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Measurement of Mono- and Polyvalent Carbohydrate-Lectin Binding by Back-Scattering Interferometry

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Abstract

Carbohydrate-protein binding is important to many areas of biochemistry. Back-scattering interferometry (BSI) is shown here to be a convenient and sensitive method for obtaining quantitative information about the strengths and selectivities of such interactions. The surfaces of glass microfluidic channels were covalently modified with extravidin, to which biotinylated lectins were subsequently attached by incubation and washing. The binding of unmodified carbohydrates to the resulting avidin-immobilized lectins was monitored by BSI. Dose-response curves, generated within several minutes and highly reproducible in multiple wash/measure cycles, provided adsorption coefficients that showed mannose to bind to concanavalin A with 3.7 times greater affinity than glucose, in line with literature values. Galactose was found to bind selectively and with similar affinity to the lectin BS-1. The avidities of polyvalent sugar-coated virus particles for immobilized conA were far higher than monovalent glycans, with increases of 60–200 fold per glycan when arrayed on the exterior surface of cowpea mosaic virus or bacteriophage Q β . Sugar-functionalized PAMAM dendrimers showed size-dependent adsorption consistent with the expected density of lectins on the surface. The sensitivity of BSI matches or exceeds that of surface plasmon resonance and quartz crystal microbalance techniques, and differs in its sensitivity to the number of binding events rather than changes in mass. Its operational simplicity, generality, and the near-native conditions under which the target binding proteins are immobilized make it an attractive method for the quantitative characterization of the binding functions of lectins and other proteins.

Keywords

carbohydrates; lectins; interferometry; binding; viruses; polyvalency

Introduction

Carbohydrate-protein interactions transmit an immense amount of information during intracellular and extracellular processes.^{1–7} As a result, functional glycomics has taken its place among genomics and proteomics as a vital area of investigation for the understanding and treatment of cellular biology and disease.⁸ A fundamental datum in the study of any

carbohydrate-protein interaction is the binding constant. Because of the size mismatch in the binding partners and the fact that carbohydrates do not usually contain functional groups that induce large changes in protein absorbance or fluorescence, such quantitative determinations of binding affinities are often quite difficult to obtain. The installation of labels (fluorophores, spin labels, crosslinking agents) on the carbohydrate, while necessary in many cases, runs the risk of distorting the binding function that is being studied. The most popular label-free technique in recent years has been surface plasmon resonance (SPR),^{9–17} with quartz crystal microbalance (QCM) technology emerging as a promising alternative at lower cost.^{15,18–20} Both methods require the immobilization of one of the binding components on a chip, with the other partner incubated with or flowed over the chip surface. The only method in common use for label-free quantitation of binding constants in solution is isothermal titration calorimetry (ITC).^{21–24} However, ITC is relatively insensitive, time-consuming, and often requires large amounts of sample.

Because both SPR and QCM techniques detect changes in mass upon binding, the small carbohydrate is usually immobilized and the large protein binding partner presented in solution.^{9,12,17} Several examples of the reverse format (addition of free sugar to immobilized lectin) have appeared,^{25–29} but sensitivity and accuracy are generally limited.³⁰ One attempt has been made to address this problem for carbohydrates with a heavy linker to enhance the mass-sensitive SPR signal upon binding.³¹

We describe here the use of a fundamentally different technique, backscattering interferometry (BSI), for the quantitation of binding constants of carbohydrate-lectin interactions. BSI is highly sensitive and can be used on surface-tethered species^{32,33} or on binding events that take place in free solution.³⁴ We have previously described its use in the detection of IgG-protein A interactions and DNA hybridization³³ and recently reported the quantification of binding affinities over six decades (μM – pM) with small molecule-protein, protein-ion, protein-protein, protein-peptide, and antibody-antigen systems.³⁴ We show here that BSI can be used to obtain highly reproducible binding constants for the interactions of both monovalent and polyvalent carbohydrates with lectins that have been immobilized in a very mild manner so as to support their native structure and function.

