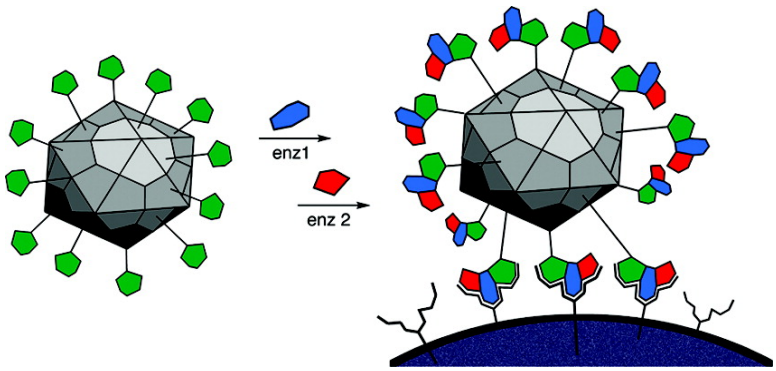
Communication

On-Virus Construction of Polyvalent Glycan Ligands for Cell-Surface Receptors

Eiton Kaltgrad, Mary K. O'Reilly, Liang Liao, Shoufa Han, James C. Paulson, and M. G. Finn

J. Am. Chem. Soc., **2008**, 130 (14), 4578-4579 • DOI: 10.1021/ja077801n



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 4 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 03/15/2008

On-Virus Construction of Polyvalent Glycan Ligands for Cell-Surface Receptors

Eiton Kaltgrad,[†] Mary K. O'Reilly,[‡] Liang Liao,[‡] Shoufa Han,[‡] James C. Paulson,*[,‡] and M. G. Finn*[,†]

Department of Chemistry and The Skaggs Institute for Chemical Biology, and Departments of Chemical Physiology and Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received October 10, 2007; E-mail: mgfinn@scripps.edu

Cell surface receptors that recognize glycans as ligands mediate a vast range of events in cellular biology through low-affinity, multivalent (simultaneous multipoint) binding interactions.[1] The development of ligand-based probes of these receptors must therefore address both the challenges of glycan synthesis and the need for multivalent platforms. A polyvalent display of glycan ligands is usually achieved by synthesis of the desired glycan with a linker containing a functional group at the reducing end, allowing conjugation to a polyvalent platform bearing the complementary reactive group.[2] An alternative approach of "immobilizing" glycan reaction precursors to the platform of choice and "building out" from such surfaces to make polyvalent oligosaccharides would provide a way to make complex and expensive oligosaccharides precisely placed, as has recently been demonstrated using gold nanoparticles.[3] Here we report the implementation of this strategy for labeling the exterior surface of a virus capsid, taking advantage of the compatibility of the protein particles with glycosyltransferase enzymes and chemoenzymatic reaction conditions.[4] The resulting structures comprise multivalent arrays of glycan ligands favorable for binding to a cell surface glycan receptor in a highly specific manner.

Our target to probe the viability of on-virus oligosaccharide synthesis was CD22 (Siglec-2), a receptor specifically expressed on B cells and B lymphoma cells that is involved in regulation of B cell signaling. The natural ligand of CD22 is Siaα2-6Gal, which is found at the termini of N-linked glycans on B and T cells.[5] CD22 is bound by ligands presented by neighboring molecules on the same B cell surface, an interaction "in *cis*" that promotes strong binding even though the ligand−receptor pair has low intermolecular affinity (K_d = ca. 0.1−0.2 mM).[6] Binding of other cells to the CD22 receptor is thereby inhibited unless the competing sialosides are presented in an effective polyvalent fashion.

