Supramolecular Structures with Cyclodextrins for Biomedical Applications

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Supramolecular structures by the host-guest interaction between cyclodextrins and polymers, inclusion complexes, have been investigated and developed to apply to biomedical fields. An example of the supramolecular structure is anionic polyrotaxanes with sulfonyl or carboxyl group, showing blood compatibility such as anticoagulant activity and antithrombin III binding activity as well as other biocompatible properties. Second example is supramolecular network systems with temperature- and/or pH-sensitive properties, giving biomedical hydrogels. In addition, a simple supramolecular structure was electrospun to fabricate a new nanofiber for practical uses as biomaterials. These studies demonstrated that supramolecular structures with cyclodextrins are potential candidates of ‘smart’ biomaterials with functions, such as stimuli-sensitivity or biocompatibility, in various biomedical applications.

Key words: Supramolecular structure, Cyclodextrin, Inclusion complex, Polyrotaxane, Biocompatibility, Heparin

Introduction

Supramolecular structures have been recently attracted to design functional smart materials constructed by a lot of types of supramolecular assemblies due to non-covalent interactions such as metal-ligand coordination, van der Waals interaction, hydrogen bonds, and hydrophobic interaction, often seen in rotaxane systems and supramolecular polymers.1-3 After discovered inclusion phenomenon of PEG into α-cyclodextrin (α-CD) by A. Harada and coworkers in 1990, many species of such inclusion complexes have been discovered and investigated.4,5 As one of these inclusion complexes, an inclusion complex consisting of a linear polymer with two or more cyclodextrins (CDs), called pseudorotaxane, has an advantage of making more complicated supramolecular architectures which change their functions in response to external stimuli as well as show bio-mimetic activities, contrary to that of monomeric guest molecules. In early stage, our systematic studies were focused on the modification of cyclodextrins in the inclusion complexes for achieving multivalent interactions6-7 and biocompatibility.8,9 From these studies, we demonstrated that ligand-conjugated structures and anionic group-appended structures show multivalent interaction and bio-mimetic properties, respectively. Our studies were also concentrated on supramolecular cross-linked structures by inclusion complexation between cyclodextrins and polymers appended with polysaccharides, resulting in thermo-responsive hydrogels.10-15 Discovering the inclusion of cyclodextrins into cationic polymers provided advanced functions in the structures. Our studied revealed that linear poly(ethyleneimine) (LPEI) forms inclusion complexes with α-CD and γ-cyclodextrin (γ-CD) in two different modes: γ-CD can include double-strands of LPEI chains while α-CD can include only single-strand of LPEI.16-19 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 had 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. However, these studies are just emerging stage of developing advanced biomaterials applicable to biomedical areas, limited to scientific interests yet.

In this review, therefore, we focus on introducing supramolecular structures of which the potential and applicability were attested in various fields. Originally, our studies show that supramolecular structures consisting of cyclodextrins and polymers can be developed to utilize in biomedical fields.
Inclusion Complexes between Cyclodextrins and Linear Polymers

In an aqueous solution, the slightly apolar cyclodextrin cavity is occupied by water molecules which are energetically unfavored (polar-apolar interaction), and therefore can be readily substituted by appropriate “guest molecules” which are less polar than water as shown in Figure 1.\textsuperscript{20,21} The dissolved cyclodextrin is the “host” molecule, and the “driving force” of the complex formation is the substitution of the high enthalpy water molecules by an appropriate “guest” molecule.\textsuperscript{22} One, two, or three cyclodextrin molecules contain one or more entrapped “guest” molecules. Most frequently the host:guest ratio is 1:1. This is the essence of “molecular encapsulation”. This is the simplest and most frequent case. However, 2:1, 1:2, 2:2, or even more complicated associations, and higher order equilibria exist, almost always simultaneously.

