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Glycolipids as attachment sites on animal cells for bacteria, bacterial toxins and viruses: aspects on identification and characteristics

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Abstract - The abundance of glycoconjugates at the animal cell surface is a probable explanation why microbes appear to have selected primarily carbohydrates as essential attachment sites for normal colonization or infection. A novel solid-phase assay based on microbe binding to a thin-layer chromatogram with separated host cell glycolipids has facilitated a preliminary mapping of the character of the receptors. The often low-affinity binding, a recognition of internally placed sequences, and the preference of certain sequences before others, appear to be of biological value. The binding to internal parts of a chain is of technical importance when assigning narrow binding epitopes, facilitating chemical synthesis of efficient receptor analogues. The use of receptor knowledge for clinical diagnosis and therapy of infections and within biotechnology is a promising new field for industrial investments. Receptor specificities for several infections of global importance have already been revealed although their biological relevance in most cases remains to be proven.

INTRODUCTION

The development in recent years of carbohydrate technology in a broad sense has allowed in intensified analysis of the carbohydrate architecture of animal cell surfaces. This is a challenge to the biochemist since the surface sugars are virtually unknown concerning precise functions. They may provide more unspecific functions like protection of surface peptide against enzyme or antibody attack or contribute to macromolecular conformation and cell surface charge (e.g. NeuAc). This may refer to the bulk of surface carbohydrate which does not vary much between different animals or cells (e.g. Man-containing oligosaccharides). On the other hand there are sophisticated variations, often for minor sequences, suggesting recognition phenomena based on specific carbohydrate-protein interactions, possibly of importance for multicellular patterning during tissue formation and development (ref. 1).

Although there are several research groups facing this recognition problem, the biological complexity makes some of the questions in part inaccessible to informative experimentation. Also, carbohydrate is not only the most abundant substance on animal cell surfaces, but represents the most complex of biosubstances. The illustrative theoretical calculation on three different monosaccharides combining into more than thousand separate trisaccharides, compared to three amino acids producing six tripeptides, emphasizes the need for technical sophistication to reveal subtle changes in surface structure which may be biologically relevant.

In my view the abundance (and also character, see further below) of animal cell surface carbohydrate and its variation between cells may explain the selective evolution of carbohydrate-binding proteins among microorganisms requiring for their survival (reproduction) attachment to (and invasion of) the animal cell as a host providing metabolic energy and reproductive machinery. It appears that the majority of specific and essential attachment sites known so far is carbohydrate rather than peptide, as reviewed for viruses (refs. 2,3), bacteria (refs. 4,5) and bacterial toxins (ref. 6),

and also shown for protozooan parasites (see ref. 7). The most likely collision partner for a ligand approaching the host cell is carbohydrate, which may direct the selection.

The first case where binding to surface carbohydrate was proven essential was influenza virus. Burnet and co-workers showed in the 1940s (ref. 8) that an enzyme fraction produced by the cholera bacterium, Vibrio cholerae, was able to eliminate virus binding after action on the host cell surface, the "receptor-destroying enzyme". The substance cleaved off was later identified as NeuAc (ref. 9). Thus it was early shown that binding (and infection after invasion) was absolutely dependent on carbohydrate. Today there is advanced information on influenza virus binding to NeuAC-containing oligosaccharides (ref. 3). In the majority of known cases of microbes binding to carbohydrate (refs. 2-7) the evidence is however mostly indirect through elimination of binding by treatment of host cells with periodate or glycosidases.

Concerning lipid-bound oligosaccharide receptors (glycolipids) van Heyningen and co-workers discovered the receptor for cholera toxin (producing diarrhoea) as the GM1 ganglioside (ref. 10) which is a specifically lipid-bound pentasaccharide not yet found in protein-bound form (ref. 6). Haywood showed a binding of Sendai virus to liposomes containing brain gangliosides (ref. 11). In more recent time E.coli causing urinary tract infection of man was demonstrated to specifically recognize Galα1-4Gal in lipid-bound form (refs. 12,13) and this finding was the stimulus for the present more systematic approach on primarily lipid-linked oligosaccharides as receptors for microbiological ligands. The purpose of the present paper is not to review known carbohydrate receptors since these have been summarized elsewhere (refs. 2-7). Rather I will discuss some aspects which are partly novel and of more general interest to the carbohydrate chemist. They have appeared from our 4 year old project where the central tool is a novel type of binding assay. However, the more biologically relevant data will generally not be considered.

