CARBOHYDRATE-PROTEIN INTERACTIONS IN VASCULAR BIOLOGY

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ABSTRACT
Carbohydrate-protein interactions participate in a wide variety of biological and pathological events. In recent years, particular attention has been paid to
the carbohydrate-protein interactions that occur in vascular biology. Sialylated oligosaccharides are ligands of a structurally diverse group of proteins that include the selectins and members of the immunoglobulin superfamily. Various glycosaminoglycans can be recognized by an overlapping set of proteins that include two of the selectins and CD44. Emerging knowledge of carbohydrate-protein interactions in human pathophysiology are discussed.

INTRODUCTION

The past few years have brought intense interest in the role of carbohydrates in numerous biological processes. Nowhere has the scientific effort been more active than in vascular biology. Notable recent advances include discovery of carbohydrate-binding proteins that support leukocyte adhesion to the vessel wall and demonstration of the fundamental contribution of vessel wall proteoglycans to the regulation of hemostasis, growth factors, and chemotactic cytokines. Today much of the research on carbohydrates emphasizes their interactions with proteins. This review focuses on recent advances in our knowledge of carbohydrate-protein interactions that occur at cell-cell interfaces in vascular biology. To provide a methodological background, we survey current analytical techniques for quantitative characterization of carbohydrate-protein-binding interactions. We discuss proteins that bind N- and O-linked carbohydrates, as well as those that bind to glycosaminoglycans. Asialo- and glycoprotein receptors, macrophage scavenger receptors, collectins, glectins, CD69, and NKR-P1 are not addressed, and the reader is referred to several recent reviews (Lodish 1991, Zhou & Cummings 1992, Drickamer & Taylor 1993, Krieger et al 1993, Barondes et al 1994, Holmskov et al 1994).

ANALYTICAL METHODS

Many important advances in our knowledge of carbohydrate-protein interactions have resulted from studies in vivo and in cell-based systems in vitro. However, quantitative characterization of isolated lectin-ligand binding events is often best accomplished using cell-free systems in vitro. The binding of carbohydrates to proteins in cell-free systems can be measured using a variety of spectroscopic and nonspectroscopic methods. Many are also applicable to study of other macromolecular interactions such as protein-protein and protein-DNA binding. However, because the monovalent binding of carbohydrates to proteins is often of relatively low affinity, special considerations apply. For example, many interactions are most easily observed using systems that allow multivalent binding. In addition, because chemical modification of small carbohydrate ligands may significantly alter their binding properties, methods that do not involve traditional labeling techniques are favored. Meth-
ods for quantitative analysis of binding interactions can be divided into two
groups: those in which one reactant is immobilized (mixed-phase methods)
and those in which both binding partners are in solution (solution-phase meth-
ods). Individual methods within these two groups differ in the type of infor-
mation that they afford, as well as in their experimental complexity and the
amounts and types of samples needed. No single method is ideal and a
combination of complementary methods often represents the best approach for
characterizing carbohydrate-protein interactions. Table 1 presents salient fea-
tures of the most common methods in use today for the quantitative analysis
of carbohydrate-protein binding interactions.

PROTEINS THAT BIND N- AND O-LINKED
CARBOHYDRATES

Mammalian lectins that bind sialic acid-containing carbohydrates form a struc-
turally heterogeneous group of molecules, including the selectin family of
leukocyte-endothelial adhesion molecules, factor H of the alternate comple-
ment pathway, and a subset of immunoglobulin (Ig) superfamily members
(Varki 1992). Most Ig superfamily cell surface receptors are involved in
protein-protein interactions; ligands are typically other members of the Ig
family or integrins (Springer 1990, Bevilacqua 1993, Carlos & Harlan 1994).
It is now evident that certain Ig family members (e.g. CD22, sialoadhesin,
myelin-associated glycoprotein) can bind sialic acid-containing sugars even
though they lack classical lectin domains. The functional consequences of this
binding are the subject of exciting research efforts.

Selectins

The selectins are a family of three cell surface glycoproteins unified by their
domain structure and by their function (Bevilacqua & Nelson 1993). Each
selectin contains an N-terminal C-type (Ca²⁺-dependent) lectin domain, an
epidermal growth factor-like (EGF) module, a variable number of short con-
sensus repeats (approximately 60 amino acids each), a single-pass transmem-
brane domain, and a short cytoplasmic tail. E-selectin is expressed (peak 4–6
h) on cytokine- or endotoxin-activated endothelium. P-selectin is rapidly
(minutes) distributed to the cell surface from storage granules in platelets and
endothelial cells in response to stimulation with thrombin, histamine, H₂O₂,
TNFα, or complement (Foreman et al 1994). L-selectin is constitutively ex-
pressed on neutrophils, monocytes, and most lymphocytes and is shed upon
cell activation. Each of the selectins can support leukocyte adhesion to vas-
cular endothelium, and together they help regulate leukocyte recruitment to
sites of inflammation. Detailed reviews on the discovery and characterization
of the selectins and their carbohydrate ligands can be found elsewhere
<table>
<thead>
<tr>
<th>Method</th>
<th>Type</th>
<th>Principle</th>
<th>Range of $K_a$ (M)</th>
<th>Material needed</th>
<th>References</th>
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<tbody>
<tr>
<td>Surface plasmon resonance</td>
<td>M</td>
<td>Mass-induced refractive index change in real time for direct measurement of association and dissociation rate constants</td>
<td>$10^{-6}$-$10^{-9}$</td>
<td>$&lt;1$ mg</td>
<td>Mach et al 1993, Hasegawa et al 1993</td>
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<tr>
<td>Two-dimensional affinity</td>
<td>M</td>
<td>Gel retardation: affinity electrophoresis of charged carbohydrate through protein-impregnated gel followed by gradient PAGE</td>
<td>$10^{-5}$-$10^{-9}$</td>
<td>$&lt;1$ mg</td>
<td>Edens et al 1995, Linhardt et al 1995</td>
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<td>resolution electrophoresis</td>
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<tr>
<td>Affinity chromatography</td>
<td>M</td>
<td>Immobilized ligand on column matrix</td>
<td>$&lt;10^{-5}$</td>
<td>$&gt;1$ mg</td>
<td>Denton et al 1981, Honda et al 1988, Olson et al 1991</td>
</tr>
<tr>
<td>Competition ELISA</td>
<td>M</td>
<td>Solution-phase and solid-phase ligands compete for binding to lectin</td>
<td>$10^{-5}$-$10^{-9}$</td>
<td>$&lt;1$ mg</td>
<td>Dawes 1988, Nelson et al 1993b, Bae et al 1994</td>
</tr>
<tr>
<td>Scintillation proximity</td>
<td>M</td>
<td>Proximity of radiolabeled ligand to immobilized lectin results in emitted photon from scintillant</td>
<td>$&lt;10^{-6}$</td>
<td>$&lt;1$ mg</td>
<td>Hart &amp; Greenwald 1979, Bosworth &amp; Towers 1989</td>
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<tr>
<td>Method</td>
<td>Description</td>
<td>Range</td>
<td>Limit</td>
<td>Reference</td>
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<tr>
<td>Nuclear magnetic resonance spectroscopy</td>
<td>Chemical shift, coupling constants, and nuclear Overhauser effect allows</td>
<td>$10^{-3}$–$10^{-5}$</td>
<td>&gt;&gt;1 mg</td>
<td>Bae et al 1994</td>
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<td></td>
<td>calculation of contact points, distances, and conformation</td>
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<td>Fluorescence spectroscopy</td>
<td>Conformational change with ligand binding induces change in fluorescence</td>
<td>$10^{-5}$–$10^{-9}$</td>
<td>&lt;1 mg</td>
<td>Rice et al 1991, Bae et al 1994</td>
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<td>properties of intrinsic or extrinsic fluorophore</td>
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<td>Circular dichroism</td>
<td>Change in rotation of plane-polarized light upon binding to measure lectin</td>
<td>NA</td>
<td>&gt;1 mg</td>
<td>Coffman-Lelouch &amp; Lansbury</td>
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<td>conformational change</td>
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<td>1992, Evans et al 1992</td>
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<tr>
<td>Fourier transform infrared spectroscopy</td>
<td>Measures protein and carbohydrate vibrational, stretching, and bending</td>
<td>NA</td>
<td>&gt;1 mg</td>
<td>Prestrelski et al 1992</td>
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<td>Isothermal microtitration calorimetry</td>
<td>Measures enthalpy of binding directly</td>
<td>$10^{-4}$–$10^{-8}$</td>
<td>&gt;1 mg</td>
<td>Thompson et al 1994, Wiseman</td>
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<td>et al 1989, Freire et al 1990,</td>
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<td>JR Fromm et al, submitted</td>
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<td>Equilibrium dialysis</td>
<td>Semi-permeable membrane partitions protein but not carbohydrate ligand</td>
<td>$&lt;10^{-3}$</td>
<td>&lt;1 mg</td>
<td>Atha et al 1984</td>
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<td>Analytical ultracentrifugation</td>
<td>Equilibrium sedimentation at different carbohydrate:protein ratios yields</td>
<td>NA</td>
<td>&gt;1 mg</td>
<td>Mach et al 1993</td>
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<td>stoichiometry of complex</td>
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<td>Laser light scattering</td>
<td>Intrinsic scattering intensities of carbohydrate:protein complex used to</td>
<td>NA</td>
<td>&gt;1 mg</td>
<td>Mach et al 1993</td>
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<td></td>
<td>calculate stoichiometry</td>
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M, mixed-phase; S, solution-phase (see text); NA, not applicable to $K_d$ determination. All mixed-phase methods are subject to surface-immobilization artifacts. Because the lectins under consideration are cell surface molecules, this may be less problematic than for molecules that normally interact in solution-phase.
sialyl Lewis^x^  sialyl Lewis'^x^  sialyl Lewis'^b^  sialyl Lewis'^a^  sialyl Lewis'\( ^a \)  

