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Selenium-Containing Polymers: Promising Biomaterials for Controlled Release and Enzyme Mimics

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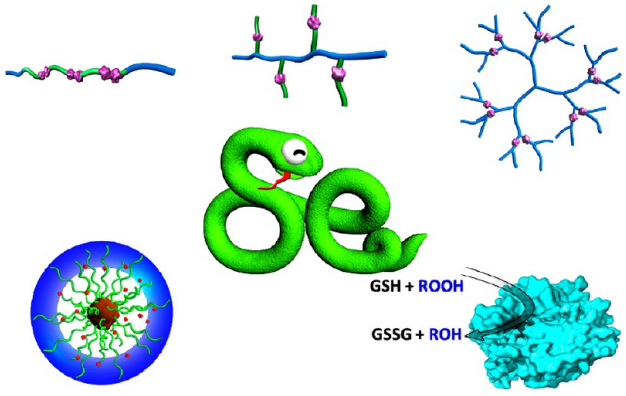
CONSPECTUS

Although researchers have made great progress in the development of responsive polymeric materials for controlled drug release or diagnostics over the last 10 years, therapeutic results still lag behind expectations. The development of special materials that respond to physiological relevant concentrations, typically within the micromolar or nanomolar concentration regime, remains challenging. Therefore, researchers continue to pursue new biomaterials with unique properties and that respond to mild biochemical signals or biomarkers.



Selenium is an essential element in human body with potential antioxidant properties. Because of selenium's electronegativity and atomic radius, selenium-containing compounds exhibit unique bond energy (C—Se bond 244 kJ mol^{-1}; Se—Se bond 172 kJ mol^{-1}). These values give the C—Se or Se—Se covalent bonds dynamic character and make them responsive to mild stimuli. Therefore, selenium-containing polymers can disassemble in response to changes under physiological relevant conditions. This property makes them a promising biomaterial for controlled release of drugs or synthetic enzyme mimics.

Until recently, few researchers have looked at selenium-containing polymers as novel biomaterials. In this Account, we summarize our recent research on selenium-containing polymers and show their potential application as mild-responsive drug delivery vehicles and artificial enzymes. We begin by reviewing the current state of the art in the synthesis of selenium-containing main chain block copolymers. We highlight the dual redox and gamma-irradiation behaviors of diselenide-containing block copolymers assemblies, discussing the possibility of their use in a combination of chemotherapy and actinotherapy. We also describe the coordination of platinum with monoselenide containing block copolymers. Such structures offer the possibility of fabricating multidrug systems for cooperative chemotherapy. In addition, we summarize the methods for the covalent and noncovalent preparation of selenium-containing polymers with side chains, which highlight the opportunity to reversibly tune the amphiphilicity of selenium-containing polymers. Finally, we present strategies for the design of highly efficient selenium-containing dendritic polymers that can mimic enzymes. This field is still in its infancy period, and further research can only be limited by our imagination.

1. Introduction

Selenium, a semimetallic chemical element and a member of the group XVI of the periodic table, was first discovered in 1817 by Jöns Jacob Berzelius. In chemical activity and physical properties, it resembles sulfur and tellurium.[1] Yet studies on selenium were rather limited until selenium was recognized as one of the necessary elements in human body in 1957. Later in the 1970s, it became clear that selenium is incorporated in proteins to make selenoproteins which prevent cellular damage from free radicals. As free radicals are natural byproducts of oxygen metabolism that may cause chronic diseases such as cancer and heart disease to human beings, great effort has been put to synthesize numerous antioxidant compounds based on selenium-containing small molecules.[2−6]

Apart from beneficial biology effects, selenium also possesses unique chemical properties owing to its special electronegativity and atomic radius. The radius of the

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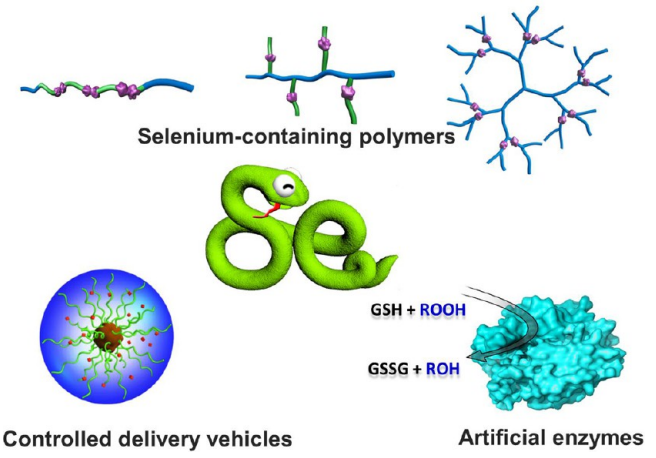
SCHEME 1. Selenium-Containing Polymers of Different Topologies and Their Potential Application as Mild-Responsive Drug Delivery Vehicles and Artificial Enzymes[a]



[a]The symbol of Se is represented by a snake, interestingly, because 2013 is the Year of Snake in terms of the Chinese lunar calendar.

selenium atom is bigger than that of sulfur, and the electronegativity of selenium is weaker than that of sulfur. This leads to lower bond energy of C−Se and Se−Se than those of C−S and S−S (C−S 272 kJ mol^{-1}; S−S 240 kJ mol^{-1}; C−Se 244 kJ mol^{-1}; Se−Se 172 kJ mol^{-1}) and makes it easier for low valence state selenium compounds to be oxidized than low valence state sulfur compounds.[7] Despite the progress made in selenium-containing compounds, literature on selenium-containing polymers is rather scarce.[8] Among them, polyselenophenes have been studied as important members of the conducting polymers with decent photovoltaic performances.[9,10] Despite their potential advantages and applications in the field of optoelectronic materials, these selenium-containing polymers are not our focus here. In addition, selenium-containing small molecules bound on polymer substrates for organic reaction catalysis and selenium-containing molecules as photoiniferters for radical polymerization will also not be discussed here because of the limited space. This Account is to highlight the most recent advance on the design and synthesis of new kinds of selenium-containing polymers and their potential bioapplications.

