Chemistry & Biology, Vol. 11, 875–881, June, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.chembiol.2004.04.010

Oligosaccharide and Glycoprotein Microarrays as Tools in HIV Glycobiology: Glycan-Dependent gp120/Protein Interactions

Eddie W. Adams,[1,5] Daniel M. Ratner,[1,5] Heidi R. Bokesch,[2] James B. McMahon,[3] Barry R. O'Keefe,[3] and Peter H. Seeberger[1,4,*]
[1]Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
[2]Basic Research Program
SAIC-Frederick, Inc.
[3]Molecular Targets Development Program
Center for Cancer Research
NCI-Frederick
Frederick, Maryland 21702
[4]Laboratorium für Organische Chemie
ETH Hönggerberg/HCI F 315
Wolfgang-Pauli-Strasse 10
CH-8093 Zürich
Switzerland

Summary

Defining HIV envelope glycoprotein interactions with host factors or binding partners advances our understanding of the infectious process and provides a basis for the design of vaccines and agents that interfere with HIV entry. Here we employ carbohydrate and glycoprotein microarrays to analyze glycan-dependent gp120-protein interactions. In concert with new linking chemistries and synthetic methods, the carbohydrate arrays combine the advantages of microarray technology with the flexibility and precision afforded by organic synthesis. With these microarrays, we individually and competitively determined the binding profiles of five gp120 binding proteins, established the carbohydrate structural requirements for these interactions, and identified a potential strategy for HIV vaccine development.

Introduction

The HIV-1 envelope glycoproteins gp120 and gp41 are important molecular targets for developing therapies aimed at decreasing patients' viral loads and for prophylaxis in preventing viral transmission. gp120 noncovalently associates with gp41 which resides in the external viral membrane. This heterodimer further associates to form a functional trimer (for review of the HIV infectious process, see [1] and [2]). These trimers initiate viral entry by interactions with CD4 molecules and members of the chemokine receptor family (primarily CCR5 and CXCR4) present on T lymphocytes, macrophages, dendritic cells, and brain microglia. Binding of CD4 induces conformational changes in gp120 that reveal a chemokine receptor binding domain. Subsequent gp120-chemokine receptor interactions unmask gp41 and enable the exposed fusion peptide to insert into the host cell membrane. This sequence of events actuates internalization of HIV by the host cell.

The high spatial density of gp120 on the virion surface makes it the primary target of the humoral immune response to HIV [2, 3]. Accordingly, most vaccine design efforts to generate effective, neutralizing antibodies against HIV have been based on monomeric and oligomeric forms of gp120. However, the steric masking of potential neutralization-sensitive epitopes of gp120 by *N*-linked high-mannose oligosaccharides frustrates both natural and vaccine-promoted humoral responses [4, 5]. In addition, these oligosaccharides are found on numerous host proteins, and are likely to be regarded as "self" or nonimmunogenic, thereby preventing a rigorous antibody response. A notable exception to this immunological tolerance of high-mannose oligosaccharides is the human monoclonal antibody 2G12, which is capable of binding Manα1-2Man-presenting oligosaccharide clusters with nanomolar affinity [6, 7].

Besides immunological means of decreasing viral spread, small molecule inhibitors and HIV binding proteins are being explored as prophylactic measures to prevent HIV entry [8, 9]. High-throughput screens of natural products derived from *Cyanobacteria* have yielded a number of promising anti-HIV agents capable of inhibiting viral entry into host cells. One of these compounds, cyanovirin-*N*, an 11 kDa protein, achieves its antiviral activity by binding the high-mannose oligosaccharides present on HIV gp120 [10, 11]. This interaction is thought to prevent gp120's receptor binding domains from interacting with their targets; alternatively, conformational changes in the glycoprotein subsequent to CVN binding may render these binding domains functionally inactive. As most cell-surface and secreted glycoproteins undergo processing in the Golgi, extensively modifying *N*-linked oligosaccharides, CVN is likely to target virus-associated oligosaccharide but not endogenous glycoprotein. This has been demonstrated by in vivo prophylaxis studies with CVN that have not shown any adverse effects upon host physiology [12].

While the density of carbohydrate present on gp120 prevents efficient generation of potent, neutralizing antibodies, these same oligosaccharides may be viewed as targets for a new class of anti-HIV agents. Thus, a detailed analysis of HIV-glycans will help define the immunology of HIV as well as guide efforts toward prophylaxis. Tools that easily identify additional biologically relevant carbohydrate/protein interactions will aid these investigations. We and others have recently developed high-density carbohydrate microarrays for the high-throughput analysis of carbohydrate-protein interactions [14, 15]. (For a review of different carbohydrate arrays described to date, see [13].) These miniaturized assays substantially decrease the amount of carbohydrate required for immobilization, in addition to the volume and quantity of analyte to be studied. Furthermore, fluorescence-based detection allows multiple binding events to be analyzed simultaneously. Here, we exploit oligosaccharide and glycoprotein microarrays to study

*Correspondence: seeberger@org.chem.ethz.ch
[5]These authors contributed equally to this work.