Results and Discussion

Chips with 90 μm channels isotropically etched in borosilicate glass were modified by the method of Matsunaga and coworkers.³⁵ After soaking the channels in 10% KOH in methanol for 30 min, mercaptopropyltriethoxysilane (**1**, Figure 1) was introduced to give a thiol-modified surface. Condensation with the bifunctional linker **2** then provided surface-tethered N-hydroxysuccinimide ester groups, which were used to capture extravidin, as indicated by wash-resistant changes in surface wetting and interferometry. The activity of the immobilized avidin, deposited under conditions in which it is expected to be a tetramer in solution, was verified by fluorescent labeling of the channels using biotinylated fluorescein (Supporting Information). Biotinylated lectins were then introduced to arrive at the fully charged channel. The use of an extravidin layer was designed to make the system as modular as possible, and also to install the binding protein of interest in a less denaturing environment than it would experience if tethered directly to the glass surface.

The interaction of biotinylated concanavalin A (conA), immobilized in the above manner, with its natural ligands was measured as a function of concentration, with the results shown in Figure 2A,B. The phase change in the interferometry signal was found to vary in a manner consistent with dose-dependent saturation of a single binding site. The apparent Langmuir adsorption coefficients calculated from these curves ($K_{\text{ads}} = 2.4 \pm 0.3 \times 10^4 \text{ M}^{-1}$, $1/K_{\text{ads}} = 42 \pm 5 \mu\text{M}$ for D-mannose; $K_{\text{ads}} = 6.5 \pm 5.4 \times 10^3 \text{ M}^{-1}$, $1/K_{\text{ads}} = 155 \pm 88 \mu\text{M}$ for D-glucose) show the same relative

trend, but stronger apparent binding, than true solution-phase equilibria (K_D) that have been measured by ITC for the free sugars (K_d = approx. 450 μ M for mannose and 1800 μ M for glucose at room temperature).³⁶ The same type of discrepancy (but to a greater degree, 1000-fold vs. 10-fold) has been observed by Kiessling, Corn, and coworkers for immobilized glycosides in SPR measurements ($1/K_{ads}$ = 0.18 ± 0.06 μ M vs. K_d = 100–200 μ M for α -Me-mannose).¹³ Galactose induced no change in interferometry signal, consistent with its inability to bind to conA (Figure 2A). In contrast, biotinylated BS-1 lectin used in the same manner responded to added galactose ($1/K_{ads}$ = 30.2 ± 2.8 μ M), but not mannose, consistent with its known glycan affinities (Figure 2C).^{37–41}

The observed K_{ads} for the conA-mannose interaction was independent of both the source of the biotinylated lectin and its loading on the channel surface (Figure 2B). The number of conA molecules immobilized on the channel was reduced by treating the avidin-coated chip with a 1:1 mixture of biotin-conA and biotinylated bovine serum albumin (BSA). BSI analysis of mannose binding in the two cases gave a diminished maximum signal in the mixed conA/BSA case, reflecting the expected dilution of glycan-binding protein on the surface. However, the same binding constant was found in both cases, suggesting that each molecule of immobilized protein acts independently from the others, although the existence of binding sites having different affinities within each conA tetramer⁴² cannot be discerned from these data. It is also noteworthy that once the channel is charged with lectin, the BSI measurements are made within minutes and are highly reproducible. Added carbohydrate can be washed out with buffer and the immobilized lectins reused with no loss in signal or change in observed binding constant over at least 30 repetitions.

