J|A|C|S COMMUNICATIONS

Published on Web 10/08/2010

High-Affinity Glycopolymer Binding to Human DC-SIGN and Disruption of DC-SIGN Interactions with HIV Envelope Glycoprotein

C. Remzi Becer,[†] Matthew I. Gibson,[†] Jin Geng,[†] Rebecca Ilyas,[‡] Russell Wallis,[§] Daniel A. Mitchell,*[,‡] and David M. Haddleton*[,†]

Department of Chemistry, University of Warwick, CV4 7AL Coventry, U.K., Clinical Sciences Research Institute, Warwick Medical School, University of Warwick, CV2 2DX, Coventry, U.K., and Department of Biochemistry, University of Leicester, LE1 9HN Leicester, U.K.

Received July 7, 2010; E-mail: d.mitchell@warwick.ac.uk; d.m.haddleton@warwick.ac.uk

Abstract: Noncovalent interactions between complex carbohydrates and proteins drive many fundamental processes within biological systems, including human immunity. In this report we aimed to investigate the potential of mannose-containing glycopolymers to interact with human DC-SIGN and the ability of these glycopolymers to inhibit the interactions between DC-SIGN and the HIV envelope glycoprotein gp120. We used a library of glycopolymers that are prepared via combination of copper-mediated living radical polymerization and azide−alkyne [3+2] Huisgen cycloaddition reaction. We demonstrate that a relatively simple glycopolymer can effectively prevent the interactions between a human dendritic cell associated lectin (DC-SIGN) and the viral envelope glycoprotein gp120. This approach may give rise to novel insights into the mechanisms of HIV infection and provide potential new therapeutics.

Noncovalent interactions between complex carbohydrates and proteins drive many fundamental processes within biological systems, including human immunity.[1−4] DC-SIGN (dendritic cell specific ICAM-3 grabbing nonintegrin) is a C-type lectin of significant medical interest that is expressed on the surface of dendritic cells: highly specialized cells that govern immune responses.[5−7] DC-SIGN recognizes oligosaccharide structures on host glycoproteins and regulates immune functions such as cytokine production and antigen presentation. Importantly, DC-SIGN also binds to carbohydrates on the surfaces of lethal opportunistic pathogens, notably HIV-1, enhancing their adhesion, infectivity, and persistence in patients.[5,8] Solid-phase competition assays have demonstrated monosaccharide mannose binding to DC-SIGN with millimolar affinity, and that affinity for oligosaccharide ligands such as $Man_9GlcNAc_2$ is greatly enhanced.[9] High-resolution structural studies have shown that DC-SIGN binds preferentially to complex mannose oligosaccharide structures and the primary carbohydrate-binding site selectively accommodates the equatorial stereochemistry of the C3 and C4 hydroxyls found in the mannopyranosides.[10] Furthermore, it has been determined previously that DC-SIGN exists at the cell surface as a tetramer of identical polypeptide subunits and that the oligomerization of sugar-binding lectin domains within these tetramers is fundamental to achieving the high affinity required for selective binding to densely clustered carbohydrate ligands.[9,11] An important example of such a ligand is the highly glycosylated gp120 envelope glycoprotein of HIV-1. Considering these, DC-SIGN is an attractive target for synthetic mannose-containing glycoconjugates.

In recent years, the use of synthetic glycopolymers to interact with lectins or for other biotechnological applications has gained interest.[12−15] The presentation of multiple carbohydrate moieties along a polymer backbone often results in increased binding to the complementary lectin compared to the individual sugars, due to the cluster glycoside effect.[16−20] Calorimetry studies indicate that this is an entropy-driven process, but questions remain over the exact mechanisms.[21] This increase in avidity has been exploited for anti-adhesion therapies whereby the glycopolymer competes for microbial binding to lectins to prevent infection or induces an anti-microbial cellular response.[22,23] For example, we recently demonstrated that glycopolymer−protein conjugates could interact with mammalian mannose-binding lectin with high affinity.[24] Considering this, synthetic glycopolymers with high affinity for DC-SIGN represent attractive materials that could offer important anti-microbial adhesion properties and provide novel therapeutic strategies for HIV treatment.[25]

