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Supramolecular Mobility in Polyrotaxanes Exploits Biomedical Functions

Nobuhiko Yui[*1,2]

Summary: The mobility of cyclodextrins (CDs) threaded onto a linear polymeric chain and the dethreading of the CDs from the chain are the most fascinating features seen in polyrotaxanes. These structural characteristics are very promising to their possible applications in biomedical use. Enhancing multivalent interaction between ligand-receptor systems by using ligand-polyrotaxane conjugates is one of the excellent properties related to the CD mobility. Gene delivery using cytocleavable polyrotaxanes is more practical but highly crucial in drug delivery. Such a supramolecular approach using CD-containing polyrotaxanes is extensively expected to exploit a new paradigm of advanced biomaterials for future medicines.

Keywords: biomaterials; gene delivery; multivalent interaction; polyrotaxane

Introduction

Nature always gives us fascinating ideas how hierarchical and dynamic architectures in cells and tissue are much related to regulate the achievement of several biological functions in living body. Supramolecular approach is one of the most outstanding features to mimic such biological functions, and has explored new paradigm of materials science.[1] By using intermolecular forces in building-blocks, supramolecular architectures can be spatially and temporally modulated as to fit any changes in the external condition. The representative of the supramolecular architectures designed in the last century is a polyrotaxane, and it is defined as a molecular assembly, in which many cyclic compounds are threaded onto a linear polymeric chain capped both terminals with bulky end-groups. Now a day, polyrotaxanes have been extensively studied as new class of polymeric assemblies, which can be expected to exhibit novel functionality. For instance, in order to prepare polyrotaxanes, intermolecular forces such as van der Waals interaction and intermolecular hydrogen bonds can be requested in a combination between cyclic compounds and a linear polymeric chain. Cyclodextrins (CDs) have been utilized as a representative of cyclic compounds in such polyrotaxanes. In the last decade, we have studied water-soluble polyrotaxanes including CDs in order to propose new ideas to apply for advanced biomaterials.

The mobility of cyclic compounds along a linear polymeric chain will be one of the greatest characteristics expected in polyrotaxanes. We have especially focused on this mobility related to modulating a variety of biomedical functions.[2] Strong evidence to support this concept is our previous achievements that the mobility of ligands linked with α-CDs threaded onto a poly(ethylene glycol) (PEG) chain in polyrotaxanes is much related to enhancing multivalent interaction with receptor proteins. In order to expand this concept to more practical applications, we have studied cytocleavable polyrotaxanes as a non-viral vector for gene delivery (Figure 1). In this paper, our recent achievements on the biomedical functions of polyrotaxanes are highlighted.

[1] School of Materials Science, Japan Advanced Institute of Science and Technology, Nomi, Ishikawa 923-1292, Japan
Fax: (+81)761 51 1645;
E-mail: yui@jaist.ac.jp
[2] JST CREST, Chiyoda-ku, Tokyo 102-0075, Japan
Fax: (+81)3 3222 2066;
E-mail: yui@jaist.ac.jp

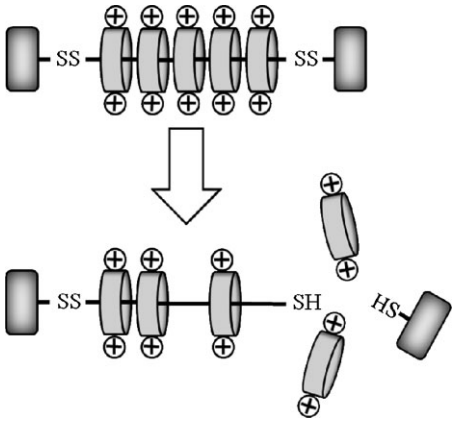

Figure 1.
Image of supramolecular dissociation of cytocleavable polyrotaxanes for DNA delivery.

Perspectives of Polyrotaxane Preparation

The structure of rotaxanes was first predicted in early 20th century, and finally prepared in the late 1960's.[3] The first report on polypseudorotaxanes (inclusion complexes) using CDs was carried out by N. Ogata and his coworkers in 1976, and they prepared them through polycondensation reaction between an inclusion complex of an aromatic or aliphatic diamine with β-CD and a dicarboxylic acid derivative.[4] Recent progress in polyrotaxane preparation has been derived from the remarkable efforts of A. Harada and his coworkers since 1990.[5] They succeeded to prepare polypseudorotaxanes directly from mixing aqueous solution of CDs with a linear polymer. One of their representatives is a polypseudorotaxane consisting of α-CDs and PEG. α-CD has been approved by FDA as a food and medical additive, and PEG is a well known water-soluble polymer for protein drug delivery. Thus, it is easily accepted that the polyrotaxanes consisting of this combination are promising if their final application for biomedical uses is considered. From these perspectives, we initiated to study biodegradable and/or water-soluble polyrotaxanes as biomaterials in 1993. For instance, we proposed a design concept for biodegradable polymers using polyrotaxanes, in which bulky end-groups are introduced into both terminals of PEG via a biodegradable linkage. Polyrotaxanes consisting of α-CDs and a PEG chain is prepared from their inclusion complex, and the process of the inclusion complexation involves the precipitation of the inclusion complex under aqueous environment due to strong intermolecular hydrogen bonds between neighboring α-CDs. In order to prepare water-soluble polyrotaxanes, any chemical modification of α-CD for reducing these hydrogen bonds is required after the capping reaction of the inclusion complex. α-CDs in the polyrotaxanes can be freely mobile in aqueous condition, once such a chemical modification is carried out. Regarding the diffusion of α-CDs along a PEG chain, K. Ito and his coworkers clarified in details in terms of quasi-elastic light scattering measurements.[6]