GLYCOLIPIDS VERSUS OTHER CELL SURFACE GLYCOCONJUGATES IN RELATION TO ASSAYING FOR RECEPTORS

The carbohydrate at the animal cell surface (Fig. 1) appears conjugated to lipid as glycolipids (ref. 14), or to peptide as glycoproteins (ref. 15) and proteoglycans (ref. 16). In addition to strictly membrane-bound glycoconjugates there are more loosely associated glycoproteins (mainly mucins, see ref. 17) and proteoglycans that are secreted from cells and may dominate the mass at the surface, especially at mucous membranes, the part of entry of most infections. When considering attachment of microbes to surface membranes as a requirement for colonization or infection this secreted part may be of decisive importance. If the secreted glycoconjugate carries the specific binding site for a microbe there may be a competitive inhibition of the association to membrane-bound sites and the microbe may be eluted in secretions and the infection avoided (see discussion on glycoproteins and influenza virus in ref. 3). Therefore, the adequate analysis of the microecological relevance of a carbohydrate binding property of a microbe should include a separate consideration of membrane-bound and secreted fractions. Today this is technically too complex to accomplish (see also below).

At the present stage of our project we are assaying for glycolipids as receptors for various microbes. Firstly, glycolipids are with some exceptions (e.g. blood plasma, ref. 15, or extruded epithelial cells in feces, ref. 18) strictly membrane-bound. Secondly, a technical advantage is that glycolipids carry one single oligosaccharide per molecule compared to glycoproteins or proteoglycans which have many, often different, oligosaccharides linked to the same peptide. This means on assaying and processing that an optimized resolution finally should yield a pure receptor glycolipid for structural analysis (see further below). A glycoprotein at this stage is still heterogenous concerning carbohydrate, which has to be chemically or biochemically released and further fractionated (with several technical drawbacks). Therefore, combined with the novel assay technology to be illustrated below glycolipids are a superior choice at this stage of development.

The convenience of using glycolipids has an important limitation. Particular oligosaccharide sequences may appear selectively bound to protein or lipid. As an example Man of higher animals is found exclusively in N-linked chains of glycoproteins (not in mucins) but not in glycolipids. Limiting the aproach to natural glycolipids therefore means that a binding specificity carried by a major group of bacteria (ref. 4) is excluded from analysis. On the other hand there are lipid-linked sequences not yet detected in glycoproteins. This problem will be further discussed below in relation to an extended technology.

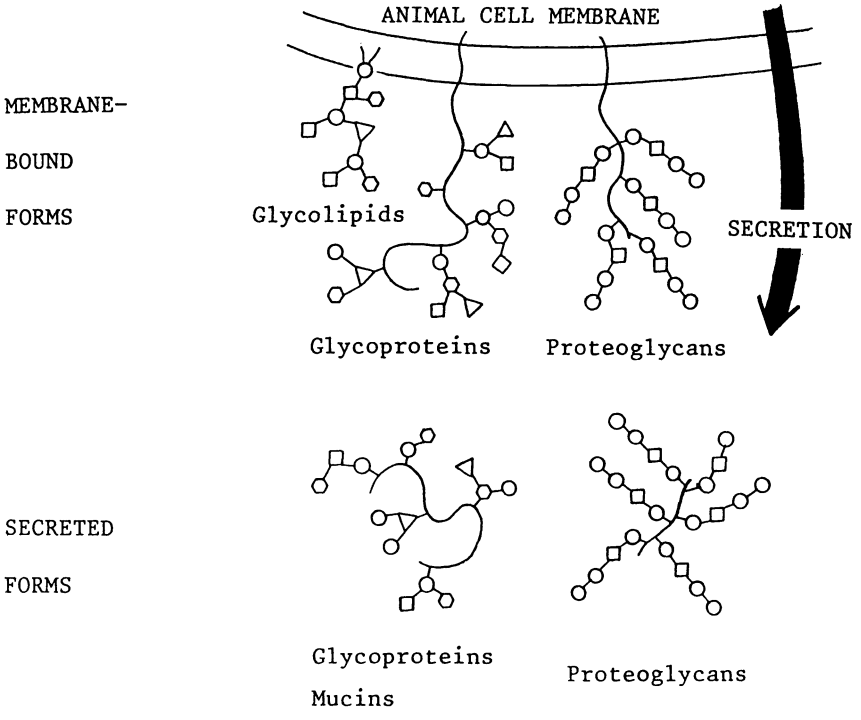

Fig. 1. Various cell-associated glycoconjugates of importance for the outcome of microbe attachment to carbohydrate. If the ligand requires a binding to strictly membrane bound receptor (e.g. viruses which have to invade the cell for reproduction) then the existence of receptor carbohydrate on secreted glycoconjugates may cause inhibition of the infection (see ref. 3). Glycolipids are not present in secretions and a selection of specifically lipid-linked saccharide as a receptor (e.g. lactose for several bacteria) assures membrane binding. Attachment to glycolipid may also give the proximity required for insertion into the membrane (e.g. cholera and Shiga toxins).