The 9-biphenyl carbonyl (BPC) derivative[7] of the natural sialoside ligand, Siaα2-6Galβ1-4GlcNAc, was selected as our target ligand for construction of multivalent capsid-based structures.[5] GlcNAc, the intermediate Galβ1,4-GlcNAc (LacNAc), and the final BPC sialoside were prepared as O-linked azidoethyl derivatives **1**, **2**, and **3**, respectively (Figure 1).[4c,8] The conveniently accessible azide groups were used to array the carbohydrates on alkyne-decorated polyvalent particles by the Cu^I-catalyzed azide−alkyne cycloaddition (CuAAC) reaction.[9] Two particles were employed: the cowpea mosaic virus (CPMV)[10] and bacteriophage Qβ coat protein,[11] both icosahedral structures approximately 30 nm in diameter.[12] Alkyne groups were introduced by acylation of surface lysine side chains with alkynyl *N*-hydroxysuccinimide ester **4**.[13] Wild-type Qβ, having more subunits per particle (180) than CPMV (60) and approximately the same number of surface-accessible lysines per subunit, displays a greater number of reactive sites. To make a particle in which the location of the attachment points is precisely known, the Qβ sequence was modified by the replacement of lysine at position 16 with methionine (K16M). This protein was

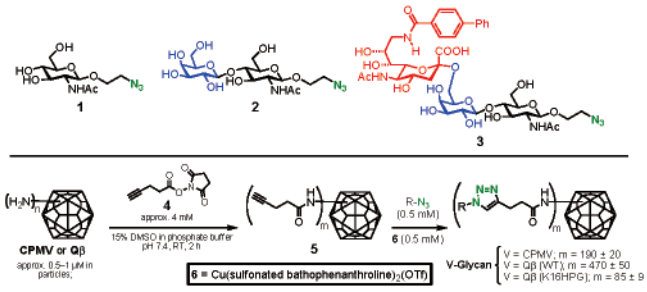


Figure 1. (Top) Structures of glycan azides used to make the "preformed" virus conjugates. (Bottom) Preparation and use of alkyne-labeled CPMV and Qβ.

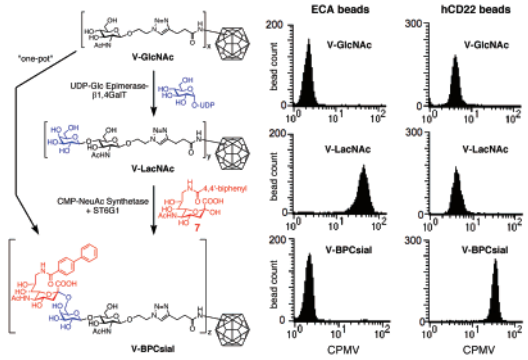


Figure 2. Sequential enzymatic synthesis of **CPMV-BPCsial**, monitored by lectin recognition properties. **CPMV-GlcNAc** (top), **CPMV-LacNAc** (center), or **CPMV-BPCsial** (bottom) were incubated with magnetic beads bearing *Erythrina cristagalli* agglutinin (ECA, specifically binds terminal galactose[16]) or a recombinant CD22-Fc immunoglobulin chimera (hCD22, specifically binds BPCsial) and analyzed by flow cytometry.

expressed in a methionine auxotroph of *E. coli* with addition of the alkyne-containing unnatural amino acid homopropargyl glycine (HPG), resulting in incorporation of HPG at approximately 90 of the 180 newly introduced Met sites at position 16. This chimera, designated K16HPG, is described more fully elsewhere.[14]

Compounds **1**, **2**, and **3** were arrayed on the virus scaffold by azide−alkyne cycloaddition under the influence of precatalyst **6**, in a method previously shown to give complete coverage of the displayed alkyne groups.[9] The resulting glycan-derivatized particles were designated **V-GlcNAc**, **V-LacNAc**, and **V-BPCsial**, respectively, where "V" is either CPMV or Qβ. The number of attached glycans in each case was estimated by analogy to reactions performed under identical conditions using a selenium-containing molecule that can be quantitatively detected after attachment.[15]

The alternative strategy of carrying out the synthesis directly on the **V-GlcNAc** particle was implemented as shown in Figure 2. Enzymatic condensation of **CPMV-GlcNAc** with uridine diphospho-galactose (UDP-galactose, generated *in situ* by rapid galactoepimerase-catalyzed conversion of the inexpensive UDP-glucose)

[†] Department of Chemistry and The Skaggs Institute for Chemical Biology.
[‡] Departments of Chemical Physiology and Molecular Biology.

