
Chapter 5.5.2

Mammalian Carbohydrate-Lectin Interactions

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Selectins

The selectins are a family of lectins that mediate the movement of leukocytes from the bloodstream into sites of inflammation. This multistep cascade proceeds by initial attachment leading to the rolling of leukocytes along endothelial vasculature via selectin-carbohydrate interactions. Subsequent firm adhesion requires chemoattractant activation of leukocyte integrins which mediate arrest through interaction with endothelial receptors. Leukocytes then extravasate between endothelial cells in the direction of chemoattractants as indicated in Fig. 3 [37].

There are three selectin molecules: E (endothelial), P (platelet), and L (leukocyte), [reviewed in 38] named for their cellular localization. P-Selectin is released to the cell surface from storage vesicles in endothelial cells and platelets minutes after stimulation by a number of activators, such as thrombin or histamine [37]. E-Selectin expression on the endothelial cell surface requires *de novo* mRNA transcription, peaking 3–6 h after stimulation with activators such as tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), or bacterial lipopolysaccharide [39]. These cytokines also upregulate the transcription of P-selectin, resulting in increased expression 2–4 h following stimulation [37].

All three selectins bind sialyl Lewis^x (SLe^x, NeuAc(α 2-3)Gal(β 1-4)[Fuc(α 1-3)]GlcNAc) which is proposed to be the minimal carbohydrate ligand for all three selectins, although sialyl Lewis^a (NeuAc(α 2-3)Gal(β 1-3)[Fuc(1-4)]GlcNAc), 3"-SO₄-Le^a and 3"-SO₄-Le^x bind with affinities similar to SLe^x [40, 41, 42, 43].

Pharmacophore studies utilizing ligands with addition or deletion of hydroxy groups and modifications to sialic acids have produced a foundation for under-

1) Rolling → 2) Activation → 3) Firm Adhesion → 4) Transmigration

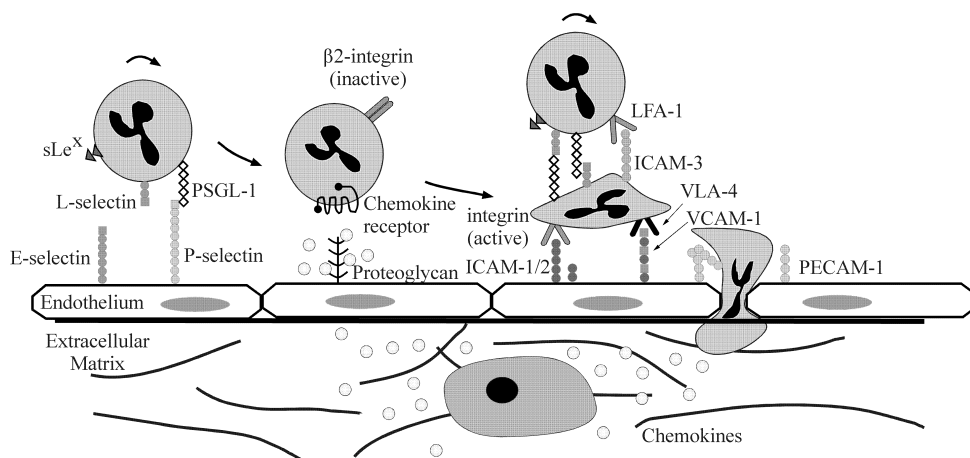


Fig. 3. Leukocyte rolling and adhesion. The migration of leukocytes to sites of inflammation begins with attachment (1a), followed by rolling (1b), activation (2), cessation of movement and flattening against the vessel wall (3), and trans-endothelial migration (4). Steps 1a–2 are mediated by selectins, 1b–4 by chemoattractants, and 3–4 by β_2 integrins. Adapted with permission from Springer (1994) Cell 76:301. Copyright 1994 Cell Press

