Polymer Micelles Stabilization on Demand through Reversible Photo-Cross-Linking

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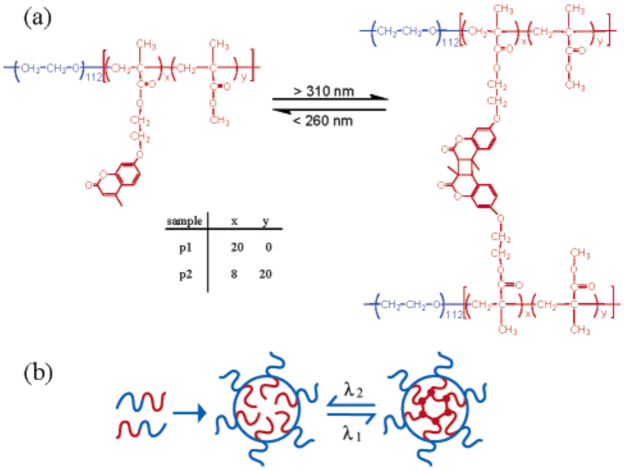
The stability of polymer micelles, which affects the stability of encapsulation of guest molecules, may be a crucial condition for some controlled delivery applications. It is possible that polymer micelles are disintegrated after being administrated in the body due to an extreme dilution to below the critical micelle concentration of the polymer. If this happens, the entrapped guest such as a drug can leak out quickly, which renders any strategy for site-specific transport of the micelles useless.[1] Therefore, various approaches have been proposed to stabilize polymer micelles and thus the encapsulation. Of them, micelle core cross-linking[2] or shell cross-linking[3] is particularly attractive owing to the structural integrity of chemically cross-linked micelles. However, a more stable encapsulation of a guest may render its release more difficult at a later time after micelles arrive at the target. Kataoka and co-workers have demonstrated an elegant method to resolve the problem of the conflicting effect.[4] They succeeded in stabilizing their polyion complex micelles by cross-linking the core through disulfide bonds and, subsequently, dissociating the micelles by cleaving the disulfide bond with a reducing reagent. A more recent report showed a similar approach in which the thiol/disulfide exchange reaction was used to reversibly cross-link and de-cross-link the shell of a thermosensitive triblock copolymer micelle.[5] In this paper, as part of our ongoing research on light-controllable polymer micelles,[6] we propose a new all-optical strategy for the stabilization on demand of polymer micelles, which differs from the previous chemical approaches.[4,5] Our proof-of-concept study shows that, through the use of reversibly photocontrolled dimerization of coumarin moieties[7] incorporated in a diblock copolymer, micelles can be cross-linked for stabilization and subsequently de-cross-linked by light illumination at two different wavelengths, with tunable cross-linking density. As this optical approach can be adapted to other chromophores displaying a reversible photoreaction[8] and to various micellar aggregates, we believe our study suggests a potentially useful new route to polymer micelles that afford both structural stabilization for stable encapsulation and subsequent destabilization for controlled release at a required time and location, through the use of light.

Figure 1a shows the chemical structures of the new amphiphilic diblock copolymers we have synthesized using atom transfer radical polymerization (ATRP), which contain pendent coumarin moieties for the reversible photodimerization and cleavage (details on synthesis and characterization in the Supporting Information). While poly(ethylene oxide) (PEO) is the hydrophilic block, the hydrophobic block varies. It is either poly(coumarin methacrylate) (PCMA) or a random copolymer of poly(methyl methacrylate) (PMMA) and PCMA. In both cases, the photodimerization of coumarin moieties at $\lambda > 310$ nm can result in interchain cross-linking, while the cleavage of cyclobutane at $\lambda < 260$ nm leads to the de-cross-linking. The depicted head-to-head photodimerization should be the main cycloaddition reaction under our experimental conditions.[9] Figure 1b is a schematic illustration of using coumarin-containing block copolymers to prepare core cross-linkable and de-cross-linkable micelles using two different wavelengths. Here it is worthwhile to mention that even though UV light was utilized in this study, coumarins are known to be able to undergo the reversible photodimerization and cleavage through two-photon absorption of visible light.[10]



sample	x	y
p1	20	0
p2	8	20

Figure 1. (a) Chemical structure of the diblock copolymer and the photodimerization and photocleavage of coumarin side groups. (b) Schematic illustration of the reversible cross-linking of micelles.

