Channels in Micelles

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Formation of Complex Micelles with Double-Responsive Channels from Self-Assembly of Two Diblock Copolymers**

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The establishment of an effective method to prepare desirable nanostructures and to eventually convert them into designed architectures is of increasing interest in nanotechnology, chemistry, and biology. Proteins that are located in the phospholipid bilayer of cell membranes are important in forming transient pores or channels to achieve ion transport, ion regulation, energy transduction, signal recognition, and other biological processes. Numerous functional materials, including nanoporous membranes and synthetic transmembrane channels, have been designed based on these important gating structures in order to mimic biological processes.[1–4]

It is now well established that amphiphilic block copolymers can self-assemble into lipid-like membranes with tunable channels, which considerably expand on the properties of natural biomembranes.[5–8] However, few studies have involved block copolymer micelles with channels. Typical polymeric micelles consist of a compact core formed by the insoluble blocks of the polymer and a stretched shell formed by the soluble blocks so that the inner core can serve as a nanocontainer for various substances.[9,10] Many efforts have been made to broaden the range of potential applications by altering the properties of the core and the shell and to fabricate novel types of micelles with special and controllable structures.[11–15] For example, Jiang and co-workers reported core-stabilized polymeric micelles with a mixed shell made from two incompatible copolymers.[13] Liu and co-workers prepared water-soluble porous nanospheres from block copolymer micelles and nano- or microspheres bearing small hemispherical bumps with surface-segregated chains.[14,15] If the multifunctionality of channels is considered, more advantages would be offered if we combined the properties of polymeric micelles with tunable channels.

Environmental stimuli-responsive polymers are an interesting class of materials since their physical and chemical properties can be adjusted by external stimuli, such as temperature, pH value, and ionic strength; the design of these polymers has been based on lipid-like bilayer membranes.[16–19] We present herein a simple and effective method to prepare complex micelles with tunable channels from the self-assembly of two diblock copolymers, namely, poly(*tert*-butyl acrylate)-*b*-poly(*N*-isopropylacrylamide) (P*t*BA-*b*-PNIPAM) and poly(*tert*-butyl acrylate)-*b*-poly(4-vinylpyridine) (P*t*BA-*b*-P4VP).

The diblock copolymers P*t*BA$_{45}$-*b*-PNIPAM$_{91}$ (polydispersity index (PDI) = 1.25) and P*t*BA$_{60}$-*b*-P4VP$_{80}$ (PDI = 1.23) were synthesized by atom-transfer radical polymerization (ATRP). Both are molecularly dispersed in *N*,*N*-dimethylformamide (DMF). With the addition of acidic water (pH 2.5), opalescence appeared, which indicates the occurrence of micellization in the solutions. Since P4VP is protonated and soluble in aqueous solution at low pH values and PNIPAM is soluble at room temperature, the hydrophobic P*t*BA blocks of the two polymers associate together to form a dense core, protected by the mixed soluble P4VP/PNIPAM blocks acting as a shell. With an increase in temperature or pH value, the core–shell micelles convert into a new type of micelle, where soluble chains stretch out from the core through the now collapsed shell. The formation of the complex micelles is shown in Figure 1.

Dynamic light scattering (DLS) and static light scattering (SLS) are used to measure the scattered light intensity, which can indicate the aggregation of polymers in solution. The

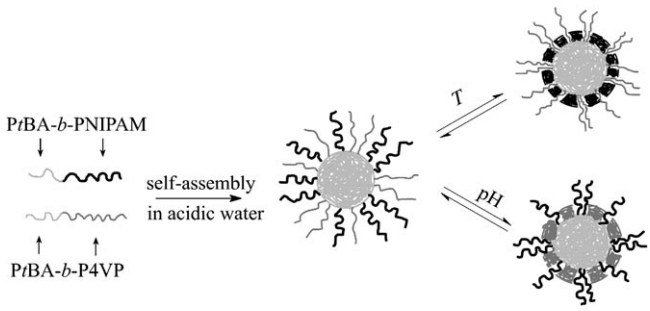

Figure 1. Formation of the complex micelles from self-assembly of P*t*BA$_{45}$-*b*-PNIPAM$_{91}$ and P*t*BA$_{60}$-*b*-P4VP$_{80}$.