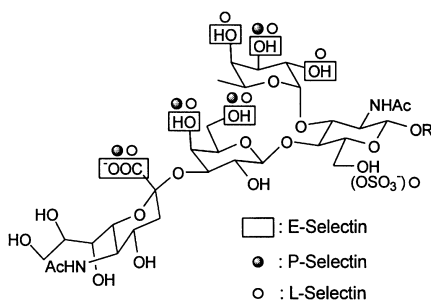


Fig. 4. Selectins bind to different functional groups of the sialyl Lewis^x tetrasaccharide. Functional groups considered to be important for binding to each of the selectins are indicated. Reprinted with permission from Simanek et al. (1998) Chem Rev 98:833. Copyright 1998 American Chemical Society

standing the nature of the interactions between SLe^x and each selectin as shown in Fig. 4 [44]. Selectins do not tolerate the deletion of Fuc hydroxy groups, while the only moiety of NeuAc necessary for binding is the C1 carboxylate. Gal C4 and C6 hydroxy groups are important for binding, while the remainder of Gal(1-4)GlcNAc is considered to function primarily as a scaffold on which Fuc and NeuAc (or sulfate) are positioned for receptor binding [44].

A variety of pathological consequences is the result of malfunctions related to the selectins. Patients with a rare disease known as leukocyte adhesion deficien-

cy type II (LAD-II, whereas LAD-I is a defect in the common $\alpha 2$ integrin subunit) lack the appropriate *de novo* fucosylation pathway to biosynthesize SLe^x, and consequently suffer from repeated severe bacterial infections in their early youth [45, 46]. Selectin "knockout" mice have been genetically engineered and demonstrate abnormal leukocyte rolling, as well as an increased susceptibility to infection [47]. It has also been proposed that the selectins mediate tumor metastases via tumor cell expression of SLe^x [43].

The SLe^x epitope is expressed on inflammation-induced acute phase proteins which serve as endogenous competitors for selectins and mediate a dampening of the immune response, returning inflammation to homeostasis [48]. Inhibitors of the selectins have been proposed to be useful therapies for treating inflammatory disorders including respiratory distress [49], hypersensitivity responses [50], and surgically-induced myocardial ischemic reperfusion injury [51]. Animal studies have shown that the inhibition of selectin-carbohydrate interactions can alleviate these inflammatory responses.

E-, P-, and L-selectins each consist of an N-terminal C-type lectin carbohydrate recognition domain (CRD), an epidermal growth factor-like (EGF) subunit, a number of short consensus repeat (CR) units, a membrane spanning region, and a C-terminal cytoplasmic tail. There is approximately 72% homology for analogous selectin CRDs across species, and ~52% homology between different selectins within a species [38]. Notably, the selectins are unique among C-type lectins as they have only one calcium binding site [52]. While the 3-dimensional structures of E-selectin CRD and EGF domains without bound ligands have been elucidated by X-ray crystallography [52], there is not yet an unambiguous understanding of the binding interactions between selectins and their ligands. The interactions of a mutant MBP with its binding specificity subtly altered to mimic E-selectin (as described above) have been elucidated by X-ray crystallography as shown in Fig. 5 [53].

As expected, this structure shows that calcium complexes directly to Fuc hydroxy groups rather than serving a purely structural role. Gal C4 and C6 hydroxy groups are hydrogen bonded to lectin side chains, while the acetyl and backbone groups of GlcNAc participate in hydrophobic bonding. Interestingly, this structure fails to demonstrate interactions between the lectin and the C1 carboxylate of NeuAc [53]. Lys 213 [52, 54] (in MBP numbering), or alternatively Arg 97 [55] have been independently identified as likely electrostatic binding partners with the negatively charged carboxylate. The NMR structure of SLe^x bound to E-selectin suggests a binding pocket where Lys 111 is positioned to hydrogen bond to the C7 hydroxy group of NeuAc, and Lys 113 is an electrostatic binding partner for the C1 carboxylic acid of NeuAc [56]. E-Selectin mutants Lys113Ala (residue 213 in MBP numbering) and Arg97Ala demonstrate a complete loss of affinity for SLe^x [52, 54]. Conservative mutation of these residues (e.g., Lys113Arg [55] and Arg97Lys [52]) failed to restore SLe^x binding activity, while mutant Lys113Glu bound as well as the wild-type [55].