The micelles of sample p1 could be obtained by adding water in its DMSO solution (DMSO is a good solvent for the two blocks), while micelles of p2 could be formed upon addition of water in its THF solution. In the latter case, the comonomer units of MMA rendered the coumarin-containing block soluble in THF. Aqueous solutions of micelles were prepared by removing the organic solvents through dialysis in water (see details on the preparation conditions in the Supporting Information). Figure 2 shows the reversible photo-cross-linking using micelles of p1 as an example. When the micellar solution (0.05 mg mL^{-1} × 2 mL) was exposed to UV light at $\lambda > 310$ nm (2000 mW from a UV−vis spot curing system), the absorption of coumarin moieties at around 320 nm decreased continuously with time, indicating the occurrence of dimerization (cross-linking) in the micelles (Figure 2a);[7] the inset gives the increase in the dimerization degree as estimated from the change in absorbance at 320 nm. Assuming the interchain dimerization of PCMA inside the micelle core, the dimerization degree should reflect the cross-linking degree. Subsequently, when the core cross-linked micellar solution was illuminated by a UV lamp ($\lambda_{max} = 254$ nm, 1.25 W, and placed at ∼7 cm from the solution), the opposite process, i.e., photocleavage of coumarin dimers, took place as indicated by the recovery of the absorption at 320 nm (Figure 2b);[7] the decrease in the dimerization degree

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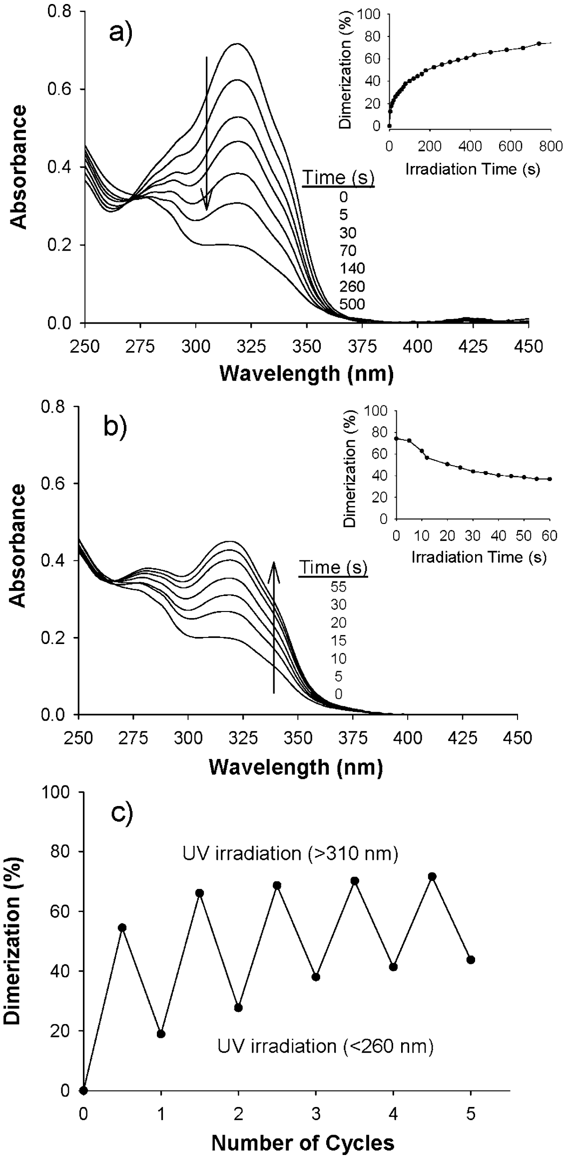

Figure 2. UV−vis spectra of a micellar aqueous solution of sample p1: (a) under UV irradiation at $\lambda > 310$ nm, the inset showing the increase in photodimerization (cross-linking) degree; (b) under subsequent UV irradiation at $\lambda < 260$ nm, the inset showing the decrease in photodimerization degree; and (c) changes in the photodimerization degree with the solution subjected to alternating irradiations at two wavelengths.

