J|A|C|S COMMUNICATIONS

Published on Web 12/18/2009

# Dual Redox Responsive Assemblies Formed from Diselenide Block Copolymers

Ning Ma, Ying Li, Huaping Xu,* Zhiqiang Wang, and Xi Zhang*

*Key Lab of Organic Optoelectronics and Molecular Engineering, Department of Chemistry, Tsinghua University, Beijing 100084, People's Republic of China*

Received September 29, 2009; E-mail: xi@mail.tsinghua.edu.cn; xuhuaping@mail.tsinghua.edu.cn

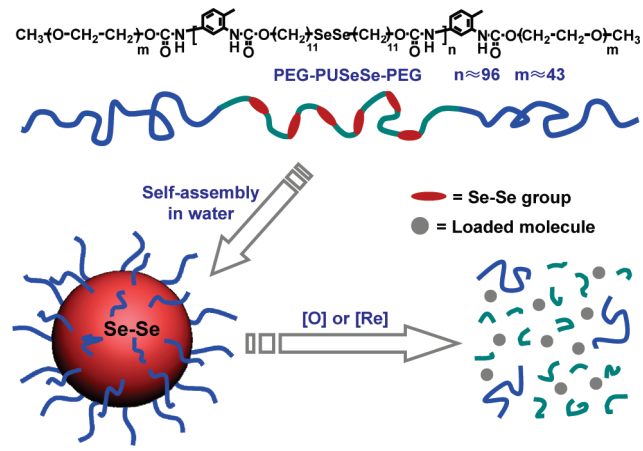


Stimuli-responsive block copolymers are defined as copolymers whose blocks can undergo relatively large and abrupt, physical or chemical changes in response to small external stimuli. Generally, slight changes in the structures and functions of block copolymer aggregates can result in the release of an incorporated species, which has great potential in the fields of controllable drug transport and gene delivery.[1] It has been proven that the aggregation behaviors of amphiphiles and amphiphilic polymers in aqueous media are determined to a great extent by their chemical composition, e.g. the ratio of the hydrophobic part to the hydrophilic part.[2] This property enables precise control of the aggregating structures of stimuli-responsive polymers by altering their chemical composition via external stimuli such as pH,[3] light,[4] temperature,[5] oxidation,[6] and reduction.[7] Among these functionalities, redox responsive polymers have attracted wide interest for their promising applications in controllable encapsulation and delivery in physiological environments, where the redox process is constantly and widely present. In terms of the utility of multiresponsive assemblies for the programmed release of functional species in different environments, however, it remains important to develop new types of block copolymer aggregates that are responsive to both oxidants and reductants (i.e., those that show dual redox responses) under mild conditions.

Selenium-containing compounds have been widely used in the pharmacochemistry as antioxidants for the well-known glutathione peroxidase (GPx) activity,[8] among which diselenide is a promising candidate for a dual redox response due to its good activity in the presence of either oxidants or reductants. Normally, Se−Se bonds are cleaved and oxidized to seleninic acid in the presence of oxidants and reduced to selenol in a reducing environment.[9] To achieve dual redox responsiveness, a block copolymer with one water-insoluble diselenide-containing block and two water-soluble polyethylene glycol (PEG) blocks was designed and synthesized and its self-assembly behavior in water was studied. It was expected that the Se−Se bonds would undergo a structural dissociation, inducing the disassembly of the aggregates in the presence of oxidants or reductants, as shown in Scheme 1.

To date, only a few successful examples have been shown for synthesis of diselenide-containing polymers by condensing organic diselenocyanates or diselenosulfates with an acid or base initiator.[10] These systems generally involved a low solubility of the polymer. In this study, the diselenide group was first introduced into a diol structure, which possessed the desirable solubility. The diselenide-containing polyurethane (PUSeSe) blocks were then synthesized via polymerization of toluene diisocyanate (TDI) in slight excess with diselenide-containing diols and finally terminated by PEG monomethyl ether. Thus, an ABA-type diselenide-containing triblock copolymer with good solubility in common solvents was obtained, denoted by PEG-PUSeSe-PEG ($M_w$ 6.85 × $10^4$ g/mol by $^1$H NMR. For details of the synthesis and characterization, see Supporting Information).

***Scheme 1.*** Structure of PEG-PUSeSe-PEG and Schematic of the Redox Responsive Disassembly of PEG-PUSeSe-PEG Micelles




PEG-PUSeSe-PEG is a typical amphiphilic block copolymer that can self-assemble in an aqueous environment. The critical aggregate concentration (CAC) of PEG-PUSeSe-PEG was ~3.1 × $10^{-4}$ mg/mL, measured by the fluorescent method, using pyrene as the probe. The size of the aggregates was determined by dynamic light scattering (DLS) to be of an ~76 nm average diameter. To further investigate the specific structure of the PEG-PUSeSe-PEG aggregates, cryo-transmission electron microscopy (cryo-TEM) was used to observe the aggregates. The results in Figure 1a clearly show that the PEG-PUSeSe-PEG block copolymer has an aggregating structure with a solid core in aqueous solution, which is generally considered to be a micellar structure. It is worth noting that these micelles were quite stable in an ambient environment, indicating that the active Se−Se groups were buried in the cores of the micelles and that the oxidation of Se−Se groups by oxygen had been greatly inhibited. Therefore, the micellar structures were stable for more than 1 month (Supporting Information, Figure S3).

