Current Topics

Effects of Clustered Epitopes in Multivalent Ligand−Receptor Interactions[†]

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ABSTRACT: Many biological ligands are composed of clustered binding epitopes. However, the effects of clustered epitopes on the affinity of ligand−receptor interactions in many cases are not well understood. Clustered carbohydrate epitopes are present in naturally occurring multivalent carbohydrates and glycoproteins, which are receptors on the surface of cells. Recent studies have provided evidence that the enhanced affinities of lectins, which are carbohydrate binding proteins, for multivalent carbohydrates and glycoproteins are due to internal diffusion of lectin molecules from epitope to epitope in these multivalent ligands before dissociation. Indeed, binding of lectins to mucins, which are large linear glycoproteins, appears to be similar to the internal diffusion mechanism(s) of protein ligands binding to DNA, which have been termed the "bind and slide" or "bind and hop" mechanisms. The observed increasing negative cooperativity and gradient of decreasing microaffinity constants of a lectin binding to multivalent carbohydrates and glycoproteins result in an initial fraction of lectin molecules that bind with very high affinity and dynamic motion. These findings have important implications for the mechanisms of binding of lectins to mucins, and for other ligand−biopolymer interactions and clustered ligand−receptor systems in general.

Many biological processes involve binding interactions between molecules with clustered and/or repeated epitopes (*1–3*). Examples of biological molecules with clustered and repeated epitopes include biopolymers such as polysaccharides, proteoglycans, filamentous proteins, and DNA. Multidomain proteins such as C-type lectins that possess multiple epidermal growth factor-like domains and complement regulatory domains possess clustered and repeated epitopes (*4*). Proteins that undergo post-translational modification, including the addition of multiple phosphate groups (cf. ref *5*) and asparagine-linked (N-)[1] and Ser/Thr-linked (O-) carbohydrates (*6*), also possess clustered and repeated epitopes. Thus, understanding the effects of epitope clustering on the affinity of ligand−receptor interactions is required to understand the structure−activity properties of these molecules in biological systems.

The effects of epitope and receptor clustering on the affinity of ligand−receptor interactions have been of interest for some time. For example, binding of different classes of antibodies that possess different valences (IgG, IgM, etc.) to clustered antigenic determinants has been considered from a theoretical point of view (*7, 8*). Theoretical calculations predict enhanced affinities for antibodies bound to antigens with clustered epitopes due to slow dissociation rates. The effects on the forward rate constants of ligands binding to multiple receptors on a cell (receptor density) have been calculated using diffusive intake models (*9, 10*). Theoretical calculations for both the association and dissociation constants of ligands from density-dependent receptors have also been considered, with several order of magnitude differences predicted relative to dispersed soluble receptors (*11*). Differences in the dissociation rates for clustered receptors were attributed to recapture of the ligand before complete dissociation of the complex, a conclusion supported by theoretical calculations on chemoreception (*9*).

Experimental confirmation of the recapture effect of ligands with clustered or repeated epitopes binding to receptors has been largely limited to the binding of protein ligands to DNA (cf. ref *12*). Evidence indicates that many DNA regulatory proteins bind at nonspecific base pair sites and then through Brownian motion diffuse along the backbone until they reach a high-affinity site (operator) (cf. ref *13*). The recapture of diffusing protein ligands by weak affinity base pair sites on DNA has generally been termed the "bind and slide" or "bind and hop" mechanisms. Such mechanisms are important in terms of the kinetics and affinity

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[1] Abbreviations: N-, asparagine-linked; O-, serine/threonine-linked; ASF, asialofetuin; ITC, isothermal titration calorimetry; ConA, concanavalin A; DGL, *Dioclea grandiflora* lectin; SBA, soybean agglutinin; VML, *Vatairea macrocarpa* lectin; PSM, porcine submaxillary mucin; Tn-PSM, porcine submaxillary mucin containing only α-GalNAc residues; CRD, carbohydrate recognition domain; LacNAc, *N*-acetyl-D-lactosamine.

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of formation of specific DNA−ligand complexes that include vicinal DNA (*12*).

Experimental evidence for the internal diffusion mechanism in biological systems other than nucleic acid polymers has generally been lacking. However, recent thermodynamic studies of the binding of lectins, which are multivalent carbohydrate binding proteins, to synthetic multivalent carbohydrates and globular and linear glycoproteins have suggested that the bind and slide mechanism occurs in these interactions, and that this mechanism is more general than previously considered.

