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MINIREVIEW

Microbial Adherence to and Invasion through Proteoglycans

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INTRODUCTION

A large array of glycoproteins, glycolipids, and proteoglycans decorate the surfaces of animal cells. These glycoconjugates mediate many fundamental cellular processes, including cell-cell and cell-matrix adhesion, motility, growth, and signaling (28, 110). Over time, many pathogenic microorganisms have learned to exploit cell surface glycoconjugates as receptors for attachment, a process which ultimately facilitates tissue colonization and invasion. The interaction of specific proteins on the surface of microorganisms (adhesins) with carbohydrate chains on the glycoconjugate (receptors) enables the microbes to take their first step towards establishing an infection.

This review concentrates on proteoglycans as adhesion receptors for bacteria, viruses, and parasites. Microbial binding to glycolipids and glycoproteins also occurs and has been discussed elsewhere (27, 40, 61–63, 85, 94). Proteoglycans are ubiquitous among animal cells, and as discussed below, their carbohydrate chains (glycosaminoglycans) bind many different protein ligands. Different experimental criteria have been used to establish a role for proteoglycans in attachment and invasion of host cells, including direct binding measurements, identification of microbial carbohydrate-binding proteins, competition studies with defined polysaccharides, loss of adhesion upon enzymatic removal of host glycans, and altered adherence to animal cell mutants with defective glycosaminoglycan biosynthesis. Overall, the experimental evidence for microbial adhesion to host cell proteoglycans is compelling, and future molecular studies may provide a basis for designing therapeutic strategies based on these interactions.

PROTEOGLYCAN STRUCTURE

Proteoglycans consist of a protein core and one or more covalently attached glycosaminoglycan chains (Fig. 1). Their assembly initiates in the endoplasmic reticulum and ends in the Golgi apparatus, where the various glycosyltransferases and sulfotransferases reside. Proteoglycans are present in intracellular secretion granules (67), in the extracellular matrix (55), and on the cell surface (12). Many membrane proteoglycans have a single membrane-spanning segment in a type I orientation (66). The prototypical membrane proteoglycan in this class is syndecan-1, which belongs to a family of structurally related proteoglycans with four members (syndecan-1, fibroglycan, *N*-syndecan, and ryudocan) (Table 1). Each member has conserved attachment sites for three to five glycosaminoglycan chains (12). The syndecans exemplify hybrid proteoglycans since they contain mixtures of the two major types of glycosaminoglycan chains found in animal cells, heparan sulfate and chondroitin sulfate (Fig. 1). Other cell surface proteoglycans include the hybrid molecules betaglycan and a splice variant of CD44 (epican) and the pure chondroitin sulfate proteoglycans NG2 and thrombomodulin. The other major family of membrane proteoglycans are called glypicans since they contain glycosylphosphatidylinositol anchors instead of a membrane-spanning segment (glypican-1, OCI-5, K-glypican, and cerebroglycan). In contrast to the syndecans, the glypicans appear to contain only heparan sulfate chains (26). In general, the expression of these proteoglycans occurs in a tissue-specific and temporally regulated manner during development, although their expression overlaps in some cell types (12, 66) (Table 1). Their localization to apical and basolateral membranes of polarized cells varies as well (26).

Much of the chemistry of proteoglycans is dominated by the glycosaminoglycan chains, which consist of alternating residues of an amino sugar and an uronic acid (Fig. 1). Chondroitin sulfate is based on the disaccharide repeat (GlcUAβ1-3GalNAcβ1-4)$_n$, and heparan sulfate is based on the disaccharide (GlcUAβ1-4GlcNAcα1-4)$_n$. As the chains polymerize, they undergo various sulfation and epimerization reactions, which result in the addition of sulfate groups to different sugars and conversion of a portion of the D-glucuronic acid (D-GlcUA) residues to L-iduronic acid (L-IdUA). Proteoglycans exhibit tremendous structural heterogeneity due to substoichiometric glycosylation, variation in the lengths of the glycosaminoglycan chains, and variation in the extent and pattern of sulfation and epimerization. Thus, a preparation of proteoglycans really consists of hundreds of thousands of distinct molecular species, or glycoforms. Determining if specific glycoforms have unique biological properties is a major goal for structural biologists in the field.

