Design of Stimuli-Responsive Supramolecular Assemblies Based on Double-Axle Inclusion into Cyclodextrin for Artificial Muscle

（人工筋肉を目指したシクロデキストリンへの二本軸包摂に基づいた刺激応答性超分子会合体の設計）

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Background

Supramolecular networks have been recently attracted to design functional 'smart materials' constructed via various types of supramolecular assemblies due to non-covalent interactions such as metal-ligand coordinations, van der Waals interaction, hydrogen bonds, and hydrophobic interaction, often seen in rotaxane systems and supramolecular polymers. As one of these supramolecular structures, an inclusion complex consisting of a linear polymer with two or more cyclodextrins (CDs) has advantages of making more complicated supramolecular architectures which change their functions in response to external stimuli, contrary to that of monomeric guest molecules. Our systematic studies have revealed the pH-dependent inclusion complexation phenomena of linear cationic polymers. Throughout these studies, we found that linear poly(ethyleneimine) (LPEI) forms inclusion complexes with α-cyclodextrin (α-CD) and γ-cyclodextrin (γ-CD) in two different ways: γ-CD can include double-strands of LPEI chains while α-CD includes single-strand of LPEI. Furthermore, we clarified that the inclusion complexation with the triblock-copolymer (PEI–PEG–PEI) consisting of LPEI and poly(ethylene glycol) (PEG) under pH variation and the pH-dependent control of α-CD mobility along the PEI–PEG–PEI capped with bulky end-groups (polyrotaxane). Eventually, our great interests have been focusing on novel supramolecular networks formed via inclusion of γ-CD with double-strands of pH-responsive block-copolymers grafted onto water-soluble polymers.

Aims

Inspired to the contractile actuation system of artificial muscle, we designed a new contracting system based on supramolecular assembling systems for applications toward artificial muscles. We introduce a new concept for the supramolecular network, herein named double-axle intrusion (DI) system, using macrocycles and linear diblock-copolymers, which can modulate rheological properties in response to pH changes. The formation of the DI system at high pH (~10) induces a drastic increase in the rheological properties (about 10^3 in the magnitudes) in spite of semi-diluted fluids (3 wt.-%). Further, the DI system shows the significantly reduced properties at low pH (~4). From all viscoelastic results and our literatures, we demonstrate that such macroscopically rheological changes are results of molecularly mechanical actuations of the DI system in supramolecular networks.

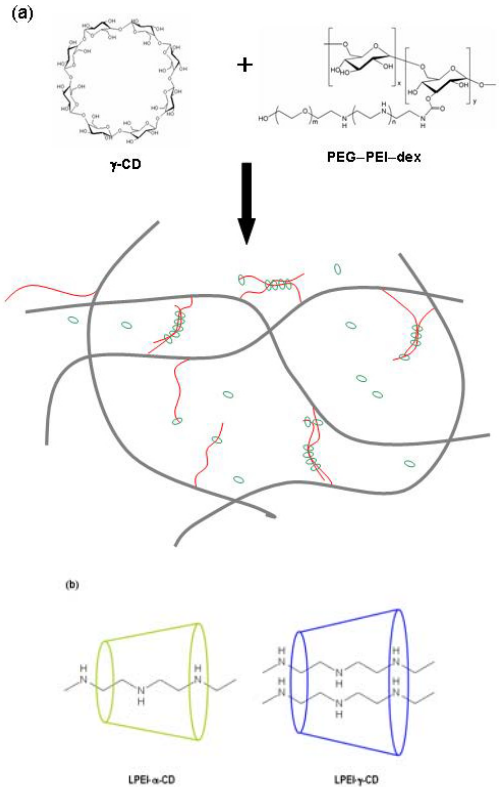

Figure 1 Schematic representation for the formation of Dex-PEI-PEG·γ-CD network. (a) A supramolecular network is formed by the DI complex between γ-CDs and double-strands of the PEG–PEI chains. The DI complexes have either parallel or anti-parallel type. (b) α-CD and γ-CD include single- and double-strand of LPEI, respectively. Stoichiometric ratios of α-CD and γ-CD to the repeating unit of LPEI are 1:2 and 1:4, respectively.

