pH-Responsive Shell Cross-Linked Nanoparticles with Hydrolytically Labile Cross-Links

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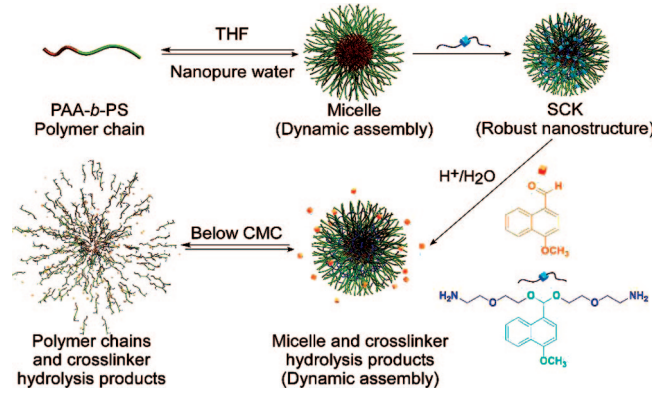
pH-responsive polymers, as intelligent materials, have drawn attention in drug delivery due to their abilities to sense changes and rapidly stimulate structural or morphological responses.[1] Considering the mildly acidic conditions found in endosomal and lysosomal compartments (pH 5−6), tumors, and inflammatory tissues (pH ≥ ca. 5),[2] pH-sensitive assemblies are attractive because they can integrate well-defined 3-D structures, desired loading capacities, various functionalities, and reduced toxicity.[3]

Over the past several years, we and others have been advancing shell cross-linked knedel-like (SCK) nanoparticles toward multifunctional imaging and therapeutic agents. SCKs are constructed by a procedure that involves the supramolecular assembly of amphiphilic block copolymers into micelles, followed by covalent cross-linking throughout the shell layer.[4] SCKs have been investigated as candidates for targeted biomedical applications due to their variable compositions, tunable sizes (typically over the size range from 10 to 100 nm), polyvalency, and exceptional stability to avoid spontaneous disassembly at concentrations below the critical micelle concentration (cmc) or under challenging conditions as occur in vivo.[5] The robust characteristics of SCKs are due, in large part, to the covalent cross-linking.[6] Conventionally, SCKs contain nondegradable cross-linkers. In our current work, we intended to develop a hydrolytically degradable SCK. In doing so, we found several interesting results. The incorporation of chromophore-linked degradable cross-linkers into the shells of SCKs provides for programmed disassembly of the nanostructures and also allows for release of reporter molecules. Moreover, at pH 7.4, the rate of hydrolysis was observed to be accelerated significantly by the local effects within the SCK polymer shell. In this Communication, therefore, we report a fundamental study that advances the structural features and potential utility of SCKs by incorporation of pH-sensitive cross-linkers.

The strategy for the development of SCKs containing hydrolytically degradable cross-links began with amphiphilic block copolymers of poly(acrylic acid) (PAA) and polystyrene (PS) that, after being allowed to assemble supramolecularly into micelles in water, were cross-linked through amidation reactions with a unique acetal-containing cross-linker (Scheme 1). Since these amide bonds are quite stable, we designed the cross-linker to have a labile central acetal linkage. Reliance on disassembly of the micelles at concentrations below the cmc after hydrolysis of the acetals would provide only a crude measure of the integrity of the nanoparticle system and not a quantifiable determination of the extent of cross-link hydrolysis. Therefore, an acid-sensitive cross-linker was prepared with a UV−vis reporter unit that is released upon hydrolysis of the acetal linkage. This approach was inspired by a combination of Fréchet's[7] use of the escape of Nile red from the core of exploding block copolymer micelles and Sames' redox-sensitive optical switch-based fluorogenic metabolic probes for readout of enzyme activity in mammalian cells.[8] Our pH-sensitive cross-linker relied upon an acetal−aldehyde conversion, readily observed by UV−vis spectroscopy, to monitor the kinetics for hydrolysis of the shell cross-links within the SCKs in water as a function of the solution pH.

