Characterization of the dynamic structures and interactions of Lewis X-carrying oligosaccharides and their clusters

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Abstract

Oligosaccharides and their clusters are involved in a variety of biological processes exemplified by cellular communications and play crucial roles in multicellular organisms through carbohydrate-protein and carbohydrate-carbohydrate interactions. However, the physicochemical studies toward elucidation of the functional mechanisms of oligosaccharides have been precluded because their interactions are generally weak and transient and conventional recombinant techniques are not available for sample preparations.

It is widely supposed that the flexible property of oligosaccharides enables their conformational adaptability to various binding proteins, and in parallel, cause a loss of the conformational entropy upon the interaction coupled with their weak binding. Therefore, controlling the dynamic processes of oligosaccharides by designing their conformational spaces is a promising approach not only for improving binding affinities and specificities but also for better understanding of the detailed processes in biomolecular interactions involving oligosaccharides. The weak interactions of oligosaccharides also can be enhanced through formation of their clusters with multivalent binding ability as exemplified by multiple carbohydrate–carbohydrate interactions in their clustered states that mediate cell–cell interactions. To shed light on such dynamical interactions in the cell surface events as objectives in molecular science, it is necessary to employ appropriate models of the oligosaccharide clusters.

In this thesis, I addressed the dynamic interaction processes involving the Lewis X-derived oligosaccharides, including their conformational adaptation to the binding

partners as well as the multivalent recognition, by hybridizing biophysical, synthetic, and biochemical approaches. Lewis X is a trisaccharide, Galβ1-4(Fucα1-3)GlcNAcβ, displayed on various cell surfaces as a functional determinant. For example, Lewis X-carrying glycoproteins play a vital role in maintaining the stemness of neural stem cells. It has also been supposed that Lewis X clusters on cell surfaces can mediate cell–cell interactions through their homophilic binding.

In chapter 2, I chemically modified the dynamic conformations of the Lewis X oligosaccharide to improve its protein binding affinity for the cognate lectins. This has been successfully achieved based on exploration of the conformational space by employing NMR techniques combined with the molecular dynamics (MD) simulation. For adequately exploring the conformational space occupied by the Lewis X trisaccharide, I performed replica-exchange MD simulation in explicit water. To experimentally evaluate the simulation results, I obtained paramagnetism-assisted NMR data of this trisaccharide. Upon addition of Tm^{3+} ions to the chemically synthesized trisaccharide attached with a lanthanide-chelating tag, the NMR spectral change was observed due to pseudocontact shift (PCS), which can provide long-distance information in conformational characterization. The observed PCS data of the Lewis X trisaccharide were in excellent agreement with those back-calculated from the conformational ensemble derived from the replica-exchange MD simulation, thereby providing atomic descriptions of dynamic behaviors of this oligosaccharide in solution. The results in conjunction with the previously reported crystallographic data revealed the lectins selected rare conformers of Lewis X during binding processes.

This finding motivated me to re-design its conformational space for controlling its protein-binding properties. Indeed, chemical modification of the Lewis X trisaccharide to populate the bound conformations successfully improved the protein binding affinity. Thus, remodeling of the conformational spaces of oligosaccharides is an effective methodology for designing artificial oligosaccharides with improved efficacy through better understanding their conformational dynamics.

In chapter 3, I hybridized the Lewis X oligosaccharide with the self-assembled complex for creating neoglycoclusters, which possess structural homogeneity suitable for structural analyses and also potential functional ability through multivalent interaction. The glycosylated organic bidentate ligand was converted to glycoclusters displaying 24 Lewis X sugar moieties on its spherical scaffold through forming a metal-organic complex in the presence of Pd^{2+} ion. I demonstrated that the self-assembled glycocluster exhibited hyper-assembly through homophilic carbohydrate-carbohydrate interactions upon addition of Ca^{2+} ion. Furthermore, the well-defined Lewis X clusters enabled detailed NMR characterization of their interactions mediated by the oligosaccharides moieties. I successfully probed metal binding to the Lewis X-containing glycoclusters by observing paramagnetic relaxation enhancement. The NMR data revealed that the specific carbohydrate structure as well as their clustering form are prerequisite for the Ca^{2+}-mediated carbohydrate-carbohydrate interaction.

Moreover, in chapter 4, I created the novel glycoclusters composed of Lewis X-carrying neoglycolipids, in which the functional oligosaccharide units were

combined to acyl chains, as tools for controlling cellular functions. Using the synthetic Lewis X-carrying neoglycolipids, cell viability assays of neural stem cells before and after differentiation were performed. In this approach, it was demonstrated that the functional Lewis X glycotope connected to the fatty acid can evoke selective apoptosis of the neural stem cells before differentiation, while leaving the differentiated neuronal cells alive. The observed apoptosis was suppressed by the removal of lipid moiety or the fucose residue from the Lewis X-carrying neoglycolipid, indicating that the functional Lewis X group in a clustered form is a prerequisite for its apoptotic activity.

Thus, I employed synthetic approach integrated with biopysical techniques including NMR spectroscopy. Consequently, I could successfully design and create the neo-glycomolecules by hybridizing biomolecules and artificial molecules. The dynamical structures and assembly states of these neo-glycomolecules are artificially controlled in attempt to endow them with the higher affinity for target proteins, the Ca^{2+}-mediated hyper-assembling property, and the selective apoptotic activity. It is expected that these neo-glycomolecules can be useful tools for probing protein-carbohydrate interactions, carbohydrate-carbohydrate interactions, and cellular functional processes. It is also expected that the strategy I developed for creation and characterization of the neo-glycomolecules can be applicable for other biomolecules with structural flexibility and assembling properties.