Optically Triggered Dissociation of Kinetically Stabilized Block Copolymer Vesicles in Aqueous Solution

Bin Yan, Jie He, Patrick Ayotte, Yue Zhao*

We demonstrate a strategy for using an optical stimulus to trigger the dissociation of block copolymer (BCP) vesicles in aqueous solution. The BCP, comprising hydrophilic poly(ethylene oxide) (PEO) and a block of poly(methacrylic acid) bearing a number of spiropyran methacrylate comonomer units (P(MAA-co-SPMA)), was allowed to firstly self-assemble into large vesicles in aqueous solution at pH = 3 with protonated carboxylic acid groups, and then become kinetically stable at pH = 8 due to the glassy vesicle membrane of P(MAA-co-SPMA). Fast dissociation of the vesicles was achieved through a cascade of events triggered by UV-induced isomerization from neutral spiropyran to charged merocyanine in the membrane.

Introduction

Research in recent years has established an amphiphilic block copolymer (BCP) design principle for photo-dissociable core–shell micelles or vesicles (polymersomes). These stable self-assembled structures can become thermodynamically unstable in aqueous solution if the photo-reaction of the photochromic moieties in the BCP can shift sufficiently the hydrophilic–hydrophobic balance toward their destabilization. Following this principle, reversible and irreversible photoinduced dissociation of BCP micelles or vesicles have been demonstrated by making use of reversible photoisomerization and irreversible photocleavage, respectively. A distinct feature of some BCP micelles or vesicles is that even under thermodynamically unstable conditions, they can be kinetically stable for quite long due to the crystallization or high glass transition temperature $T_g$ of the micelle core- or vesicle membrane-forming polymer. It is of fundamental interest to develop strategies that allow the use of light to trigger the dissociation of kinetically stabilized BCP micelles or vesicles. In this Communication, we report a study that shows how, through rational BCP design and controlling the vesicle preparation conditions, an optical trigger can induce a cascade of events leading to dissociation of the vesicles formed by a BCP composed of poly(ethylene oxide) (PEO) and a block of poly(methacrylic acid) bearing a number of spiropyran methacrylate comonomer units (P(MAA-co-SPMA)). The spiropyran–merocyanine photoisomerization inside the vesicles membrane and their optically triggered dissociation are directly observable in aqueous solution using an optical microscope. This study is a significant step forward in developing photoresponsive BCP nanocarriers for controlled delivery applications.

Experimental Section

Details on the BCP synthesis using atom transfer radical polymerization (ATRP) and its characterizations are given in Supporting Information.

Results and Discussion

Figure 1 shows the chemical structure of the BCP, a schematic of the vesicle preparation procedure and a typical
TEM image of representative vesicles. The BCP comprises a hydrophilic block of PEO and a block of poly(methacrylic acid) bearing a number of spiropyran methacrylate comonomer units (P(MAA-co-SPMA)), which can be hydrophilic or hydrophobic depending on the pH. To prepare the vesicles, the BCP was dissolved in THF, which is a good solvent for both blocks, at a concentration of 5 mg·mL⁻¹. 50 wt.-% of water at pH = 3 was then added slowly into the THF solution (water/THF, 1/2 in weight) to induce aggregation of the BCP chains. The choice of pH = 3 at this stage is important because it keeps carboxylic acid groups unionized (pKₐ between 5 and 6 for PMAA), making the P(MAA-co-SPMA) block hydrophobic and allowing it to form the vesicle membrane. Afterwards, fourfold of water at pH = 10 (with respect to the volume of the initial solution) was added quickly to quench the solution. After removal of THF by evaporation, a BCP concentration of ca. 0.5 mg·mL⁻¹ was obtained. The final pH of the aqueous solution was ≈8. At this basic pH, carboxylic groups should be ionized, rendering the P(MAA-co-SPMA) block hydrophilic. However, the vesicles were kinetically stable due to a Tₘ of the polymer forming the vesicle membrane. Indeed, a bulk sample of P(MAA-co-SPMA) exhibited a Tₘ ≈ 130 °C (Figure S3). Even though the actual Tₘ of the thin vesicle membrane may vary, it is likely to remain high. Therefore, once the BCP vesicle is formed in acidic solution at pH = 3, it has a glassy and compact membrane that, in basic solution at pH ≈ 8, could prevent water molecules from diffusing inside. Under these conditions, the giant vesicles were stable for 2–3 h, as can be seen from the TEM image in the dry state (Figure S4).

