Self-assembled multivalent carbohydrate ligands†

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Materials that display multiple carbohydrate residues have gained much attention due to their potential to inhibit or modulate biological multivalent interactions. These materials can be grouped accordingly to the way they are prepared, as unimolecular or as self-assembled systems. Both systems take advantage of the fact that multivalent interactions have significantly higher binding affinity than the corresponding monovalent interactions. The self-assembled system is a more recent field of research compared to the unimolecular system. In this review, we describe current efforts to realize multivalent carbohydrate interactions from the perspective of synthetic self-assembled systems. We limit the scope to self-assembled systems that are stable, soluble in aqueous solution and morphologically discrete. We grouped them into two separate categories. In the first category carbohydrate ligands self-assemble onto a pre-organized nanostructure, and in the second carbohydrate-conjugated block molecules spontaneously assemble to construct morphologically distinct nanostructures.

1. Introduction

Multivalent interactions, which are characterized by simultaneous binding of multiple ligands on multiple receptors, are prevalent and essential in many biological recognition events.[1–7] Multivalent interaction is one of the nature's many ingenious creations and controls the onset of many biological processes. It is a concentration dependent process within the microenvironment where the increase in the binding constant is far higher than the simple arithmetic sum of respective binding constants of each binding partner. Numerous examples of biological multivalent interactions include antibody–antigen interaction, pathogen recognition of the host cell, ligand–receptor interactions on the cell surface, and a number of intracellular processes such as transcriptional regulation.

Protein–protein, nucleic acid–protein, and carbohydrate–protein interactions are the most prevalent biological multivalent interactions. Among them, large proportions of currently discovered multivalent interactions are mediated by carbohydrate–protein interactions.[3–5] They achieve their specificity by exploring the wide structural diversity of carbohydrates. Carbohydrate molecules on the mammalian cell surface are the targets of many pathogenic viruses and bacteria in their initial cell recognition events. The pathogens utilize multivalent interactions for tight binding and specific recognition of the cells, which is then followed

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† The HTML version of this article has been enhanced with a colour image.

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by infection of the host cells. A number of bacterial toxin–cell interactions also occur by multivalent interactions.[8,9] For example, shigalike and cholera toxins are members of the AB_5 family of bacterial toxins. They are composed of one enzymatic A subunit as a lethal factor and five B subunits. The five B subunits, galactose binding proteins, are organized symmetrically such that the pentamer interacts with the cell surface sugar ligands in multivalent binding mode before they initiate intracellular invasion.

Self-assembly, a powerful approach for the construction of novel supramolecular architectures, is mediated by noncovalent interactions including hydrogen bonds, electrostatic interactions, and hydrophobic interactions.[10–16] Block molecules that mimic lipid amphiphilicity have been promising scaffolds for self-assembly into nanometer-sized objects. Depending on the molecular structure of the amphiphilic components, it has been possible to construct various supramolecular architectures such as spherical micelles, vesicles, fibers, and nanotubes. Since the intrinsic nature of self-assembly is a building-up of higher-order structure by the repetitive interactions of monomeric building blocks, self-assembled structures are excellent platforms for constructing multivalent ligands.

Chemically-synthesized multivalent molecules that can disrupt pathological carbohydrate-dependent processes have been regarded as potential therapeutic agents. In the past, these multivalent inhibitors were prepared mostly by synthesizing polymers with multiple pendant carbohydrates. However, the recent interest in supramolecular chemistry is fuelling the effort directed toward the utilization of the self-assembly process for generating multivalent carbohydrate ligands.

2. A unimolecular system *versus* a self-assembled system

Consideration of a scaffold or framework that serves as a molecular anchoring system where multiple carbohydrate ligands can be attached is the first step in constructing a multivalent system. A traditionally considered scaffold is a molecule with high-valency reactive functional groups to which all the carbohydrate ligands can be linked covalently.[17–23] A number of diverse covalent scaffolds have been used for multivalent presentation. Molecules of low valency, generally from di- to octavalent, have been constructed from a one-dimensional linear chain or a two-dimensional round molecules such as macrocycles. For generating molecules of high valency, polymer or dendrimer scaffolds have been used. Construction of multivalent molecules by a self-assembly process, a more recent technology, utilizes the fact that monovalent units spontaneously assemble to form higher order structures. There are advantages and disadvantages of both systems, *i.e.*, the unimolecular system *versus* the self-assembled system. The advantages of the self-assembled multivalent system that can be considered are as follows. First, it is an energy efficient and cost-effective way; instead of making one big molecule with multivalency, which often demands multiple synthetic steps, all one needs to synthesize is a simpler monovalent building block. Second, using a self-assembly process is better, especially when it comes to making an object of extremely high valency. Synthesis of polymers, for example, with more than several thousands repeating units is not practically easy and moreover, high molecular weight polymers are generally insoluble. Synthesis of dendrimers of higher generation is also challenging. In addition, high efficiency surface functionalization of the high generation dendrimer periphery is often not easy due to the steric crowding. Third, the conformation of polymer chain is generally globular rather than extended, which might act disadvantageously for multivalent interaction with large objects. By contrast, it has been possible to construct self-assembled molecules of various shapes and sizes, which extends the repertoire of possible choice. Examples of the extended self-assembled nanostructures are cylinders and tubes, and condensed ones are micelles and vesicles.