A NOVEL SOLID-PHASE OVERLAY ASSAY

The classical approach for chemical identification of receptor substances on cell surfaces (for e.g. hormones, antibodies, microbes) includes a solubilization of membrane substance and use of it (after fractionation) in inhibition studies (preincubation of ligand with actual substance to test the effect on binding to intact cells). This is usually connected with serious problems in part explaining a relatively slow progress. Membrane substances are amphipathic with aggregation properties making them difficult to purify and producing falsely positive and negative results. In case of glycoconjugates the oligosaccharide may be released for use in homogeneous solution avoiding the mentioned drawbacks. However, our experience from a few years of work in the field indicates that the majority of microbes recognizing carbohydrates binds in a low-affinity mode requiring multivalency for a firm binding. This means that a soluble univalent saccharide is without inhibitory effect (see further below).

We have eliminated part of these problems by using the thin-layer plate (ref. 19) as an assay surface (artificial cell surface) for viruses (ref.

20) and bacteria (ref. 21). Glycolipids of target cells are first separated into molecular species or groups of molecules. The developed plate is then carefully treated with plastic (a very thin film into which the glycolipids are supposed to stick hydrophobically with their paraffin chains) followed by coating of excess hydrophobic surface with serum albumin. While still wet the plate in horizontal position is overlayered with ligand suspension for a few hours, washed and bound ligand is finally detected in various ways (refs. 20-22). A typical result for a radiolabelled bacterium is shown in Fig. 2. There is a selective binding to compounds in the 3-5 sugar region. Major rapid-moving or slow-moving glycolipids are completely negative for binding. Thus there is specificity in the binding and accuracy of the method with no background staining.

Fig. 2 Thin-layer chromatogram detected with anisaldehyde (left) and autoradiogram (right) after binding of 35-S-labeled uropathogenic E. coli (typical for strain J96) to non-acid glycolipids of the following sources: Human erythrocytes (1), human meconium (2), intestine from Macaca cynomolgus(3), dog small intestine (4), and rabbit small intestine (5). Numbers to the left indicate the approximate number of sugars. The solvent was chloroform-methanol-water 60:35:8, by vol. Autoradiography for 24 h. The binding specificity is for Galα1-4Gal in terminal or internal position of the oligosaccharide chain (compare Table 1).

Among the advantages of the technique is the multivalent presentation (see problem defined above) with no solubility problems. Furthermore a large number of receptor candidates can be added in one single assay allowing an efficient screening for receptors. This is facilitated by a technique of preparing total glycolipids of various sources free of non-glycolipid contaminants (ref. 23).

This easy detection of a receptor-active substance is then used to assay the laborious isolation of receptors for structural analysis including high-technology mass spectrometry and NMR spectroscopy (see ref. 14).

CHARACTER OF SELECTED RECEPTOR SACCHARIDES AND RECEPTOR INTERACTIONS

In the following I want to summarize some characteristics of carbohydrate receptors for microbiological ligands that have become evident during use of the novel overlay assay described above. Many of our results are still being processed for publication and the space does not allow a penetrating description of new binding specificities revealed for bacteria, bacterial toxins and viruses.

Binding to internal sequences

The E. coli strain used for illustration in Fig. 2 is frequently found in urine of patients with urinary tract infection and we have studied its binding specificity in detail (ref. 24) including the target cells of the urinary tract (ref. 25). As is summarized in Table 1 the common denominator

for positive binders is Galα1-4Gal (underlined), which is confirming the proposals done in the original contributions (refs. 12,13). No glycolipids shown to lack this sequence are able to bind the bacteria using the overlay assay (compare Fig. 2). On the other hand all glycolipids carrying the disaccharide are binding with about the same strength regardless of the location of the sequence along the chain, being terminal or placed internally with different neighbouring sequences. In other words, there is a number of isoreceptors defined as molecules carrying the same binding epitope in different locations. The pattern of isoreceptors differs between different tissues which is easy to demonstrate using the overlay assay (see Fig. 2 and refs. 20-22, 24-31).