Multivalent Carbohydrate−Lectin Interactions. Clustered carbohydrate epitopes are present in many naturally occurring carbohydrates and glycoproteins (cf. ref *6*). Indeed, the majority of cell surface receptors are glycoproteins that possess clustered carbohydrate epitopes, with many of the receptors involved in cell signaling mechanisms, including apoptosis (*14*), cell growth (*15*), and transport activities (*16*). The interactions of endogenous lectins with glycoprotein receptors are associated with many of these signaling events. Studies have shown that binding of lectins to glycoprotein receptors that possess multiple copies of the same carbohydrate epitope often leads to cross-linking, clustering, and activation or stabilization of the receptors (*14–17*). While the mechanisms of formation of noncovalent cross-linked lattices of lectins with multivalent carbohydrates and glycoproteins are relatively well understood (*18, 19*), the mechanisms associated with the enhanced affinities of lectins binding to multivalent carbohydrates and glycoproteins have been less well understood in certain cases (*20, 21*).

It is known that the affinity enhancements of lectins binding to multivalent carbohydrates and glycoproteins depend on the type of interactions that occur between the molecules (*22, 23*). These interactions include face-to-face binding of a lectin that possesses multiple subsites or multiple subunits to a carbohydrate or glycoprotein with clustered epitopes as shown in Figure 1A. The affinity enhancements in this case are large and relatively well understood. For example, binding of the asialoglycoprotein receptor to a trivalent carbohydrate with terminal LacNAc residues results in an $\sim 10^6$-fold increase in affinity relative to that of LacNAc (*24*). In this case, the affinity of the receptor for the trivalent carbohydrate is the sum of the free energies of binding of the three subsites of the receptor as shown in Figure 1A. Studies by Bundle and co-workers including binding of the Shiga toxin, which is a carbohydrate binding protein, to multivalent glycoconjugates also fit the face-to-face model with similar large increases in affinities (*25*).

Alternatively, the interactions can be due to binding of different lectin molecules to the epitopes of a multivalent carbohydrate or glycoprotein (Figure 1B). The affinity enhancements in this case are smaller than those in Figure 1A (*26, 27*) and have not been well understood until recently. For example, the affinity of two Man-specific lectins, concanavalin A (ConA) and *Dioclea grandiflora* lectin (DGL), for a synthetic tetraantennary clustered glycoside are ~35- and 50-fold greater, respectively, than that of the corresponding monovalent carbohydrate (*28*). However, the affinities of two GalNAc-specific lectins, the soybean agglutinin (SBA) and *Vatairea macrocarpa* lectin (VML), for a linear chain glycoprotein (mucin) that possesses ~2300 GalNAc residues have recently been reported to be $\sim 10^6$-fold greater than that of the corresponding monovalent carbohydrate (*29*) (Figure 1C). The enhancement in affinity of the lectins for the mucin analogue is nearly the same as that for face-to-face interactions between a lectin and a trivalent carbohydrate described in Figure 1A (*24*).

