Photo/pH dual-responsive behavior of azopyridine-containing copolymer vesicles

Jia Wei, Zeng Yan, Li Lin, Junsu Gu, Ze Feng, Yanlei Yu *  

Department of Material Science, State Key Laboratory of Molecular Engineering of Polymers, Fudan University, 220 Handan Road, Shanghai 200433, PR China

Article info

Article history:  
Received 7 February 2013  
Received in revised form 26 April 2013  
Accepted 15 May 2013  
Available online 23 May 2013

Keywords:  
Azopyridine  
Diblock copolymer  
Dual-responsive  
Self-assembly  
Vesicles

Abstract

The amphiphilic diblock copolymer composed of poly(ethylene oxide) as the hydrophilic chain and polymethacrylate with azopyridine moieties in the side groups as the hydrophobic chain self-assembled to vesicles in the mixture of THF/water. The dual photo- and pH-responsive behavior of the copolymeric vesicles was investigated by UV–Vis absorption spectra and optical microscope. It was found that the trans–cis photoisomerization of azopyridine in the vesicles was influenced by the water content in the mixture and the intensity of light irradiation. Regard to the pH-responsive behavior, the copolymer vesicles dissociated into solution gradually triggered by acid, while they reformed in a basic environment. Furthermore, the UV light irradiation accelerated the process of pH-induced dissociation of the vesicles.

1. Introduction

Amphiphilic block copolymers are able to self-assemble in aqueous solution into well-defined nanoscale and microscale colloidal aggregates such as different types of micelles or vesicles [1–3]. More attention in the vesicles made of the amphiphilic block copolymers is currently attracted to prepare the stimuli-responsive vesicles which have the potential application in medicine, pharmacy, and biotechnology [4–6]. Much effort has been directed toward the development of the intelligent vesicle responding to stimuli like temperature, pH, ionic strength, and light [7,8]. Compared to those investigations of single-stimuli responsive polymers, dual or multi responsive systems, resembling the living systems in their complex behavior as a response to the applied stimuli, still represent a significant challenge [9]. The development of these smart materials would possess more variable to be manipulated in designing the controlled release of active agents entrapped in vesicles.

Until now the most studies about the dual stimuli responsive behavior of the polymeric assemblies are the combination of the temperature and pH [10–16]. For the preparation of these block or grafted polymer, two different components are generally needed to be involved, that is thermo-responsive segments and pH-responsive moieties. For example, benefited from the thermo-sensitivity of poly(N-isopropylacrylamide) (PNIPAm) and the pH-sensitivity of poly(N,N-(dimethyl amino)ethyl methacrylate) (PDMAEMA), the amphiphilic star block copolymer PMMA-b-P(NIPAm-co-DMAEMA) undergoes thermo/pH dual-response [11].

In the recent years, self-assembly of photo-responsive amphiphilic copolymers has been widely investigated [17] since light has the advantages of long-distance control and being conveniently controllable, in which much work concern the azobenzene-containing polymers based on the trans–cis photoisomerization in response to UV and visible light [18–24]. At the same time, the light responsiveness of azobenzene has been extended to the area of dual stimuli responsive system, in which another stimulus could be temperature or pH. There have been several reports on temperature/photo dual-responsive copolymer which synthesized by copolymerization of the azobenzene-containing monomers and polyacrylamide derivate [25–27]. However, the study on photo/pH dual-responsive copolymer is insufficient relatively, which can be realized by involving azobenzene-containing monomers and polyacids/polybases [28]. Agarwal et al. found that the azobenzene-containing block copolymer poly(ethylene oxide)-b-poly(2-(diethylamino)ethyl methacrylate-co-6-(4-phenylazo phenoxo)hexyl methacrylate) [PEO-b-P(DEAEMA-co-PPHMA)] underwent reversible morphological transition from micelles to vesicles in aqueous solution induced by the multistimuli, because of the pH- and photo-responsiveness of DEAEMA and 6-(4-phenylazo phenoxo) hexyl methacrylate (PPHMA) units, respectively [28].