As with any surface-based technique, BSI lends itself to measurements of polyvalent binding.¹⁰ To demonstrate this, we turned to icosahedral virus particles that have been previously employed for the presentation of polyvalent glycans to lectins, cell surfaces, and the avian immune system,^{43–45} and to polyamidoamine (PAMAM) dendrimers decorated with monosaccharides, known to have a strong generational (size and valency) dependence on interactions with cognate lectins.^{46–48}

Cowpea mosaic virus (CPMV)^{49,50} and bacteriophage Q β virus-like particles,^{51,52} both approximately 30 nm in diameter but very different in their structural details,^{53,54} were decorated with monosaccharides as shown in Figure 3. CPMV displays 240 lysine amine side chains on its exterior surface in solvent-accessible positions.^{55,56} Two forms of Q β were used: the wild-type assembly domain sequence of the coat protein which displays up to 900 amine groups per particle, and the K16M mutation of this sequence which eliminates the most accessible lysine and therefore bears 720 surface amines.⁵⁷ In both Q β structures, some steric hindrance exists among sets of symmetry-related residues, diminishing the maximum number of acylations that can be performed.

Each capsid particle was reacted with a large excess of an NHS ester-alkyne reagent to acylate most of the surface lysine side chains. The isolated alkyne-derivatized particles were then condensed with azidoethyl derivatives of α -mannose or β -galactose⁴¹ (**3** and **4**, respectively) using complex **6** as a precatalyst for the copper-catalyzed azide-alkyne cycloaddition (CuAAC) “click” reaction that has been developed for the purpose.^{45,58} This conjugation methodology allows for complete coverage of the alkyne groups with modest concentrations of the desired azide under mild conditions, irrespective of the other functional groups in the reaction partners. The numbers of attached glycans were estimated by performing reactions under identical conditions with the selenomethionine azide derivative **5** instead of a glycan-azide. Selenium, not present in detectable levels as background, can be quantified at sub- μ M concentrations using inductively coupled plasma optical emission spectroscopy (ICP-OES). Along with the independent determination of protein concentration in each purified sample by a modified

Lowry assay, this provides a measurement of the average number of attachments made per virion by the CuAAC reaction. An experimental error of 10% is typical for independent reactions under identical conditions. Compound **5** is designed to replace dyes such as fluorescein that we have previously used to determine loading on capsid scaffolds,^{43,55} since **5** more closely resembles the hydrophilic character of carbohydrates and thereby provides a closer analogy to their attachment.

Polyvalent CPMV-glycan structures **7**, **8**, and **11** were prepared, bearing approximately equal numbers of α -mannose molecules but with different linkers. In addition, Q β structures **9** and **10** were made to test higher densities of sugar loading on the shortest linker arm. The binding of these particles to immobilized conA was measured by BSI, with the results shown in Figure 4. The unmodified wild-type virions showed no interaction with the conA-derivatized surfaces, whereas the mannosylated particles were tightly bound. On a per-mannose basis, the measured average adsorption coefficients were approximately 60 times better for the CPMV-displayed sugar than for free mannose in solution (and thus likely to be 20–30 times better than α -Me-mannoside, which binds conA 2–3 times more tightly than mannose³⁶). On a per-particle basis, the virus adducts achieved avidities in the low nanomolar range. For the same surface glycan density, the use of different linkers made no difference. When displayed at a significantly higher density on the Q β scaffold, binding was further improved, with affinity approximately 200 times that of free mannose on a per-sugar basis, and sub-nM in terms of particle concentrations.

Changes in polyvalent affinity were also conveniently determined by BSI for CPMV particles bearing different ratios of mannose and galactose, with the same overall loading of sugar. These structures were prepared using different ratios of mannose and galactose azides **3** and **4**, assuming that the rates of CuAAC condensation are insensitive to the identity of the monosaccharide (Figure 3). As shown in Figure 5A–B, particles **11** (bearing only mannose) and **15** (bearing only galactose) were not bound by immobilized BS-1 and conA lectins, respectively, ruling out contributions from nonspecific adsorption by the linker and triazole moieties added to the coat proteins in the bioconjugation process. In each case, the installation of mannose or galactose at 25% of the approximately 200 virus surface sites gave rise to highly potent binding to conA and BS-1, respectively, with modest increases in avidity of the particles observed as the percentage of active glycan was increased (Figure 5C–D, blue). On a per-glycan basis, however, the affinities (while still much higher than the free sugars) were found to either decrease throughout the series for mannose-conA or decrease to an approximate plateau for galactose-BS-1 (Figure 5C–D, black) as the loading of the active sugar on the virus surface was increased. The magnitude of the increase in per-glycan affinity (60–200 times) suggests that true polyvalent binding (simultaneous multipoint interactions with more than one anchored receptor) is taking place.^{46,47}