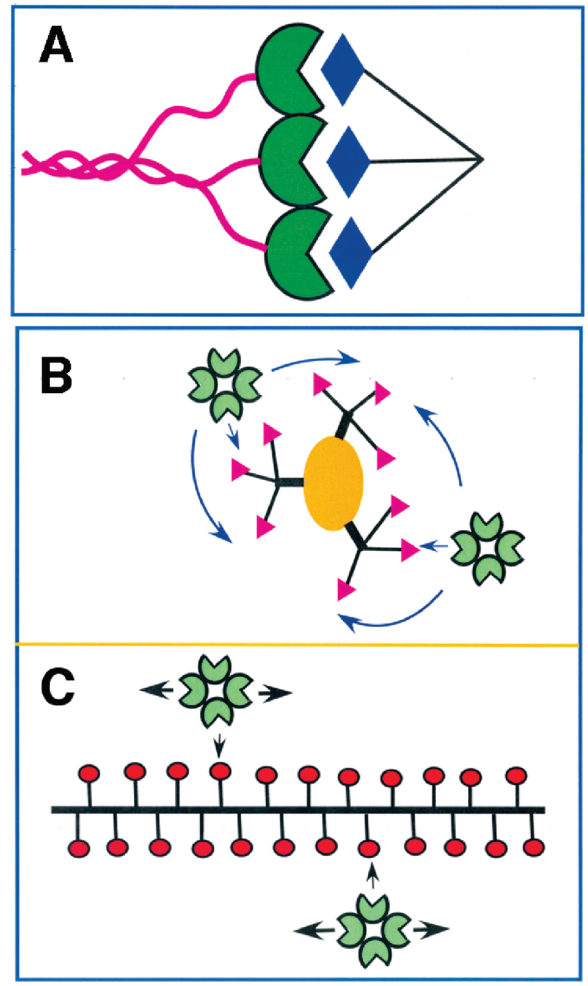

FIGURE 1: Schematic representations of (A) binding of a lectin with three subsites (green) to a trivalent carbohydrate (blue), (B) binding of a nonavalent glycoprotein (orange, black, and pink) to two lectin molecules (green), and (C) binding of a linear glycoprotein (black and red) to two lectin molecules (green).

This work will review thermodynamic studies of the binding of lectins to small clustered glycosides (*28, 30*), a multivalent globular glycoprotein (*31*), and mucins, which possess different numbers of carbohydrate epitopes, epitope densities, and polypeptide chain lengths using ITC (*29*). The results indicate that lectins bind to these multivalent carbohydrates and glycoproteins by a common mechanism that involves "binding and sliding" from carbohydrate to carbohydrate epitope in the same molecule that results in a gradient of decreasing microaffinity constants. In the case of lectins binding to mucins, the bind and slide mechanism is similar to protein ligands binding to DNA, another biopolymer with clustered epitopes (*12, 32*). The results are important for understanding the dynamics of binding of lectins to multivalent globular and linear glycoprotein receptors on the surface of cells and have important implications for the effects of clustered epitopes on ligand−receptor interactions in other biological systems.

Binding of Lectins to Synthetic Cluster Glycosides. Enhanced affinities and gradient binding mechanisms have been

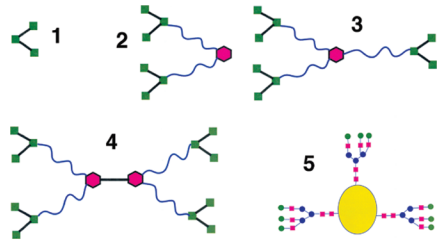

FIGURE 2: Schematic representations of trisaccharide **1** [methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside], cluster glycosides **2**, **3**, and **4** (*28*), and asialofetuin (ASF) (**5**).

reported in hemagglutination inhibition and ITC studies of the binding ConA and DGL to synthetic clustered glycosides **2**−**4** in Figure 2, which are di-, tri-, and tetravalent structural analogues of trisaccharide **1** [methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside), respectively (*28, 30*). Trisaccharide **1** is a monovalent ligand for both lectins and is present in the core region of N-linked carbohydrates. ConA binds to **2**, **3**, and **4** with 6-, 11-, and 35-fold greater K_a values, respectively, than **1**, and DGL binds to **2**, **3**, and **4** with 5-, 8-, and 53-fold greater K_a values, respectively (*28*). Thus, both lectins show small increases in affinity for the synthetic cluster glycosides with an increase in structural valence.

Importantly, the ITC-derived *n* value, the number of binding sites per monomer of the lectin, was shown to be inversely proportional to the valence of the carbohydrate (*28*). This result is important for analyzing the thermodynamic binding parameters for multivalent carbohydrates. For example, the *n* values for ConA binding to the synthetic cluster glycosides indicated that **2** and **4** possessed bivalent and tetravalent binding activities, while **3**, which is structurally trivalent, binds the lectin as a divalent glycoside. Analogue **3**, on the other hand, shows a mixture of bivalent and trivalent activities for DGL.

Evidence that ConA and DGL bind to synthetic cluster glycosides **2**−**4** by an internal diffusion mechanism is derived from Hill plots of the raw ITC data (*30*). Hill plots show increasing negative cooperativity for the binding of both lectins to all three glycosides (data for ConA binding to **2** and **4** are shown in Figure 3A). Progressive three-point tangent slopes for **4** are shown in Figure 3B, with a bar graph of the data shown in Figure 3C. Importantly, Hill plots of the binding of both lectins to monovalent **1** show straight lines with slopes close to 1.0 (Figure 3A). Thus, the negative cooperativity observed for the lectins binding **2**, **3**, and **4** is due to the cluster glycosides and not the lectins.

