Polymeric Micelles

Light-Induced Reversible Formation of Polymeric Micelles**

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Highly efficient photoreversible micellization of amphiphilic block copolymers is attractive for drug delivery and separation systems, since reversible control of encapsulation and release of hydrophobic materials incorporated in the interior of polymeric micelles can be achieved by an external light source.

Polymeric micelles are formed in aqueous media by self-assembly of amphiphilic block copolymers.[1–7] Light-sensitive micelles are generally composed of a hydrophilic shell and a responsive hydrophobic core with tunable solubility that depends on the wavelength of light with which it is irradiated.[8] In these systems, the core-forming block plays a crucial role in the formation and dissociation of the micelles. The block copolymers initially aggregate spontaneously to form micelles with a hydrophobic core. After UV irradiation, the hydrophobic core becomes hydrophilic and the copolymer can dissolve in water. Recently, photoreversible morphological changes of block copolymer micelles based on azobenzene have been reported.[8] Azobenzene units undergo *cis–trans* isomerization on alternating irradiation with UV and visible light, which induces changes in the polarity of the units; the *cis* isomer of azobenzene is more polar and therefore less hydrophobic than the *trans* isomer.[9,10] However, in this system complete micellar disruption and regeneration were not achieved, since the *cis* azobenzene units are not sufficiently water soluble.

Photochromic spiropyran units respond to light and undergo a reversible isomerization between colorless spiropyran (SP) and colored merocyanine (ME).[11,12] The reversible behavior of these materials has led to their evaluation in several applications: optical and electrical switching,[13] data recording,[14] reversible solubility control of enzymes,[15] and light-actuated nanovalves.[16] As opposed to azobenzene moieties, the incorporation of SP units into block copolymer micelles leads to reversible micelles with improved light responsiveness, since the difference in polarity between hydrophobic SP units and hydrophilic, zwitterionic ME units is greater.[17]

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Herein we demonstrate that polymeric micelles can be completely disrupted by UV irradiation and reversibly regenerated by irradiating with visible light. When a hydrophobic dye is encapsulated within the hydrophobic core of a polymeric micelle, its release can be induced by exposure to UV light, which disrupts the micellar structure and facilitates block-copolymer dissolution. Moreover, a portion of the released hydrophobic dye can be re-encapsulated on regeneration of the micelles by irradiating with visible light.

The strategy employed in our study is schematically illustrated in Scheme 1. The hydrophobic SP block was
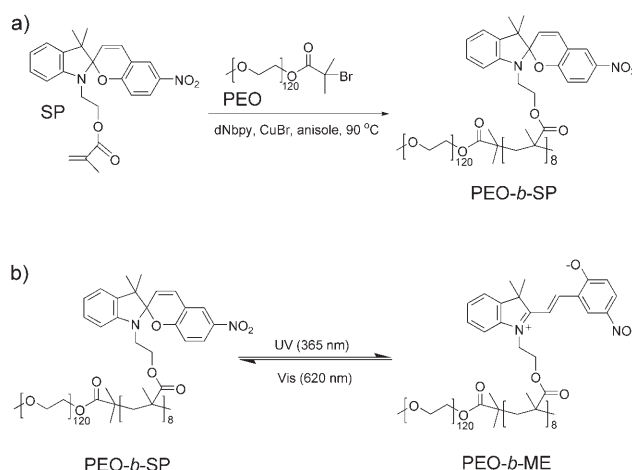
Scheme 1. a) Synthesis of a PEO-*b*-SP diblock copolymer; dNbpy = 4,4′-bis(5-nonyl)-2,2′-bipyridine. b) Light-induced isomerization between PEO-*b*-SP (colorless aqueous solution) and PEO-*b*-ME (purple aqueous solution) by alternating irradiation of UV and visible light.

prepared by atom-transfer radical polymerization (ATRP)[18,19] from a hydrophilic poly(ethylene oxide) (PEO) macroinitiator. Both the PEO macroinitiator ($M_n = 8.0 \times 10^3$ g mol^{-1}, PDI = 1.04) and spiropyran-containing methacrylate monomer (SP) were synthesized according to methods described in the literature.[20,21] Polymerization was carried out at 1:3 ratio by weight of SP monomer in anisole to provide a completely homogeneous solution. The resulting PEO-*b*-SP ($M_n = 1.24 \times 10^4$ g mol^{-1}, PDI = 1.16) was composed of 120 units of EO and 8 units of SP, as determined by ^{1}H NMR spectroscopy. In aqueous solution, this block copolymer self-assembled to form micelles in which the hydrophobic SP block forms the inner core, and the hydrophilic PEO the outer shell. Irradiation with UV light (365 nm) led to photoisomerization and subsequent conversion of hydrophobic SP to hydrophilic ME, which completely dis-

rupted the micelles due to the nonselective nature of the surrounding aqueous medium. Reversible micellization was demonstrated by photochemical reversion from ME to SP, which occurred as a result of irradiation with visible light (620 nm). This reversed isomerization resulted in regeneration of the block copolymer in its amphiphilic form and reformation of the polymeric micelles.