One way to fractionate proteoglycans is based on the charge characteristics of the glycosaminoglycan chains. Chondroitin sulfates usually contain one sulfate residue per disaccharide unit, attached to the hydroxyls at C-4 (chondroitin sulfate A) or C-6 (chondroitin sulfate C) of GalNAc residues (Fig. 1). A third type of chondroitin sulfate (B, or dermatan sulfate) contains IdUA and somewhat more sulfate, with groups attached to hydroxyls at C-4 or C-6 of GalNAc residues and to C-2 of IdUA residues. Typical dermatan sulfate preparations from porcine skin contain ~1.1 sulfate groups per disaccharide and as much as 80% IdUA (82). Heparan sulfate chains vary in sulfate content from 0.8 to 1.4 sulfate groups per disaccharide (79, 81). Heparin, which is found in intracellular granules of mast cells, is the most highly sulfated glycosaminoglycan. Commercial preparations have been prefractionated by charge and contain, on average, ~2.4 sulfate groups per disaccharide (38). In heparan sulfate and heparin, the sulfate residues occur at the amino groups and the C-6 hydroxyl groups of glucosamine

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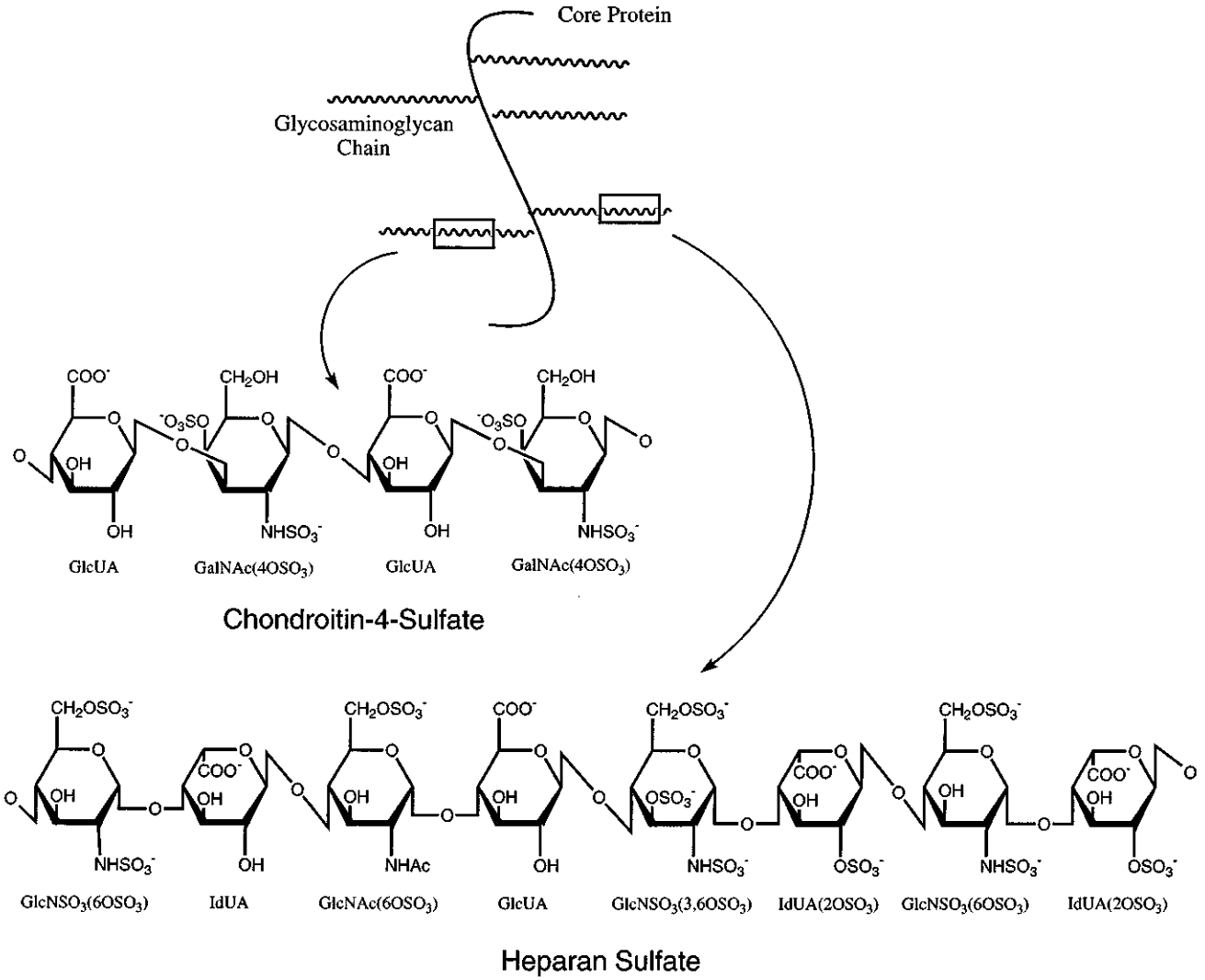

