The Role of Valence on the High-Affinity Binding of *Griffonia simplicifolia* Isolectins to Type A Human Erythrocytes†

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ABSTRACT: The *Griffonia simplicifolia-I* (GS-I) isolectins have been used to probe the effect of lectin valence on their high-affinity binding to human erythrocytes. These tetrameric lectins are composed of A and B subunits and constitute a series of five isolectins (A_4, A_3B, A_2B_2, AB_3, B_4). The A subunit is specific for α-D-GalNAc end groups and binds to the blood type A determinant GalNAcα1, as well as to terminal α-D-Gal groups found on type B cells. The B subunit is specific for α-D-Gal end groups, and binds very specifically to type B erythrocytes. This series of isolectins is tetravalent (A_4), trivalent (A_3B), divalent (A_2B_2), and monovalent (AB_3) for type A erythrocytes; thus, this system provides the opportunity to examine the effect of lectin valency on the association constants of these GS-I isolectins binding to cells. Cell binding experiments carried out using ^{125}I-labeled GS-I isolectins and type A human erythrocytes allowed us to demonstrate that (1) the association constant of the isolectin monovalent for α-D-GalNAc (AB_3) is virtually identical to its association constant for the haptenic sugar methyl-*N*-acetyl-α-D-galactosaminide, reported previously, and (2) the association constant of the GS-I isolectins for human type A erythrocytes increases with increasing valency of the isolectin. These results indicate that the increased affinity displayed by the GS-I isolectins for human type A erythrocytes is dependent on their multivalency, and not on an extended binding site nor on nonspecific, or noncarbohydrate, interactions of the lectin with the cell surface. These findings should be of general relevance to understanding the high-affinity interactions observed between other multivalent proteins and multivalent ligands (e.g., cell surfaces).

The seeds from *Griffonia* (*Bandeiraea*) *simplicifolia* (GS) contain three distinct families of lectins. The series of lectins designated GS-I consists of five isolectins, each of which is a tetramer composed of two distinct subunits: A and B, which are noncovalently associated (*1*). Although the A (M_r = 32K) and B subunits (M_r = 33K) have very similar amino acid compositions and size, they differ in that the B subunit has one methionine residue not found in the A subunit (*1*, *2*). In addition, N-terminal sequencing has shown that 21 of 25 N-terminal residues are identical. Tryptic peptide mapping of the subunits demonstrated that approximately 30% of the peptides were common to both subunits (*2*). Despite their structural similarities, these subunits differ in affinities for haptenic sugars. The A subunit has been shown to interact with high affinity with the blood group type A determinant GalNAcα1 as well as with α-D-galactosyl end groups. The B subunit is highly specific for the blood group type B determinant α-D-galactosyl end groups that occur in the human blood group B substance. The B subunit does not interact with terminal GalNAcα1 (*2*, *3*).

The association constants of the A and B subunits for their corresponding haptenic sugars have been determined by equilibrium dialysis as 1.87×10^5 and 2.06×10^4 M^{-1}, respectively (*3*). The apparent association constant determined for the cell surface lectin receptors was reported to be $(1-3) \times 10^7$ M^{-1} for the mixture of GS-I isolectins (*4*).

Several explanations have been advanced to account for the often dramatic increase in affinity that lectins display for cells over the simple sugar haptens to which they bind. These include the following: (1) nonspecific interactions with components of the cell surface or interactions unrelated to the carbohydrate binding interactions of the lectin; (2) an extended carbohydrate binding site of the lectin that has not been fully determined; while not fully elucidated, these oligosaccharide structures may exist on the cell and allow the lectins to bind with high affinity; (3) the multivalent nature of lectins and of the cells to which they bind produces a cooperative effect resulting in an increase in the apparent affinity of the lectin for the cell due to the multiple attachment of the lectin to the cell.

It has been shown previously that the multivalency of immunoglobulins increases the observed affinity for a hapten 10^3-10^6-fold over the intrinsic affinity for the hapten (*5*). In this paper we report the use of the GS-I isolectins to provide lectins which are mono (AB_3), di (A_2B_2), tri (A_3B), and tetra (A_4) valent for human type A erythrocytes. Using these lectins, we have been able to demonstrate the dramatic role that multivalency plays in high-affinity lectin−cell interactions.

