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Commentary

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‘‘Multivalent’’ saccharides: development of new approaches for inhibiting the effects of glycosphingolipid-binding pathogens

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Abstract

A number of diseases are initiated by the adherence of viruses, bacteria, or bacterial toxins to cell surface carbohydrates, a number of which are components of glycosphingolipids (GSLs). Studies of the binding of lectins indicated that many adhered weakly to monomeric carbohydrate ligands. The seminal observation that lectins adhered more strongly to a ligand with multiple carbohydrate binding sites initiated a plethora of studies designed to identify effective ‘‘multivalent’’ carbohydrate ligands for pathogens expressing multiple carbohydrate-binding sites. In addition to more completely defining ligand specificity of the carbohydrate-binding pathogen, identification of ‘‘multivalent’’ carbohydrate ligands has led to studies of their efficacy as pathogen inhibitors. This commentary focuses on pathogens that recognize the carbohydrate portion of GSLs. Because many GSL-binding pathogens have been shown to bind ‘‘multivalent’’ saccharides, approaches for identifying and preparing them as well as methods for characterizing their effectiveness as ligands are reviewed. Also discussed are areas of promise that should be investigated and pitfalls that might be encountered in the development of ‘‘multivalent’’ saccharides as pharmacologic agents.

Keywords: Glycosphingolipids; Gangliosides; Dendrimers; Multivalent saccharides; Glycosphingolipid-binding pathogens

1. Introduction

Three problems in the treatment of infectious diseases are: (a) the emergence of antibiotic-resistant strains of bacteria, (b) the existence of bacteria that secrete toxins, the activity of which is not affected by antibiotics, and (c) the lack of effective anti-viral agents. The first step in infection of a cell by viruses, bacteria, or bacterial toxins is their adherence to ligands on the cell surface. Over the past several decades, a number of pathogens have been shown to adhere to cell surface GSLs, and these interactions are the focus of this commentary. Table 1 contains examples of pathogens that adhere to GSLs, and Table 2 shows the saccharide portion of the GSLs discussed. So, how were the GSL ligands listed for the pathogens shown in Table 1 identified?

2. Identification of potential GSL ligands

A simple method for determining whether a GSL is needed for a pathogen to act upon its target cell is to determine the effect of inhibition of GSL synthesis on its ability to act upon cells that are normally susceptible to it. Treatment of cells with fumonisin B1, an inhibitor of sphingosine *N*-acyltransferase [1], blocks the synthesis of all GSLs. In contrast, inhibitors of ceramide:UDP glucosyltransferase, such as *N*-butyldeoxygalactonojirimycin [2], *d,l-threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol·HCl [3], or *d,l-threo*-1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol·HCl [4], block the synthesis of GSLs derived from glucocerebroside. By comparing the amount of infectivity seen when each type of inhibitor is used, one can determine whether sphingolipids or glucosylcerebroside-derived GSLs, or both, are needed.

Once it is determined that GSLs are necessary for infectivity, the next step is to identify those necessary for infection. After extracting GSLs from the cells [5], they can be separated by TLC and then overlaid with the pathogen [6,7]. Adherent pathogen can be visualized by indirect immunoassay or, if a labeled pathogen was used,

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Abbreviations: GSL, glycosphingolipid.

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Table 1
Pathogens identified as binding to a glycolipid receptor