To extend the observation of polyvalent binding, generation 4 (G4) and generation 6 (G6) PAMAM dendrimers decorated with varying numbers of α -linked mannose and galactose units were prepared as previously described (Figure 3).⁴⁸ BSI measurements with immobilized conA (Figure 5E–H) were again sensitive and highly reproducible, with signal magnitude depending on the number of mannose units loaded onto each dendrimer, and the measured adsorption coefficients sensitive to dendrimer size. The G4-based particles showed per-mannose affinities ($1/K_{\text{ads}} \approx 20 \mu\text{M}$) approximately twice the measured values for mannose alone, and thus exactly what one would expect for monovalent α -alkylmannoside. Neither the per-mannose nor the per-particle affinity ($1/K_{\text{ads}} \approx 0.5 \mu\text{M}$) changed appreciably with variation in mannose loading from 18 to 42 per dendrimer (Figure 5G). In contrast, the G6 dendrons did show a modest improvement in per-mannose adsorption coefficient from $10.0 \pm 3.4 \mu\text{M}$ (for **24**, with 31 mannoses) to $2.8 \pm 0.4 \mu\text{M}$ (for **21**, with 111 mannoses) as mannose loading increased. Similarly, the per-dendrimer association constants improved through the G6 series (Figure 5H; **24**, $1/K_{\text{ads}} = 84 \pm 29 \text{ nM}$, to **21**, $1/K_{\text{ads}} = 25.8 \pm 4.1 \text{ nM}$). The “proximity effect”

from the presentation of high local concentrations of glycan ligands on the dendrimer surface to individual immobilized lectins can be expected to contribute to the improved avidity of dendrimer-glycan conjugates.^{48,59,60} However, the fact that even the most lightly-loaded G6 structure shows better binding than the G4 dendrimers that are more densely decorated with glycan suggests that the G6 particles interact with the immobilized conA in a different manner than G4. Such interactions are presumably bi- or polyvalent, either with individual conA lectins (G6 being able to reach two binding sites better than G4) or adjacent conA molecules.

It has previously been demonstrated by agglutination and precipitation assays that both the G4 and G6 dendrimers used here, but not G3 or smaller structures, are able to engage conA units in polyvalent binding interactions in solution.⁴⁸ In the BSI measurements described above, however, G4 glycan dendrimers are monovalent binders and G6 dendrimers only begin to show polyvalent-style affinities, whereas the virus-based structures bind much more tightly. The most obvious distinguishing characteristic among these polyvalent ligands is their size: G4 dendrimers, G6 dendrimers, and the virus capsids have approximate diameters of 5, 7, and 30 nm, respectively. The avidin tetramer occupies a volume of approximately $5 \times 5 \times 6$ nm,⁶¹ and is likely to be affixed to the glass-NHS ester surface in an orientation that blocks at least two of its biotin binding sites from solution.⁶² The density of surface attachment points for biotinylated lectin should therefore be quite low, and adjacent lectins would be reachable for polyvalent binding only by the larger glycosylated structures. Multivalent binding to individual adsorbed conA tetramers will be difficult for these dendrimers and virus particles which have short tethers connecting the sugar to the platform.⁴⁸