Photochemical isomerization of spiropyran moieties was demonstrated by UV/Vis absorption spectroscopy. An aqueous micellar solution was prepared by adding water to a solution of the polymer in THF; a 1.0-mg sample of PEO-*b*-SP was dissolved in 1 mL of THF, and then 10 mL of water was added dropwise overnight to form micelles while THF was allowed to completely evaporate. The solution was filtered through a 0.22-μm filter before use. The initial aqueous solution of the block copolymer micelles (0.01 wt%) was exposed to visible light (620 nm) for 1 h to ensure that the ring-closed SP groups were dominant. Then the solution was irradiated with either UV light (365 nm; Figure 1a) or visible light (620 nm; Figure 1b) until photostationary states were reached. On UV irradiation, a strong absorption band at 560 nm appeared, which is characteristic of the ME form. During this process, the colorless SP solution became pink. Irradiation with visible light isomerized the ME form back to the SP form, as was confirmed by the spectra gradually reverting to the original absorption profile. The reverse ME to SP isomerization took twice as long as the

original SP to ME isomerization, as has been previously observed.[23] While the same general isomerization trends were observed in homogeneous THF solutions, the isomerizations were almost 20 times faster than those in aqueous solutions.

The photoinduced formation, disruption, and regeneration of the polymeric micelles were confirmed by atomic force microscopy (AFM, Figure 2). The samples were prepared from solutions with the same concentrations as those employed for the UV/Vis investigations. The AFM images of the samples deposited directly after micellization revealed the presence of uniform, well-dispersed individual globular micelles (Figure 2a) with an average height of 3.5 nm (Figure 2a1). The main mode of the apparent volume distribution of the original micelles was about 1000 nm³; some tailing towards larger volumes was caused by the presence of infrequent aggregates (Figure 2a2). The micelle solution was then exposed to 365-nm UV light for 30 min and quickly spin-coated onto a mica surface for AFM analysis. This time, the characteristic well-defined globular micelles gave way to smaller, ill-defined aggregates surrounded by the irregular "halos" of nearly molecularly resolved individual polymer chains (Figure 2b, see also inset), consistent with disruption of polymer micelles by UV irradiation. The same solution was then exposed to 620-nm visible light for 30 min. The AFM images of the samples deposited after this step again showed the presence of well-defined globular structures; this indicates regeneration of the micelles. This time, however, the micelles tended to aggregate into branched, chainlike structures (Figure 2c). Interestingly, continuing exposure to visible light for 120 min induced micelles with more uniform size distribution (Figure 2d). Over the UV/Vis irradiation cycle, the average height changed from 3.5 to 6.1 nm (Figure 2d1). The apparent volume of the micelles exhibited nearly threefold increase to about 3000 nm³ (Figure 2d2). The observed evolution of the morphology of the micelles after UV and Vis treatment can be attributed to annealing/equilibration accompanying the regeneration process.