Fuc_{\alpha1\rightarrow3} Neu5Ac_{2\rightarrow3} Gal_{\beta1\rightarrow4} GlcNAc

Fuc_{\alpha1\rightarrow4} Neu5Ac_{2\rightarrow3} Gal_{\beta1\rightarrow4} GlcNAc

Fuc_{\alpha1\rightarrow3} O_{\beta}SO_{\rightarrow3} Gal_{\beta1\rightarrow4} GlcNAc

sulfo Lewis'^x^  sulfo Lewis'^b^  sulfo Lewis'^a^  sulfo Lewis'

Fuc_{\alpha1\rightarrow4} Gal_{\beta1\rightarrow4} GlcNAc

Figure 1  Sialyl Lewis^-x^, sialyl Lewis^-a^ and related carbohydrate structures. Neu5Ac, sialic acid; Gal, galactose; GlcNAc, N-acetylgalactosamine; Fuc, fucose.


The selectins, true to their lectin character, can bind to multiple carbohydrate ligands. Perhaps the best-studied ligands of the selectins are those containing sialyl Lewis^-a^ (sLe^-a^), sialyl Lewis^-x^ (sLe^-x^) (Figure 1), and related oligosaccharides. Human neutrophils, monocytes, and a minority of lymphocytes express complex carbohydrates containing a terminal sLe^-a^ that can be recognized in a Ca\(^{2+}\)-dependent manner by E-selectin. Studies using synthetic sLe^-a^-containing oligosaccharides indicate that both fucose and sialic acid are important for E-selectin binding. Fucose is critical: Its alteration or substitution diminishes binding (Brandley et al 1993, Nelson et al 1993b; Ramphal et al 1994). Modification or replacement of sialic acid can be tolerated: Substitution of a sulfate group for sialic acid in either sLe^-a^ or sLe^-x^ maintains binding to E-selectin (Yuen et al 1994). Both sLe^-a^ (Nelson et al 1993b) and sulfo-Le^-a^ (Yuen et al 1994) structures bind to E-selectin with higher affinity than the corresponding Le^-a^-based molecules, with the sulfo derivatives appearing to bind with highest affinity. Binding of most sLe^-x^-related synthetic ligands is enhanced with increasing chain length and can also be increased by the presence of a hydrophobic aglycone (Nelson et al 1993b). Studies using recombinant
forms of E-selectin, including direct binding assessed by NMR (Cooke et al 1994) and competition ELISA (Nelson et al 1993b), suggest that the binding affinity of the sLe\(^a\) tetrasaccharide is approximately 1 mM; in competition studies, some of the sLe\(^a\) derivatives described above bind E-selectin with higher affinities. Multivalent sLe\(^a\) ligands also display stronger binding: Sialyl Le\(^a\) dimers were several-fold more active than monovalent sLe\(^a\) (deFrees et al 1995), and a bovine serum albumin (BSA) neoglycoconjugate with 16 sLe\(^a\) residues per protein molecule was several hundred-fold more active, yielding IC\(_{50}\) values near 1 \(\mu\)M in assays of selectin-dependent cell adhesion (Welplky et al 1994).

Multiple studies using synthetic derivatives of sLe\(^a\) and the isomeric sLe\(^a\) have demonstrated that the three selectins do not bind to these related ligands equally well. For example, whereas E-selectin binds well to sLe\(^a\), L-selectin does not (Nelson et al 1992), instead preferring sulfo-Le\(^a\) derivatives (Green et al 1995). Addition of sulfate to the GlcNAc of an sLe\(^a\)-galactose pentasaccharide increases inhibitory potency against L-selectin binding to the peripheral node addressin fourfold (Scudder et al 1994).

More complex carbohydrate structures have been isolated from human neutrophils and U937 cells that bind E-selectin with relatively high affinity (\(K_d\) <1 \(\mu\)M). They have been identified as complex N-linked tetra-antennary difucosyl oligosaccharides of the type shown in Figure 2A (Patel et al 1994). A di-antennary complex oligosaccharide isolated from recombinant human protein C (Figure 2B) inhibits the adhesion of U937 cells to activated HUVEC.
with an IC\textsubscript{50} of 70 μM (Grinnell et al 1994). Unexpectedly, Neu5Ac (sialic acid) is absent from this structure, the terminal portion of which is an analogue of the neutral trisaccharide Le\textsuperscript{a}, with galactose replaced by N-acetylgalactosamine. The origin of the potency of this inhibitor is unclear.

The selectins can also bind ligands unrelated to sLe\textsuperscript{a}. For example, P- and L-selectin, but not E-selectin, bind to heparin, heparin oligosaccharides (Nelson et al 1993a; Green et al 1995), as well as to other polysulfated or phosphorylated molecules such as inositol polyaniolins (Cecconi et al 1994). In addition, a computer pharmacophore search yielded several molecules that displayed selectin-inhibitory activity (Rao et al 1994). Modification of one of these with fucose produced the most active compound of the group, a C-fucoside of glycyrrhetic acid, with IC\textsubscript{50} values <0.5 mM for E-, P- and L-selectin binding to immobilized sLe\textsuperscript{a}.