$H_2O_2$ was chosen as an oxidant to investigate the cleavage of the Se−Se bonds in the aggregates and the consequent disassembly of the PEG-PUSeSe-PEG micelles. This was added into an aqueous solution of PEG-PUSeSe-PEG aggregates to investigate the oxidation responsive behavior of the aggregates. As shown in Figure S3, the micellar structure of PEG-PUSeSe-PEG was converted into irregular aggregates after 2 h of oxidation and these were subsequently decomposed into tiny aggregates with sizes of several nanometers 3 h later, indicating that the oxidation stimulus had indeed induced the cleavage of the PEG-PUSeSe-PEG micelles. Cryo-TEM observation also indicated that no block copolymer aggregates existed. Therefore, the Se−Se bonds in the PEG-



10.1021/ja908124g 

PUSeSe-PEG micelles could undergo cleavage in the presence of peroxides, which resulted in oxidation responsive disassembly. It is interesting to note that the micelles of PEG-PUSeSe-PEG are not only oxidation responsive but also reduction responsive. Because the Se−Se bonds were quite sensitive and underwent a cleavage in the presence of reductants such as thiols,[9] reduced glutathione (GSH) was used as a reductant to detect the reductant responsiveness of the above aggregates. Similar results were obtained when GSH was added to a final concentration of 0.1 mg/mL: the micelles became irregular and, subsequently, tiny aggregates formed during the process of reduction, as shown by TEM and cryo-TEM observations (Supporting Information, Figure S5). Both the oxidation and reduction procedures were shown to have the capability to destroy Se−Se bonds so that the redox responsiveness of the Se-containing block copolymer aggregates was realized. It was therefore confirmed that the introduction of diselenide groups into the polymer backbone can endow the formed micelles with dual redox responsiveness.

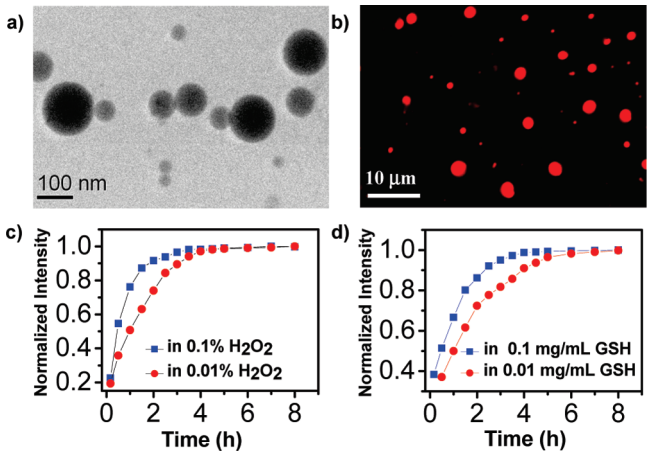



***Figure 1.*** (a) Cryo-TEM image of PEG-PUSeSe-PEG micelles. (b) FM image of RB-loaded micelles, and the release behavior of RB in (c) $H_2O_2$ and (d) GSH.

As demonstrated, this kind of dual redox responsive block copolymer micelles can be employed to incorporate and release small molecules. Taking fluorescent Rhodamine B (RB) as a model, RB-loaded PEG-PUSeSe-PEG micelles were prepared to perform fluorescence microscopy (FM) and controlled release experiments. The micellar solution was dialyzed against the deionized water until the water outside the dialysis tube exhibited negligible fluorescence emission of RB. This dialysis not only guaranteed the removal of the unentrapped RB molecules but also indicated that the RB-loaded micelles were quite stable in aqueous solution before the introduction of the redox stimuli. As shown in Figure 1b, the RB-loaded PEG-PUSeSe-PEG micelles were found as spherical aggregates on the substrate, while the aggregates disappeared upon the addition of 0.1% $H_2O_2$ solution, indicating disassembly of the micelles and the release of RB from the aggregates. Similarly, collapse of the micelles was also observed after the addition of GSH. To understand the release behavior of the RB molecules upon the addition of oxidant and reductant, a solution of RB-loaded micelles was kept in a dialysis tube and then placed into a solution of $H_2O_2$ or GSH at a certain concentration. Thus, the release of RB was monitored by the increasing fluorescence of the solution outside the dialysis tube. From the curves in Figure 1c and 1d, an abrupt release of RB molecules was initially observed upon the addition of oxidant and reductant. More importantly, the release rate of RB could be further tuned through variation of the concentration of oxidants and reductants. RB exhibited a slower release in a diluted solution of $H_2O_2$ or GSH. It should be pointed out that even in a solution with a relatively low concentration of oxidants and reductants, such as 0.01% v/v and 0.01 mg/mL, the PEG-PUSeSe-PEG micelles could still undergo evident collapse in response to the external redox stimuli, indicating good sensitivity and mild operation conditions. Thus, the conclusion can be drawn that the PEG-PUSeSe-PEG micelles can be used to load functional molecules and that these molecules can be released in a controllable manner through either oxidation or reduction stimuli.