Recently, we prepared a new amphiphilic diblock copolymer (PEO-b-PAP) composed of a hydrophilic poly(ethylene oxide) (PEO) and a hydrophobic polymethacrylate with azopyridine (PAP) moieties in side groups [29]. In this system, the azopyridine...
moieties render copolymer with dual responsiveness of light and pH simultaneously. It was found the copolymeric vesicles showed photoinduced circular morphological transition process including fusion, damage and defect formation, disruption, disintegration and rearrangement in THF/water, which mimics some biological processes [30]. In this research, we are very interested in the dual stimuli-responsive behavior of these azopyridine-containing copolymer vesicles, especially the pH-responsive property is focused.

2. Experimental section

2.1. Synthesis of PEO-b-PAP

PEO-b-PAP was synthesized via the atom transfer radical polymerization (ATRP) of azopyridine monomer, 6-[(4-(4-pyridylazo)phenyloxy)hexyl methacrylate] (AP), using PEO-Br as macroinitiators (Fig. 1a) [29]. $M_n$(SEC) = 2.2 x 10^4, $M_w$/Mn = 1.25, $M_n$(NMR) = 1.5 x 10^4, which is calculated by the ratio of the integration value of CH$_2$CH$_2$O in PEO segment to the pyridine protons of azopyridine at meso-position to N-N of PAP segment from $^1$H NMR spectrum (Fig. 1b). Accordingly, the mass ratio of the PEO to PAP is about 1:5.5.

2.2. Preparation of PEO-b-PAP vesicles

PEO-b-PAP was dissolved in THF to obtain a solution with an initial concentration range from 1.0 to 6.0 mg mL$^{-1}$. The solution was stirred overnight at room temperature. To get the self-assembly aggregates, the required amount of deionized water was added into the THF solution at a rate of 5 mL L$^{-1}$ s$^{-1}$ with a durative stirring.

To obtain the vesicles encapsulated of Nile Red, PEO-b-PAP (1 mg) and Nile Red (0.05 mg) was dissolved into THF (1 mL). With a durative stirring, water was added at a rate of 5 mL s$^{-1}$ until the water content reached 50 vol.% to induce the vesicles formation and the simultaneous encapsulation of Nile Red. Finally, the THF was evaporated to get the aqueous vesicles solution and water was added to dilute the solution to 3 mL.

2.3. Measurements

$^1$H NMR spectra were recorded on a Bruker DMX-500 MHz spectrometer with TMS as the internal standard. Size exclusion chromatograph (SEC) measurements were performed on Shimadzu LC-10ADvp using THF as eluent (0.8 mL/min) with polystyrene as calibration. The self-assembly morphology was observed by the cryo-scanning electron microscope (cryo-SEM, Hitachi S-4800). Photo- and pH-responsive behaviors of the copolymeric vesicles in solution were observed with an optical microscope (OLYMPUS BH-2) and the images were recorded with a digital camera (OLYMPUS SC5050ZOOM). To investigate the photo-responsive process of the vesicles, the vesicle solution (2 mL) was placed in a quartz cuvette with a stopper and irradiated with a UV-LED irradiator (OMRON ZUV-C30H) with a wavelength of 365 nm under the stirring. Once the UV light was turned off, the vesicle solution was immediately transferred into a glass cell (17 μm x 6 μm x 200 μm) for the optical microscopic observations. UV-Vis absorption spectra were recorded on a JASCO V650 spectrometer with 1 cm quartz cell. And the turbidity measurement was carried out by UV–Vis Spectrophotometer (TU-1800), according to the method of the literature reported [31]. The Nile Red release experiment was detected by the fluorescence spectra (Shimadz RF-5301PC Fluorescence spectrometer).

3. Results and discussion

3.1. The influence of water content and irradiation intensity on the photo-responsive behavior of the PEO-b-PAP

The amphiphilic diblock copolymer PEO-b-PAP, composed of hydrophilic PEO and hydrophobic PAP, self-assembled to vesicles...
in the mixture of THF/water [29]. The typical morphology of the vesicles was observed by cryo-SEM as shown in Fig. 2, the sizes of the vesicles are about 100–600 nm. The hydrophobic azopyridine groups generate compact packing in the membranes of the vesicles due to the non-covalent acromatic–acromatic interaction. When the vesicles are irradiated with UV light, the azopyridine groups isomerize from the rod-like trans form to the bent cis form, which disrupts the original relatively tight packing and brings a loose stacking formation in the vesicular membrane. In our previous work, it was found that the copolymeric vesicles showed morphological transition induced by trans–cis photoisomerization of the azopyridine groups in THF/water with fixed water content during the irradiation of UV light [29]. Herein, we revisit the photoresponsive behavior of the PEO-b-PAP vesicles focusing on the two influencing factors: the water content in the solution and the UV-light intensity.

