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Review

Glycoconjugates and Glycomimetics as Microbial Anti-Adhesives

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Microbial adhesion is an essential step in infection and is mediated primarily by protein–carbohydrate interactions. Antagonists of such interactions have become a promising target for anti-adhesive therapy in several infective diseases. Monovalent protein–sugar interactions are often weak, and most successful anti-adhesive materials consist of multivalent glycoconjugates. Although often very effective in hampering microbial adhesion, natural epitopes often show limited resistance to enzymatic degradation. The use of carbohydrate mimics (glycomimetics) as a replacement for natural sugars potentially allows higher metabolic stability and also higher selectivity towards the desired protein target. In this review we describe the state of the art in the design and synthesis of glycoconjugates and glycomimetics employed for the construction of anti-adhesive biomaterials.

Trends

Increasing antibiotic resistance is drawing attention to anti-adhesive approaches for the treatment of infections.

Microbial adhesion to host cells can be antagonized by disrupting the sugar–protein interactions that drive it.

Antagonists can be targeted either to bacterial adhesins or to host glycan receptors.

Effective anti-adhesive biomaterials have been created using multivalent glycoconjugates and glycomimetics.

Microbial Adhesion and Carbohydrates

Bacterial adhesion to host cells is essential for microbial pathogenesis and is initiated by molecular recognition events at the host–guest interface. Efficient adhesion permits the pathogen to escape the natural cleansing mechanisms of the host and allows access to nutrients, delivery of toxins, and ultimately colonization and invasion of the host, including biofilm formation [1]. In most cases, molecular recognition is triggered by specific carbohydrate–protein interactions involving glycoconjugates and sugar-binding protein (lectins). The lectin involved can be displayed either on the bacteria or on the host surface. Bacterial **adhesins** (see Glossary), located on the bacterial surface or on pili and fimbriae, interact with specific glycans on host tissues to initiate colonization. Bacteria also use adhesins to adhere to other microbial cells as a prerequisite for biofilm formation. Conversely, specific host lectins, particularly in the immune system, recognize pathogens by interaction with their surface glycans. This step, designed for pathogen processing and clearance, is often exploited by the pathogen to colonize the host. Thus, it has been proposed that appropriate neo-glycoconjugates able to interfere with carbohydrate–protein recognition could be used to inhibit microbial adhesion in the very early stages of an infection. This approach strives to prevent colonization and possibly even to reverse biofilm formation, but does not aim to kill the invading pathogen, and therefore should not exert selective pressure leading to resistance [2]. Because bacterial resistance to antibiotic treatment is increasing, and is becoming a pressing public-health concern, anti-adhesive therapies are emerging as a valuable alternative or complementary approach.

Individual interactions between proteins and sugars are usually very weak, a flaw often overcome in living systems by multivalency on both the glycan and the lectin side, so that high avidity is reached. This effect, often referred to as 'the glycan cluster effect' or 'the velcro effect', largely depends on the specific features of lectin binding sites, which tend to be flat, large, and exposed to the solvent. Effective anti-adhesive therapy requires either high-affinity monovalent lectin

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ligands capable of outperforming glycan clusters, or multivalent structures incorporating several copies of ligands of moderate affinity on a polyvalent scaffold (dendrimer, polymer, nanoparticle). Depending on the nature of the lectin involved, a large number of glycomimetic and neo-glycoconjugate structures have been proposed as antagonists of sugar–protein interaction events initiating bacterial and viral infections, and recent comprehensive reviews are available [3–6]. Among the many reported examples, in this paper we will focus on two applications of the anti-adhesive approach targeting the recognition of host glycans by a bacterial adhesin or the recognition of microbial glycans by a human lectin. These two examples, which have been thoroughly investigated in recent years, illustrate how an anti-adhesive approach can be used against different types of pathogens exploiting the same fundamental (bio)chemical mechanism and targeting either host or microbial sugar receptors. In the first case, the development of inhibitors of **adherent-invasive** (AIEC) and **uropathogenic** (UPEC) ***Escherichia coli*** represents an alternative and/or complementary antibacterial strategy against widespread and difficult-to-treat infections, which are increasingly becoming resistant to classical antibiotic therapies. With the second paradigmatic example we aim to illustrate the progress made towards prophylactic antiviral agents targeting **dendritic cell-specific ICAM-3 grabbing non-integrin** (DC-SIGN), a receptor of the human immune system that, although discovered only 15 years ago, is rapidly becoming an established target for microbial infections.