Pathogen	Ligand	References
Botulinum neurotoxin	GT1b	[49]
Campylobacter jejuni-enterotoxin	GM1	[50,51]
Cholera toxin	GM1	[10,21]
Clostridium perfringens δ toxin	GM2	[52]
Heat-labile enterotoxin of *Escherichia coli*	GM1 > GM2, GD3, GD1b,	[53]
	Asialo-GM1, and paragloboside	[54]
Heat-labile enterotoxin of *Salmonella typhimurium*	GM1	[55]
Shiga toxin	Gb3, and galα1-4galβ1-containing lipids	[56]
Tetanus neurotoxin	GT1b	[57]
Verotoxin 1 and 2c	Gb3	[58]
Influenza virus hemagglutinin	GM3, GD1a, IV^3(NeuAc)n-Lc4Cer, IV^6(NeuAc)nLc4Cer, i and I-active ganglioside	[59]
Influenza virus	$Poly_{(->50)}$glycosylceramides	[60]
	Gb4, SSEA-3, SSEA-4 and paragloboside	[61]
Rotavirus (all 3 strains)	GM2, GD1a, asialo-GM2	[62]
	and -GM1	
SA11	and NeuGc-GM3	
NCDV	and NeuGc-GM3	
UK	and NeuAc-GM3 and GM1	
Sendai virus	GD1a	[63]
Actinomyces naeslundii	Ga1ß1–3GalNAc, GalNAcß1–3Gal	[64]
Borrelia burgdorferi	Galβ1-cer, LC, Gb3, GD1a, and GT1b	[65]
Borrelia hermsii	Galβ1-cer	[66]
Chlamydia pneumonia	Asialo-GM2 and -GM1	[67]
Chlamydia trachomatis	Asialo-GM2 and -GM1	[67]
Entamoeba histoytica HM1-IMSS	Galβ1-4GlcNAc	[68]
E. coli VJ1	Asialo-GM2 and -GM1	[69]
E. coli 6883	Asialo-GM2 and -GM1	[69]
E. coli K99 fimbriae	NeuGc-GM3	[70,71]
E. coli P-fimbriade HB101/pPIL219-15	Galα1-4Gal	[72]
Helicobacter pylori	GM3, $poly_{(11–41)}$glycosylceramides	[73,74]
Haemophilus influenzae	Asialo-GM2 and -GM1	[69]
H. parainfluenzae	Asialo-GM2 and -GM1	[69]
Klebsiella penumoniae	Asialo-GM2 and -GM1	[69]
Lactobacillus johnsonnii La1	Asialo-GM1	[75]
Mycoplasma pneumoniae M129	$Gal(3SO_4)$β1–	[76]
Neisseria gonorrhoeae MS11, ML1, and LP1	Asialo-GM2 and -GM1, lac-cer, isoglobotriaosyl-cer	[77]
Propionibacterium	Galß1–44Glcß–	[78]
Pseudomonas aeruginosa	Asialo-GM2 and -GM1	[69]
Pseudomonas cepacia	Asialo-GM2 and -GM1	[69]
Salmonella typhimurium	Asialo-GM2 and -GM1	[79]
Streptococcus pneumoniae	Asialo-GM2 and -GM1	[69]
	Globoside	[80]

by monitoring the label. As shown in Table 1, a pathogen can often recognize more than one ligand. If this occurs, it may be possible to determine whether the pathogen prefers one to another by repeating the experiment using equivalent amounts of each GSL. Variants of ELISAs in which the GSL ligand is immobilized on plastic microtiter wells have also been used to identify potential GSL ligands (e.g. Ref. [8]). However, the TLC-overlay procedure has the advantage of eliminating the need to purify each GSL prior to testing its ability to function as a ligand.

Three approaches have been used to confirm the identification of a GSL as a potential ligand. In one, the ability of the GSL to inhibit binding/infection of cells by the pathogen is determined. Another is to determine whether the pathogen can infect GSL^- cells, and the third is to grow GSL-depleted cells in the presence of medium containing the potential GSL ligand for a period of time prior to adding the pathogen and monitoring binding/infectivity. A combination of these approaches was used in studies of the adherence of cholera toxin to its cell surface ligand, ganglioside GM1 [9]. Initially it was found that incubation of cholera toxin with GM1 prior to its addition to liver cell membranes inhibited its adherence to the membranes [10]. Subsequently, it was observed that when cholera toxin-resistant, $GM1^-$ cells were grown in medium containing added GM1, the exogenous GM1 was incorporated into their plasma membranes and made the cells susceptible to the toxin [11].

3. Identification of the need for "multivalent" saccharides for optimum binding by a pathogen

Why should a "multivalent" saccharide be needed to optimize binding by a pathogen whose natural ligand is a

Table 2
Structure of the saccharide portion of glycolipids discussed in this review