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MATERIALS AND METHODS

Purification and Iodination of Lectins. *Griffonia simplicifolia* I isolectins were purified as previously described (*6*, *7*). Purity was determined by the presence of a single protein band on nondenaturing PAGE[1] at pH 8.9 as previously reported by Murphy and Goldstein (*8*). Each purified isolectin was radiolabeled with ^{125}I using the method of Fraker and Speck (*9*) and Tolan et al. (*10*), in the presence of protective haptenic sugar (50 mM). Following iodination, the active isolectins were purified from inactive protein and excess isotope by affinity chromatography using melibionate-Sepharose-4B. Bound lectin was eluted with 50 mM methyl α-D-galactopyranoside in PBS, and dialyzed against PBS. Specific activity was calculated from the protein concentration by the absorbance at 280 nm, and radioactivity was determined by gamma counting (1191 GammaTrac, TmAnalytic, Elk Grove Village, IL). Typical specific activities were 300−400 000 dpm/μg.

Binding of GS-I Isolectins to Human Type A Erythrocytes. Fresh human type A_1, A_2, and O erythrocytes were obtained from single donors from John Judd, University of Michigan Hospitals Blood Bank. The cells were collected, washed 3 times with Dulbecco's PBS (140 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 5 mM Na PO_4, pH 7.4), and resuspended in Dulbecco's PBS at a concentration of approximately 10^9 cells/mL. The incubation mixtures containing lectin solution were prepared in Dulbecco's PBS to give a range of lectin concentrations as follows: A_4, 5−100 μg/mL; A_3B, 5−100 μg/mL; A_2B_2, 5−200 μg/mL; AB_3, 5−400 μg/mL. The incubations were initiated by the addition of 200 μL of a freshly prepared suspension of human type A erythrocytes to each tube. Tubes were sealed and mixed continuously at 4 °C for 4 h. All data points were performed in triplicate. Nonspecific binding was determined by a parallel set of incubations containing 50 mM methyl α-D-galactoside as haptenic sugar. Cell concentrations were determined using a hemocytometer.

At the end of the incubation period, the unbound lectin was separated from the erythrocyte-bound lectin according to the method of Barak Briles et al. (*11*). Briefly, the tubes were centrifuged at top speed in an Eppendorf microfuge for 5 min at 4 °C. The supernatant solutions were removed by aspiration, 400 μL of Dulbecco's PBS and a mixture of *n*-butyl phthalate/*n*-octyl phthalate (1/1 v/v) (200 μL) was added to each tube, and the samples were centrifuged. The water and oil supernatant layers were removed, and the bottom of the tube containing the lectin-bound erythrocytes was cut into a vial for gamma counting.

To demonstrate that equilibrium binding of the isolectins to the erythrocytes was achieved during the incubation period, the radioactivity bound to the cells was determined at 4 °C at 1, 2, 3, 4, and 5 h of incubation. To ascertain that saturable binding of the isolectin to the cell had been reached, a plot of micrograms of bound lectin versus Log [micrograms of free lectin] was made according to Klotz (*12*).

[1] Abbreviations: GS-I-A_4, *Griffonia simplicifolia-I* isolectin A_4; GS-I-A_3B, *Griffonia simplicifolia-I* isolectin A_3B; GS-I-A_2B_2, *Griffonia simplicifolia-I* isolectin A_2B_2; GS-I-AB_3, *Griffonia simplicifolia-I* isolectin AB_3; GS-I-B_4, *Griffonia simplicifolia-I* isolectin B_4; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline, pH 7.2.

Scatchard Analysis. Scatchard analysis was performed as reported (*13*). Specific binding was determined by subtracting the amount of radioactivity in the samples containing haptenic sugar from the radioactivity in the corresponding samples without haptenic sugar. The percentage of nonspecific binding was determined as the radioactivity bound in the presence of haptenic sugar divided by the specifically bound radioactivity and expressed as a percentage of isolectin bound. The association constants for the GS-I isolectins were determined from curve fitting using Mathematica Software (Wolfram Research, Inc.) and Prizm Software (GraphPad, Inc) using the equation:

$$\text{bound ligand} = \sum_{i=1}^{4} \frac{n_i[\text{free ligand}]}{K_{d_i} + [\text{free ligand}]}$$

For comparison, the association constants for the monovalent (GS-I-AB_3) and divalent (GS-I-A_2B_2) isolectins were also determined from the slope of the Scatchard plot as described by Klotz and Hunston (*14*). The curve-fitting results of each experiment were used to determine the mean number of sites and the mean association constants.

