ACS Macro Letters

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Redox-Responsive Amphiphilic Macromolecular [2]Pseudorotaxane Constructed from a Water-Soluble Pillar[5]arene and a Paraquat-Containing Homopolymer

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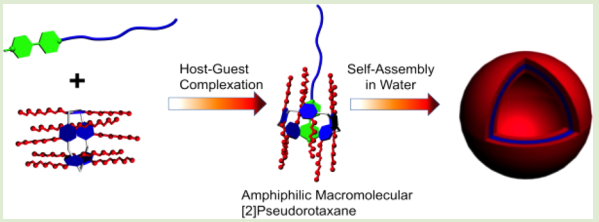
Supporting Information

ABSTRACT: Here we report a redox-responsive host−guest complex between a new water-soluble pillar[5]arene (**WP5**) and a paraquat derivative. Compared with the neutral form of the paraquat derivative that binds **WP5** weakly, its dication form binds **WP5** much more strongly. Furthermore, we utilize this new water-soluble redox-responsive molecular recognition motif to construct the first pillararene-based amphiphilic macromolecular [2]-pseudorotaxane, which self-assembles into redox-responsive polymeric vesicles in water. Such pillararene-based supramolecular vesicles were further used to construct a drug delivery system to encapsulate and controlled release DOX·HCl, an anticancer drug. The uptake of these DOX·HCl-loaded supramolecular vesicles by cancer cells was studied with confocal laser scanning microscopy. Meanwhile, DOX·HCl-loaded supramolecular vesicles showed anticancer activity in vitro comparable to free DOX·HCl under the examined conditions.



Polymeric vesicles[1] have been of great interest recently because of their potential applications in biomaterials,[2] controlled release,[3] drug delivery,[4] and so on. These applications utilize the unique stimuli-responsive cavities constructed by spontaneous organization of amphiphiles. Various molecular building blocks have been used for the fabrication of polymersomes.[5] Among them stimuli-responsive macromolecular supra-amphiphiles are more promising building blocks to make vesicles[3,6] since they undergo conformational transitions in response to environmental stimuli. Various stimuli-responsive supramolecular vesicles that can respond to photo-, thermo-changes, pH-changes, and/or redox have been reported so far.[7] Among them, redox-responsive systems are of special interest. In a membrane system, a redox-responsive assembly can be easily achieved through initiating lipid bilayer activities by changing the membrane potential.[3] Therefore, it is essential to develop new redox-responsive assemblies, which can elucidate and biomimic the biological activities of the bilayers. Furthermore, artificial redox-responsive supramolecular vesicles are well-suited to the encapsulation and controlled release of drugs since it is easy to realize the redox stimulation in cells and the human body.[3]

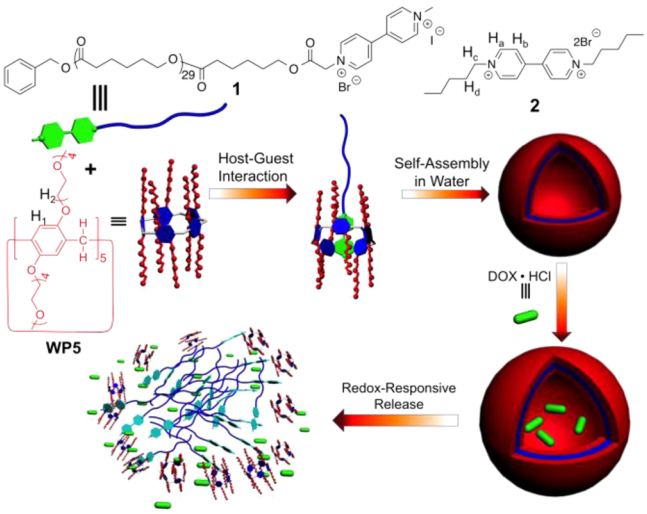
Pillar[*n*]arenes,[8] a new type of macrocyclic hosts next to crown ethers,[9] cyclodextrins,[10] calixarenes,[11] cucurbiturils,[12] and other important macrocycles,[13] have shown excellent abilities to selectively complex various guests and offered a good platform for the construction of different kinds of interesting supramolecular systems, such as daisy chains,[14] supramolecular polymers,[8b] functional vesicles,[7c,d] transmembrane channels,[8d] and other interesting supramolecular systems.[11] Recently, stimuli-responsive self-assemblies constructed from pillar[*n*]arene-based supra-amphiphiles have been actively employed, and various topological morphologies, such as micelles, vesicles, and nanotubes, have been observed. For example, Wang and co-workers reported pH-responsive supramolecular vesicles that were prepared from self-assembly of a novel host−guest complex based on a water-soluble pillar[6]arene and a ferrocene derivative in water.[7c] However, most of these studies focused on small molecular supra-amphiphiles,[15] which exhibit lower thermodynamic stability and durability compared with macromolecular supra-amphiphiles,[16] restricting their application. To the best of our knowledge, pillar[*n*]arene-based amphiphilic macromolecular [2]-pseudorotaxanes have not been reported. Furthermore, redox-responsive self-assemblies formed from pillar[*n*]arene-based supra-amphiphiles have not been explored either. Therefore, macromolecular supra-amphiphiles constructed from pillararene-based molecular recognition need to be explored.

