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論文の要旨

Summary (Abstract) of doctoral thesis contents

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.

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博士論文審査結果の要旨

Summary of the results of the doctoral thesis screening

糖鎖およびそのクラスターは、細胞間のコミュニケーションをはじめ、様々な生命現象において不可欠な存在だと考えられている。しかしながら、糖鎖の機能メカニズムに関する分子科学的な研究は、未だ十分に行われていない。その要因の一つは、糖鎖の認識機構が、弱く過渡的な相互作用を通して生じることにある。本論文はこうした状況に鑑みて、糖鎖およびそのクラスターの動的な構造や相互作用様式を、合成化学と物理化学の手技を駆使した統合的アプローチによって明らかにしたものである。

申請者は、ルイスX糖鎖の溶液中における詳細な動的立体構造解析を行うとともに、化学的構造改変によるコンフォメーション制御を通してタンパク質との親和性を向上させ、糖鎖− タンパク質相互作用様式の解明を図った。ルイスX糖鎖は、神経細胞において分化前の細胞に特異的に発現しており、その幹細胞性の維持を担っている。また、ルイスX糖鎖は互いを認識し、糖鎖間相互作用を示すことが知られている。ルイスXの溶液中でのコンフォメーションが、どのようにタンパク質に認識されているかを調べるため、ルイスX糖鎖を化学合成し、常磁性NMR計測を行った。さらに、これら一連の糖鎖の構造アンサンブルモデルを、レプリカ交換分子動力学計算によって求め、NMR実験データに基づいて計算結果を評価した。得られた立体構造情報を基に、ルイスXの化学的構造改変によって、タンパク質結合状態に見られる開いたコンフォマーを増大させ、タンパク質との親和性を向上させることに成功した。

また申請者は、ルイスX糖鎖を人工分子骨格とハイブリッドした糖鎖クラスターを合成し、これを用いて集積化した糖鎖の糖鎖− 糖鎖間相互作用メカニズムの構造基盤解明を行った。ルイスXを含む糖鎖構造を化学合成した後、これを有機配位子と連結した。合成した配位子分子とパラジウムイオンとの錯形成反応により自己組織化錯体を調製した。これにより、直径およそ40 Åの一義構造をもった球状分子表面に、24個のルイスX糖鎖を集積することに成功した。調製したルイス Xクラスターのマクロ挙動を、NMRおよび動的光散乱法によって観測したところ、この球状分子はカルシウムイオン依存的な糖鎖間相互作用によって、水中でより大きな会合体を形成することを明らかにした。さらに、常磁性NMR法を用いたミクロ計測によって、糖鎖−金属イオン結合の構造基盤情報を収集した。その結果、クラスター間会合を誘起するために必要なルイス Xとカルシウムイオンとの相互作用には、糖鎖の化学構造のみならず、その集積形態が重要であることを見出した。

さらに、糖鎖が関わる細胞機能の制御を目指し、人工設計に基づく機能性糖脂質の創生に取り組んだ。申請者は、Lewis X糖鎖にエフェクター部位を連結したネオ糖脂質を調製し、その生理活性評価を行った。合成したネオ糖脂質は水中でミセルを形成し、集積化することを明らかにした。さらに得られたネオ糖脂質クラスターと細胞との相互作用を評価した結果、ネオ糖脂質が神経幹細胞に対して特異的に細胞死を誘導することを見出した。

以上のように、申請者は主体的に研究に取り組み、ルイスX糖鎖およびそのクラスターの動的な相互作用機構に関する構造基盤を明らかにすることに成功している。さらに、生体分子と人工分子の融合による機能分子科学を進展させている意義も大きい。また、本論文の成果は、申請者を筆頭著者とする英文論文として国際学術雑誌1報に発表済みである。

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以上のことから、本論文は、博士（理学）の授与にふさわしいものであると審査委員全員一致で結論した。