The most important difference between the BSI technique and methods such as SPR and QCM concerns the nature of the signal: BSI detects changes in refractive index,³⁴ and is thus sensitive to the number of binding events rather than the change in mass brought about by binding. For example, the maximum (saturation) signal for polyvalent particles such as viruses and dendrimers was found to be proportional to the number of binding ligands attached to the scaffold (mannose vs. galactose, for example, in Figures 5A,B,E, and F), even though the maximum number and mass of particles that can access the surface was the same for all members of the series. In contrast, one could not distinguish by SPR at surface saturation between particles bearing different numbers of binding ligands. The mass change in such a measurement would be equal in all such cases, regardless of how many receptor-ligand binding events occur, except for water displaced from each protein binding site upon interaction with the ligand. Because BSI detects changes in refractive index, it is sensitive to that water displacement. For this reason, the magnitudes of the phase changes observed for the saturation of the immobilized proteins with monovalent sugars are *greater* than those observed for binding of the massive virus- or dendrimer- displayed structures (Figure 2 vs. Figures 4 and 5).

This difference in the nature of the signal also manifests itself when species are removed from the surface. If one washes a large molecule off an SPR chip, the signal invariably decreases due to the loss of attached mass. With BSI detection, the signal depends on which species is used to do the washing, as shown in Figure 6. When soluble conA was used to remove CPMV-mannose (**11**) from the standard extravidin-conA channel, a dose-dependent decrease in signal was observed, consistent with the competitive stripping away of the virion from the surface by the soluble receptor (Figure 6A). One could in principle use this phenomenon to measure solution-phase association constants by competitive binding, as has been done with SPR imaging.¹³ In contrast, the addition of soluble mannose (Figure 6B) gave rise to an invariant increase in signal, even when the mannose concentration (20 μ M) was well below the K_D value for free mannose-conA binding (42 ± 6 μ M). At that concentration (and probably higher concentrations as well), mannose cannot be expected to dislodge virus particles that bind with much greater affinity ($K_D = 2.2 \pm 0.36$ nM in virions; 0.42 ± 0.07 μ M per mannose). The interferometry signal increases because mannose occupies empty conA binding sites that are

underneath virus particles attached to the surface. In this case, it is impossible to tell when virus is outcompeted by added mannose for surface conA sites, since the signal does not change. Figure 6C summarizes in graphical form the various states achieved when large polyvalent ligand particles compete with soluble ligand or receptor for surface receptor sites, highlighting the dependence of BSI signal on the number of receptor-ligand binding events occurring at the surface of the channel.

Conclusions

Back-scattering interferometry uses simple hardware to achieve highly sensitive measurements of protein binding events on very small amounts of material in a reusable format. We demonstrate here its application to the quantitative determination of adsorption coefficients, and the relative determination of binding constants and polyvalent avidities, for glycan-lectin interactions, one of the most important classes of interactions in biochemistry. We have attached the receptor to the BSI channel by a general method involving complexation with an intervening layer of avidin, providing relatively large spacing between attachment sites and an environment conducive to the maintenance of native structure and function.

The interferometric response detects the act of complexation without direct regard to the size of the species doing the binding. This is consistent with BSI's known sensitivity to changes in refractive index.³⁴ We presume that conformational changes in the tethered binding partner and/or expulsion of bound water caused by ligand binding contribute to refractive index modulation. The technique therefore gives rise to very different types of responses to polyvalent interactions, detecting the total number of binding events whereas SPR and QCM report on the fate of the polyvalent structure as a whole. Given its technical simplicity, high sensitivity, and label-free nature, we expect that BSI will find use in the quantitative exploration of glycan-receptor interactions in a variety of contexts.