Glycolipid	Saccharide structure
Lactosylceramide	Galβ1–4Glcβ1–
Trihexosylceramide (CTH, Gb3)	Galα1–4Galβ1–4Glcβ1–
Isoglobotriaosylceramide	Galα1–3Galβ1–4Glcβ1–
Paragloboside (nLc4)	Galß1–4GlcNAcß1–3Galβ1–4Glcβ1–
Globoside (Gb4)	GalNAcß1–3Galα1–4Galß1–4Glcß1–
SSEA-3	Galß1–3GalNAcß1–3Galα1–4Galß1–4Glcß1–
SSEA-4	NeuAcα2–3Galß1–3GalNAcß1–3Galα1–4Galß1–4Glcß1–
GM3	NeuAc/NeuGcα2–3Galβ1–4Glcβ1–
GD3	NeuAcα2–8NeuAcα2–3Galβ1–4Glcβ1–
GM2	GalNAcβ1–4(NeuAcα2–3)Galβ1–4Glcβ1–
Asialo-GM2	GalNAcβ1–4Galβ1–4Glcβ1–
GM1	Galβ1–3GalNAcβ1–4(NeuACα2–3)Galβ1–4Glcβ1–
IV^3(NeuAc)nLc4Cer	NeuAcα2–3Galß1–4GlcNAcß1–3Galß1–4Glcß1–
IV^6(NeuAc)nLc4Cer	NeuAcα2–6Galß1–4GlcNAcß1–3Galß1–4Glcß1–
i-active ganglioside	NeuAcα2–3Galß1–4GlcNAcß1–3Galß1–4GlcNAcß1–3Galß1–4Glcß1–
I-active ganglioside	Galα1–3Galß1–4GlcNAcß1 (6) Galß1–4GlcNAcß1–3Galß1–4Glcß1– ; NeuAcα2–3Galß1–4Glcß1 (3)
Asialo-GM1	Galβ1–3GalNAcβ1–4Galβ1–4Glcβ1–
GD1a	NeuAcα2–3Galβ1–3GalNAβ1–4(NeuAcα2–3)Galβ1–4Glcβ1–
GD1b	Galβ1–3GalNAcβ1–4(NeuAα2–8NeuAcα2–3)Galβ1–4Glcβ1–
GT1b	NeuAcα2–3Galβ1–3GalNaβ1–4(NeuAcα2–8NeuAcα2–3)Galβ1–4Glcβ1

GSL? The answer was provided, in part, by the observation that GSLs are found in clusters in lipid rafts on the cell surface [12]. The concept of multiple carbohydrate binding sites providing the basis for strong adherence to carbohydrate receptors evolved from studies of lectin binding. Those studies indicated that even though binding of a lectin to a single saccharide might be weak, its adherence to multiple saccharides resulted in a strong association of the lectin with its ligand [13,14]. Similarly, while the K_D for the adherence of hemagglutinin to α-sialoside monomeric derivatives was ~2 mM, its attachment to the surface of an erythrocyte expressing many sialosyl residues was strong with a K_D of $<10^{-12}$ M [15]. Combining these observations led to the hypothesis that, for pathogens adhering to the carbohydrate portion of a GSL, it might be possible to synthesize "multivalent" saccharides that could inhibit their adherence to cell surface GSLs, thereby preventing infection.

Early evidence supporting the use of "multivalent" ligands to inhibit binding was provided by studies with cholera toxin and the heat-labile enterotoxin of *Escherichia coli*. Both are AB_5 toxins characterized by the presence of an A-subunit and a homopentameric B-subunit that contains five binding sites [16,17]. These two toxins also have 80% homology in their amino acid sequences [18]. In 1974, it was reported that the oligosaccharide portion of GM1 (oligo-GM1) was not as effective a ligand for cholera toxin as intact GM1 [19,20]. Subsequently, it was shown that a "divalent" oligo-GM1 saccharide was a better ligand than oligo-GM1 but still not as good as GM1 [21]. Binding analyses using "multivalent" oligo-GM1 ligands prepared by linking oligo-GM1 to poly-L-lysine indicated that a "multivalent," non-lipid, oligosaccharide was an effective inhibitor of the binding of both toxins to GM1 immobilized on plastic [8].

