectra of: (a) polymer **1a** as a solid film; (b) polymer **1a** in $CHCl_3$ solution as microspheres; and (c) monomeric 4-benzyloxy-3,5-dipropamidopyridine in $CHCl_3$ solution.

Table 1. Average Mean Diameters and Standard Deviations of Microspheres and Vesicles

polymer	M_n	$D_{microsphere}$ (μm)[a]	$D_{vesicle}$ (μm)[a]
1a	5k	2.9 ± 2.0^b	2.4 ± 1.1^b
1b	16k	6.2 ± 3.5^b	6.3 ± 2.5^b
1c	30k	9.6 ± 5.9^b	9.0 ± 4.0^b

[a] Average mean diameters determined from DIC images. [b] Size distribution (standard deviation) obtained from ~400 aggregates.

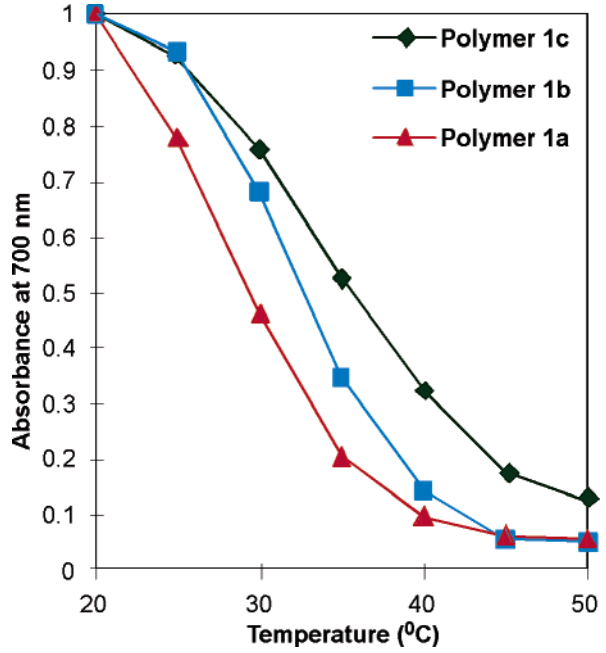

Figure 4. Thermal stability of microspheres obtained from polymer **1a**−**c**.

The thermal stability of the microspheres was determined using turbidometry (Figure 4). The microspheres were gradually heated in a sealed vessel from 20 to 50 °C, with dissociation followed by monitoring relative solution absorbance at 700 nm, a wavelength at which none of the polymers absorb. Increasing molecular weight increased the stability of the microspheres, presumably due to the cooperativity of DAP−DAP interactions.

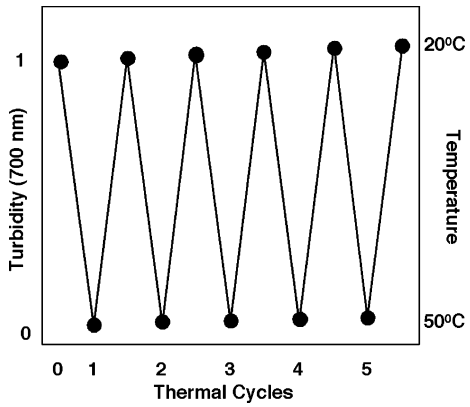

Figure 5. Solution turbidity (700 nm) of microspheres generated from polymer **1c** during heat−cool cycles.

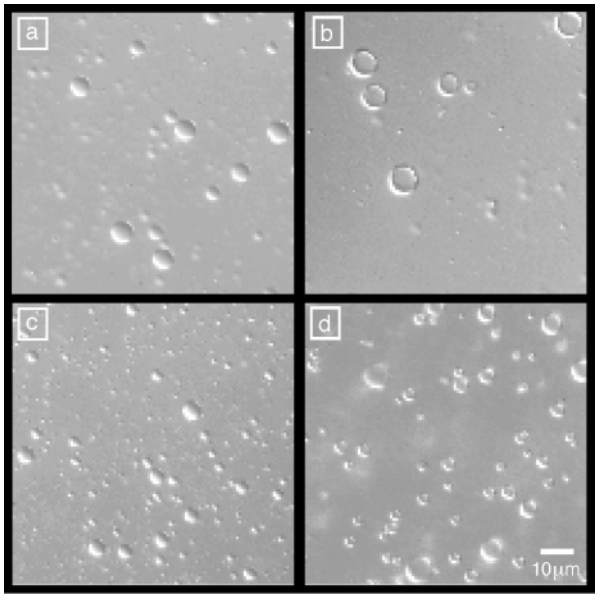

Figure 6. DIC micrographs (×40) of (a) spherical aggregates formed by polymer **1c**, (b) vesicular structures with addition of polymer **2a**, (c) microspheres re-formed by adding polymer **1c** to the preformed vesicle solution, and (d) vesicular structures reversibly formed with addition of polymer **2a** into the microsphere solution shown in (c).