TABLE 1. Examples of glycolipids tested for binding of uropathogenic *E. coli* and the Shiga toxin. Different binding preferences of the two ligands to internally placed Galα1-4Gal indicate separate binding epitopes on the disaccharide.

No.		*E. coli*	Shiga toxin
1	Galα1-4GalβCer	+	+
2	Galα1-4Galβ1-4GlcβCer	+	+
3	GalNAcβ1-3Galα1-4Galβ1-4GlcβCer	+	(+)
4	GalNAcβ1-3GalNAcβ1-3Galα1-4Galβ1-4GlcβCer	+	-
5	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4GlcβCer	+	-
6	Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4GlcβCer	+	-
7	Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4GlcβCer	+	-
8	GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4GlcβCer	+	-
9	NeuAcα2-3Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4GlcβCer	+	-
10	NeuAcα2-6(NeuAcα2-3)Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4GlcβCer	+	-
11	Galα1-3Galα1-4Galβ1-4GlcβCer	+	+
12	$(Gal\alpha1\text{-}3)_{2\text{-}5}$Galα1-4Galβ1-4GlcβCer	+	-
13	GalNAcβ1-3$(Gal\alpha1\text{-}3)_{1\text{-}5}$Galα1-4Galβ1-4GlcβCer	+	-
14	Galα1-3Galβ1-4GlcβCer	-	-
15	Galα1-4Galβ1-4GlcNAcβ1-3Galβ1-4GlcβCer	+	+

The recognition of internally placed sequences deviates from e.g. most antibodies directed against the same substances (e.g. those of Table 1, see further discussion below) and appears to be a rule for microbial proteins. Thus we have found it for other bacteria (refs. 21, 28-30), for bacterial toxins (refs. 27, 29-31) and for viruses (ref. 30).

Low-affinity binding

A property repeatedly appearing is a low-affinity binding defined here for the Shiga toxin with the same specificity (refs. 27,31) as for *E. coli* discussed above (Table 1). This toxin is produced by *Shigella dysenteriae type 1* causing bacillary dysentery of man. In contrast to *E. coli* it is not possible to inhibit the attachment to target cells by preincubation of the ligand with the disaccharide (ref. 31). However, by coupling the saccharide multivalently to bovine serum albumin a good inhibition was obtained at a fairly sensitive level (ref. 31). In our experience so far the requirement for multivalency to establish binding is a rule rather than exception for microbial systems and a possible biological meaning of this will be discussed below. Evidently ligands interacting in this low-affinity mode are not possible to detect with classical inhibition methods (ref. 4) and explains why earlier known systems are high-affinity binders, e.g. Man-binding bacteria (ref. 4).

Nature of the binding epitope

As will be further outlined below the property of recognizing internal sequences is of decisive help when locating a binding epitope (a limited sur-

face on e.g. a disaccharide directly interacting in the binding). This can be approached by molecular modelling of active and inactive isoreceptors, preferably using the computer-based HSEA calculation (ref. 32), which has been done for E.coli and Galα1-4Gal-containing isoreceptors in collaboration with Klaus Bock (refs. 24,29). The bacterium binds to all isoreceptors regardless of the nature of neighbouring groups (Table 1) and with similar strength. As there is an almost 90 degrees bend of the chain at Galα1-4Gal the binding epitope must reside on the convex side of this bend (Fig. 3), otherwise various neighbour substituents would sterically interfere with binding. This side has a continuous non-polar surface indicated by ring hydrogens (H-1 and H-2 of Galα and H-1, H-3, H-4 and H-5 of Galβ) and this is surrounded by polar oxygens. Interestingly, this fits with a generalized binding epitope on carbohydrate for antibodies and plant lectins as worked out by Lemieux and collaborators (ref. 33 and references therein). The formulation is an extended non-polar surface (contributing to the overall strength of the interaction) ending in key oxygens (providing the specificity of the binding). In this respect it has been shown that the two OH-6s may be key polar groups, since synthetic saccharides lacking these (D-Fucα 1-4Gal or Galα1-4DFuc) were inactive as inhibitors of E. coli binding (ref. 34).