4. Carriers for the preparation of "multivalent" saccharides

A number of proteins, e.g. bovine serum albumin, α-amylase, and lysozyme [22], have been used for the preparation of "multivalent" saccharides. Dendrimers, hyperbranched polymers having a known structure, were discovered in the early 1990s (e.g. Ref. [23]). These have provided researchers with more defined carriers for "multivalent" saccharide preparation. A number of different types of divergent dendrimers (dendrimers prepared outwardly from a central core) have been synthesized and used for this purpose (for a recent review, see Ref. [24]). Two divergent dendrimers are commercially available (Aldrich). Poly(propylene imine) dendrimers (DAB-Am) are synthesized from 1,4-diaminobutane and acrylonitrile, and Starburst® (polyamido amine, PAMAM) is synthesized from ethylenediamine and propionic acid. PAMAM dendrimers have been shown to be nontoxic [25]. The first three generations (a generation indicates the number of branches: e.g. generation 1 has 4 arms, 2 has 8, 3 has 16, etc.) of DAB-Am dendrimers have been shown to be defect-free and generations four and five to be relatively homogeneous [26]. Examples of other compounds used in the synthesis of dendrimer cores are *N,N′*-bis(acrylamido)acetic acid [27] and hyperbranched L-lysine [28]. See Fig. 1 for examples of these structures.

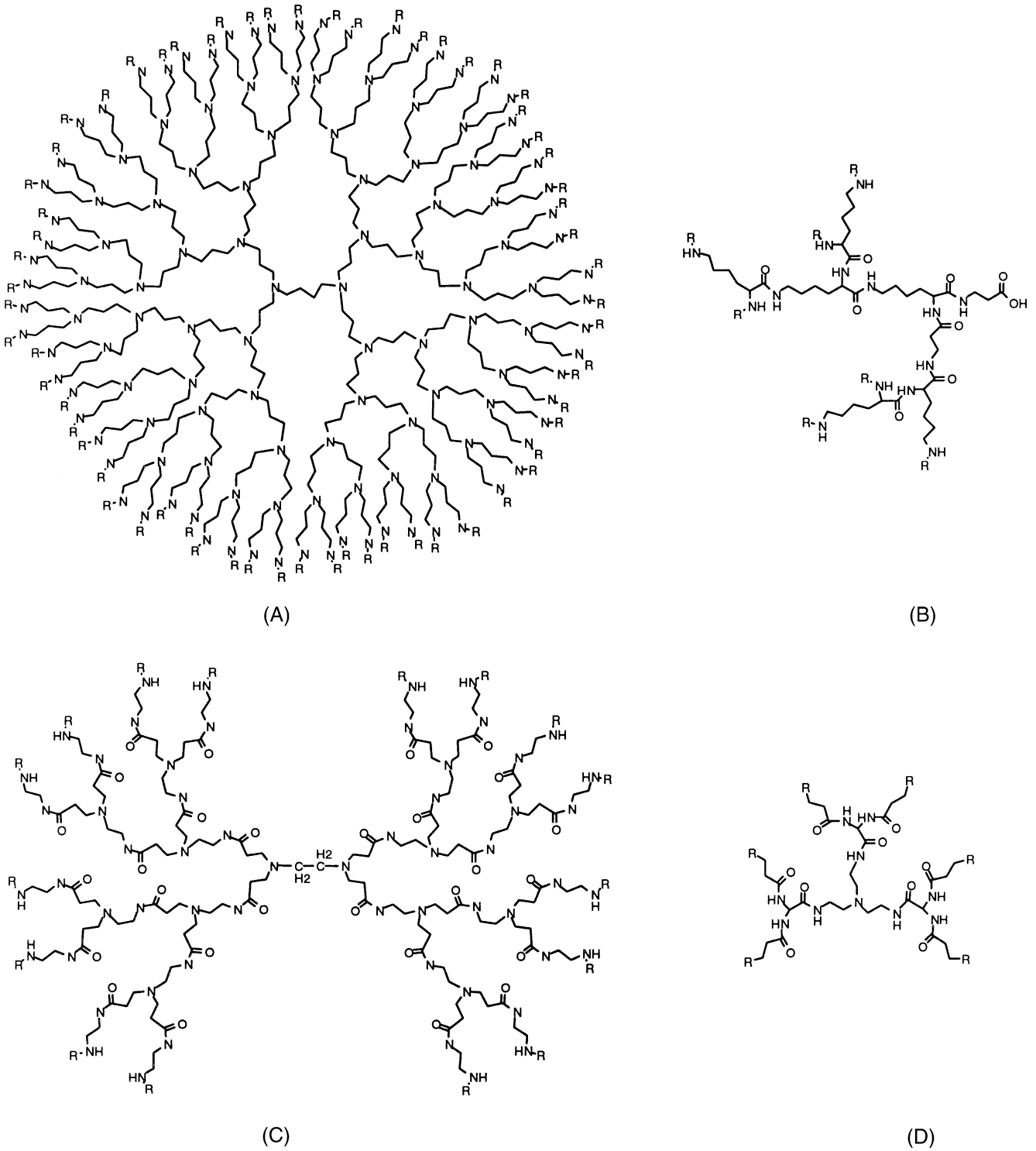

Fig. 1. Structures of some common dendrimer cores. (A) DAB-Am, generation 5, (B) hyperbranched L-lysine core, (C) Starburst® (PAMAM), generation 2, and (D) *N,N′*-bis(acrylamido) acetic acid core. R = saccharide covalently linked to each terminus.