is also shown in the inset. These results demonstrate that for the same polymer micelle an easy regulation of the cross-linking density can be obtained by adjusting the irradiation time (or the intensity). Although the photocleavage of coumarin dimers appeared incomplete, a reversible photocontrol of the cross-linking density could be achieved to a certain degree, as shown in Figure 2c. With the micellar solution repeatedly exposed to alternating UV light at $\lambda > 310$ nm (5 min) and $\lambda < 260$ nm (1 min) for five cycles, the reversible change in the dimerization degree (cross-linking density) can be noticed. Under the used preparation conditions, dynamic light scattering (DLS) measurements of the aqueous micellar solutions found small variations in the distribution of sizes and the average sizes of the micellar aggregates before cross-linking (141 nm), after cross-linking (136 nm), and after the subsequent de-cross-linking (139 nm). The DLS results basically are consistent with the TEM observations made on the same solutions, which revealed that most small core−shell micelles ($\sim$10 nm) coalesced into the larger micellar aggregates observed on DLS (Supporting Information). Note that we have also used TEM to obtain an additional evidence for the occurrence of micelle core cross-linking. An aqueous micellar solution of p1 after exposure to UV light at $\lambda > 310$ nm was first dried under an air stream for 2 days and then redissolved in DMSO with stirring for 2 h. After casting on a grid and drying in a vacuum oven, TEM observations found that micelles were not dissolved in the organic solvent due to the cross-linking induced structural integrity (Supporting Information).

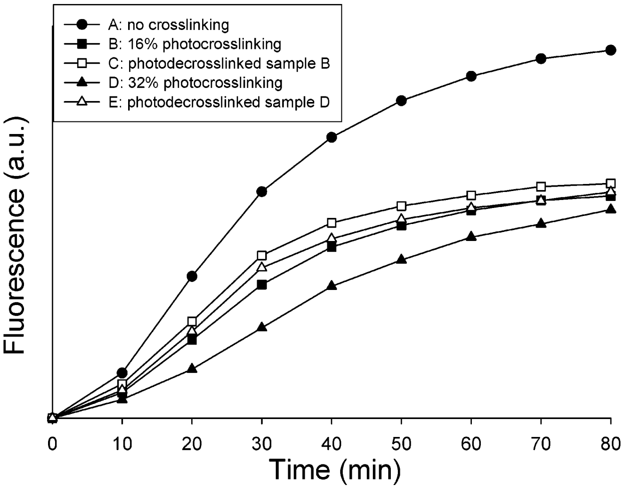

Figure 3. Plots of fluorescence intensity vs time showing different rates of release of Nile Red loaded in un-cross-linked, photo-cross-linked, and subsequently photo-de-cross-linked micelles of sample p1 subjected to dialysis in THF/water (2/3, v/v).

We also investigated the effect of photo-cross-linking and photo-de-cross-linking on the stability of encapsulation under various conditions. Figure 3 shows an example of the results obtained with micelles of p1 in which the hydrophobic dye of Nile Red (NR) was loaded; the experiment was designed to accelerate the leak of NR from the hydrophobic core of micelle and to compare the rate of release for different micellar solutions. To this end, five aqueous solutions of micelles, whose cores were respectively un-cross-linked, photo-cross-linked to two different dimerization degrees (16 and 32%), and subsequently photo-de-cross-linked, were subjected to a dialysis in THF/water (2/3, v/v) under the same conditions (1 mL of micellar solution in 15 mL of THF/water). Since NR is soluble in the mixed solvent, loaded guest molecules could leak out the micelles quickly. The rate of release was monitored by measuring the fluorescence of NR in the mixed solvent. The results in Figure 3 indicate that core cross-linking slowed down the release of NR from the micelles and that the effect is more important with increasing the cross-linking density. Following the photo-de-cross-linking, the rate of release indeed increased, but it could not recover to the level for the un-cross-linked micelles. The incomplete photocleavage of coumarin dimers is likely a cause for the behavior, since a photostationary state can be reached due to the occurrence of also some dimerization reaction under our used irradiation wavelength at $\lambda < 260$ nm.

To summarize, we have designed and synthesized a novel coumarin-containing amphiphilic diblock copolymer, whose micelles can be reversibly cross-linked and de-cross-linked using light at two different wavelengths. The reversible photoisomerization and photocleavage of coumarin derivatives has been explored for functional materials,[7,11] but to our knowledge, our study is the first attempt to apply this property to design photocontrollable polymer micelles. Even though the current system showed limited efficacy and reversibility degree, in view of the generality of the approach, which makes it easily adaptable to many polymers and chromophores, we believe this study demonstrated the potential interest of a new concept that consists in using light to first afford the stability of polymer micellar aggregates (and their encapsulation) through photo-cross-linking and then to allow the release of encapsulated guest through photo-de-cross-linking-induced disruption of the micelles. More studies are underway in our laboratory to improve the efficacy of the approach.

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Supporting Information Available: Block copolymer synthesis and characterization, UV−vis, fluorescence, DLS, and TEM measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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