RESULTS

Time and Concentration Dependence of Reversible Binding. Figure 1A demonstrates that the binding of the GS-I isolectins A_4 and A_3B is time dependent and maximum binding is achieved during the 4 h period of incubation. To demonstrate that the concentration range of lectin utilized in this study was appropriate to achieve saturation of cell surface binding sites, the amount of specifically bound lectin was plotted versus log [free lectin] according to the method of Klotz (*12*) (Figure 1B). This figure shows that the GS-I-AB_3 isolectin approaches saturation of binding sites while the GS-I-A_4 and -A_2B_2 isolectins display at least 50% saturation of binding sites. Attempts to achieve higher levels of receptor saturation were not possible due to cell lysis during the incubation with these isolectins. Only the GS-I-AB_3 isolectin could be used at the highest concentrations without noticeable cell destruction.

Greater than 97% of the bound radiolabled lectin was removed when the cells were incubated with methyl α-D-galactoside, indicating that lectin binding to the cells is a reversible interaction under the experimental conditions used.

Lectin Binding to Cells and Scatchard Analysis. Scatchard analysis of [^{125}I]lectin binding to cells is shown in Figure 2A−C. In Figure 2A, we observe that the binding of the monovalent GS-I-AB_3 isolectin to type A red blood cells shows a simple linear Scatchard plot with an association constant $K_a = 4.2\ (\pm 1.6) \times 10^5\ M^{-1}$ and total sites $n =$ 311 000. Curve fitting of these data gave an association constant $K_a = 7.5\ (\pm 1.4) \times 10^5\ M^{-1}$ and total sites $n =$ 311 000. These association constants are similar to that reported for the binding of the haptenic sugar GalNAc to the GS-I-A_4 isolectin ($K_a = 1.87 \times 10^5\ M^{-1}$) (*3*). These results imply that binding of the lectin to the cell surface receptors is dependent entirely on the recognition of the terminal α-linked GalNAc unit and not to a more complex oligosaccharide structure. This result also militates against the involvement of other nonspecific or noncarbohydrate interactions.

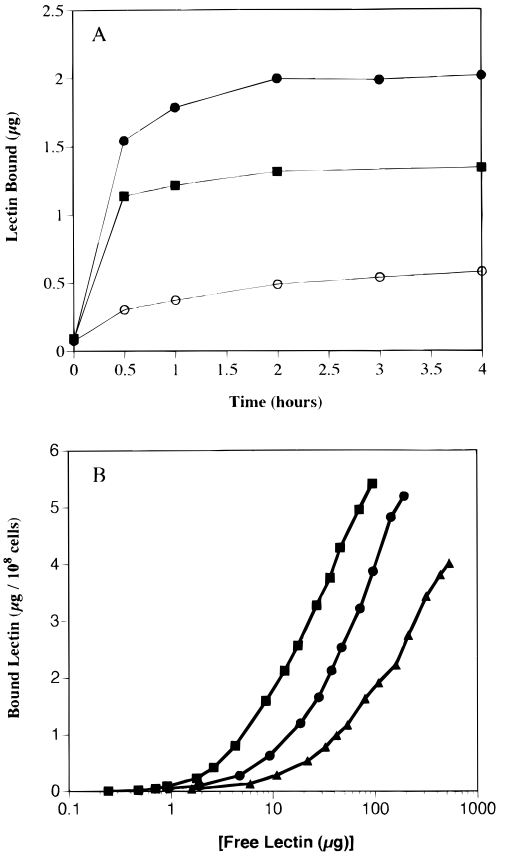

FIGURE 1: (A) Time dependence of binding. The incubation time used in all experiments was determined following the examination of the time dependence of binding. Types A_1 and A_2 human red blood cells were incubated at a single concentration of either GS-I-A_4 (15 μg/mL) or GS-I-A_3B (22 μg/mL). At the indicated times, cells were collected by centrifugation, and total binding was determined. Shown are binding of GS-I isolectin A_4 to blood group A_2 erythrocytes (○), binding of GS-I isolectin A_4 to blood group A_1 (●), and binding of GS-I isolectin A_3B to A_1 erythrocytes (■). (B) Saturation of binding sites. Binding of GS-I isolectins AB_3 (▲), A_2B_2 (●), and A_4 (■) to type A_1 human red blood cells was determined. The lectin used was that indicated under Materials and Methods for the cell binding experiments.

