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Selected Papers

Reductive Glutathione-Responsive Molecular Release Using Water-Soluble POSS Network Polymers

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We describe in the manuscript reductive glutathione (GSH)-responsive molecular release using polyhedral oligomeric silsesquioxane (POSS) network polymers. A series of the POSS network polymers with the different crosslinking ratios containing disulfide linkers were synthesized, and molecular encapsulation by the polymers was executed. We found that the POSS polymers encapsulated the molecules in aqueous solutions. On the contrary, the reduced polymers hardly retained the guest molecules. By using the change of the encapsulation ability of the polymers via reduction, we demonstrated that the dye-loaded network polymers released the encapsulated molecules. Finally, we were able to detect GSH with intercellular concentration by the increase of the intensity of fluorescence emission.

Hydrophilic network materials, which can encapsulate guest molecules, are a versatile platform as a biosensor or a vessel for drug release because of their flexibility in molecular design.[1–4] Since the size, internal properties, and surface functionality can be tuned by changing the chemical structure, molecular weight, and crosslinking ratio of the polymers, the target selectivity, the stimuli-responsiveness, and site- or time-specificity of drug delivery can be readily modulated.[5–7] Thus, such network materials have large potentials to satisfy various demands from biological studies or the practical purposes in vivo.[8–10]

We have previously reported water-soluble polymers composed of polyhedral oligomeric silsesquioxane (POSS).[11] The POSS moiety inside these materials can create a distinct space because of the strong hydrophobicity and rigidity of POSS and sustain hydrophobic guest molecules inside the network.[12–15] Consequently, the loaded fluorescent molecules in the POSS-containing polymeric materials can show different optical properties from those in bulk water.[11,16–20] Our next interest is directed to apply the changes of optical properties as a bioprobe. Hence, stimuli-responsiveness for molecular release by recognition of biologically significant molecules is desired.

Herein, we report a molecular releasing system using POSS-containing network polymers triggered by reduction. Water-soluble network polymers containing disulfide linkers were prepared, and the change of the encapsulation ability via reduction was evaluated with reductive glutathione (GSH) which is a class of biological reducing agent and plays a key role in maintaining the cellular reductive environment.[21] Finally, we demonstrate that the dye-loaded network polymers were able to work as a GSH sensor which increased the intensity of fluorescence emission.

Experimental

General. ^{1}H NMR and ^{13}C NMR spectra were measured with a JEOL EX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer. ^{29}Si NMR spectra were measured with a JEOL JNM-A400 (80 MHz) spectrometer. Coupling constants (J value) are reported in Hertz. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual dimethyl sulfoxide ($\delta = 2.50$ in ^{1}H NMR, $\delta = 39.5$ in ^{13}C NMR) as an internal standard. Emission from the samples was monitored using a Perkin-Elmer LS50B at 25 °C using 1 cm path length cell. The dynamic light scattering (DLS) measurements were carried out at 90° scattering angle and 25 ± 0.2 °C using a FPAR-1000 particle analyzer with a He-Ne laser as a light source. The CONTIN program was used for data analysis to extract information on the average hydrodynamic size. MASS spectra were obtained on a JEOL JMS-SX102A.

Polymerization. The typical protocol for the polymerization reaction is described here. To a solution of octaammonium-POSS chloride[22–24] (117.4 g, 0.10 mmol) in 5 mL of water, the premixed solutions containing 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM, 90%), 3,3′-dithiopropionic acid, and 2 equivalent of triethylamine to 3,3′-dithiodipropionic acid in water (5 mL) were added, and the reaction mixture was stirred at room temperature. After stirring for 24 h, the resulting mixture was poured into acetonitrile containing 0.1% HCl, and the precipitation was washed with acetonitrile. The POSS polymers were obtained as a white powder after drying in vacuo. The yields are shown in Table 1 in the content.

Determination of the Crosslinking Ratio. From ^{1}H NMR spectra of the polymerization products in DMSO, the integral ratio was determined from the peaks at 1.7 and 1.5 ppm

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assigned as the 2-position of the hydrogen atoms before (H_a) and after (H_b) the amide bond formation, respectively. The crosslinking ratio was calculated according to the following eq 1.

$$\text{The crosslinking ratio (\%)} = (H_b - H_a)/H_b \times 100 \quad (1)$$

Fluorescence Measurements. Samples were prepared by mixing the polymers (10 mg mL^{-1}) with the guest molecules (×10 concentration) at 25 °C, and then water and PBS (×10) were added. All measurements were executed within 10 min after the sample preparation. Fluorescence measurements were executed with the aqueous solutions containing the POSS polymer (1 mg mL^{-1}) and respective concentrations of the guest molecules in PBS at 25 °C. The quantum yields were calculated as a relative value.