Multivalent Interaction between Receptor Proteins and Ligand-Polyrotaxane Conjugates

Functional groups introduced into CDs in polyrotaxanes enables us to link any biologically active agents or ligands, which are to bind with receptor sites of plasma proteins in cell surfaces. Controlling the binding of biological ligands with receptor sites of proteins on plasma membranes of cells is a crucial event on modulating receptor-mediated cellular metabolism as well as endocytosis for drug targeting. How effectively and specifically the binding on membrane proteins using very limited amount of the agents or ligands can be enhanced is one of the most important subjects in this issue. The term of ''multivalency'' is defined as a way to bind multiple copies of ligands with the receptor sites simultaneously, and the multivalency has been believed to be much effective to enhance the binding constant between the ligands and receptors.[7] In the last few decades, a variety of multifunctional polymers have been studied for the multivalent interactions, and include synthetic water-soluble polymers such as poly(acrylic

acid), poly(amino acid) such as poly(aspartic acid), polysaccharides such as dextran, and dendritic polymers. However, an increase in the binding constant using such multifunctional polymers is not striking as expected. This is mainly due to thermodynamically unfavorable situation in the interaction: increasing the number of ligands in the polymer eventually causes excessively increased density of the ligands. It is easily imagined that many ligands introduced to the polymeric chain can contribute to increasing the enthalpic gain, however, their binding to the polymeric chain derives the entropic loss at the same time.

From these perspectives, we postulated that the mobility of ligands linked to α-CDs in polyrotaxanes contributes to preventing such an entropic loss, leading to an increase in the multivalent interaction with protein receptors. We have believed that polyrotaxanes are advantageous to derive thermodynamic benefits on enhancing the multivalent interaction with biological systems: freely mobile ligands linked to CDs in polyrotaxanes can effectively bind receptor proteins in a multivalent manner. One of our representative studies using polyrotaxanes on the multivalent interaction is to clarify the effect of maltose-polyrotaxane conjugates on interaction with lectin (Concanavalin A).[8–10] The maltose-polyrotaxane conjugates were prepared by condensation reaction between β-maltosylamine and carboxypropanoyl-α-CDs in the polyrotaxanes consisting of α-CDs and PEG ($M_n = 20{,}000$). The extent of the multivalent interaction was examined by Con A-induced hemagglutination test using erythrocyte suspension. In fact the introduction of maltose into the polyrotaxanes enhanced the binding with Con A, up to 3,000 times larger than maltose itself. Special attention should be paid to the fact that the extent of the enhancement was dependent upon the number of α-CDs in the polyrotaxanes as well as the number of maltose groups. Pulse NMR experiments revealed that the mobility of maltose linked with α-CDs estimated from spin-spin relaxation time is much influenced by the number of α-CDs in the polyrotaxanes. Finally, we found that the extent of multivalent binding between maltose and Con A in the polyrotaxanes was closely proportional to the mobility of maltose (Figure 2). This finding strongly suggests that the mobility of ligands in polymers can dominate the binding with receptor proteins and the polyrotaxane is a suitable tool which can reduce spatial mismatches of the binding due to their mobility along the polymeric chain.

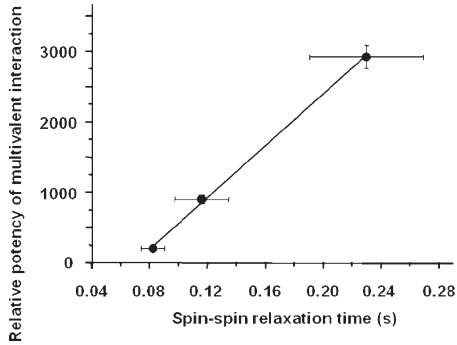

Figure 2.
Enhanced multivalent interaction between Con A and maltose-polyrotaxane conjugates in relation to the mobility of maltose (spin-spin relaxation time of maltose C1 proton).