FIG. 1. Partial structure of a membrane proteoglycan.

residues, at C-2 of IdUA and GlcUA residues, and occasionally at the C-3 hydroxyl of glucosamine residues (Fig. 1). The various modifications (epimerization and sulfation) occur to a greater extent in heparin than in heparan sulfate. Thus, 80 to 85% of the glucosamine residues in heparin are N sulfated, compared to 40 to 60% in heparan sulfate. The content of IdUA and the degree of O sulfation are also much greater in heparin.

The enzymes involved in heparan sulfate and heparin biosynthesis are coordinated, resulting in regions rich in IdUA and sulfated sugars, interspersed among unmodified sections of the chain (79, 81). The reactions tend to be incomplete, giving rise to structural heterogeneity within the modified regions of the chains. These domains can have a very high charge density due to the clustering of sulfate groups, which can result in nonspecific binding of proteins, such as a cation-exchange resin. However, several examples exist in which unique sequences of sugars in the glycosaminoglycan chain define a binding site for a ligand. For example, different subpopulations of heparin bind to fibronectin, type I collagen, and laminin (98); different heparin oligosaccharides bind to acidic fibroblast growth factor and basic fibroblast growth factor (43, 80);

TABLE 1. Cell surface proteoglycans

Proteoglycan	Glycosaminoglycan(s)	Cell type(s)
Syndecan family Syndecan 1 Fibroglycan *N*-syndecan Ryudocan	Chondroitin sulfate, heparan sulfate	Epithelial cells, endothelial cells, fibroblasts
Glypican family Glypican OCI-5 K-glypican Cerebroglycan	Heparan sulfate	Epithelial cells, endothelial cells, fibroblasts
Betaglycan	Chondroitin sulfate, heparan sulfate	Fibroblasts
Thrombomodulin	Chondroitin sulfate	Endothelial cells
NG2	Chondroitin sulfate	Melanocytes
CD44	Chondroitin sulfate	Lymphocytes

TABLE 2. Methods for producing modified heparin

Modification	Reference
$GlcNSO_3$ N desulfation	54
GlcN re-N sulfation	75
GlcNAc N deacetylation	100
GlcN re-N acetylation	95
Iduronic acid/glucuronic acid 2-O desulfation	74
Glucosamine 3-O desulfation	
Glucosamine 6-O desulfation	86
Carboxyl reduction (GlcUA and IdUA)	106
Periodate oxidized, borohydride reduced (nonsulfated GlcUA and IdUA residues)	47
Aminomethylsulfonic acid addition (carboxyl groups)	25

and a distinct pentasaccharide with a uniquely positioned 3-O-sulfated glucosamine residue binds with high affinity to antithrombin III (13, 66). Binding sequences often consist of combinations of modified and unmodified residues (Fig. 1). A major goal is to establish if microbial binding depends on specific types of proteoglycans and if unique arrangements of sugar residues in the glycosaminoglycan chains play a role.

METHODOLOGICAL CONSIDERATIONS

In order to study microbial adhesion in vitro, adherence assays have been developed for many microorganisms with the use of cultured cells, tissue sections, or plastic surfaces coated with proteoglycans or glycosaminoglycans. Inhibition of adherence by the addition of glycosaminoglycans is usually the first indication that receptors on the mammalian cells might be proteoglycans. If heparin inhibits adhesion and other glycosaminoglycans (e.g., chondroitin sulfate and dermatan sulfate) do not, then adhesion or binding is generally considered specific for heparan sulfate proteoglycans. However, this interpretation may not be correct since heparin may compete nonspecifically due to its high charge density. To circumvent this problem, one can cleave heparin into fragments of specific length and chemically alter the number and position of sulfate groups (Table 2). These derivatives (heparinoids) can then be used to determine if competition, and, by inference, adhesion, depends on specific functional groups or a specific sugar sequence(s). Some tissues contain adequate amounts of heparan sulfate to prepare native material for competition studies (e.g., liver contains about 1 mg of glycosaminoglycan/g [dry weight] of tissue). For direct binding measurements, the chains can be radiolabeled by using the techniques described in Table 2. Alternatively, radioactive proteoglycans can be prepared from cultured cells by supplementing the growth medium with radioactive precursors (e.g., [1-^{3}H]galactose, [6-^{3}H]glucosamine, or $^{35}SO_4$, [5]).