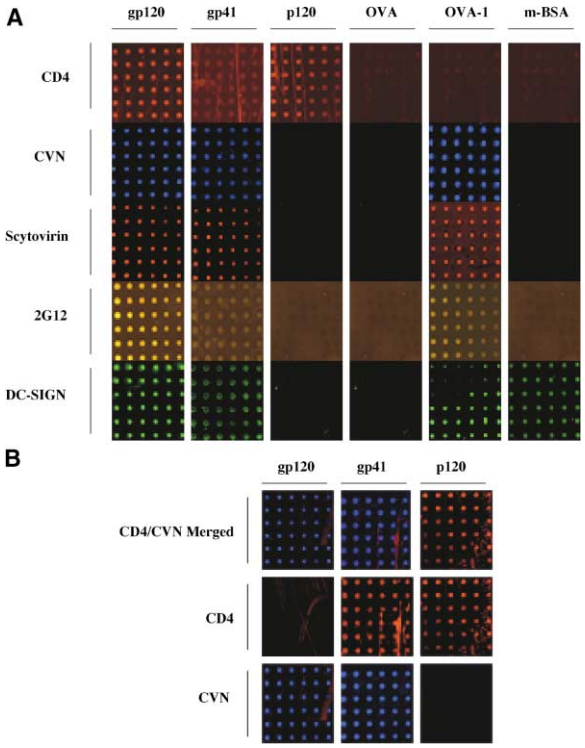

Figure 1. Analyzing Glycoprotein Binding Individually and Competitively

(A) Glycoprotein microarrays (including deglycosylated gp120, p120) were incubated with fluorophore-labeled proteins, and binding events were detected with a DNA-microarray scanner.

(B) A glycoprotein array (plus p120) incubated with CD4 (25 μg/ml) and then coumarin-CV-N (25 μg/ml) analyzed for displacement of CD4 binding by CV-N.

the glycan-dependent binding interactions of four gp120 binding proteins: the dendritic cell lectin DC-SIGN [16], the antibody 2G12, cyanovirin-*N*, and a recently identified anti-HIV protein, scytovirin [17]. We also investigate the nonglycan-dependent interactions of CD4 with gp120 in the presence of these potential inhibitors.

Results and Discussion

Initially, we sought to determine if the glycan binding profiles of the above proteins are dependent on the polypeptide backbone to which the high-mannose oligosaccharides are appended. To evaluate the role of the polypeptide backbone, microarrays bearing natural and modified glycoproteins as well as neoglycoproteins were fabricated by reacting amine-modified glass slides with ethylene-glycol disuccinimide to form a hydrophilic, amine-reactive surface. Proteins were arrayed at high density on these functionalized surfaces with a contact-printing robot and incubated overnight in a humidity chamber. The slides were subsequently quenched in a solution of bovine serum albumin to inactivate remaining succinimidyl groups. Each slide was incubated with one fluorophore-labeled protein, washed, and scanned to establish binding (Figure 1A).

As anticipated, gp120 is bound by each of the proteins. Coincubation of each protein with $(Man)_9(GlcNAc)_2$ successfully inhibited the respective interactions with gp120 (data not shown) in accordance with the known specificities of these proteins. Coincubation of these same carbohydrate structures did not affect CD4 binding to gp120 (data not shown). All five proteins were also shown to interact with gp41. These interactions were inhibited by coincubation with $(Man)_9(GlcNAc)_2$ except in the case of CD4. While recognition of the high-mannose oligosaccharides on gp41 [18] has been described for CVN [19] and scytovirin [17], this is the first demonstration of gp41 binding by DC-SIGN and 2G12. The physiological relevance of the observed g41 binding by DC-SIGN requires further study as it has not yet been determined if gp41 is exposed upon DC-SIGN-gp120 interactions. It also remains to be determined whether interactions between 2G12 and exposed gp41 can effectively inhibit viral entry. Pending further experimentation, speculation on the biological significance of these findings would be premature.

In order to determine if any of the aforementioned glycan-dependent gp120 binding proteins could inhibit CD4-gp120 interactions, we took advantage of our fluorescence-based detection system to conduct a series of sequential incubations with fluorophore-labeled binding partners. In these experiments, soluble CD4 was incubated with the glycoprotein microarray followed by one of the potential inhibitors. Thereafter, CD4 was detected with a fluorophore-labeled anti-CD4 monoclonal antibody (independent control incubation with anti-CD4 revealed little to no nonspecific binding; data not shown). Alternatively, soluble CD4 was added to the arrays only after preincubation with potential inhibitors. In agreement with previous studies, we observed that CD4 bound gp120 pretreated with CVN, indicating that CVN does not block or disrupt the CD4 binding site on gp120 [20]. Remarkably, however, we observed that CVN completely displaced bound CD4 from gp120 pretreated with soluble CD4 (Figure 1B). CD4 bound to nonglycosylated gp120 (p120) was not disrupted by incubation with CVN, indicating the glycan-dependent nature of CVN's mode of action. Previous mechanistic explanations for CVN's potent inhibitory properties postulated that CVN may form aggregates on the virion surface that prevent membrane-bound receptors from interacting with their respective gp120 binding domains [20]. Our findings suggest a new mechanism in which treatment of cell-associated (i.e., CD4-bound) gp120 with CVN induces conformational changes in gp120 that disrupt existing gp120-CD4 interactions. As our experiments were conducted with soluble CD4 and immobilized gp120, additional inhibition experiments with cell-associated CD4 and whole virion are needed to support this new mechanism.

