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# A Tubular Biocontainer: Metal Ion-Induced 1D Assembly of a Molecularly Engineered Chaperonin

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Cylindrical nanocarriers sometimes exhibit better pharmacokinetics and efficiency in drug delivery than spherical ones. A recent example highlighted the excellent ability of long filamentous polymeric micelles to deliver paclitaxel into tumor cells.[1] Here we report a novel protein-based soft "nanotube" formed by supramolecular polymerization of chaperonin GroEL, a barrel-shaped tetradecameric protein assembly with an inner diameter of 4.5 nm.[2] In biological systems, GroEL utilizes its ATP-induced mechanical motion to assist refolding of denatured proteins. GroEL used for the present study ($GroEL_{SP/MC}$) was site-specifically modified in the entrance parts of its cavity with a number of photochromic [spiropyran (SP)/merocyanine (MC)] units (Figure 1a), allowing the switching of certain functions of GroEL by ATP and light.[3] In the course of this study, we noticed that $GroEL_{SP/MC}$ sometimes displays a higher molecular-mass fraction in size-exclusion chromatography (SEC) and later confirmed that this fraction is caused by one-dimensional (1D) assembly of $GroEL_{SP/MC}$ triggered by divalent metal ions. In this communication, we also highlight that this cylindrical hollow assembly can serve as a novel biocontainer for proteins (Figure 1b).

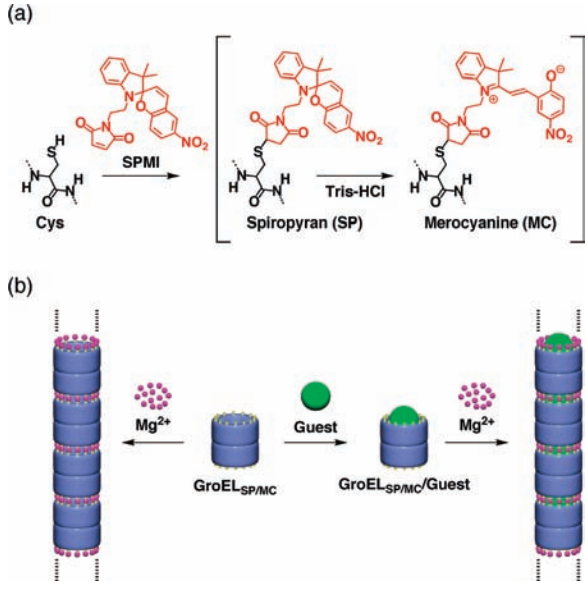


***Figure 1.*** (a) Preparation of $GroEL_{SP/MC}$ by modification of mutant $GroEL_{Cys}$ with 1′-(maleimidoethyl)spirobenzopyran. (b) Schematic illustrations of $Mg^{2+}$-induced 1D assembly of $GroEL_{SP/MC}$ (left) without and (right) with guest.

To obtain $GroEL_{SP/MC}$, we prepared mutant GroEL ($GroEL_{Cys}$: C → A; $K^{311}$ → C, $L^{314}$ → C) bearing 14 Cys residues in each entrance part of the cavity. Next, the Cys residues were allowed to react with spirobenzopyran-appended maleimide (SPMI) in 25 mM tris-HCl buffer (pH 7.4). During incubation for 12 h at 4 °C, the colorless mixture gradually turned light-purple because of partial isomerization of SP to MC, a known spontaneous process occurring in buffers.[4,5] The reaction mixture was subjected to rapid gel filtration with a Sephadex G-25 column (50 mM tris-HCl buffer) to remove unreacted SPMI. The filtrate, containing a protein fraction, was analyzed by Ellman's test. In comparison with $GroEL_{Cys}$, the filtrate showed hardly any residual SH, indicating nearly complete conversion of $GroEL_{Cys}$ into $GroEL_{SP/MC}$.[5]

When a 0.6 $\mu$M tris-HCl buffer solution of $GroEL_{SP/MC}$ was subjected to SEC using a 100 mM tris-HCl buffer (50 mM, pH 7.4) solution of KCl as an eluent, a small shoulder at a shorter retention time was observed along with a major peak due to $GroEL_{SP/MC}$ (red, Figure 2a). To our surprise, addition of $MgCl_2$ to the above solution gave rise to a significant change in the SEC. For example, when $GroEL_{SP/MC}$ was immersed for 0.5 h in $MgCl_2$ solution (5 mM) at 37 °C, a broad elution curve (blue) with a peak top elution volume of 2.5 mL emerged at the expense of the peak due to $GroEL_{SP/MC}$ (elution volume 3.2 mL). Dynamic light scattering (DLS) analysis (Figure 2b) of the resulting mixture indicated the presence of ~7 $\mu$m-sized particles. As observed by TEM (Figure 3a), the mixture contained very long cylindrical nanofibers with a uniform diameter of 15 nm. The typical pattern of lateral stripes in Figure 3b and the reported dimensional features of GroEL[2] indicate that the cylinders were composed of a large number of $GroEL_{SP/MC}$ units connected via their