Although NeuAc (or an appropriately spaced negatively charged carboxylate isostere) is required for binding to the selectins [57], the model of an electro-

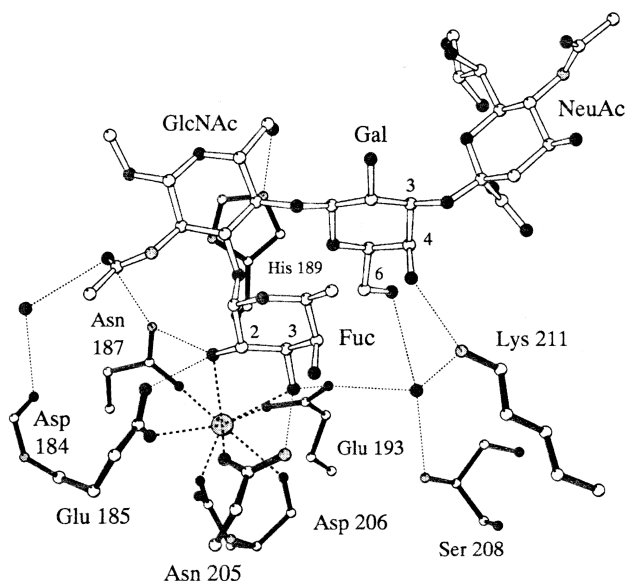


Fig. 5. X-ray crystal structure of the binding site of a mutant mannose binding protein with specificity for sialyl Lewis^x with bound ligand. See text for details. Reprinted with permission from Ng and Weis (1997) *Biochemistry* 36:979. Copyright 1997 American Chemical Society.

static interaction between the C1 carboxylate and binding site lysine(s) has not been confirmed. A short sequence of lysine and arginine residues shown to be conserved in six bacterial sialic acid binding lectins [*H. pylori* hpaA, *E. coli* fimbrial adhesion (SfaS), *E. coli* K99 fibrillar adhesion protein (K99), *E. coli* colonization factor antigen I (CFA/I), *V. cholerae* heat-labile toxin B subunit (CT-B), and heat-labile *E. coli* enterotoxin (LT-I-B)] was proposed to comprise a sialic acid binding consensus sequence, and the positive charge of these residues in the binding pocket prompted the theory that they were electrostatic binding partners for the C1 carboxylate of sialic acids [58]. The "basic consensus sequence" residues of the K99 and SfaS lectins were mutated to neutral residues, and resulted in a loss of binding [59,60]. It has been shown that selective chemical modification of lysine and arginine residues of CT-B blocks binding to sialic acids [61]. It was not until the X-ray crystal structures of the CT-B and LT-I-B bound to ligands ganglioside GM1 and lactose [62,63] were published that this theory was questioned. The conserved lysine and arginine residues are not part of the binding site, and their roles are structural rather than important for binding.

There is evidence that biologically relevant selectin receptors are multimeric. Gel permeation chromatography of P-selectin isolated from human platelets showed an apparent molecular weight of 2000 kDa which was shifted to an apparent molecular weight of 470 kDa (the apparent molecular weight of P-selectin by SDS-PAGE is 120 kDa) when eluted with detergent [64]. Analytical ultra-

centrifugation of P-selectin has indicated that it forms higher ordered species from dimers to hexamers [65]. Likewise, E-selectin demonstrates the formation of high molecular weight species by size-exclusion chromatography [66].