Nowadays, biomaterials are extensively used every day in dental applications, surgery, and drug delivery. The development of the area of self-assembly has led to deeper understanding of the functions of building blocks such as proteins and enzymes and their cooperative effects in physiological processes. It comes to a golden period to develop new types of biomaterials to meet the development of material science and life science.[11−15] In recent decades, sulfur-containing polymers have been widely studied as redox-responsive nanocarriers for active anticancer drug release or self-healing materials.[16] The reports on selenium-containing polymers for bioapplication are far behind. In this Account, we seek to elucidate recent advances in the field of selenium-containing polymers and their potential application as physiological condition-responsive drug delivery vehicles and artificial enzymes. The Account is divided into several major sections guided by the topology of the polymers: main chain selenium-containing block copolymers, covalent or noncovalent side-chain selenium-containing polymers, selenium-containing hyperbranched polymers, and dendrimers (Scheme 1). It is hoped that the novel selenium-containing polymers can provide a new platform for the next generation of biomaterials, enriching the field of stimuli-responsive materials and opening new avenues for programmable responsive systems of controlled release.

2. Main-Chain Selenium-Containing Polymers

Stimuli-responsive polymers for the transport and delivery of materials such as drugs or genes to a given location at a specific time are highly valuable in various applications.[11−16] Slight changes in the structures and functions of polymer aggregates can result in the release of encapsulated species.[17] 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.[16,18] Due to the unique redox property of selenium element, selenium-containing polymers have proven to be ideal candidates for responsive disassembly at mild conditions, which may be more benign for clinical use.

2.1. Diselenide-Containing Main Chain Block Copolymers. It is still a challenge to utilize multiresponsive assemblies for the programmable release of functional species in different environments. Diselenide is a promising candidate for a dual redox response due to its good activity in the presence of either oxidants or reductants. Normally, diselenide bonds can be oxidized to selenic acid in the presence of oxidants and reduced to selenol in a reducing environment. We successfully synthesized a dual redox responsive block copolymer with diselenide bonds located at the polymer main chains which can be used as drug delivery vehicles in a controlled manner.[19] To date, only a few examples have been shown for the synthesis of selenium-containing main chain polymers by

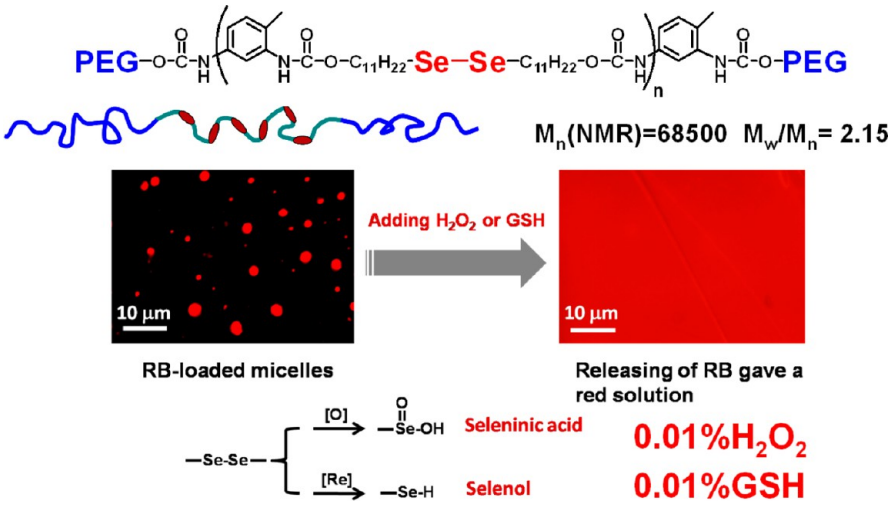


FIGURE 1. Dual redox responsive assemblies formed from PEG-PUSeSe-PEG. PEG-PUSeSe-PEG can self-assemble into micelles in water, and in mild oxidative or reductive stimuli, disassemble and release the cargos loaded.

stepwise polymerization because of the solubility issue.[8] In our study, the diselenide group was first introduced into a diol with long alkyl chains, which possessed the desirable solubility in organic solvent. The dialkyl diselenide containing polyurethane (PUSeSe) blocks were then synthesized via stepwise polymerization of toluene diisocyanate 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 as PEG-PUSeSe-PEG.

PEG-PUSeSe-PEG, a typical amphiphilic block copolymer, can self-assemble in an aqueous environment to form micelles with an average diameter of 76 nm. The micelles exhibit unique disassembly behavior upon the addition of oxidant or reductant, as shown in Figure 1. The encapsulated Rhodamine B (RB) can be released within 5 h in response to a very dilute concentration of oxidant (H_2O_2, 0.01%, v/v) or reductant (glutathione (GSH), 0.01 mg/mL). Since oxidative stress is often caused predominantly by accumulation of H_2O_2 and thought to be involved in the development of many diseases, H_2O_2 can potentially be useful as a stimulus for targeted drug delivery to diseased tissue. However, current polymeric systems are not sensitive enough to biologically relevant concentrations of H_2O_2 (50−100 μM). Our work may open a new avenue for the preparation of block copolymer micelles capable of undergoing backbone cleavage and thus release of loaded cargoes upon exposure to such low concentrations of hydrogen peroxide. It should be further noted that intracellular reduction-response is exceedingly fast and efficient due to presence of a high concentration of GSH (approximately 1−10 mM) in the cytosol and cell nucleus. Thus PEG-PUSeSe-PEG block copolymer micelles could be an ideal burst release system in physiological environment of cell.

Chemotherapeutic drugs are normally toxic and may cause side-effects by the nonspecific uptake of healthy cells. Current research on cancer treatments is still far away from targeted drug delivery. The amount of drug delivered to targeted tumors is much less than 5%.[20] On the other hand, radiotherapy refers to the treatment of disease (especially cancer) by exposure to a radioactive substance. In order to obtain better tumor control with a higher dose, the normal tissues often receive radiation injury caused mostly by the aqueous free oxidative radicals generated by the radiation on water.[21] In order to diminish the side-effects of chemotherapy and radiotherapy, novel drug-delivery systems for the combination of radiotherapy and chemotherapy need to be developed.[21] Breaking covalent bonds in the polymer backbone normally needs high radiation dose of high-energy rays, such as γ-radiation. For example, disulfide bonds can be broken with γ-radiation as high as a few kGy. The high doses of radiation present little potential for the application of this technique in biological or medical fields because they greatly exceed the dose limit that living organisms can survive. Therefore, it is desirable for new kinds of radiation-sensitive aggregates to be developed, which are able to respond to a low radiation dosage close to that used on human subjects.