To investigate the influence of water content on the photoresponsive behavior, the vesicles in H2O/THF with different water contents were prepared. The content of cis-isomer was used to represent the degree of photoisomerization, which could be calculated from UV–Vis absorption spectra using the method reported by literature [32]. Under the fixed UV light intensity of 36 mW/cm², the cis-isomer content increases with the irradiation time and reaches photostationary state finally (Fig. 3a), as the water content in these systems is 50, 70, and 90 vol.% respectively. However, the content of cis-isomer at the photostationary state decreases with the increase of the water content, which indicates the degree of the photoisomerization is lower with higher water content. This is because water, that restricts the movement of the hydrophobic block chain PAP, makes the chain frozen and inhibits the trans–cis photoisomerization of the azopyridine moieties.

On the other hand, the effect of the UV-light intensity on the trans–cis photoisomerization behavior of the azopyridine moieties was investigated with the fixed water content (70 vol.%). It was found that under different intensity of the UV light irradiation, the content of cis-isomer varies with the same irradiation time before the photostationary state is obtained. Fig. 3b shows that although the UV-light intensity is different, when the system reaches the photostationary state, the content of cis-isomer is almost the same, retaining about 70%. It illustrates that the thermodynamic equilibrium state does not depend on the UV-light irradiation intensity, while the time to make the system getting to the thermodynamic equilibrium state is highly dependent on the intensity of the irradiation because higher content of cis-isomer is induced by higher UV-light intensity with the same irradiation time.

In order to demonstrate potential of the vesicles as photo-triggered carrier, Nile Red was chosen to be as the fluorescence probe to study their release behavior under the UV irradiation. The fluorescence spectra of the PEO-b-PAP vesicles solution encapsulated with Nile Red under different irradiation time from 0 to 100 s are shown in Fig. 4. There is a characteristic peak of Nile Red at 600 nm on the fluorescence spectra at 0 s, showing Nile Red has been entrapped in the hydrophobic chains of the PEO-b-PAP. A significant decrease in the fluorescence intensity over time was obtained when the solution of loaded vesicles was exposed to UV. It is because under the UV light irradiation, the copolymer vesicles experience photo-induced circular process, including damage, defect formation, disruption, disintegration [29], all of which induce the release of Nile Red. As the irradiation time goes by, the peak at 600 nm becomes weaker until the time gets to 70 s, when the system reaches the photostationary state and the release of Nile

![Fig. 2. The cryo-SEM images of the PEO-b-PAP vesicles. The initial polymer concentration is 6.0 mg mL⁻¹ and the solvent is H₂O/THF with 50 vol.% water content.](image_url)

![Fig. 3. The content of cis-isomer of azopyridine groups vs the UV-irradiation (365 nm) time: (a) water content of the solution is 50, 70, and 90 vol.% with fixed irradiation intensity of 36 mW cm⁻²; and (b) the irradiation intensity is 18, 36, and 72 mW cm⁻², with the water content of 70 vol.%. The concentration of the copolymer solution is 0.4 mg mL⁻¹.](image_url)
chloric acid. In the neutral environment, the uniform vesicles were
was observed by the optical micrographs as shown in Fig. 5. Of the vesicles solution, the different morphology of the vesicles
was possible to dissolve/disintegrate the vesicle based on the hydro-
phobic as the pH value increases owing to deprotonation. Thus, it
mer that is more hydrophilic at low pH values owing to
protonation of the azopyridine groups. With the pH change
phobicity–hydrophilicity change as a response to the protonation
is a kind of Lewis base which has the ability to accept pro-
groups are one of the most studied stimuli-sensitive systems because of
the presence of pH variations within the body[4]. The azopyridine
The fluorescence spectra of the PEO-b-PAP vesicles solution encapsulated
with Nile Red under the UV-light irradiation (365 nm, 50 mW cm
Red ceases. Therefore, the light irradiation can serve to control the release process.