Experimental results

Synthesis of poly(ethylene glycol)–*b*–poly(ethylenimine) copolymer and grafting onto dextran

Novel supramolecular structure, poly(ethylene glycol)–*b*–poly(ethylenimine) (PEG-PEI) copolymer-grafted dextran, was synthesized through cationic ring-opening polymerization of oxazoline (EtOz) was polymerized using PEG monotosylate as a macroinitiator, followed by acid hydrolysis of poly(oxazoline) chain to obtain a linear PEI block, characterized as shown in Fig. 2. As a result, the resulting polymer was

capable of forming an inclusion complex with cyclodextrins and showing the contractile motion of CDs on block copolymer against pH variation. It is expected that PEG-PEI copolymer-grafted dextran will be a good candidate as a pH-responsive supramolecular assembly in aqueous solution. Practically, we could observed the inclusion complexation of our prepared polymer with CDs in terms of turbidity as shown in Fig. 3.

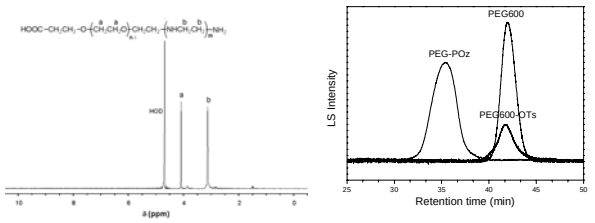

Figure 2 ^{1}H NMR spectrum of PEG-PEI in D_2O at 80 °C and GPC traces of PEG-POz, PEGOTs, and PEG.

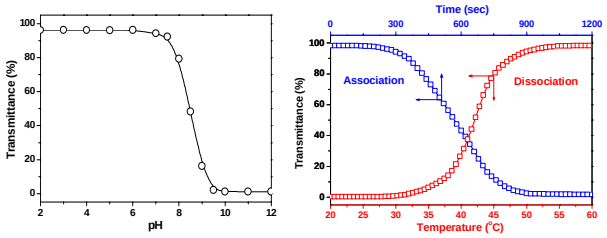

Figure 3 The inclusion complexation between Dex-PEI-PEG and γ-CD: Association and dissociation behaviors of γ-CD and Dex-PEI-PEG (a) as temperature and time and (b) pH.

<u>^{1}H NMR titration study of stimuli-responsive supramolecular network using naphthalene-appended γ-CD</u>

We have previously prepared a stimuli-responsive inclusion complex between dextran–*g*–PEI–*b*–PEG graft copolymer (Dex-PEI-PEG) and γ-CD in order to investigate unique inclusion phenomena, double-axle inclusion. For further study, a γ-CD derivative, mono-6-*O*-(2-sulfonato-6-naphthyl)-γ-CD (SN-γ-CD) was additionally synthesized for ^{1}H NMR titration study, which is expected to induce the competition of pendant naphthyl group with external polymer guests. Consequently, ^{1}H NMR titration results of the inclusion complex of Dex-PEI-PEG with SN-γ-CD showed stoichiometric changes, temperature-dependence, and reversibly pH-responsive properties of the inclusion complexes in terms of chemical shift variation.

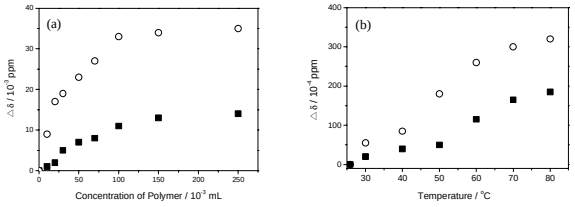

Figure 4 ^{1}H NMR titration of the inclusion complexation between Dex-PEI-PEG and SN-γ-CD. (a) as increasing Dex-PEI-PEG and (b) as increasing temperature. Opened circle: PEG–PEI and closed square: Dex-PEI-PEG.