Experimental Section

Production, Isolation, and Derivatization of Virus Particles

CPMV particles were produced in cowpea plants and isolated using previously published procedures.⁶³ Briefly, CPMV was isolated from infected leaves of black-eye cowpea plants. Primary leaves from 10-day old cowpea plants were first dusted with carborundum and inoculated with homogenized infected leaves in phosphate buffer. Symptoms of infection appear within a week and a systemic infection is observed after three weeks. Leaves were collected, weighed and frozen for future purification of CPMV. Blended leaf tissue was separated from virus as previously described.⁶³ Expression of the Q β coat protein from a recombinant plasmid has been previously reported;⁵¹ we created our own vector to allow for more convenient genomic manipulation, as will be described in detail elsewhere. A 133-amino acid version of the Q β coat protein gene was cloned into the vector pQE-60 and expressed under IPTG control in M15MA cells in SOB media. After expression, collected cells were lysed by sonication and lysozyme treatment and then centrifuged to remove insoluble cell components. Assembled particles were precipitated from the resulting supernatant using 8% PEG 8000. Following further centrifugation, the isolated pellet was resuspended in 0.1M potassium phosphate pH 7.0. The virus-like particles then underwent a final purification by ultracentrifugation through 10–40% sucrose gradients followed by ultrapelleting and resuspension in 0.1M potassium phosphate pH 7.0.

Final purification of all viruses was performed by ultracentrifugation through 10–40% sucrose gradients; we find that this is more reliable than size-exclusion “spin columns” previously employed (and still used for preliminary cleanup in some cases). It may be possible to improve upon the maximum recovery of 70–80% from sucrose gradients with the use of molecular

weight cutoff filtration (resin or membranes), but this was not attempted in the studies described here. CPMV concentrations were determined by absorbance at 260 nm (0.1 mg/mL virus sample gives an absorbance of 0.8). Q β concentrations were determined using the modified Lowry protein assay.⁶⁴ Unless otherwise indicated, all virus samples were handled in 0.1 M potassium phosphate buffer (pH 7.0).

Acylation reactions were performed as shown in Figure 3, followed by purification by sucrose-gradient ultracentrifugation. At each stage (acylation and CuAAC coupling), only intact particles were observed, as characterized by sucrose gradient sedimentation and size-exclusion chromatography.

Synthesis of Glycan Azides

Compounds **3** and **4** were synthesized as previously described from α -mannose pentaacetate or β -galactose pentaacetate.⁶⁵ A solution of pentaacetate (316 mM) and 2 equiv (633 mM) 2-azidoethanol (CH₂Cl₂) was placed under dry nitrogen atmosphere, cooled to 0°C, and treated with freshly distilled BF₃·Et₂O (2 equiv) in dropwise fashion. The mixture was stirred for 1 hour and the cooling bath was removed to allow stirring overnight at room temperature. The reaction was followed by silica gel thin layer chromatography (TLC, 2:3 EtOAc:hexanes), with the product showing R_f=0.4. The mixture was neutralized with solid sodium bicarbonate, filtered, and evaporated. The residue was purified by flash chromatography on silica gel (gradient of 10–50% EtOAc in hexanes) to obtain the intermediate pentaacetate azidoethyl adducts in 60–80% yields as colorless oils. EI-MS (M+H⁺) 417.

Each protected azide-carbohydrate was dissolved in MeOH with 3 Å molecular sieves under a nitrogen atmosphere. NaOMe (1% in MeOH, approximately 1 equiv with respect to acetate groups) was added dropwise and the reaction was stirred for 45 minutes at room temperature, with monitoring by TLC (2:3 EtOAc:hexanes, product R_f=0.1). Dowex 50W x2-200 resin was added to neutralize the reaction, followed by filtration and concentration by rotatory evaporation. The product was purified by flash chromatography on silica gel (9:1 CH₂Cl₂:MeOH) to obtain pure **3** or **4** in approximately 80% yield as a colorless oil. The NMR spectra of **3** matched the published data.⁶⁶ The α -anomer of azidoethylgalactose has been reported;⁶⁷ compound **4** is assigned as the β -configuration due to its very different anomeric proton chemical shift and coupling constant (4.22 ppm, J=10 Hz vs. 4.67 ppm, J=3.3 Hz for the α -anomer). ¹H NMR spectra of **3** and **4** are shown in Supporting Information.