We have successfully used γ-radiation to destroy the aggregates formed by PEG-PUSeSe-PEG and release the encapsulated anticancer drugs Doxorubicin (Dox) based on

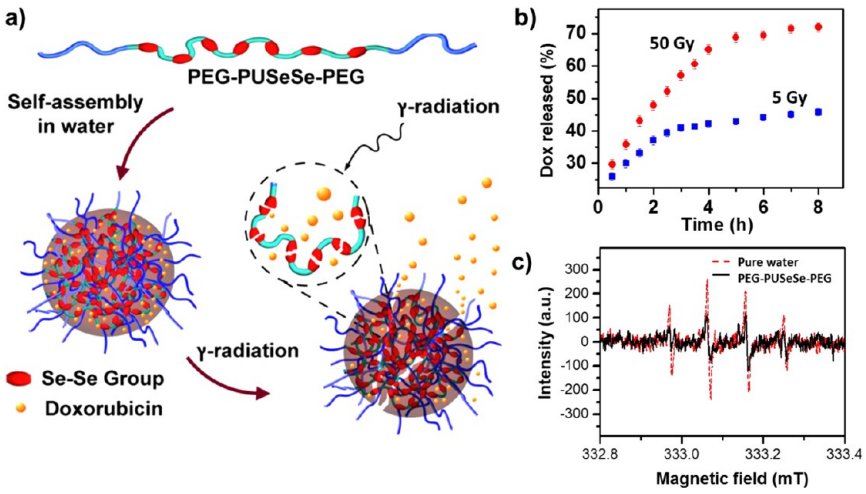


FIGURE 2. (a) Disassembly and drug release of PEG-PUSeSe-PEG aggregates under exposure to γ-radiation. (b) Release plots of Dox under exposure to different γ-radiation doses (^{60}Co as radiation source). (c) ESR signals of pure water and the polymer solution to detect the reaction between free radicals and the polymers. Reproduced from ref 22. Copyright 2011 the American Chemical Society.

the active nature of the diselenide bonds, as shown in Figure 2.[22,23] The morphology of PEG-PUSeSe-PEG micelles varies with the radiation dose. They were slightly swollen after a radiation dose of 5 Gy. When the radiation dose was increased to 50 Gy, the aggregates began to collapse into irregular aggregates. When the radiation increased to 500 Gy, all of the spherical aggregates collapsed into irregular aggregates, indicating that the micelle has been completely destroyed by the radiation. The loaded Dox in the polymer micelles realized a control release profile with different radiation dose. PEG-PUSeSe-PEG absorbs the oxidative radicals formed by the irradiation of γ-ray in water and undergoes a structural variation on the polymer backbone, resulting in the radiation-sensitive disassembly. It should be noted that the release of the loaded drug does not rely on the complete collapse of aggregates. Even with a small dose (5 Gy) of γ-radiation, the aggregates can still release about 45% of the loaded Dox. This is important because 5 Gy is close to the radiation dosage that patients receive during a single radiotherapy treatment. Thus, this study greatly enhances the possibility of biological and medical applications for these diselenide-containing block copolymer aggregates.

Light-responsive polymers usually contain azobenzene unit, spiropyran unit, malachite green unit, and so forth, which are all UV-responsive.[24−26] However, UV light cannot penetrate deep enough and is harmful to organisms, which limits its application in biological area. Long-wavelength light such as red light and near-infrared light can meet the criteria for biological usage and is highly desirable for application clinically.[27,28] Herein we have introduced a way of tuning the self-assembly of PEG-PUSeSe-PEG with red light (wavelength from 600 to 780 nm).[29] PEG-PUSeSe-PEG is sensitive to singlet oxygen, which can be produced in solution by porphyrin derivatives when irradiated with red light. The singlet oxygen oxidizes PEG-PUSeSe-PEG and cleaves the diselenide bonds. After oxidation, the self-assembly of PEG-PUSeSe-PEG is disrupted and the encapsulated Dox can be released (Figure 3). Besides, we also demonstrate that the red light response behaviors can be tuned by the PEG length as revealed by IR spectra (Figure 3b): as PEG chain length increases, it is more difficult for singlet oxygen to oxidize the diselenide bonds. The longer PEG chain may prevent the singlet oxygen from approaching the diselenide bonds or quench the singlet oxygen totally.

Although selenium is an essential micronutrient for humans, for a new potential drug release system, the cytotoxicity of the selenium-containing block copolymer is still an important factor that needs to be evaluated. To address this problem, we used L-02 cells to investigate the cytotoxicity of PEG-PUSeSe-PEG. From Figure 3c, it can be seen that, at high concentrations, such as 0.1 and 0.01 mg mL^{-1}, all of the PEG-PUSeSe-PEG polymers with different PEG lengths had no inhibitory effect on the L-02 cells. Interestingly, at a concentration of 1×10^{-3} mg mL^{-1}, the growth of L-02 cells was slightly accelerated, which may be related to the nutritive effects of the selenium element at low concentrations.[35] MTT assays of HepG2 cells show similar trends.[22] The cytotoxicity experiments indicate that PEG-PUSeSe-PEG possesses good biocompatibility and may be clinically used in the future.