Scheme 1. Illustration of SCKs That Contain Hydrolytically Labile Cross-Links To Allow for pH-Triggered Hydrolysis and Nanostructure Disassembly



The acetal-based degradable cross-linker was prepared from 4-methoxy-1-naphthaldehyde and azidoethoxyethanol, followed by reduction. The aldehyde exhibits distinct UV absorption in comparison to the acetal (Figure 1a). Real-time monitoring of the degradation process was accomplished by observing the UV absorbance in the region of 275−400 nm and comparing the absorbance at 350 nm, at which wavelength the only absorption is from the aldehyde hydrolysates, giving the relative amount of aldehyde generated (relative to 100% aldehyde hydrolysate production).

pH-sensitive SCKs were constructed from amphiphilic diblock copolymer PAA_{60}-*b*-PS_{30}, followed by cross-linking using the acetal cross-linker. Purification was accomplished by centrifugation using Amicon ultracentrifugal filter devices (30 kDa MWCO), repeatedly, until no absorption was observed for the filtrates in the region of 275−400 nm. Fluorescence studies using pyrene[9] in water determined the cmc of the micelle to be 3.68×10^{-7} M (see Supporting Information, Figure S3), whereas the SCK exhibited no cmc. Using the molar extinction coefficient (ε) of the acetal cross-linker (3.96×10^3 cm^{-1} M^{-1}, pH = 7.4, λ_{max} = 295 nm), it was determined that ca. 30 mol % was incorporated into the SCKs, relative to the available acrylic acid residues. Importantly, the preparation and purification steps were performed at 0 °C, and the samples were maintained at this reduced temperature, which minimized premature hydrolysis so that only ca. 8% of the cross-linker underwent degradation prior to the kinetic studies (Figure 1b). At pH 7.4, the number-average hydrodynamic diameters ($D_{h,n}$) of the micelles, SCKs, and hydrolyzed SCKs were each very similar, at 18 ± 2, 15 ± 1, and 17 ± 1 nm, respectively, as measured by dynamic light scattering (DLS). The number-average diameters (D_{av}) of the

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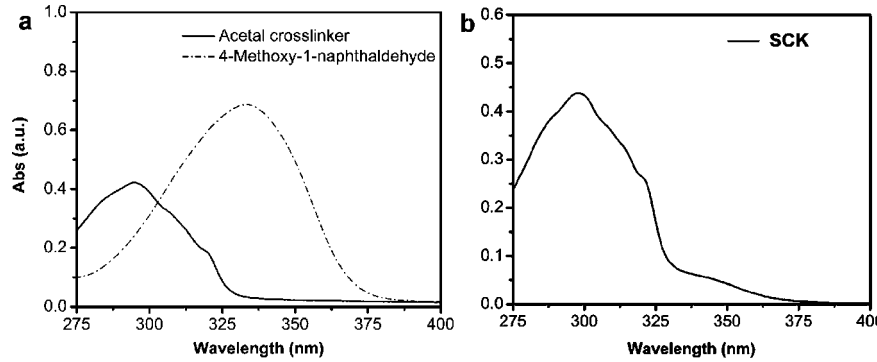

Figure 1. UV−vis spectra of the acetal cross-linker and 4-methoxy-1-naphthaldehyde (a) and pH-sensitive SCK maintained at 0 °C, 30 min after purification, illustrating the incorporation of the cross-linker with only ca. 8% hydrolysis (b), each in aqueous PBS buffer, with 5% DMSO added to the acetal cross-linker solution.

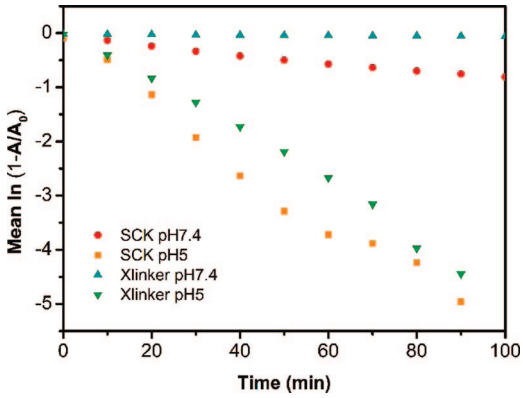

Figure 2. Arrhenius plots for the hydrolysis kinetics (average of triplicate) of acetal cross-linker as a small molecule and after its incorporation into SCKs.

micelles and SCKs were 10 ± 1 and 9 ± 1 nm, respectively, as measured by transmission electron microscopy (TEM).