Design of Cytocleavable Polyrotaxanes for Gene Delivery

Inventing non-viral gene vectors is one of the important challenges for advanced medicines, since viral vectors are not approved due to their lack of safety in living body.[11] Gene vectors are generally required to form a polyplex (polyion complex) with DNA, which should be stable during systemic circulation and before cellular uptake via endocytosis, escape from endosom/lysosome to cytoplasm (prevent from lysosomal digestion) after the endocytosis, and release DNA at nucleus. Cationic polymers such as poly(L-lysine) and poly(ethyleneimine) have been recently studied as a candidate for non-viral gene vectors, however, their low transfection efficiency and high cytotoxicity have not been elucidated. In general stable

polyplex formation of cationic polymers with anionic DNA requires excess amount of cationic groups, which causes a difficulty in the DNA release and an increase in the cytotoxicity. In order to solve this controversy, we have proposed the use of cytocleavable polyrotaxanes as a gene vector: the cytocleavable polyrotaxanes consist of cationic α-CDs and a PEG chain capped with tyrosine via disulfide (SS) linkage.[12–14] Cationic groups of α-CDs are expected to participate into the polyplex formation with anionic DNA, and this polyplex can be dissociated to release DNA by the intracellular cleavage of SS linkage in response to high cytoplasmic glutathione concentration.

The cytocleavable polyrotaxanes were prepared by the following steps: 1) the capping of PEG ($M_n = 4{,}000$) with cystamine to obtain a SS-introduced PEG-bisamine (SS-PEG), 2) the formation of an inclusion complex between α-CDs and the SS-PEG, 3) the capping reaction of z-protected L-tyrosine to both terminals of the inclusion complex, and 4) the introduction of dimethylaminoethylcarbamoyl (DMAEC) groups into α-CDs in the polyrotaxane. The polyrotaxanes were found to form a polyplex with plasmid DNA (pDNA) at relatively low N/P ratio, which may be due to the mobility of CDs along a PEG chain in the polyrotaxane through preventing spatial mismatch between DMAEC groups and phosphate groups in pDNA. Furthermore, it was confirmed that this polyplex was dissociated under reduced condition to release pDNA via the SS cleavage and intermolecular ionic exchange with anionic polymers. In the *in vitro* experiments using cultured NIH3T3 cells, the cytocleavable polyrotaxane/pDNA polyplex was found to exhibit much greater transfection activity than the reference polyplex using a non-cytocleavable polyrotaxane, and the magnitude reached over 500 times higher than the reference (Figure 3). This indicates the SS cleavage of cytocleavable polyrotaxanes plays an important role in intracellular pDNA release for gene expression. Also, the cytocleavable polyrotaxane/pDNA polyplex showed little cytotoxicity, although the cytotoxicity of the reference polyplex increased with the N/P ratio (Figure 4). This is considered due to the dissociation of the polyrotaxane into building-blocks contributes to the prevention of toxicity generally seen in cationic polymers. These results obviously demonstrate the efficiency of the cytocleavable polyrotaxanes as a gene vector, and suggest the feasibility of our design concept which can cope with two critical issues in gene delivery: the catch and release of pDNA at appropriate biological environments and

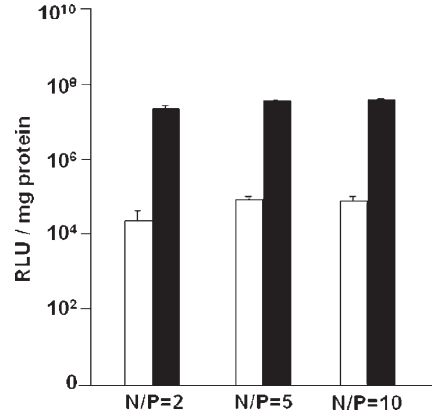

Figure 3.
DNA transfection by: closed bar = cytocleavable polyrotaxane, open bar = non-cytocleavable polyrotaxane.

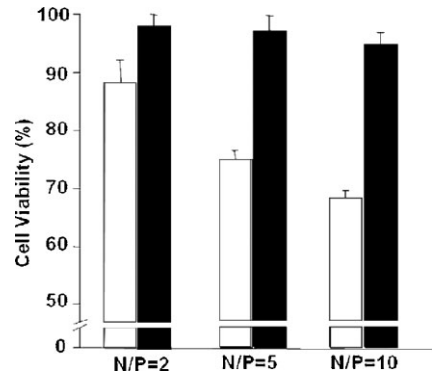

Figure 4.
Cellular viability test by MTT assay for; closed bar = cytocleavable polyrotaxane, open bar = non-cytocleavable polyrotaxane.

the prevention of cytotoxicity observed for conventional cationic polymers.

Conclusions

We prepared a series of ligand-polyrotaxane conjugates and cytocleavable polyrotaxanes and succeeded to clarify the importance of the mobility of cyclic compounds threaded onto a linear polymeric chain in modulating their biomedical functions. Modulating multivalent interaction with biological systems is much related to this unique feature of the polyrotaxanes. Alternatively important issue in the polyrotaxanes is its ability of dissociating the supramolecular structures into pieces if one of the both terminals is cleaved. This enables us to modulate binding constant with biological substrates, and in fact exploits outstanding properties in the design of non-viral DNA vectors.

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