Thermal reversibility is one of the key characteristics of self-assembled materials, making the resultant structures both processable and recyclable, and potential leads for the development of delivery and transport systems.[16] Heating the microspheres formed using polymer **1c** to 50 °C resulted in total loss of turbidity, indicating complete dissolution of the aggregates. Upon cooling to 20 °C, microspheres spontaneously reformed, restoring the turbidity. This process could be repeated multiple times with little or no change in turbidity at either temperature (Figure 5).

On the basis of the three-point interactions possible with the thymine side chains of polymer **2a**, we expect this polymer to

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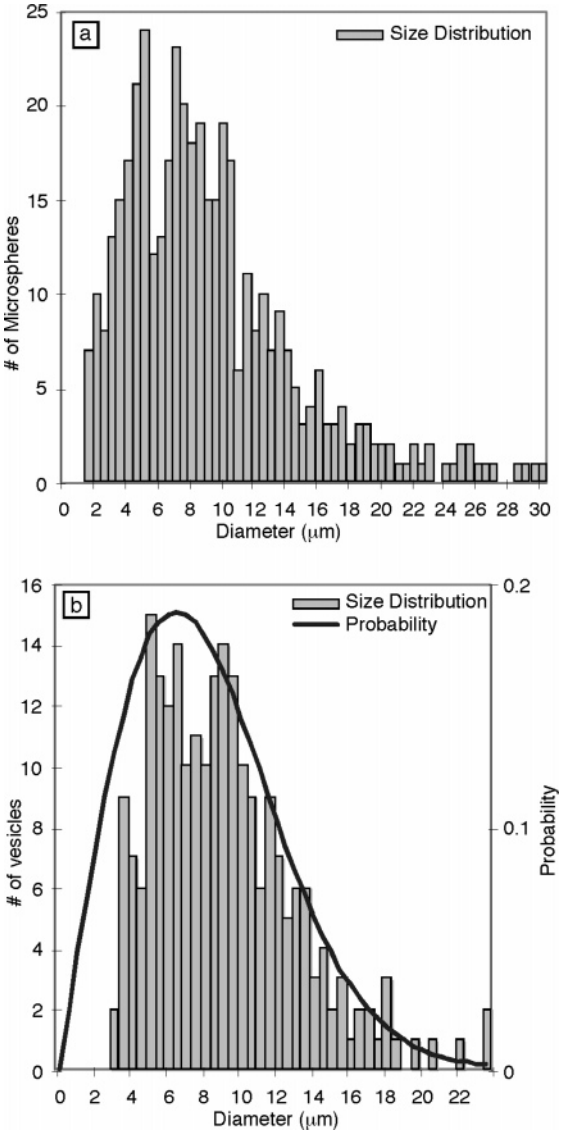

Figure 7. Size distributions of (a) the initial solution of microspheres formed by polymer **1c**, and (b) the resulting vesicles by adding polymer **2a** with an overlay of the probability histogram.

interact preferentially to the 2-point DAP−DAP interaction. Addition of the complementary Thy-polymer **2a** (3 mg/mL) to a solution of the self-assembled DAP-polymer **1c** in $CHCl_3$ resulted in a spontaneous change in morphology from microspheres to hollow spheres. The morphology change was visualized with DIC (Figure 6a,b). IR spectroscopy of polymer **1c** (see Supporting Information) revealed a decrease in the free N−H stretching modes at 3424 cm^{-1}, with the C═O stretching mode of Thy becoming much broader and less intense at 1755 cm^{-1}, upon addition of polymer **2a**, thereby suggesting an increase in the H-bonding interactions upon vesicle formation. The increase in interchain cross-linking, which originates from the outer surface of the microspheres upon complementary hydrogen-bonding interactions, may be responsible for the microsphere−vesicle transformation. The units introduced into the polymer form stronger hydrogen bonds with the DAP groups in the microspheres. These interactions result in the formation of the vesicular aggregates by incorporation of DAP polymers from the microspheres into the newly emerging vesicular structure.