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diameter distributions of P*t*BA$_{45}$-*b*-PNIPAM$_{91}$ micelles, P*t*BA$_{60}$-*b*-P4VP$_{80}$ micelles, and the complex micelles are shown in Figure 2. The average hydrodynamic diameters (D_h) of these micelles are 79, 60, and 84 nm, respectively.

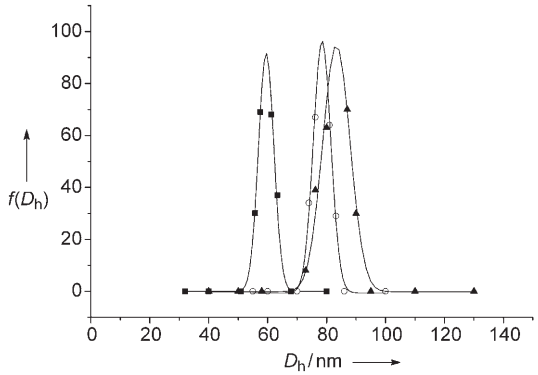

Figure 2. The hydrodynamic diameter distribution, $f(D_h)$, for P*t*BA$_{45}$-*b*-PNIPAM$_{91}$ micelles (○), P*t*BA$_{60}$-*b*-P4VP$_{80}$ micelles (■), and the complex micelles (▲) at pH 2.5 and 25 °C.

Clearly, each of the micelles shows a narrow diameter distribution, while the average diameter of the complex micelles is somewhat larger than either of the two individual micelles.

From the fitted lines of the Berry plots for P*t*BA$_{45}$-*b*-PNIPAM$_{91}$ micelles, P*t*BA$_{60}$-*b*-P4VP$_{80}$ micelles, and the complex micelles at pH 2.5 and 25 °C (see the Supporting Information), the radii of gyration (R_g) of these micelles are calculated to be 36, 26, and 31 nm, respectively. The R_g/R_h ($R_h = 0.5D_h$) value can reveal the morphology of particles dispersed in solutions.[19] The values of R_g/R_h for these micelles are 0.91, 0.87, and 0.74, respectively, results suggesting that the micelles are spherical. Moreover, the R_g/R_h value of the complex micelles is much lower than that of either individual micelle, which indicates that the structure of the complex micelles is more compact.

With increasing temperatures, water progressively becomes a poor solvent for PNIPAM blocks, so the stretched chains collapse from an extended-coil conformation to a shrunken conformation. Figure 3 shows the temperature dependence of the R_g value during one cycle of the heating-and-cooling process when the polymer solution was equilibrated for about two hours at each temperature. The values of R_g/R_h at different temperatures were also calculated, with results shown in the insert of Figure 3. The decrease of the R_g/R_h value from 0.74 to 0.60 indicates that the structure of the micelles has changed to a more compact state. The values of R_g in the cooling process reveal the reversible globule-to-coil transition of the PNIPAM chains, although there is a slight hysteresis as compared to the results of the heating process.

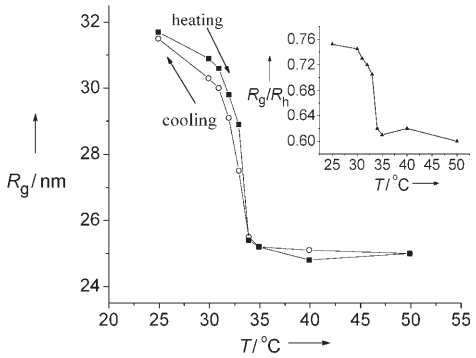

Figure 3. Temperature dependence of R_g (and of R_g/R_h, inset) for the complex micelles.

For individual P*t*BA$_{45}$-*b*-PNIPAM$_{91}$ micelles, large aggregates form when the temperature rises above 33 °C due to the insolubility of both P*t*BA and PNIPAM. However, the D_h value for the complex micelles remains nearly constant with increasing temperatures because the hydrophilic P4VP chains can stabilize the micelles at pH 2.5. This result further confirms the formation of complex micelles between P*t*BA$_{45}$-*b*-PNIPAM$_{91}$ and P*t*BA$_{60}$-*b*-P4VP$_{80}$. The values of D_h, R_g, and R_g/R_h for the complex micelles measured under different temperature conditions are listed in Table 1.

Table 1: DLS and SLS data for the complex micelles under different conditions.