Another line of evidence for the multimeric organization of selectins comes from increased binding of multivalent ligands. Selectin-ligand interactions are noted to be weak (K_D =mM), consistent with the interactions of many lectins with monovalent ligands [67]. Bivalent SLe^x ligands show a five-fold increase over monovalent ligands in blocking neutrophil binding to immobilized E-selectin [68, 69]. Bovine serum albumin (BSA) bearing 7, 11, or 16 SLe^x moieties displayed a 20-, 900-, or 1000-fold increase in avidity, respectively, in an assay measuring leukocyte binding to immobilized E-selectin [70]. Monovalent, divalent, and tetravalent SLe^x moieties on linear and branched carbohydrate scaffolds inhibited L-selectin-dependent adhesion of lymphocytes with potencies increasing in order of valency to an IC_{50} value of 1 nM for tetravalent structures [71, 72]. Liposomes bearing SLe^x have shown enhanced inhibition of human umbilical cord vein endothelial cells (HUVECs) binding to immobilized E-selectin by seven orders of magnitude compared to the monovalent ligand [73]. One explanation for the increased potency of multivalent ligands is that a cluster of appropriately spaced ligands is in a unique position to interact with the multiple binding sites of a cluster of lectins (the "multivalency effect" as discussed below). Another recently offered explanation suggests that the apparent avidity of lectin-carbohydrate interactions depends upon whether the lectin or the carbohydrate is immobilized [74]. When a small carbohydrate ligand is immobilized and the lectin free, binding as measured by surface plasmon resonance is five orders of magnitude greater than if the same lectin is immobilized while the same ligand is free. It has been proposed that the "multivalency effect" of lectin-carbohydrate interactions often attributed to lectin oligomerization can sometimes be attributed to an artifact of a slower k_{off} for ligands which are either larger than monosaccharides or bear multiple ligands [74].

Although SLe^x , SLe^a , or their sulfated derivatives are widely considered to be "the ligands" for the selectins, appropriate sialylation/sulfation and fucosylation for biologically relevant binding to selectins could potentially occur on any number of N- or O-linked oligosaccharides, carbohydrate polymers, or glycolipids. A number of candidate selectin ligands has been identified [reviewed in 38, 75, 76]. The best characterized ligand for P-selectin is dimeric PSGL-1, presenting SLe^x -type determinants on the non-reducing end of O-linked oligosaccharides [77, 78, 79]. Three candidate ligands for L-selectin have been isolated. They are GlyCAM-1, CD34, and MAdCAM-1 presenting SLe^x on the non-reducing end of O-linked oligosaccharides [80, 81]. Ligands for E-selectin have been advanced as either glycolipids [82, 83] or the glycoprotein ESL-1, the latter presenting relevant carbohydrate determinants on N-linked oligosaccharides [84, 85, 86]. Dissociation constants for the binding of selectins to multivalent ligands and potential ligand-carriers are typically in the range of nM [65, 87, 88, 89], while binding to monovalent sialylated or sulfated Lewis antigens are in the range of mM [90].

4.2

Oligomerization of Galectins

The dimeric nature of galectins 1 and 2 was first demonstrated by size-exclusion chromatography and crosslinking experiments [166]. Their 3-dimensional structures have more recently been characterized as dimers of individual 14 kDa CRDs, demonstrating two-fold axes of symmetry [142, 160, 161]. The CRDs of a single dimer are oriented away from each other such that binding sites cannot each interact with antennae of a single biantennary oligosaccharide [142], but can serve as a cross-linker for α -galactosides. Non-covalent dimeric association is observed in galectins 1, 2, and 7 [167]. Galectins 4, 6, 8, and 9 contain two CRDs in a single compact protein, oriented apart as seen in galectins 1 and 2 [168, 169, 170].

It has not been clearly demonstrated that the biologically relevant form of galectin 3 is either dimeric or oligomeric. Residues corresponding to the N-terminal region of galectins 1 and 2 which have been implicated as important for dimerization are absent in galectin 3 [162]. However, it has been shown that full-length galectin 3 forms oligomers which can be trapped by chemical cross-linking, while galectin 3 expressed as only the C-terminal lectin domain is primarily monomeric [171]. Additionally, full-length galectin 3 demonstrates hemagglutination activity while the C-terminal fragment is devoid of this activity [171]. Galectin 3 also shows positive cooperativity in binding to laminin, giving rise to speculation that both subunits of this possibly dimeric lectin can simultaneously bind Gal on the antennae of a biantennary N-glycans [172]. It is unclear if galectin 5 is functionally a monomer or an oligomer. While it does not show the presence of higher ordered species by size exclusion chromatography, its ability to hemagglutinate erythrocytes suggests that its functional state is oligomeric [167].