Having demonstrated the reversible, light-induced transitions of the PEO-*b*-SP micelles, we attempted to utilize the system for reversible capture and release of the hydrophobic dye coumarin 102. To incorporate coumarin 102 into the core of the micelles, 1.0 mg of PEO-*b*-SP and 0.2 mg of coumarin 102 were dissolved in 1 mL of THF followed by dropwise addition of water (10 mL) overnight. The photochemical control of encapsulation of coumarin 102 was assessed by fluorescence spectroscopy. Emission spectra in Figure 3a provide evidence of reversible encapsulation and release of the dye following different irradiation treatments of the micellar solution. An excitation wavelength of 420 nm was chosen, since no photoconversion between SP and ME occurs at this wavelength.[22] The initial micellar solution showed the strong emission of coumarin 102 with maximum at 491 nm. After UV irradiation, the emission intensity of coumarin 102 decreased drastically, since released coumarin 102 is insoluble in water. Subsequent visible-light irradiation of the solution, while vigorously stirring, caused regeneration of polymeric micelles which, remarkably, was accompanied by re-encap-
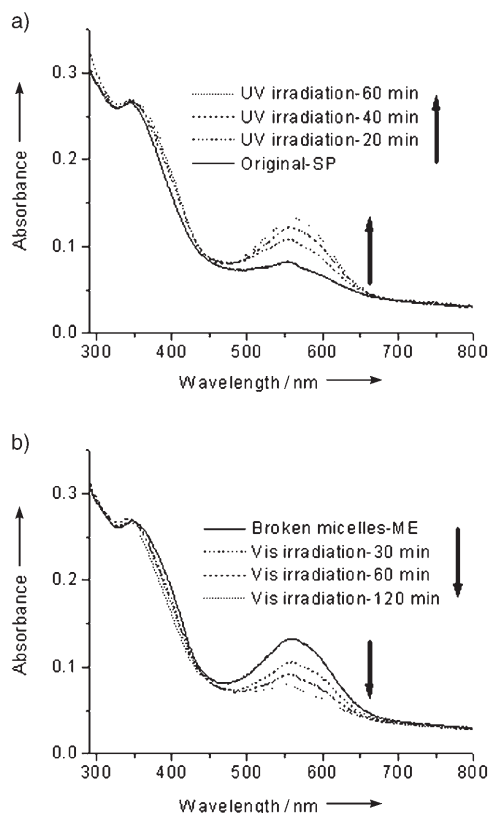

Figure 1. UV/Vis spectra of the polymeric micelles upon a) UV (365 nm) and b) visible-light (620 nm) irradiation in a 0.01-wt% aqueous solution.

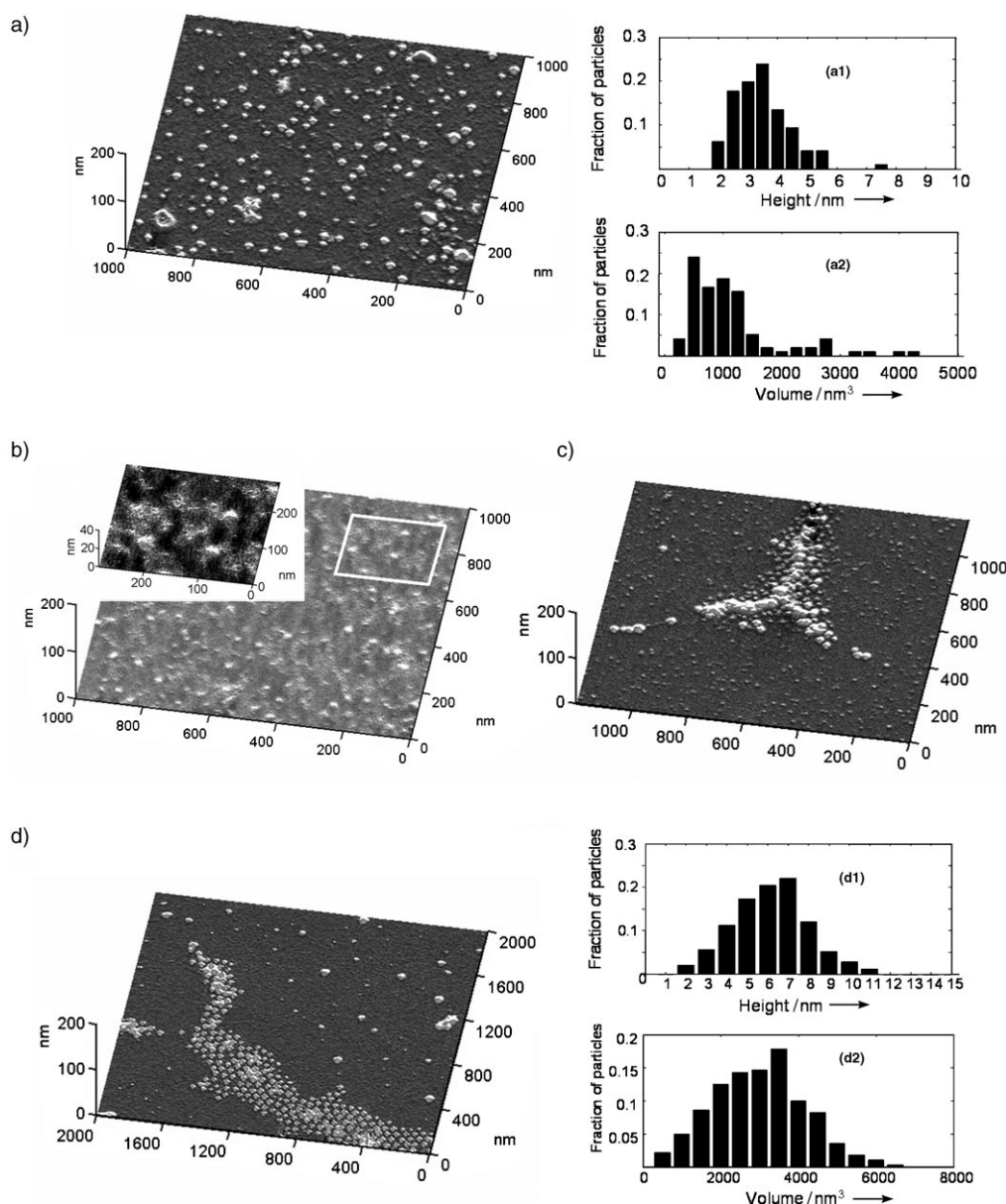© 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Figure 2. AFM height images of PEO-*b*-SP solutions spin-coated on mica under various conditions: a) PEO-*b*-SP micelles (a1) height distribution, a2) volume distribution); b) PEO-*b*-SP micelles after 365-nm UV exposure for 30 min; inset: magnification of the area indicated by the rectangle; c) PEO-*b*-SP micelles after 365-nm UV exposure for 30 min followed by 620-nm visible-light exposure for 30 min; d) PEO-*b*-SP micelles after 365-nm UV exposure for 30 min followed by 620-nm visible-light exposure for 120 min (d1) height distribution, d2) volume distribution).