10.1021/ja077801n CCC: $40.75

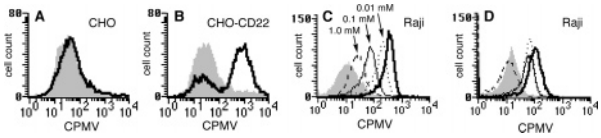


Figure 3. Flow cytometry analyses of the binding of CPMV-glycans with native cells in a CD22- and sialic acid-dependent manner. (A and B) **CPMV-LacNAc** (gray filled) or **CPMV-BPCsial** (black) incubated with CHO or CHO-CD22 cells. (C) CPMV bearing LacNAc (gray filled) or BPCsial (thick black solid) incubated with native Raji cells. BPCsial-bearing viruses were also incubated in the presence of the indicated concentrations of free BPC-sialoside **3** as inhibitor. (D) Raji cells incubated with wild-type CPMV (light gray filled), **CPMV-LacNAc** (dashed), **CPMV-BPCsial** from preformed trisaccharide (thick black solid), **CPMV-BPCsial** made enzymatically on the virus from preformed **CPMV-LacNAc** (dotted), or **CPMV-BPCsial** made in a one-pot reaction on the virus (thin black solid).

gave **CPMV-LacNAc**. The product was purified away from the small molecule and enzymatic reagents by ultracentrifugation through a 10−40% sucrose gradient, which is typical for virus particles of this size and nearly as convenient as filtration of polystyrene resin beads. Similarly, reaction of **CPMV-LacNAc** with BPC-sialic acid **7** gave the final product **CPMV-BPCsial**, which was again purified by sucrose gradient sedimentation. The yield of recovered virus for each step was approximately 70%, consistent with the normal loss of material sustained in manipulations of this kind.

The viruses derived from conjugation of presynthesized and sequentially formed glycans were found to be intact with no indication of decomposition or instability.[15] The binding properties of the particles following enzymatic conversion were assessed by flow cytometry using magnetic beads coated with glycan binding proteins that recognize the precursor and product of each reaction (Figure 2). Complete selective switching between the expected binding properties was observed with each enzymatic transformation. Very similar results were obtained with the analogous Qβ particles,[15] showing that polyvalent glycan-lectin binding was independent of the platform.

The virus conjugates were also found to bind to CD22 expressed on cell surfaces. **CPMV-BPCsial**, but not **CPMV-LacNAc**, displayed preferential binding to Chinese hamster ovary (CHO) cells expressing recombinant CD22 (Figure 3B) and to Raji lymphoma cells, which naturally express CD22 (Figure 3C), over native CHO cells that do not express CD22 (Figure 3A). Competition for binding to Raji cells between virus conjugates of BPCsial and free BPC-sialoside **3** showed partial disruption of the virus−cell interaction at 100 μM of the monomeric ligand, and greater, but still incomplete inhibition at 1 mM (Figure 3C and Supporting Information). These values are 4−40 times that of the total BPC-sialoside concentration presented on **V-BPCsial** (ca. 25 μM) and far greater than the conf-centration of glycosylated particles (ca. 130 nM), consistent with the expectation that interaction of virus-displayed ligands with CD22 on cells is polyvalent in nature. Similar tight-binding behavior was observed for BPC-sialoside adducts of wild-type and K16HPG forms of Qβ, which differ in the number of attached glycans.[15] Such a result suggests that the valency presented on these constructs exceeds that needed to form a stable complex with CD22 on the cell surface.

The maximum binding of **CPMV-BPCsial** produced in these experiments was slightly but measurably below that of **CPMV-BPCsial** produced by conjugating preformed azide **3** to the virus (Figure 3D), suggesting that the enzymatic reaction on the virus-bound substrates was able to address most, but not all, of the 190 GlcNAc molecules on the CPMV surface. The same observation was made for preformed and enzymatically synthesized **CPMV-LacNAc** binding to ECA beads.[15]

After identification of the amounts of transferase enzymes required for optimal glycosylation in each separate step,[15] **CPMV-GlcNAc** was converted to **CPMV-BPCsial** in an efficient "one-pot" reaction (Figure 2). The particles obtained after purification from the cascade reaction were nearly as effective in binding to B cells as the particles obtained by CuAAC conjugation of presynthesized BPC-sialoside to the virus scaffold (Figure 3D).