In this report we aimed to investigate the potential of mannose-containing glycopolymers to interact with human DC-SIGN and the ability these glycopolymers to inhibit the interactions between DC-SIGN and the HIV envelope glycoprotein gp120. We used a highly efficient post-polymerization modification approach[26,27] based on the alkyne−azide cycloaddition reaction ("click reaction")[28] to create a library of glycopolymers.[29−32] This method allows control over the density of the carbohydrate binding moiety along the polymer chain, while ensuring that each polymer has identical chain length and chain length distribution, which is not possible to achieve by direct polymerization of glycosylated monomers. The well-defined clickable polymer backbone was synthesized by copper-mediated living radical polymerization of trimethylsilyl propargyl methacrylate[33] to give a polymer with a number-average degree of polymerization of ca. 58. Following the removal of the trimethylsilyl group, copper(I)-catalyzed click reaction was used to synthesize five glycopolymer species containing varying densities of α-D-mannose and β-D-galactose, Figure 1.

Multichannel surface plasmon resonance (MC-SPR) was used to investigate the binding affinity of this library of glycopolymers with bacterially expressed soluble recombinant human DC-SIGN tetramers. DC-SIGN was immobilized onto a SPR sensor chip, Figure 1a, and the interactions between DC-SIGN and the glycopolymers were probed as a function of glycopolymer concentration. Figure 2 shows representative SPR sensorgrams of the 100% mannose glycopolymer, **P1**, and HIV gp120 analytes flowed over the immobilized DC-SIGN. This recombinant form of the gp120 envelope glycoprotein of HIV-1 is a biological DC-SIGN ligand known to carry substantial amounts of *N*-linked high mannose oligosaccharides. The sensorgrams demonstrate that both **P1** and gp120 bind to the DC-SIGN with high (nanomolar) affinity, in a dose-dependent fashion. Furthermore, the presentation of mannose in clustered polymeric form was essential for high-affinity

[†] Department of Chemistry, University of Warwick.
[‡] Clinical Sciences Research Institute, Warwick Medical School.
[§] Department of Biochemistry, University of Leicester.

10.1021/ja1056714 © 2010 American Chemical Society

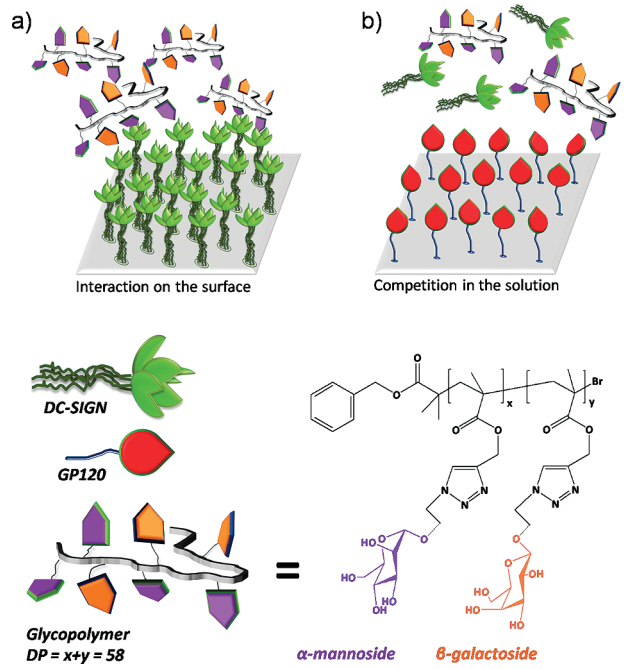


Figure 1. Experimental design. (a) DC-SIGN functionalized surface for evaluating glycopolymers binding affinity. (b) gp120 functionalized surface for competitive binding studies. (Bottom) Schematic structures of DC-SIGN and gp120 and the glycopolymer chemical structure.

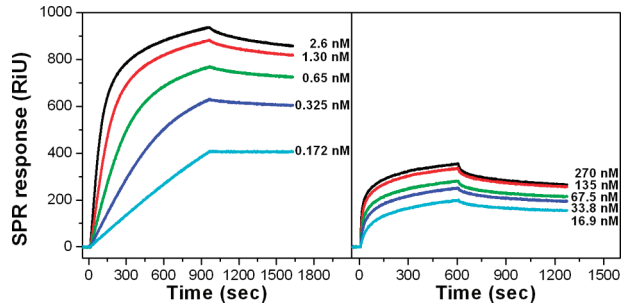


Figure 2. SPR sensorgrams showing the binding of gp120 (left) and **P1** (right) onto DC-SIGN functionalized surfaces. The concentration ranges for **P1** and gp120 were 16.9−270 and 0.172−2.6 nM, respectively.