Vesicles of large sizes can be observed with an optical microscope. This is the case with the BCP vesicles prepared under the conditions used in the present study, which made it possible to observe directly their photoinduced dissociation in aqueous solution by means of an optical microscope. We emphasize that all micrographs shown in Figure 2 were recorded with vesicles in solution (not in the dry state). To this end, a vesicle solution (1.5 mL) in a small flask was exposed to UV light (365 nm, 85 mW·cm⁻²) for various cumulative durations under gentle stirring, and after each sequential exposure, a drop of the solution was deposited on a glass slide, followed immediately by microscopic observation. The vesicles had a uniform size prior to UV exposure (Figure 2a). After 1 min irradiation, some vesicles already started to dissociate (Figure 2b). The occurrence of the spiropyran (SP)-to-merocyanine (MC) photoisomerization can easily be noticed from the color change of the vesicles. They turned red as a result of the formation of MC moieties that absorb visible light around 560 and 400 nm, as can be
seen from the UV–Vis spectra also presented in Figure 2. Since the irradiation with UV light was performed from the top of the solution through the open flask, even under gentle stirring for mixing, vesicles close to the surface should absorb more photons and have a greater SP-to-MC isomerization degree than vesicles located deeper in the solution. Those more extensively photo-reacted vesicles are expected to dissolve first. The low resolution available by optical microscopy does not allow one to assess the degree of photoisomerization based on the perceptible differences in color. Over time, all vesicles could eventually reach the threshold degree of photoisomerization required for their dissociation. Indeed, with increasing irradiation time, the UV–Vis spectra show an increased concentrations of MC groups, which is accompanied by the dissociation of an increased number of vesicles in solution. Under these experimental conditions, most vesicles were totally disrupted after 17 min. of irradiation. We note that the vesicles in solution viewed by an optical microscope (Figure 2) appear bigger than those observed by TEM (Figure 1). One reason is that TEM observation was made with vesicles in the dried state while the optical micrograph was taken with vesicles equilibrated with the solution. In the optical microscope image, the thick dark circle line (before irradiation) may be caused by an optical contrast effect, which also contributes to making the vesicles look big in solution.

Some other observations are also worth mentioning. Firstly, both intact and dissolved vesicles tend to aggregate upon photoisomerization. This may arise from electrostatic interactions between the charged, zwitterionic MC moieties at pH ≈ 8.[8] Secondly, despite the photoinduced destruction of the vesicular membrane structure, the BCP is not dissolved quickly, which is normal for entangled polymer chains. The optically destroyed vesicles remain in the form of a loose and hydrated entity. After 17 min irradiation by UV light followed by vigorous stirring in the dark overnight, most of the BCPs was dissolved in the aqueous solution. Thirdly, the photoinduced dissociation process is not reversible. This is understandable given the mechanism at play. Obviously, after UV-induced dissociation in aqueous solution at pH = 8, even if subsequent visible irradiation converts charged MC back to neutral SP, carboxylic acid groups remain ionized and, consequently, no vesicles can be reformed.

In addition to the direct observation of the photoreaction and vesicle dissociation in aqueous solution with an optical micrograph, Figure 2 shows the BCP vesicles in aqueous solution before and after UV irradiation for various durations (image size: 100 μm × 100 μm) and the absorption spectra of the corresponding BCP solution.
microscope for the first time, the present system also demonstrates a pathway to using an optical stimulus to trigger a cascade of events, a phenomenon also found in living systems, and which is fundamentally challenging to design and control in artificial systems. As mentioned above, using this BCP design and these preparation conditions, the vesicles are kinetically stable in aqueous solution at pH = 8 because of the difficulty for water molecules (aqueous hydroxides) to diffuse into the membrane of P(MAA-co-SPMA) and for protons to diffuse out of the membrane. At this state, the applied UV light can trigger a series of sequential events: (i) neutral SP is converted to charged MC which makes the vesicle membrane more soluble in water, (ii) the membrane becomes hydrated as water molecules flow in, (iii) carboxylic acid groups “feel” the basic pH = 8 medium and are ionized, (iv) the P(MAA-co-SPMA) block switches to being water-soluble, and (v) the vesicle membrane is dissolved. This example may help design and understand supramolecular systems that are able to undergo a cascade of events in response to a single stimulus.