In summary, a diselenide-containing block copolymer was synthesized and its dual redox responsive disassembly upon addition of oxidants or reductants was investigated. The results show that the redox responsive PEG-PUSeSe-PEG micelles were quite stable under ambient conditions but could exhibit very good sensitivity to external redox stimuli. The incorporated species could be released from the micelles when some effective oxidants or reductants were added in a mild environment. Considering the active nature of the Se−Se bonds existing in the block copolymer, attempts were made to use some other stimuli, such as $\gamma$ rays, to destroy the aggregates and release the loaded species. It is fully expected that this kind of multiresponsive block copolymer aggregate may function as a controlled drug delivery system, enabling a combination of chemotherapeutics and actinotherapy.

**Acknowledgment.** This work was financially supported by the National Basic Research Program (2007CB808000), National Natural Science Foundation of China (20944001, 20904028, 50973051, 20974059), Science Foundation of China Postdoctor (20080440362), and the NSFC-DFG joint grant (TRR 61). The authors thank Prof. Zhibo Li (ICCAS, Beijing) for his help on cryo-TEM and helpful discussions.

**Supporting Information Available:** Synthesis, characterization, and other experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Reviews for example: (a) Rodríguez-Hernández, J.; Chécot, F.; Gnanou, Y.; Lecommandoux, S. *Prog. Polym. Sci.* **2005**, *30*, 691. (b) Rijcken, C. J. F.; Soga, O.; Hennink, W. E.; von Nostrum, C. F. *J. Controlled Release* **2007**, *120*, 131. (c) Meng, F.; Zhong, Z.; Feijen, J. *Biomacromolecules* **2009**, *10*, 197.
(2) Wang, Y.; Xu, H.; Zhang, X. *Adv. Mater.* **2009**, *21*, 2849.
(3) (a) Gillies, E. R.; Jonsson, T. B.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 11936. (b) Bronich, T. K.; Keifer, P. A.; Shlyakhtenko, L. S.; Kabanov, A. V. *J. Am. Chem. Soc.* **2005**, *127*, 8236.
(4) (a) Jiang, J.; Tong, X.; Zhao, Y. *J. Am. Chem. Soc.* **2005**, *127*, 8290. (b) Goodwin, A. P.; Mynar, J. L.; Ma, Y.; Fleming, G. R.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2005**, *127*, 9952. (c) Wang, Y.; Ma, N.; Wang, Z.; Zhang, X. *Angew. Chem., Int. Ed.* **2007**, *46*, 2823.
(5) (a) Lambeth, R. H.; Ramakrishnan, S.; Mueller, R.; Poziemski, J. P.; Miguel, G. S.; Markoski, L. J.; Zukoski, C. F.; Moore, J. S. *Langmuir* **2006**, *22*, 6352. (b) Qin, S.; Geng, Y.; Discher, D. E.; Yang, S. *Adv. Mater.* **2006**, *18*, 2905.
(6) (a) Napoli, A.; Valentini, M.; Tirelli, N.; Müller, M.; Hubbell, J. A. *Nat. Mater.* **2004**, *3*, 183. (b) Wang, X.; Wang, H.; Coombs, N.; Winnik, M. A.; Manners, I. *J. Am. Chem. Soc.* **2005**, *127*, 8924. (c) Wang, C.; Guo, Y.; Wang, Y.; Xu, H.; Zhang, X. *Chem. Commun.* **2009**, 5380.
(7) (a) Cerriteli, S.; Velluto, D.; Hubbell, J. A. *Biomacromolecules* **2007**, *8*, 1966. (b) Dong, W.; Kishimura, A.; Anraku, Y.; Chuanoi, S.; Kataoka, K. *J. Am. Chem. Soc.* **2009**, *131*, 3804. (c) Klaikherd, A.; Nagamani, C.; Thayumanavan, S. *J. Am. Chem. Soc.* **2009**, *131*, 4830.
(8) (a) Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G.; Hoekstra, W. G. *Science* **1973**, *179*, 588. (b) Zhang, X.; Xu, H.; Dong, Z.; Wang, Y.; Liu, J.; Shen, J. *J. Am. Chem. Soc.* **2004**, *126*, 10556. (c) Xu, H.; Gao, J.; Wang, Y.; Smet, M.; Dehaen, W.; Zhang, X. *Chem. Commun.* **2006**, 796.
(9) (a) Fredga, A. *Ann. N.Y. Acad. Sci.* **1972**, *192*, 1. (b) Trahanovsky, W. S. *Oxidation in Organic Chemistry, Part C*; Academic Press: 1978.
(10) Günther, W. H. H.; Salzman, M. N. *Ann. N.Y. Acad. Sci.* **1972**, *192*, 25.

JA908124G