Recently, several specific glycoprotein ligands of the selectins have been characterized. Ca\textsuperscript{2+}- and sialic acid-dependent ligands specific for P-selectin (Norgard et al 1993, Sako et al 1993, Moore et al 1994, Lenter et al 1994, Ma et al 1994) and for E-selectin (Steegmaier et al 1995) have been identified on human and murine myeloid cells. The high-affinity glycoprotein ligand of P-selectin (PSGL-1) cloned from HL-60 cells contains sLe\textsuperscript{a} and binds P-selectin with 50-fold higher affinity than it binds E-selectin (Moore et al 1994). The murine P-selectin ligand is 160 kDa and appears to be O-glycosylated (Lenter et al 1994). These molecules are mucin-type, which means they contain regions heavily decorated with O-linked carbohydrates. Expression of functional (adhesion-supporting) PSGL-1 in COS cells requires cotransfection with a cDNA encoding α1→3/4-fucosyltransferase (Sako et al 1993). Cloning of a murine E-selectin ligand (ESL-1) reveals that it is a variant of the chicken cyssteine-rich fibroblast growth factor receptor (Steegmaier et al 1995). This 150-kDa, N-linked glycoprotein supports E-selectin-dependent cell adhesion when transfected into CHO cells cotransfected with α1→3/4-fucosyltransferase.

Mucin-type molecules found on high endothelial venules (HEV), which contain complex fucosylated, sialylated oligosaccharides and require expression of sulfate groups, have been identified as ligands for L-selectin: GlyCAM-1 (Sgp50) and CD34 (Sgp90) (Imai et al 1993, Baumheter et al 1993, Hemmerich & Rosen 1994). A tetrasaccharide derivative of sLe\textsuperscript{a} possessing a sulfate group on the 6-position of galactose, Neu5Acα2→3 (6-O-SO\textsubscript{4}H\textsuperscript{−}) Galβ1→4 [Fucα3→3] GlcNAc, has been identified as a major capping group of the L-selectin ligand GlyCAM-1 (Hemmerich & Rosen 1994), although its affinity for L-selectin has not been reported. An antibody raised against a purified complex glycolipid detected a separate L-selectin ligand on HEV of human lymph nodes (Sawada et al 1993). An unrelated molecule, bovine submaxillary mucin, inhibits the binding of all three selectins to immobilized
BSA-sLe\(^*\) with IC\(_{50}\) values between 75 and 200 nM (R Nelson, unpublished data).

Selectins bind to endogenous ligands with high affinity compared to their binding to small, synthetic ligands. For example, two recombinant truncated forms of P-selectin bound myeloid cells with a \(K_d\) of about 70 nM (Ushiyama et al 1993). Recombinant E-selectin lacking the transmembrane domain binds a ligand on HL-60 cells with a \(K_d\) of 1 \(\mu\)M (Hensley et al 1994). As suggested by the combined data on selectin-ligand binding interactions, ligand multivalency, complexity, and spatial presentation may all contribute to affinity and avidity. It has been proposed that selectins recognize an epitope formed by clustered patches of oligosaccharides on the cell surface (Varki 1994). Full characterization of protein and carbohydrate portions of natural ligands for E-, P-, and L-selectin in human and animal species will help define the structural basis for their high-affinity binding. In addition, X-ray structure and NMR studies of selectin/ligand complexes will clarify our understanding of binding and lead to the development of more potent synthetic compounds.

**CD22**

CD22 (Figure 3) is a B cell lineage-restricted cell surface glycoprotein of 130–150 kDa whose pattern of expression is related to that of IgD. In the pre-B cell stage of development, CD22 expression is intracellular; surface IgM (slgM) expression precedes that of surface CD22. Interestingly, slgM+/CD22- cells appear to be unresponsive to stimulation by anti-IgM antibodies, and acquisition of responsiveness to anti-IgM appears to coincide with expression of surface IgD and surface CD22 (Pezzutto et al 1988).

Two cDNA clones encoding human CD22 have been isolated and predict an integral membrane polypeptide member of the Ig superfamily (Stamenkovic & Seed 1990, Wilson et al, 1991). The smaller isoform, CD22\(\alpha\), possesses an extracellular region composed of five Ig domains; the larger isoform, CD22\(\beta\), is identical except for two additional extracellular Ig domains (domains 3 and 4) positioned between Ig domains 2 and 3 of CD22\(\alpha\). CD22\(\beta\) appears to be the dominant isoform expressed on B cells and will hereafter be referred to as CD22. The extracellular portion of CD22 is highly related to several known adhesion molecules that include myelin-associated glycoprotein (MAG), neural cell adhesion molecule (N-CAM), and vascular cell adhesion molecule (VCAM-1) (Stamenkovic & Seed 1990, Wilson et al 1991).

Evidence that CD22 may function as an adhesion molecule came from observations that COS cells expressing human CD22 and BHK cells transfected with the murine homologue promote erythrocyte as well as T and B cell adhesion (Stamenkovic et al 1991b; Torres et al 1992). Binding of CD22-
Ig$^1$ to its candidate glycoprotein ligands is completely abrogated by pretreatment of ligand expressing cells with sialidase (Stamenkovic et al 1991b; Sgroi et al 1993, Sgroi & Stamenkovic 1994).

A clue to the nature of CD22-ligand interaction was provided by the observation that CD22-Ig binds to COS cells transfected with a human β-galactoside α2→6-sialyltransferase (α2→6ST). Like several other glycosyltransferases, α2→6ST expression is confined to the Golgi (Munro 1991, Bast et al 1992), suggesting that CD22 recognizes cell surface glycoproteins (and possibly glycolipids) containing sialic acid in α2→6 linkage. This notion was further supported by the observation that CD22-Ig specifically recognizes a 115-kDa cell surface glycoprotein in α2→6ST-transfected, but not α2→3ST-transfected, COS cells (Sgroi et al 1993). Because β-galactoside α2→6ST adds sialic acid to a receptor disaccharide Galβ1→4GlcNAc commonly associated

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$^1$Immunoglobulin fusion proteins (here designated CD22-Ig) have also been called receptor globulins (designated CD22-RG).
with N-linked oligosaccharides, CD22-Ig-binding glycoprotein-derived N-linked oligosaccharides were analyzed for CD22 binding independently of the underlying protein core (Powell et al. 1993). These experiments confirmed that oligosaccharides, which contain $\alpha2\rightarrow6$-linked sialic acid residues, are required to bind CD22 and that the greater the number of $\alpha2\rightarrow6$-sialyl residues per sugar chain, the higher is the binding affinity. Selective removal of exocyclic sialic acid side chains by mild periodate treatment results in abrogation of ligand recognition, indicating that intact sialic acid side chains are required for binding (Sgroi et al. 1993, Powell et al. 1993).

Potential determinants of the CD22-ligand affinity were examined by testing the ability of several naturally and enzymatically sialylated oligosaccharides and sialoglycoproteins to bind to a CD22-Ig-protein-A-sepharose column (Powell & Varki, 1994). These studies demonstrate that Neu5Ac$\alpha2\rightarrow6$Galβ1$\rightarrow4$Glc(NAc) is the minimal structure that binds CD22, with $K_d = 32 \mu$M (Powell et al. 1995) and that reduction of the glucose residue of Neu5Ac$\alpha2\rightarrow6$Galβ1$\rightarrow4$Glc weakens the interaction (Powell & Varki 1994). Branched oligosaccharides with two $\alpha2\rightarrow6$-sialyl residues enhances binding, whereas $\alpha2\rightarrow3$-sialyl residues have no effect on binding. Increasing the number of $\alpha2\rightarrow6$-sialyl residues on multi-antennary oligosaccharides progressively improves binding, whereas the oligosaccharide core does not affect binding (Powell & Varki 1994). Other studies suggest that binding of any given $\alpha2\rightarrow6$-sialylated glycoprotein to CD22 cannot be predicted on the basis of the oligosaccharide composition alone (Powell et al. 1995). Structural features of the underlying polypeptide core, therefore, are likely to play a role in determining binding affinity and may explain the highly variable binding of different $\alpha2\rightarrow6$-sialylated glycoproteins to CD22. A second regulatory factor appears to be a 9-$O$-acyl group on the sialic acid side chain, which diminishes the binding of CD22 (Sjoberg et al. 1994).