Neoglycoproteins were employed to study the peptide context in which glycans are presented. Ovalbumin, a glycoprotein bearing both hybrid and complex-type oligosaccharides, is not bound by any of the proteins. In contrast, ovalbumin modified with high-mannose oligosaccharide **1** (ova-**1**) via a nonnatural linkage is bound by DC-SIGN, CVN, scytovirin, and, to a lesser extent, 2G12. This observation suggests that carbohydrate recognition by these proteins is largely insensitive to the

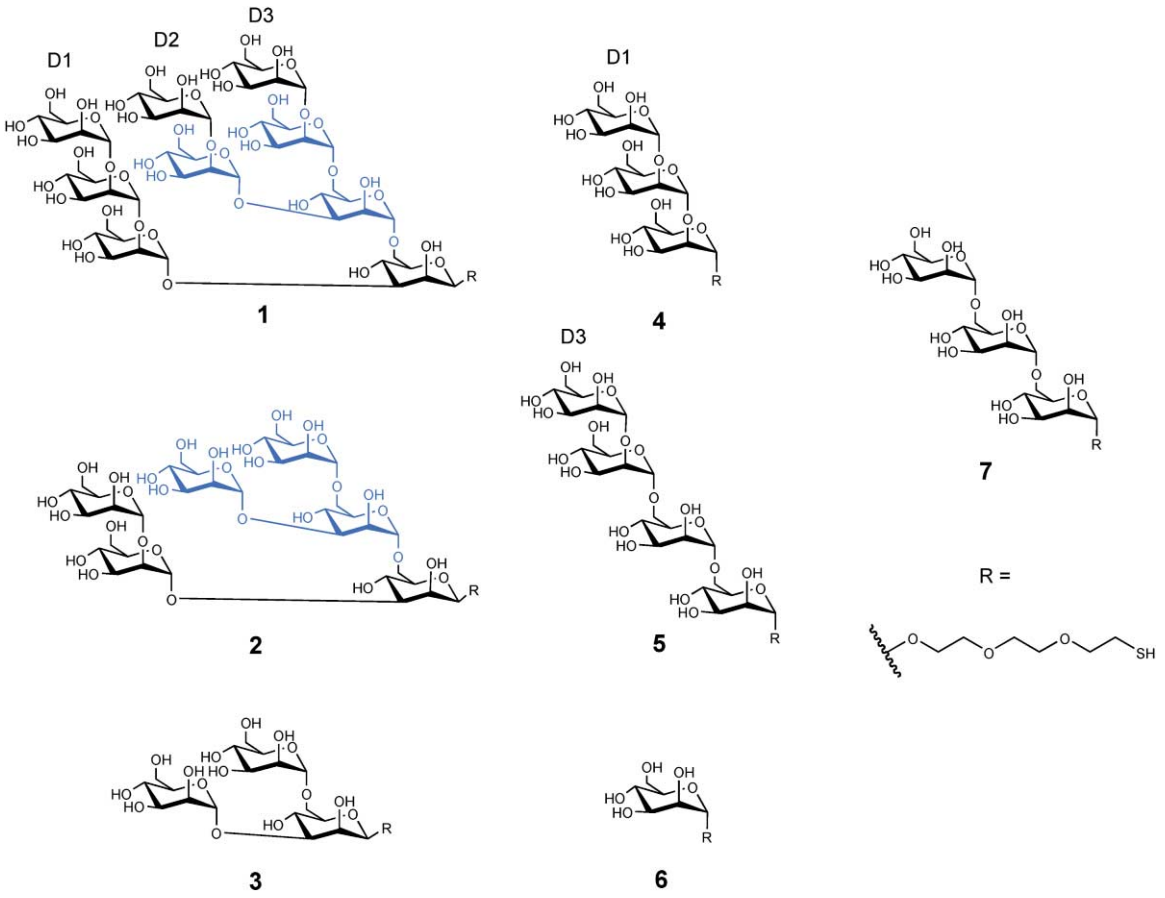

Figure 2. High-Mannose Oligosaccharide **1** and Synthetic Substructures Utilized in This Study

Stereochemistry as indicated at reducing end. The branched outer-trimannoside unique to high-mannose oligosaccharides is highlighted in blue. Reducing-end stereochemistry accurately represented by –R.

underlying polypeptide chain, supporting the notion that the density of the displayed carbohydrate determines binding [21].

The observed binding of 2G12 to ova-**1** prompted us to investigate whether 2G12 would bind arrays of oligosaccharides. Epitope mapping studies with 2G12 have shown that it binds a conserved group of *N*-linked high-mannose oligosaccharides present on gp120, making it an effective neutralizing antibody against a number of primary HIV isolates [6]. If 2G12 could bind high-mannose oligosaccharides in the absence of a peptide backbone, a vaccine composed of clusters of these oligosaccharides might generate a 2G12-like response to gp120 glycans.