An explanation for the increasing negative cooperativity is shown in Figure 4 for ConA binding to **4** (*30*). Binding is described as a series of microequilibrium constants involving sequential lectin binding to the four epitopes of **4**. The microaffinity constant of the first lectin that binds to the first unbound epitope of **4** is greater than that of the second lectin binding to the second epitope, which is greater than that of the third lectin binding to the third epitope, which is greater than that of the fourth lectin binding to the fourth epitope. Thus, to a first approximation, the microaffinity constants are determined by the number of unbound carbohydrate epitopes in the complex that are involved in the binding and sliding (recapture) mechanism. The binding and sliding mechanism for each complex leads to an increase in its

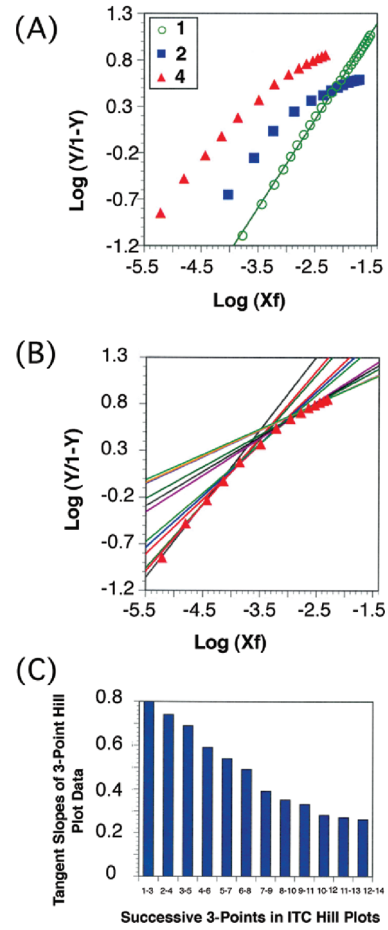

FIGURE 3: (A) Hill plots of the ITC data for ConA binding to glycosides **1**, **2**, and **4** (*32*). Hill plots are $\log\{Y(i)/[1 - Y(i)]\}$ vs $\log[X_f(i)]$. where $Y(i)$ is $[X_b(i)] \times$ (functional valency of ligand)/$M_t(i)$, which is a modified version of the Hill plot (*32*) that takes into account the functional valence of the ligand. $X_b(i)$ and $X_f(i)$ are the fraction of ligand bound and free, respectively, after the *i*th injection. The functional valences of **1**, **2**, and **4** were determined by ITC to be 1, 2, and 4, respectively (*28*). (B) Tangent slopes of progressive three-point intervals of the Hill plot of ConA binding to analogue **4**. (C) Bar graphs of the three-point tangent slopes of the Hill plots of ConA binding to analogue **4**.

residence time, and hence, the off rate is reduced and the affinity increased. Figure 4 thus provides a mechanism for the observed negative binding cooperativity of the lectins to the cluster glycosides in Figure 3.

Importantly, the range of microaffinity constants for lectin molecules binding to the first unbound epitope versus the last unbound epitope of **4** was calculated to be ∼1000-fold for ConA and ∼2500-fold for DGL (*30*). Thus, there is a large difference in the affinity of lectin molecules binding to the first epitope as compared to the last epitope of **4**. This is important since it indicates large gradients of decreasing microaffinity constants associated with lectins binding to multivalent carbohydrates by the bind and slide mechanism.

Direct experimental demonstration of different microscopic affinity constants for ConA binding to the carbohydrate epitopes in analogues **2** and **3** was performed by "reverse" ITC in which the lectin is titrated into a solution of the carbohydrate (*33*). The results provided the complete thermodynamics for binding of ConA to the two epitopes of **2**, and the two functional epitopes of **3**, which exhibits bivalent binding activity with respect to ConA (*28*). The data indicate

an 18-fold difference in the microaffinity constants of the two epitopes of **2** and a 53-fold difference in the microaffinity constants of the two functional epitopes of **3**. The difference in microaffinity constants of the two binding epitopes of **2** and **3** was shown to be due to differences in their entropy of binding in both cases (*33*).