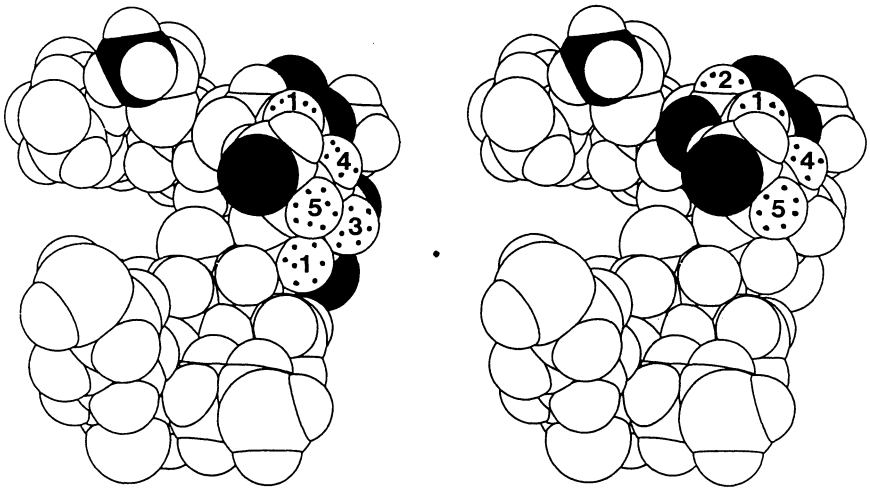

Fig. 3. Hypothetical difference in binding epitope on Galα1-4Gal for E. coli (left) and the Shiga toxin (right). The HSEA-calculated models of substance 5 of Table 1 (ref. 24) show the non-polar ring hydrogens numbered on the disac charide which forms a bend of about 90 degrees (H-1 and H-2 of Galα and H-1, H-3, H-4 and H-5 of Galβ). Both ligands recognize specifically the disaccharide but with different preferences of isoreceptors (Table 1). It is likely that the epitope for Shiga toxin has a smaller non-polar surface (weaker binding) and is shifted upwards (binding inhibited by e.g. extensions with GalNAcβ, see Table 1) compared to E. coli. In the models the methyl carbon of NAc in GalNAcβ has been indicated in black. Oxygens of the disaccharide proposed to participate in binding are also black, and the non-polar ring hydrogens are dotted.

We have performed analogous dissections for binding epitopes concerning other specificities which confirm that microbial carbohydrate-binding proteins have selected epitopes similar in character to proteins of higher organisms.

Variants with closely related specificities

The Galα1-4Gal specificity is carried by several other microbiological ligands including the Shiga toxin as noted above. In addition to being a low-affinity binder (see discussion above) the toxin differs from E. coli in its binding preferences to isoreceptors as summarized in Table 1. This indicates that the complementary binding site on the protein must differ

between the bacterial lectin and the toxin, probably only slightly, by one or two amino acids. This is analogous to the interesting variants of influenza virus which recognize NeuAcα2-3 and NeuAcα2-6, respectively, and where an amino acid change from Gln to Leu has been proven by crystallography and molecular genetical methods (ref. 3). The postulated difference between the two Galα1- 4Gal binders is not only producing a weaker binding for the toxin, which in itself may explain the differences shown in Table 1. In case of substances no. 2 and 3 *E. coli* is slightly preferring 3 before 2 (ref. 24) but the situation for the toxin is reversed (Table 1). Hypothetically therefore the toxin may have a less extended non-polar interaction (weaker binding) over the two sugars and this is shifted more towards Galα in its epitope (explaining a steric interference from GalNAcβ) compared to *E. coli* (Fig. 3). To prove this one would need synthetic analogues of Galα1-4Gal with modifications at the indicated differences producing differential changes in the binding of the two ligands. This is a very laborious task comparable to the pioneering work by Lemieux and co-workers on saccharide binding epitopes for antibodies and plant lectins (ref. 33).

We have other examples of similar variants, including Sendai virus and binding to gangliosides (ref. 30), and *Propionibacterium* and binding to lactosylceramide (ref. 28).

Certain saccharides preferred as receptors

A large number of bacteria recognize Man (refs. 4,5) and both bacteria, bacterial toxins and viruses often bind to NeuAc-containing structures (refs. 1-6). One reason may be the abundance of these binding sites on cell surfaces. In other cases there is a selection of lipid-linked oligosaccharides, as GM1 for cholera toxin (ref. 6), Galα1-4GalCer for Shiga toxin (ref. 31) or lactosylceramide for a number of bacteria (refs. 21, 28, 30). Possible reasons for this are discussed below.

REFLECTIONS ON RECEPTOR CHARACTERISTICS

As noted in the INTRODUCTION one reason why carbohydrate appears to have been selected by microbes before proteins for the essential attachment to host cells may be the abundance of carbohydrate at the animal cell surface (primary collision partner). A characteristic variation in surface carbohydrate between animals and tissues may in addition explain why certain infections are restricted to distinct cells (tropism). There is another general property (great variability) that I will come to in the end of this section.

