Hydrogen Peroxide-Responsive Micelles Self-Assembled from Peroxalate Esters-Containing Triblock Copolymer

Journal:	*Biomaterials Science*
Manuscript ID	BM-COM-09-2015-000391.R2
Article Type:	Communication
Date Submitted by the Author:	02-Dec-2015

Complete List of Authors:	Liu, Chao; Chinese Academy of Medical Science, Institute of Biomedical Engineering Zhu, Xiaowei; Chinese Academy of Medical Science, Institute of Biomedical Engineering Wang, Xiaoli; Chinese Academy of Medical Science, Institute of Biomedical Engineering Miao, Dandan; Chinese Academy of Medical Science, Institute of Biomedical Engineering Liang, Xiaoyu; Chinese Academy of Medical Science, Institute of Biomedical Engineering Wang, Cuiwei; Chinese Academy of Medical Science, Institute of Biomedical Engineering Pang, Liyun; Chinese Academy of Medical Science, Institute of Biomedical Engineering Sun, Hongfan; Chinese Academy of Medical Science, Institute of Biomedical Engineering Kong, Deling; Chinese Academy of Medical Science, Institute of Biomedical Engineering Yang, Jing; TianJin key Laboratory of Biomaterial Research, Institute of Biomedical Engineering, Chinese Academy of Medical Science,

COMMUNICATION

Hydrogen Peroxide-Responsive Micelles Self-Assembled from Peroxalate Esters-Containing Triblock Copolymer†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Chao Liu, Xiaowei Zhu, Xiaoli Wang, Dandan Miao, Xiaoyu Liang, Cuiwei Wang, Liyun Pang, Hongfan Sun, Deling Kong, Jing Yang*

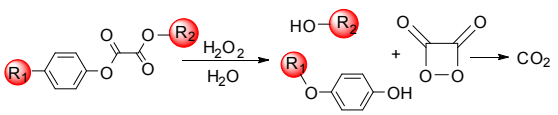
A novel ABA triblock copolymer by using peroxalate esters as linkages was synthesized. In water, this amphiphilic copolymer self-assembled into micelles which were used as drug delivery carriers. When stimulated by hydrogen peroxide, micelles were disassembled to release drugs since the responsive peroxalate ester linkages would be cleaved after reaction with hydrogen peroxide.

Reactive oxygen species (ROS), a special type of cellular signalling molecules, is the key factor which maintains cellular homeostasis and communication in physiological processes[1]. However, excessive ROS can cause severe cellular damage[2]. Hydrogen peroxide (H_2O_2), as a major component of ROS, plays a key role in mediating cell growth and apoptosis[3]. Similarly, its abnormal overproduction usually indicates some diseases, such as cancer[4], ischemia/reperfusion (I/R) injury[5], inflammatory triggered by oxidative stress[6] and so on. Therefore, the preparation of drug delivery carriers by using hydrogen peroxide responsive polymers to achieve targeted drug release and scavenge redundant hydrogen peroxide in pathological location is meaningful and necessary.

To date, responsive polymers, which are sensitive to environmental stimuli (redox[7], temperature[8], pH[9], or light[10], etc.), have recently triggered extensive research. Redox responsive polymers containing disulfide bonds[11], thioether linker[12], arylboronic esters[13], diselenide bonds[14], etc. have been developed in recent years. Drug delivery carriers prepared by these polymers can achieve targeted drug release in redox conditions. However, these oxidation sensitive polymers were sensitive to not only hydrogen peroxide but also other ROS[11-14] Therefore, it is urgently required to prepare a novel specific hydrogen peroxide responsive polymer, achieving targeted drug delivery and treating hydrogen peroxide overproduction relative diseases.