3. Carbohydrate ligands assembled onto the pre-organized nano-structures

Carbohydrate-coated gold nanoparticles

A gold surface offers an opportunity for attaching thiolated molecules to form self-assembled monolayers. Gold nanoparticles functionalized with thiolated carbohydrates have been prepared to explore cell–cell interactions. The surface of mammalian cells is covered with a dense coat of carbohydrates named the glycocalyx. It has been shown that in addition to protein–protein and protein–carbohydrate interactions, cells utilize attractive interactions between carbohydrates as an initial step for adhesion and recognition. The carbohydrate–carbohydrate and carbohydrate–protein interactions are often mediated by divalent cations such as calcium ion.[24]

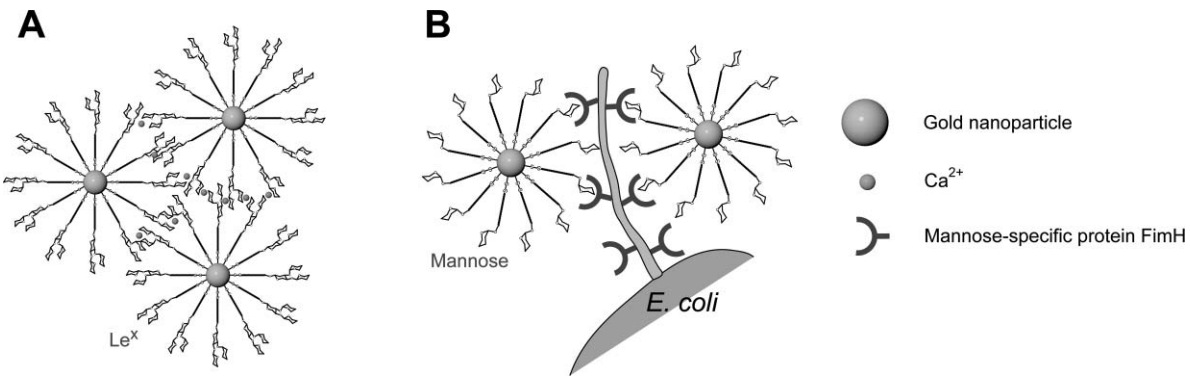

Fig. 1 (A) Calcium ion mediated recognition of Le^x-functionalized gold nanoparticles. (B) Binding of mannose-functionalized gold nanoparticles onto the mannose binding proteins in the *E. coli* pili.

As one example, gold nanoparticles coated with trisaccharide Lewis^x (Le^x) and disaccharide lactose were reported.[25] Le^x at the embryonic cell surface has been shown to interact specifically with Le^x on the opposing homotypic cell surfaces, providing a basic mechanism for cell recognition during early development.[24] The gold nanoparticles were obtained by *in situ* functionalization of carbohydrate disulfide, $HAuCl_4$and $NaBH_4$. The mean diameter of the gold nanoparticles was around 2 nm and they were soluble in aqueous solution. The Le^x functionalized gold nanoparticles showed a selective ability for self-recognition through a specific carbohydrate–carbohydrate interaction in the presence of Ca^{2+}. The self-recognition resulted in the aggregation of the nanoparticles and was dependent on the calcium ion concentration (Fig. 1A).

The biomedical application of the carbohydrate functionalized gold nanoparticles was found in antiadhesive therapy of cancer cells.[26] In metastasis, tumoral cells detach from the primary tumor and travel through the lymphoid and blood vessels until they arrive at a specific target location. The adhesion of tumor cells to the vascular endothelium for the creation of new foci is a critical step in metastasis and is promoted by interactions between tumor-associated carbohydrate antigens and epithelial cell surface carbohydrates. Penadés and collaborators prepared lactosylated gold nanoparticles with 70 lactose molecules per nanoparticle. The nanoparticles were able to inhibit the interaction between ganglioside GM3 (NeuNAc2α3Galβ4GlcβCer) of a murine melanoma cell line (B16) and lactosylceramide of endothelium cells in *ex vivo* mouse model.