Next, examining the Scatchard analysis of the GS-I-A_2B_2 isolectin (Figure 2B), we observe evidence of positive cooperativity at very low lectin concentrations. In this analysis, the association constant increases to 2.5 ($\pm$1.3) $\times$ 10^6 M^{-1}, while the number of binding sites (n = 334 000) is essentially the same. Curve fitting of these data gave an association constant K_a = 2.9 ($\pm$1.1) $\times$ 10^6 M^{-1} and total sites n = 347 000. The observed positive cooperativity must be attributed to changes at the cell surface since hapten binding to the GS-I-A_2B_2 isolectin did not show any evidence of cooperativity (*3*). These results suggest that after the first low-affinity binding event occurs, which places the isolectin at the cell surface, the second binding event is more favorable. It is possible that this cooperativity is due to the lectin being held in close proximity to the cell surface or due to organization of the cell surface receptors into clusters of receptors more readily accessible to additional binding events by the subunits of the same lectin molecule. This association constant does not imply an increase in the individual site constants. The increase may also reflect the fact that the likelihood of the second binding event is

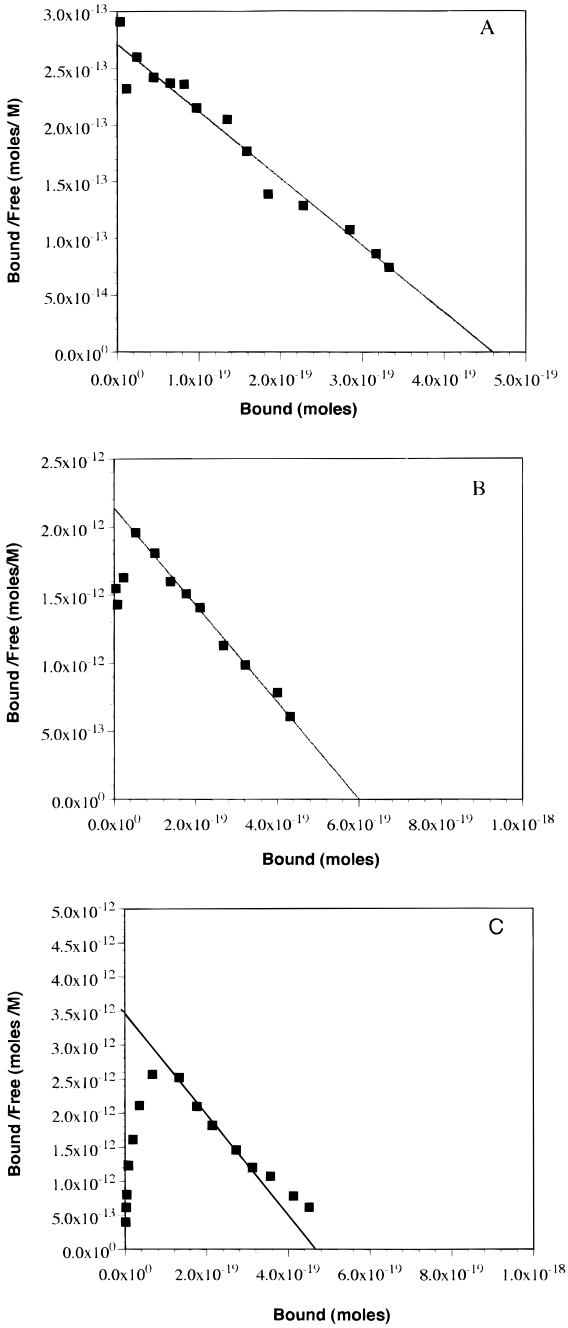

FIGURE 2: Scatchard analysis of GS-I lectin binding to human RBC. Binding of GS-I isolectins to type A_1 human red blood cells was analyzed according to the method of Scatchard (*13*). Shown here are the monovalent AB_3 (panel A), divalent A_2B_2 (panel B), and tetravalent A_4 (panel C).

increased following the first binding and that in order to bind to the cell only a single event need occur, while the dissociation requires the simultaneous breaking of two interactions. The results of curve fitting are reported in Table 1 because errors in the determination of the lowest levels of ligand binding would produce a larger effect on the fitting of a line to a Scatchard analysis than to the result of curve fitting to a plot of bound versus free ligand. The results of both analyses are discussed here to demonstrate that both methods generate similar results.