3.2. pH-Responsive behavior of the PEO-b-PAP

The pH-responsive systems have attracted great attention and are one of the most studied stimuli-sensitive systems because of
the presence of pH variations within the body[4]. The azopyridine
group is a kind of Lewis base which has the ability to accept pro-
tons. As a result, PEO-b-PAP is a pH-responsive amphiphilic poly-
mer that is more hydrophilic at low pH values owing to
protonation of the azopyridine groups and becomes more hydro-
phobic as the pH value increases owing to deprotonation. Thus, it
is possible to dissolve/disintegrate the vesicle based on the hydro-
phobicity–hydrophilicity change as a response to the protonation or deprotonation of the azopyridine groups. With the pH change
of the vesicles solution, the different morphology of the vesicles was observed by the optical micrographs as shown in Fig. 5. Fig. 5a gives the morphology of the vesicles before adding hydro-
chloric acid. In the neutral environment, the uniform vesicles were
presented as the hydrophobic azopyridine groups generate comp-
act packing in the bilayer membranes of the vesicles due to the
strong non-covalent aromatic-aromatic interaction. When the hydrochloric acid was added to make the solution in acid environ-
ment, there was no vesicle observed in Fig. 5b. However, with add-
ing the equivalent amount of sodium hydroxide solution into the
solution, the vesicles reformed again as shown in Fig. 5c. This phe-
nomenon is because the azopyridine groups are protonated with
the addition of hydrochloric acid and the hydrophobicity of PAP
block decreases, leading to the dissociation of the vesicles. Never-
theless, after the sodium hydroxide solution was added, the proton-
tated azopyridine groups deprotonated and the vesicles reformed.

As the degree of the protonated azopyridine groups depends on the amount of the acid used, the details of the acid-induced
dissociation behavior of the vesicles were investigated further as
shown in Fig. 6. Initially, the morphology of the vesicles was well maintained under the neutral solution (Fig. 6a). When 10 µL hydrochloric acid (0.5 mol L
was added to the 2 mL initial solution, the defects and folds appeared in the membrane of the vesicles (Fig. 6b). Because that the protonated azopyridine groups make the PAP blocks less hydrophobic, the protonated segments tend to solute into the solution and the stability of the membrane is damaged and the folds come out. When more acid was added (20 µL), the edge of the vesicles became obscure (Fig. 6c). As the acid amount increased to 25 µL, almost all vesicles disappeared, which means the vesicles cannot obtained from the totally protonated PEO-b-PAP in the THF/water mixture with 50 vol.% water.

With the addition of acid or base, the effect of pH on the turbid-
ity of the vesicles solution of the PEO-b-PAP is shown in Fig. 7. With the pH decreasing, the turbidity of the solution decreased drastically, showing that the vesicles begin to dissociate. When the pH reached to 2.5, the percentage of the protonation came to 50%, thus the solution was clear and turbidity went to 0.18, show-
ing the vesicles are dissociated totally. This phenomenon was also proved by the change of the protonation percentage of the azopyri-
dine chromophores: the higher the protonation percentage is, the lower the turbidity is. In other words, pH values determine the

![Fig. 4. The fluorescence spectra of the PEO-b-PAP vesicles solution encapsulated with Nile Red under the UV-light irradiation (365 nm, 50 mW cm
2) with different irradiation time from 0 to 100 s.](image)

![Fig. 5. Optical micrographs of pH stimuli-responsive behavior of the PEO-b-PAP vesicles in H2O/THF solution with 50 vol.% water content: (a) the morphologies of vesicles before adding hydrochloric acid, (b) after adding hydrochloric acid (0.5 mol L
1), 25 µL) and then (c) with sodium hydroxide solution (0.5 mol L
1, 25 µL) added. The initial copolymer concentration was 6 mg mL
1 and the volume of vesicle solution was 2 mL.](image)