Evaluation of the Amount of the Encapsulated Guest Molecules into the Polymers. The solutions containing the guest molecules and polymers (1 mg mL^{-1}) were prepared in PBS buffer and allowed to equilibrate at ambient conditions for 30 min. The samples were filtered through Nanosep 3K centrifugal devices (Pall Life Sciences) by centrifugation (2000*g*, 30 min, 25 °C), and the concentration of the dyes was determined from the light absorption of the filtrates.

Reduction of POSS Polymers with GSH. The reduction reactions of POSS polymers were executed in the presence of 5 mM GSH in PBS at 25 °C. The data of fluorescence and DLS measurements were obtained immediately after adding GSH into the solutions. The amounts of the encapsulated molecules were determined with the same procedure described above.

Table 1. Physicochemical Properties of the POSS Polymers

Polymer	Feed ratio of dithiodipropionic acid/equiv	Yield /%	Crosslinking ratio /%[a)]	Solubility in water /mg mL^{-1}
SS2	2	41	29	10
SS3	3	51	39	10
SS4	4	67	62	<1

a) All values were determined from the ^{1}H NMR spectra in Figure 1 according to the eq 1.

Results and Discussion

Scheme 1 illustrates the synthesis of the POSS-containing water-soluble network polymers. The polymerization reactions with octaamino-POSS[22–24] which promised to be the capturing unit for various guest molecules by the hydrophobic interaction and various equivalents of 3,3′-dithiodipropionic acid were executed in water in the presence of triethylamine and DMT-MM as a condensation reagent. After stirring for 24 h at ambient temperature, reprecipitation in acetonitrile with 0.1% HCl and washing with acetonitrile afforded the chloride salts of the polymers as a white powder.

To evaluate the crosslinking ratios in the network polymers, we determined the conversion of amino groups to amide groups from the comparison with the integration areas of hydrogen atoms at the 2-position in the POSS moieties in the ^{1}H NMR spectra (Figure 1). The conversion correspondingly increased by an increase of the feed ratios of dithiodipropionic acid

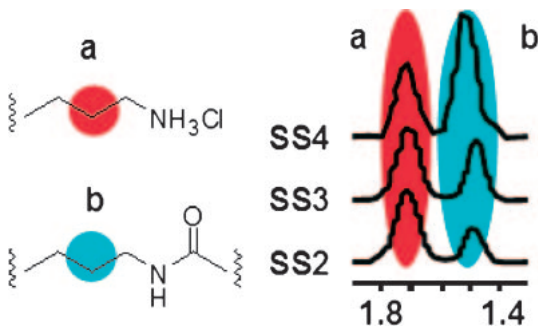
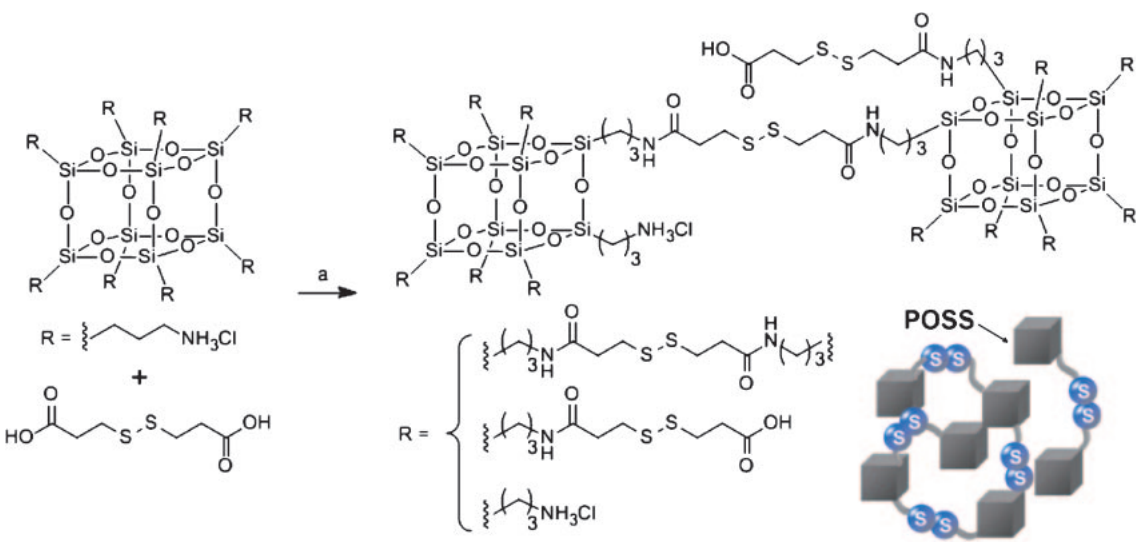


