論 文 の 内 容 の 要 旨

論文題目　Functional Nanostructures From Chemically And Genetically Engineered Proteins

（タンパク質の超分子集積化による機能性構造体の構築と応用）

氏　名　ビスワス　シュヴェンドゥ

Introduction:

In Nature, proteins assemble via multivalent supramolecular interactions that allow them to assemble tenderly and reversibly to form functional nanostructures and retain their functions even in the assembled form. Nucleic acid helicase, tubulin, myosin, kinesin, molecular chaperon, ATP-synthase, channel protein and ribosome are some perfect examples of functional nanostructures of proteins in Nature which are built up from supramolecular assembly of their subunits. Structural and functional excellence of proteins has inspired scientists to synthetically manipulate them and design protein-based nanostructures with a view to achieve novel functional materials. In the present study, I have developed a chemical approach of using multivalent supramolecular interactions to develop nanotubes by assembling cylindrical molecular chaperons one-dimensionally. Innate biological functions of the chaperons, such as,

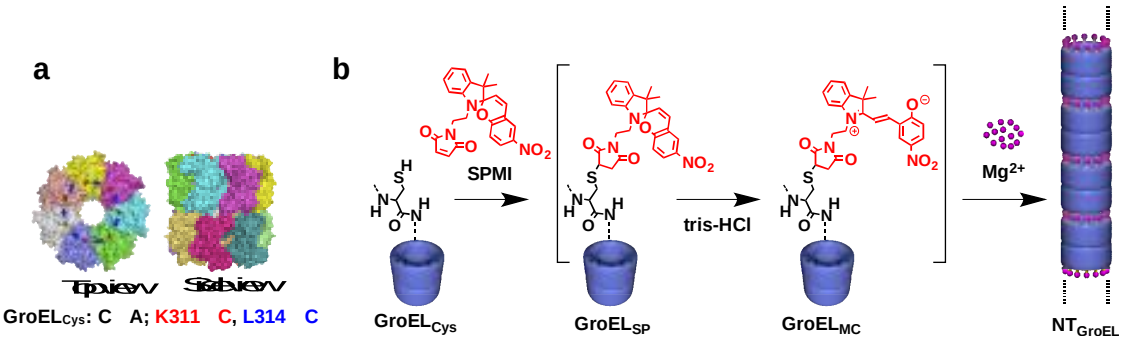

Figure 1. (a) A molecular model of mutant chaperonin $GroEL_{Cys}$. (b) Schematic illustrations for Mg^{2+}-induced one-dimensional assembly of $GroEL_{SP/MC}$.

trapping of denatured proteins and their release by adenosine triphosphate (ATP)-fueled machine-like open/close motion were successfully retained in the nanotubes. As a result, the synthetically developed chaperone nanotubes exhibits some novel functions

and properties based on the functions and properties of its constituent chemical and biological building blocks. Such functional nanotubes have bright prospects to trigger a paradigm shift in the filed of nanomaterial and nanomedicine.

One-dimensional assembly of chaperonin GroEL:[1]

GroEL is a barrel-shaped tetradecameric protein assembly with an inner diameter of 4.5 nm (Fig. 1a). In biological systems, GroEL traps denatured proteins into its hollow cavity and utilizes its ATP-induced mechanical motion to assist refolding of denatured proteins. GroEL used for the present study ($GroEL_{SP/MC}$) is modified in the entrance parts of its cavity by a number of photochromic (spiropyran/merocyanine) units site-specifically; thereby one may switch certain functions of GroEL by ATP and light. In course of this study, we noticed that $GroEL_{SP/MC}$ sometimes displays a higher molecular-mass fraction in size-exclusion chromatography (SEC) and confirmed later that this fraction is caused by one-dimensional (1D) assembly of $GroEL_{SP/MC}$ in the presence of divalent metal ions. For obtaining $GroEL_{SP/MC}$, we prepared mutant GroEL ($GroEL_{Cys}$: C→A; K^{311}→C, L^{314}→C), which bears 14 cysteine (Cys) residues in each entrance part of the cavity (Fig. 1a). Then, the Cys residues were allowed to react with spirobenzopyran-appended maleimide (SPMI) in 25 mM tris-HCl buffer (pH 7.4; Fig. 1b). During incubation for 12 h at 4 °C, the colorless mixture gradually turned light purple, due to partial isomerization of SP to MC, a known spontaneous process occurring in buffers, to give $GroEL_{SP/MC}$. When a 0.6 μM tris-HCl buffer solution of $GroEL_{SP/MC}$ was subjected to size exclusion chromatography (SEC), a small shoulder at a shorter retention time was observed along with a major peak due to $GroEL_{SP/MC}$ (red, Fig. 2a). To our surprise, addition of $MgCl_2$ to the above solution gave rise to a significant change in SEC. For example,