Binding of Galectins to the Multivalent Glycoprotein Asialofetuin. Enhanced affinities and gradient binding mechanisms due to increasing negative cooperativity were also observed in the ITC binding of galectins-1, -2, -3, -4, -5, and -7, and truncated, monomeric versions of galectins-3 and -5, which are members of a family of β-Gal-specific animal lectins, to asialofetuin (ASF) (**5** in Figure 2), a naturally occurring 48 kDa glycoprotein that possesses nine LacNAc epitopes (*31*). Galectins-1, -2, -5, and -7 are prototype galectins that exist as a monomeric or homodimeric carbohydrate recognition domain (CRD); galectin-3 is a chimera-type galectin that contains a non-lectin N-terminal short sequence segment followed by 8−12 collagen-like repeats of nine amino acids connected to the C-terminal CRD, and galectin-4 is a tandem repeat type galectin composed of two distinct CRDs in a single polypeptide chain connected by a linker peptide (*6*). The observed K_a values for ASF binding to the galectins and the two truncated forms are 50−80-fold greater than that of LacNAc, a monovalent ligand.

Hill plots of the ITC data for all of the galectins, including truncated galectin-3 and -5 (*31*), showed increasing negative cooperativities that were similar to those observed for ConA and DGL binding to cluster glycosides **2**, **3**, and **4** (*30*). Hill plots of the binding of the galectins to monovalent LacNAc are straight lines with slopes close to 1.0, indicating that the negative cooperativity is due to ASF and not the galectins. Importantly, the ITC-derived *n* value showed that all nine LacNAc epitopes of ASF were involved in binding the galectins. Hence, the increasing negative cooperativity observed in the Hill plots of the binding of the galectins to ASF was assigned to sequential binding of galectin molecules to the first, second, and eventually ninth LacNAc epitope of ASF, with a gradient of decreasing microaffinity constants associated with this sequence of binding. The enhanced affinity of the first galectin binding to the first epitope of ASF was ascribed to a binding and sliding (recapture) mechanism (Figure 1B), which is similar to that observed for ConA and DGL binding to glycosides **2**−**4** (*30*).

Importantly, the observation that truncated monovalent galectins-3 and -5 exhibited increasing negative cooperativity similar to that of the intact galectins (*31*) reduced the possibility that lectin−carbohydrate cross-linking interactions are responsible for the observed negative cooperativity as well as the results for ConA and DGL binding to cluster glycosides **2**−**4**.

Calculations indicated that the galectins bound to the first epitope of ASF with microaffinity constants 3000−6000-fold higher than that for binding to the last epitope of ASF (*30*). These gradients of decreasing microaffinity constants for the galectins binding to ASF are slightly larger than those calculated for ConA and DGL binding to glycoside **4** (*30*). These findings suggest similar bind and slide mechanisms for galectins binding to the nine LacNAc epitopes of ASF as observed for ConA and DGL binding to **2**−**4**.

Binding of Lectins to Mucins. Large increases in affinity and gradient binding mechanisms have recently been reported

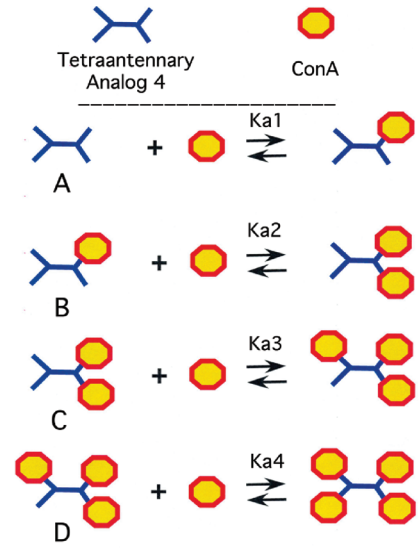

FIGURE 4: Schematic representation of ConA binding to glycoside **4**. Four microequilibrium constants of the tetravalent glycoside **4** are represented by K_{a1}, K_{a2}, K_{a3}, and K_{a4} for binding of ConA to the first epitope of **4** (species A), to the second epitope of **4** (species B), to the third epitope of **4** (species C), and to the fourth epitope of **4** (species D) to give the fully bound complex.