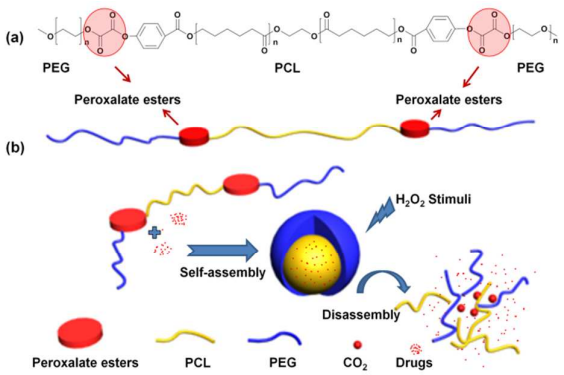
It has been reported that the peroxalate derivatives can only react with hydrogen peroxide[15]. At present, peroxalate esters (PO) were largely used at the chemiluminescent field[15, 16]. Specifically, mixture of peroxyoxalate and fluorescent dyes could emit visible light when react with hydrogen peroxide. Peroxalate esters used for chemiluminescence were mainly aromatic esters such as diphenyl oxalate and trichlorophenyl oxalate[16]. As shown in scheme 1, the aromatic peroxalate esters can be oxidized by hydrogen peroxide to an unstable intermediate, and the intermediate can be degraded to carbon dioxide[15]. It was reported that micelles/nanoparticles loading peroxyoxalate and fluorescent dyes were able to detect hydrogen peroxide *in vivo*[17]. Lee et al. firstly prepared the fluorescent dyes-loaded polyoxalate nanoparticles for specifically imaging hydrogen peroxide[18]. Afterwards, Lee et al. prepared drug delivery carriers with the polyoxalate polymer to treat the disease of oxidative injury[19]. However, block copolymer linked by peroxlate esters have not been reported yet.

In our study, a new method was established to synthesize specifically responsive block copolymer, poly (ethylene glycol)-PO-poly(caprolactone)-PO-poly(ethylene glycol) (PEG-PO-PCL-PO-PEG), by using peroxalate esters (PO) as hydrogen peroxide-responsive linkages. And the micelles used as drug delivery carriers were obtained by the self-assembly of the amphiphilic copolymer in aqueous solution. When stimulated by hydrogen peroxide, the copolymer was cleaved into three fragments accompanying by the disassembly of the micelles due to the strong reactivity of peroxalate esters with hydrogen peroxide. Hence,



Scheme 1 Schematic diagram of reaction between aromatic peroxalate esters and hydrogen peroxide.

TianJin key Laboratory of Biomaterial Research, Institute of Biomedical Engineering, Chinese Academy of Medical Science, Tianjin 300192, P.R. China.
Email: yangjing37@hotmail.com
Tel: +86 22 87891191
†Electronic Supplementary Information (ESI) available: Experiments for synthesis and characterization. See DOI: 10.1039/x0xx00000x



Scheme 2 The structure of as-synthesized hydrogen peroxide responsive PEG-PO-PCL-PO-PEG copolymer (a) and Schematic diagram of H_2O_2-responsive disassembly of PEG-PO-PCL-PO-PEG Micelles (b).

the micelles self-assembled by the responsive copolymer achieved targeted drug release as well as the scavenging of hydrogen peroxide in the surrounding environment, as shown in Scheme 2.

The synthetic route of PEG-PO-PCL-PO-PEG was depicted in Scheme S1 (ESI†). Firstly, PCL was synthesized by ring-opening polymerization, then PCL was modified by phenolic hydroxy group in its terminal hydroxyl. And the block copolymer was obtained when the modified PCL was linked with PEG by oxalyl chloride. To identify the chemical structure of the copolymer, Fourier-transform infrared (FTIR) spectrometer and ^{1}H NMR spectrometer were conducted. Gel permeation chromatography (GPC) revealed that the molecular weight (Mn) of the copolymer was 18134 g mol^{-1} with a polydispersity index (PDI) of 1.6. (For details of the synthesis and characterization of the copolymer, see ESI†). The self-assembly behaviour of the copolymer was examined in water. The amphiphilic copolymer aggregated spontaneously in aqueous environment, then micelles were obtained after solvent evaporation.

The critical aggregate concentration (CAC) of the copolymer was ~3.98×10^{-6} mg mL^{-1}, measured by the method of pyrene fluorescent probe. The size of micelles was about 114.9 nm with a PDI of 0.18 before lyophilisation, measured by dynamic light scattering (DLS). After lyophilisation, the size changed to 172.2 nm with a PDI of 0.224, as shown in Fig.1a. TEM was employed to investigate the micro-nanostructures of these micelles. TEM image (Fig.1c) clearly indicated that the micelles exhibited spherical morphologies and intact core-corona structure[20]. (The preparation and characterization of micelles, see ESI†).