2G12-carbohydrate interactions were evaluated with microarrays of **1** and five other synthetic substructures related to **1** (Figure 2). By printing the synthetic oligosaccharides across a wide range of concentrations, it is possible to establish the saturation point for observed binding by a fluorescently-labeled protein (E.W.A. and D.M.R., unpublished results). At this concentration, a carbohydrate binding profile can be made for a given protein by comparing the integrated fluorescence between the spots of different immobilized oligosaccharide. Incubation of 2G12 with the microarray revealed antibody binding at spots corresponding to oligosaccharides **1**, **2**, **4**, and **5**, but not to the branched trimannoside **3** or mannose **6** (Figure 3A). The only structural motif in common for oligosaccharides **4** and **5** is the Manα1-2Man linkage, suggesting that this glycosidic linkage alone is necessary for recognition by 2G12. Based on the observation that incubation of gp120 with a α1-2 mannosidase greatly diminished 2G12 binding, previous studies [6, 22] concluded that 2G12 recognizes the Manα1-2Man linkages present in **1**. A single microarray allowed for rapid confirmation of this structural requirement for 2G12 recognition based on the diversity of glycosidic linkages attained by chemical synthesis. In addition, the microarray enabled direct verification of 2G12 binding to carbohydrates in the absence of a polypeptide backbone.

We used our microarrays to study the carbohydrate recognition profile of the cyanobacterial protein, scytovirin [17]. Scytovirin is a 9.7 kDa protein isolated from aqueous extracts of the cyanobacterium *Scytonema varium*. The protein binds gp120, gp160, and gp41 and has potent anticytopathic activity against primary isolates of HIV-1. Initial studies demonstrated that scytovirin binds HIV gp120 through a carbohydrate-dependent mechanism that is blocked by soluble $(Man)_9(GlcNAc)_2$, and

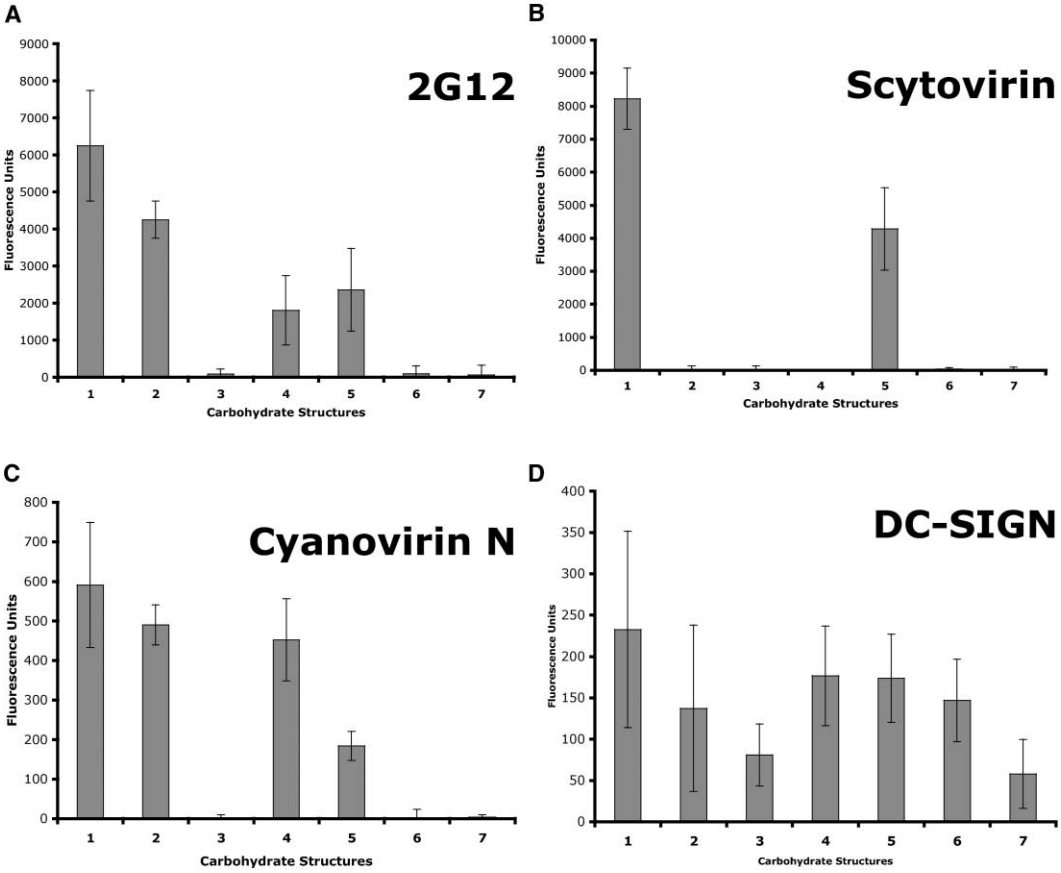

Figure 3. Analysis of High-Mannose Binding Proteins with Carbohydrate Microarrays Reveals Structural Requirements for Binding

(A) 2G12, (B) Scytovirin, (C) CVN, (D) DC-SIGN. Each protein was incubated with microarrays bearing carbohydrates **1–7** and analyzed as described (Experimental Procedures).

$(Man)_8(GlcNAc)_2$, but not $(Man)_7(GlcNAc)_2$. However, structural determination has not established which specific mannose residues are missing from the truncated high-mannan $(Man)_7(GlcNAc)_2$.

Analysis of scytovirin's carbohydrate binding profile with our microarrays revealed that of all the structures present, scytovirin bound only **1** and **5** (Figure 3B). This finding suggests that the terminal α1-2 mannose linkage is necessary for recognition of the underlying α1-6 trimannoside moiety unique to the D3 arm. If the Manα1-6Man linkages alone were sufficient for scytovirin binding, oligosaccharides **2** and **3** also should have been bound. Likewise, if Manα1-2Man glycosidic linkages alone were sufficient for recognition by scytovirin, structures **2** and **4** would have been bound. These results suggest that recognition of high-mannose oligosaccharides by scytovirin occurs by a markedly different mechanism than that observed for both 2G12 and CVN. Both 2G12 and CVN bind terminal Manα1-2Man independent of the underlying linkages found in the D1, D2, and D3 arms (Figures 3A and 3C).