Figure 1. ^{1}H NMR spectra (400 MHz, DMSO) of the polymerization products. The signal peaks were assigned as the 2-position of the hydrogen atoms at the aminopropyl linker. After the amide bond formation of the amino groups in octaammonium-POSS, the peak was shifted from 1.7 to 1.5 ppm. The crosslinking ratio was calculated from the integral ratio of these peaks.



Scheme 1. Synthetic scheme of the POSS monomers and polymers. Reagents and conditions: (a) DMT-MM, triethylamine, water, 25 °C, 24 h.

Table 2. Optical Properties of CCVJ Entrapped into the POSS Polymers[a)]

POSS compounds	Emission intensity /arb unit	Φ_F[b)]
No	37	0.0024
Octaamino-POSS	39	0.0028
SS2	110	0.0086
SS3	160	0.0104
SS4	—[d)]	—[d)]
SS3+GSH[c)]	138	0.0090

a) Emission intensities at peak tops and quantum yields were obtained from the solution containing 10 μM CCVJ with 1 mg mL^{-1} the POSS polymers in PBS with the excitation at 430 nm at 25 °C. b) Quantum yields were calculated as a relative value. c) GSH concentration was 5 mM. d) The poor solubility of SS4 in water inhibited obtaining data.

(Table 1). These data indicate that the crosslinking ratios can be tuned at the polymerization. The increase of the crosslinking ratio reduces the solubility of the polymers. It is likely that the conversion of amino groups by the formation of amide bonds should decrease water-solubility of the polymers.

To investigate the mobility of the loaded guest molecules in the polymers, the change of optical properties of carboxy julolidine (CCVJ) (Figure 2a)[25,26] was monitored. By using the size-exclusive filtration experiments for evaluating the amount of noncaptured CCVJ according to the experimental protocols, it was confirmed that most CCVJ molecules can be encapsulated into the polymers. Fluorescence emissions from CCVJ in PBS after encapsulation into the polymers were greatly enhanced in the presence of POSS network polymers (Figure 2b and Table 2). In contrast, the emission spectrum was less significantly influenced in the presence of octaamino-POSS. In addition, it was found that most of the CCVJ was suspended into the polymers. This shows that the formation of POSS networks can significantly enhance the ability for the encapsulation and the suppression of the rotation of the encapsulated molecules. From the comparison of the fluorescence intensities between the complexes of CCVJ with SS2 and SS3, the increase of the crosslinking ratio suppressed the molecular rotation of CCVJ. The relatively higher condensed POSS moieties could effectively disturb the free motion of the encapsulated molecules.

Next, we investigated the reactivity of the network polymers to GSH. Signal intensity immediately decreased by the addition of GSH with 5 mM concentration for mimicking the cellular environments (Figure 2c). However, the amount of the loaded CCVJ inside the network polymers was not influenced during the reduction. This indicates that the linker scission can decrease the packing density of the networks, and then the recovered molecular rotation of CCVJ was monitored by the decrease of fluorescence emission.