Surface Immobilization of Lectins

BSI chips were manufactured by Micronit, Inc. The chips were isotropically etched in borosilicate glass to give a cross section described by two quarter-circles of 40 μ m radius connected by a 10 μ m flat region. We chose to first immobilize a layer of avidin, to which biotinylated lectins could be attached by simple mixing. The channel surface was cleaned with 10% KOH in methanol for 30 minutes, then rinsed with deionized water and dried in air. The channel was then filled with a toluene solution of 3-mercaptopropyl triethoxysilane (**1**, 2% in toluene) for 60 minutes to introduce surface thiol groups, then rinsed again with deionized water and air dried. The chip was then filled with 1 mM *N*-[γ -maleimidobutyryloxy]succinimide ester (**2**) in absolute ethanol for 30 minutes, rinsed, air dried, and then soaked in a solution of ExtrAvidin (1 mg/mL in PBS) overnight. After rinsing with PBST (PBS with 0.05% Tween-20) and PBS, the biotinylated lectin was then attached to the surface by filling the channel with a 1 mg/mL solution for 60 minutes, followed by washing with sodium acetate buffer. The activity of the immobilized avidin was independently verified by fluorescent labeling of the channels using biotinylated fluorescein (Supporting Information).

For the experiment shown in Figure 2, conA was biotinylated by mixing it with a 10-fold molar excess of *N*-hydroxysuccinimidobiotin (CAS# 35013-72-0) for one hour at room temperature, followed by filtration three times through 10,000 MW cutoff microcentrifuge filtration tubes to remove the excess reagent, rinsing with 50 mM sodium acetate buffer containing 1 mM Ca^{2+} and Mn^{2+} (pH = 6.8).

Backscattering Interferometry

BSI has been described in detail previously;^{32,34} we briefly summarize the apparatus and method here. The instrument consists of a red helium-neon (HeNe) laser ($\lambda = 632.8$ nm) to illuminate the microfluidic channel and a camera for transduction of the signal contained in the fringe pattern. In a simple optical train, the laser is coupled to a collimating lens through a single-mode fiber, producing a 100 μm diameter beam and a probe volume of approximately 300 picoliters. When the laser beam impinges the channel and interacts with its surface, a set of high-contrast interference fringes is produced. The spatial position of these fringes depends upon the refractive index of the fluid within the channel and is monitored in the direct backscatter region. The change in the fringe position is quantified using a CCD array in combination with Fourier analysis methodology that allows the positional shift to be interpreted as a change in phase, calculated in the Fourier domain. All data was collected in real-time utilizing an in-house program written in LabViewTM.

To perform the binding studies, the lectin was immobilized onto the surface of the channel utilizing the scheme outlined above. A reference solution of sodium acetate buffer was introduced into the channel by pipetting 1 μL of solution into the inlet reservoir and applying a vacuum to the outlet well. Once the solution had filled the channel, the flow was stopped and the backscatter signal, or binding event, was monitored for one minute. This process was repeated iteratively for increasing concentrations of the carbohydrate from 10–100 M. The channel was rinsed with sodium acetate buffer between each analyte sample to remove any bound sugar from the Con A; the BSI signal was always observed to return to the baseline value after such rinsing. The same experiments were performed with CPMV particles (0–40 nM in capsids, 0–8 μM in attached sugar), Q β virus-like particles (0–5 nM in capsids, 0–2.5 μM in attached sugar), generation-4 PAMAM dendrimers (0–2.5 μM in dendrimer, 0–100 μM in attached sugar), and generation-6 PAMAM dendrimers (0–60 nM in dendrimer, 0–5 μM in attached sugar). Wild-type CPMV and Q β particles, as well as G4 and G6 dendrimers bearing only galactose, were used as controls in order to rule out contributions from non-specific adsorption.