for the binding of GalNAc-specific lectins to modified forms of porcine submaxillary mucin, a large linear glycoprotein (*29*). ITC and hemagglutination inhibition measurements demonstrate that a chemically and enzymatically prepared form of porcine submaxillary mucin that possesses a molecular mass of $\sim 10^6$ Da and ~2300 α-GalNAc residues (Tn-PSM) (Figure 5B) binds to the soybean agglutinin (SBA) with a K_d of 0.2 nM, which is an affinity enhanced $\sim 10^6$-fold relative to that of monovalent GalNAcα1-*O*-Ser, the Tn pancarcinoma carbohydrate antigen (*34*). The enzymatically derived 81-amino acid tandem repeat domain of Tn-PSM containing ~23 α-GalNAc residues (81-mer Tn-PSM) (Figure 5C) binds with $\sim 10^3$-fold enhanced affinity, while the enzymatically derived 38/40-amino acid cleavage product(s) of Tn-PSM containing ~11−12 α-GalNAc residues (38/40-mer Tn-PSM) (Figure 5D) shows $\sim 10^2$-fold enhanced affinity. A natural carbohydrate-decorated form of PSM (Fd-PSM) containing 40% of the core 1 blood group type A tetrasaccharide, and 58% peptide-linked GalNAcα1-*O*-Ser/Thr residues, with 45% of the peptide-linked α-GalNAc residues linked α(2,6) to *N*-glycolylneuraminic acid (Figure 5A), shows an $\sim 10^4$-fold enhanced affinity for SBA. *Vatairea macrocarpa* lectin (VML), which is also a GalNAc binding lectin, displays a pattern of binding similar to that of the PSM analogues, although there are differences in its affinities compared to those of SBA (*29*). The higher affinities of SBA and VML for Tn-PSM relative to Fd-PSM indicate the importance of carbohydrate composition and epitope density of the mucins on their affinities for the lectins. The higher affinities of both lectins for Tn-PSM relative to its two shorter chain analogues demonstrate that the length of a mucin polypeptide and hence total carbohydrate valence determine the affinities of the lectins for the three Tn-PSM analogues. Kiessling and co-workers (*35*) have reported a similar increase in the inhibitory activity of synthetic polymers of increasing lengths that possess Man residues in a hemagglutination assay with ConA. The authors concluded that the enhanced inhibitory activities of the longer chain polymers

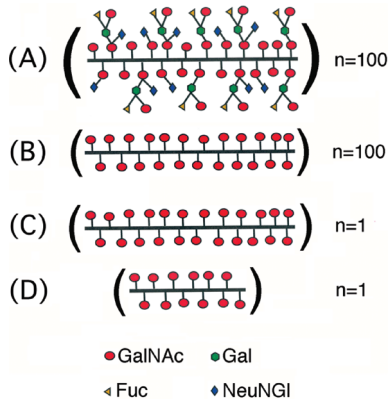

FIGURE 5: Structural representations of (A) the fully carbohydrate-decorated form of the 100-repeat 81-residue polypeptide O-glycosylation domain of PSM (Fd-PSM), (B) the 100-repeat 81-residue polypeptide O-glycosylation domain of PSM containing only peptide-linked α-GalNAc residues (Tn-PSM), (C) the single 81-residue polypeptide O-glycosylation domain of PSM containing peptide-linked α-GalNAc residues (81-mer Tn-PSM), and (D) the 38/40-residue polypeptide(s) derived from the 81-residue polypeptide O-glycosylation domain of PSM containing peptide-linked α-GalNAc residues (38/40-mer Tn-PSM). The number of glycan chains in Fd-PSM and Tn-PSM is ∼2300. The number of α-GalNAc residues in 81-mer Tn-PSM is ∼23, while the number of α-GalNAc residues in 38/40-mer Tn-PSM is ∼11−12. The number of polypeptide repeats is defined as *n* for each mucin.

were "largely due to a combination of statistical and chelation effects" and therefore slower dissociation rates.

The results of the ITC mucin study also included the complete thermodynamics of binding of SBA and VML to the PSM analogues, including their stoichiometries of binding (*29*). Only a fraction of the total α-GalNAc residues on each mucin analogue are bound, presumably due to steric effects associated with the bound lectin molecules. Importantly, the heat of binding (enthalpy) of each GalNAc epitope of the mucin analogues is the same as that of monovalent GalNAcα1-*O*-Ser. Thus, the entropy of binding is responsible for the increased affinity of the lectins for the mucins relative to GalNAcα1-*O*-Ser/Thr.