In addition to competition and binding experiments, one can examine how removal of glycosaminoglycans from target cells affects adhesion. Treatment of cells with bacterial heparin or chondroitin lyases removes cell surface heparan sulfate or chondroitin sulfate, respectively (73). These enzymes are commercially available and are quite reliable. Care should be taken to ensure that the enzymes were effective by measuring the loss of cell surface glycosaminoglycans (e.g., after trypsin treatment).

Another way to modify the expression of proteoglycans is to grow cells in medium containing an inhibitor of glycosaminoglycan formation. One class of useful compounds contains xylose covalently linked to an hydrophobic aglycone (β-D-xylosides). These compounds resemble a natural biosynthetic intermediate and act as primers of free glycosaminoglycan chains, which are then secreted from cells (29). Growth of cells in the presence of a β-D-xyloside inhibits the formation of proteoglycans by diverting assembly of the chains from the core proteins, causing underglycosylated proteoglycans to appear on the cell surface.

Another tactic is to reduce the extent of sulfation of the glycosaminoglycan chains by growing cells in medium deficient in inorganic sulfate and supplemented with sodium chlorate, an inhibitor of sulfation (2). These conditions lead to variable undersulfation of the glycosaminoglycan chains, depending on the concentration of inhibitor (6, 53). One problem with inhibitors is the lack of specificity. β-D-Xylosides can affect the formation of glycolipids in some cells (34), and chlorate inhibits the addition of sulfate to other glycoconjugates, lipids, and proteins (2). However, when these characteristics are com-

TABLE 3. Mutant CHO cell lines used in microbial adherence studies

Cell line	Biochemical deficiency	Glycoaminoglycan produced	Microorganism(s) studied (reference)
Wild type	None	Heparan sulfate, chondroitin-4-sulfate	
pgsA-745	Xylosyltransferase	None	*Bordetella pertussis* (45), *H. influenzae* (89), *Borrelia burgdorfeii* (69), *Trypanosoma cruzi* (49), HSV (101), CMV (24, 88), *Neisseria gonorrhoeae* (109)
pgsB-761	Galactosyltransferase I	~5% of wild-type heparan sulfate, chondroitin sulfate	*Bordetella pertussis* (45), *C. trachomatis* (114), *T. cruzi* (49), HSV (101)
pgsB-618	Galactosyltransferase I	~15% of wild-type heparan sulfate, chondroitin sulfate	*N. gonorrhoeae* (109), *H. influenzae* (89), *Leishmania amazonensi* (77)
pgsB-650	Galactosyltransferase I	~30% of wild-type heparan sulfate, chondroitin sulfate	None
pgsD-677	*N*-Acetylglucosaminyl transferase, glucuronosyltransferase	No heparan sulfate, threefold-higher levels of chondroitin sulfate	*H. influenzae* (89), *Borrelia burgdorfeii* (69), *L. amazonensi* (77), *T. cruzi* (49), HSV (101), CMV (24, 88), *N. gonorrhoeae* (109)
pgsF-17	2-O-Sulfotransferase	2-*O*-Sulfate-deficient heparan sulfate, normal levels of chondroitin sulfate	*P. falciparum* (102a)