In addition to lymphocytes and erythrocytes, CD22-Ig binds cultured human endothelial cells (HEC) (Hanasaki et al. 1994) and dermal microvascular endothelial cells (Hanasaki et al. 1995b) stimulated with IL-1, TNFα, or LPS. Cytokine-treated HEC display enhanced $\alpha2\rightarrow6$ST expression and activity, and several sialoglycoproteins, including ICAM-1, VCAM-1, and E-selectin, were specifically immunoblotted using the Sambucus nigra lectin (SNA), which recognizes $\alpha2\rightarrow6$-linked sialic acid in preference to $\alpha2\rightarrow3$-linked sialic acid (Hanasaki et al. 1994). TNFα treatment led to a twofold increase in $\alpha2\rightarrow6$-linked sialic acid on N-linked carbohydrates (Hanasaki et al. 1994) and doubled the proportion of total cellular N-linked oligosaccharides capable of binding to CD22 (Hanasaki et al. 1995b). Interestingly, the relatively minor change in total $\alpha2\rightarrow6$-linked sialic acid yielded a substantial increase in the cell surface binding of CD22-Ig (~ fivefold) and in the adhesion of CHO cells stably transfected with human CD22 cDNA (Hanasaki et al. 1995b). The biological
role of CD22 interactions with the glycoconjugates of activated endothelium remains to be determined.

Sialic acid-dependent interaction between CD22 and its ligands on lymphocytes and endothelial cells can be inhibited by serum and lymphoid cell culture supernatants (S Braesch-Andersen & I Stamenkovic, unpublished data) as well as by plasma (Hanasaki et al 1995a). Several sialoglycoproteins in human plasma bind immobilized CD22 (Hanasaki et al 1995a). Only three of these sialoglycoproteins, with molecular weights of 74, 44, and 25 under reducing conditions, were specifically eluted with α2→6-sialyllactose. Two of these proteins were identified as subunits of human IgM and of haptoglobin, and interaction with both proteins requires α2→6-linked sialic acid. Together these observations demonstrate that CD22 is capable of selective recognition of plasma sialoglycoproteins carrying α2→6-linked sialic acid. Interestingly, both IgM and haptoglobin are involved in immune and inflammatory responses.

CD22 can bind multiple ligands expressed in different cell types. However, a possible clue as to how CD22 might function in lymphocyte activation is provided by the discovery that one ligand of CD22 is CD45. Evidence for a CD22-CD45 interaction was the immunoprecipitation of a 180-kDa cell surface protein from T cell lysates, which was identified as CD45RO in immunoblot assays with the UCHL-1 antibody (Aruffo et al 1992). Recent work has revealed that CD22-Ig also binds other isoforms of CD45 (Sgroi & Stamenkovic 1994). It is noteworthy that CD45—a highly sialylated molecule—contains sialic acid exclusively in α2→6 linkage (Sato et al 1993). Recent observations suggest that the interaction of CD22 with CD45 influences lymphocyte activation (Sgroi et al 1995).

Experiments in vitro have shown that COS cells expressing CD22 bind T and B lymphocytes. However, coexpression of CD22 and α2→6ST in CHO and COS cells abrogates CD22-mediated adhesion (Hanasaki et al 1995b, Braesch-Andersen & Stamenkovic 1994). Interestingly, B cells that express CD22 are also decorated with N-linked oligosaccharides sialylated in α2→6 linkage, including on CD22 itself (Schwartz-Albiez et al 1991). These observations raise the possibility that cellular expression of α2→6-linked sialic acid may modulate the function of CD22 expressed by the same cell.

Sialoadhesin

A second cell surface sialic acid-binding molecule known as sialoadhesin has been shown to have carbohydrate-binding properties similar to those of CD22. Sialoadhesin is expressed on macrophages and—like CD22—recognizes sialylated ligands on erythrocytes, T cells, and B cells without a requirement for divalent cations (van den Berg et al 1992, Sgroi et al 1993). Although both molecules bind sialic acid, sialoadhesin recognizes α2→3-linked sialic acid,
whereas CD22 recognizes α2→6-linked sialic acid. A human sialoadhesin cDNA predicts a type I integral membrane protein with 17 extracellular Ig domains, making sialoadhesin the largest known Ig superfamily member (Crocker et al 1994). The first four Ig domains of sialoadhesin are closely related to those in CD22, as are domains 2–7 of CD22 and 9–14 of sialoadhesin. A soluble recombinant molecule consisting of Ig domains 1–4 of sialoadhesin possesses the necessary structural elements to promote adhesion of erythrocytes and lymphocytes (Kelm et al 1994). Exposure of lymphoid cells and erythrocytes to sialidase abolishes adhesion, which can be reconstituted by resialylation with two distinct sialyltransferases: Galβ1→3GalNAccα2→3ST (3STO), which sialylates O-linked glycans, and Galβ1→3(4)GlcNAccα2→3ST (3STN), which sialylates N-linked glycans (Crocker et al 1994).

The physiologic function of sialoadhesin remains to be elucidated. The observations that sialoadhesin is expressed on the surface of resident bone marrow macrophages and is involved in interactions with developing myeloid cells suggests a role in hematopoiesis. Because it appears to facilitate macrophage interactions with T and B lymphocytes, sialoadhesin may also participate in antigen presentation and in lymphocyte-macrophage costimulatory pathways.

**Myelin-Associated Glycoprotein**

MAG is expressed on neural cells where it is believed to play a role in myelin formation and to be involved in homotypic adhesion. A carbohydrate epitope associated with MAG, called HNK-1 (Kunemund et al 1988), has been proposed to be involved in MAG-mediated adhesion (Kruse et al 1984, Kunemund et al 1988). Like CD22 and sialoadhesin, to which it is closely related (Crocker et al 1994, Kelm et al 1994), MAG recognizes ligands on erythrocytes, neutrophils, and neural cells in a sialic acid-dependent manner. The functional ligand is generated by 3STO sialyltransferase (Kelm et al 1994). Consistent with a neurological function in vivo, recombinant MAG-Ig fusion protein binds in a sialic acid-dependent fashion to dorsal root ganglion neurons with extended myelinated axons and to cerebellar neurons, the majority of which have unmyelinated axons (Kelm et al 1994).

CD22, MAG, and sialoadhesin clearly constitute a novel subgroup of the Ig superfamily, i.e. calcium-independent sialic acid-binding lectins. It is interesting that these molecules may recognize ligands expressed on the same cell or on apposing cells. Further study will be required to illuminate the physiologic roles of these molecules.

**PROTEINS THAT BIND GLYCOSAMINOGLYCANS**

Proteoglycans are a diverse group of proteins unified by their possession of one or more covalently attached glycosaminoglycan (GAG) chains, un-
Figure 4 Glycosaminoglycan structures. IdoA sugars are in L-configuration, all other sugars are in D-configuration. Hyaluronan, heparin, and heparan sulfate are glycosaminoglycans; chondroitin 4- and 6-sulfate and dermatan sulfate are galactosaminoglycans; and keratan sulfate is a sulfated polylactosamine. Heparin is more highly and regularly N- and O-sulfated than heparan sulfate and contains more IdoA; the pentasaccharide example shown is the classical sequence from heparin (also present, although less frequently, in heparan sulfate) that binds with high affinity to antithrombin III. Keratan sulfate can be sulfated at the 6-position of the GlcNAc (as shown), the 6-position of the Gal, or both. GlcA, glucuronic acid; IdoA, iduronic acid; GalNAc, N-acetyl-galactosamine.

branched polysaccharides consisting of repeating disaccharide units (reviewed in Kjellén & Lindahl 1991). These macromolecules frequently contain N- and/or O-linked branched carbohydrates in addition to their GAG polysaccharide and constitute a significant portion of the extracellular matrix. Proteogly-
Carbohydrate-Protein Interactions

Carbohydrates are synthesized by most cells during some part of their life cycle. There are seven known GAG types (Figure 4), five of which are synthesized as part of proteoglycans. They are distinguished by their backbone disaccharide composition, as well as by the extent and nature of post-synthetic modification (N-sulfation, O-sulfation, epimerization). Most proteoglycans contain one or more of the following GAGs: chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate, and heparan sulfate. Heparin, a highly sulfated GAG, is synthesized as part of a proteoglycan in granules of mast cells but is typically found as free carbohydrate after degranulation. Keratan sulfate is a common component of connective tissue and cornea. The seventh GAG, hyaluronan, is synthesized independently of a protein component, and structurally is the simplest GAG, composed of repeating disaccharides of glucuronic acid and N-acetylgalactosamine in alternating β1→3 and β1→4 linkage and containing no sulfate groups.