To confirm the role of the terminal Manα1-2Man glycosidic linkage in binding of the D3 arm by scytovirin, we took advantage of our linear synthetic strategy [23] to prepare **7**, a truncated derivative of the D3 arm lacking the terminal Manα1-2Man linkage. Microarrays were prepared with structures **1–7** and incubated with scytovirin, CVN, and 2G12. None of these proteins bound oligosaccharide **7** (Figures 3B and 3A), confirming the specificity of 2G12 and CVN for Manα1-2Man linked saccharides and the necessity of this linkage for recognition of the D3 arm by scytovirin. This mechanism of high-mannose oligosaccharide recognition by scytovirin has not been described for any other high-mannose binding protein. On-going NMR and crystallographic studies should elucidate how scytovirin's tertiary structure dictates specific interactions with the D3 arm.

The dendritic cell lectin DC-SIGN was the last gp120 binding protein we chose to analyze. This 44 kDa C-type lectin is expressed by dermal dendritic cells (DCs) in mucosal tissue, by interstitial DCs, and on DCs in the lymph nodes [24]. While initially shown to be a CD4-independent receptor for gp120, recently its role in HIV pathology became clear. DC-SIGN binds gp120 in a carbohydrate-dependent manner via the glycoprotein's high-mannose oligosaccharides [24, 25]. This interaction promotes internalization of virus by the DC to a nonlysosomal compartment, where HIV appears to be protected from degradation and remains infectious for several days [26]. Finally, DC-$CD4^+$ T cell interaction leads to productive infection of the recipient lymphocyte.

Some studies of DC-SIGN's carbohydrate binding profile, including X-ray analysis, suggested that DC-SIGN specifically recognizes the branched outer trimannoside motif of high-mannose oligosaccharides (Figure 2) [25, 27]. However, this lectin is also capable of high-affinity interactions with branched fucosylated structures, such as the Lewis blood group determinants [28].

We used our arrays to further analyze DC-SIGN-carbohydrate interactions and determine the importance of branched glycosidic linkages for carbohydrate recognition. Incubation of the labeled extracellular domain of DC-SIGN with carbohydrate arrays bearing structures **1–7** revealed that all structures were bound by DC-SIGN (Figure 3D). The integrated fluorescence signals from each structure suggests that DC-SIGN binds these carbohydrates in the following order: **1** > **4** ≥ **5** > **6** > **2** > **3** > **7**. The observation that **3** is bound with less affinity than any of the other branched oligosaccharides is likely due to the stereochemistry at the reducing end. As noted in the crystal structure of DC-SIGN, a β-linkage at the anomeric carbon of the central mannose residue would cause substituents at this center to clash with Phe^{325} [25]. This structural constraint is relieved in **1** and **2** where the core mannose of the branched outer trimannoside moiety (Figure 2) (Manα1-6[Manα1-3]Man) is in the α-anomeric conformation.

DC-SIGN's binding of unbranched oligosaccharides must be explained by additional protein-carbohydrate interactions. All C-type lectins studied to date bind carbohydrates through contacts promoted via calcium chelation by the 3′ and 4′ hydroxyl groups of the nonreducing terminal carbohydrate residue [29]. If the unbranched oligosaccharides **4**, **5**, and **7** were bound by DC-SIGN in this manner, it would be expected that these structures plus monosaccharide **6** would be bound with similar affinities. However, the observed fluorescence intensities (Figure 3D) indicate that other contacts between DC-SIGN's carbohydrate recognition domain and oligosaccharides **4**, **5**, and **7** must occur and account for the higher affinities observed here.

The inhibition of viral entry is a promising avenue for combating HIV infection [9, 30, 31]. Identifying proteins and small molecules that interfere with HIV host entry is vital to this pursuit. The glycans of HIV have long been studied for their involvement in the infectious process, their antigenicity, and their ability to neutralize the humoral response. It is postulated that an evolving glycan-shield aids HIV's evasion of the immune response [32]. Therefore, the study of agents that bind these glycans, irrespective of their placement on the polypeptide backbone, can facilitate circumvention of HIV's evasion mechanisms. Here, we demonstrated the use of carbohydrate and glycoprotein microarrays for the study of gp120 binding proteins, and defined the carbohydrate structural requirements sufficient for binding.

Using our microarray platform we have defined the binding profile of a novel HIV-inactivating protein, scytovirin, and identified a new mechanism of recognition of high-mannose oligosaccharides. Our study of DC-SIGN has elaborated its carbohydrate binding profile to include unbranched oligosaccharide structures. Analyses of CVN and 2G12 have confirmed the necessity of Manα1-2Man linkages for carbohydrate recognition. Furthermore, our study of 2G12 has definitively demonstrated that 2G12 can bind high-density arrays of Manα1-2Man-containing oligosaccharides in the absence of a polypeptide backbone. We feel this finding and the synthetic derivatives of **1** employed in the analysis of 2G12 will prove particularly important in aiding the design of carbohydrate-based vaccines aimed at eliciting a 2G12-like response.