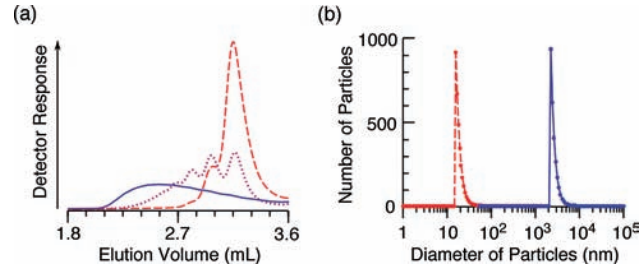


***Figure 2.*** (a) SEC traces (absorption at 280 nm) of 0.6 $\mu$M $GroEL_{SP/MC}$ without (red, dashed) and with (blue, solid) 5 mM $MgCl_2$ before mixing with 25 mM EDTA and after EDTA treatment (violet, dotted). (b) DLS profiles of $GroEL_{SP/MC}$ without (red, dashed) and with 5 mM $MgCl_2$ (blue, solid) before EDTA treatment.

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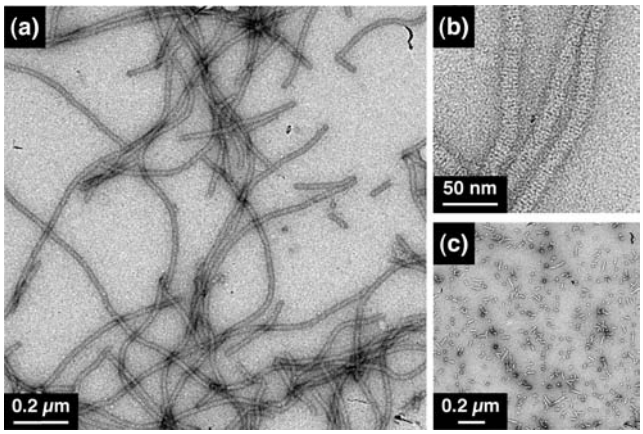


***Figure 3.*** TEM micrographs of one-dimensionally assembled $GroEL_{SP/MC}$ after mixing with 5 mM $MgCl_2$ (a, b) before and (c) after mixing with 25 mM EDTA.

entrance parts.[6] Notably, the longest nanotube was ~2.5 $\mu$m long, corresponding to a 170-mer of $GroEL_{SP/MC}$ (MW $\approx 1.4 \times 10^8$).[5] In sharp contrast, $GroEL_{Cys}$ did not polymerize upon mixing with $MgCl_2$ but remained unassembled,[5] suggesting that the photochromic units in $GroEL_{SP/MC}$ play a critical role in its 1D assembly.