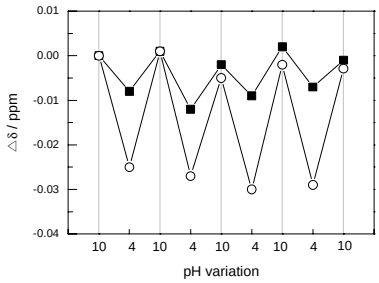

Figure 5 Reversible properties of the inclusion complex between Dex-PEI-PEG and SN-γ-CD on pH, which was shown by the variation of chemical shifts versus repeated changes in pH from 10 to 4. Closed squares: Dex-PEI-PEG and SN-γ-CD, opened circles: PEG–PEI and SN-γ-CD.

<u>Rheological measurements of the supramolecular networks of Dex-PEI-PEG with γ-CD</u>

Figure 6 shows frequency sweep data for the supramolecular network at different pH. In terms of the zero shear viscosity (η_0), the addition of γ-CD to Dex-PEI-PEG induced a drastic increase of 10^3 in the magnitude, indicating the presence of rather tight and extensive association between the PEG–PEI–dex chains via the DI complex by γ-CDs (Figure 6a). After lowering the pH to 4, all parameters moved down to the level as low as about 10^2 of the magnitudes, although the profiles were maintained (Figure 6b). As shown in Figure 7, these results were derived from characteristic DI complex system.

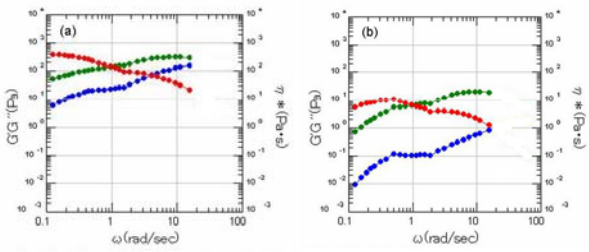

Figure 6 Dynamic viscoelastic parameters of semi-diluted fluids. The storage modulus (G′, blue), the loss modulus (G″, green), and the complex viscosity (η*, red) for 3 wt.-% aqueous solutions of Dex-PEI-PEG·γ-CD at (a) pH 10 and (b) pH 4 as a function of angular frequency (ω) with the range of 0.01–100 at 25 °C.

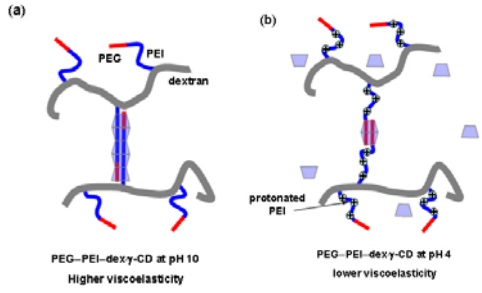

Figure 7 Proposed structures of supramolecular networks under different conditions. (a) Dex-PEI-PEG·γ-CD network is formed via full DI complex at pH 10, (b) The full DI complex of Dex-PEI-PEG·γ-CD network is transformed to partial DI

complex at pH 4 due to the protonation of PEI chains.

DLS study of the supramolecular networks between Dex-PEI-PEG and γ-CD

Figure 8 shows the distribution data for sizes in aqueous solution (hydrodynamic radii) of all samples. Dynamic light scattering (DLS) data supported our assumption, pH-dependent transformation of network as shown in Figure 7, by supplementary evidences. In order of size, hydrodynamic radius R_{h1}, R_{h2}, and R_{h4} are assigned to monomeric γ-CD, Dex-PEI-PEG, and self-aggregated γ-CD respectively. For the inclusion complex, the peak (R_{h3}) assigned to supramolecular structures was observed at larger size than one at pH 10. This result suggests that the supramolecular network was transformed to looser structure by lowering pH to 4.