The formed inclusion complexes can be isolated as stable crystalline substances (Figure 2). Upon dissolved these complexes, as equilibrium is established between dissociated and associated species, and this is expressed by the complex stability constant $K_a$\textsuperscript{23}. The association of the CD and guest (D) molecules and the dissociation of the formed CD/guest complex are governed by a thermodynamic equilibrium. Poly pseudorotaxane formation is defined as the inclusion complexation between many cyclic molecules and a linear polymer chain in a certain solvent.\textsuperscript{24} Many combinations of cyclic molecules and polymers have been reported.\textsuperscript{25} The representative cyclic molecules are cyclodextrins (CDs)-homochiral cyclic oligosaccharides, most of which are composed of 6, 7, or 8 $\alpha$-1,4-linked D-glucopyranose units. CDs composed of 6, 7, and 8 glycopyranose units are usually referred to as $\alpha$-, $\beta$-, and $\gamma$-CDs, respectively. Poly pseudorotaxane formation has been extensively studied by Harada et al.,\textsuperscript{26} Weinz et al.,\textsuperscript{27} Baglioni et al.,\textsuperscript{28} and Li et al.\textsuperscript{29} Generally, CDs are threaded onto a linear polymer chain in water. Poly pseudorotaxanes are often obtained as white crystals after precipitation and filtration from water dispersions.

Sulfonated Polyrotaxanes as Blood Compatible Biomaterials

We demonstrated synergistic effect of supramolecular structure of the polyrotaxanes and sulfonation of many hydroxyl groups of $\alpha$-CDs in the polyrotaxanes on anticoagulant activity (Figure 3).\textsuperscript{9} $\alpha$-CD and PEG-b-PPG-b-PEG triblock copolymers (Pluronic) were selected for the main component of the polyrotaxanes because 1) $\alpha$-CDs can be threaded onto the PEG segment of Pluronic and 2) the PPG segment of Pluronic can physically be immobilized on conventional polymer surface. An inclusion complex of $\alpha$-CDs and PEG-b-PPG-b-PEG triblock copolymer was capped with Z-L-Phe (Z-L-phenylalanine) to prevent de-threading of $\alpha$-CDs, and the obtained polyrotaxane was sulfonated by 1,3-propane sultone. The anticoagulant activity of sulfonated polyrotaxanes was investigated by APTT in comparison with non-sulfonated polyrotaxanes and Pluronic itself. These types of polyrotaxanes with sulfonated groups may be useful in the surface modification of clinically used polymers as one of new materials for blending or coating. Preliminary results showed the decrease of protein adsorption and bacteria adhesion, and the prevention of thrombin-induced platelet activation on the sulfonated polyrotaxane-modified polyurethane surfaces.\textsuperscript{30}

Sulfonated Polyrotaxanes Coated PU Films as Biocompatible Surfaces

Sulfonated $\alpha$-cyclodextrins ($\alpha$-CDs) were threaded onto the poly(ethylene glycol) (PEG) segments in a PEG-b-poly(propylene glycol) (PPG)-b-PEG triblock copolymer (Pluronic) capped with benzylloxycarbonyl (Z)-L-phenylalanine (Z-L-Phe), were prepared as a novel surface-modifying biomaterial. Surface modification of the polyurethane (PU) was carried out by blending the PRx-SO$_3$'-s with a PU solution, followed by solution casting. The incorporated PRx-SO$_3$'-s led to the enhanced hydrophilicity by changing the surface properties of the PU matrix. Modified PUs showed the stable entrapment of the

![Figure 1. Schematic representation of CD inclusion complex formation. P-Xylene is the guest molecule; the small circles represent the water molecules.\textsuperscript{23}](image-url)
PRx-SO₃'s with little extraction into water and enhanced mechanical properties after exposure to water compared to the PU control. The incorporated PRx-SO₃'s repelled the proteins and kept them from closely approaching the surface areas, prevented platelet activation by thrombin, and effectively repelled bacteria (shown in Figure 4). These results suggest that both the supramolecular structure of the polyrotaxanes and exposure of the sulfonated groups onto the surfaces contribute to these phenomena. Thus, surface modification with PRx-SO₃'s is suggested to be useful for the fabrication of biocompatible medical devices.