Herein, we report a redox-responsive host−guest complex between a new water-soluble pillar[5]arene (**WP5**) and a paraquat derivative (Scheme 1). Compared with the neutral form of the paraquat derivative that shows weak binding affinity, its dicationic form binds **WP5** much more strongly

Received: July 28, 2015
Accepted: August 25, 2015
Published: August 27, 2015

DOI: 10.1021/acsmacrolett.5b00525
ACS Macro Lett. 2015, 4, 996−999

Scheme 1. Chemical Structures of WP5, 2, and Polymer 1 and Schematic Illustration of the Preparation of Polymeric Vesicles and the Process of Redox-Controlled Release of DOX·HCl Molecules



because of the efficient charge-transfer interactions between the host and the guest. Furthermore, we utilize this new water-soluble redox-responsive molecular recognition motif to construct the first pillararene-based amphiphilic macromolecular [2]pseudorotaxane, which self-assembles into polymeric vesicles in water. Moreover, due to the redox responsiveness of this inclusion complex, the polymeric vesicles are used for the controlled release of a water-soluble anticancer drug.

The synthetic methods for **WP5** and paraquat-containing homopolymer **1** are shown in Schemes S1 and S2. **WP5** was prepared by etherification of the *per*-hydroxylated pillar[5]arene, which was prepared according to a reported procedure.[17]

The complexation between **2** and **WP5** was studied by ^{1}H NMR spectroscopy. When 1.0 equiv of **WP5** was added to a solution of **2**, the resonance peaks corresponding to protons H_a, H_c, and H_d on **2** shifted upfield by 0.58, 0.72, and 0.26 ppm, respectively (Figure 1). Moreover, the peaks of protons on **WP5** also exhibited slight chemical shift changes in the presence of **2** (Figure 1b and 1c). All these chemical shift changes demonstrated that the complexation of **WP5** with **2** happened in aqueous solution. The 2D NOESY spectrum (SI, Figure S9) of an equimolar mixture of **WP5** and **2** shows correlation signals between protons H_1 and H_2 on **WP5** and protons H_a, H_c, and H_d of **2** (SI, A, B, and C in Figure S9), indicating that the paraquat derivative threaded into the cavity of **WP5**. By isothermal titration calorimetry (ITC), the stoichiometry of the complexation between **WP5** and **2** was shown to be 1:1 in water, and the association constant for the complexation was determined to be $(4.2 \pm 0.3) \times 10^4$ M^{-1} (SI, Figure S10).