Another type of biological application for carbohydrate coated gold nanoparticles can be found in selective bacterial recognition (Fig. 1B).[27] Mannose coated gold nanoparticles (m-AuNP) were synthesized using a similar *in situ* functionalization method to that described above. The m-AuNP was a multivalent one with approximately 200 attached mannose residues per nanoparticle. The m-AuNP was very stable in deionized water and buffered saline solution, and its stability was independent of ionic strength and pH values in the range from 1.5–12. Electron microscopy studies showed that m-AuNP was able to bind to mannose-specific protein FimH of type 1 pili in *Escherichia coli.* The type 1 pili are filamentous proteinaceous appendages that extend from the surface of many Gram-negative bacteria. The specificity of the interaction was manifested by the use of an *E. coli* strain without FimH where no attachment of m-AuNP on the pili was observed. The interaction was shown to be very strong, as 2000 times excess monovalent mannose was required to compete out 90% bound nanoparticles, which indicates that the interaction between m-AuNP and FimH is multivalent.

With a similar strategy of using thiolated molecules, as in construction of the carbohydrate-coated gold nanoparticles, the preparation of carbohydrate-coated quantum dots has been reported.[28] The advantage of the quantum dots is that they are useful for cellular imaging. More in depth description of relevant topics can be found in recent reviews.[29,30]

Carbohydrate-coated carbon nanotubes

Carbon nanotubes (CNTs) represent a unique class of one-dimensional nanostructures that possess characteristics suitable for a variety of possible applications. The chemical modification or functionalization of their surfaces allows the attachment of various bioactive molecules including carbohydrates. The CNTs are suitable for multivalent display because of their high surface area-to-weight ratio. Bertozzi and collaborators recently described the preparation of CNTs coated with a biomimetic carbohydrate polymer for the purpose of mimicking cell surface mucin glycoproteins.[31] The polymer comprised a poly(methylvinyl ketone) backbone decorated with α-*N*-acetylgalactosamine (α-GalNAc) residues. The C_{18} lipid tail provided a hydrophobic anchor for the CNT surface. By taking advantage of the *Helix pomatia* agglutinin (HPA), a hexavalent lectin that is specific for α-GalNAc residues, the mucin mimic CNTs were able to bind specifically to Chinese hamster ovary (CHO) cells (Fig. 2). One notable advantage of the glycopolymer-coated CNTs was that they did not show any sign of cytotoxicity whereas unmodified CNTs did. Thus, the glycopolymer coating renders the CNTs nontoxic while simultaneously providing a means for specific cell surface binding.

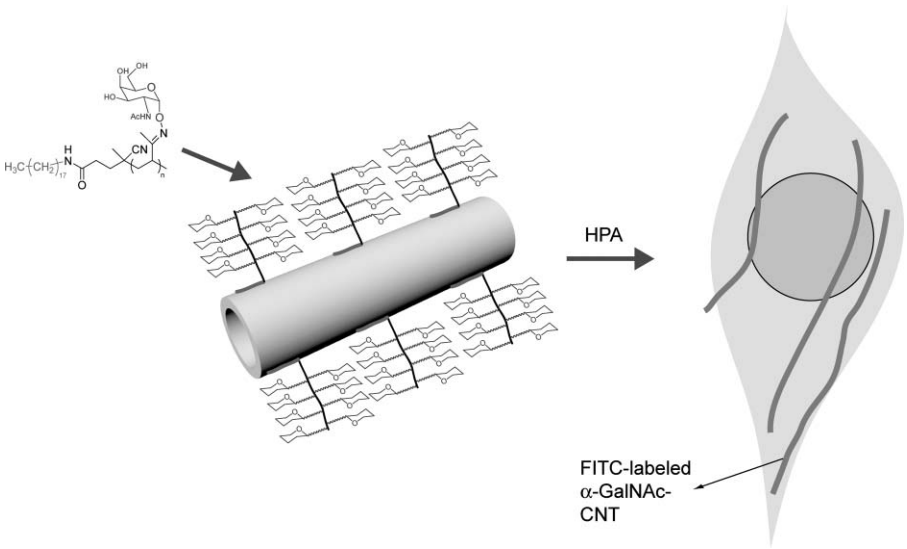

Fig. 2 A CNT coated with biomimetic carbohydrate polymers to mimic cell surface mucin glycoproteins. The CNTs can bind to mammalian cells specifically with the aid of a hexavalent lectin, HPA.

4. Self-assembly from disorganized carbohydrate-conjugated block molecules

In the self-assembled systems described above, monovalent carbohydrate units are attached to the pre-organized nanostructures, gold nanoparticles or carbon nanotubes. Next, we describe another type of self-assembly system in which disorganized block molecules of a carbohydrate and an assembly molecule self-associate to form organized nanostructures.