Significance

Utilizing chemistries that permit the precise immobilization of synthetically defined oligosaccharide structures, this study continues the expansion of microarray technology into the burgeoning field of glycobiology. In an effort to go beyond proof-of-concept-type applications, we demonstrate the potential of carbohydrate microarray analysis by probing the carbohydrate affinity and structural requirements of four well-known and relevant gp120 binding proteins, and the novel HIV-inactivating protein scytovirin. The total synthesis of several unique structural constituents of high-mannose oligosaccharides enabled a microarray-based profiling of the binding requirements of these proteins. By including synthetic neoglycoproteins in the microarrays, additional information was gleaned regarding the importance of the underlying peptide context in which glycans are presented to their binding partners. The successful application of carbohydrate microarrays to real world problems in glycobiology will be further enhanced as additional linkage chemistries provide a wider range of glycans available for study in the microarray format. This will enable large-scale screening of interactions where complex carbohydrates are thought to play a role, akin to the whole-proteome screening assays envisaged for protein microarrays.

Experimental Procedures

Preparation of Oligosaccharides 1–7

Sulfhydryl-containing ethylene glycol derivatized oligosaccharides **1–7** (Figure 2) were prepared by methods analogous to those described in the literature [15, 23]. Structural confirmation was achieved with NMR, ESI mass spectrometry, and MALDI-TOF mass spectrometry.

Maleimide-Functionalization of Amine-Coated (GAPS) Slides and Carbohydrate Microarray Fabrication

Sulfhydryl-reactive slides were prepared in batches of two GAPS slides (Corning) incubated overnight at room temperature in 45 ml anhydrous *N,N*-dimethylformamide (DMF, Aldrich), 10 mg succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC, Pierce Endogen) and 880 μl *N,N*-diisopropylethylamine (Aldrich). Slides were washed with 3 volumes methyl alcohol, dried under a stream of nitrogen, and stored in a dessicator prior to printing. Thiol-containing oligosaccharides **1–7** were incubated at room temperature with 1 equivalent tris-(carboxyethyl)phosphine hydrochloride (TCEP, Pierce Endogen) in 1× PBS for 1 hr. The structures were printed at concentrations ranging from 0.1 mM to 2 mM on maleimide-derivatized GAPS slides using a MicroGrid TAS array printer (30% humidity, 120 μm spots with 300 μm spacing). Printed slides were incubated 12 hr in a humidity chamber, washed 2 times with distilled H_2O, and then incubated for 1 hr in 1 mM 2-(2-(2-mercaptoethoxy)ethoxy)ethanol in PBS (50 ml) to quench reactive maleimide groups. Slides were rinsed with distilled H_2O (3 × 50 ml), 95% ethanol (3 × 50 ml), and stored in a dessicator prior to use.

Succinimidyl-Functionalization of Amine-Coated (GAPS) Slides and Glycoprotein Microarray Fabrication

Nonglycosylated gp120 and gp120 were obtained through the NIH AIDS Reference and Reagent Program. All other glycoproteins were obtained from Sigma-Aldrich except for gp41 (Advanced Biotechnologies, Inc.) Amine-reactive GAPS slides were prepared by treatment with ethylene-glycol-bis(succinimidylsuccinate) (EGS, Pierce Endogen) in anhydrous DMF similar to preparation of maleimide-functionalized slides (see above). Glycoproteins (100 μg/ml in PBS) were printed on amine reactive GAPS slides with a MicroGrid TAS array printer (30% humidity, 120 μm spots with 300 μm spacing). Printed slides were incubated 12 hr in a humidity chamber, washed 2 times with distilled H_2O, and then incubated for 2 hr in 1% BSA in PBS to quench reactive succinimidyl groups. Slides were rinsed with PBS (3 × 50 ml), dried under a stream of nitrogen, and stored in a dessicator prior to use.

Oligosaccharide Modification of Ovalbumin

1.9 mg sulfosuccinimidyl 4-[*N*-maleimidomethyl]-cyclohexane-1-carboxylate (Sulfo-SMCC, Pierce Endogen) was dissolved in 88 μl DMF and added to 5 mg ovalbumin (Sigma) in 315 μl PBS. The reaction solution was mixed for 1 hr at room temperature. Maleimide-activated ovalbumin was purified from nonreacted Sulfo-SMCC by gel filtration on a NAP-25 desalting column preequilibrated in PBS. The ovalbumin fractions were collected and mixed with thiol-containing oligosaccharide **1** (900 μg), which had been previously reduced with 1 equivalent TCEP. This reaction proceeded for 12 hr at room temperature with constant mixing. Modified ovalbumin was purified from excess oligosaccharide by multiple rounds of centrifugal ultrafiltration with Vivaspin 10,000 MW cut-off cartridges (Vivascience). Purified protein was lyophilized and stored at −20°C until use.