sulation of the released coumarin 102, as demonstrated by the increase of its emission intensity. Systematic changes of coumarin 102 emission intensity on irradiation are illustrated in the graph of normalized emission intensity versus irradiation time (Figure 3b), which points to about 35% recovery of emission intensity on regeneration of the micelles. Incomplete re-encapsulation of the dye could be due to the relatively high rate of micelle regeneration, which would prevent the released molecules of coumarin 102 from interacting with the hydrophobic block in a manner sufficient to re-encapsulate all of them.

The photograph displayed in Figure 4a shows distinct color changes of micellar solutions upon light irradiation. The green micellar solution in which the dye was encapsulated

(left) changed to pink after UV irradiation, and this confirms the conversion of the SP form to the ME form and release of coumarin 102. After irradiation with visible light, the color of the solution changed back to pale green, that is, the ME form reverted to the SP form and coumarin 102 was partially re-encapsulated. Additional evidence for reversible release and re-encapsulation of coumarin 102 by irradiation was provided by the fluorescence microscopy images taken for the three solutions shown in Figure 4a. The image of the initial micellar solution encapsulating coumarin 102 (Figure 4a1) showed the presence of bright fluorescent spots, indicative of the encapsulated dye. After UV irradiation, these spots disappeared due to dye release from the micelles (Figure 4a2). The bright fluorescent spots that reappeared after irradiation with visible
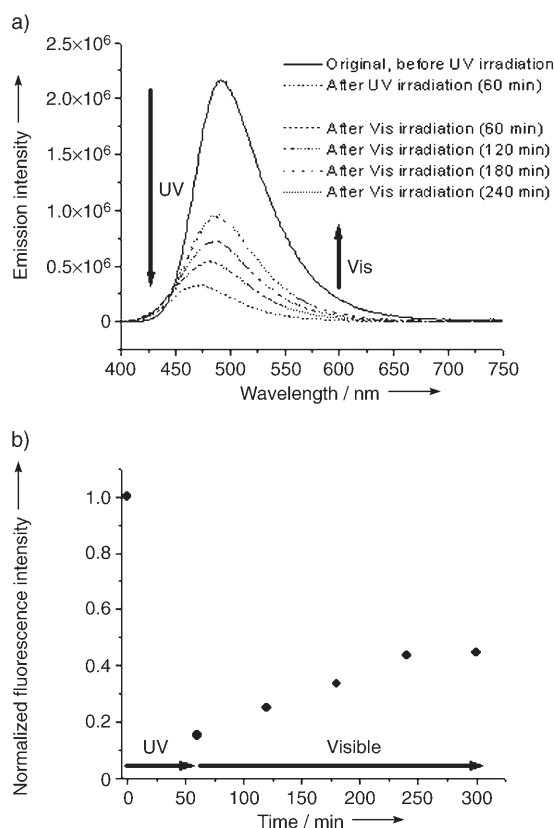
a)

b)

Figure 3. a) Emission spectra of an aqueous micellar solution with encapsulated coumarin 102, coumarin 102 released from disrupted micellar solution after UV irradiation, and regenerated micellar solution with coumarin 102 partially re-encapsulated after irradiation with visible light. b) Normalized fluorescence intensities based on the emission maximum from each curve in spectra.