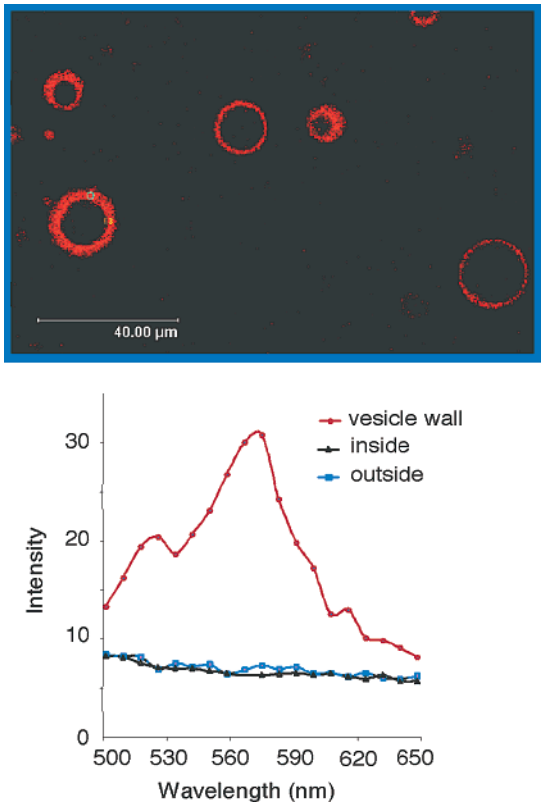

Figure 8. Vesicular morphology: with the addition of **2a** labeled with Rhodamine B (E_{max} = 570 nm).

The combination of polymer aggregation with the reversible nature of molecular recognition processes furnishes an attractive means of constructing recyclable materials and allows further control in the morphology of the formed structures.[17] To test the reversibility of this morphology transformation, one molar equivalent of functional units of polymer **1c** (relative to the original polymer **1c** solution) was added into the preformed solution of vesicles, formed using polymers **1c** and **2a**, restoring the microsphere morphology.[18] Further addition of an equivalent of polymer **2a** into the microspheres transformed them back into the vesicles (Figure 6c,d). In a control study, *N*-methyl thymine polymer **2b**, which does not participate in hydrogen bonding, was mixed with the microspheres: no change in morphology confirmed the specific recognition dependence of the process.

DIC microscopy was used to obtain the size distribution of these aggregates (Figure 7). The mean diameter of these vesicles remained similar to that of the initial microspheres (Table 1). The mean diameter of the reformed microspheres (made from vesicles of **1c** and **2a** through addition of polymer **1c**) was 8.2 ± 4.2 μm, and the diameter of the resultant vesicles after addition of polymer **2a** was 7.8 ± 3.5 μm, indicating retention of size upon cycling. In contrast to the microspheres formed using polymers **1**, heating of the vesicles resulted in an insoluble gel formation on the sides of the quartz cuvette.

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(18) The transition is quite sensitive to stoichiometry; the addition of one-half of a molar equivalent of polymer **1c** to polymer **1a** resulted in the partial transition of the morphology from microsphere to vesicle.

The hollow structure of these vesicles was further verified, and localization of individual polymers was determined by LCSM. In these experiments, discrete vesicles were obtained by mixing the microspheres derived from flavin-labeled DAP-polymer **1c** (E_{max} = 525 nm) with a rhodamine B-labeled Thy-polymer **2a** (E_{max} = 570 nm). Both of the dyes were excited at 488 nm, and the emission spectrum was collected from 500 to 650 nm. The colocalization of polymers **1c** and **2a** is demonstrated by the emission maxima observed at 525 and 570 nm in the walls of the vesicle (Figure 8).

Conclusions

In summary, we have demonstrated that microspheres cooperatively assembled through self-complementary interactions can be reversibly converted into vesicular architectures by using recognition-specific interactions and the size of these structures can be controlled by the molecular weight of the polymer. Further studies aimed at the incorporation of additional functional groups on these polymers to impart desired properties to these RIPs are under investigation and will be reported in due course.

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Supporting Information Available: Size distributions, synthesis, GPC traces, and NMR spectra of polymers, and IR spectra of the polymers individually and collectively. This material is available free of charge via the Internet at http://pubs.acs.org.

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