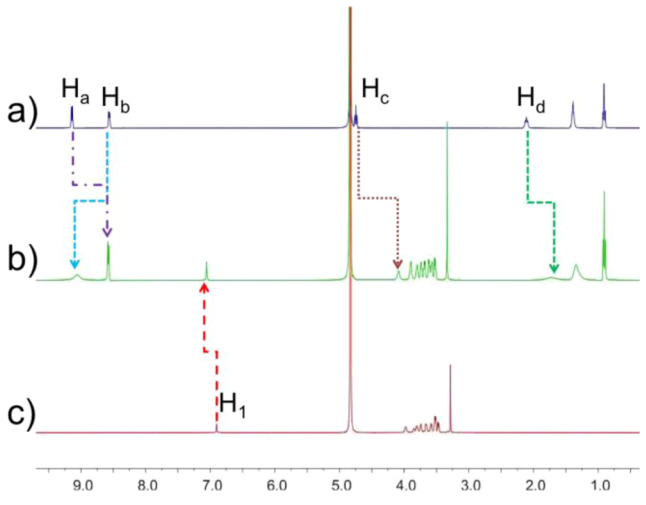


Figure 1. Partial ^{1}H NMR spectra (400 MHz, 298 K, D_2O): (a) 2.00 mM **2**; (b) 2.00 mM **WP5** and **2**; (c) 2.00 mM **WP5**.

After confirmation of the complexation between **WP5** and **2**, redox-responsiveness was explored by cyclic voltammetry. The relative binding abilities of the guest in different redox states to the host can be reflected through the changes of half-wave potentials.[18] Cyclic voltammetry curves of **2** obtained in the absence and presence of **WP5** are shown in Figure 2. As shown in Figure 2, in the absence of **WP5**, **2** shows two reversible one-electron reductions. A pronounced effect on the cyclic voltammogram of **2** happened after **WP5** was added to **2**. The relatively small negative shift observed in the $E_{1/2}$ value of the first reduction in the presence of **WP5** demonstrates that the radical cation binds **WP5** less strongly compared with the initial dicationic form.[18] Besides, the much larger negative shift observed in the $E_{1/2}$ value of the second reduction indicates that the complexation ability of the neutral species to **WP5** is considerably reduced.[18] The electrochemical study reveals that **WP5** binds the charged species more strongly. The association constants between the reduced species (radical cation and neutral form) and **WP5** estimated by the potential shifts are listed in Table 1. In comparison with the binding constants of the corresponding β-cyclodextrin (β-CD) and cucurbit[7]uril (CB[7]) inclusion complexes, we can find that the relative complexation abilities of **WP5** toward paraquat derivatives in the different redox states show almost the same trend as those of CB[7] complexes,[18] but opposite to those of β-CD complexes.[19]

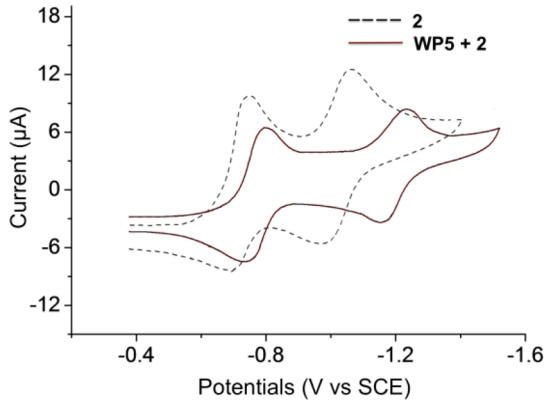


Figure 2. Cyclic voltammetry curves (0.1 V/s) of 1.0 mM **2** in the presence of equimolar **WP5** (red solid line) and in the absence of **WP5** (black dashed line). Here SCE means saturated calomel electrode.

After establishing the **WP5**⊃**2** supramolecular inclusion complex as a recognition motif in aqueous solution, paraquat-functionalized polymer **1** and **WP5** were utilized to construct redox-responsive polymeric vesicles. Assemblies with a final concentration of 2 mg/mL were made by a dialysis method due to the hydrophobic PCL chain. UV/vis spectroscopy was used

DOI: 10.1021/acsmacrolett.5b00525
ACS Macro Lett. 2015, 4, 996−999

Table 1. Association Constants (M^{-1}) for the Inclusion of Paraquat Derivative 2 in WP5 in Different Redox States and Comparison with Those for β-CD and CB[7][18,19aa]

