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Facile synthesis of reduction-responsive amphiphilic triblock polymer via selective thiol-disulfide exchange reaction

Xiangyu Liu, Jingwen He, Ding Hu, Yile Niu, Xinnian Xia, and Yanbing Lu

A reduction-responsive amphiphilic triblock copolymer mPEG-b-PDS-b-mPEG was synthesized via polycondensation between a dithiol and dipyridyl disulfide, followed by selective thiol-disulfide exchange reaction. The reduction-triggered release of Nile Red by DTT was further demonstrated using fluorescence spectroscopy. The disulfide bond (−S−S−) has an extremely value in a variety of chemical and biological agents due to the advantages of the disulfide bond in terms of biocompatibility, stability in the bloodstream, and responsiveness to redox stimuli. It can be reduced by the abundance of free thiols including glutathione in the cells, especially cancer cell, which has an enhanced glutathione level. Hence, researchers have developed various disulfide-containing polymers as nanocarriers. Sun reported the preparation of redox responsive micelles via linking up poly(ethylene glycol) and poly(ε-caprolactone) by disulfide bond. The DOX-loaded micelles released DOX rapidly in the presence of 10 mM DTT in pH 7.4 PB buffer at 37 °C, and reached virtually quantitative release after 12 h. Cheng reported the synthesis of disulfide-based cross-linked micelles via the ring opening polymerization of 5-(4-(prop-2-yn-1-ylxy)benzyl)-1,3-dioxolane-2,4-dione and click chemistry. The cross-linked micelles could keep the structural stability of it under extreme dilution conditions and released the payload quickly in the presence of dithiothreitol (DTT). However, they all used small disulfide compound as the linker. Recently, a mild and versatile synthetic route for making poly(disulfide)s was developed by Ghosh. Telechelic poly(disulfide)s (PDS) were synthesized according to same strategy reported by Ghosh. Condensation reaction between 2,2′-dithiodipyridine (M1) and 1,6-hexanethiol (M2) with three different mole ratios gave solid powder PDS in about 70% yield. H NMR end group analysis showed that the obtained PDS(1) with monomer mole ratio at 1.04:1 had a number average molecular weight (Mn) of 5.4 kDa (Table 1 and Figure 1). GPC measurement demonstrated a unimodal distribution with a Mn of 6.4 kDa (polystyrene standards) and a moderate polydispersity index (PDI) of 1.66 (Table 1 and Figure 2). Thiol-terminated hydrophilic polymer mPEG-SH was obtained by esterification of monomethoxy poly(ethylene glycol) (Mn= 1.9 kDa) with mercaptaoacid acid using p-toluenesulfonic acid as catalyst. Then, triblock copolymer mPEG-b-PDS-b-mPEG was readily prepared via thiol-disulfide exchange reaction between PDS and mPEG-SH. Using the similar strategy, we have developed a facile route for synthesis of ABA type amphiphilic triblock copolymer via the thiol-disulfide exchange reaction of telechelic pyridyl disulfide terminated poly(disulfide)s with a hydrophilic thiol-terminated polymer (Scheme 1). The molecular structures, self-assembly, and reduced-triggered drug release were thoroughly investigated by means of NMR, gel permeation chromatography (GPC), dynamic light scattering (DLS), transmission electron microscope (TEM), and fluorescence spectrophotometer.

Scheme 1 Synthesis of ABA triblock copolymer
Table 1 Molecular characteristics of PDS and PEG-b-PDS-b-PEG

<table>
<thead>
<tr>
<th>Entry</th>
<th>M₁:M₂ ratio</th>
<th>Yield (%)</th>
<th>Mₘ/Mₙ́</th>
<th>Mₘ,GPC (kDa)</th>
<th>Mₘ,NMR (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDS(1)</td>
<td>1.045:1</td>
<td>73</td>
<td>1.66</td>
<td>6.4</td>
<td>5.4</td>
</tr>
<tr>
<td>PDS(2)</td>
<td>1.025:1</td>
<td>71</td>
<td>1.42</td>
<td>11.6</td>
<td>8.6</td>
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<tr>
<td>PDS(3)</td>
<td>1.01:1</td>
<td>75</td>
<td>1.30</td>
<td>17.9</td>
<td>13.7</td>
</tr>
<tr>
<td>mPEG-b-PDS(1)-b-mPEG</td>
<td>-</td>
<td>88</td>
<td>1.29</td>
<td>12.1</td>
<td>9.1</td>
</tr>
<tr>
<td>mPEG-b-PDS(2)-b-mPEG</td>
<td>-</td>
<td>84</td>
<td>1.25</td>
<td>15.6</td>
<td>12.3</td>
</tr>
<tr>
<td>mPEG-b-PDS(3)-b-mPEG</td>
<td>-</td>
<td>86</td>
<td>1.23</td>
<td>20.8</td>
<td>17.5</td>
</tr>
</tbody>
</table>

* Both molecular weight (Mₘ,GPC) and the polydispersity (Mₘ/Mₙ́) of the polymers were determined by GPC. * Mₘ,NMR was determined by ¹H NMR.