In vascular biology, much of the GAG-related research has focused on endothelial proteoglycans, although these molecules are also expressed on the surfaces of most circulating cells, including lymphocytes, neutrophils, monocytes, and macrophages (Steward et al 1990). On the vascular endothelium, common proteoglycans include syndecan, ryudocan, and thrombomodulin, whose major GAG components are dermatan sulfate, chondroitin sulfate, and heparan sulfate (Kjellén & Lindahl, 1991; Rapraeger 1993). Recent reviews have provided substantial information on the biochemistry and biology of proteoglycans (Ruoslanti 1989, Gallagher et al 1990, Kjellén & Lindahl, 1991, Yanagishita & Hascall 1992, Bernfield et al 1992, Hardingham & Fosang 1992); here we focus primarily on the endothelial GAGs and their binding interactions with cell surface adhesion molecules, growth factors, and cytokines.

Increasing evidence points to the specificity of GAG-protein binding interactions. Structural diversity in GAGs, which imparts specific recognition by proteins, depends on both the backbone disaccharide composition and the post-synthetic modifications. In heparan sulfate, heparin, and dermatan sulfate, structural diversity can be increased by epimerization of glucuronic acid to iduronic acid. Furthermore, component sugars of GAGs can be N- and O-sulfated, contributing additional intrachain heterogeneity. It should be pointed out that structural diversity exists not only between different GAG chains of the same type, but also along the length of a single GAG chain. Thus, while many proteins may bind to heparin, they do not necessarily recognize the same sugar sequence. The clearest demonstration of this is the observation that antithrombin III binds with high affinity to a specific pentasaccharide structure present in some heparan sulfate and heparin chains (Bourin & Lindahl 1993). Increasing evidence suggests that other heparin-binding proteins will also demonstrate sequence specificity.
Molecules that Bind Heparin and Heparan Sulfate

Heparan sulfate proteoglycans (HSPG) are expressed on the surface of many cell types and are also found in the extracellular matrix, where they play an important structural role. More recently, important regulatory functions of these molecules have been identified (see below). Regulation of endothelial cell surface GAG expression, like regulation of selectins, can occur at the level of both synthesis and cellular distribution (Ihrcke et al 1993). For example, activation of cultured human umbilical cord endothelial cells with endotoxin, IL1, TNF, or interferon-γ results in increased GAG levels in the medium and decreased cell surface GAGs (Klein et al 1992). The released GAGs appear not to be newly synthesized and may result from release of intracellular GAG stores, as well as from cleavage of cell surface molecules (Klein et al 1992). Although GAG synthesis is decreased early, by 48 h overall GAG synthesis in response to these mediators is increased over baseline. The potential effects of proteoglycan shedding on endothelial cell properties and function include increased adhesiveness, increased thrombogenicity, and increased permeability. Other stimuli that alter synthesis and secretion of cultured endothelial cell GAGs include exposure to heparin itself (Finhal et al 1994), X-irradiation (Pye et al 1994), and hypoxia (Karlinsky et al 1992).

Neutrophils have been shown to release products, including elastase (Key et al 1992), which sculpt and degrade HSPG on endothelial cell surfaces (Key et al 1992, Klein et al 1992, 1993). In addition, endo-β-d-glucuronidase released by activated platelets, neutrophils, and T cells can digest heparan sulfate to small fragments. The ability of lymphocytes to extravasate and reach sites of infection also appears to require their elaboration of enzymes that cleave GAGs and proteoglycans (Lider et al 1990); this has obvious utility for cells migrating through the extracellular matrix but could also be important for the initial extravasation through the endothelial cell layer. Activated T cells also precipitate release of endothelial HSPG, which may exacerbate ischemic injury associated with transplant rejection (Geller et al 1994). The ability of cells to modulate HSPG synthesis and expression by itself suggests that the presence or absence of cell surface HSPG can have significant effects on cell behavior. The interactions of HSPG with the molecules discussed below provide further evidence in support of this thesis.

L-SELECTIN, P-SELECTIN, AND PECAM-1 Most studies on the carbohydrate ligands of selectins have focused on sialylated fucosylated structures, such as sLe^a (see above). However, recombinant soluble versions of L-selectin and P-selectin also bind heparin and heparin-like molecules (Bevilacqua & Nelson 1993). Exogenous heparin, and to a lesser extent heparan sulfate, inhibits the binding of P- and L-selectin to sLe^a and sLe^a (Nelson et al 1993a). Recent
evidence suggests that an endogenous endothelial cell ligand for L-selectin is a heparan sulfate proteoglycan, whose GAG chains are enriched with unsubstituted amino groups (Norgard-Sumnicht et al 1993, Norgard-Sumnicht & Varki 1995). The GAG-binding sites on L- and P-selectin have not been defined. Interestingly, E-selectin, which shares a high degree of identity to P- and L-selectin (approximately 60% in the lectin and EGF domain), does not bind heparin or other GAGs (Nelson et al 1993a). Whether binding of GAGs to P- and L-selectin, and not to E-selectin, is a function of the C-type lectin domain or other parts of the molecules, or both, remains to be determined. The relative importance of P- and L-selectin interactions with heparan sulfate compared to sLeâ-related ligands in vivo is unclear.

PECAM-1 (CD31), a member of the immunoglobulin superfamily, is found on platelets, leukocytes, and localized at endothelial cell junctions where it may contribute to endothelial cell-endothelial cell integrity via homotypic (PECAM-PECAM) and/or heterotypic (PECAM-heparan sulfate) interactions (DeLisser et al 1993). In purified component assay systems using recombinant protein, PECAM-heparin binding is substantially stronger than PECAM-PECAM binding (R Delmendo & R Nelson, unpublished data). Other molecules that participate in cell adhesion and interaction of lymphocytes and also bind polyanionic polysaccharides include CD2 and CD4 (Parish et al 1992) and Thy-1 (Hueber et al 1992).

VEGF AND FGF There has been substantial progress in the past several years toward elucidation of interactions between the FGF family of growth factors, their receptors, and HSPG. Although much of this work has focused on basic FGF (bFGF or FGF-2), recent attention has been paid to vascular endothelial growth factor (VEGF); both proteins are angiogenic. Human VEGF is secreted as a homodimer by many cell types, including macrophages, monocytes, vascular smooth muscle cells, vascular endothelial cells, and many solid tumor cells. Five molecular weight forms of VEGF have been described that result from alternative splicing of mRNA transcribed from a single gene. Unlike PDGF, with which it shares 15% (A chain) to 18% (B chain) identity, VEGF is mitogenic in vitro only for endothelial cells. In vivo, VEGF is a potent inducer of angiogenesis and blood vessel permeability whose function may be required for wound healing and solid tumor growth. Like PDGF, VEGF is only active as a disulfide-linked homodimer. VEGF binds to tyrosine kinase receptors on endothelial cells encoded by the flt-1 and KDR/flk genes; receptor dimerization is required for receptor autophosphorylation and signal transduction. VEGF binding to aortic arch-derived bovine endothelial cells is inhibited by heparin fragments of 16–18 sugars and potentiated by fragments of 24 sugars and longer (Soker et al 1994). The angiogenic activity of VEGF in vivo may be held in check by â2-macroglobulin, an abundant plasma
proteinase inhibitor that binds and inhibits the activity of VEGF, TGFβ, bFGF, and PDGF (Soker et al 1993). Heparin and heparan sulfate, but not chondroitin sulfate or dextran sulfate, inhibit VEGF and TGFβ binding to α2M. Interestingly, maximal inhibition occurs with heparin chains of 14–16 sugars, a similar range for inhibition of VEGF binding to endothelial cells.

