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Controlled Release of Guest Molecules from Spherical Assembly of Trigonal Gultathione by Disulfide Recombination

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Nanospheres formed by self-assembly of trigonal glutathione (**TG**) underwent disulfide recombination upon addition of dithiothreitol. Guest molecules encapsulated in the nanospheres were gradually released during the recombination process.

In biological systems, many redox reactions involving disulfide bonds play pivotal roles in protein folding and consequently in the control of protein functions.[1] Since cytoplasm is generally a reductive environment, many disulfide bonds exist as the reduced form (thiol group) in cells. In the past decade, many methodologies for controlled release of drugs based on the cleavage of disulfide bonds have been developed.[2–4] For example, release of nucleic acids[2] and drugs[3] from polymer micelles has been regulated by the reduction of disulfide bonds. Controlled gating systems to release drugs from mesoporous silica using the redox reaction of disulfide bonds have been also reported.[4]

It has become feasible to reversibly control the structures of polymers or nanoassemblies by applying dynamic covalent bonds such as disulfide and Schiff base.[5–9] Recently, Kataoka and co-workers reported a structural transition from polyion complex micelles bearing poly(ethylene glycol) into polyion complex polymersomes (PICsome) by reduction of disulfides.[8] Otsuka and co-workers reported chain recombination of disulfide-containing polyesters by photoirradiation.[9] Whereas the controlled release of guest molecules based on the reductive cleavage of disulfide bonds has been widely studied as mentioned above, structural transition of nanoassemblies by recombination of disulfides and their application to controlled molecular release are much less common.

We have developed C_3-symmetric peptide conjugates mimicking spherical viruses and clathrin and reported their self-assembling behavior in water.[10–12] It has been reported that a C_3-symmetric conjugate bearing glutathione (trigonal glutathione, **TG**), which is an ubiquitous natural tripeptide, spontaneously self-assembled into 100–250 nm nanospheres in water and that uranine was encapsulated into the nanosphere.[11a] In this paper, we report controlled release of guest molecules from the **TG** assemblies by disulfide recombination (Figure 1).

TG was synthesized from trimesoyl chloride according to our previous report, and its chemical structure was confirmed by MALDI-TOF-MS and ^{1}H NMR.[11a] **TG** was dissolved in 10 mM Tris-HCl buffer (pH 7) without heating and sonication. After the **TG** solution was equilibrated at 25 °C for at least 30 min, the self-assembling behavior in the buffer was investigated. The dynamic light scattering (DLS) of 0.33 mM **TG** in the buffer showed formation of monodispersed assemblies with the size of

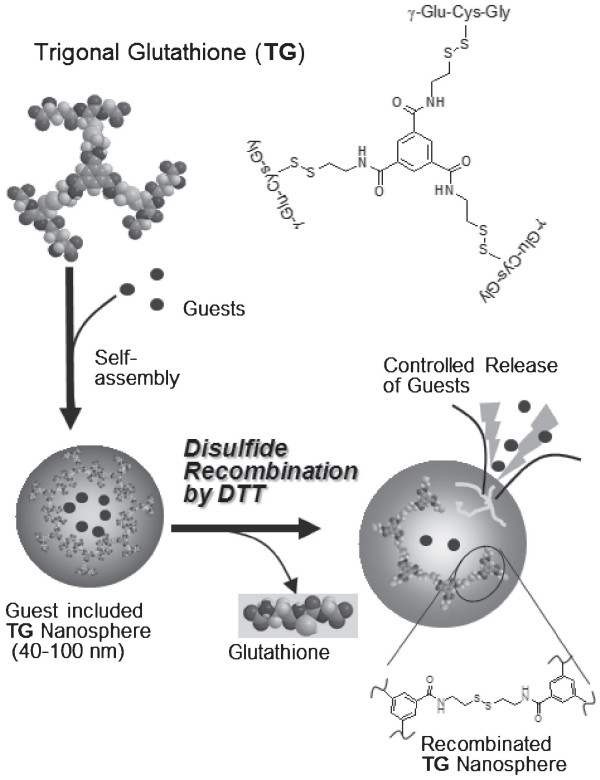

Figure 1. Schematic illustration of disulfide recombination in spherical assembly of trigonal glutathione (**TG**).

68 ± 13 nm (Figure 2A).[13] The SEM also showed the existence of 50–100 nm spherical assemblies (Figure 2B). The **TG** assemblies in 10 mM Tris-HCl buffer (pH 7) were smaller than those in pure water (173 ± 32 nm) at the same concentration (Figure S1[16]). The observed shrinkage of **TG** assemblies is likely to be caused by the electrostatic interaction between Tris-HCl cations and anionic **TG**. Figures 2C and 2D, respectively, show size distribution obtained from DLS and SEM images of the **TG** assemblies two days after addition of 1.0 mM dithiothreitol (DTT, 1 equivalent to disulfide bonds of **TG**). Nanoparticles with average diameter of 72 ± 15 nm were observed, with a small fraction of these with increased diameter of 234 ± 73 nm. The large particles might be caused by the fusion among **TG** assemblies through the formation of interparticle disulfide bonds.