In contrast to divergent dendrimers, convergently synthesized dendrimers are prepared by synthesizing carbohydrate-containing wedges and linking them to additional branching components prior to their addition to the dendrimer core. This approach was used for the synthesis of the decameric STARFISH ligand that was found to be a very effective inhibitor of verotoxin binding [29]. Although the convergent approach may yield a more structurally homogeneous product than the divergent approach, the divergent approach is straightforward, and the polydispersity of the product can be determined using matrix-assisted laser desorption/ionization time-of-flight

mass spectrometry (MALDI-TOF MS) [30]. The variety of methods available for the synthesis of dendrimers provides a means for tailoring the number and spacing of the saccharides to the receptor site(s) on the pathogen of interest.

5. Synthesis of "multivalent" saccharides

To facilitate the linkage of saccharides and to retain the internal sugar in its cyclic conformation, a number of reactive groups have been added to, or left on the C(1) position of that sugar. Examples include the addition of phenylisothiocyanate [31], 3-mercaptopropionic acid [32], squaric acid diester [33], or allyl alcohol [28], and retention of a portion of the ceramide during formation of a "ceramide" acid [34]. Reductive amination [35] has also been used to link saccharides through their reducing sugars directly to primary amines. The drawback with reductive amination is that it opens the ring of the reducing sugar, which may affect the ability of the saccharide to function as a ligand. The average number of saccharides linked per dendrimer using a specific procedure can be determined using MALDI-TOF MS [30].

As mentioned when discussing the selection of a dendrimer, one of the factors to consider is the space around the sites to which each saccharide will be linked. The linker used to covalently bind each carbohydrate residue will also affect the spacing around the saccharide moieties. Therefore, conformational space of glycodendrimers reflects the structural features of the core component, the saccharide residues, and the structure of the component used to link the saccharides to the core [36]. When the structure of the pathogen for which the saccharide is a ligand is known, the availability of different cores and linking components makes it possible to design potential "multivalent" inhibitors that can be adhered to by the multiple binding sites on the pathogen.

Cholera toxin and the heat-labile enterotoxin of *E. coli* can be used as examples of how knowledge of the crystal structure of the binding entity can be used to explain its adherence to a "multivalent" saccharide. The binding sites of both toxins are formed by the interaction of one B-peptide with another. The interactions between the five identical monomers cause the pentameric binding subunit to have a ring-like structure with five identical binding sites formed by the interaction of each peptide with its adjacent neighbor [16,17] (Fig. 2). This may explain why poly-L-lysine, derivatized with an average of 7 or 8 saccharide residues isolated from GM1 (oligo-GM1), was a better inhibitor of the adherence of both toxins to GM1 than the same poly-L-lysine derivatized with an average of 2 or 3 oligo-GM1 residues [8]. Interestingly, a comparison of the predicted structure of an oligo-GM1-derivatized DAB-Am-8 dendrimer with the structure of each toxin indicated that the saccharides should be available for binding by more than one of the toxin binding sites. However, if that occurred, the core of the dendrimer could block the center of the ring formed by the pentameric B-subunit. That would be predicted to block activity of the toxins because it is believed that the A-subunit of each moves through that area prior to acting on its target cell [37]. Using the crystal structure for the heat-labile enterotoxin as a guide, Fan *et al.* [38] synthesized a pentavalent ligand having just galactose residues. A terminal galactose is an essential component of ligands adhered to by the heat-labile enterotoxin of *E. coli* [17]. Fan *et al.* [38] found that, by adjusting the length of the spacer arms, significant changes were obtained in the concentration of the pentavalent ligand needed to inhibit, by 50% (IC_{50}), binding of the toxin to the disialoganglioside GD1b [39] immobilized on plastic. While the "multivalent" galactose ligand was not as efficient an inhibitor as the oligosaccharide portion of GM1, their results indicate that it may be possible to make effective inhibitors using key components of a native ligand.