![Fig. 6. Optical micrographs of acid-induced dissociation of the PEO-b-PAP vesicles in H2O/THF solution with 50 vol.% water content: (a) the morphologies of vesicles before adding hydrochloric acid, (b) after adding 10 µL, (c) 20 µL, and (d) 25 µL of hydrochloric acid (0.5 mol L
1). The initial copolymer concentration was 6 mg mL
1 and the volume of vesicle solution was 2 mL.](image)
induced by the reversible protonation behavior of the azopyridine
the hydrophilic-hydrophobic balance of the block copolymer
disruption and reformation process is explained by the change in
solution. The reversibility was not reduced after 10 cycles. This
xyl solutions alternately causes the turbidity change of the vesicles
demonstrates the addition of hydrochloric acid and sodium hydro-
change of turbidity of the PEO-
properties and the relationship between these two stimuli was also
investigated here. With the addition of the hydrochloric acid, the
degree of the protonation, and the latter one determines that the
PAP segments perform as hydrophilic or hydrophobic ones.
Moreover, with the addition of the base into the solution men-
tioned above to neutralize the hydronium, the deprotonation of the
azopyridine chromophore happened. Simultaneously the percent-
age of the protonation decreased, implying that the amphiphility
of the block copolymer was recovered. With the increasing of pH,
the turbidity of solutions increased. It confirms that the PEO-b-
PAP could reform aggregates through the self-assembly progress.
This pH-responsive behavior showed excellent reversibility. Fig. 8
demonstrates the addition of hydrochloric acid and sodium hydro-
xyl solutions alternately causes the turbidity change of the vesicles
solution. The reversibility was not reduced after 10 cycles. This
disruption and reformation process is explained by the change in
the hydrophilic-hydrophobic balance of the block copolymer
induced by the reversible protonation behavior of the azopyridine
chromophores.

3.3. The effect of UV-irradiation on pH-induced dissociation

The PEO-b-PAP vesicles possess photo/pH dual-responsive
properties and the relationship between these two stimuli was also
investigated here. With the addition of the hydrochloric acid, the
change of turbidity of the PEO-b-PAP vesicles solution with and
without the irradiation of under UV light is shown in Fig. 9. Com-
paring the two curves, it is found the turbidity of the irradiated
solution decrease faster with the added acid than that of the
non-irradiated solution, which means pH-induced dissociation of
aggregates is accelerated by the UV light irradiation. Furthermore,
the totally volume of acid added to dissociate all the aggregates
with the UV light irradiation was less than that without light irra-
diation. The addition of acid led to the enhancement of the hydro-
phillicity of the copolymer chains as a result of the protonation of
the azopyridine chromophore, at the same time, the aggregates
dissociated gradually and the turbidity of solutions decreased. If
this process was companied with the photoisomerization of azo-
pyridine groups induced by the UV light irradiation, the hydrophi-
llicity of the polymer chains was enhanced both by the
photoisomerization and the protonation of the azopyridine groups.
Thus the UV light irradiation can accelerate the process of pH-in-
duced dissociation.

4. Conclusion

We have introduced the dual pH- and photo-responsive
copolymer vesicles which were induced by the protonation or
deprotonation, and the photoisomerization of the azopyridine
groups respectively. Under UV irradiation, the trans–cis photoiso-
ermerization of azopyridine in vesicle is suppressed by water and
facilitated by higher irradiation intensity. It is also found the UV
light induces release of the encapsulated Red Nile from the vesi-
cles, indicating these vesicles are promising as photo-triggered
carrier.

On the other side, with the decrease of the pH value of solution,
the protonation of azopyridine enhances the hydrophilicity of
diblock and make the vesicles dissociated. It is noted that this pro-
cess could be accelerated by the UV light irradiation which
induces the trans–cis transition and improves the hydrophilicity of
diblock. Utilizing this synergistic effect, the speed of dissociation
induced by acid can be adjusted by light. Conversely, the addition
of base brings the recovery of hydrophobicity of the PAP block
due to the deprotonation of the azopyridine groups and therefore the
vesicles reform. The reversible dissociation and reformation of
the vesicles of the PEO-b-PAP were controlled through the addition
of the acid or base alternately without obvious reduction through
several cycles.

This photo/pH-responsive amphiphilic diblock copolymer is a
promising multifunctional system, in particular in the therapeutic
applications acting as drug carriers. The dual-responsive property
makes them release encapsulated substance more controllably
and suitable to more applications.
Acknowledgements

Financial supports from National Natural Science Foundation (51225304, 21273048, 21134003, and 51203023), National Research Fund for Fundamental Key Projects (2009CB930400), Shanghai Rising-Star Program (11QH1400400), and Shanghai Natural Science Foundation (12ZR1401600) are greatly acknowledged.

References