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Stimuli-Sensitive Assemblies of Homopolymers

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Two homopolymers assemble into nanoparitcles in a common solvent of water through ionic complexation. These nanoparticles reversibly and rapidly respond to both pH and temperature, and are particularly promising as intelligent systems.

Introduction

Polymer aggregates have been widely studied for many years due to their promising applications in numerous fields.[1−4] These aggregates are typically synthesized by micellization of block or graft copolymers in selective solvents.[5] However, the use of organic solvents in most cases may limit their applications, e.g., as drug-delivery vehicles. As a result, aqueous polymer assemblies driven by electrostatic interactions rather than hydrophobic interaction have recently attracted increasing attention. Three main families of these polyelectrolyte aggregates have been extensively explored: (1) two oppositely charged polyelectrolytes form aggregates through a layer-by-layer deposition or as a result of the use of dendrimers;[6] (2) polyelectrolytes and multivalent metal ions (e.g., Cu^{2+} or Ca^{2+}) aggregate into supramolecular structures through intermolecular or intramolecular bridging;[7] (3) complexes of polyelectrolytes and aromatic organic dyes assemble into nanostructures induced by $\pi-\pi$ stacking of aromatic dye moieties.[8] For the above systems, structures of assemblies can be further conveniently controlled by tuning ionic strengths. For instance, pH-sensitive nanoparticles have been previously synthesized and are very important for uses in intelligent systems. However, in all cases, size distributions of above aggregates are usually broad,[9] which may limit their applications such as drug-delivery vehicles. Naturally, it is of great interest to fabricate polymer aggregates with relatively narrow distributions. Here we report that two homopolymers directly assemble into narrowly distributed nanospheres in water through ionic interactions among side chains between a rigid polyelectrolyte and a flexible counterionic polyelectrolyte. The rigidity of building complexes may be responsible for the uniform size. In addition, these assemblies reversibly and rapidly respond to both pH and temperature, and may be applied as intelligent drug-delivery vehicles.

Experimental Section

Material. Polyallylamine and poly(ethyleneimine) were purchased from Simga-Aldrich, and their average molecular weights are 65000 and 750000, respectively. Poly(*N*,*N*-dimethyl acrylamide) (average molecular weight of 148200) was provided by Polymer Source, Inc. Sulfonated aromatic poly(ether ether ketones) were synthesized via the nucleophilic aromatic substitution reactions of sodium 5,5-carbonylbis(2-fluorobenzene-sulfonate) and 3,3,5,5-tetramethyl-4,4-biphenol. Synthetic details for two monomers and polymers have been previously reported.[9,10] Four sulfonated aromatic poly(ether ether ketones) samples with repeating units of 8, 12, 16, and 17 were used.

Complexation. Water is a common solvent for polyallylamine and sulfonated aromatic poly(ether ether ketones), as no aggregation was detected by dynamic laser light scattering in their respective solutions, even at concentrations up to 200 mg/mL. However, aggregation immediately took place when 1 mg/mL sulfonated aromatic poly(ether ether ketone) solution and 1 mg/mL polyallylamine solution were mixed at room temperature with a molar ratio of SO_3Na groups in sulfonated aromatic poly(ether ether ketones) to NH_2 groups in polyallylamine value between 1/30 and 4/5. The NH_3 asymmetric stretching vibration that occurs at 1590 cm^{-1} for pure polyallylamine shifts to 1537 cm^{-1} for the complexes.[11]

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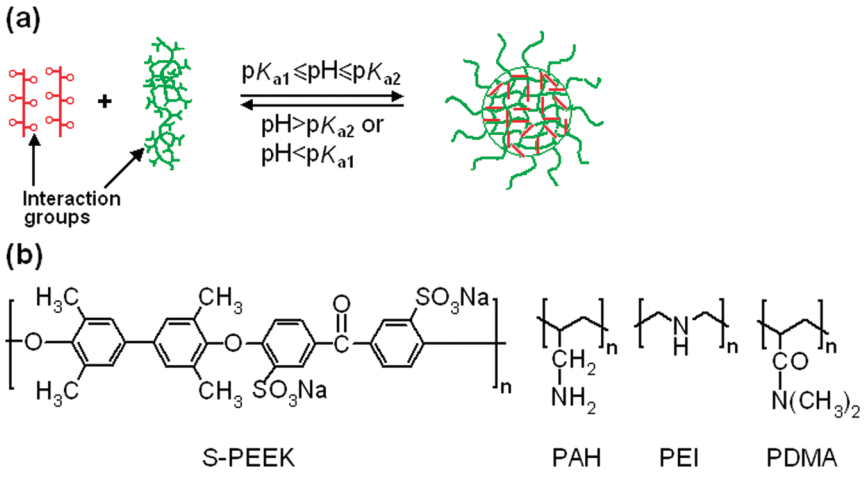