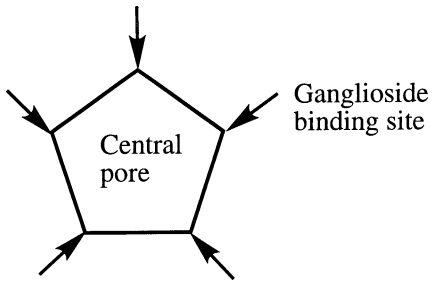

Fig. 2. Schematic drawing of the binding subunit of cholera toxin or the heat-labile enterotoxin of *E. coli*. The arrows indicate that the five binding sites, present on the same side of the pentavalent binding subunit, are formed by interactions between adjacent subunits [16,17].

6. Characterization of a "multivalent" ligand as a potential inhibitor of pathogen adherence

The efficacy of a "multivalent" ligand as a potential inhibitor of the binding of a pathogen is usually determined by measuring the affinity of the pathogen for it or by determining its IC_{50}. Surface plasmon resonance (SPR), which optically measures changes in mass concentration of molecules at a biospecific interface, can be used to obtain real time measurements of binding affinities. Advantages of this approach are that measurements can be made using unlabeled protein, and only small amounts of material are needed. The K_D found using SPR to monitor the adherence of cholera toxin to GM1 was 4.61×10^{-12} M [40], while that for the adherence of the C-terminal portion of tetanus toxin to its ligand trisialoganglioside GT1b was 1.7×10^{-7} M [41]. ELISAs provide a simple approach for determining the IC_{50} of a potential inhibitor. ELISA assays were used to determine the efficacy of the multivalent galactose compounds discussed above at inhibiting

the adherence of the heat-labile enterotoxin of *E. coli* to GD1b [38,39].

7. Promising results

Rhesus monkeys are susceptible to colonization by *Helicobacter pylori* and can be experimentally colonized using strains of human origin. Over time, they develop symptoms similar to those seen in people [42]. Using monkeys colonized with *H. pylori* for a year or more, Mysore *et al.* [43] were able to cure 2 of 6 monkeys using oral administration of 3′-sialyllactose. Interestingly, bovine serum albumin carrying ~20 3′-sialyllactose residues was found to have an IC_{50} that was about a thousand-fold less than 3′-sialyllactose when used to inhibit adherence of *H. pylori* to monolayers of epithelial cells [44]. This observation suggests that determining the efficacy of the multivalent ligand at inhibiting *H. pylori* colonization *in vivo* would be therapeutically relevant.

Results of experiments to determine the effectiveness of multivalent saccharides at inhibiting the adherence of pathogens to their target cell surface GSL receptors are encouraging. For example, three different types of dendrimers were sialylated and used to inhibit adherence of two different strains of influenza virus [X-31 (A/Aichi/2/68 × A/PR/8/34) influenza A H3N2 and influenza virus (A/AA/6/60) H2N2, mouse passaged] to Madin Darby canine kidney (MDCK) cells [45]. The amount of inhibition varied from 55 to over 90% for the X-31 strain, while that for influenza A H2N2 ranged from 23 to 35%. Because the actual concentration of sialic acid residues was kept constant when different ‘‘multivalent’’ saccharides were used, the variation in the degree of inhibition observed for a given strain reflected the effect of the core on the overall structure. Variation between strains may reflect differences in spacing between the sialic acid-binding hemagglutinin molecules present on the surface of the virions. The presence of more sialic acid on the surface of a ligand enhanced its effectiveness as a viral inhibitor and reduced the probability of its having a toxic effect on the cells.

Studies with cholera toxin and the heat-labile enterotoxin of *E. coli* [46] indicated that ‘‘multivalent’’ saccharides, prepared by covalently linking the phenylisothiocyanate derivative of oligo-GM1 to the arms of an octa(propylene imine) dendrimer, inhibited cellular adherence by both toxins. Less than a 100 nM concentration of the derivatized dendrimer was able to inhibit binding of the toxins to GM1-containing cells. Exposure of the cells to a 500 nM concentration of derivatized dendrimer for 24 hr had no effect on their viability, as monitored by trypan blue exclusion. These examples underscore the need for studying the adherence of viruses, bacteria, and bacterial toxins to saccharides and for identifying effective inhibitors of those interactions.