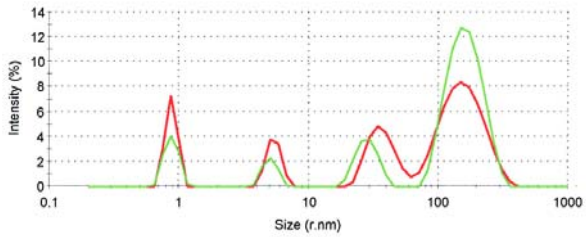

Figure 8 Size distribution data by DLS measurement for aqueous solutions of (a) γ-CD (green line) and Dex-PEI-PEG (red line), and (b) Dex-PEI-PEG·γ-CD at pH 10 (green line) Dex-PEI-PEG·γ-CD at pH 4 (red line). The concentration of solutions: [γ-CD] and [Dex-PEI-PEG] = 1.0 mg/ml, [Dex-PEI-PEG·γ-CD] = 2.0 mg/ml.

Prospects

We can improve and modulate macroscopic properties from the supramolecular network by changing various parameters such as the grafting yield and length of block chains, the feed ratio of γ-CD to block chains, the end-capping of grafted chains to prevent CD dethreading, and molecular weight of dextran backbone, and so on. Interesting examples of electronic devices reminiscent of very primitive computers have recently been reported by several researchers. The molecular components of these devices are catenanes or rotaxanes able to undergo intramolecular motions by various chemical groups, the motions permitting the molecules to act as electronic switches.

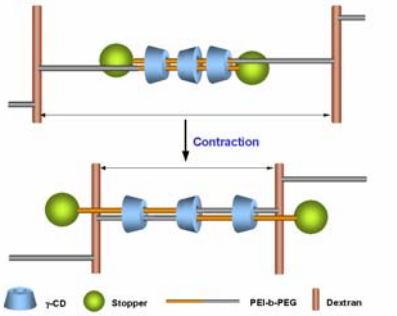

Figure 9 Schematic representation of the expected contracting system consisting of inclusion complex between Dex-PEI-PEG and γ-CD.

Additional Research

Anticoagulant activity of taurine-conjugated CEE-polyrotaxanes

Polyrotaxanes with both sulfonyl and carboxyl groups were synthesized and characterized for mimicking the anticoagulant activity of heparin. A polyrotaxane consisting of α-cyclodextrins (α-CDs) and poly(ethylene glycol) (PEG) was synthesized, and carboxyethylester (CEE) groups and taurine were successively conjugated with the polyrotaxane to obtain taurine-conjugated carboxyethylester-polyrotaxanes (TAU–CEE–PRxs). The number of α-CDs and the anionic groups could be varied by changing synthetic conditions. The results of Activated Partial Thromboplastin Time (APTT) test revealed that structural factors required for higher anticoagulant activity were (i) relatively lower threading percentage of α-CDs, (ii) the ratio of anionic groups similar to heparin, and (iii) lower molecular weight of PEG. The TAU–CEE–PRx that sufficiently meet the mentioned requirements showed enhanced antithrombin III (AT III) activity, indicating that the TAU–CEE–PRx interacts with AT III and/or thrombin. Therefore, these results suggest that free α-CD sliding and rotation for anionic groups as to fit the binding with AT III without any sterical hindrance contributes to enhancing anticoagulant activity.

Minor-Research Theme

Fabrication of supramolecular nanofibers based on inclusion complexes between PEO and α-CD by electrospinning technique

A new nanofiber from polypseudorotaxanes (PPRx) between poly(ethylene oxide) (PEO) and α-cyclodextrin (α-CD), PPRx nanofiber, was fabricated by electrospinning technique. For the formation of PPRx, the aqueous mixtures of α-CD and PEO (M_n = 100,000), with various stoichiometric ratio from 0 to 10, were sonicated and stirred for making homogeneous solutions and then the solutions were heated and cooled repeatedly for achieving maximum threading percentage of α-CD. The prepared solutions were spun under various conditions containing the voltage range of 15–20 kV, the electric field distance of 20 cm, and the temperature range of 20–45 °C. The spun nanofibers were characterized by Field emission–Scanning electron microscope (FE–SEM), Atomic force microscope (AFM). The average diameter of the electrospun nanofibers was shown at the range of 100 –150 nm by observing images of SEM.