Not only $Mg^{2+}$ but also other divalent metal ions such as $Ca^{2+}$, $Mn^{2+}$, $Co^{2+}$, and $Zn^{2+}$ triggered 1D assembly of $GroEL_{SP/MC}$,[5] while monovalent cations such as $Na^+$, $K^+$, and $Cs^+$ hardly induced the assembly. Trivalent cations such as $Fe^{3+}$, $In^{3+}$, $Ce^{3+}$, and $Eu^{3+}$ caused the assembly of $GroEL_{SP/MC}$ but gave only ill-defined aggregates.[5] Divalent metal ions are known to coordinate with MC, affording their 1:2 complexes under certain conditions.[7] Hence, they possibly coordinate with MC in $GroEL_{SP/MC}$. In fact, when $MgCl_2$ was added at 37 °C to a tris-HCl buffer solution of $GroEL_{SP/MC}$, the emission from MC at 629 nm ($\lambda_{ext}$ = 562 nm) immediately showed a 7 nm blue shift.[5] Furthermore, when EDTA, a strong metal ion chelator, was added to the preassembled system at 37 °C, the long cylinders were cut into short-chain oligomers and eventually into monomeric $GroEL_{SP/MC}$ [Figures 2a (violet) and 3c]. Since $GroEL_{SP/MC}$ possesses multiple MC units, such metal coordination can take place in a "multivalent" manner to ensure the 1D assembly. In relation to this important issue, we investigated the $Mg^{2+}$-induced assembly of $GroEL_{SP/MC}$ under illumination with visible light (>400 nm).[5] As already described, SP spontaneously isomerizes to form an SP/MC mixture in tris-HCl buffer.[4,5] However, upon continuous visible-light irradiation, the reverse isomerization can be induced concomitantly, reducing the content of MC in $GroEL_{SP/MC}$. In agreement with our expectation, the 1D assembly of $GroEL_{SP/MC}$, as observed by SEC, was less pronounced than in the absence of illumination.[5] Finally, we point out that the assembly is highly temperature-dependent. When the mixture of $GroEL_{SP/MC}$ and $MgCl_2$ was maintained at a low temperature (e.g., 4 °C), the assembly of $GroEL_{SP/MC}$, as observed by SEC, proceeded very sluggishly.[5] However, heating the mixture to 37 °C clearly accelerated the assembling event.[5] We assume that the 1D assembly of $GroEL_{SP/MC}$ may be completed by dehydration, as generally observed for protein assembly: metal ligation of the MC units certainly allows a primary assembly of $GroEL_{SP/MC}$ that is is still dynamic but can be greatly stabilized when thermal dehydration of the protein facets follows.

Similar to GroEL, $GroEL_{SP/MC}$ can bind denatured proteins, even under the 1D assembly conditions containing $Mg^{2+}$. We added an aqueous solution of denatured α-lactalbumin, fluorescently labeled with lucifer-yellow (α-$Lac_{FL/denat}$; 0.6 $\mu$M),[5] to a 0.6 $\mu$M tris-HCl buffer solution of $GroEL_{SP/MC}$. After incubation for 15 min at 4 °C, 5 mM $MgCl_2$ was added, and the mixture was immersed at 37 °C. When monitored by absorption at 280 nm, the ternary mixture provided a broad SEC profile (Figure 4a, blue curve) typical of 1D-assembled

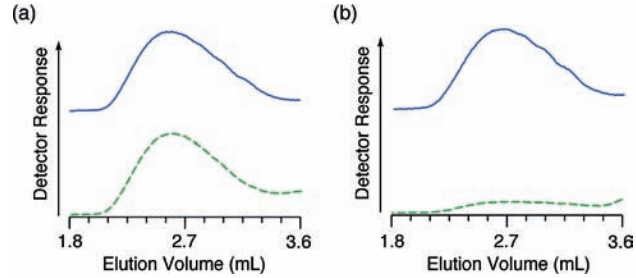


***Figure 4.*** SEC traces of $GroEL_{SP/MC}$ (a) after mixing with α-$Lac_{FL/denat}$ followed by $MgCl_2$ and (b) after mixing with $MgCl_2$ followed by α-$Lac_{FL/denat}$. Blue solid curves were obtained by monitoring the absorption at 280 nm and green dashed curves by monitoring the emission at 531 nm ($\lambda_{ex}$ = 426 nm).

$GroEL_{SP/MC}$. A virtually identical chromatogram resulted when the SEC was followed by emission at 531 nm due to lucifer-yellow ($\lambda_{ex}$ = 426 nm) (green curve), showing that $Mg^{2+}$ allows $GroEL_{SP/MC}$ to undergo 1D assembly without losing the α-$Lac_{FL/denat}$ guest. In sharp contrast, when α-$Lac_{FL/denat}$ was added to the system after mixing with $MgCl_2$, the resulting assembly in SEC showed hardly any fluorescence response (Figure 4b). For such a remarkably low dynamic nature of the 1D hollow assembly of $GroEL_{SP/MC}$, we assume that both multivalent metal coordination and hydrophobic interactions between the dehydrated protein facets must be responsible.

In conclusion, through a serendipitous observation, we found a molecular design strategy to allow 1D assembly of a group I chaperonin protein into micrometer-long hollow cylinders with a remarkably high mechanical stability.[8] As demonstrated by trapping of α-$Lac_{FL/denat}$, these cylinders could take advantage of unique biological functions of molecular chaperons and may serve as a novel biocontainer for delivering a variety of guest molecules, including proteins.

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**Supporting Information Available:** Details of experimental procedures, including preparation of $GroEL_{Cys}$ and $GroEL_{SP/MC}$, and Figures S1−S14. This material is available free of charge via the Internet at http://pubs.acs.org.

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