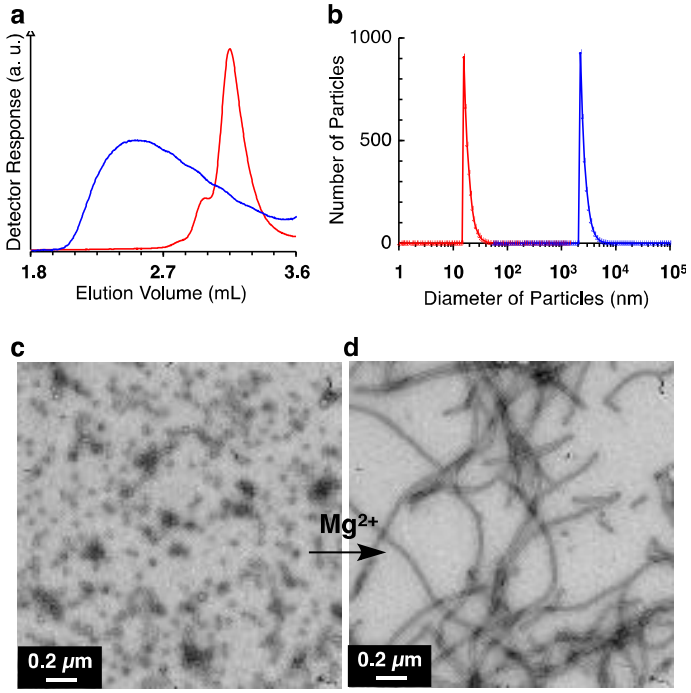

Figure 2. (a) SEC traces (observed at λ = 280 nm) of $GroEL_{SP/MC}$ (0.6 μM) without (red) and with $MgCl_2$ (5 mM). (b) DLS profiles of $GroEL_{SP/MC}$ without (red) and with $MgCl_2$ (5 mM; blue). TEM images $GroEL_{SP/MC}$ (c) without and (d) with $MgCl_2$ (5 mM).

when $GroEL_{SP/MC}$ was immersed for 0.5 h with $MgCl_2$ (5 mM) at 37 °C, a broad elution curve (blue, Fig. 2a) 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 (Fig. 2b) of the resulting mixture (blue) indicated the presence of ca. 7 μm-sized particles (without $MgCl_2$; red). As observed by TEM (Fig. 2d), the mixture contained very long cylindrical nanofibers with a uniform diameter of 15 nm (without $MgCl_2$; Fig. 2c). We found that the supramolecular polymerization of $GroEL_{SP/MC}$ takes place via multivalent MC••metal ion bridging.

Stimuli responsive properties of GroEL nanotubes (NT_{GroEL}):[1], [2], [3]

NT_{GroEL} consists of three components mainly: (1) divalent metal ions that bridges the MC units via MC••metal ion complex, (2) photochromic MC units that act as linkers at both ends of cylindrical GroEL and (3) GroEL units that undergoes machine-like open/close motion when fueled by ATP. Due to the stimuli responsiveness of respective components, NT_{GroEL} can exhibit unique and novel stimuli responsive properties.

[a] Dissociation of NT_{GroEL} in presence of EDTA:[1]

When EDTA, a strong metal ion chelator, was added to the pre-assembled system of GroEL and incubated for 30 min at 37 °C, the long nanotubes, were cut into short-chain oligomers and, eventually, monomeric $GroEL_{SP/MC}$. Dissociation of the nanotubes is confirmed with SEC and TEM.