Inhibitors of AIEC and UPEC

Infections of the urinary tract are most frequently caused by UPEC adhering to α-mannopyranosyl ligands on the surface of the host urothelial cells. The adhesin involved is called **FimH** and is located on the tip of bacterial type 1 fimbriae, rod-like fibers 1–2 μm in length and 7 nm in thickness, that protrude from the bacterium body. FimH contains a mannose-specific lectin domain which adheres to terminal mannosides of two glycoproteins, uroplakin and $\alpha_3\beta_1$-integrin, that are abundantly expressed by urothelial cells. FimH also appears to control the adhesion of AIEC isolated from Crohn's disease patients.

It was established early on that mannosides bearing aromatic aglycones can be used as antagonists of FimH-mediated bacterial adhesion [7]. Crystallographic studies [8] (PDB 1UWF, 1TR7) showed that the lectin domain of the adhesin contains a relatively deep sugar-binding pocket lined by aromatic lipophilic residues at the rim (the tyrosine gate, formed by Tyr48, Tyr137, and Ile52) and established butyl α-D-mannoside **1** (Figure 1A) as a strong antagonist. Optimization of the aglycone chain length led to the discovery of *n*-heptyl α-D-mannoside (α-D-HM, **2**, Figure 1A) as a nanomolar FimH antagonist [9]. Recent structural studies [10] have called into question the importance of the tyrosine gate in the recognition process of mannosides by the full FimH protein. Indeed, NMR relaxation analysis and small-angle scattering X-ray studies revealed that α-D-HM **2** promotes dimerization of FimH **carbohydrate recognition domain** (CRD), a process that may lead to overestimation of its binding affinity in *in vitro* studies. In addition, a Y48A mutant of FimH was found to possess binding properties very similar to the wild-type protein. However, the selectivity of aryl mannosides for FimH and against a panel of human mannose-binding lectins, a key element for *in vivo* applications, was shown to be determined by the structure of the aglycone [11,12].

Dissociation constants in the nanomolar range have been routinely achieved with both polyvalent and monovalent compounds, and, depending on the application, both monovalent mannosides and mannosylated high-valency materials are being developed as FimH antagonists. Prevention and treatment of urinary tract infection by UPEC requires orally-active FimH antagonists, absorbed in the intestine and renally excreted, with finely tuned pharmacokinetic properties, to achieve an optimal balance between affinity and duration of the therapeutic effect in the bladder. This is typically pursued with monovalent aryl mannosides [13–17] targeted to the FimH binding site by their mannose component and carrying extended aromatic aglycones that

Glossary

Adherent invasive *Escherichia coli* (AIEC): bacteria found in the small intestine of patients with Crohn's disease.
Adhesin: Components of bacterial surface that facilitate adhesion to other cells or surfaces. Adhesins are generally proteins in Gram-negative bacteria and polysaccharides in Gram-positive bacteria.
Atomic force microscopy (AFM): a scanning probe microscopy technique with very high resolution (fractions of nanometers) that can measure local properties of sample surfaces
C-lectins: also called C-type lectins, Ca^{2+}-dependent glycan-binding proteins that share structural homology in their CRDs.
Carbohydrate recognition domain (CRD): in a lectin, contains the sugar binding site
Dendritic cell-specific ICAM-3 grabbing non-integrin (DC-SIGN): also called CD209, DC-SIGN is a PRR membrane receptor of dendritic cells with specificity for mannosylated and fucosylated oligosaccharides.
Dynamic light scattering (DLS): a photophysical technique that allows the hydrodynamic radius of a particle to be determined.
FimH: bacterial adhesin located at the tip of bacterial fimbriae and that is responsible for mannose-mediated adhesion.
Glyco-nanodiamonds (GND): nanodiamonds modified on their surface with carbohydrates or glycomimetics
gp120: glycoprotein of the HIV envelope. It is recognized by DC-SIGN through an interaction with its high-mannose glycans.
ICAM3: a member of the intercellular adhesion molecule (ICAM) family.
Langerin: transmembrane C-type lectin, specifically expressed on Langerhans cells. It is also known as CD207.
Nanodiamond (ND): a carbon-based nanoparticle obtained by detonation; they are available in large quantities at reasonable price.
Pattern recognition receptors (PRRs): protein receptors expressed by cells of the innate immune system. They recognize invading pathogens binding to so-called pathogen-associated molecular patterns that are often glycans displayed at the pathogen surface.