TABLE 4. Microorganisms bind proteoglycan receptors on eukaryotic cells

Microbe	Target tissue(s)	Type of cells used or activity assayed in vitro	Reference(s)
Gram-negative bacteria			
Bordetella pertussis	Ciliated epithelium in respiratory tract	HeLa cells, CHO cells	45, 83
Chlamydia trachomatis (obligate intracellular organism)	Eyes, genital tract, lymphoid tissues	HeLa cells, CHO cells, L929 mouse fibroblasts, binding of ^{125}I-heparan sulfate	17, 65, 114
Helicobacter pylori	Gastric mucosa	Binding of ^{125}I-heparan sulfate	1, 20, 50
Haemophilus influenzae	Respiratory epithelium	Hep-2, mouse fibroblast, human foreskin fibroblasts, CHO cells	89
Borrelia burgdorfeii	Endothelium, epithelium, extracellular matrix	HeLa cells, Vero cells, CHO cells	56, 69
Nesseria gonorrhoeae	Genital tract	Chang cells, CHO cells	19, 109
Gram-positive bacteria			
Staphylococcus aureus	Connective tissues, endothelial cells	Binding of ^{125}I-heparin to bacteria	70, 71
Streptococcus pyogenes	Cardiac and kidney tissues	Binding of ^{125}I-heparin to bacteria, binding to heparin-agarose, kidney and cardiac tissue sections	11, 41, 99, 111
Streptococcus mutans	Cardiac and kidney tissues	Binding to heparin-agarose, kidney and cardiac tissue sections	21, 22
Streptococcus gordonii	Cardiac tissue	Human umbilical vein endothelial cells	108
Parasites			
Plasmodium falciparum (CSs)	Hepatocytes, placenta	HepG-2 cells, primary mouse hepatocytes, human placenta, brain capillaries, coated plastic	35, 36, 91, 97
Leishmania amazonensi (amastigotes)	Macrophages, fibroblasts, epithelium	CHO cells, mouse peritoneal macrophages, mouse fibroblasts	77
Leishmania donovani (promastigotes)	Macrophages, fibroblasts, epithelium	Mouse macrophages	14
Trypanosoma cruzi	Heart, tract, nervous system, extracellular matrix	Vero cells, CHO cells	49, 90
Viruses			
HSV	Mucosal surfaces of mouth, eyes, genital tract, respiratory tract; latent in nerve ganglia	Vero, HepG-2, CHO, mouse L cells	42, 78, 101
CMV	Neutrophils, monocytes	Human foreskin fibroblasts, human embryonic lung fibroblasts	24, 88
HIV-1	T lymphocytes	T-cell lines MT-4 and H9	92

bined with the results of the other strategies described above, a clear picture regarding the role of proteoglycans in adhesion emerges.

Many studies have utilized mutant cell lines defective in glycosaminoglycan biosynthesis (Table 3). A large collection of biochemically characterized Chinese hamster ovary (CHO) cell mutants lacking glycosaminoglycans have been reported (3–6, 30, 31, 72). Some lack both heparan and chondroitin sulfate glycosaminoglycans (*pgsA* and *pgsB* mutants). Other strains lack only heparan sulfate (*pgsD*) or chondroitin sulfate (*ldlD*) cells when starved for GalNAc. Some are defective in polymer modification reactions, e.g., GlcNAc N deacetylation/N sulfation (*pgsE*) or IdUA 2-O sulfation (*pgsF*). Mutant mouse L cells have also been described previously (7, 8, 42). These strains provide the opportunity for studying microbial adhesion to living cells with defined alterations in proteoglycan expression.

HEPARAN SULFATE MEDIATES ADHESION

An impressive body of literature documents that many bacteria, parasites, and viruses exploit proteoglycans as adhesion receptors (Tables 3 and 4). Most adherent microorganisms apparently bind to heparan sulfate, but as discussed above, this predilection may be nonspecific. The most thoroughly studied example of specific oligosaccharides that appear to be involved in microbial adherence is the binding of herpes simplex virus (HSV) glycoproteins gpB and gpC. Studies that involved competition experiments, lyases, and CHO cell mutants have shown that HSV depends on cell surface heparan sulfate for adherence and penetration (101, 102, 113). HSV binding to host cells is inhibited by heparin fragments composed of five disaccharide units and containing at least 1.5 sulfates per disaccharide (78). Using chemically modified heparin with a defined structure, Herold et al. (47) demonstrated that both gpC and gpB promote adherence by binding to heparan sulfate but that the individual glycoproteins interact with different structural motifs within the chain. Sulfate groups at C-2 of the uronic acids and the carboxyl groups were critical for gpB binding to heparin, but they were not required for gpC binding. If these studies can be extended to natural heparan sulfate chains on cells, then the differential expression of sequences rich in 2-O-sulfated uronic acids on various cells could partly explain the tissue tropism of HSV-1 and HSV-2 (39, 46). This issue is controversial since the results of other studies suggest that chondroitin sulfate receptors may exist as well (7, 8, 42).