We also used pyrene, a fluorescent probe molecule, in order to get more insight into the change in hydrophilicity of the vesicle membranes upon UV irradiation. In this experiment, 50 μL of a THF solution of pyrene (1.5 mg·mL−1) was added in 0.5 mL of an aqueous vesicle solution (0.5 mg·mL−1) at acidic pH under stirring. The vesicle membrane could be plasticized by the presence of THF,[10] which allows the preferential partitioning of pyrene inside the hydrophobic membrane. After removal of THF, the solution was diluted to 0.025 mg·mL−1 and the pH of the solution was adjusted to pH = 8. Figure 3 reports the pyrene fluorescence emission ratio \( I_2/I_3 \), measured at 374 and 384 nm respectively, as a function of irradiation time, while the inset shows the emission spectra of pyrene before and after UV irradiation. The \( I_2/I_3 \) ratio reflects the polarity of the environment in which pyrene molecules are located.[11] Before UV irradiation, the \( I_2/I_3 \) ratio is about 1.3 indicating that pyrene molecules are solubilized by the hydrophobic P(MAA-co-SPMA) membrane.[11c] Upon irradiation, the \( I_2/I_3 \) ratio increases continuously to reach a value of 1.6 after 20 s, which indicates pyrene molecules experience an increasingly polar environment. As compared to the experimental results presented in Figure 2, the photoreaction rate appears to be much faster. This is interpreted as being due to the much diluted vesicle concentration in the fluorescence experiments. Since dissolved vesicles still form loose aggregates, many pyrene molecules should remain in a hydrated polymer environment. This explains why the \( I_2/I_3 \) ratio is smaller than the value expected for pure water, namely \( \approx 1.9. \)[11] This fluorescence probe experiment clearly shows the photoinduced hydration of the vesicle membrane, which brings pyrene molecules to be in contact with water.

**Conclusion**

We demonstrated that through rational BCP design and by controlling the vesicle preparation conditions to render them kinetically stable, an optical signal inducing the switching from neutral SP to charged MC in the vesicle membrane could trigger a cascade of events and lead to dissociation of BCP vesicles. For the first time, the photoreaction inside the giant BCP vesicles and their concomitant dissociation in aqueous solution were directly observed by means of an optical microscope.

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Figure 3. Fluorescence emission ratio \( I_2/I_3 \) of pyrene as a function of UV irradiation time, the inset showing the fluorescence emission spectra \( \lambda_{ex} = 339 \text{ nm} \) before and after UV irradiation \( \lambda_{em} = 365 \text{ nm}, 85 \text{ mW·cm}^{-2} \) of the pyrene-loaded vesicle solution \( 0.025 \text{ mg·mL}^{-1}, 2.5 \text{ mL, pH} = 8 \).
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1. Synthesis of the Block Copolymers

Materials. All reagents were purchased from Aldrich and used without further purification. 2-(3', 3'-dimethyl-6-nitro-3'H-spiro [chromene -2, 2'-inol]-1'-yl)-ethanol (SP) was synthesized using a published method.\textsuperscript{[S1]} Poly(ethylene glycol) methyl ether with number-average molecular weight about 2000 g mol\textsuperscript{-1} was used to prepare poly(ethylene oxide) (PEO) macroinitiator according to a reference.\textsuperscript{[S2]}