The cross-linker and SCK were incubated in pH 7.4 and 5.0 buffers at 37 °C, and the hydrolyses were monitored by UV−vis spectroscopy in situ. Analysis of the data (Figure 2) gave the hydrolysis rate constant (k) and half-life ($t_{1/2}$) values. The cross-linker was hydrolyzed ca. 120-fold faster at pH 5.0 ($k = 0.049$ min^{-1}, $t_{1/2} = 14$ min) than at pH 7.4 ($k = 0.000\,40$ min^{-1}, $t_{1/2} = 29$ h). After incorporation into the SCK, hydrolysis occurred ca. 7-fold faster at pH 5.0 ($k = 0.054$ min^{-1}, $t_{1/2} = 13$ min) than at pH 7.4 ($k = 0.0075$ min^{-1}, $t_{1/2} = 1.5$ h). At pH 7.4, the hydrolysis rate of the cross-linker increased 19-fold after incorporation into the nanoparticle, whereas it was similar at pH 5.0 as a small molecule in solution. Since the pH of the cross-linker solution was adjusted from 7.4 to 5.0 by addition of a small volume of concentrated acetic acid solution, salt effects are negligible. The apparent dissociation constants of polyelectrolytes, such as PAA, are not constant but instead are a function of the degree of ionization.[10] At pH 5.0, sufficient protons are found in the bulk buffer solution, so that there is no obvious influence of the acrylic acid residues of the SCKs. However, at pH 7.4, the PAA shell could provide protons within the nanoscopic confines of the nanoparticle shell layer, resulting in the acceleration of the cross-linker hydrolysis.

Since the micelle, SCK, and hydrolyzed SCK sizes were similar, tapping-mode atomic force microscopy (AFM) was applied to demonstrate the disassembly of the acid-labile SCKs. After evaporation of the water in vacuo and addition of dichloromethane (DCM), samples of the intact SCKs and those that had been undergone hydrolysis of the cross-linkers were drop deposited onto mica and allowed to dry under ambient conditions. The SCKs remained robust and appeared as well-defined nanoparticles (Figure 3a) with an average height (H_{av}) of 4 ± 1 nm, which was in agreement with the original SCK height of 4 ± 1 nm, as deposited from aqueous solution. In contrast, the micelles, as dynamic assemblies resulting from degradation of the cross-linkers, disassembled into polymer chains upon evaporation of water and then reassembled into ill-defined aggregates in DCM, with a H_{av} of ca. 6 ± 5 nm (Figure 3b, also see Supporting Information, Figure S6). When the hydrolyzed SCK samples were redispersed into tetrahydro-

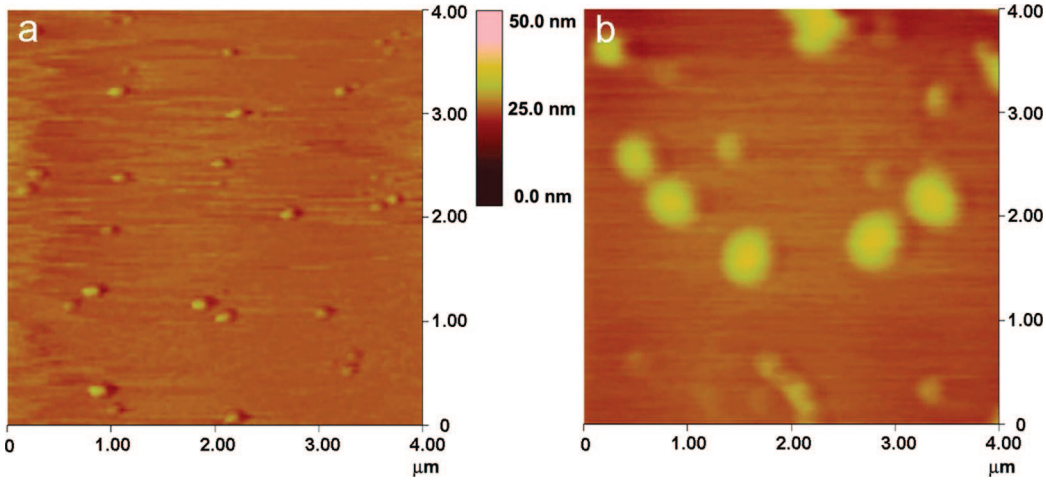

Figure 3. Tapping-mode AFM images of the SCKs (a) and reassembled nanoparticles (b) in DCM (drop deposition on mica).

furan, a solvent for both block segments, no nanostructures were observed, as expected due to the SCK degradation into single polymer chains.

In summary, a pH-sensitive cross-linker has been synthesized and incorporated into supramolecular block copolymer assemblies. The inclusion of a UV−vis active probe within the cross-linker provided direct and sensitive detection of the cross-link cleavage events. Accelerated degradation was observed at lysosomal pH, in comparison to physiological pH, for this cross-linker in general, which might provide an attractive approach for controlled drug delivery. More importantly, the local environment within the shell of the nanostructure promoted hydrolysis of the cross-linker. Studies to investigate further tuning of the rate of cleavage of these cross-linkers, and also designing higher levels of complexity, with various modes for inclusion of biologically active agents (drugs) for potential burst release and nanostructure disassembly are underway.

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Supporting Information Available: Measurements, synthesis, and characterization of the cross-linker, amphiphilic block copolymer, SCK nanoparticles, and hydrolysis procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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