Table 1. Binding Data of gp120 and Glycopolymers Obtained from SPR Measurements

			DC-SIGN binding		
code	Man (%)	Gal (%)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (nM)
gp120	na	na	8.89×10^{6}	3.77×10^{-5}	0.004
P1	100	−	4.99×10^{5}	2.48×10^{-4}	0.496
P2	75	25	2.98×10^{5}	1.79×10^{-4}	0.602
P3	50	50	2.42×10^{5}	2.01×10^{-4}	0.832
P4	25	75	2.37×10^{5}	4.75×10^{-4}	2.01
P5	−	100	na	na	na

binding, as the equivalent mass of free D-mannose gave no binding signal. The homo glycopolymer of mannose, **P1**, bound with a K_D of 4.96×10^{-10} M. However, no significant galactose glycopolymer **P5** binding was observed, highlighting the specificity of the binding site for terminal mannose residues with equatorial C3 and C4 hydroxyl groups. The glyco copolymers **P2**−**P4** were tested in the same system. As expected, it was shown that increased galactose density correlated negatively with binding affinity to DC-SIGN. The calculated apparent K_D values for the full glycopolymer series are indicated in Table 1. As a comparison, the calculated K_D value for gp120 was found to be 4.24×10^{-12} M. This low K_D value highlights the strong binding provided by the exquisite presentation of branched carbohydrates in gp120, which contributes toward the high affinity of HIV-1 for human dendritic cells. Furthermore, binding studies using DC-SIGN transfected cells yield apparent K_D values within the same order of magnitude.[35] Therefore, it can be concluded that the SPR assay offers a good reflection of binding events at the cell surface.

A therapeutic role for mannose-containing glycopolymers would lie in their potential to interrupt viral adhesion to host receptors, a process referred to as anti-adhesion therapy. We therefore established a competition assay within the SPR system to examine whether the glycopolymers could inhibit DC-SIGN interactions with HIV gp120, Figure 1b. Recombinant HIV gp120 was immobilized on a sensor chip surface, and soluble DC-SIGN at a fixed concentration of 2 nM was flowed over the surface in the presence of the glycopolymers at a range of concentrations. The concentration of polymer required to prevent 50% of the DC-SIGN from binding to the gp120 surface (IC_{50}) was determined. Figure 3 shows representative SPR sensorgrams used to calculate the IC_{50} value; further details are given in the Supporting Information. An increase in the mannose content of the polymers from 25 to 100% reduces the IC_{50} value from 1453 to 37 nM, highlighting the enhanced avidity due to multivalent presentation of the binding epitopes, i.e., the cluster glycoside effect, Figure 4. When expressed as the relative inhibition per mannose residue, **P1** (100% mannose) has almost 10 times the avidity compared to **P4** (25% mannose). These values are in good agreement with the calculated values of K_D, Table 1. **P5** showed only a weak inhibitory effect, and a value of IC_{50} could not be calculated using the concentration range tested.

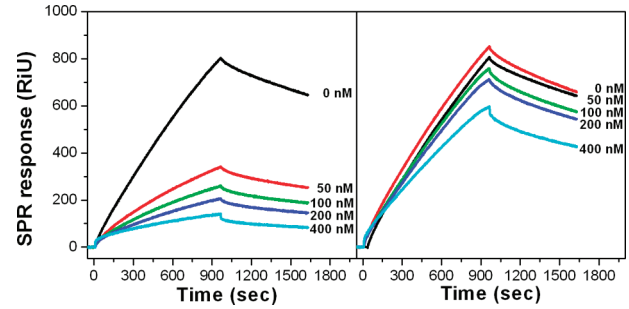
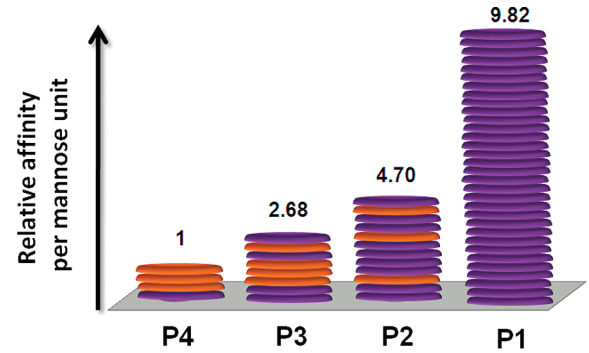


Figure 3. Competition experiments on gp120 functionalized surface between DC-SIGN and homo Man-polymer **P1** (left) or homo Gal-polymer **P5** (right) at a concentration range of 0−400 nM for the glycopolymers and 4 nM DC-SIGN.