Plasmodium falciparum sporozoites bind to heparin and heparan sulfate in a tissue-specific manner (35), with preferred binding to the basolateral surface of hepatocytes and the basement membrane of kidney tubules. Binding is mediated by the circumsporozoite (CS) protein that covers the sporozoite cell surface, but the heparan sulfate proteoglycans that interact

TABLE 5. Microbial heparin-binding proteins

Microorganism	Protein	Binding domain(s)	Reference
Plasmodium falciparum	CS protein	C-terminal region II+ (PCSVTCGNGIQVRIK)	103
HIV	gp120	V3 loop (NNTRKSIRIQRGPGRAEVTIGKIG), C4 region	96
Trypanosoma cruzi	Penetrin	Unknown	49
HSV	gpC	Region SP-1 (RxxxRCFRxxxR), region SP-4	107
Bordetella pertussis	Filamentous hemagglutinin	C-terminal region	76

with CS have not been identified. It is known that hepatocytes produce a heparan sulfate highly enriched in N-sulfated glucosamine residues and 2-O-sulfated uronic acids, with most of the modifications clustered in three heparin-like domains at the nonreducing ends of the chains (79). Perhaps this structure facilitates the selective binding of CS protein by hepatocytes (16). Recent studies indicate that binding also depends on the charge imparted by the carboxyl groups of the uronic acids (102a), consistent with the idea that the CS protein binds mostly through electrostatic interactions with highly charged regions of the chains. Subpopulations of *Plasmodium* also bind to chondroitin sulfate A, but the identity of the chondroitin receptor has not yet been established (36, 97).

Recent studies of *Chlamydia trachomatis* indicate a complex mode of adhesion. In this organism, heparan sulfate acts as a bridge, binding both host cell receptors and *Chlamydia trachomatis* (18, 114). At minimum, a decasaccharide is needed, and based on competition studies with chemically modified heparin, the binding sequences for host and microbial receptors apparently differ (18). This situation is reminiscent of the coreceptor activity of heparan sulfate in binding basic fibroblast growth factor and its high-affinity signal-transducing receptor (60). Interestingly, *Chlamydia* produces its own sulfated heparin-like molecule (114), which may provide the opportunity to infect cells with low levels of endogenous heparan sulfate or with heparan sulfate that lacks appropriate binding sequences.

HEPARIN-BINDING ADHESINS

Information regarding the adhesins expressed on microbial cells that bind to glycosaminoglycans is accumulating. The heparin-binding adhesins associated with intracellular pathogens are probably the best-studied proteins. These include the filamentous hemagglutinin (FHA) of *Bordetella pertussis*, the CS surface protein of *Plasmodium falciparum*, gp120 from human immunodeficiency virus (HIV), gpB and gpC of HSV, and the trypanosome adhesin penetrin (Table 5). Several high-molecular-weight proteins on *Haemophilus influenzae* show significant homology to FHA and bind to the epithelium through heparan sulfate proteoglycans (89). A common motif has emerged from studying the primary sequence of these adhesins. Their binding domains generally include a region rich in basic amino acids flanked by hydrophobic residues (Table 5). In some cases two distant regions of primary sequence may be brought together by a secondary structure to form the binding site (96).

In malarial sporozoites, the CS protein interacts with heparan sulfate proteoglycans on hepatocytes (15) through a cluster of positively charged amino acids and adjoining hydrophobic residues at the C-terminal end of the protein (91, 103). A synthetic peptide that inhibits adherence to cells and CS protein clearance by the liver in mice has been constructed (103). The sequence is also present in thrombospondin, a glycosaminoglycan-binding protein present in the extracellular matrix, and several other heparin-binding proteins (52, 103). Evidence indicates that this region may interact preferentially with heparan sulfate and with lower affinity to chondroitin sulfate (84).

A number of viruses utilize cell surface proteoglycans as receptors, including HIV, HSV, and cytomegalovirus (CMV) (Tables 3 and 4). The glycoproteins involved in HSV binding to heparan sulfate have been well studied (105, 107), and protein sequences involved in this interaction are conserved and functional in other alphaherpesvirus glycoproteins (104). CD4 constitutes the primary receptor on T cells for the HIV-1 envelope glycoprotein gp120. However, attachment of the virus also appears to involve heparan sulfate (92). The V3 and C4 domains of gp120 contain positively charged regions that interact with heparin. These regions may be brought together in oligomeric gp120 to form a binding site for heparan sulfate (96).