Figure 3 shows the time course of reversed phase HPLC observed for the reaction of **TG** and DTT in 10 mM Tris-HCl

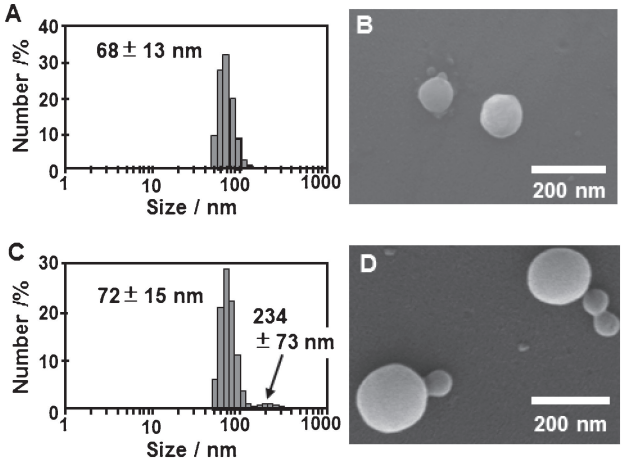

Figure 2. SEM images (B, D) and size-distributions obtained from DLS (A, C) for solutions of **TG** (0.33 mM) in 10 mM Tris-HCl buffer (pH 7) at 25 °C. A, B: in the absence of DTT. C, D: two days after addition of 1.0 mM DTT. SEM samples were coated with ca. 3 nm Pt.

buffer. 15 min after addition of DTT, new three peaks (**1**–**3**) together with a peak of **TG** appeared in the HPLC chart. Peaks **1** and **2** were assigned by MALDI-TOF-MS (Figure S2[16]) to the products in which one and two glutathiones were eliminated from **TG**, respectively.[14] Peak **3** was also assigned to a disulfide product formed from two eliminated products **2**. Six hours after addition of DTT, the peaks assigned to **TG**, **1**, **2**, and **3** disappeared, and only peak **4** was detected at the retention time of 49 min. The products **3** and **4** probably originate from the disulfide bond formation between core molecules of **TG**. The newly formed disulfide bonds of products **3** and **4** might be hardly reduced by DTT due to the consumption of DTT. These results indicate that disulfide recombination (thiole–disulfide exchange) in the **TG** assembly occurred together with the elimination of glutathiones from **TG** (Scheme S1[16]). It is noteworthy that the spherical morphology of **TG** assemblies remained even after the completion of disulfide recombination, although the size distribution was affected (Figure 2). It indicates that the oligomeric cores of **TG** can be dispersed in water as spherical assemblies even without glutathione units, probably due to the presence of hydrophilic groups. It seems that the spherical assemblies after the recombination (Figure 2D) were dissociated to the oligomeric cores under the HPLC condition.

The binding of dyes (Rhodamine 6G, Methyl Orange, and Uranine) to the **TG** nanospheres was investigated by equilibrium dialysis in 10 mM Tris-HCl buffer solution (pH 7) at 25 °C. Aqueous solutions of dye were added to the powdery samples of **TG**, and the mixtures were dispersed in buffer ([**TG**] = 0.33 mM, [dye] = 30 μM). The amount of dye bound into **TG** nanospheres was quantitatively estimated by measuring the concentration of the dialyzed solutions. As a result, all dyes were bound to nanospheres regardless of the charge (Figure 4A, black bar). It is probable that dyes bound to powdery excess **TG** via hydrophobic interactions and then were effectively included into the **TG** nanospheres during the dissolution of solid into water. In contrast, when the solutions of dye were mixed with the **TG** solution (0.33 mM), these dyes were minimally bound to