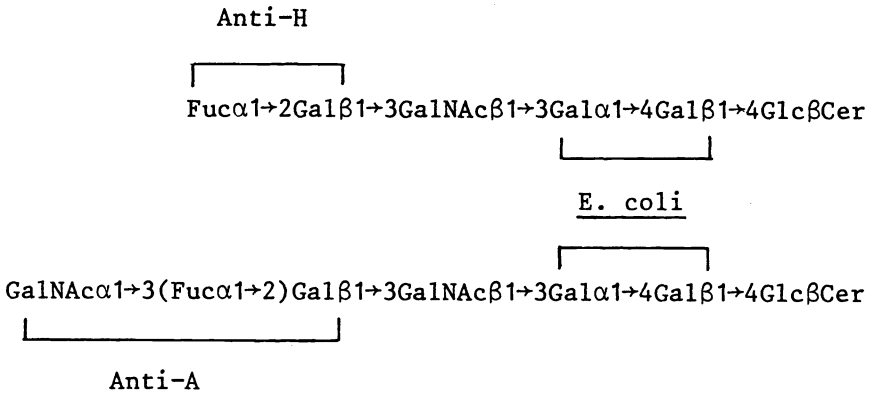

Fig. 4. Different locations in the cell surface saccharide chain of binding epitopes for antibodies and uropathogenic *E. coli* as selected for two substances of Table 1. Immunodominant groups are most often terminal sequences which differ between individuals (antibodies have been created to distinguish non-self from self). Microbiological ligands have on the other hand selected a binding to internal core sequences, which means that they avoid the differences between individuals. This may be of decisive biological value for the microbes in their attachment to the host animal tissues.

The recognition of internally placed sequences (Table 1) is a property of microbes in general and deviates from e.g. antibodies (produced by animals) which mostly recognize terminal parts (Fig. 4). This property may have been selected to avoid differences between e.g. host individuals, which reside in terminal parts, and which the antibodies in part have been invented to detect (non-self versus self).

The low-affinity binding as defined above for the Shiga toxin in relation to E. coli (inability to be inhibited by free receptor-carrying saccharides) appears to be a rule among microbes. At first glance this may seem inefficient since a stronger binding should improve attachment. On the other hand the Shiga toxin acting in the large intestine may have some benefit of avoiding being inhibited in its multivalent attachment to its host cell by free Galα1-4Gal-containing saccharides that may be a consequence of enzymatic detachment from membrane-bound receptors. In case of cholera toxin the interacton is of the high-affinity type since free GM1 pentasaccharide is a potent inhibitor of binding in vitro (ref. 6). However, in this case the free saccharide is not expected to appear outside the target cell of the small intestine in vivo as the physiological degradation of GM1 is stepwise and does not produce intact receptor saccharide. Therefore the selection of affinity level may depend on the nature of the chosen receptor or the ecological conditions.

The preference of a certain receptor saccharide may depend on its abundance on the cell for attachment (e.g. Man, NeuAc) or its unique appearance if a certain host cell is required. It may also depend on where the glycoconjugate is presented (membrane-bound versus secretions). One may note that Man is not known to be present in mucins, the most abundant secreted glycoconjugate for epithelial cells (refs. 15,17). A distinct choice of lipid-bound oligosaccharide may have at least two reasons. In case of cholera toxin and the GM1 ganglioside (refs. 6,10) the required pentasaccharide is not present in peptide-bound form. Also, the mechanism postulated for the membrane penetration of the toxin requires a bilayer-close epitope to have the protein inserted. Similarly, the recent formulation (ref. 31) of Galα1-4GalβCer as the functional receptor for the Shiga toxin is based on the same principle: proximity to the bilayer membrane. In this case HeLa cells resistant to toxin action were namely shown to bind the toxin on the surface as good as sensitive cells (inhibition of protein synthesis after uptake). It was shown that both cell types carried Galα1-4Gal-containing glycolipids but only the sensitive cells had the disaccharide directly linked to ceramide (compare substances of Table 1).

Relatively many lactose-binding bacteria (refs. 28,30) have assured a binding to membranes since this disaccharide is not known in other glycoconjugates than glycolipids (strictly membrane-bound). Free lactose is however present in urine and in relatively large amounts in milk but a low-affinity binding for these bacteria avoids an inhibition of cell attachment (see discussion above).