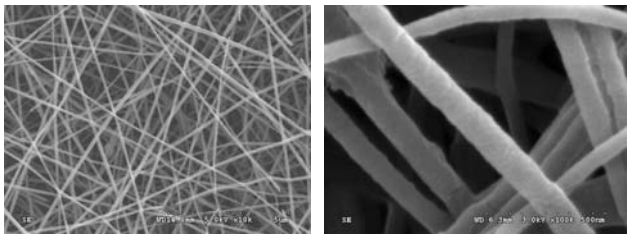

Figure 10 SEM images of electro-spun nanofiber from polypseudorotaxanes between PEO and α-cyclodextrin.

Table of Contents for Dissertation

Accomplishments

PUBLICATIONS

1. Y. K. Joung, Y. Sengoku, T. Ooya, K. D. Park, N. Yui, Anticoagulant supramolecular-structured polymers: synthesis and anticoagulant activity of taurine-conjugated carboxyethylester-polyrotaxanes, *Science and Technology of Advanced Material*s **2005,** *6*, 447-451.
2. Y. K. Joung, T. Ooya & N. Yui, Modulating rheological properties of supramolecular networks by pH-responsive double-axle intrusion into γ-cyclodextrin, *Advanced Materials* **2006**, in publication.
3. Y. K. Joung, H. S. Choi, T. Ooya & N. Yui, ^{1}H NMR titration of stimuli-responsive polymeric networks: inclusion complexes between PEG−*b*−PEI copolymer-grafted dextran and γ-cyclodextrins, *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 15 May **2006,** submitted.

PROCEEDINGS

1. Y. K. Joung, T. Ooya, N. Yui, "Stimuli-responsive supramolecular networks formed by double-axle intrusion of PEG-*b*-PEI copolymer-grafted dextran into γ-cyclodextrin", 55(2), The Society of Polymer, Japan, Toyama, Japan, (September 20-22, **2006**).
2. Y. K. Joung, H. S. Choi, T. Ooya, N. Yui, "Stimuli-Responsive Polymeric Networks by Inclusion Complexation between PEG−*b*−PEI Copolymer-Grafted Dextran and α- or γ-Cyclodextrins", XIII International Cyclodextrin Symposium, Torino, Italy, (May 14-17, **2006**).
3. Y. K. Joung, H. S. Choi, T. Ooya, N. Yui, "pH-Driven contractile supramolecular structures: assemblies of cyclodextrins and poly(ethylene glycol)-*b*-poly(ethylenimine) copolymer-grafted dextran", 54(2), The Society of Polymer, Japan, Yamakata, Japan, (September 20-22, **2005**).
4. Y. K. Joung, H. S. Choi, T. Ooya, N. Yui, "pH-Responsive contractile motion of supramolecular structure: polyrotaxanes of poly(ethylene glycol)-*b*-poly(ethylenimine) copolymer-grafted dextran with cyclodextrins", 54(1), The Society of Polymer, Japan, Yokohama, Japan, (May 25-27, **2005**).
5. Y. K. Joung, H. S. Choi, T. Ooya, N. Yui, "Synthesis of amphiphilic poly(ethylene glycol)–*block* –poly(ethylenimine) copolymers for supramoelcular assembling system", 229th ACS National Meeting, San Diego, CA, USA (March 13-17, **2005**).
6. Y. K. Joung, Y. Sengoku, T. Ooya, N. Yui, K. D. Park, "Anionic groups-conjugated supramolecular assemblies for anticoagulant activity", JAIST International Symposium on Nano Technology 2004, Ishikawa, Japan (September **2004**).
7. Y. K. Joung, Y. Sengoku, T. Ooya, N. Yui, K. D. Park, "Synthesis and anticoagulant activity of anionic polyrotaxanes with heparin-like activity", 53(1), The Society of Polymer Science, Japan, Kobe, Japan (May **2004**).