Synthesis of PEO-{\it b}-poly(tert-butyl methacrylate) (PEO-{\it b}-PtBMA). PEO macroinitiator (37 mg, 0.0185 mmol), Cu(I)Br (5.6 mg, 0.037 mmol) and tert-butyl methacrylate (tBMA) (0.524 g, 3.7 mmol) were mixed in a 5 mL ampoule equipped with a magnetic stir bar. The whole system was bubbled with nitrogen for 10 min. Then, the ligand, N, N, N', N', N"–pentamethyldiethylentriamine (PMDETA) dissolved in anisole (1 mL) was added by using a syringe. The mixture was degassed three times using a freeze-pump-thaw procedure and filled with nitrogen. The polymerization was conducted at 60 °C for 12 hr. The reaction was stopped by exposing the ampoule to air, and the mixture was passed through a basic alumina column. The polymer was precipitated in pentane and filtered. The purification procedure by dissolving-precipitation was repeated three times and the product was dried under high vacuum at room temperature for 12 h. $M_n$ (GPC) = 34,000, PDI = 1.12 (see Figure S1). The sample was characterized by $^1$H NMR, and based on the molecular weight of PEO, the block copolymer composition obtained was PEO$_{45}$-{\it b}-PtBMA$_{184}$, where the subscripts indicate the numbers of the EO and tBMA units.

Preparation of PEO-{\it b}-Poly(methacrylic acid) (PEO-{\it b}-PMAA).\textsuperscript{[S3]} Trifluoroacetic acid (0.9 mL) was added in a dichloromethane solution of the diblock copolymer (300 mg in 3 mL). The reaction solution was stirred at room temperature overnight for the hydrolysis reaction. The solvent was carefully removed by evaporation; the residue was then redissolved in methanol and precipitated in cold ethyl ether. The product was collected by filtration and dried in vacuum at 45 °C for 24 hr. Complete hydrolysis of tBMA was confirmed by $^1$H NMR. This gave rise to the block copolymer PEO$_{45}$-{\it b}-PMAA$_{184}$.

Preparation of spiropyran (SP)-containing block copolymer. PEO$_{45}$-{\it b}-PMAA$_{184}$ (300 mg, 0.0190 mmol) and SP (1.23 g, 3.50 mmol) was dissolved in anhydrous DMSO (10 mL). After the solution became homogenous upon stirring, a solution of 4-dimethylaminopridine (DMAP) (0.576 g, 0.52 mmol) and dicyclohexylcarbodiimide (DCC) (1.26 g, 6.24 mmol) in DMSO (5 mL) was added. The mixture was stirred at room temperature for 48 hr. After filtration, the solvent was eliminated under reduced pressure. The residue was dissolved in THF, and the solution was filtrated before being poured into methanol. The precipitated product was collected by filtration and the whole procedure was repeated twice. The product was dried in vacuum at room temperature for 2 days. $M_n$ (GPC) = 52,800, PDI = 1.75 (see Figure S1). The relative broad dispersity is due to the strong interaction between PMAA and the polystyrene column and the low solubility of PMAA in THF. From $^1$H NMR spectrum (see Figure S2), the block copolymer composition was estimated to be PEO$_{45}$-{\it b}-P(MAA$_{146}$-co-SPMA$_{38}$).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1}
\caption{GPC curves of the PEO macroinitiator, the block copolymers PEO$_{45}$-{\it b}-PtBMA$_{184}$ and PEO$_{45}$-{\it b}-P(MAA$_{146}$-co-SPMA$_{38}$).}
\end{figure}
2. Characterizations