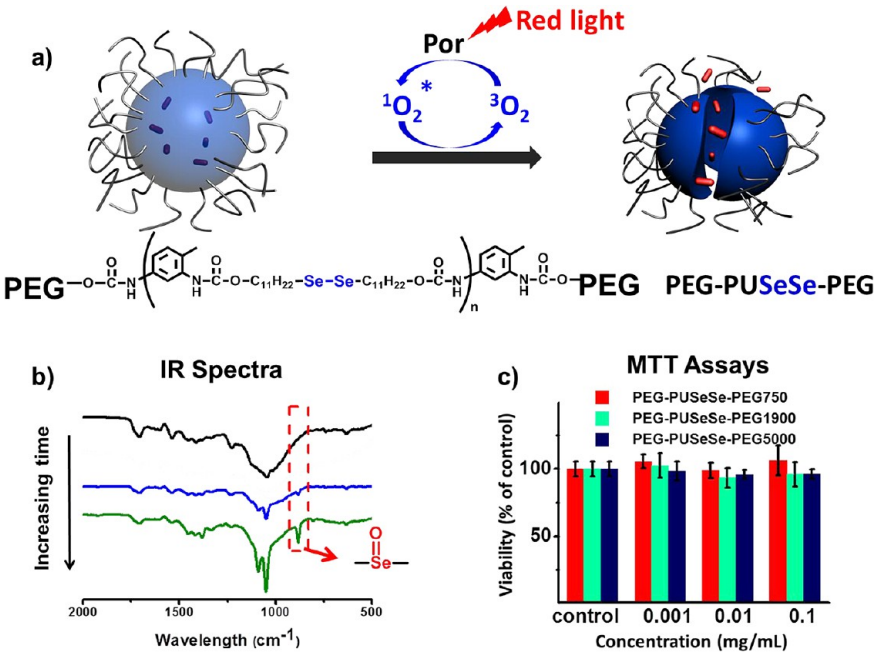


FIGURE 3. (a) Red light responsive PEG-PUSeSe-PEG micelles: red light can trigger the disassembly by producing singlet oxygen in the presence of porphyrin derivatives, thus achieving controlled release of Dox. (b) IR spectra of PEG-PUSeSe-PEG750 under red light of increasing time. (c) MTT assays using L-02 cells after 24 h exposure to the polymer solution.

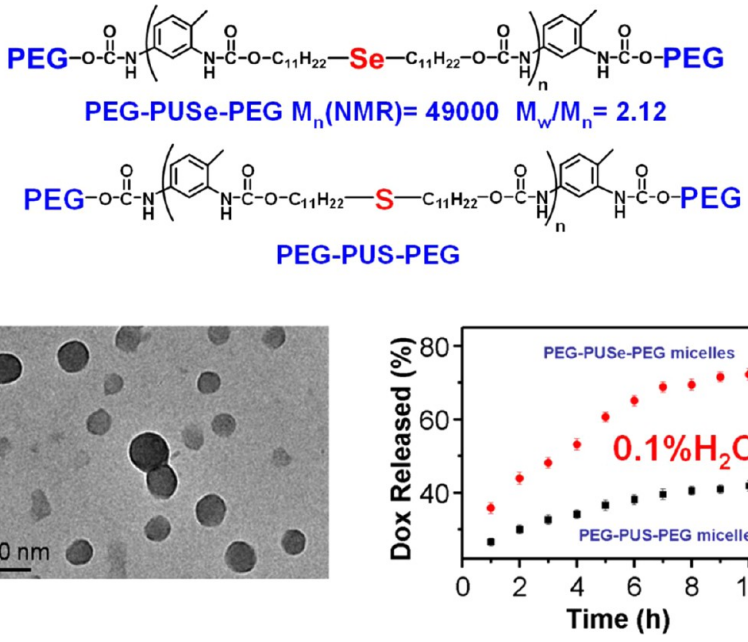


FIGURE 4. Oxidation-responsive aggregates formed from PEG-PUSe-PEG and PEG-PUS-PEG. The lower left of the figure is the TEM image of aggregates formed by PEG-PUSe-PEG. The lower right is oxidation-responsive release studies of Dox from PEG-PUSe-PEG micelles and PEG-PUS-PEG.

2.2. Monoselenide-Containing Main Chain Block Copolymers. Amphiphilicity is one of the molecular bases for self-assembly. By tuning the amphiphilicity of building blocks, controllable self-assembly and disassembly can be realized.[17] In the above section, the diselenide bonds on the polymer backbone can be cleaved upon redox response or irradiation. Thus, the amphiphilicity of the diselenide-containing block copolymer has been completely changed and the encapsulated drugs can be released upon the structure disruption. In fact, it is not necessary to have bond cleavage on the polymer backbone to realize controlled drug release. Hydrophobic dialkyl selenide groups intend to

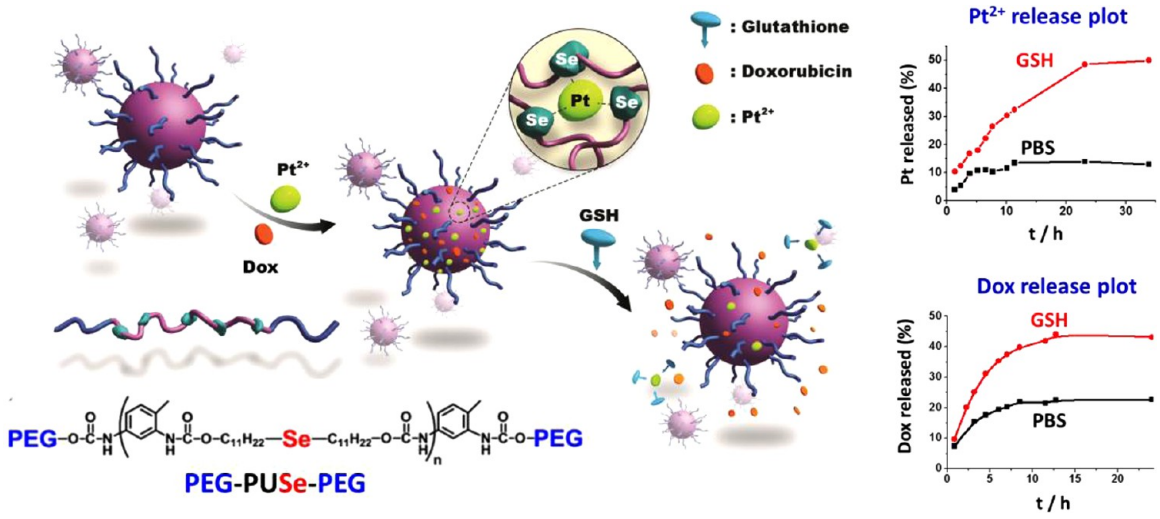


FIGURE 5. Coordination of Pt^{2+} to PEG-PUSe-PEG micelles and controlled release of Dox triggered by the disassembly of Pt^{2+} through competitive coordination with glutathione. Reproduced from ref 32. Copyright 2012 The Royal Society of Chemistry.

form hydrophilic selenoxide or selenone groups in an oxidative environment. Therefore, the balanced amphiphilicity of the dialkyl selenide-containing block copolymers can be destroyed and aggregates formed by the block copolymers are disassembled consequently upon oxidation.