Figure 1 ¹H NMR spectra of PDS(1) and PEG-b-PDS(1)-b-PEG

Figure 2 GPC curves of PDS and corresponding mPEG-b-PDS-b-mPEG

PEG-SH. The successful exchange reaction of thiol-disulfide bonds were demonstrated with ¹H NMR and GPC. In the ¹H NMR spectrum of mPEG-b-PDS(1)-b-mPEG (Figure 1), the disappearance of the peak of the proton in the region of 7.0 ~ 8.5 ppm (corresponding to the terminal pyridyl disulfide group) and appearance of the peaks in the region of 3.3~4.3 ppm (corresponding to the mPEG protons) confirmed the successful thiol-disulfide exchange reaction. Moreover, GPC analysis revealed that the obtained triblock copolymer also possessed a unimodal distribution with a narrow PDI value (Table 1 and Figure 2). The Mₘ of the

Figure 3 Fluorescence emission intensity vs log of micelle concentrations (a), mean size distribution (b) and TEM photograph (c) of mPEG-b-PDS(1)-b-mPEG micelles
corresponding triblock copolymer mPEG-b-PDS(1)-b-mPEG from PDS(1) increased from 6.4 kDa to 12.1 kDa and the PDI changed from 1.66 to 1.29.

The amphiphilic nature of the triblock copolymer mPEG-b-PDS-b-mPEG provided an opportunity to form micelles in water. Micelles of mPEG-b-PDS(1)-b-mPEG were prepared by dialysis method. Hydrophobic dye Nile Red (NR) was chosen as a fluorescence probe. It fluoresces when solubilized by the hydrophobic micelle core, but the emission intensity decreases when the micelle is disrupted bringing the dye molecule in contact with water. The critical micelle concentration (CMC) could be calculated by tracking the fluorescence intensity of NR as a function of the sample concentration.26,27 As shown in Figure S2, the fluorescence intensity of the NR remained nearly constant below a certain concentration and then increased rapidly, which reflected that the dye was encapsulated into the hydrophobic region of micelles. The corresponding concentration of the inflection point in the Figure 3(a) was identified as the CMC of mPEG-b-PDS(1)-b-mPEG, which was about 0.024 mg mL$^{-1}$.

Both the morphology and the average size of the self-assembled PEG-b-PDS-b-PEG micelles were investigated by means of TEM and DLS. DLS measurements (Figure 3(b)) showed that mPEG-b-PDS(1)-b-mPEG formed micelles with a number-average hydrodynamic diameter of 29.9 nm and a polydispersity of 0.021, which indicated that the micelles had a narrow size distribution. As shown in Figure 3(c), TEM micrograph confirmed that micelles of mPEG-b-PDS(1)-b-mPEG had a spherical morphology with an average size of ~25 nm. The average size determined from TEM analysis is smaller than that determined by DLS, which was most likely led by shrinkage of the PEG shell upon drying.

The reducible moiety, PDS on the micelles can be degraded in the presence of reducing reagents, which had been demonstrated by Ghosh et al.25 They used $^1$H NMR to monitor the degradation of triblock copolymer poly(lactide)-b-PDS-b-poly(lactide) in presence of reducing reagent DTT and confirmed the cleavage of the polydisulfide. The demicellization process of the mPEG-b-PDS(1)-b-mPEG micelles induced by DTT was investigated by a fluorescence spectroscopy using NR as probe.28,31 As shown in Figure 4, in the presence of DTT, the fluorescence emission intensity of NR loaded micelles (0.2 mg mL$^{-1}$) decreased rapidly and about 48% NR was released from the micelle core into the aqueous solution within the first 8 h. After 48 h, the fluorescence intensity remained about 30%, which was probably because the precipitation of short hydrophobic PDS chain from aqueous solution would enclose some NR molecules and keep them in hydrophobic environment during the chain scission process. In contrast, fluorescence emission intensity did not change obviously in the absence of DTT and only about 6% NR was released from the micelles. The results indicated that encapsulated guest release from mPEG-b-PDS-b-mPEG micelles can be triggered by reduction.

Conclusions

In summary, we reported a facile synthetic method for the preparation of reduction-responsive amphiphilic triblock copolymers composed of two hydrophilic block of mPEG and a hydrophobic block of PDS. The molecular structures, selfassembly, and reduction-triggered release of mPEG-b-PDS-b-mPEG were thoroughly investigated. mPEG-b-PDS(1)-b-mPEG could self-assemble into spherical micelles in water with an average size of ~30 nm and a critical micelle concentration of 0.024 mg mL$^{-1}$. Fluorescence spectroscopy showed that the encapsulated NR can be released while reductive DTT was introduced. The reduction-responsive micelles are promising for efficient delivery and release of potential hydrophobic anticancer drugs.

Notes and references

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Electronic Supplementary Information (ESI) available: [Experimental details, including polymer synthesis and NMR spectra.]. See DOI: 10.1039/c000000x/

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