There is a final aspect on the selection of carbohydrate before protein as receptors and this is of higher sophistication and may prove to be an advantage for microbes mutating their lectin-like proteins to optimize a selective attachment. As remarked in the INTRODUCTION there is a very high theoretical variability (information) per unit mass in carbohydrate compared to protein (variation not only in sequence but in binding position and configuration as well as ring size and branching). This in combination with the property of the ligand to bind to internally placed epitopes (see above) may produce efficient "receptor shifts" or "epitope drifts" through mutations producing single amino acid substitutions in the site of the binding protein. For example, a probably very small change (one amino acid) of the lectin of Propionibacterium variants noted above to recognize separate lactose epitopes (ref. 28) may shift the binding from epithelial to non-epithelial cells. This is due to the fact that the two separate epitopes on lactose reside on isoreceptors being present in different tissue compartments (see ref. 35). This hypothesis on receptor shifts made more efficient on carbohydrate than peptide will be explained elsewhere.

PERSPECTIVES ON FUTURE DEVELOPMENTS OF TECHNOLOGY AND APPLICATIONS

Although evidence is gathering for carbohydrates as essential receptors on host cells for various microbes (refs. 1-7) the field is still in its beginning concerning precise technology and application procedures. The complexity of carbohydrates in various conjugated forms at and around cells in different animal tissues, with a dynamic change with different functional states, requires improvements of present day techniques although basic structural methods like mass spectrometry and NMR spectroscopy have advanced considerably in recent time. The following are some areas for consideration.

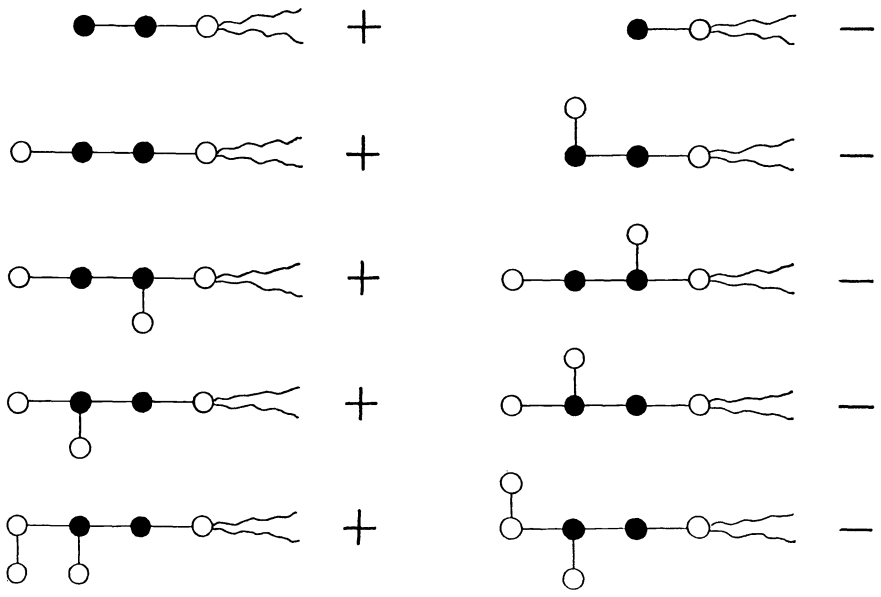

Fig. 5. Illustration of the possibility of obtaining partial information on the binding epitope on receptors for microbes using natural isoreceptors in form of glycolipids. The essential requirement is the general property of microbes of recognizing internally placed sequences. In the hypothe ticalexample the specificity is restricted to the disaccha ride indicated in black. If there is a binding to the series shownon the left but not to that on the right (both shown in conformations obtained by e.g. HSEA calculation) this means that the epitope (substructure) on the disaccharide involved in binding should be located on top of the conformations as presented various substitutions on this side, but not on the opposite side, inhibit the binding). This information is not accessible in case of only terminally recognized sequences as for antibodies (compare Fig. 4). An approximated binding epitope for a microbe may thus be designed from binding pre ferences to a series of natural isoreceptors, facilitating the planning of the laborious organic synthesis of receptor analogues for practical applications.

Assaying of various glycoconjugates

The dramatic facilitation of assaying for carbohydrate receptors as described above has a serious limitation to natural lipid-linked oligosaccharides (hydrophobic anchoring on the assay surface is essential). One project we are working with is to release the multivalently linked oligosaccharide chains from peptide and couple them univalently to simple lipid chains, thus taking advantage of the solid-phase assay as discussed in the beginning of this paper, and also use fractionation and structural characterization of detected receptor-active spots as for the natural glyco-

lipids. In this way various glycoconjugates, membrane-bound or secreted (see Fig. 1), may be adequately assayed for binding with molecular resolution. However, a large volume of work is required to optimize these procedures.