	K_1	K_2	K_3
β-CD	0	30	1.4×10^3
CB[7]	2.0×10^5	8.5×10^4	2.5×10^2
WP5	$(4.2 \pm 0.3) \times 10^4$	$(4.3 \pm 0.3) \times 10^3$	$(1.6 \pm 0.2) \times 10^2$

[a]Here K_1 is the association constant between the host and the dicationic species; K_2 is the association constant between the host and the radical cationic species; and K_3 is the association constant between the host and the neutral form.

to elucidate the formation of the inclusion complex. The appearance of the charge transfer band of **WP5⊃1** indicated the formation of the host–guest complex (SI, Figure S11). By using Nile red as a probe, the critical aggregation concentration (CAC) was determined to be 0.15 mg/mL, indicating the formation of assemblies.[20] Dynamic light scattering (DLS) and transmission electron microscopy (TEM) were conducted to determine the morphology and size of the obtained aggregates. The size of the aggregates was determined to be ~189 nm in the average diameter by DLS (Figure 3c). The TEM image in

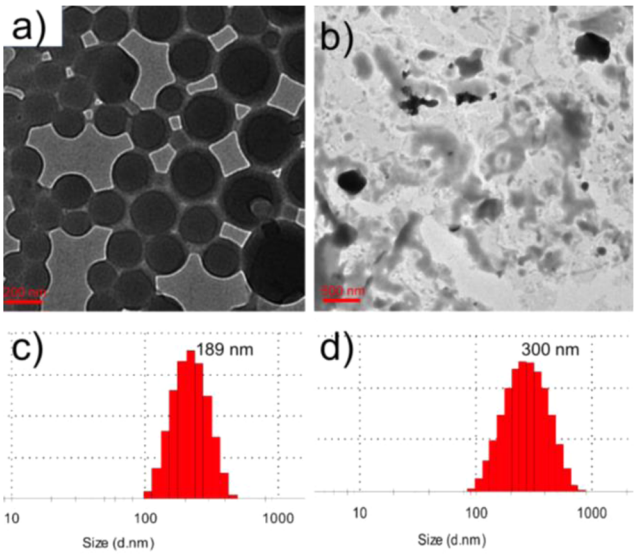


Figure 3. TEM images: (a) **WP5** + **1** aggregates; (b) **WP5** + **1** aggregates after $Na_2S_2O_4$ was added; (c) DLS data of **WP5** + **1** aggregates; (d) DLS data of **WP5** + **1** aggregates after $Na_2S_2O_4$ was added.

Figure 3a reveals that the assemblies have a spherical morphology with the average diameter of ca. 200 nm. The wall thickness of these vesicles was about 15 nm.

As discussed above, the host–guest complexation of the paraquat unit with **WP5** can be controlled by the redox chemistry of the paraquat unit. Therefore, sodium dithionite ($Na_2S_2O_4$) as a reducing agent was added into a solution of supramolecular assemblies to study their redox responsiveness. The size of the assemblies estimated by DLS gradually increased from 189 to 300 nm after addition of the reducing agent (Figure 3c and 3d). From the TEM image in Figure 3b, we can see that the aggregates underwent big morphological change after 3 mg/mL of $Na_2S_2O_4$ was added.

To study the drug release behavior and evaluate the encapsulation efficiency of the redox-responsive polymeric vesicles, doxorubicin hydrochloride (DOX·HCl), an anticancer drug, was used as a model. Unencapsulated drug molecules were removed by dialysis against phosphate buffer solution (PBS). The drug loading content and efficiency were calculated to be 7.8% and 17%, respectively (SI, page S18), which are close to the drug delivery systems constructed from cyclodextrin- and cucurbituril-based host–guest complexes.[20,21] The release behavior of DOX·HCl from the drug-loaded vesicles was controlled by adding a reducing agent. As shown in Figure 4, the drug-loaded vesicles showed significant sustained release