Examination of the Scatchard plot for the trivalent GS-I-A_3B isolectin (data not shown) demonstrates the presence of both positive cooperativity already observed for the

Table 1

isolectin	K_{assoc} (M^{-1})	total sites/ cell, *n*	nonspecific binding (% of total binding)
A_4 for GalNAc[a]	1.87×10^5	4	−
B_4 for GalNAc[a]	not detectable	0	−
AB_3	$K = 7.5\ (\pm 1.4) \times 10^5$	311000	<3
A_2B_2	$K = 2.9\ (\pm 1.1) \times 10^6$	347000	<3
A_3B	$K = 1.4\ (\pm 0.1) \times 10^7$	331000	<3
A_4	$K = 1.2\ (\pm 0.5) \times 10^7$	343000	<3

[a] From Goldstein et al. (*4*). K_{assoc}'s (M^{-1}) and the total sites n were calculated by curve fitting from a minimum of three experiments as described (Materials and Methods). K_{assoc}'s are reported as mean (±SD); the total number of sites is reported as the average of the total sites of all experiments.

divalent lectin and negative cooperativity or the presence of a second site. In attempting to fit the data to current mathematical models, these data show a very good fit to the two-site model. The assumption is that the trivalent lectin has n_1 sites equal to zero, meaning that all bound lectin interacts through two or more sites and that there are approximately zero lectin molecules bound only via a single site. The results of curve fitting for the trivalent GS-I-A_3B isolectin are reported in Table 1 with $K_a = 1.4\ (\pm 0.1) \times 10^7\ M^{-1}$ and $n = 331\ 000$.

Examination of the Scatchard plot for the GS-I-A_4 isolectin displays the same pattern observed for GS-I-A_3B, i.e., both positive and negative cooperativity. Making the assumptions described for the trivalent lectin (that $n_1 = n_2 = 0$, meaning that there is approximately zero lectin bound through only one or two binding sites), the calculated $K_a = 1.2\ (\pm 0.5) \times 10^7\ M^{-1}$ and $n = 343\ 000$ (Table 1).

These results, summarized in Table 1, indicate that (1) binding of the lectin to erythrocytes can be effectively inhibited by the monosaccharide with which the lectin is known to interact (all isolectins exhibit less than 3% nonspecific binding). Although single sets of data are shown in the figures for each isolectin, we have repeated these experiments with type A_2 erythrocytes from additional donors with identical results. The results from experiments utilizing type A_2 erythrocytes demonstrated that A_2 cells possessed approximately 30% as many cell surface binding sites for the GS-I isolectins as found on the A_1 erythrocytes (data not shown). Our results also indicate that neither nonspecific nor noncarbohydrate interactions contribute significantly to the GS-I lectin binding to cells. Additionally, the GS-I-A_4 isolectin does not bind to type O erythrocytes. (2) We observed that the K_{assoc} for the monovalent isolectin AB_3−cell interaction (Figure 2A) is essentially the same as the K_{assoc} observed for the lectin−hapten interaction, which is an additional argument against an extended binding site for this lectin. (3) The increase in the K_{assoc} as the lectin valence increases further supports our hypothesis that affinity is dependent on the valence of the lectin.

The model we propose is that each lectin binding site represents a cluster of 3−4 ligands on the cell surface. A single lectin subunit binds to a single ligand in the cluster and prevents the binding of a second lectin to this cluster, probably due to steric hindrance. Thus, we observe that the number of binding sites does not change between isolectins. Further, we propose that each site binds only to the terminal GalNAc residue and not an extended oligosaccharide structure, which is supported by the similarity between the binding of the hapten to the isolectin and the binding of the monovalent GS-I-AB_3 isolectin to the cells.

Positive cooperativity was observed in the three multivalent isolectins. This cooperativity may be due to the fact that binding of the lectin requires that a single bond is formed, while dissociation requires that multiple bonds be broken simultaneously. Additional explanations may involve the organization of the cell surface ligands that facilitates or increases the probability of additional binding interactions. We have been unable to find an adequate model that accounts for the binding of multivalent ligands to multivalent receptors.

The negative cooperativity observed for both the trivalent and tetravalent isolectins may be due to a deformation in the cell surface receptors that is required for binding of the third or fourth lectin subunits. The 'cost' of binding the third or fourth isolectin subunits is manifested as negative cooperativity. It is significant that the association constants for both the trivalent and tetravalent lectins are identical. This result indicates that for these isolectins the fourth subunit does not contribute to the binding of the lectin to the cell and only three subunits are required for the high-affinity interaction of both GS-I-A_3B and GS-I-A_4 isolectins with the cell surface.