From obtained results, though it was difficult to elucidate the effect of PRx itself, due to the poor solubility of PRx in the PU substrate, the obtained results suggest that adding a small amount of supramolecular-structured PRx-SO₃'s induces the changes in surface/bulk properties. The reduced protein adsorption on the modified surfaces is strongly influenced by the physicochemical interactions of the PRx-SO₃'s primarily governed by the supramolecular structure of PRx, which may be correlated with the inhibition of platelet activation and bacterial adhesion. It is well known that plasma protein may have several important roles, including coagulation cascade and platelet activation. The correlation of platelet activation with plasma protein adsorption, as well as with the physico-

**Figure 2.** Crystal structure of inclusion complex of β-cyclodextrin with poly(trimethylene oxide). Intermolecular hydrogen bonds are shown as dotted lines. Solvent molecules that form direct hydrogen bonds with β-CDs shown as open circles with hydrogen bonds [Figure is cited a reference³³].

**Figure 3.** Chemical structure of a sulfonated polyrotaxane.
chemical interaction of the PRx-SO₃'s on the polymer substrates, may need to be more precisely investigated. This study demonstrated that surface modification with sulfonated polyrotaxanes can be useful in the fabrication of biocompatible medical devices. In a preliminary study to enhance the biocompatibility by the introduction of sulfonated PEG (PEG-SO₃) to PRx, we have recently synthesized the sulfonated PEG-grafted PRx (PRx-PEG-SO₃), next to PRx-SO₃. The PRx-PEG-SO₃ showed an enhanced anticoagulant activity. Further studies related to the surface modification of biomaterials using PRx-PEG-SO₃ and relevant biocompatibility are now under investigation.

**Anionic Polyrotaxanes with Heparin-Like Structure**

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 that of heparin (~3), and (iii) lower molecular weight of PEG. Lower threading percentage of α-CDS could maintain average space between adjacent α-CD molecules, resulting in the high anticoagulant activity. Both the conclusions (ii) and (iii) strongly suggest that the structural factors required for high anticoagulant activity should mimic heparin. The TAU-CEE-PRx satisfied with the requirements is considered to bind with AT III, which enhances further complexation with thrombin. It is assumed that synergistic effect of mobile anionic groups by sliding motion of α-CDs in TAU-CEE-PRxs contributes to specific binding with AT III. Further experiments about AT III binding are now in progress and will be reported in our forthcoming paper.

**Supramolecular Networks as Stimuli-Sensitive Hydrogels**

Supramolecular structures can be developed to prepare stimuli-sensitive hydrogels using inclusion complex formation of polymers and cyclodextrins. Our group also demonstrated a
new PIC structure that can introduce a novel type of gelation in aqueous media. It is hypothesized that IC formation of the polymers grafted to hydrophilic polymers, here represented as dextran, can induce crystalline PIC domains, which act as physical cross-linking. It is significant that the physical gelation is introduced by a specific host-guest interaction, providing supramolecular-structured hydrogels. PEG grafts were found to form inclusion complexes with \( \alpha \)-CD molecules, resulting physical junction. The thermo-reversible gelation is based on supramolecular assembly and dissociation. The hydrogel structure of GC ICs is schematically represented in Figure 5.

From this result, we hypothesized that the gel properties can be tailor-made by varying the combination of the host and the guest molecules, as well as changing the average number and length of the grafted PEG side-chains. Therefore, poly(propylene glycol) (PPG)-grafted dextran was synthesized as a building block to construct rapidly induced hydrogel systems together with \( \beta \)-CD. The \( \beta \)-CD and PPG grafts comprised crystalline IC domains, which act as physical junctions in GCIC hydrogels to hold together hydrated dextran chains. These hydrogels also showed a thermo-reversible, sol-gel transition induced by reversible supramolecular assembly and dissociation. The induction time for gelation was relatively short, and varied depending on the concentration or the feed ratio of the host and guest molecules. From the results, we could conclude that the physical properties of the hydrogels could be modulated by changing the number of hydrophobic moieties per chain or the combination of PPG-grafted dextrans and \( \beta \)-CD molecules.