The synthetic strategy of monoselenide-containing amphiphilic block copolymers PEG-PUSe-PEG is similar with that of PEG-PUSeSe-PEG.[30] PEG-PUSe-PEG is able to self-assemble in aqueous solution to form micellar aggregates, as shown in Figure 4. The aggregates have a good oxidation-responsiveness and undergo a structural dissociation in a mild oxidative environment (such as 0.1% H_2O_2 v/v) due to the unique sensitivity of selenide groups in presence of oxidants. The oxidation induces the formation of selenone groups, which has greatly changed the amphiphilicity of the selenium-containing block copolymer and enhanced its solubility in water, thus resulting in the oxidation-responsive disassembly of the formed aggregates. Compared with the dialkyl sulfide-containing analogue PEG-PUS-PEG, PEG-PUSe-PEG is more sensitive to oxidants. Most of the selenide groups can be converted to selenones (O=Se=O), but only a very small portion of sulfide groups can be converted to low oxidation state (S=O) at the same condition within 5 h oxidation. The drug release profile also shows that the PEG-PUS-PEG aggregates exhibit a slower release rate and a lower release percentage of Dox, as compared with the PEG-PUSe-PEG block copolymer. In other words, selenium-containing block copolymers are advantageous in terms of mild responsive to oxidants compared with sulfur-containing block copolymers.

Since there are no bond cleavages upon the oxidation like PEG-PUSeSe-PEG, we are able to employ atomic force microscopy-based single molecular force spectroscopy (SMFS) to give further insight into the oxidation induced disassembly mechanism of PEG-PUSe-PEG polymer micelles.[31] SMFS experiments on PEG-PUSe-PEG and PEG-PUSeox-PEG in water and DMSO suggest that the variation from selenide to oxidized selenone contributes significantly to the change in amphiphilicity, which leads to the disappearance of the supramolecular micellar structure. This line of research provides new information about how much small changes in the chemical structure can significantly influence the amphiphilicity as well as the assembling behavior of selenium-containing amphiphilic block copolymers on the single-molecule level.

Apart from unique redox properties, selenium is also a good coordination ligand. By utilizing a mediator of Pt^{2+} that performs coordination to PEG-PUSe-PEG micelles and subsequent release of Pt^{2+} by competitive coordination with GSH, as shown in Figure 5, we have successfully prepared a coordination-responsive system for controlled release of Dox.[32] Pt^{2+} and selenium are found to coordinate together with a 3:1 stoichiometry by employing di-(1-hydroxylundecyl) selenide as a model compound. The PEG-PUSe-PEG polymers can coordinate with Pt^{2+} in a similar manner with the small model compound and form spherical micelles in aqueous media. In the presence of 10 mM GSH, the platinum can be released from the micelles in a controlled manner through competitive coordination with GSH. The coordination-responsive micelles can be used to load Dox and release them under

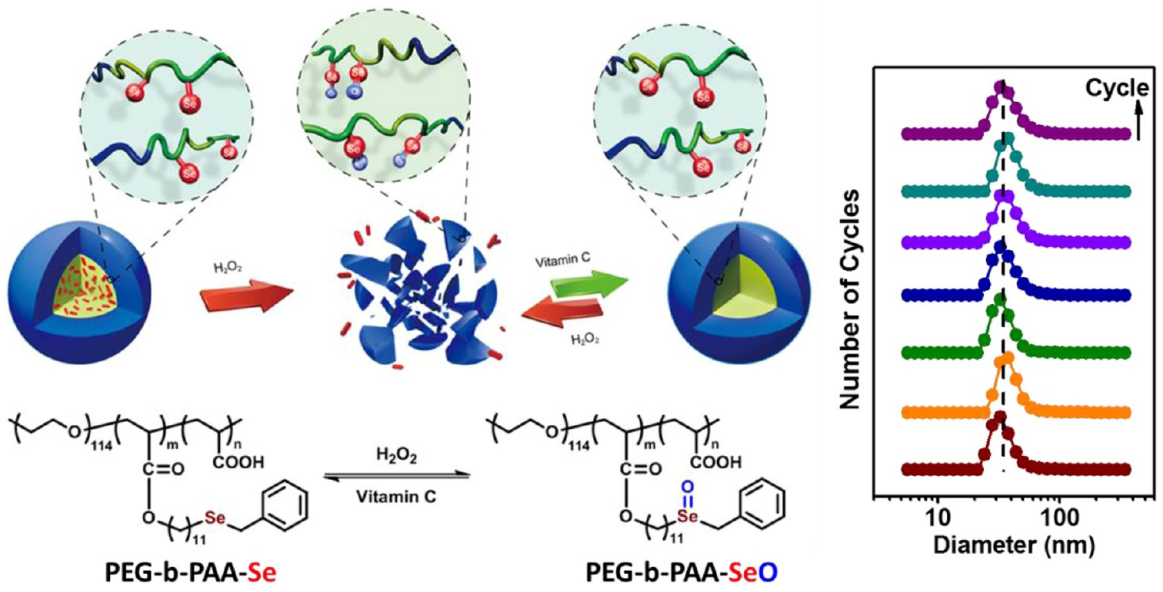


FIGURE 6. Reversible redox-controlled self-assembly and disassembly of PEG-b-PAA-Se. The right of the figure is the reversibility of the micelles over seven oxidation−reduction cycles based on DLS results. Reproduced from ref 33. Copyright 2012 The Royal Society of Chemistry.

the stimuli of GSH. With a stronger coordination ligand dithiothreitol (DTT) to Pt^{2+}, the release percentage of encapsulated Dox will be higher and the release speed will be much faster, further confirming the coordination-responsive release mechanism. In addition, the coordination micelles are biocompatible as shown by MTT assays in HUVEC cells and HepG2 cells. To the best of our knowledge, few successful coordination responsive examples have been reported previously for controlled drug release. With platinum-containing drugs coordinated with the block copolymer, for example, cisplatin, multidrug systems for cooperative chemotherapy may be achieved by encapsulating other anticancer drugs simultaneously.