In one example, sialic acid (SA) was conjugated to a lipid chain, and the conjugate was mixed with natural lipids during liposome preparation.[32] In the resulting SA-coated liposomes, the SA moieties can, in principle, move by lateral diffusion to optimize their binding to multiple hemagglutinin proteins on the influenza virus surface. It was found that the liposome display of multivalent SA molecules resulted in 500–10^4 fold better efficiency in hemagglutination inhibition (HAI) assay. A similar conformationally-flexible self-assembly strategy has been explored by Thoma *et al*.[33] They synthesized carbohydrate coupled dendrons with a hydrophobic core expecting that the self-assembled aggregates would optimize their size and shape to fit in their multivalent binding partners. Kataoka and coworkers have prepared sugar-installed poly(ethylene glycol) (PEG)/poly(DL-lactide) block copolymers.[34] The block copolymer formed a core–shell type micelle with hydrophilic sugar and PEG groups at the surface. The micelle was able to recognize a cognate lectin protein in a multivalent manner. Surface plasmon resonance studies have been utilized to investigate the details of interactions between self-assembled micelles and lectins.[35]

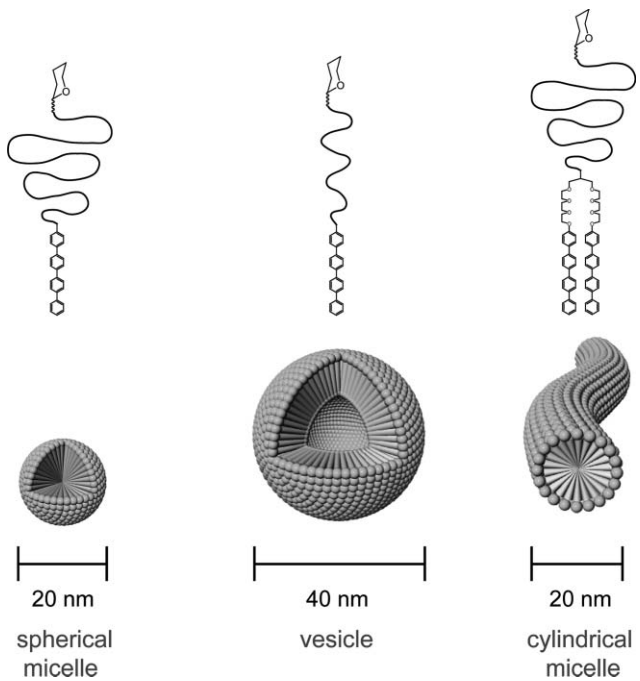

Fig. 3 The mannose-coated supramolecular nanostructures of predefined size and shape.

Rather different strategies utilized the supramolecular nanostructures of predefined size and shape (Fig. 3). We have reported several rod–coil amphiphilic molecules with attached mannose residues that can form vesicles, spherical or cylindrical micelles depending on the molecular architectures of the rod and the coil segment.[36,37] The supramolecular assemblies bound specifically to cognate binding proteins on the type 1 pili of *E. coli* as revealed by transmission electron microscope (TEM) analysis. All the supramolecules showed their ability to efficiently inhibit concanavalin A (Con A)-promoted erythrocyte agglutination in HAI assay. Depending on the size and shape of the supramolecular assemblies, the efficiency varied from 800–1800 fold, where the spherical micellar object was the highest among them. These results imply that the biological activity of carbohydrate-functionalized supramolecular objects is critically dependent on their size and shape.

5. Prospect for the self-assembled multivalent carbohydrate ligands

We have described representative examples of carbohydrate-displayed and shape-persistent self-assembled systems. Myriad biological multivalent interactions are mediated in different physiological settings. Therefore, it can be considered that the ways to modulate these biological interactions should be different depending on their specific characteristics. The field of supramolecular chemistry is rapidly expanding, making it possible to generate self-assembled objects with various sizes, shapes and stimuli responsive functions at the designer's discretion. In addition, the supramolecular building blocks display the range of repertoire that spans from organic or inorganic molecules to biological molecules such as peptides or nucleic acids. The combined versatility of supramolecular systems should be useful in exploring biological multivalent interactions (and this is just the beginning). We envision that the self-assembled carbohydrate display system will be useful in many areas of biomedical application such as inhibition of malignant cell–cell or pathogen–cell adhesion, gene delivery, drug delivery and cellular imaging. To achieve these goals, the specificity of carbohydrate ligands needs to be improved and the characteristics of self-assembled nanostructures should be controllable to meet the demands of specific biological circumstances.

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