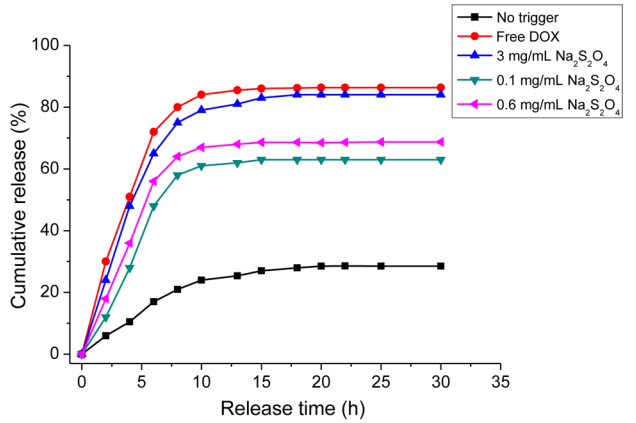


Figure 4. Drug release profiles of DOX-loaded assemblies with or without different concentrations of the reducing agent.

behavior of less than 30% without a trigger. However, the assemblies released faster after adding the reducing agent $Na_2S_2O_4$ (Figure 4). From the curve in Figure 4, a sudden release (~5 h) appeared upon addition of $Na_2S_2O_4$. More importantly, the release content of DOX·HCl was tuned through variation of the concentration of $Na_2S_2O_4$. As a consequence, this slow release behavior with no external stimuli and fast release behavior under a redox stimulus make DOX·HCl-loaded vesicles good candidates for drug delivery systems.

Then we studied whether the drug-loaded vesicles could be internalized by cancer cells by confocal laser scanning microscopy (CLSM). As shown in Figure S16, compared with DOX fluorescence of free DOX·HCl, which mainly cumulated in the cell nucleus (SI, Figure S16a), DOX·HCl-loaded vesicles appeared mainly in the cytoplasm of cells after 4 h incubation, demonstrating that vesicles had been internalized and drug was released inside the cells (SI, Figure S16b). Furthermore, with the incubation time of drug-loaded vesicles with HepG2 cells extended to 24 h, strong DOX fluorescence appeared in the nucleus of cells, indicating that more and more DOX·HCl molecules were released from drug-loaded vesicles into the cells, followed by diffusing into the cell nucleus (SI, Figure S16c).

To evaluate the anticancer efficiency of DOX·HCl-loaded vesicles, HepG2 cells were incubated with free DOX·HCl and DOX·HCl-loaded vesicles for 24 and 48 h, respectively (SI, Figure S17). After 24 h incubation, the cell viability showed that drug-loaded vesicles had lower cytotoxicity than free drug, implying that the encapsulation of the drug in the vesicles could reduce the toxicity of the drug. However, after 48 h incubation, in comparison with the drug efficacy of the free drug, drug-loaded vesicles showed enhanced drug efficacy and had similar drug efficacy as that of the free drug.

DOI: 10.1021/acsmacrolett.5b00525
ACS Macro Lett. 2015, 4, 996–999

In conclusion, we successfully established a new water-soluble redox-responsive pillararene-based molecular recognition motif. It was utilized to construct the first pillararene-based macromolecular amphiphilic [2]pseudorotaxane, which self-assembled in water to form polymeric vesicles with redox responsiveness. Significantly, a drug delivery system was constructed using such pillararene-based supramolecular vesicles to encapsulate and controlled release DOX·HCl. Furthermore, the cellular uptake of these DOX·HCl-loaded vesicles by cancer cells was investigated by CLSM. DOX·HCl-loaded vesicles exhibited anticancer activity *in vitro* comparable to free DOX·HCl. This study indicates that such polymeric vesicles can be used to fabricate controlled drug delivery systems. Moreover, considering the wide application of water-soluble redox-responsive cyclodextrin- and cucurbituril-based molecular recognition motifs in supramolecular chemistry,[22] the new water-soluble redox-responsive pillararene-based molecular recognition motif established here can be further employed in the construction of functional redox-responsive supramolecular systems with applications in various fields.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.5b00525.

Full experimental details and characterization data (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Basic Research Program (2013CB834502), the NSFC/China (91027006, 21125417, 21434005), and the Key Science Technology Innovation Team of Zhejiang Province (2013TD02).

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DOI: 10.1021/acsmacrolett.5b00525
ACS Macro Lett. 2015, 4, 996−999