3. Side-Chain Selenium-Containing Block Copolymers

Besides selenide-containing main-chain polymers, there are side-chain selenium-containing block copolymers which can be fabricated in a covalent or noncovalent (supramolecular) approach. In the meantime, there is an increasing interest in developing reversible methods for tuning amphiphilicity, and by these methods the molecular amphiphilicity is expected to be reversible tuned by stimuli-responsive groups attached on the amphiphiles, thus providing ways to realize the control of self-assembly.

3.1. Covalent Side-Chain Selenium-Containing Polymers. In order to combine the advantage of structure control and stability, we have designed and synthesized a new type of side-chain selenide-containing amphiphilic poly(ethylene oxide-*b*-acrylic acid) block copolymers, PEG-*b*-PAA-Se in short, as shown in Figure 6.[33] In aqueous solution PEG-*b*-PAA-Se can self-assemble into uniform and stable micelles. One oxygen atom can be added to selenium to form selenoxide upon the oxidation by dilute hydrogen peroxide, leading to the disassembly of the micelles. Due to the steric hindrance of the benzyl group, PEG-*b*-PAA-Se cannot be further oxidized to selenone. More interestingly, this oxidation process can be reversed upon the addition of mild reductant such as vitamin C, which endows it with good reversibility. This reversible oxidation and reduction process can be repeated at least seven cycles as indicated by DLS studies. Vitamin C is an essential nutrient for humans and naturally present in the human body. Therefore, it is highly desired that this type of side-chain selenide-containing amphiphilic block copolymers can be potentially used as regenerable and benign biomaterials.

3.2. Noncovalent Side-Chain Selenium-Containing Polymers. Besides the covalent strategies, there is a supramolecular approach that can be used to prepare side-chain selenium-containing polymers on the basis of noncovalent interactions. In the noncovalent approaches, double-hydrophilic block copolymers with ionic and nonionic water-soluble segments and oppositely charged polyions or surfactants are mixed together. A polymeric supramolecular amphiphile or supra-amphiphile is then formed (Figure 7). The advantages of this supramolecular approach include the avoidance of organic solvents, a simple preparation

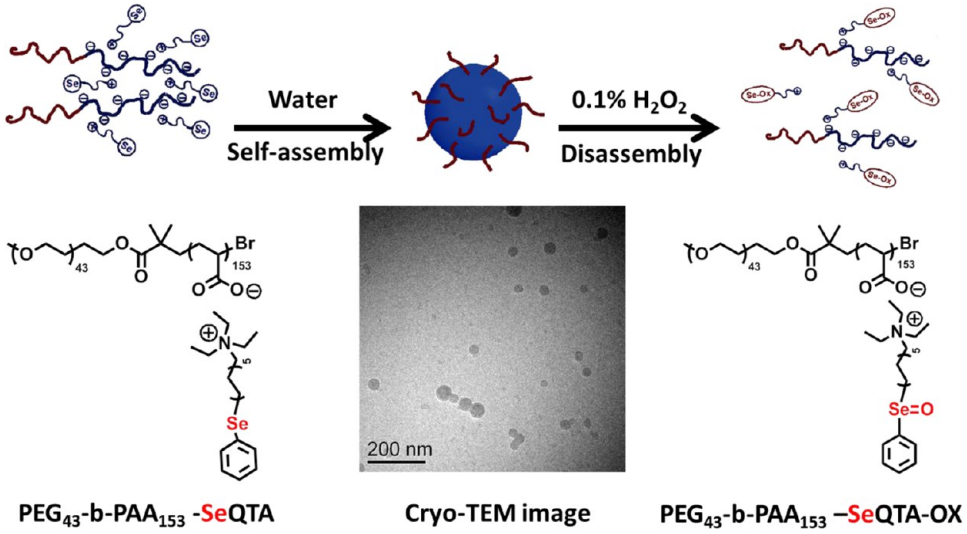
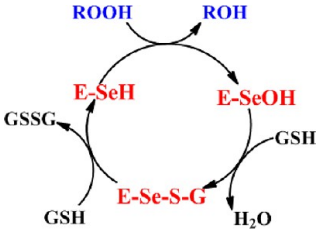


FIGURE 7. Oxidation-responsive micelles based on a side-chain selenium-containing polymeric supra-amphiphile formed through electrostatic interaction. Reproduced from ref 39. Copyright 2010 the American Chemical Society.

SCHEME 2. Scheme of the GPx Catalyzed Reduction of a Variety of Hydroperoxides (ROOH), Using Glutathione (GSH) as the Reducing Substrate



procedure, and the decrement of surfactants.[34−38] The selenium-containing side-chain polymeric supra-amphiphile can be fabricated on the basis of electrostatic interaction of PEG-*b*-PAA and a selenium-containing surfactant (SeQTA).[39] PEG-*b*-PAA is highly water-soluble and cannot form aggregates in aqueous solution. However, upon the complexation with SeQTA to form the polymeric supra-amphiphiles, it is able to self-assemble to form micelles in solution. The micelles can be disassembled with the addition of oxidant because SeQTA is very sensitive to oxidation, inducing amphiphilicity variation from hydrophobic −Se− to relative hydrophilic −SeO−. Moreover, the simple backbone of selenium-containing surfactants allows us to control the function of the obtained aggregates precisely by varying the structure or amount of surfactants.

4. Dendritic Selenium-Containing Polymers

Glutathione peroxidase (GPx) is a mammalian antioxidant seleno-enzyme that protects biomembranes and other cellular components from oxidative damage by catalyzing the reduction of a variety of hydroperoxides (ROOH), using GSH as the reducing substrate (Scheme 2).[4−6] The catalytically active center of GPx, selenocysteine, is located in the hydrophobic cavity of the protein surface. Inspired by the structure of native GPx, one of the strategies to design GPx mimics with high efficiency is to consider the substrate binding and mimic the catalytic hydrophobic environment. Dendritic polymers, including dendrimers and hyperbranched polymers (HBPs), are the fourth major polymer architecture following the linear, branched and cross-linking polymers.[40−42] By deliberately introducing selenium into the dendritic polymers and taking full use of the microenvironment provided by the dendritic structures, highly efficient GPx mimics can be developed.