Figure 1. (a) Schematic illustration to pH-responsive assembly of acidic and basic homopolymers. (b) Chemical structures of homopolymers.

Cellular Uptake of Nanoparticles. Human monocytic THP1 cells were treated with ~ 20 μg nanoparticles in 1 mL of RPMI medium. After incubation of 2 h, the treated THP1 cells were fixed for 1 h in 2% paraformaldehyde/Dulbecco's phosphate buffered saline at room temperature. Control samples of untreated THP1 cells were cultured, washed, and incubated for the same time in each experiment. The 4′,6-diamidino-2-phenylindole dye was used to stain the cell nuclei.

Characterization. Dynamic light scattering measurements were performed on a Malvern Zetasizer Nano ZS with a laser wavelength of 633 nm. The detection system was placed at a scattering angle of 173°. Average hydrodynamic diameter and polydispersity index of aggregates were analyzed by Dispersion Technology Software. Morphologies of polymeric aggregates were characterized by transmission electron microscopy (TEM, JEOL 2010 operated at 120 kV).

Results and Discussion

Formation of such responsive assemblies relies on cooperative assembly of acidic and basic polymeric building blocks and their tunable electrostatic interactions. Figure 1a schematically shows pH-responsive assembly beginning with acidic building blocks (red line, proton dissociation constant pK_{a1}) and basic building blocks (green line, proton dissociation constant pK_{a2}). When the media pH is lower than pK_{a1} (pH < pK_{a1}), the basic assembling blocks are positively charged, while the acidic blocks are neutral, hindering the formation of acid/base assemblies. Increasing the pH between pK_{a1} and pK_{a2} negatively charges the acidic polymer and positively charges the basic polymer, which results in their assembly into aggregates through electrostatic interactions. As the electrostatic interaction strengths between the acidic and basic building blocks vary with the media pH (between pK_{a1} and pK_{a2}) and temperature, aggregates may be tuned with desired sizes by controlling the above two stimuli. Further increasing pHs above pK_{a2} neutralizes basic building blocks, while acidic polymers remain negatively charged, which results in dissolution of aggregates.

On the basis of this design strategy, we choose sulfonated poly(ether ether ketones) (S-PEEK, pK_a = 0.7) as the acidic building block, polyallylamine (PAH, pK_a = 10.7), poly(ethyleneimine) (pK_a = 8.5), and poly(*N*,*N*-dimethyl acrylamide) (pK_a = −0.5) as the basic building blocks (see Figure 1b), and have examined their assemblies at different pHs. S-PEEK and PAH were first mixed at a molar ratio (MR) of 1/30 (ratio of SO_3Na groups in S-PEEK to NH_2 groups in PAH) and concentration of 1 mg/mL. The formation of aggregates was monitored using dynamic light scattering (DLS). It was found that no aggregates were formed when pH was higher than 12. Decreasing pH to less than 10 induced aggregation as indicated by abruptly increased light scattering intensity. During the decrease of pH from 7 to 1, the average hydrodynamic diameter ($\langle D_h \rangle$) of nanoparticles systematically decreased from 450 to 275 nm (see Figure 2), and distributions of apparent sizes varied between 0.15 and 0.35. Low pH decreases the association capability of two homopolymers, so sizes of nanoparticles are reduced. Upon increasing the solution pH, $\langle D_h \rangle$ quickly returned to the original value, indicating a reversible process. A volume change of ~338% was observed for the media pH from 1 to 7. Different from hydrogels typically with very slow responses, the increasing/decreasing size cycle of such nanoparticles proceeds in seconds, which provides many advantages in practical applications. $\langle D_h \rangle$ slightly and systematically increases/decreases with decreasing/increasing pH between 10 and 7 (e.g., 429 nm for pH of 9.6 and 450 nm for pH of 6.8). With the further decrease of pH values to lower than 1, no stable nanoparticle solutions were available based on DLS measurements (note that ionic effect may also contribute to this change).