DISCUSSION

Numerous reports in the literature cite the fact that lectins bind with several orders of magnitude greater affinity to cells than to the haptenic sugars for which they are specific: *Helix pomatia* (*15*), *Griffonia* (*Bandeiraea*) *simplicifolia* (*4*), lentil lectin (*16*, *17*), *Phaseolus vulgaris* (*18*), and wheat germ agglutinin (*19*) are examples of this phenomenon.

Several plant lectins have been reported to have extended binding sites: *Maackia amurensis* (*20*), elderberry bark lectin (*21*), *Datura stramonium* agglutinin (*22*, *23*), tomato lectin (*24*), concanavalin A (*25*, *26*), and *Phaseolus vulgaris* lectin (*27*) provide examples. Several animal lectins, such as murine CBP-35 (*28*) and the selectin family (*29*), have also been reported to possess extended binding sites. However, it should be noted that all lectins need not have an extended site in order to explain their enhanced interactions with cells.

Hydrophobic, noncarbohydrate binding sites have been reported for the lima bean and other lectins (*30*). Currently, it is unknown what role these binding sites might play in the interactions of lectins with cell surface ligands. In addition, the possibility of nonspecific binding of a lectin to the cell surface must be considered.

Hornick and Karush (*5*) observed that a divalent antibody had a 10^3-fold increased affinity in its interaction with a polyvalent ligand compared to a monovalent ligand. They also reported that a monovalent antibody fragment showed a 100-fold decrease in affinity to the same polyvalent ligand. Studies with a decavalent IgM showed a 10^6-fold enhancement of affinity over the affinity observed for the monovalent ligand. The authors reasoned that IgM must have an attachment to at least three points in order to explain its increased affinity compared with IgG.

Phaseolus vulgaris isolectins (*31*) showed an increase in affinity for human erythrocytes (30-fold) as one proceeds in the series from the monovalent EL_3 to the tetravalent E_4 isolectin. However, the authors did not report a change in

the number of receptors and so argued against multipoint attachment. The clustering effect produced by these lectins is most probably due to their valence. Also of relevance are the reports of Schechter and co-workers (*32*) and Ruddon et al. (*33*) that aggregated octavalent soybean agglutinin was a good mitogen for pig lymph node cells whereas the native tetravalent lectin was not, and that octavalent lima bean lectin was a significantly better lymphocyte mitogen than the tetravalent protein.

Surolia et al. (*34*) have observed greater than 100-fold affinity for the binding of the *Ricinus communis* agglutinin to GM1 ganglioside containing liposomes than to the monovalent hapten. Ketis and Grant (*35*) have also invoked the idea of clustering of membrane receptors to explain positive cooperative binding of Con A to model membranes. Lee et al. (*36*) reported that the affinity of galactose-terminated glycoconjugates for the hepatic asialoglycoprotein receptor increased 5000-fold from the monovalent to the trivalent ligand. Similarly, the human mannose binding protein is reported to display almost 10 000-fold greater affinity for a hexavalent ligand than for the divalent ligand (*37*).

In the present GS-I isolectin system, the monosaccharide haptens have been shown to be effective inhibitors of the isolectin−erythrocyte interaction. This result militates against a substantial contribution to binding affinity from either nonspecific or noncarbohydrate interactions. This also suggests that the isolectin is unlikely to have an extended binding site, inasmuch as we would not expect a monosaccharide to effectively displace lectin binding to a complex oligosaccharide structure. Examples of this include the fact that the sialic acid-specific lectin *Maackia amurensis* leukoagglutinin is not inhibited by Neu5Ac whereas the presence of the terminal sialic acid residue is critical for the binding of the *Maackia amurensis* leukoagglutinin lectin to its ligand (sialic acid α2,3Gal*β*1,4Glc/GlcNAc) (*20*), and the *N*-acetyllactosamine-specific lectin CBP-35, which is poorly inhibited by galactose, but at the same time requires galactose as the terminal unit of its oligosaccharide ligand (*28*).