8. Problems that need to be addressed

From the foregoing discussion, it can be seen that a number of questions need to be addressed when designing a ‘‘multivalent’’ saccharide inhibitor. First and foremost is the problem of ‘‘multivalent’’ receptors. The molecules that mediate adherence of bacteria and viruses to their target cells are present in multiple copies on their surfaces, and toxins often express multiple binding sites. For a monovalent ligand to inhibit their adherence, it is necessary to have the ligand present at a concentration great enough to be competitively bound by the proteins that mediate pathogen binding to the GSLs on target cells. In the case of the influenza virus, the amount of soluble monomeric sialic acid needed to inhibit its binding to the surface of erythrocytes would be nonphysiological and possibly toxic. The monomeric sialic acid would also be susceptible to enzymatic degradation [45]. The problem of monomer concentration is also seen in the study in which 3′-sialyllactose was used to treat monkeys infected with *H. pylori*. The lowest amount of orally administered 3′-sialyllactose used was 100 mg/kg/day for 28 days and the greatest 500 mg/kg/day for 56 days [43]. For a 150-lb. human, the comparable minimum dose would be more than 6 g/day. The observation that the multivalent ligand was about 1000-fold more effective an inhibitor of the adherence of *H. pylori* to cells [44] indicates that it should be more effective than 3′-sialyllactose *in vivo*.

Another problem is specificity. A ‘‘multivalent’’ saccharide that is an effective inhibitor of the cellular adherence of one strain of virus or bacteria may not be as effective at preventing the adherence of another. This was observed in studies of the effectiveness of sialylated dendrimers at inhibiting the uptake of two different strains of influenza virus by MDCK cells [45]. To design effective ‘‘multivalent’’ ligands, detailed information is needed about the native structure of the proteins that adhere to them. Perhaps the best example of how detailed structure analysis led to the development of an exceptionally effective ‘‘multivalent’’ ligand is seen in the synthesis of the decameric STARFISH dendrimer used to inhibit the adherence of verotoxins [29].

An unanswered question is whether administration of a ‘‘multivalent’’ saccharide, shown to inhibit binding when given with the pathogen, will be effective at dislodging bound pathogen. The results obtained in a study of monkeys infected with *H. pylori* indicated that this should be possible [43]. While initial infection of cells may not be prevented, it may be possible, where there is release of newly synthesized pathogen, to inhibit subsequent infection of additional cells, thereby ameliorating the symptoms. For diseases such as cholera and Travelers’ diarrhea, it should even be possible to develop ‘‘multivalent’’ saccharides that could be taken prophylactically. In these instances, the toxin produced by the bacteria would be bound by the multivalent ligands, and if the affinity of the

interaction was high, the toxin would subsequently be excreted.

A major problem in the past has been obtaining enough saccharide for the preparation of ''multivalent'' ligands. Despite significant advances made toward automating the chemical synthesis of saccharides (see Ref. [47] for a recent review), it is still not a trivial matter to prepare specific tri- or greater oligosaccharides. However, progress in identifying and cloning bacterial glycosyltransferases has made it possible to engineer bacteria to produce large quantities of specific oligosaccharides (see Ref. [48] for a recent review). As more complex oligosaccharides become more readily available, it will be possible to use them for the synthesis of ''multivalent'' ligands. Use of the actual saccharide recognized by the pathogen instead of just a sialic acid or galactose residue should result in the formation of much more effective ligands. This hypothesis is supported by the observation that STARFISH dendrimers carrying ten terminal Gb3 trihexoside moieties were extremely effective inhibitors of verotoxins [29].

9. Conclusions

The use of ''multivalent'' saccharides to inhibit adherence of pathogens to their target cells is an area of research that has come into its own. This is due, in part, to advances in structure analysis and computer modeling that help enormously in designing effective ''multivalent'' ligands able to antagonize pathogen–host interactions (e.g. Refs. [29,37]). The development of automated approaches to the chemical synthesis of oligosaccharides, coupled with the use of bioengineered bacteria to produce large quantities of specific oligosaccharides, should make it easier in the future to synthesize ''multivalent'' ligands using the actual saccharide recognized by the pathogen. With these advances, it is anticipated that research in this area will grow and that within a few years there will be clinical trials testing the therapeutic efficacy of specific ''multivalent'' saccharides.

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