Array Incubation and Analysis

2G12 and soluble CD4 were obtained from the NIH AIDS Reagent and Reference Program. 2G12 was labeled with NHS-activated Cy3 (Amersham). FITC-labeled extracellular domain of DC-SIGN was a kind gift of Professor Kurt Drickamer (Oxford, UK). Cyanovirin-*N* was labeled with NHS-activated coumarin (Pierce Endogen) and scytovirin was labeled with NHS-activated Cy5 (Amersham). All proteins were purified by gel filtration to remove nonreacted dye and used at a concentration of 20 μg ml^{-1} in 50 mM HEPES buffer (pH 7.5), 0.1 M NaCl, and 1% BSA. DC-SIGN incubations were performed in the same buffer, with the addition of $CaCl_2$ to a final concentration of 1mM. Array incubations were performed as follows: 5 μl of fluorophore-labeled protein were placed between array slides and plain glass coverslips and incubated for 30 min at room temperature. The arrays were washed with 50 mM HEPES(pH 7.5), 1% Tween 20, 0.1% BSA (3 × 50 ml), twice with distilled water (50 ml), and then centrifuged at 200 × g for 5 min to ensure dryness. For detection of bound CD4, arrays were incubated with an Alexa-647-labeled monoclonal antibody against CD4 (Molecular Probes) and then washed as above. Microarrays were scanned on an ArrayWoRx fluorescence slide scanner (Applied Precision) and fluorescence intensities from these scans were integrated on Digital Genome (MolecularWare).

Acknowledgments

We thank Sean Milton of MIT's Biomicroarray Center for assistance in array fabrication. We also thank Professor Kurt Drickamer of the University of Oxford for his generous gift of fluorescene-labeled DC-SIGN. This research was supported by an NIH Biotechnology Training Grant (for D.M.R) and an NDSEG Fellowship (for E.W.A.). P.H.S. thanks GlaxoSmithKline (Scholar Award), the Alfred P. Sloan Foundation (Fellowship), and Merck for financial support. This publication has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract number NO1-CO-12400. The content of this article does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade name, commercial products, or organization imply endorsement by the U.S. Government.

Received: February 7, 2004
Revised: April 23, 2004
Accepted: April 26, 2004
Published: June 25, 2004

References

1. Wyatt, R., and Sodroski, J. (1998). The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science *280*, 1884–1888.
2. Poignard, P., Saphire, E.O., Parren, P.W., and Burton, D.R. (2001). gp120: Biologic aspects of structural features. Annu. Rev. Immunol. *19*, 253–274.
3. Burton, D.R. (1997). A vaccine for HIV type 1: the antibody perspective. Proc. Natl. Acad. Sci. USA *94*, 10018–10023.
4. Kwong, P.D., Doyle, M.L., Casper, D.J., Cicala, C., Leavitt, S.A., Majeed, S., Steenbeke, T.D., Venturi, M., Chaiken, I., Fung, M., et al. (2002). HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. Nature *420*, 678–682.
5. Wyatt, R., Kwong, P.D., Desjardins, E., Sweet, R.W., Robinson, J., Hendrickson, W.A., and Sodroski, J.G. (1998). The antigenic structure of the HIV gp120 envelope glycoprotein. Nature *393*, 705–711.
6. Scanlan, C.N., Pantophlet, R., Wormald, M.R., Ollmann Saphire, E., Stanfield, R., Wilson, I.A., Katinger, H., Dwek, R.A., Rudd, P.M., and Burton, D.R. (2002). The broadly neutralizing anti-human immunodeficiency virus type 1 antibody 2G12 recognizes a cluster of α1→2 mannose residues on the outer face of gp120. J. Virol. *76*, 7306–7321.
7. Calarese, D.A., Scanlan, C.N., Zwick, M.B., Deechongkit, S., Mimura, Y., Kunert, R., Zhu, P., Wormald, M.R., Stanfield, R.L., Roux, K.H., et al. (2003). Antibody domain exchange is an immunological solution to carbohydrate cluster recognition. Science *300*, 2065–2071.
8. Chan, D.C., and Kim, P.S. (1998). HIV entry and its inhibition. Cell *93*, 681–684.
9. Howard, O.M., Korte, T., Tarasova, N.I., Grimm, M., Turpin, J.A., Rice, W.G., Michejda, C.J., Blumenthal, R., and Oppenheim, J.J. (1998). Small molecule inhibitor of HIV-1 cell fusion blocks chemokine receptor-mediated function. J. Leukoc. Biol. *64*, 6–13.
10. Boyd, M.R., Gustafson, K.R., McMahon, J.B., Shoemaker, R.H., O'Keefe, B.R., Mori, T., Gulakowski, R.J., Wu, L., Rivera, M.I., Laurencot, C.M., et al. (1997). Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. Antimicrob. Agents Chemother. *41*, 1521–1530.
11. Bolmstedt, A.J., O'Keefe, B.R., Shenoy, S.R., McMahon, J.B., and Boyd, M.R. (2001). Cyanovirin-N defines a new class of antiviral agent targeting N-linked, high-mannose glycans in an oligosaccharide-specific manner. Mol. Pharmacol. *59*, 949–954.
12. Tsai, C.C., Emau, P., Jiang, Y., Tian, B., Morton, W.R., Gustafson, K.R., and Boyd, M.R. (2003). Cyanovirin-N gel as a topical microbicide prevents rectal transmission of SHIV89.6P in macaques. AIDS Res. Hum. Retroviruses *19*, 535–541.
13. Drickamer, K., and Taylor, M.E. (2002). Glycan arrays for functional glycomics. Genome Biol. *3* (reviews), 1034.
14. Adams, E.W., Ueberfeld, J., Ratner, D.M., O'Keefe, B.R., Walt, D.R., and Seeberger, P.H. (2003). Encoded fiber-optic microsphere arrays for probing protein-carbohydrate interactions. Angew. Chem. Int. Ed. Engl. *42*, 5317–5320.
15. Ratner, D.M., Adams, E.W., O'Keefe, B., Mrksich, M., and Seeberger, P.H. (2004). Probing protein-carbohydrate interactions with microarrays of synthetic oligosaccharides. Chembiochem *5*, 379–383.
16. Geijtenbeek, T.B., Torensma, R., van Vliet, S.J., van Duijnhoven, G.C., Adema, G.J., van Kooyk, Y., and Figdor, C.G. (2000). Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell *100*, 575–585.
17. Bokesch, H.R., O'Keefe, B.R., McKee, T.C., Pannell, L.K., Pat-

