Abstract of Doctoral Dissertation

Supramolecular Design for Multivalent Interaction: Preparation and Evaluation of Ligand-Density Controlled Polyrotaxanes for Efficient Binding with the Receptor Proteins

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In a ligand-receptor binding system, multivalent ligands are essential to induce strong and specific recognition events. Ligand placing, polymer folding, and active adjustment of ligand positioning are important subjects that need to be addressed for the generation of multivalent systems and the dynamic rearrangement of the biological interactions. In this respect, azidated polyrotaxanes controlling the ligand density were designed that can be utilized for demonstrating how the molecular motion of α-cyclodextrins (α-CDs) in the polyrotaxane affects the binding affinity with receptors.

As a feasible way for controlling density of ligands in polyrotaxanes, the azidated polyrotaxanes consisting of poly(ethylene glycol) (MW; 3,000 and 20,000 g/mol) and mono-, di-, or tri-azidated α-CDs were prepared in an optimized water/DMSO solution in a one-pot synthesis. Subsequently, mono-, di-, or tri-azidated polyrotaxanes were allowed to conjugate with propargyl-modified mannose as a ligand via click chemistry. As proven by the FT-IR spectroscopy and ^{1}H NMR integration, mannose molecules were efficiently introduced into all the azide moieties of the polyrotaxanes. These results verify the achievement of ligand-density controlled polyrotaxanes. This well-functionalized polyrotaxanes can be utilized for a variety of biological applications through opting propargyl-modified ligands.

The well-defined polyrotaxanes in terms of the number of mannose molecules were utilized for investigating the relation to multivalent interactions between the mannose moiety and *concanavalin A* (*Con A*) immobilized surfaces. As the results of surface plasmon resonance (SPR) spectroscopy, the mannose-conjugated polyrotaxanes (Man-PRxs) showed higher response units than any other mannose conjugates in both surfaces of high- and low-density *Con A*. From the kinetic analysis of their binding pattern, the binding affinities were mostly dependent on the association rate constant due to the high accessibility of Man-PRxs to the *Con A* surfaces. Moreover, the flexibility and movability of Man-PRxs were also examined to understand the effect of molecular motions of α-CDs in the polyrotaxane through dynamic light scattering (DLS) and fluorescence resonance energy transfer (FRET) methods. The results of the intermolecular FRET analysis suggest that the movability of α-CDs in the polyrotaxane more efficiently contribute to their binding interactions in a multivalent manner. This well-defined polyrotaxanes system provides control over ligand density, mobile ligands, and efficient response to the biological interaction receptor, which has not been easy to achieve in covalently bound polymeric systems.

As the practical application, macrophage response to the Man-PRxs was investigated in terms of the morphological change of macrophage-like J774.1 cells and their inflammation-related cytokine expressions. The obtained results were compared with those by mannose-conjugated α-CD, mannan, and mannose-conjugated polyacrylamide (Man-PAAm). The largest percentage of the morphological change was observed for Man-PRxs, which is presumably induced by mannose receptor-mediated endocytosis. In the cytokine expression experiments, anti-inflammatory cytokine was expressed by Man-PRxs, however, pro-inflammatory cytokine level was increased by Man-PAAm. Therefore, it is concluded that Man-PRxs has a great potential as anti-inflammatory biomaterials.