The dependence of the affinity of the lectins on the length of the Tn-mucin chains together with the thermodynamic data suggests that SBA and VML bind and slide from α-GalNAc residue to α-GalNAc residue along the polypeptide chain of the mucin before dissociating. Figure 6A shows this mechanism for SBA binding to Tn-PSM. This mechanism of binding is similar to that observed for the binding of ConA and DGL to cluster glycosides **2**−**4** (*30*) as well as galectins-1, -2, -3, -4, -5, and -7, and truncated, monomeric versions of galectins-3 and -5, to ASF (*31*). Hence, the internal diffusion jumps of the lectins on the mucin chains increase the lifetime of the complex and, hence, the binding affinity. This mechanism explains the dependence of the observed affinities of the lectins on the lengths of the Tn-PSM analogues and is consistent with the enhanced entropy of binding of the lectins to the mucins.

Curved Hill plots of the ITC data for SBA and VML binding to Tn-PSM were reported but not shown by the authors (*29*). Inspection of the model for SBA binding to Tn-PSM in panels A and B of Figure 6 shows that the path length for internal diffusion of the lectin along the mucin chain decreases with an increased total number of bound lectin molecules. This suggests that the affinity of SBA (and

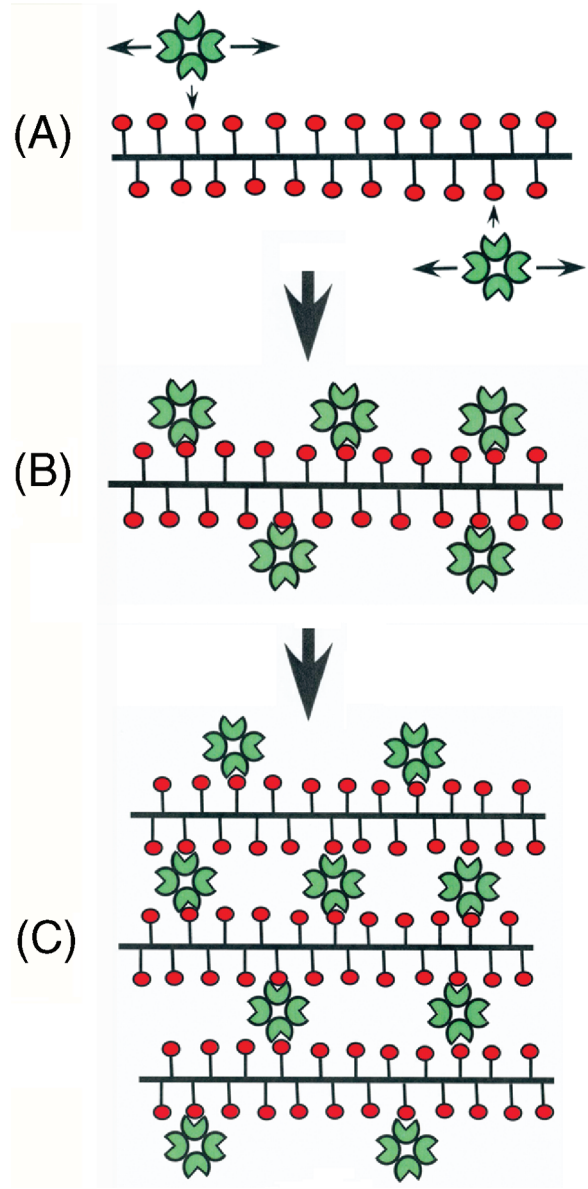

FIGURE 6: Schematic representations of (A) SBA binding at low fractional occupancy to Tn-PSM, (B) SBA binding at higher fractional occupancy to Tn-PSM, and (C) SBA binding at saturation that involves cross-linking of Tn-PSM.

VML) for Tn-PSM decreases with increased fractional binding of the lectin to the mucin chain (Figure 6B). Eventually, saturation binding and cross-linking of the complex occur as shown in Figure 6C. The negative binding cooperativity predicted by Figure 6 is in agreement with the dependence of the observed macroscopic affinity contants of SBA and VML on the lengths of the polypeptide chains of Tn-PSM, 81-mer Tn-PSM, and 38/40-mer Tn-PSM. Hence, the bind and slide mechanisms observed for ConA and DGL binding to cluster glycosides **2**−**4**, galectins binding to ASF, and SBA and VML binding to Tn-PSM analogues of different chain lengths are associated with increasing negative cooperativity and a gradient of decreasing affinity constants. This suggests that the first SBA and VML molecules that bind to Tn-PSM possess dissociation constants several orders of magnitude lower than the observed K_d values of 0.2 and 0.1 nM, respectively, and with dynamic motion along the complete mucin chain.