The stability of micelles was of great vital for its application and availability. Consequently, the mean size of micelles was measured after storage for different time in aqueous solutions at 4 °C. As shown in Fig.1b, no obvious changes were observed in mean size as well as PDI, which indicated that the micelles were quite stable in water. The cytotoxicity of micelles was investigated using T/G HA-VSMCs cells by tetrazolium salt reagents (WST-1) reduction assay (For detailed operation method, see ESI†). As shown in Fig.S5 (ESI†), micelles with different concentration possessed no cytotoxicity compared with the control groups, suggesting their good biocompatible as drug delivery applications.

Cryo-TEM was performed to observe the structure changes of micelles after treated with hydrogen peroxide solution (50 μM). As shown in Fig.1d, the spherical micelles converted to irregular aggregates due to the burst of as-synthesized micelles in hydrogen peroxide solution. Because the peroxalate esters-containing copolymer could react with hydrogen peroxide, the hydrogen peroxide-scavenging ability of as-synthesized micelles was studied (For detailed operation method, see ESI†). The hydrogen peroxide concentration was examined by Amplex Red Assay. As shown in Fig.2a, the scavenging ability of micelles was more remarkable in comparison with that of the PCL nanoparticles and PEG solution at the same polymer concentration of 1 mg·mL^{-1}. As demonstrated, the hydrogen peroxide-responsive micelles could be employed to load and targeted release drugs. Liposoluble rapamycin (RPM) was used as model drugs and RPM-loaded micelles were prepared. Before lyophilisation, the micelles were washed three times with water to remove free RPM molecules in the surface of micelles, then the drug release behaviour was investigated (For detailed operation method, see ESI†). As shown in Fig.2b，the drug release speed of the micelles was accelerated by hydrogen peroxide stimuli as a result of the micelles disassembly.

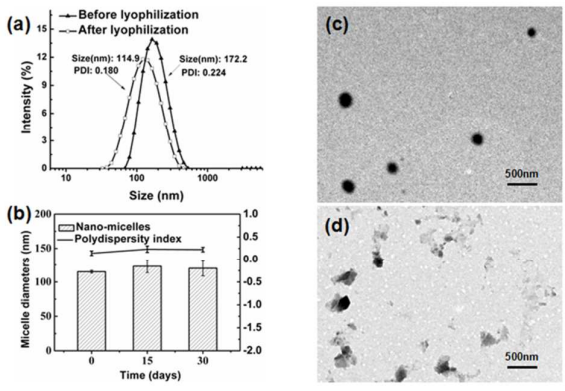


Fig. 1 (a) Dynamic light scattering of PEG-PO-PCL-PO-PEG micelles before and after lyophilisation (b) PEG-PO-PCL-PO-PEG micelles size within 1 month and cryo-TEM image of micelles before (c) and after (d) H_2O_2 stimuli.

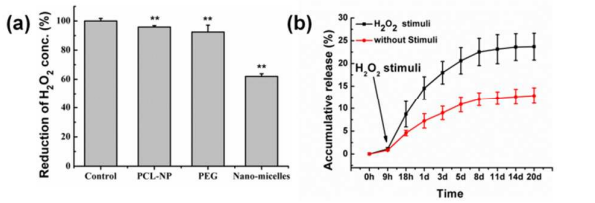


Fig. 2 (a) H_2O_2 (0.1 μM) scavenging activity of the as-synthesized micelles determined by the Amplex Red Assay. **p<0.05 vs. control group (b) The release behaviour of RPM with and without H_2O_2 (5 μm) stimuli.

In summary, a novel ABA triblock copolymer containing peroxalate esters was successfully synthesized. The copolymer can self-assemble into micelles in aqueous solution. With hydrogen peroxide stimuli in a mild condition, the micelles disassembled and released incorporated drugs due to the oxidation of peroxalate esters by hydrogen peroxide. Meanwhile, the hydrogen peroxide concentration in surrounding environment was also reduced. Considering the biocompatibility and hydrogen peroxide responsive, the as-synthesized micelles could be used as drug delivery carriers to achieve targeted drug release and treat hydrogen peroxide overproduction related diseases.