4.1. Hyperbranched Selenium-Containing Polymers. HBPs, an important member of the dendritic polymer family, have attracted increasing scientific and industrial attention in recent years due to their unusual chemical and physical properties.[40] A novel hyperbranched polyselenide with selenide at the branching units HBPSe was successfully prepared by our group.[43] We used a direct polymerization of NaHSe (as the AA′ monomer) and 1,3,5-tris-bromomethyl-2,4,6-trimethyl-benzene (as the B3 monomer) in a ratio of 1:1, leading to new hyperbranched polyselenides HBPSe as shown in Figure 8. Due to the multicatalytic sites at the branching units, the novel hyperbranched polyselenide HBPSe as GPx mimic are more efficient than the small analogs. The initial reduction rate of the hyperbranched polyselenides is around 3.7 times that of the comparative organic monoselenide BDB-Se.

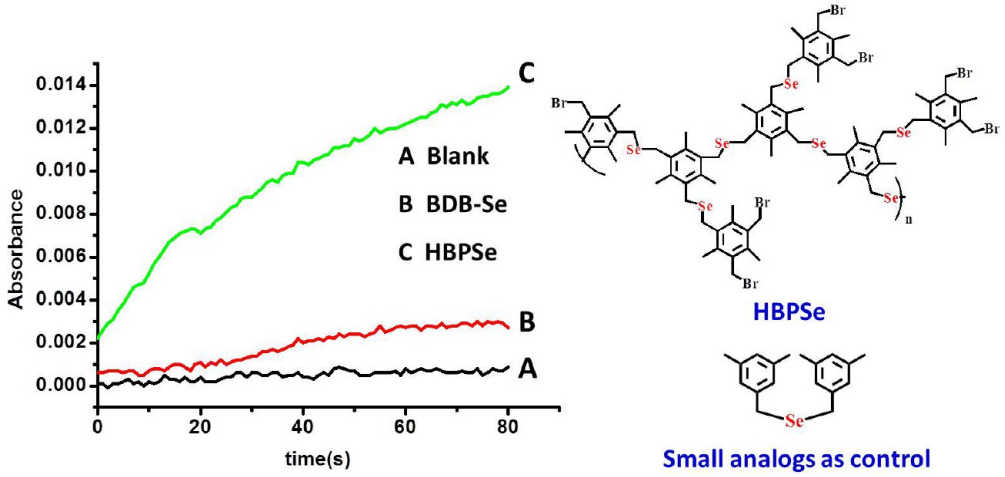


FIGURE 8. Hyperbranched polyselenides as glutathione peroxidase mimics. Plots of the absorbance at 305 nm vs time during the catalytic reduction of H_2O_2 (0.25 mM) by PhSH (1 mM) in a solvent mixture of 1:9 chloroform−methanol. Catalysts: (A) Control; (B) BDB-Se 1.32 mg per 100 mL; (C) HBPSe 1.32 mg per 100 mL.

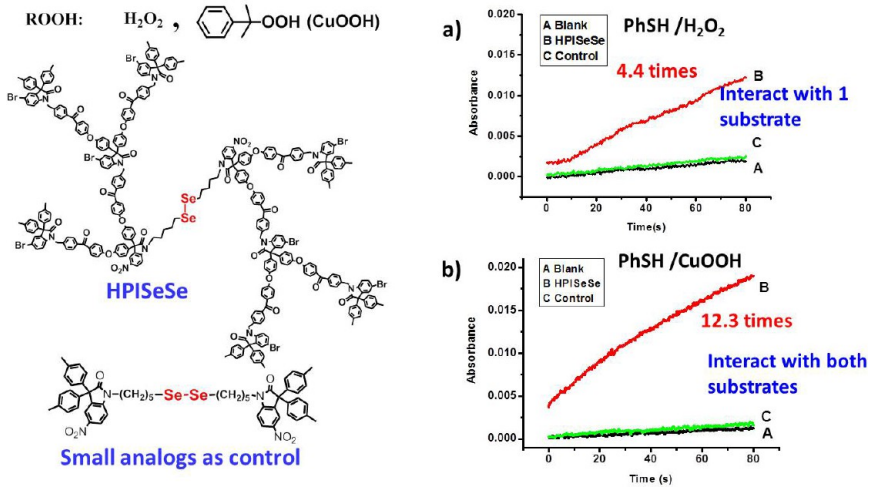
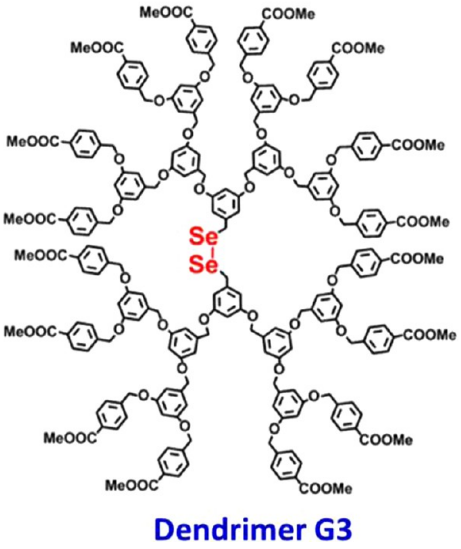
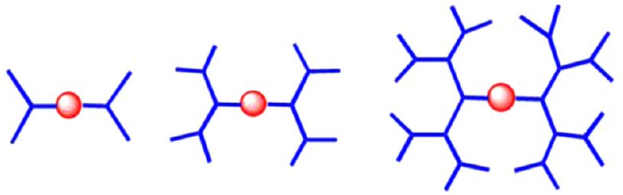


FIGURE 9. Fully branched hyperbranched polymer with a diselenide core as glutathione peroxidase mimics. The right of the figure is plots of the absorbance at 305 nm vs time during the catalytic reduction of ROOH (2 mM) by PhSH (1 mM) in a solvent mixture of 4:6 chloroform/methanol. ROOH: H_2O_2 (a) and CuOOH (b).