Conclusions. The bind and slide model for lectins binding to multivalent glycosides, globular, and linear glycoproteins is distinct from the classical "lock and key" model for ligand−receptor interactions. The bind and slide (internal diffusion) model allows a small fraction of bound lectin molecules to dynamically move from carbohydrate to carbohydrate epitope in globular and linear glycoproteins. This, in turn, can facilitate lectin-mediated cross-linking of such glycoproteins on the surface of cells (*17*). In this regard, as few as three galectin-1 molecules bound to ASF has been observed to lead to cross-linking of the complex (*36*). For transmembrane mucins such as CD-43, fractional high-affinity binding of lectins such as galectin-1 can lead to homogeneous cross-linked receptors (*37*) or, in the case of galectin-3, to heterogeneous cross-linked receptors (*38*). Such cross-linked receptors, in turn, trigger signal transduction mechanisms such as apoptosis in susceptible T cells as observed for galectin-1 (*39*) and galectin-3 (*40*). The fractional high affinities of multivalent globular and linear glycoproteins associated with their gradient binding mechanisms also facilitate the uptake and storage of low concentrations of lectin molecules released from the surface of cells. The presence of affinity gradients prevents overoccupancy of the receptor glycoproteins by lectin molecules that would inhibit cross-linking interactions.

Importantly, the internal diffusion model of a lectin binding and sliding from carbohydrate epitope to epitope along a mucin chain is similar to that for a variety of protein ligands binding to DNA (cf. ref *12*). Single-molecule tracking experiments show that a base repair binding protein binds to DNA lacking a high-affinity operator site by one-dimensional diffusion and oscillation along the full length of the DNA molecule (*13*). Indeed, these results suggest that binding and sliding is a general mechanism of binding of ligands to biopolymers, including glycosaminoglycans, polysaccharides, filamentous proteins, and nucleic acid polymers. Interestingly, the formation of lectin cross-linked complexes with mucin-type receptors such as CD43 (*39*), the formation of growth factor−receptor complexes on heparan sulfate chains of proteoglycans (*41*), and the formation of protein initiation and termination complexes on DNA (*12*) all involve binding of ligands to biopolymers that mediate the formation of functionally active complexes. In the case of proteoglycans, the multiple attached glycosaminoglycan chains can function as dynamic capacitors in storing growth factor ligands for release to growth factor receptors during morphogenesis (*41*). Binding of ligands to filamentous proteins such as the antitumor drug Taxol to microtubules may well involve the bind and slide mechanism, which would help to explain stabilization of the polymeric form of the protein by Taxol (*42*). Interestingly, the mechanisms of translocation of molecular motor transport proteins such as myosin V on actin filaments are related to the bind and slide mechanism but with an energy-dependent bias in the direction of Brownian diffusion by presumed conformational transitions in the molecule (*43*). Thus, these studies suggest that biopolymers provide fractional high-affinity binding and rapid one-dimensional diffusion of ligands (and receptors) in the non-energy-dependent examples given above.

Lastly, these results suggest a paradigm shift in defining the specificity of ligand−receptor interactions. The large increase in affinity of $\sim 10^6$-fold (or more in terms of microaffinity constants) for SBA and VML binding to Tn-PSM indicates the importance of epitope clustering in a molecule. This presumably holds true for two- and three-dimensional clustering of receptors, since diffusion equations predict such effects (*44, 45*). Indeed, a large number of transmembrane receptors are found clustered, including those in supramolecular assemblies such as the immunological synaspse (*46*), neuronal synaspse (*47*), and glycosynapse (*48*). Many of these clustered receptors, in turn, are found in lipid rafts, which form microdomains on the surface of cells (*49*). Clustered receptors also often undergo reversible regulation such as the acetylcholine receptor in neuromuscular gap junctions (*50*). Thus, the affinity and hence specificity of ligand−receptor interactions may be regulated by epitope and receptor clustering in many biological systems.

NOTE ADDED AFTER ASAP PUBLICATION

An incorrect version of this paper was published ASAP on July 25, 2008. The corrected version includes a new paragraph on page 8472 that describes the data presented in Figure 3. From that point forward in the corrected version, each mention of Figures 3-5 has been altered. The corrected version was published August 12, 2008.

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