The changes of the hydrodynamic radii (r_H) of the network polymers in PBS before and after the addition of 5 mM GSH were monitored from DLS measurements, and the results are shown in Figure 3. The r_H values of the network polymer were significantly reduced by the addition of GSH. These data

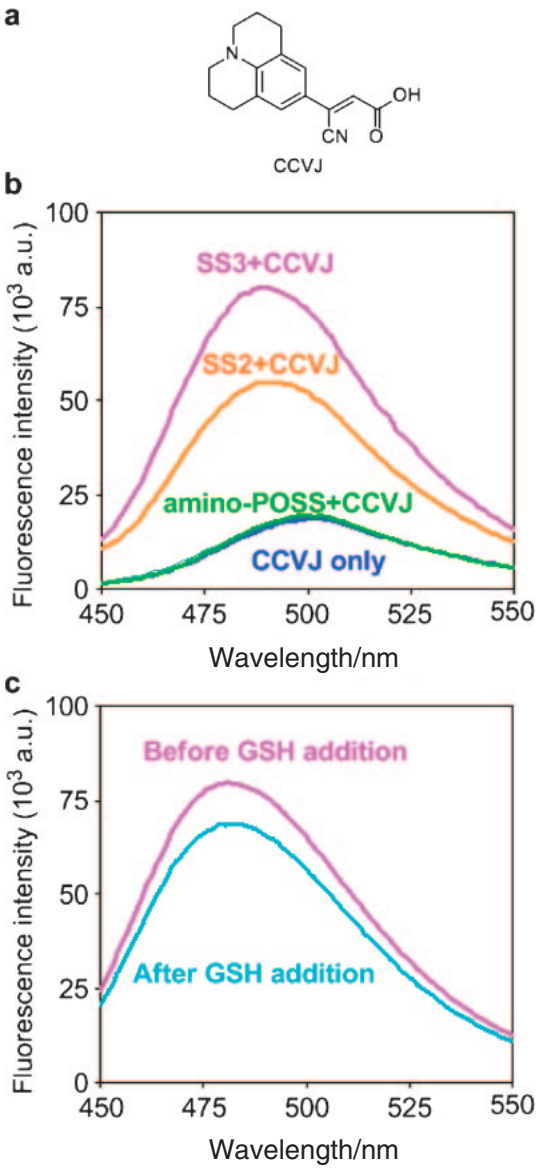


Figure 2. (a) Chemical structure of CCVJ. (b) Fluorescence spectra of the sample containing 10 μM CCVJ (blue line) and 1 mg mL^{-1} of octaamino-POSS (green line), SS2 (yellow line), and SS3 (magenta line) in PBS, measured at 25 °C with the excitation at 430 nm. (c) Fluorescence spectra of the complex with SS3 and 10 μM CCVJ in the absence (magenta line) and presence (light blue line) of 5 mM GSH.

suggest that the disulfide bonds in the linkers should be cleaved by the reduction with GSH.

The chemosensors for monitoring the GSH concentration should be feasible for further understanding GSH metabolism and for developing conventional diagnostic or research tools on these significant biological events.[27–29] The imaging probes which show the increase of the intensity of the fluorescence emission after the recognition of the targets are favorable because the background noise can be excluded by adjusting the threshold level.[30] Therefore, in order to simplify the detection of GSH, we designed the system for monitoring GSH by a positive signal illustrated in Scheme 2. Ferrocene carboxylic acid (Fc–COOH) can work as a quencher for the emission from

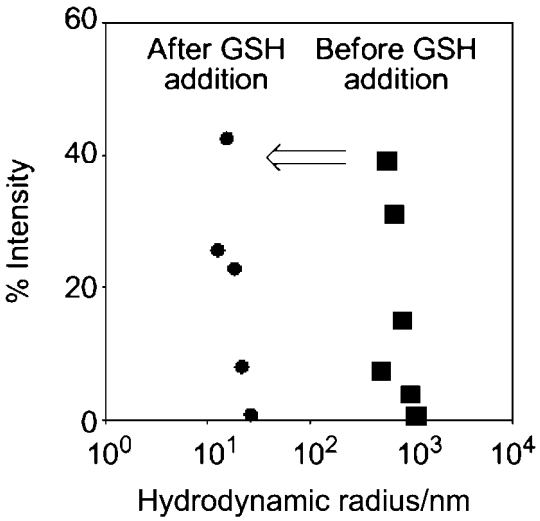


Figure 3. The hydrodynamic radii (r_H) of the network polymers determined from the DLS measurements at 25 °C in the absence (square dots) and presence (circular dots) of 5 mM GSH in PBS.