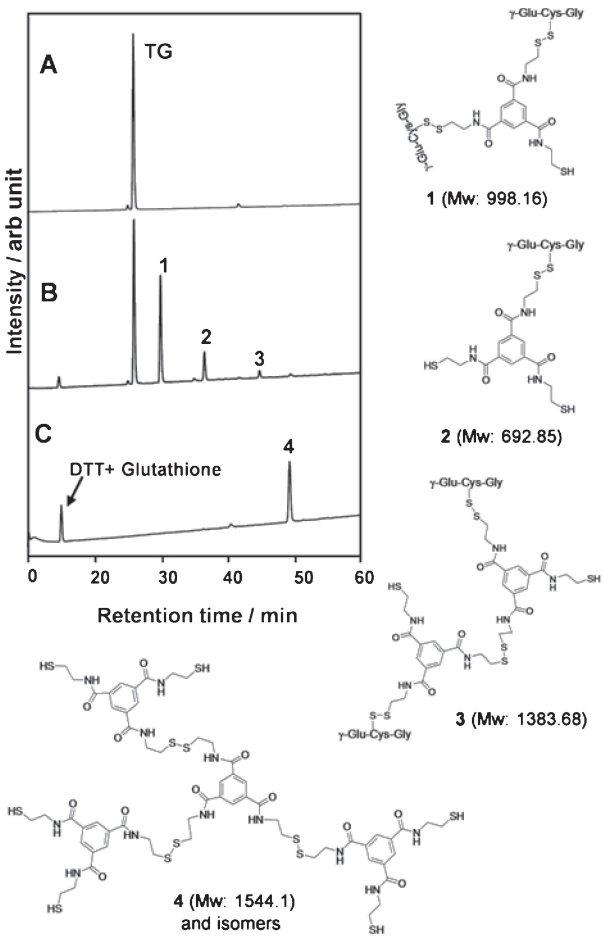

Figure 3. Reversed phase HPLC time course for the disulfide recombination reaction of **TG**. [**TG**] = 0.33 mM, [DTT] = 1.0 mM, in 10 mM Tris-HCl (pH 7) at 25 °C. (A) Before addition of DTT, (B) 15 min and (C) 6 h after addition of 1.0 mM DTT. Inertsil ODS-3 column (20 × 250 mm); flow rate, 10 mL min^{-1}; detected at UV 220 nm; eluted with a linear gradient of CH_3CN/water (10/90 to 50/50 over 90 min) containing 0.1% TFA. The products were assigned by MALDI-TOF-MS (see Supporting Information[16]).

nanospheres (Figure 4A, gray bar), indicating that **TG** nanospheres in aqueous solutions scarcely include dyes. The size distribution (50–75 nm) of dye-included **TG** assemblies obtained by DLS is similar to that of **TG** without dyes (Figure S3[16]). Methyl Orange included in **TG** assembly was scarcely released in 10 mM Tris-HCl buffer over 24 h (Figure 4B, square). On the other hand, when 1 mM DTT was added, the included Methyl Orange was gradually released from **TG** assembly, reaching equilibrium within 12 h (Figure 4B, circle). Rhodamine 6G and Uranine were also released from **TG** assembly by adding DTT, although the rate was slower than that observed for Methyl Orange (Figure S4[16]). The slower release of cationic Rhodamine 6G from anionic **TG** assembly at pH 7 might arise from electrostatic interactions. It is probable that Uranine is strongly bound to **TG** assembly by hydrophobic interactions. These results indicate that the included dyes can be released by disulfide recombination in aqueous **TG** assemblies.[15]

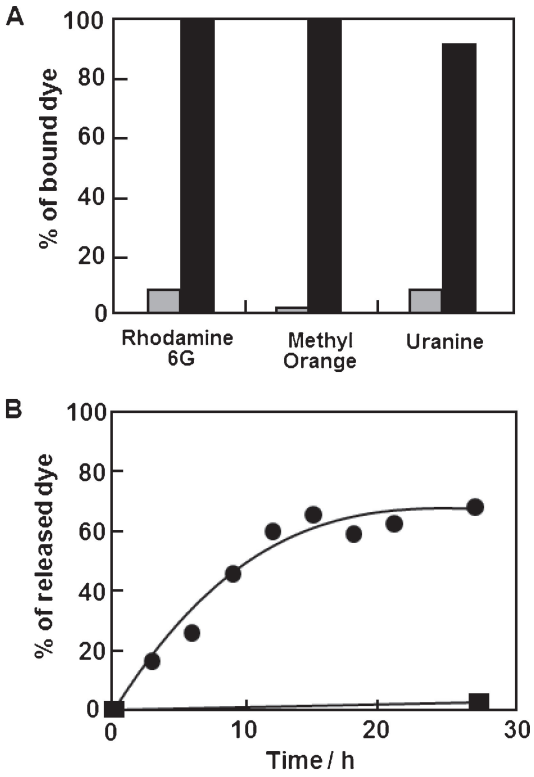

Figure 4. (A) The proportion of dyes bound to **TG** assemblies ([**TG**] = 0.33 mM and $[dye]_{total} = 30\,\mu M$) after dialysis in 10 mM Tris-HCl. Black bar: solutions of dye were added to the powdery **TG**. Gray bar: solutions of dye were mixed with solution of **TG**. (B) The time course of controlled release of Methyl Orange from **TG** assemblies by the disulfide recombination reaction: (circle) in the presence of 1 mM DTT (DTT was added at time = 0 h); (square) in the absence of DTT in 10 mM Tris-HCl buffer (pH 7) at 25 °C.

In conclusion, we have demonstrated that addition of DTT to **TG** nanospheres caused disulfide recombination while maintaining spherical morphology, and guest molecules were gradually released in response to the recombination. To date, there have been no reports on a controlled release system based on bond recombination of nanoassemblies. The present molecular system provides a novel guideline for the molecular design of drug delivery systems and functional soft materials.

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13 In previous report,[11a] we have shown the apparent association constant of **TG** ($K_a = 6.36 \times 10^3\,M^{-1}$) in pH 3 water at 25 °C. We expect that the K_a in Tris-HCl buffer would be higher than that in pH 3 water due to the interaction between **TG** and Tris-HCl. This suggests that at least 76% or more amount of **TG** self-assembles at 0.33 mM in Tris-HCl buffer at 25 °C.

14 We could not quantify the amount of glutathione released from **TG** nanospheres by reverse phase HPLC, because the peak of glutathione would overlap with the peak of DTT.

15 It seems that the release of dyes arises from the loose structure of assembly formed after recombination.

16 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