We gratefully acknowledge the financial support by grants from the National Natural Science Foundation of China (81271706), the Tianjin Research Foundation Advanced Technology Program (13JCZDJC30700), and the Technology Foundation for Selected Overseas Chinese Scholars, Ministry of Personnel of China.

Notes and references

1 K. Rutault, C. Alderman, B. M. Chain and D. R. Katz, *Free Radical Bio. Med.*, 1999, **26**, 232; K. Bedard and K.H. Krause, *Physiol. Rev.*, 2007, **87**, 245; A. Görlach, E. Y. Dimova, A. Petry, A. Martínez-Ruiz, P. Hernansanz-Agustín, A. P. Rolo, C. M. Palmeira and T. Kietzmann, *Redox Bio.*, 2015.

2 T. Finkel and N. J. Holbrook*, Nature*, 2000**, 408**, 239; C. E. Thomas and V. Darley-Usmar*, Free Radical Bio. Med.*, 2000, 28, 1449.

3 S. G. Rhee, *Science*, 2006, **312**, 1882; M. Giorgio, M. Trinei, E. Migliaccio and P. G. Pelicci, *Nat. Rev. Mol. Cell Bio.*, 2007, **8**, 722.

4 S. Toyokuni, K. Okamoto, J. Yodoi and H. Hiai, *Febs lett.*, 1995, **358**, 1; D. Trachootham, J. Alexandre and P. Huang, *Nat. Rev. Drug Discovery*, 2009, **8**, 579.

5 M. Aragno, J. C. Cutrin, R. Mastrocola, M. G. Perrelli, F. Restivo, G. Poli, O. Danni and G. Boccuzzi, *Kidney Int.*, 2003, **64**, 836.

6 S. Reuter, S. C. Gupta, M. M. Chaturvedi and B. B. Aggarwal, *Free Radical Bio. Med.*, 2010, **49**, 1603; J. Kwon, J. Kim, S. Park, G. Khang, P. M. Kang and D. Lee, *Biomacromolecules*, 2013, **14**, 1618; D. Yoo, K. Guk, H. Kim, G. Khang, D. Wu and D. Lee, *Int. J. Pharm.*, 2013, **450**, 87.

7 G. H. Jiang, Y. Wang, R. Zhang, R. J. Wang, X. H. Wang, M. Zhang, X. K. Sun, S. Y. Bao, T. Wang and S. Wang, *ACS Macro Lett.*, 2012, **1**, 489; Y. Wang, H. B. Wang, G. Y. Liu, X. S. Liu, Q. Jin and J. Ji, *Macromol. Biosci.*, 2013, **13**, 1084.

8 G. Y. Li, L. Q. Shi, R. J. Ma, Y. L. An and N. Huang, *Angew. Chem.*, 2006, **118**, 5081; Q. Yan, J. Y. Yuan, Y. Kang, Z. N. Cai, L. L. Zhou and Y. W. Yin, *Chem. Commun*., 2010, **46**, 2781.

9 S. Binauld, W. Scarano and M. H. Stenzel, *Macromolecules*, 2012, **45**, 6989; Y. Wang, H. B. Wang, Y. J. Chen, X. S. Liu, Q. Jin and J. Ji, *Chem. Commun.*, 2013, **49**, 7123.

10 W. Xiao, W. H. Chen, X. D. Xu, C. Li , J. Zhang, R. X. Zhuo and X. Z. Zhang, *Adv. Mater.*, 2011, **23**, 3526.

11 F. H. Meng, W. E. Hennink and Z. Y. Zhong, *Biomaterials*, 2009, **30**, 2180; H. Wang, L. Tang, C. L. Tu, Z. Y. Song, Q. Yin, L. C. Yin, Z. H. Zhang and J. J. Cheng, *Biomacromolecules*, 2013, **14**, 3706; L. L. Dai, J. H Li, B. L Zhang, J. J. Liu, Z. Luo and K.Y. Cai, *Langmuir* 2014, **30**, 7867.

12 J. R. Kramer and T. J. Deming, *J. Am. Chem. Soc.*, 2012, **134**, 4112.

13 E. W. Miller, A. E. Albers, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2005, **127**, 16652; K. E. Broaders, S. Grandhe and J. M. J. Fréchet, *J. Am. Chem. Soc.*, 2010, **133**, 756.