Dissection of binding epitopes

In the impressive project carried out by Lemieux and collaborators (ref. 33 and references therein) on epitope designation on carbohydrates that bind antibodies and plant lectins a systematic synthetic approach is used to modify the carbohydrate and thus successively reveal the required interaction surface. This is an enormous amount of effort for each single epitope. For microbial carbohydrate receptors an interesting simplification is however appearing, using a primary dissection on natural isoreceptors. The property of recognizing internally placed sequences (see Table 1) is decisive in this respect. By analyzing binding preferences to a large number of natural isoreceptors the effect on binding of various neigbouring groups to the active binding part is mapped. By relating these data to the conformation of the actual isoreceptors (through e.g. HSEA calculation, ref. 32) a fairly good idea about a rough epitope should be obtained (ref. 36 and Fig. 3). This is an important facilitation and may bring down the starting alternatives for the more laborious organic synthesis to finally establish the detailed epitope. This is obviously not possible to the same extent in case of the ligands studied by Lemieux and collaborators which bind terminal sequences lacking most of the informative neighbouring groups (Fig. 5).

Receptor analogues

If low-affinity sites have been selected as a survival benefit for the microbes (see discussion above) this has some interesting consequences for technical developments. Consider the unique crystal conformation of L-Ara and the transport protein of E. coli (ref. 37). This protein has an extremely high affinity for the sugar, to be able to pick it up from a diluted external solution. The crystal structure indicates an optimized interaction between the two substances, using various forces including bridges of water. So there is a maximal fit between the sugar and the binding pocket of the protein. The much lower affinity of e.g. Shiga toxin for Galα1-4Gal compared to this transport protein must mean a less good fit between the postulated epitope and the protein. One consequence of this is the possibility to improve this fit, and thereby the binding strength, by modifying the receptor structure into non-physiological analogues. This may be done by chemical or biochemical treatment of natural receptor sequences, or by total organic synthesis of suitable candidates predicted on basis of the dissected binding epitope (see discussion above).

Applications

Because of its early infancy the field has not yet produced commercial applications from receptor findings. Logical developments may be anticipated within medicine and biotechnology. Present-day diagnosis of an infection often includes cultivation of the infectious agent from a sample, requiring several days for growth. A realistic supplement may be to pick up the ligand on a stick to which a specific receptor-active substance has been linked, and rapidly detect a bound ligand by antibody or a nucleotide probe. For therapy an interesting supplement may be to use soluble receptor analogues to inhibit attachment (and thereby infection) in those cases where rapid mutations of the microbe develop resistance to antibiotics (e.g. bacterial infections) or produce surface antigenic changes that invalidate important vaccinations. The receptor-binding property is however highly conserved for microbe survival. This supplementation may be especially interesting for viral infections (where chemotherapy is still lacking) and in those cases where long-term vaccination has failed (e.g. influenza, aids). Within biotechnology one may use the receptor knowledge for purification purposes or for solid-phase association of e.g. bacteria as catalysts in fermentation tanks for continuous processing of biomass. The receptor substance may be prepared from natural sources or synthetized in modified form for optimized binding (see above).

GENERAL CONCLUSIONS

Concerning proteins that specifically recognize and bind carbohydrates those carried by microbes seem to be especially fruitful to process for several reasons. Firstly, there is abundance and diversity of these lectin-like proteins since they appear to have been designed for microbe survival through attachment to the coat of carbohydrates at natural surfaces including host animal cells. Secondly, the possibility of attacking infections of man and animals at this level of association opens up industrial developments. Thirdly, the development of technology around both the identified receptor epitopes and the binding proteins affords promising applications within biotechnology. Finally, some unique properties of these proteins (e.g. recognition of internally placed sequences) make them technically supplementary to e.g. antibodies which are widely used carbohydrate-directed reagents today.

The novel assy procedure discussed in some of its consequences in the present paper may after suitable extensions to various other glycoconjugates than natural glycolipids prove to be a very efficient tool for a primary picking-up of receptor specificities and a help for elucidating the biological relevance of these. The decisive improvement in the planning of organic synthesis of receptor analogues that has appeared from this approach may reduce part of the hesitations before this laborious and expensive technology.

For the basic biomedical scientist engaged in this field the carbohydrate-mediated interaction between microbes and animals is technically and biologically complex but a challenging science.

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