Our observations, together with the reports cited above, suggest that (1) the multivalent isolectins must occupy more than a single ligand on the cell surface and (2) the multivalent lectin organizes the cell surface receptors into a cluster to which it attaches and is able to sterically prevent other lectin molecules from binding to this cluster of receptors. In this way we might consider the increase in K_{assoc} as the result of several available ligands to which the lectin must bind such that, as one lectin−ligand interaction is broken, another is readily formed, while the lectin is held in place by its other lectin−ligand interactions. In the case of a monovalent lectin, the disruption of the single interaction results in the dissociation of lectin and ligand, while the multivalent lectins require multiple bonds to be broken simultaneously.

It is necessary to address an additional issue: how can the positive and negative cooperativity in this system be explained? What do these interactions tell us regarding lectin−cell interaction?

We observed positive cooperativity in the binding of GS-I-A_3B and -A_4 to erythrocytes. We did not observe cooperativity in the binding of GS-I-AB_3 to cells (Figure 2A), nor does this isolectin display cooperative binding to the haptenic sugars (*4*). Hammarström (*15*) reported negative cooperativity for the binding of *Helix pomatia* to cells. Positive cooperativity indicates that the K_{assoc} increases as the occupancy of the lectin binding sites increases. Prujansky et al. (*38*) suggested this was due to an increase in the affinity of the binding sites of the lectin, or unmasking of cryptic sites on the cell. As stated earlier, the binding of monovalent haptens to GS-I isolectins does not result in cooperative binding. Neither concanavalin A nor wheat germ agglutinin (*39*, *40*) displays cooperativity in the binding of their haptenic sugars, but they do show positive cooperativity in binding to membranes. It is important to recall that in these experiments the lectin is the ligand and that the positive cooperativity must be due to changes at the cell surface and not to molecular events involving only the lectin. This would suggest that the site constant, the affinity of the lectin subunits for the receptors, does not change. However, there may be an increase due to the statistical increase in available sites to bind to the cell and a seemingly lower affinity site due to the deformation of the receptor at the membrane.

Several investigators have attempted to explain cooperative binding of lectins to cells or to model membranes. Ketis and co-workers (*41*) advanced several explanations for positive cooperativity: lectin-induced exposure of cryptic sites; restructuring of membrane components; or involvement of cellular machinery in some way that enhanced binding of the lectin to the cell surface. However, since positive cooperativity occurs at 4 °C, it is unlikely to involve massive change or reorganization of the cell surface ligand molecules. Positive cooperativity can be observed in model membrane systems; therefore, the involvement of cellular processes (*34*, *35*, *41*) can be ruled out.

Both positive cooperativity and negative cooperativity (or two sites) in our system for GS-I-A_4 and GS-I-A_3B must be explained. At very low lectin concentrations, we observed positive cooperativity in the binding of GS-I-A_4 to type A_1 erythrocytes. At higher lectin concentrations, we observed that two-thirds of the sites for these lectins are high affinity and one-third of the sites are of lower affinity. The explanation we propose is that once the lectin binds to the first site, binding to the second site is more rapid because the lectin is held in close proximity to other receptors; this appears as positive cooperativity since the first binding event facilitates the second event. In addition, this second binding site increases the affinity by 4-fold. In order for the third binding event to occur for GS-I-A_3B, the lectin may cause a deformation of the cell surface ligands. This deformation could produce a strain in the binding that results in a less favorable binding of the third lectin subunit to the third cell surface receptor. This is observed as a lower affinity binding or negative cooperativity. This third site also adds only a 5−6-fold increase in the affinity of the interaction. The tetravalent lectin offers an additional site for binding but does not result in an increase in affinity over that observed for the trivalent lectin. The presence of the additional binding site must be responsible for the increased affinity. While the increase in the association constant is certainly due to the increased valence of the GS-I isolectins, the observed increase is less than those observed for other carbohydrate−protein interactions, as discussed above. Currently, it is not understood why the change in affinity is less in this system. It is possible that those other systems also have contributions due to extended oligosaccharide binding sites.

An important practical consideration is that whereas the oligosaccharide−lectin interaction may be relatively weak ($K_{assoc} = 10^4$), lectins are still capable of acting effectively in their immobilized forms as affinity resins. Cuatrecasas et al. (*42*) reported even weak interactions ($K_{assoc} = 10^4$) could be useful for enzyme purification if a high density of ligand was immobilized. More recently this same observation has been reported for wheat germ agglutinin (*43*) and for the *Griffonia simplicifolia* lectin (*44*). In summary, it is reasonable to expect that an increase in the K_{assoc} of lectin binding to cells due to the multivalency of the lectin should be considered to be a general phenomenon.

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