14 X. Zhang, H. P. Xu, Z. Y. Dong, Y. P. Wang, J. Q. Liu and J. C. Shen, *J. Am. Chem. Soc.*, 2004, **126**, 10556; N. Ma, Y. Li, H. P. Xu, Z. Q. Wang and X. Zhang, *J. Am. Chem. Soc.*, 2009, **132**, 442; J. X. Ding, C. S. Xiao, L. Yan, Z. H. Tang, X. L. Zhuang, X. S. Chen and X. B. Jing, *J. Control. Release*, 2011, **152**, e11; L. Wang, W. Cao, Y. Yi and H. P. Xu, *Langmuir*, 2014, **30**, 5628.

15 C. V. Stevani, S. M. Silva and W. J. Baader, *Eur. J. Org. Chem.*, 2000, **2000**, 4037; S. M. Silva, K. Wagner, D. Weiss, R. Beckert, C. V. Stevani and W. J. Baader, *Luminescence*, 2002, **17**, 362.

16 L. J. Bollyky, R. H. Whitman and B. G. Roberts, *J. Org. Chem.*, 1968, **33**, 4266; D. Lee, V. R. Erigala, M. Dasari, J. Yu, R. M. Dickson and N. Murthy, *Int. J. Nano.Nanomed.*, 2008, **3**, 471.

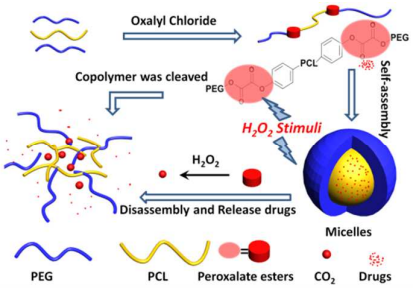
17 R. Chen, L. Z. Zhang, J. Gao, W. Wu, Y. Hu, and X.Q. Jiang, *J. Biomed Biotechnol.*, 2011, **2011**, 679492; Y. D. Lee, C. K. Lim, A. Singh, J. Koh, J. Kim, I. C. Kwon and S. Kim, *ACS nano*, 2012, **6**, 6759.

18 D. Lee, S. Khaja, J. C. Velasquez-Castano, M. Dasari, C. Sun, J. Petros, W. R. Taylor and N. Murthy, *Nat. Mater.*, 2007, **6**, 765; I. Lee, O. Hwang, D. Yoo, G. Khang and D. Lee, *B. Kor. Chem. Soc.*, 2011, **32**, 2187.

19 H. Park, S. Kim, S. Kim, Y. Song, K. Seung, D. Hong, G. Khang, and D. Lee, *Biomacromolecules*, 2010, **11**, 2103; S. Kim, H. Park, Y. Song, D. Hong, O. Kim, E. Jo, G. Khang and D. Lee, *Biomaterials*, 2011, **32**, 3021; D. Lee, S. Bae, D. Hong, H. Lim, J. H. Yoon, O. Hwang, S. Park, Q. Ke, G. Khang and P. M. Kang, *Sci. Rep.*, 2013, **3**, 2233; D. Lee, S. Bae, Q. Ke, J. Lee, B. Song, S. A. Karumanchi, G. Khang, H. S. Choi and P. M. Kang, *J. Control. Release*, 2013, **172**, 1102; J. Kwon, J. Kim, S. Park, G. Khang, P. M. Kang and D. Lee, *Biomacromolecules*, 2013, **14**, 1618; S. Park, J. Yoon, S. Bae, M. Park, C. Kang, Q. Ke, D. Lee and P. M. Kang, *Biomaterials*, 2014, **35**, 5944; E. Ko, D. Jeong, J. Kim, S. Park, G. Khang and D. Lee, *Biomaterials*, 2014, **35**, 3895.

20 Q. Yan, J. Y. Yuan, W. Z. Yuan, M. Zhou, Y. W. Yin and C. Y. Pan, *Chem. Commun*., 2008, 6188; Q. Yan, J. Hu, R. Zhou, Y. Ju, Y. W. Yin and J.Y Yuan, *Chem. Commun.*, 2012, **48**, 1913.

Table of contents



A novel copolymer was synthesized by using peroxalate esters as linkage and the formed micelles possessed specific H_2O_2 responsive reactivity.