Man content	0.25	0.50	0.75	1.00
IC_{50} (nM)	1453	271	103	37

Figure 4. IC_{50} and relative affinities per mannose (cylinders) of the glycopolymers in solution obtained from SPR competitive binding measurements. Purple and orange represents the relative ratio of mannose and galactose in the copolymer, respectively.

It is clear that exploitation of the carbohydrate-binding of human cells by pathogens is a significant mechanistic feature of several devastating infectious diseases. In addition to supporting HIV adhesion and infection, DC-SIGN is also strongly implicated in the uptake and chronic infectivity of other lethal viruses, including hepatitis C virus and Ebola virus, pathogens that collectively cause disease in millions of human beings.[35,36] Thus, the power to unravel these adhesion mechanisms, and positively interfere with them, carries the potential to provide substantial global healthcare benefit. Herein, we have shown that a relatively simple glycopolymer can effectively prevent the interactions between a human dendritic cell-associated lectin (DC-SIGN) and the viral envelope glycoprotein gp120. This approach may give rise to novel insights into the mechanisms of HIV infection and provide potentially important components to new therapeutics, notably novel topical barrier therapeutics.

Acknowledgment. C.R.B. is funded by a European Union Marie Curie Fellowship scheme (235999). M.I.G. is a Birmingham Science City Interdisciplinary Research Fellow. D.A.M. and R.W. are Research Councils UK Academic Fellows, with additional support from the Warwick Research Development Fund (D.A.M.) and Wellcome Trust Grant 077400 (R.W.). Equipment used was supported by the Innovative Uses for Advanced Materials in the Modern World (AM2), with support from Advantage West Midlands and partially funded by the European Regional Development Fund.