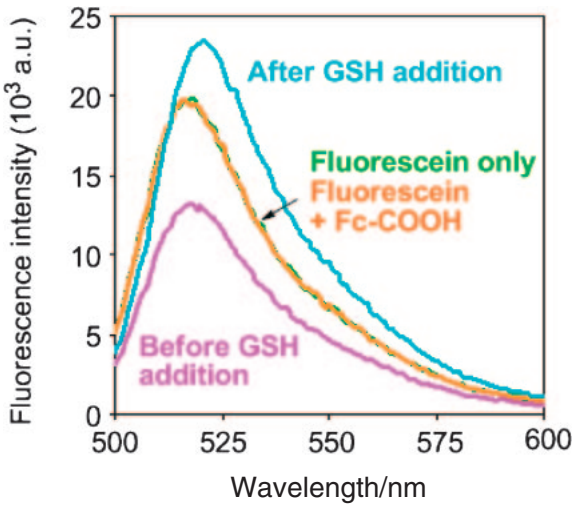
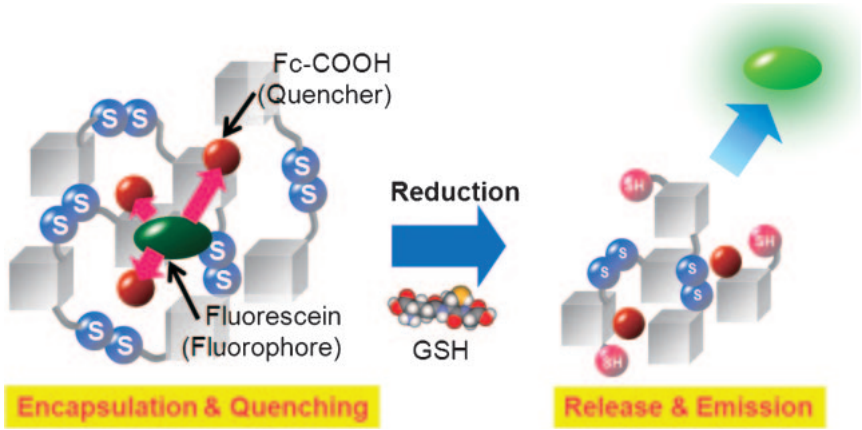


Figure 4. Fluorescence spectra of the samples containing 100 μM fluorescein (green line), 2 mM Fc–COOH (yellow line), and 1 mg mL^{-1} of SS3 in the absence (magenta line) and presence (light blue line) of 5 mM GSH in PBS, measured at 25 °C with the excitation at 490 nm.



Scheme 2. Schematic illustration for the detection of GSH.

fluorescein.[31] Thereby, in the polymers containing both fluorescein and Fc–COOH, fluorescence emission from fluorescein can be strongly suppressed. The quenching process could occur by electron transferring in which the quenching efficiency should be significantly dependent on the intermolecular distances.[32] Thus, it can be expected that the fluorescence emission from fluorescein could be recovered after releasing because of long intermolecular distances from the quencher. Fluorescence emission from the solution containing 100 μM of fluorescein with or without 2 mM Fc–COOH showed similar intensities (Figure 4). In contrast, in the presence of 1 mg mL^{-1} of the network polymers, the fluorescence intensity decreased. These results suggest that the quenching effect should be enhanced by the encapsulation of dyes and quenchers because of the condensed states inside the network polymers. By adding 5 mM GSH in the solution, the fluorescence emission increased. The amounts of loaded dyes in the network polymers were determined in the same manner. Before the reduction, 58% of fluorescein and 68% of Fc–COOH were encapsulated into SS3. The proportions of the encapsulated molecules significantly decreased to 14% and 30%, respectively. These data clearly indicate that dyes and quenchers were released after the addition of GSH. In addition, these results mean that the encapsulation ability of the network polymers should be decreased by the reduction. The distances between dyes and quenchers averagely increased, and the fluorescence emission can be recovered as we expected.

Conclusion

In conclusion, we synthesized water-soluble network polymers in which POSS moieties are linked via disulfide bonds. The dye-loaded network polymers released the encapsulated molecules triggered by the reductive scission of the disulfide linkers. Finally, we detected GSH with intercellular concentration by the increase of the intensity of fluorescence emission. POSS-based network polymers can encapsulate a variety of molecules including hydrophobic drugs, dyes, and bioactive materials. Thus, our system described here could be applicable for biosensors, site-specific bioimaging probes, and target therapeutic tools.

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