[b] Photoreversible supramolecular polymerization of GroEL:[3]

Photochromic MC units at both ends of $GroEL_{SP/MC}$ can be reversibly photoisomerized to SP. Since NT_{GroEL} is formed via a 1:2 complexation of divalent metal ions and MC units, photoisomerization of MC to nonionic SP may trigger the dissociation of the NT_{GroEL}. In fact, when a polymerized mixture containing NT_{GroEL} was exposed to visible light (λ > 400 nm) for 15 min at 25 °C, a fraction corresponding to original NT_{GroEL} in the SEC profile was considerably diminished. Instead, an oligomeric fraction of GroEL consisting mainly of 1_{mer}–4_{mer} emerged which indicates the photoreversible cleavage of long NT_{GroEL}.

[c] ATP-fueled dissociation of NT_{GroEL} due to chemomechanical bond scission:[2]

NT_{GroEL} depolymerizes readily into monomeric GroEL units when it senses ATP. As shown in the SEC traces, after a 30-min incubation with ATP, original NT_{GroEL} almost diminished, affording its oligomeric fraction consisting mainly of 1_{mer}–3_{mer} (TEM). Not only ATP but also other nucleoside triphosphates such as cytidine-5'-triphosphate (CTP) and uridine-5'-triphosphate (UTP) gave rise to the dissociation of NT_{GroEL}. In contrast, no dissociation resulted when NT_{GroEL} was treated with inosine-5'-triphosphate (ITP) and guanosine-5'-triphosphate (GTP). According to the literature, only CTP and UTP, among the four ATP analogues tested, can induce a conformational change of GroEL. Thus, it is likely that the machine-like open/close

motion of each GroEL units in the nanotube drives the dissociation of NT_{GroEL}. In fact, NT_{GroEL} displayed an ATPase activity, which is as large as that of non-polymerized $GroEL_{Cys}$.

Trapping and release of denatured protein by NT_{GroEL}:[2]

Similar to GroEL, $GroEL_{SP/MC}$ can trap denatured GFP ($GroEL_{MC}\supset GFP_{denat}$). In presence of Mg^{2+} $GroEL_{SP/MC}\supset GFP_{denat}$ can polymerize to form nanotubular $NT_{GroEL}\supset GFP_{denat}$. Importantly, $NT_{GroEL}\supset GFP_{denat}$ can disassemble too in the presence of ATP (100 μM) and release GFP_{denat}. Release of non-fluorescent GFP_{denat} can be traced from its spontaneous refolding to GFP in the neutral buffer, which fluoresces strongly at 508 nm. Importantly, dissociation of $NT_{GroEL}\supset GFP_{denat}$ and release of GFP takes place in a sigmoidal manner in response to the concentration of ATP. Such a nonlinear [ATP]-dependency of the guest-release activity is extremely advantageous for aiming at targeted drug delivery with a fail-safe mechanism. Dissociation of $NT_{GroEL}\supset GFP_{denat}$ and release of GFP was also observed in an ATP-rich intracellular environment (HeLa cell lysate; [ATP] = 125 μM), however, no such phenomena took place in an extracellular environment (fetal bovine serum; [ATP] = 2 μM). These interesting results suggest that NT_{GroEL} can differentiate environments on the basis of [ATP] and specifically release guests only to an ATP-rich environment. Furthermore, NT_{GroEL} bearing a particular type of boronic acid on the surface can smoothly penetrate into HeLa cells. It is observed that upon penetration, a major portion of the penetrated NT_{GroEL} is not trapped in lysosome. Further investigation of this interesting property of NT_{GroEL} is underway.

Conclusion:

Thus, from chemically and genetically engineered chaperonin protein, a bionanotube with unique properties and novel functions is achieved. These nanotubes can be further modified at their surface for their targeted delivery and [ATP]-responsive functions. In contrast to most nanocarriers so far reported, an ATP-fueled robotic nanocarriers such as NT_{GroEL} may provide a universal strategy for on-demand targeting, since activated immune cells such as lymphocytes and macrophages are known to release large amounts of ATP into extracellular matrices.

References: i) Wagner, C. R. *et al. J. Am. Chem. Soc.* **2006**, *128*, 7630.
ii) (a) Mougous, J. D. *et al. PNAS*, **2008**, *105*, 3733. (b) Heddle, J. G. *et al. Small*, **2009**, 5, 2077.

Publications: [1] Biswas, S. *et al. J. Am. Chem. Soc.* **2009**, *131*, 7556.
[2] Biswas, S. *et al.* submitted.
[3] Sendai, T.; Biswas, S. *et al.* submitted.