In next step, we reported how both rates are modulated for rapid gelation properties in PEG-grafted hyaluronic acid (PEG-graft-HA) systems, which can exhibit inclusion complexation with \( \alpha \)-CDs. A combination of low molecular weight PEG and high molecular weight HA in the grafted polymer system exhibits microphase-separated structure in aqueous solution. The PEG-grafted HA, exhibiting a microphase separation between the grafted PEG and HA in aqueous media, underwent a rapid gelation by adding \( \alpha \)-CD solution, because of the predominant distribution of \( \alpha \)-CDs into the PEG domains and the following inclusion complexation with the grafted PEG. We recently reported a report on supramolecular hydrogels with a familiar cationic polymer. A cationic polymer PL was introduced as a side chain grafted onto the dextran backbone to give pH sensitivity as well as thermo-sensitivity. By introducing cationic PL instead of PEG or PPG, the PL-grafted dextran and CD\( s\) show pH-sensitive hydrogelation in addition to thermo-reversible gel-sol transitions.

Recently, we developed a novel type of supramolecular network assembled by a unique system, named double-axle intrusion (DI) system, using gamma-cyclodextrin and the linear block copolymers grafted onto dextran, which can modulate rheological properties in response to pH changes. The supramolecular network induced drastic changes in the rheological properties in response to pH variation. Our knowledge based on supramolecular research demonstrates that such macroscopically rheological changes are results of molecularly mechanical actuations of the DI system in supramolecular networks. As a result, the molecular mobility allows to modulating the length of cross-linked part in polymeric network under pH changes. It is expected that our pH-tunable supramolecular network system can be utilized to achieve advanced functional biomaterials such as smart hydrogels and drug carriers.

**Electrospun Nanofibers of Supramolecular Structures**

A novel type of nanofibers can be fabricated with polypseudo-dorotaxane (PPR\( s\)) of poly(ethylene oxide) (PEO) and \( \alpha \)-cyclo-
dextrin (α-CD) by electrospinning technique. For the formation of PPRx, the aqueous mixtures of α-CD and PEO (Mn = 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) and 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. The addition of α-CD in PEO solution resulted in decreasing the average diameter of the PPRx nanofibers and increasing in roughness of the surface, supported by AFM observations. If performing further research, almost completely CD-threaded PPRx nanofiber can be obtained by changing various process conditions and solvents and by using advanced electrospinning technique.

Perspectives

Our studies suggest applicability of supramolecular structures with cyclodextrins in a few fields of biomaterials, containing blood- or bio-compatible materials and stimuli-sensitive hydrogels. Obtained results demonstrated that the materials were highly complicate and intricate to fabricate although assembled structures with cyclodextrins showed functions adequate to biomaterials. So, biomaterials based on our outcomes can be realized through many efforts to simplify fabrication process and methods of biomaterials. As another part of our researches, N. Yui and coworkers keep developing gene delivery carriers using positive charged supramolecular structures using cyclodextrins, showing promising results as advanced substitutes of established polyplexes. Our studies are expected to be utilized in the field of biomaterials as well as molecular machine. One of the most popular practical outcomes, but still hypothetical, among chemists, is that of information storage and processing. 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 6. 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.

Figure 7. SEM images of electrospun nanofiber from polypseudorotaxanes between PEO and α-cyclodextrin.
Abbreviations

APTT : activated partial thromboplastin time
AT III : antithrombin III
DI : double-axle intrusion
CD : cyclodextrin
CEE : carboxylethylester
HA : hyaluronic acid
PEG : poly(ethylene glycol)
PPG : poly(propylene glycol)
(L)PEI : (linear)poly(ethylenimine)
PU : polyurethane
PR : polyrotaxane
PPR : poly(ethylene glycol)
PU : polyurethane
Z-L-Phe : Z-L-phenylalanine

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