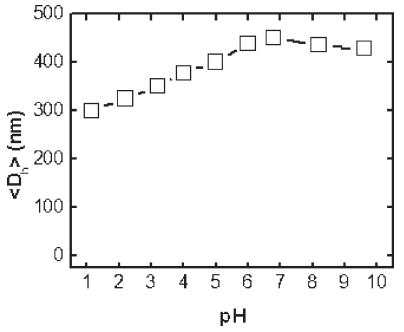

Figure 2. pH-responsive assemblies of S-PEEK (n = 17)/PAH (MR = 1/30, C = 1 mg/mL) in water. Nanoparticles are characterized by $\langle D_h \rangle$ as a function of solution pH values.

The sizes of nanoparticles can be also tuned by varying the molar ratios between two homopolymers and molecular weights of S-PEEKs. Nanoparticle diameters increased with decreasing MRs between S-PEEK and PAH, e.g., the $\langle D_h \rangle$ at MRs of 1/1 and 1/10, were 135 and 231 nm, respectively, compared to 450 nm at MR of 1/30. This result is in contrast with normal polymer micelles in which $\langle D_h \rangle$ slightly increases at higher MRs.[12,13] This

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phenomenon may be due to their unique structure in these nanoparticles, i.e., more complexes at higher MRs dramatically shrink the nanoparticle sizes. In fact, TEM observations showed that nanoparticles became denser with decrease of diameters. It is easy to understand that higher molecular weights of S-PEEKs will produce larger nanoparticles. S-PEEK is a rigid molecule that tends to build up a larger structure with increasing molecular weight. It was found that $\langle D_h \rangle$ of nanoparticles (MR of 1/30) derived from S-PEEK with a repeating unit of 8 was ~220 nm, compared to 450 nm for a repeating unit of 17.

Replacing PAH with a weaker basic homopolymer, such as poly(ethyleneimine) with a pK_a of 8.5, shifted the critical pH from 10 to 8, and similar pH-dependent $\langle D_h \rangle$ was detected by DLS measurements. In contrast, no aggregation was observed upon mixing S-PEEK with a much weaker basic homopolymer, poly(*N*,*N*-dimethyl acrylamide), with a pK_a of −0.5. Therefore, the critical pH point is adjustable by controlling the intensity of the ionic interaction between two homopolymers. Note that complexes from other rigid polyelectrolytes may form other morphologies such as a cylinder.[14]

Nanoparticles also rapidly and reversibly respond to environmental temperature. Here we mainly study a temperature range of 5−50 °C, which is close to many practical applications. The $\langle D_h \rangle$ of nanoparticles decreased from 381 to 134 nm with increasing temperatures from 5 to 50 °C (see Figure 3a). Upon decreasing solution temperature, $\langle D_h \rangle$ quickly returned to the original value, indicating a reversible decreasing/increasing change of nanoparticle sizes. A volume change of ~22 times of nanoparticles was observed for decreasing temperature from 50 to 5 °C. Distributions of apparent sizes slightly varied between 0.23 and 0.33 for the above temperature range. Figure 4a shows a typical TEM image of as-synthesized S-PEEK/PAH nanoparticles at room temperature. The calculated average size is ~100 nm, smaller than that from DLS measurement (Figure 4b). This difference may be due to two facts: (1) shrinkage of nanoparticles after evaporation of solvent during preparation of the transmission electron microscopy sample; (2) increase/decrease of pH after evaporation/dilution.

Reduced sizes of nanoparticles with increasing temperatures are due to the fact that high temperatures decrease the association capability of two homopolymers, e.g., increase of temperature can induce deprotonation of polycations. The decreasing zeta potentials with increasing temperatures further confirm this explanation (Figure 3b). Reduction of positive charges at higher temperatures decreases zeta potentials of nanoparticles. In addition, consistent with the dependence of $\langle D_h \rangle$ on temperature, their zeta potentials exhibit rapid and reversible changes in response to media temperature.