Charcot-Leydon crystal protein (CLC) has been designated galectin 10 based on its 15–30% sequence identity and structural similarity to other galectins [164]. Additionally, seven of twelve residues conserved in galectin carbohydrate binding sites are present [164]. CLC autocrystallizes in a hexameric bipyramidal arrangement [173]. CLC weakly binds to GlcNAc or LacNAc and its lectin activity has only been shown by binding to these monosaccharides immobilized on affinity columns [168]. CLC demonstrates enzymatic activity as a lysolecithin acylhydrolase and is a major constituent of total protein in eosinophils and basophils [174].

The amino acids considered to be required for ligand binding by galectins are shown for the representative galectin 2 in complex with lactose in Fig. 11 [161]. The hydrogen bonding interactions are dominated by the C4 hydroxy group of Gal which binds to the side chains of His 45, Asn 47, and Arg 49, and the C3 hydroxy group of Glc which is in contact with Arg 49, Asn 58, Glu 68, and Arg 70. Also seen are hydrogen bonds from Asn 58 and Glu 68 to the C6 hydroxy group of Gal. The multiple hydrogen bonds to the C4 hydroxy and the C6 hydroxy group suggest that ligand discrimination is accomplished through recognition of the axial C4 hydroxy group.

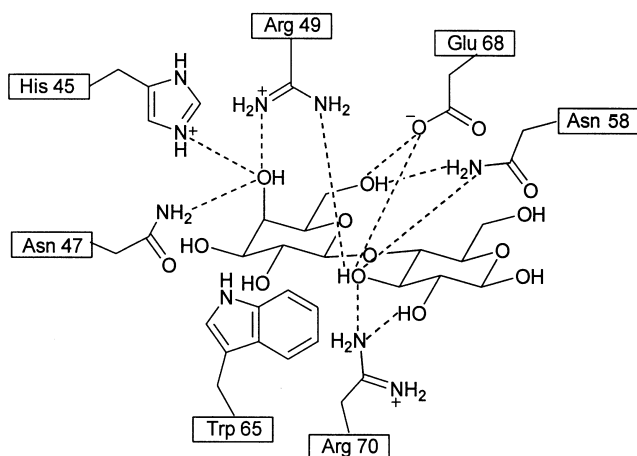


Fig. 11. X-ray crystal structure of lactose bound to galectin 2. *Dashed lines* indicate hydrogen bonds. Adapted with permission from Lobsanov et al. (1993) *J Biol Chem* 268:27034. Copyright 1993 American Society for Biochemistry and Molecular Biology

Hydrophobic interactions of the indole side chain of Trp 65 with the pyranose ring of Gal suggest a reason why the binding site cannot accommodate a monosaccharide with an equatorial C4 hydroxy group [161]. X-Ray crystal structures of galectins bound to lactose, LacNAc or biantennary N-acetyllactosamine terminating oligosaccharide show a conserved hydrogen bonding network consistent with that determined for galectin 2 [164] (Table 1).

While the terminal Gal residue is in intimate contact with the lectin binding pocket, the Glc/GlcNAc residue is oriented away from this region, presumably making a smaller contribution to binding specificity and affinity [160]. The lactose/LacNAc binding sites of galectins are located within a wide protein cleft, suggesting that the internal sugar residues of carbohydrate polymers, such as polylectosaminoglycans, can be accommodated by galectins [163]. No thiol residues participate in ligand binding and the cysteines can be either oxidized or reduced without affecting binding [160]. Additionally, the complex of galectin 1 with biantennary oligosaccharide shows the ability of galectins to crosslink molecules bearing more than one ligand in solution [142].