Clearly, the incorporation of numerous catalytic groups in the macromolecule is important for the higher activity of the hyperbranched polyselenides (HBPSe). The local concentration of selenium in the hyperbranched polymer is very high. We think the advantage of hyperbranched polyselenides during the catalysis may result from an increased chance for the substrate to get close enough to an active catalytic site. The normal way to use HBPs as catalysts is to functionalize the peripheries with catalytic groups. Few efforts have been made to introduce catalytic groups at the inner branching units. To the best of our knowledge, this may be the first successful example of incorporating catalytic sites onto the skeleton of the hyperbranched polymer. Further water solubilization can be easily achieved by postsynthetic modification of the periphery, which is important for real use as an antioxidant drug.

Apart from the hyperbranched polymers with catalytic selenide groups at the branching units, we have also synthesized hyperbranched polymer with diselenide as the core. HPISeSe with 100% branching degree was successfully prepared by introducing diselenide catalytic center at the focal point of the polymer through post synthetic modification (Figure 9).[44] HPISeSe has demonstrated prominent GPx activity as desired compared with an analogous small molecule, which can be attributed to the hydrophobic, densely branched and core−shell structure of the polymer





catalyst	v_0 (μ M.min^{-1}) CHCl3/MeOH 3:7	CHCl3/MeOH 7:3	CHCl3/MeOH/H2O 3:6.5:0.5
none	0.97 (0.06)	0.71 (0.07)	1.15 (0.13)
G1	4.07 (0.65)	3.29 (0.47)	3.91 (0.86)
G2	8.19 (1.03)	3.61 (0.53)	158.39 (27.89)
G3	2431.20 (46.50)	5.39 (0.68)	2966.48 (167.71)

FIGURE 10. Highly efficient dendrimer-based mimic of glutathione peroxidase. Initial reduction rates of H_2O_2 (2 mM) with PhSH (1 mM) in the presence of various dendrimer catalysts (0.01 mM) in different solvent mixtures.

surrounding the catalytic center. As shown in Figure 9, when the polymer has $\pi-\pi$ interaction with only thiol substrate, the initial reduction rate is 4.4 times that of the model compound. It becomes 12.3 times that of the model compound when the polymer has $\pi-\pi$ interaction with both the thiol and peroxide substrates. The study demonstrates that post modification of the focal position of the hyperbranched polymer is feasible and efficient, affording a new approach to functionalized hyperbranched polymers, though their GPx activity is not satisfactory.

4.2. Dendritic Selenium-Containing Polymers. The structures of dendrimers can be precisely controlled at the molecular level,[41,42] resulting in a well-defined microenvironment, to achieve high GPx activity.[45] For this purpose, we have designed and synthesized a series of Fréchet-type poly(aryl ether) dendrimers with a diselenide core as shown in Figure 10. We measured the catalytic activity of the synthesized dendrimer mimics of GPx using benzenethiol (PhSH) as a glutathione alternative. For the same solvent mixture of chloroform/methanol (3:7, volume ratio), dendrimers G1 and G2 show relatively low GPx activity. However, for dendrimer G3, we have found interestingly that the initial rate can be as high as 2431.20 μM min^{-1}. To the best of our knowledge, this rate is the highest among the organic systems mimicking GPx until now. The high GPx activity of G3 originates from the hydrophobic microenvironment provided by its macromolecular structure. The binding constants of dendrimers G1, G2, and G3 with the reaction substrate PhSH are 16.4, 39.4, and 252.7 M^{-1}, respectively, with a slight increase from the first to the second generation but a dramatic increase from the second to the third generation, indicating that dendrimer G3 contains a favorable microenvironment for the catalytic cycle.

The microenvironment of dendrimers can be further adjusted by solvent mixture. As shown in Figure 10, with less good solvent (chloroform), the activity of G3 reached as high as 2431.20 μM min^{-1}, indicating that G3 should adopt a more globular conformation that is more favorable for the catalytic cycle. The GPx activity can be even higher after adding 5% water into the solvent mixture. The enhanced GPx activity after adding water may indicate that hydrophobicity plays a role in the catalytic reaction. Normally, steric hindrance resulting from the encapsulation of catalytic groups in the core within the dendrimer significantly leads to slower kinetics. Our results have demonstrated a notable exception that substrate binding ability and microenvironment effects are paramount to achieving high reactivity. This is also a rare example that shows positive dendritic effect with the increase of the generation the catalytic activity increases concomitantly.

5. Conclusions and Outlook

This Account has summarized recent progress on design and synthesis of selenium-containing polymers for responsive drug delivery vehicles and artificial enzymes. It also sheds light on how the topology of selenium-containing

polymers on its molecular level can affect the functional performance of the self-assemblies on the supramolecular level.

Some basic challenges still remain unsolved before selenium-containing polymers can be put into real application. The first challenge is to find out the in vivo biological effect of selenium-containing polymers, which needs the joint effort from scientists of chemistry, biology, and medicine. As selenium-containing compounds are closely related to the concentration of reactive oxygen species (ROS) and that ROS plays an important role as regulatory mediators in signaling processes at moderate concentrations, and can induce cellular apoptosis at high concentrations, we need to pay attention to how selenium-containing polymers of different structures affect the intracellular ROS concentration. It will help elucidate a few important issues that concern the physiological metabolism, biocompatibility, and possible toxicity. In addition, it may help to gain more insight about their antioxidant properties, thus facilitating the development of efficient antitumor and other drug delivery systems. The second challenge is to deeply understand the physical and chemical nature of Se···X (X denotes nitrogen, oxygen or halogen atoms),[46] because intramolecular Se···X noncovalent interactions play important roles in realizing the function of selenoproteins. It will help the rational design of the molecular structures of selenium-containing polymers toward advanced functions. The third challenge lies on how to finely control the dynamic nature of C−Se or Se−Se bond between seleno-enzymes or seleno-peptides with other chemical species and then finely tune their biological functions. Disulfide-containing systems and Diels−Alder reaction systems are well studied dynamic covalent bond systems, showing promising application as self-healing materials. The research of Se-containing systems can learn much from them in this regard.

Selenium-containing polymers have provided advantageous features (e.g., high sensitivity, programmability, reversibility, and dynamics) for supramolecular soft materials, but we should not forget that this field is still in its infancy. It seems that this line of research can only be limited by our imagination. It will enrich the selenium chemistry from the molecular level to the supramolecular level. Moreover, it is highly anticipated that selenium-containing polymers may serve as a new bridge between material sciences and life sciences.

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FOOTNOTES

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The authors declare no competing financial interest.

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