Conditions	D_h [nm]	R_g [nm]	R_g/R_h
pH 2.5, 25 °C	84	31	0.74
pH 2.5, 50 °C	84	25	0.60
pH 7.8, 25 °C	86	27	0.63

In addition, the stretched P4VP chains collapse due to their deprotonation when the pH value is increased from 2.5 to 7.8 at 25 °C. Individual P*t*BA$_{60}$-*b*-P4VP$_{80}$ micelles would precipitate at pH 7.8, but the complex micelles remain stable and suspended because the PNIPAM block is still soluble. The values of D_h, R_g, and R_g/R_h for the complex micelles at pH 7.8 and 25 °C are also listed in Table 1. The remarkable decrease in the R_g and R_g/R_h values for the complex micelles at higher pH values reveals the collapse of the P4VP chains.

^{1}H NMR spectra recorded in D_2O at different temperatures and pH values were used to further study the thermo- and pH-responsive behavior of the complex micelles. In Figure 4 a, peaks a and b, due to the PNIPAM blocks, and peaks c and d, due to the P4VP blocks, are all evident, which means that the two blocks are completely water soluble at pH 2.5 and 25 °C. The proton signals from the P*t*BA blocks are invisible, which suggests that they form the immobile and nonsolvated micellar core. The disappearance of the PNIPAM signals at 50 °C and the P4VP signals at pH 7.8 indicates the much lower mobility and decreased solubility of PNIPAM chains at 50 °C and P4VP chains at pH 7.8.

The fact that the complex micelles remain stable at high temperatures or pH values makes us believe that the PNIPAM chains and P4VP chains are mixed in the shell. If PNIPAM and P4VP were separately attached to different regions of the core to form Janus micelles, the complex micelles would further associate into much larger aggregates, as shown in Scheme S2 in the Supporting Information.[20]

From the above discussion, we conclude that core–shell complex micelles self-assembled from mixtures of P*t*BA$_{45}$-*b*-

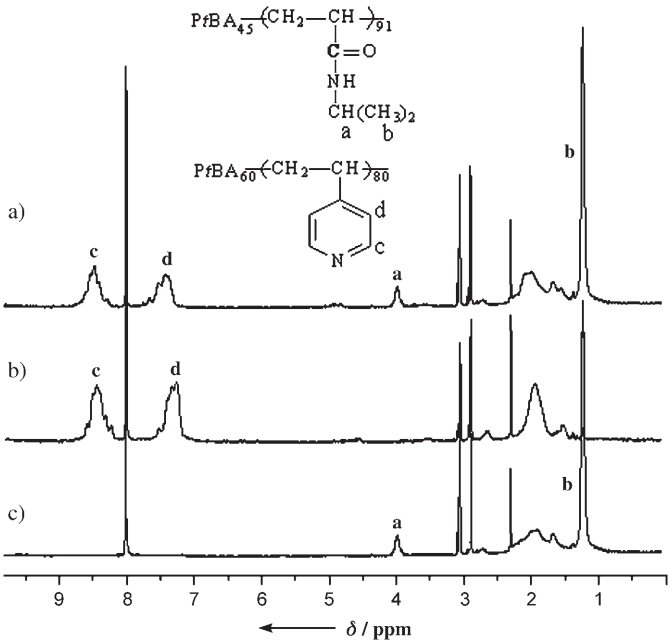

Figure 4. ^{1}H NMR spectra of the complex micelles at a) pH 2.5 and 25 °C, b) pH 2.5 and 50 °C, and c) pH 7.8 and 25 °C. The peak labels are explained in the text.

$PNIPAM_{91}$ and P*t*BA_{60}-*b*-$P4VP_{80}$ convert into a new type of micelles with increasing temperature or pH value. The resultant micelles are expected to have a structure with the hydrophobic P*t*BA blocks as a dense core surrounded by collapsed PNIPAM or P4VP blocks as the shell with soluble P4VP or PNIPAM chains stretching outside as the corona to protect the micelles (see Figure 1).