The competition data shown in Figure 6B,C were obtained from the following procedure. A 32 nM solution of particle **11** was incubated in a standard extravidin/conA-derivatized channel for one minute, during which time the signal stabilized at approximately 0.03. The value indicated by the horizontal black line in each plot represents the average of these measurements throughout the experiment (standard deviation = 0.005). A solution of conA or mannose, starting with the most dilute concentration, was flowed into the channel, displacing the solution of **11**. The change in signal was monitored for one minute; it reached the indicated value within 15 seconds. The channel was then rinsed extensively with buffer and the process was started again with a fresh 32 nM solution of **11**, and the next highest concentration of reagent.

Data Analysis

A correction must be made for the bulk refractive index change due to the presence of different concentrations of ligand in solution. This is accomplished by recording a calibration curve for the ligand in the absence of immobilized protein (data not shown). For each experiment, the observed phase change (corrected for bulk ligand effects) was plotted versus concentration in order to create a saturation binding curve. This endpoint analysis plot was then fitted to the

square hyperbolic function (Langmuir isotherm) shown in Equation 1 using Prism™ software to obtain a value for $(1/K_{ads})$.

$$(S - S_0) = \frac{(S_{max} - S_0)[L]}{(1/K_{ads}) + [L]} \quad (1)$$

where S = corrected phase change in the presence of carbohydrate ligand, S_0 = corrected phase change in the absence of ligand (buffer only), S_{max} = maximum corrected phase change in the presence of ligand (assumed to represent full binding to the immobilized protein), and $[L]$ = concentration of carbohydrate ligand in solution. Use of the Frumkin isotherm equation as described by Kiessling and coworkers¹³ gave no indication of attractive or repulsive interactions between the adsorbing molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Functional preparation of immobilized lectins in for BSI measurements.

Figure 2.

Measurement of monovalent carbohydrate binding to immobilized biotin-conA.

(A) Comparison of sugars on immobilized commercial biotin-conA. (B) Comparison of mannose binding to different sources of biotinylated conA (deposited at the same concentration): red squares = commercially available material; blue diamonds = conA biotinylated in house; green triangles = an equimolar mixture of commercial conA-biotin and biotinylated BSA. (C) Comparison of sugars on immobilized biotin-BS-1. Error bars on all plots are derived from three independent experiments using different chips, showing a high degree of reproducibility.

conjugate	Gen	glycans per particle		conjugate	Gen	glycans per particle	
		Man	Gal			Man	Gal
16	G4	42	0	21	G6	111	0
17	G4	38	9	22	G6	86	33
18	G4	31	10	23	G6	53	33
19	G4	18	31	24	G6	31	88
20	G4	0	42				

Figure 3.
Polymer virus- and dendrimer-carbohydrate adducts.

Figure 4.
Measurement of polyvalent virus-carbohydrate binding to immobilized biotin-conA.

Figure 5.

Binding of mixed mannose-galactose particles to immobilized conA and BS-1 lectins. (A, B) BSI measurements of the binding of the indicated CPMV-(sugar)₁₉₂ particles to immobilized conA and BS-1. (C, D) Plots of values of $1/K_{ads}$ derived from A and B, in terms of the overall concentrations of the indicated glycans presented on the virus surface (black) and the concentrations of the virus particles (blue). (E, F) BSI measurements of the binding of the indicated G4 and G6 dendrimer-(sugar)_n particles to immobilized conA. (G, H) Plots of $1/K_{ads}$ derived from E and F. In all cases, $1/K_{ads}$ values were calculated from each curve independently, ignoring the relative differences in signal magnitudes between curves. See the text for further discussion.

Figure 6.

(A and B) BSI measurement of the treatment of CPMV-mannose particle **11** bound to an extravidin-conA channel with increasing concentrations of (A) soluble conA and (B) mannose. (C) Cartoon representation of experiments measuring binding and competition with free sugar and receptor. For particles bearing different numbers of sugars, saturation is reached with approximately the same number of virions bound, and yet the signal intensities in the two cases are markedly different.