C. trachomatis (114) and promastigotes of *Leishmania donovani* (14) appear to utilize heparan sulfate in a unique way, as a bridging molecule between heparan sulfate-binding proteins on their surfaces and on host cells. Thus, the addition of heparan sulfate at low concentrations enhances the attachment of chlamydiae to CHO cells deficient in heparan sulfate, but at high concentrations it inhibits attachment, presumably by increasing the formation of binary complexes instead of ternary complexes (114). This is an open area for research since the heparin-binding proteins have not yet been described in any detail (77). Host cell proteins that bind heparan sulfate also have been detected (68, 93), but their role in infection has not been established. Heparin-binding proteins may be interesting targets for developing antibody-based inhibitors of adhesion.

INVASION

Although it is clear that cell surface proteoglycans act as adhesion receptors, their role in invasion is unclear. The abilities of heparin to block the initial interaction of microbes with cells and to displace freshly bound organisms suggest that proteoglycans mediate an early, probably initial, stage in adhesion and that the initial interaction is relatively weak. Adherence can become irreversible with time, suggesting that additional interactions may occur, possibly with non-heparan sulfate receptors. Some heparan sulfate-binding adhesins appear to be multifunctional. For example, in addition to a heparan sulfate-binding site, the FHA of *Bordetella pertussis* contains an RGD binding sequence that interacts with the β_2 integrin CR3 on macrophages (76). Binding to proteoglycan receptors may also result in host cell responses that lead to internalization of adherent microbes (32, 33, 57). Thus, it may be more appropriate to refer to the proteoglycans as coreceptors, where binding initiates a series of interactions that lead to tight adherence, subsequent internalization, and possibly downstream inflammatory responses characteristic of infections (51).

Recent evidence suggests a role for proteoglycans in viral invasion. Studies of HSV show that heparan sulfate is involved in fusion of the viral envelope with the host cell plasma membrane (101). The viral glycoprotein gpC appears to be primar-

ily an adhesin that promotes adherence and is not required for penetration of the cell. In contrast, gpB binds heparan sulfate and promotes both adherence and virus-cell fusion (48). gpB binding to heparan sulfate also leads to syncytium formation (102). The mechanism by which heparan sulfate facilitates membrane fusion is unknown, but perhaps it acts like a template facilitating the association of fusogenic membrane proteins.

SUMMARY AND FUTURE STUDIES

Although many microorganisms bind to cell surface heparan sulfate (Tables 3 and 4), questions about the nature of these interactions remain unanswered. Are specific oligosaccharide sequences required for binding? Do microbes bind to subsets of proteoglycans that differ in protein or glycosaminoglycan composition (64)? Does binding to the core protein as well as to the carbohydrate chains occur (44)? What is the amino acid sequence in the carbohydrate binding domains of adhesins? What is the role of heparin-binding proteins on host cells? Recent studies suggest that some proteoglycans reside in focal adhesions (112), where they participate in cell attachment to substrata and intracellular signaling (59). Thus, future studies should focus on whether microbial binding to proteoglycans also results in a signal transduction event that subsequently triggers processes helpful to the microbe (e.g., invasion).

Although the in vitro evidence for heparan sulfate-microbial cell interactions is compelling, the role of heparan sulfate in microbial pathogenesis is not well established. Examination of virulent and nonvirulent isolates may reveal a correlation with heparan sulfate-dependent adherence and expression of heparin-binding proteins on the microbial cell surface. Another approach suggested by in vitro studies is to administer fragments of heparin or heparan sulfate to infected animals and subsequently determine microbial distribution, tissue colonization, and host survival. The ability of exogenous heparin and related polysaccharides to inhibit viral replication suggests that this approach might lead to polysaccharide-based antiviral pharmaceutical agents (23, 58, 87, 88).

Another approach worth consideration is to inhibit the production of proteoglycans metabolically by using β-D-xylosides (29). Cultured cells take up β-D-xylosides rapidly and use them as primers for the assembly of single glycosaminoglycan chains, which are then secreted (37). Priming also diverts the synthesis of the chains from endogenous proteoglycans, resulting in the expression of underglycosylated proteins on the cell surface. Recent studies show that β-D-xylosides when injected into animals prime glycosaminoglycan chains and that the compounds are apparently well tolerated (9, 10). The secreted glycosaminoglycan chains may block microbial attachment by competition, and the reduction in cell surface receptors should reduce the number of binding sites on the host cells. Thus, β-D-xylosides may be a double-edged sword to fend off infection.

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