Cell surface heparan sulfate appears to be required for VEGF to bind to the endothelial cell receptors (Gitay-Goren et al 1992). Although the mechanism by which heparan sulfate regulates VEGF binding to its receptors has not been elucidated, studies using murine flk-1 receptor indicate that, unlike the case with bFGF, heparin or heparan sulfate (not chondroitin sulfate or desulfated heparin) potentiates VEGF binding by interacting only with VEGF (Tessler et al 1994) and that the effect of heparin on VEGF binding can vary with receptor type. For example, low concentrations (1–10 μg/mL) of heparin potentiate VEGF binding to a flk-1 construct expressed in NIH-3T3 cells and inhibit the binding of VEGF to endogenous receptors on this cell type. Differential regulation by heparin has also been reported for binding of acidic FGF (aFGF or FGF-1) and KGF to KGFR, with low concentrations potentiating the former and inhibiting the latter interaction (Reich-Slotky et al 1994). Further evidence for modulation of bFGF-receptor interactions by specific GAG sequences comes from studies showing differential effects of heparinases I, II, and III on neovascularization in vivo and on endothelial cell mitogenesis in vitro (Sasisekharan et al 1994). The effect in vivo could also result from impact on VEGF-receptor binding. An unrelated angiogenic molecule expressed by tumors (angiogenin) also binds heparin tightly, and it has been suggested that HSPG may be involved in tumor cell adhesion (Soncin et al 1994). Finally, hypoxia, a condition that decreases cultured bovine aortic endothelial cell surface heparan sulfate (Karlinsky et al 1992), increases VEGF and VEGF receptor gene expression in rat lungs (Tuder et al 1995).

Substantial progress has been made in the last several years in the characterization of heparin-bFGF binding using mutagenesis and binding energetics studies. Studies have indicated a requirement for HSPG as cofactor in bFGF-receptor tyrosine kinase activity (Rapraeger 1993). Several recent studies suggest that the binding of bFGF to its receptor is enhanced by HSPG but does not require it (Mason 1994). Heparin increased the affinity of the bFGF-FGFR-1 interaction in three separate cell systems; however, in the absence of heparin there was no mitogenesis (Roghani et al 1994). Enhanced affinity of bFGF for FGFR-1 in the presence of heparin was confirmed in a series of titration calorimetry studies examining interactions between growth factor, receptor, and HSPG (Pantoliano et al 1994). Moreover, the thermodynamic data combined with molecular modeling indicated that when a heparin molecule is of sufficient size to bridge growth factor and receptor (decasaccharides and larger), receptor dimerization is facilitated. Although receptor dimerization of
bFGF has not been unambiguously demonstrated to be a requirement for signaling in whole cell systems, this tantalizing observation is in accord with those of Roghani et al (1994) mentioned above.

Mutagenesis studies of bFGF agree on several of the amino acid residues that make up the FGF-binding site for heparin. Li et al (1994) demonstrated that single replacement of lysine with glutamine at position 128 or 134 resulted in a substantial decrease in heparin binding without change in receptor binding, PAI induction, or angiogenic activity. Surprisingly, data from examination of binding interactions at varying ionic strengths, assuming that heparin behaves as a polyelectrolyte, indicate that pure electrostatic interactions appear to account for only about 30% of the binding energy between bFGF and heparin (Thompson et al 1994). This conclusion is corroborated by studies demonstrating that several bFGF mutants with lysine residues replaced with glutamine displayed substantial decreases (up to one order of magnitude) in heparin binding without exhibiting a significant change in the molarity of sodium chloride required to elute them from a heparin sepharose column (Li et al 1994). When a 5-residue loop of bFGF (amino acid residues 118–122) connecting the ninth and tenth β-strands containing three lysines and a glutamic acid was replaced by analogous loops in αFGF or IL-1β, there was no change in affinity for heparin; the only difference was an acquired ability of the former to bind to KGFR (Seddon et al 1995). Specific sequences of heparan sulfate and heparin involved in promotion of FGF–FGFR binding are beginning to emerge and appear to be different for different growth factor-receptor combinations (Guimond et al 1993, Maccarana et al 1993, Ishihara et al 1993, Aviezer et al 1994). A recent report indicates that specific di- and trisaccharides sequences from heparan sulfate are capable of binding to bFGF and enabling receptor binding (Omitz et al 1995), which suggests that the heparin bridging hypothesis may not be correct. A complete description of the ternary interaction at the cell surface between HSPG, bFGF, and FGFR will require additional work to extend the fascinating studies to date.

CHEMOKINES In addition to modulation of growth factor activity as exemplified above by FGF and VEGF, the role of HSPG in the modulation of a variety of cytokines, especially chemotactic cytokines (chemokines), is becoming apparent (Tanaka et al 1993b). MIP-1β binds to HSPG on lymph node endothelial cell surfaces in vivo. In vitro, MIP-1β bound immobilized glycosaminoglycan (BSA-heparin or CD44), but triggered activation and cell adhesion of C8+ T cells only when co-immobilized with VCAM-1 (Tanaka et al 1993a). VCAM-1 immobilized alone had no effect, nor did two other heparin-binding chemokines, RANTES and GRO. In similar studies, neutrophil activation by IL-8 was enhanced by heparin or heparan sulfate (Webb et al 1993). Different
chemokines, like the growth factors and their receptors, exhibit differential binding to GAGs (Witt & Lander 1994).

**Molecules that Bind Hyaluronan**

Hyaluronan (hyaluronic acid, HA) is a high molecular mass, broadly distributed extracellular matrix glycosaminoglycan (GAG) polymer synthesized by HA synthetase, expressed in plasma membranes of fibroblasts, chondrocytes, and a variety of other cell types (Laurent & Fraser 1992) (see Figure 4). Chain extension is by addition of sugars to the reducing end as the nonreducing end is pushed into the pericellular space (Laurent & Fraser 1992). Unlike other GAGs, HA is not sulfated and is not covalently bound to a core polypeptide. Although it was discovered and characterized as early as 1934 (Meyer & Palmer 1934), HA had for a long time been considered as a space filler with little biological significance. This view began to change when it was discovered that HA is specifically bound by several cell surface and matrix proteins. HA-binding proteins, or hyaladherins (Knudson & Knudson 1993), can be subdivided into two classes: The first comprises those that are part of the extracellular matrix (ECM), including link protein, aggrecan, and versican (Knudson & Knudson 1993). The second class represents cell surface HA-binding proteins. All matrix hyaladherins contain a sequence of amino acids homologous to the tandem-repeated B loops of cartilage-link protein, a structural motif that has been predicted to contain the HA-binding domain. The HA-binding motif has recently been defined as BX_{1,2}B, where B is either Arg or Lys, and X_{1} contains no acidic residues and at least one basic amino acid (Yang et al 1994). This motif has been identified in all ECM and cell surface hyaladherins characterized to date (Yang et al 1994). At least four major cell surface receptors of HA have been identified and characterized: CD44, TSG-6, ICAM-1 (CD54), and RHAMM (receptor for hyaluronan-mediated motility). Despite structural similarities between cell surface and ECM hyaladherins, an important functional difference is that ECM hyaladherins require at least a deca- or dodecasaccharide of HA to compete for binding to polymeric HA, whereas HA hexamers constitute the minimal sequence that is recognized by cell surface HA receptors (Knudson 1993). The affinity of cell surface hyaladherins for HA increases with increasing polymer length of HA, which suggests that multivalent interaction of HA with multiple cell surface receptor molecules results in the observed high affinity (K_{d} \sim 10^{-9} M) (Toole 1990, Underhill 1992). Binding of HA to cell surface hyaladherins increases with increasing ionic strength, and binding to CD44 is resistant to mild glutaraldehyde fixation. Thus several properties facilitate the distinction between specific binding of HA to cell surface hyaladherins and specific aggregation of HA with ECM hyaladherins. Increased HA production can be observed in developing limbs, at sites of inflammation and wound healing, and in the vicinity of tumor
invasion (Toole 1981), underscoring the involvement of HA in multiple biological processes.