Gel permeation chromatography (GPC) measurements were performed using a Waters system equipped with a refractive index and a photodiode array detector. THF was used as eluent (elution rate, 1 mL/min) and polystyrene standards used for calibration. The GPC curves of the macroinitiator and the diblock copolymers were shown in Figure S1. \(^1\)H NMR spectra were obtained with a Bruker Spectrometer (300 MHz, AC 300). The \(^1\)H NMR spectra of PEO\(_{45}\)-b-PtBMA\(_{184}\) and PEO\(_{45}\)-b-P(MAA\(_{146}\)-co-SPMA\(_{38}\)) were shown in Figure S2. The units of tBMA in PEO\(_{45}\)-b-PtBMA\(_{184}\) were calculated from the signal intensity ratio between the proton of O-CH\(_2\)-CH\(_2\) at 3.9 and that of tertiary butyl at 1.4 ppm, while the units of SPMA in PEO\(_{45}\)-b-P(MAA\(_{146}\)-co-SPMA\(_{38}\)) was calculated from the intensity ratio between the proton of O-CH\(_2\)-CH\(_2\) at 3.9 and that of –O-CH\(_2\)-CH\(_2\)-N- at 4.1 ppm. UV-vis absorption spectra were recorded on a Varian Cary-50 spectrophotometer. A TA DSC Q200 differential scanning calorimeter (DSC) was used to investigate the phase transition behaviors, using indium as the calibration standard and a heating or cooling rate of 10 °C/min. Glass transition temperature (T\(_g\)) was taken as the midpoint of the change in heat capacity. The DSC heating and cooling curves (second scan) of PEO\(_{45}\)-b-P(MAA\(_{146}\)-co-SPMA\(_{38}\)) was shown in Figure S3. A high-temperature T\(_g\) around 130 °C could be noticed for this block copolymer. Optical microscopic observations were conducted on a Leitz DMR-P microscopy.

![Figure S2](image)

Figure S2. \(^1\)H NMR spectra of the block copolymers (a) PEO\(_{45}\)-b-PtBMA\(_{184}\) and (b) PEO\(_{45}\)-b-P(MAA\(_{146}\)-co-SPMA\(_{38}\)).

![Figure S3](image)

Figure S3. DSC heating and cooling curves (10 °C/min) for PEO\(_{45}\)-b-P(MAA\(_{146}\)-co-SPMA\(_{38}\)).

3. Preparation of Block Copolymer Vesicles

PEO\(_{45}\)-b-P(MAA\(_{146}\)-co-SPMA\(_{38}\)) was dissolved in THF at a concentration of 0.5 wt%. After stirring overnight, water (pH = 3, HCl) was added dropwise at a rate of 0.015 mL/min to yield the vesicle solution (water/THF, ½ in weight). The acidic water was used in order to keep all the carboxylate acid groups (pKa ~ 5-6)\(^4\) in the protonated state so that the P(MAA\(_{146}\)-co-SPMA\(_{38}\)) block was kept hydrophobic. Afterwards, the vesicle solution was quenched by adding 4-fold water (pH = 10, NaOH) to freeze the vesicles. THF was eliminated by evaporation under reduced pressure at room temperature.
The concentration of the solution was around 0.5 mg/mL. The final pH of the aqueous solution was ca. 8, which was measured by using a pH-meter (Orion 410 A+, Thermo Electron Corporation).

4. Stability of Block Copolymer Vesicles

From Figure S4, it can be seen that without UV irradiation, the BCP vesicles could be stable for 2-3 h under stirring. After 4.5 h, some vesicles started to dissolve. Because of the glassy membrane of the vesicles (kinetically stabilized), it is difficult for aqueous hydroxide to diffuse in, which explains the 2-3 h stability. As the time goes on, the slow diffusion of aqueous hydroxide begins to ionize the carboxylate acid group and the membranes become increasingly hydrophilic and get dissolved.

5. Effect of Photoisomerization on Block Copolymer Vesicles

Figure S5 shows that after UV–induced dissociation and aggregation of BCP vesicles, irradiation of the solution with visible light (400-500 nm, 35.6mW/cm²) could partially convert charged MC back to neutral SP moieties (from absorption spectra and color change). This had as effect the re-dispersion of some aggregates due to diminished number of charges.
6. Loading and Release of Pyrene
50 µL of the pyrene stock solution in THF (1.5 mg/mL) was added to an empty vial. To this vial was added 2 mL of the stock block copolymer vesicle solution (0.5 mg/mL) in acidic water. This solution was stirred vigorously at room temperature for 1 hr. After removal of THF, the vesicle solution was diluted to 0.025 mg/mL and the final pH of the system was adjusted to 8. To investigate the change in fluorescence emission of pyrene (λ<sub>ex</sub>=339 nm) upon UV light irradiation of the vesicle solution, 2.5 mL of the solution was placed in a standard cuvette and exposed to UV light (365 nm, intensity: 85 mw/cm<sup>2</sup>) for a chosen time. After turning off the irradiation, the solution in the cuvette was used to record the fluorescence emission spectrum.