Polymer aggregates have been widely studied as drug-delivery vehicles. To investigate this application, S-PEEK/PAH nanoparticles were incubated with human monocytic THP1 cells in buffer solutions. Figure 5 compares fluorescent microscopy images of THP1 cells without and after incubation with nanoparticles for 6 h. As expected, nanoparticles can readily enter cells with relatively high efficiency. Fluorescein isothiocyanate was used as the model drug in the above experiment. Particularly, these responsive nanoparticles show two desirable properties for smart delivery vehicles, i.e., targeting and controlled release.[15] NH_2 groups on their outer surfaces provide targeting functionalities by attaching ligands that recognize tumor-specific or tumor-associated antigens,[4c] while drug release from nanoparticles may be controlled by external stimuli such as pH or temperature. More research efforts are underway.

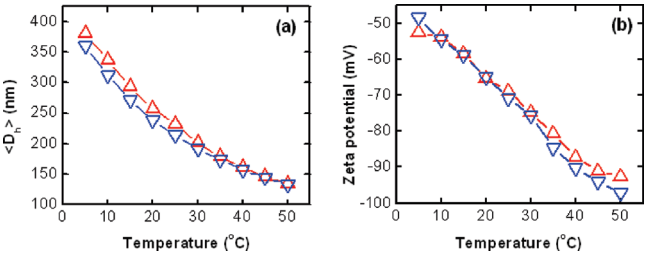

Figure 3. Temperature-responsive assemblies of S-PEEK (n = 8)/PAH (MR = 4/5, C = 1 mg/mL) in water. (a) Nanoparticles characterized by $\langle D_h \rangle$ as a function of solution temperatures. (b) Nanoparticles characterized by zeta potentials as a function of solution temperatures. Red color shows the temperature increase from 5 to 50 °C, and blue color shows the temperature decrease from 50 back to 5 °C. pH values are 10.0, 9.9, 9.8, 9.7, 9.5, 9.4, 9.3, 9.2, 9.1, and 9.0 at 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 °C, respectively.

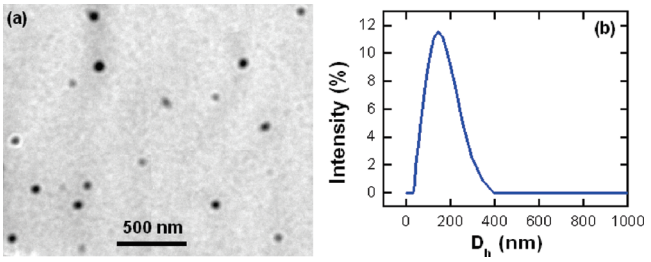

Figure 4. (a) Typical TEM image and (b) DLS measurement of nanoparticles. Nanoparticles were constructed from S-PEEK (n = 8)/PAH (MR = 4/5, C = 1 mg/mL).

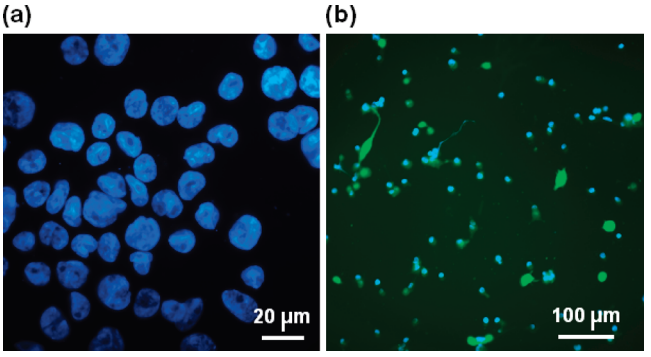

Figure 5. Fluorescein isothiocyanate-loaded nanoparticles targeting THP1 cells. (a) THP1 cells not incubated with nanoparticles. (b) THP1 cells after being incubated with nanoparticles for 6 h. The original nanoparticles were synthesized from S-PEEK (n = 8)/PAH (MR = 4/5, C = 1 mg/mL).

Conclusion

In summary, this work reports an unexpected assembly of two hydrophilic homopolymers into nanoparticles at pHs between two critical points in water. When solution pH is beyond the critical range, nanoparticles immediately disassociate. This association/disassociation process is reversible, and the critical range is tunable. Compared with polymer hydrogels typically with very slow responses, the formed homopolymer nanoparticles can rapidly and reversibly respond to two external stimuli of pH

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and temperature with tunable sizes. This research may also provide a general and efficient paradigm to fabricate a family of responsive polymer nanoparticles.

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