4.3

Biological Roles of Galectins

Galectins are conserved in a number of unrelated organisms such as marine sponges, nematodes, frogs, birds, and mammals, suggesting that α -galactoside binding lectins may be biologically important [159, 168, 170, 175]. The tissue-specific localization of several galectins is the strongest evidence for their functions. While it is clear from the examples given below that a number of galectins are involved in growth regulation, cell adhesion, cell migration, cancer, and im-

Table 1. Conservation of the hydrogen bonding pattern of galectins binding to Gal β (1-4)Glc/NAc

Sugar Atom	Bovine Galectin 1 [159]	Human Galectin 2 [160]	Human Galectin 3 [161]	Human Galectin 7 [162]	Human Galectin 10 [163]
Gal O4	His 44 2.8	His 45 2.7	His 158 2.7	His 49 2.7–29	His 53 2.9
Gal O4	Arg 48 3.0	Arg 49 3.3	Arg 162 3.1	Arg 53 2.6–3.2	–
Gal O4	Asn 46 3.4	Asn 47 3.1	Asn 160 3.2–3.3	Asn 51 3.2–33	Gln 55 2.7
Gal O5	Arg 48 2.9	–	Arg 162 3.0	Arg 53 2.8–3.1	–
Gal O5	–	–	–	Glu 72 3.0	–
Gal O6	Asn 61 2.7	Asn 58 2.7	Asn 174 2.9	Asn 62 2.4–2.9	Asn 65 3.4
Gal O6	Glu 71 2.8	Glu 68 2.6	Glu 184 3.1	Glu 72 2.5–3.1	Gln 75 2.9
Glc/GlcNAc O3	Arg 73 3.3	Arg 70 3.1	Arg 162 2.7–2.8	–	–
Glc/GlcNAc O3	Arg 48 2.8	Arg 49 2.6	–	Arg 53 3.1	Arg 61 2.9
Glc/GlcNAc O3	Glu 71 2.4	Glu 68 2.8	Glu 184 2.6	Glu 72 2.6	Gln 75 2.8
Glc/GlcNAc O3	Arg 48 3.2	–	Arg 162 3.0	–	–

Summary of the hydrogen bonding pattern of galectins bound to ligand(s). Beneath the identified binding amino acid, the interatomic hydrogen bond distance is given in Å. A range of interatomic distances indicates that the X-ray structure has been independently solved bound to different ligands. Interactions with Glc O2 have been omitted for the purpose of clarity. Adapted from [163] with permission

mune responses, it is not clear that any of these processes are mediated by galectins in non-redundant pathways. Genetically engineered "knockout" mice lacking galectins 1 and 3 show no phenotypic abnormalities, indicating that these galectins under normal conditions are not necessary for survival [176]. However, in thioglycolate-induced peritonitis, galectin 3 null mice demonstrate reduced recruitment of leukocytes to sites of inflammation [177].

Galectin 1 is proposed to mediate cell adhesion, regulate cellular proliferation, and mediate cellular apoptosis [175]. Galectin 3 has been implicated in cell adhesion, regulation of inflammation, pre-mRNA splicing, as well as protecting against induced apoptosis [175]. Cell-cell and/or cell-extracellular matrix cross-

linking functions have been proposed for galectins 4 and 6 [168]. The selective expression of galectin 5 in erythrocytes suggests roles in maturation and/or cell adhesion of erythrocytes [168]. Likewise, galectin 7 is a constitutive marker for all keratinocyte subtypes but its specific function is unclear at this time [178]. Galectin 9 may mediate clonal deletion of thymocytes, as evidenced by its role in lactose-dependent apoptosis of these cells *in vitro* [179]. An isoform of galectin 9 shows chemoattractant activity which is selective for eosinophils [180].

Several galectins have altered expression in tumor cells relative to normal cells. The high expression of galectin 1 correlates with thyroid tumors whereas high and low expression of galectin 3 has been proposed as a marker for a number of other malignancies [175]. The absence of galectin 7 in squamous carcinoma cell lines may serve a diagnostic role [178]. Likewise, the overexpression of galectin 8 may serve as a marker for prostate cancer [170]. The expression of galectin 9 is increased in Hodgkin's lymphoma (HL) patients where 50% of clinical populations possess antibodies against galectin 9 [168].

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