Figure 4. a) Photographs of solutions containing coumarin 102 and PEO-*b*-SP. a1: Initial solution with coumarin 102 encapsulated inside the PE0-*b*-SP micelles; a2: Solution after UV irradiation, which disrupted the micelles and released coumarin 102; a3: Solution in which micelles were regenerated by irradiation with visible light and coumarin 102 was partially re-encapsulated. a1–a3) Fluorescence microscopy images of solutions shown in (a). b) Schematic representation of encapsulation of a dye within the hydrophobic core of a polymeric micelle on micellization (a1), release of dye on disruption of micellar structure by UV irradiation (a2), and re-encapsulation of a portion of the released dye on regeneration of the micelles by irradiation with visible light (a3).

light (Figure 4a3) confirmed partial re-encapsulation of coumarin 102. The entire process is depicted schematically in Figure 4b.

In summary, we have shown that a PEO-*b*-SP block copolymer undergoes reversible SP/ME photoisomerization which is accompanied by transformation between an amphiphilic and a double-hydrophilic block copolymer. Consequently, polymeric micelles formed from an aqueous solution of PEO-*b*-SP were disrupted by UV irradiation and regenerated by irradiation with visible light. This block-copolymer micelle system was also successfully applied to the efficient encapsulation, release, and partial re-encapsulation of a hydrophobic dye. It was shown that an external light source can reversibly control the morphology of micelles and loading/reloading of a hydrophobic dye.

Experimental Section

Copper(I) bromide (Acros, 99%) was purified as described previously.[24] 4,4′-Bis(5-nonyl)-2,2′-bipyridine (dNbpy; Aldrich, 99%) and anisole (Aldrich, 99%) were used as received.

PEO-*b*-SP: dNbpy (49 mg, 0.12 mmol), SP monomer (1.26 g, 3 mmol), PEO macroinitiator (0.154 g, 0.03 mmol, $M_n = 8.0 \times 10^3$, PDI = 1.04), and anisole (4.0 mL) were added to a 10-mL Schlenk flask equipped with a magnetic stir bar. Oxygen was removed by three freeze–pump–thaw cycles, and CuBr (8.6 mg, 0.06 mmol) was added
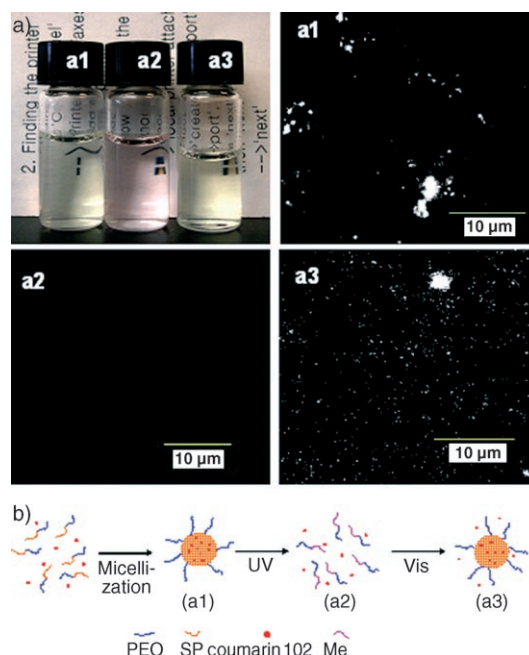
under nitrogen. Polymerization was conducted at 90°C for 20 h. The reaction was stopped by opening the flask to air, and the catalyst was removed by passing the solution through a neutral alumina column. The polymer was precipitated in methanol, filtered, and dried under high vacuum at room temperature for 12 h. M_n(GPC) = 1.24×10^4, PDI = 1.16.

Molecular weight and polydispersity were determined by GPC, conducted with a Waters 515 pump and Waters 2414 differential refractometer with PSS columns and THF as an eluent. Linear poly(methyl methacrylate) standards were used for calibration. ^{1}H NMR spectra were recorded in CDCl$_3$ on a Bruker 300 MHz spectrometer. A high-power mercury-arc lamp (200 W) emitting UV to visible light was used. UV/Vis spectra were recorded on a Cary 50 Bio UV/Vis spectrophotometer. Fluorescence emission spectra were recorded with a SPEX-fluorolog-2 spectrofluorometer. Tapping-mode AFM experiments were carried out with a Multimode Nanoscope III system (Veeco Instruments, Santa Barbara, CA) equipped with a J-type "vertical-engage" scanner.

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