Supporting Information Available: Full synthetic details, data analysis, and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Sharon, N. *Biochim. Biophys. Acta−Gen. Subjects* **2006**, *1760*, 527.
(2) Haltiwanger, R. S.; Lowe, J. B. *Annu. Rev. Biochem.* **2004**, *73*, 491.
(3) Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357.
(4) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Curr. Opin. Chem. Biol.* **2000**, *4*, 696.
(5) Geijtenbeek, T. B.; Kwon, D. S.; Torensma, R.; van Vliet, S. J.; van Duijnhoven, G. C.; Middel, J.; Cornelissen, I. L.; Nottet, H. S.; Kewal-Ramani, V. N.; Littman, D. R.; Figdor, C. G.; van Kooyk, Y. *Cell* **2000**, *100*, 587.
(6) Geijtenbeek, T. B.; Torensma, R.; van Vliet, S. J.; van Duijnhoven, G. C.; Adema, G. J.; van Kooyk, Y.; Figdor, C. G. *Cell* **2000**, *100*, 575.
(7) van Liempt, E.; Bank, C. M. C.; Mehta, P.; García-Vallejo, J. J.; Kawar, Z. S.; Geyer, R.; Alvarez, R. A.; Cummings, R. D.; Kooyk, Y. v.; van Die, I. *FEBS Lett.* **2006**, *580*, 6123.
(8) Tailleux, L.; Schwartz, O.; Herrmann, J. L.; Pivert, E.; Jackson, M.; Amara, A.; Legres, L.; Dreher, D.; Nicod, L. P.; Gluckman, J. C.; Lagrange, P. H.; Gicquel, B.; Neyrolles, O. *J. Exp. Med.* **2003**, *197*, 121.
(9) Mitchell, D. A.; Fadden, A. J.; Drickamer, K. *J. Biol. Chem.* **2001**, *276*, 28939.
(10) Feinberg, H.; Mitchell, D. A.; Drickamer, K.; Weis, W. I. *Science* **2001**, *294*, 2163.
(11) Feinberg, H.; Guo, Y.; Mitchell, D. A.; Drickamer, K.; Weis, W. I. *J. Biol. Chem.* **2005**, *280*, 1327.
(12) Spain, S. G.; Gibson, M. I.; Cameron, N. R. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 2059.
(13) Miura, Y.; Yasuda, K.; Yamamoto, K.; Koike, M.; Nishida, Y.; Kobayashi, K. *Biomacromolecules* **2007**, *8*, 2129.
(14) Gibson, M. I.; Barker, C. A.; Spain, S. G.; Albertin, L.; Cameron, N. R. *Biomacromolecules* **2009**, *10*, 328.
(15) Kiessling, L. L.; Strong, L. E.; Gestwicki, J. E. *Annual Reports in Medicinal Chemistry*; Academic Press Inc.: San Diego, CA, 2000; Vol. 35, p 321.
(16) Ambrosi, M.; Cameron, N. R.; Davis, B. G. *Org. Biomol. Chem.* **2005**, *3*, 1593.
(17) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 2348.
(18) Pukin, A. V.; Branderhorst, H. M.; Sisu, C.; Weijers, C.; Gilbert, M.; Liskamp, R. M. J.; Visser, G. M.; Zuilhof, H.; Pieters, R. J. *Chembiochem* **2007**, *8*, 1500.
(19) Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 14922.
(20) Smith, E. A.; Thomas, W. D.; Kiessling, L. L.; Corn, R. M. *J. Am. Chem. Soc.* **2003**, *125*, 6140.
(21) Ambrosi, M.; Cameron, N. R.; Davis, B. G.; Stolnik, S. *Org. Biomol. Chem.* **2005**, *3*, 1476.
(22) Joshi, A.; Vance, D.; Rai, P.; Thiyagarajan, A.; Kane, R. S. *Chem.—Eur. J.* **2006**, *14*, 7738.
(23) Pieters, R. J. *Med. Res. Rev.* **2007**, *27*, 796.
(24) Geng, J.; Mantovani, G.; Tao, L.; Nicolas, J.; Chen, G. J.; Wallis, R.; Mitchell, D. A.; Johnson, B. R. G.; Evans, S. D.; Haddleton, D. M. *J. Am. Chem. Soc.* **2007**, *129*, 15156.
(25) Schaefer, M.; Reiling, N.; Fessler, C.; Stephani, J.; Taniuchi, I.; Hatam, F.; Yildirim, A. O.; Fehrenbach, H.; Walter, K.; Ruland, J.; Wagner, H.; Ehlers, S.; Sparwasser, T. *J. Immunol.* **2008**, *180*, 6836.
(26) Gauthier, M. A.; Gibson, M. I.; Klok, H.-A. *Angew. Chem., Int. Ed.* **2009**, *48*, 48.
(27) Becer, C. R.; Hoogenboom, R.; Schubert, U. S. *Angew. Chem., Int. Ed.* **2009**, *48*, 4900.
(28) Klob, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
(29) Nurmi, L.; Lindqvist, J.; Randev, R.; Syrett, J.; Haddleton, D. M. *Chem. Commun.* **2009**, 2727.
(30) Geng, J.; Lindqvist, J.; Mantovani, G.; Chen, G.; Sayers, C. T.; Clarkson, G. J.; Haddleton, D. M. *QSAR Comb. Sci.* **2007**, *26*, 1220.
(31) Chen, G. J.; Tao, L.; Mantovani, G.; Geng, J.; Nystrom, D.; Haddleton, D. M. *Macromolecules* **2007**, *40*, 7513.
(32) Becer, C. R.; Babiuch, K.; Pilz, D.; Hornig, S.; Heinze, T.; Gottschaldt, M.; Schubert, U. S. *Macromolecules* **2009**, *42*, 2387.
(33) Ladmiral, V.; Mantovani, G.; Clarkson, G. J.; Cauet, S.; Irwin, J. L.; Haddleton, D. M. *J. Am. Chem. Soc.* **2006**, *128*, 4823.
(34) Su, S. V.; Hong, P.; Baik, S.; Negrete, O. A.; Gurney, K. B.; Lee, B. *J. Biol. Chem.* **2004**, *279*, 19122–19132.
(35) Lozach, P.-Y.; Lortat-Jacob, H.; De Lacroix De Lavalette, A.; Staropoli, I.; Foung, S.; Amara, A.; Houlès, C.; Fieschi, F.; Schwartz, O.; Virelizier, J.-L.; Arenzana-Seisdedos, F.; Altmeyer, R. *J. Biol. Chem.* **2003**, *278*, 20358.
(36) Alvarez, C. P.; Lasala, F.; Carrillo, J.; Muniz, O.; Corbi, A. L.; Delgado, R. *J. Virol.* **2002**, *76*, 6841.

JA1056714