It should be noted that P4VP and PNIPAM are attached to a common core, which means that hydrophilic P4VP chains or PNIPAM chains are stretching outside from the core through the collapsed shell. Phase separation between the hydrophobic shell and the hydrophilic corona leads to channels in the shell, as shown in Figure 5a. The hydrophilic chains are embedded in the hydrophobic shell, just as the channel proteins through which ions and other small molecules can pass are embedded in a lipid-like membrane (Figure 5b). The solubility of the hydrophilic P4VP or PNIPAM chains depends on the pH value, ionic strength, and temperature of the solutions and, as a result, the size of the channels can be regulated by changing the environmental conditions or by manipulating the composition of the two diblock copolymers. Although the functions of the channels in this case are not as perfect as those of the protein channels in cellular membranes, these novel nanostructures may prove to be useful and versatile in applications such as controlled-release devices. A preliminary study on the release of bilirubin from the micelles has been performed, as discussed in the Supporting Information.

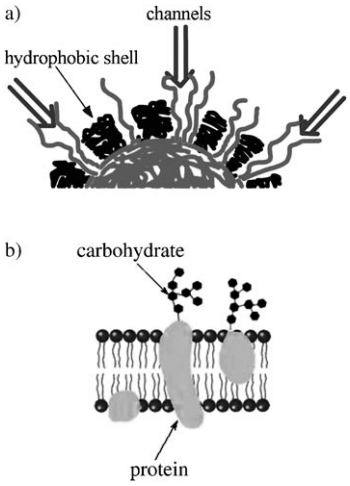

Figure 5. Illustration of double-responsive channels self-assembled from a) a complex micelle and b) a typical lipid-like bilayer membrane.

In summary, a new type of complex micelles with tunable channels is formed through the self-assembly of a binary mixture of P*t*BA_{45}-*b*-$PNIPAM_{91}$ and P*t*BA_{60}-*b*-$P4VP_{80}$ diblock copolymers upon increasing the temperature or pH value of the solution. The size and permeability of the channels may be regulated by manipulating the composition of the diblock copolymers or by changing the environmental conditions. These new complex micelles with controllable channels may be promising candidates for use in controlled-uptake/release processes. Detailed studies on the selective permeation of substances into these micelles is in progress.

Experimental Section

Preparation of block copolymers: The macroinitiator P*t*BA-Cl was prepared by ATRP by using 1-chlorophenylethane (1-PECl) as the initiator and CuCl/*N*,*N*,*N*′,*N*″,*N*″-pentamethyl diethylenetriamine (PMDETA) as the catalyst in a solvent mixture of butanone and 2-propanol (7:3 v/v).

Block copolymers of P*t*BA-*b*-PNIPAM and P*t*BA-*b*-P4VP were obtained by using P*t*BA-Cl to initialize the polymerization of NIPAM or 4VP with CuCl/tris[2-(dimethylamino)ethyl]amine (Me_6TREN) as the catalyst. A typical polymerization procedure for obtaining P*t*BA-*b*-PNIPAM is as follows: P*t*BA-Cl (5.0 g) was added to a reaction flask and then the solvent mixture of butanone and 2-propanol (6:4 v/v; 6 mL) was added. Subsequently, CuCl (0.15 g), Me_6TREN (0.35 g), and NIPAM (10.0 g) were introduced into the flask and degassed with a nitrogen purge. Polymerization was performed at 40 °C for 48 h. The product was purified by passing the mixture through an Al_2O_3 column and was then deposited in a methanol/water mixture.

The molecular weights and PDI values of P*t*BA-*b*-PNIPAM and P*t*BA-*b*-P4VP were determined by a Waters 600E gel permeation chromatography (GPC) analysis system with tetrahydrofuran or $CHCl_3$ as the eluent and polystyrene as the calibration standard. The composition of the block copolymers was determined in $CDCl_3$ by use of ^{1}H NMR spectroscopy on a Varian UNITYplus 400 MHz NMR spectrometer.

Preparation of the complex micelles: P*t*BA-*b*-PNIPAM and P*t*BA-*b*-P4VP with a weight ratio of 1:1 were first dissolved in DMF to make a polymer concentration of 0.1 mg mL^{-1}. Subsequently, a given volume of acidic water (pH 2.5) was added into the polymer solution with stirring. The formation of micelles occurred, as indicated by the appearance of opalescence in the solution, and then the solution was dialyzed in acidic water for four days to remove the DMF.

DLS and SLS measurements were performed on a laser light scattering spectrometer (BI-200SM) equipped with a digital correla-

tor (BI-10000AT) at 514 nm. All samples were first prepared by filtering solutions (about 1 mL) through a 0.45-μm Millipore filter into a clean scintillation vial and were then characterized at the given temperatures.

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