terson, G.M., Gardella, R.S., Sowder, R.C., 2nd, Turpin, J., Watson, K., Buckheit, R.W., Jr., et al. (2003). A potent novel anti-HIV protein from the cultured cyanobacterium Scytonema varium. Biochemistry *42*, 2578–2584.

18. Johnson, W.E., Sauvron, J.M., and Desrosiers, R.C. (2001). Conserved, N-linked carbohydrates of human immunodeficiency virus type 1 gp41 are largely dispensable for viral replication. J. Virol. *75*, 11426–11436.
19. O'Keefe, B.R., Shenoy, S.R., Xie, D., Zhang, W., Muschik, J.M., Currens, M.J., Chaiken, I., and Boyd, M.R. (2000). Analysis of the interaction between the HIV-inactivating protein cyanovirin-N and soluble forms of the envelope glycoproteins gp120 and gp41. Mol. Pharmacol. *58*, 982–992.
20. Esser, M.T., Mori, T., Mondor, I., Sattentau, Q.J., Dey, B., Berger, E.A., Boyd, M.R., and Lifson, J.D. (1999). Cyanovirin-N binds to gp120 to interfere with CD4-dependent human immunodeficiency virus type 1 virion binding, fusion, and infectivity but does not affect the CD4 binding site on gp120 or soluble CD4-induced conformational changes in gp120. J. Virol. *73*, 4360–4371.
21. Shenoy, S.R., Barrientos, L.G., Ratner, D.M., O'Keefe, B.R., Seeberger, P.H., Gronenborn, A.M., and Boyd, M.R. (2002). Multisite and multivalent binding between cyanovirin-N and branched oligomannosides: calorimetric and NMR characterization. Chem. Biol. *9*, 1109–1118.
22. Sanders, R.W., Venturi, M., Schiffner, L., Kalyanaraman, R., Katinger, H., Lloyd, K.O., Kwong, P.D., and Moore, J.P. (2002). The mannose-dependent epitope for neutralizing antibody 2G12 on human immunodeficiency virus type 1 glycoprotein gp120. J. Virol. *76*, 7293–7305.
23. Ratner, D.M., Plante, O.J., and Seeberger, P.H. (2002). A linear synthesis of branched high-mannose oligosaccharides from the HIV-1 viral surface envelope glycoprotein gp120. Eur. J. Org. Chem., 826–833.
24. Geijtenbeek, T.B., Kwon, D.S., Torensma, R., van Vliet, S.J., van Duijnhoven, G.C., Middel, J., Cornelissen, I.L., Nottet, H.S., KewalRamani, V.N., Littman, D.R., Figdor, C.G., and van Kooyk, Y. (2000). DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances *trans*-infection of T cells. Cell *100*, 587–597.
25. Feinberg, H., Mitchell, D.A., Drickamer, K., and Weis, W.I. (2001). Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. Science *294*, 2163–2166.
26. Kwon, D.S., Gregorio, G., Bitton, N., Hendrickson, W.A., and Littman, D.R. (2002). DC-SIGN-mediated internalization of HIV is required for *trans*-enhancement of T cell infection. Immunity *16*, 135–144.
27. Mitchell, D.A., Fadden, A.J., and Drickamer, K. (2001). A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR. Subunit organization and binding to multivalent ligands. J. Biol. Chem. *276*, 28939–28945.
28. Van Die, I., Van Vliet, S.J., Nyame, A.K., Cummings, R.D., Bank, C.M., Appelmelk, B., Geijtenbeek, T.B., and Van Kooyk, Y. (2003). The dendritic cell-specific C-type lectin DC-SIGN is a receptor for Schistosoma mansoni egg antigens and recognizes the glycan antigen Lewis x. Glycobiology *13*, 471–478.
29. Drickamer, K. (1996). Ca(2+)-dependent sugar recognition by animal lectins. Biochem. Soc. Trans. *24*, 146–150.
30. Meanwell, N.A., and Kadow, J.F. (2003). Inhibitors of the entry of HIV into host cells. Curr. Opin. Drug Discov. Dev. *6*, 451–461.
31. Pohlmann, S., and Doms, R.W. (2002). Evaluation of current approaches to inhibit HIV entry. Curr. Drug Targets Infect. Disord. *2*, 9–16.
32. Wei, X., Decker, J.M., Wang, S., Hui, H., Kappes, J.C., Wu, X., Salazar-Gonzalez, J.F., Salazar, M.G., Kilby, J.M., Saag, M.S., et al. (2003). Antibody neutralization and escape by HIV-1. Nature *422*, 307–312.