**CD44** CD44 is a broadly expressed polymorphic cell surface glycoprotein whose diversity results from alternative splicing of 10 exons encoding a portion of the extracellular domain, as well as cell-type-specific glycosylation and glycosaminoglycan substitution (Underhill 1992, Lesley et al 1994). The most commonly expressed isoform, often referred to as CD44H, is also the smallest, containing none of the variably spliced exons (Stamenkovic et al 1989, Goldstein et al 1989). Although the predicted mass of the encoded polypeptide is 37 kDa, cell surface CD44H is found to be a glycoprotein of 80–90 kDa, consistent with abundant N- and O-linked glycosylation (Stamenkovic et al 1989, Goldstein et al 1989). The extracellular domain of CD44 can be subdivided into two regions: The NH₂-terminal 135 residues form a sequence that is related to the link protein B loop motif (Stamenkovic et al 1989, Goldstein et al 1989, Nottenburg et al 1989); the membrane proximal sequence contains a large number of serine and threonine residues, which may provide multiple O-linked glycosylation sites. Insertion of sequences encoded by variably spliced exons occurs within this region, between residues 220 and 221 (Stamenkovic et al 1991a, Brown et al 1991, Sereaton et al 1992).

Using a soluble CD44-Ig fusion protein (Aruffo et al 1990) and monoclonal antibodies (Miyake et al 1990), human and murine CD44H have been shown to be major cell surface hyaluronan receptors. CD44 contains two BX₂B motifs in the extracellular domain (Yang et al 1994, Stamenkovic et al 1989), both of which are within the cartilage link protein-like region. The more NH₂-terminal of the two motifs appears to provide a greater contribution to HA binding, based on the observations that mutation of a single Arg residue within the amino terminal motif abrogates CD44 binding to HA, whereas mutation of basic residues, alone or in combination, within the second motif reduces but does not abrogate binding (Peach et al 1993).

The ability of CD44H to bind hyaluronan is itself regulated because CD44H does not invariably promote cell attachment to substrate, including HA. For example, normal lymphocytes express a relatively high level of CD44H but bind HA poorly (Murakami et al 1990, 1994; Lesley et al 1994). Upon activation by antigen, mitogens, MAbs, or certain cytokines, a several-fold increase in binding is observed (Murakami et al 1990, 1994; Lesley et al 1994). The observed enhancement in binding does not appear to be the result of an increase in receptor number, but rather some alteration of receptor conformation. Alterations in both N- and O-linked glycosylation may provide a potential mechanism for regulating the affinity of CD44H for HA (A Bartolazzi & I Stamenkovic, unpublished data).

The intracellular domain of CD44 has been proposed to be associated with
cytoskeletal proteins (Lacy & Underhill 1987). Ankyrin (Lokeshwar et al 1994) and the ezrin/moesin/radixin (EMR) family (Tsukita et al 1994) are thought to provide the most promising candidate molecules based on recent experimental evidence. The cytoplasmic domain of CD44 is required for CD44-dependent migration on HA surfaces (Thomas et al 1992), supporting a functional role for CD44-cytoskeletal interaction. Although HA-binding is a major property of CD44, recognition of other ligands has also been observed. Thus CD44 appears to bind collagens type I and VI (Carter & Wayner 1988), fibronectin (Jalkanen & Jalkanen 1992), and chondroitin sulfate (Naujokas et al 1993).


RHAMM A distinct HA receptor expressed in fibroblasts has been recently identified and given the acronym RHAMM, for receptor for hyaluronic acid mediated motility (Turley et al 1991). RHAMM is expressed as 52- and 58-kDa isoforms of a protein encoded by a single gene on the surface of ras-transformed fibroblasts where it forms a hyaluronan receptor complex (HARC) with at least two other molecules of 68 and 72 kDa (Hardwick et al 1992). RHAMM is expressed on the leading lamellae and the perinuclear region of rapidly migrating fibroblasts and has been shown to bind HA and to promote HA-associated locomotion of ras-transformed fibroblasts (Turley et al 1991, Hardwick et al 1992). Interestingly, RHAMM is not homologous to CD44 but does contain two BX3B motifs (residues 401–411 and 423–432) that contribute equally to HA binding (Yang et al 1993, 1994).

TSG-6 Fibroblasts and peripheral blood leukocytes stimulated with TNFα, IL-1, phorbol ester, or calcium ionophore A23187 express TNF-stimulated gene-6 product (TSG-6); a variety of tumor-derived or virus-transformed cell lines do not express TSG-6 in response to the same stimuli (TH Lee et al
Transfection of normal fibroblasts with SV40 large T antigen suppresses the inducibility of TSG-6 (TH Lee et al, 1992). The nucleotide sequence of TSG-6 cDNA predicts a mature protein of 29 kDa without a typical transmembrane domain. A portion of the amino terminal domain (residues 33-142) displays significant relatedness to cartilage link protein and to CD44 (36% and 37% identity, respectively). This sequence contains one BXxB motif (residues 40-48). TSG-6 binds hyaluronan-sepharose, and binding is partially blocked by soluble HA. In addition, TSG-6 can be coprecipitated with HA by cationic detergents (TH Lee et al, 1992). Thus TSG-6 appears to recognize carbohydrate motifs unique to HA.

Most of TSG-6 synthesized in transfected cells is rapidly secreted into the cell culture medium (TH Lee et al, 1992) and is seen there as a 39-kDa monomer and as a multimer of 110-120 kDa, which is possibly a trimer or a TSG-6 monomer associated with other proteins. Because TSG-6 production by fibroblasts and leukocytes is stimulated by IL-1 and TNFα, it seems reasonable to expect increased production during inflammation. TSG-6 is abundant in joints of patients with rheumatoid arthritis, sera of patients with bacterial sepsis, and sera of volunteers injected with LPS, but not in synovial fluid of non-inflammatory joints or in sera of normal individuals (Lee et al, 1993). Thus TSG-6 may play a role in inflammation; the nature and magnitude of this role awaits further study.

ICAM-1 The mechanism for clearing HA from the bloodstream is receptor-mediated endocytosis by liver endothelial cells (LEC; McGary et al, 1989). The HA receptor on LEC (also known as HARLEC) is an 85-100 kDa that is not related to CD44 (McGary et al, 1989). Corneal endothelial cells as well as nonendothelial, thymic reticular cells are specifically recognized by anti-HARLEC MAb. Purification and protein sequencing of rat HARLEC revealed that its amino acid sequence is identical to that of ICAM-1 (McCourt et al, 1994). The extracellular domain of ICAM-1 is composed of 5-Ig subdomains. So far, none of the Ig superfamily members has been found to bind HA. However, ICAM-1 contains several near-BXxB motifs, differing from the canonical motif by the presence of at least one acidic residue in the X7 sequence. This suggests that the HA-binding motif may need to be redefined, and/or that other sequences are involved in binding. Interestingly, cell surface ICAM-1 expressed in COS cells and leukocytes, as well as soluble ICAM-Ig fusion proteins overproduced in COS cells, do not appear to bind HA.

SUMMARY

It has been written that "reversible molecular interactions are at the heart of the dance of life" (Stryer 1988). The reversible carbohydrate-protein interac-
tions involved in processes as diverse and important as fertilization, morphogenesis, immunity, and inflammation animate the poetry of this statement. It is anticipated that future research on carbohydrates and the proteins they bind to will greatly increase our knowledge of many areas of biology. In addition, continuing study of carbohydrate-protein binding will provide insight into the fundamental principles governing macromolecular interactions.

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Literature Cited

CARBOHYDRATE-PROTEIN INTERACTIONS

Hanasaki K, Varke A, Powell LD. 1994b. CD22-mediated cell adhesion to cytokine-activated human endothelial cells: positive
and negative regulation by α2-6-sialylation of cellular glycoproteins. J. Biol. Chem. 270: 7533–42.


Hemmerich S, Rosen SD. 1994. 6'-sulfated sialyl Lewis x is a major capping group of GlyCAM-1. Biochemistry 33:4830–35


Li LY, Safran M, Aviezer D, Boehlen P, Seddon AP, Yayon A. 1994. Diminished hepatic binding of a basic fibroblast growth factor mutant is associated with reduced receptor binding, mitogenesis, plasminogen activator induction, and in vitro angiogenesis. Biochemistry 33:10397–100
Murakami S, Miyake K, June CH, Kincaide PW, Hodges RJ. 1990. IL-5 induces a Fgp-1 (CD44) bright B cell subpopulation that is
highly enriched in proliferative and Ig secre-
tory activity and binds to hyaluronate. J. Im-
munol. 145:3618–24
etin and inhibit acute inflammation. Blood 82:3253–58
Nelson RM, Dolich S, Aruffo A, Cecconi O, Bevilacqua MP. 1993b. Higher-affinity oligo-
Noble PW, Lake FR, Henson PM, Riches DW. 1993. Hylarourate activation of CD44 in-
duces insulin-like growth factor-I expression by a tumor necrosis factor-alpha-
Norgard-Summitt KE, Varki NM, Varki A. 1993. Calcium-dependent heparin-like li-
gands for L-selectin in nonlymphoid endothelial cells. Science. 261:480–83
Nottenburg G, Rees G, St John T. 1989. Isolation of mouse CD44 cDNA: structural fea-
tures are distinct from the primate cDNA. Proc. Natl. Acad. Sci. USA. 86:8521–25
Olson ST, Bock PE, Sheffer R. 1991. Quantitative evaluation of solution equilibrium binding interactions by affinity partitioning: application to specific and nonspecific pro-
valent ligand-receptor binding interactions in the fibroblast growth factor system produce a cooperative growth factor and heparin mechanism for receptor dimerization. Bio-
chemistry 33:14825–34
Pezzuto A, Rabonovich PS, Darken B, Mold-
enhauser G, Clark EA. 1988. Role of the CD22 human B cell antigen in B cell trigger-
ing by anti-immunoglobulin. J. Immunol. 140:1791–95
Pinhal MA, Walenga JM, Jeske W, Hoppenste
bolic agents stimulate the synthesis and modify the sulfation pattern of a heparan sulfate proteoglycan from endothelial cells. Thromb. Res. 74:143–53
gosaccharide binding by recombinant soluble and native cell-associated CD22: evidence for a minimal structural recognition motif and the potential importance of multi-
site binding. J. Biol. Chem. 270:7523–32
Powell LD, Sgroi D, Sjoberg BR, Stanenk-
ovic I, Varki A. 1993. Natural ligands of the B cell adhesion molecule CD22 beta carry N-linked oligosaccharides with al-
pha-2,6-linked sialic acids that are re-
activity relationships of sialyl Lewis x-containing oligosaccharides. 1. Effect of modi-
CARBOHYDRATE-PROTEIN INTERACTIONS

629

lications of the fucose moiety. J. Med. Chem. 37:3459-63
mimics derived from a pharmacophore search are selectin inhibitors with anti-in-
flammatory activity. J. Biol. Chem. 269: 19663-66

Rapprager AC. 1993. The coordinated regulation of heparan sulfation, syndecans, and cell
tial effect of cell-associated heparan sulfates on the binding of keratinocyte growth
factor (KGF) and acidic fibroblast growth factor to the KGF receptor. J. Biol. Chem.
269:3279-85

Rice KG, Wu RG, Brand L, Lee YC. 1991. Intermolecular distance and flexibility of a tri-
antennary glycopeptide as measured by resonance energy transfer. Biochemistry 30:
6646-55

the affinity of basic fibroblast growth factor for its receptor but is not required for binding.
J. Biol. Chem. 269:3976-84


functional glycoprotein ligand for P-selectin. Cell 75:1179-86


chains of human leukocyte common antigen CD45. Biochemistry 32:12694-704

a complex sialyl Lewis X antigen on high endothelial venules of human lymph nodes:

Schwarz-Albiez R, Dorken B, Monner DA, Moldenhauer G. 1991. CD22 antigen: bi-
synthesis, glycosylation and surface expression of a B lymphocyte protein involved in B
cell activation and adhesion. Int. Immunol. 3:623-33

Screaton GR, Bell MV, Jackson DG, Cornelis FB, Gerth U, Bell JJ. 1992. Genomic structure
of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alter-

Scudder PR, Shalhoubi K, Duffin KL, Streeter PR, Jacob GS. 1994. Enzymatic synthesis of a
G-sulfated sialyl Lewis X which is an inhibitor of L-selectin binding to peripheral
addressin. Glycoconjugate J 9:13-31

growth factor: alteration of receptor binding specificity. Biochemistry 34:731-36

Sgrò D, Kortezky GA, Stamenkovic I. 1995. Regulation of functional syndecans is

Sgrò D, Stamenkovic I. 1993. CD22 engages the B cell adhesion molecule and is regarded
as a cross-reactive reactive and recognizes distinct sialo-glycoproteins on different functional T-cell sub-

Sgrò D, Varka A, Braesch-Andersen S, Stamenkovic I. 1993. CD22, a B cell specific
immunoglobulin superfAMILY member, is a siualic acid-binding lectin. J. Biol. Chem.
268: 701-18

1265-62

size and sulfation of a receptor modulate the effect of heparin on the binding of

Soker S, Svanh CM, Neufeld G. 1993. Vascular endothelial growth factor is inactivated by
binding to α2-macroglobulin and the binding is inhibited by heparin. J. Biol. Chem.
268: 7685-91

Soncin F, Shapiro R, Pertt JW. 1994. A cell-surface proteoglycan mediates human adeno-
arcinoma HT-29 cell adhesion to human angiogenin. J. Biol. Chem. 269:8999-9005


Stamenkovic I, Amiot M, Pesando JM, Seed B. 1989. A lymphocyte molecule implicated in
lymph node homing is a member of the cartilage link protein family. Cell 56:1057-62

Stamenkovic I, Aruffo A, Amiot M, Seed B. 1991a. The hematopoietic and epithelial
forms of CDS4 are distinct polypeptides with different adhesion potentials for hy-
aluronate-bearing cells. EMBO J. 10:343-48

Stamenkovic I, Aruffo A, Sy MS, Anderson T. 1991b. The B lymphocyte adhesion
molecule CD22 interacts with leukocyte common antigen CD45R0 on T cells
and alpha 2–6 sialyltransferase, CD75, on B cells. Cell 66:1135-44

Steegmaier M, Levnovitz A, Isenmann S,