The results shown here demonstrate that virus capsids provide a platform that can be used to create a multivalent particle by en-bloc transfer of preformed ligands or used as "beads" upon which multivalent ligands can be synthesized by sequential enzymatic transformations. Their unique size, density, and chemically robust nature allow for convenient isolation away from other reaction components. Most importantly, viruses allow the precise placement of functional units, in this case the starting glycan acceptors, and therefore control of the distribution of polyvalent oligosaccharides obtained from such "on-bead" transformations. The resulting particles can be highly effective binders to specific receptor clusters, as in the present case to cell-surface CD22.

Acknowledgment. We thank the Skaggs Institute for Chemical Biology, the NIH (CA112075, AI056013, GM60938), the American Cancer Society (PF0719301), the W. M. Keck Foundation for support of this work, and Warren Wakarchuck at the National Research Council, Ottawa for galactosyltransferase.

Supporting Information Available: Synthetic and analytical procedures and data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321−327. (b) Gabius, H.-J.; Siebert, H.-C.; Andre, S.; Jimenez-Barbero, J.; Rudiger, H. *ChemBioChem* **2004**, *5*, 740−764. (c) Collins, B. E.; Paulson, J. C. *Curr. Opin. Chem. Biol.* **2004**, *8*, 617−625. (d) Dube, D. H.; Bertozzi, C. R. *Nat. Rev. Drug Discovery* **2005**, *4*, 477−488.
(2) (a) Bovin, N. V.; Gabius, H.-J. *Chem. Soc. Rev.* **1995**, *24*, 413−421. (b) Lindhorst, T. K. *Top. Curr. Chem.* **2001**, *218*, 201−235. (c) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555−578. (d) Cairo, C. W.; Gestwicki, J. E.; Kanai, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 1615−1619. (e) Woller, E. K.; Walter, E. D.; Morgan, J. R.; Singel, D. J.; Cloninger, M. J. *J. Am. Chem. Soc.* **2003**, *125*, 8820−8826. (f) Raja, K. S.; Wang, Q.; Finn, M. G. *ChemBioChem* **2003**, *4*, 1348−1351. (g) Kagan, E.; Ragupathi, G.; Yi, S. S.; Reis, C. A.; Gildersleeve, J.; Kahne, D.; Clausen, H.; Danishefsky, S. J.; Livingston, P. O. *Cancer Immunol. Immunother.* **2005**, *54*, 424−430.
(3) Nagahori, N.; Nishimura, S.-I. *Chem.—Eur. J.* **2006**, *12*, 6478−6485.
(4) (a) Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443−73. (b) Sears, P.; Wong, C.-H. *Science* **2001**, *291*, 2344−2350. (c) Blixt, O.; Allin, K.; Pereira, L.; Datta, A.; Paulson, J. C. *J. Am. Chem. Soc.* **2002**, *124*, 5739−5746. (d) Nahalka, J.; Liu, Z.; Chen, X.; Wang, P. G. *Chem.—Eur. J.* **2003**, *9*, 373−377 and references therein. (e) Seeberger, P. H. In *Carbohydrate-Based Drug Discovery*; Wong, C.-H., Ed.; Wiley-VCH: 2005; pp 103−127.
(5) Collins, B. E.; Blixt, O.; Han, S.; Duong, B.; Li, H.; Nathan, J. K.; Bovin, N.; Paulson, J. C. *J. Immunol.* **2006**, *177*, 2994−3003 and references therein.
(6) Han, S.; Collins, B. E.; Bengston, P.; Paulson, J. C. *Nat. Chem. Biol.* **2005**, *1*, 93−97.
(7) Zaccai, N. R.; Maenaka, K.; Maenaka, T.; Crocker, P. R.; Brossmer, R.; Kelm, S.; Jones, E. Y. *Structure* **2003**, *11*, 557−567.
(8) Blixt, O.; Brown, J.; Schur, M. J.; Wakarchuk, W.; Paulson, J. C. *J. Org. Chem.* **2001**, *66*, 2442−2448.
(9) (a) Sen Gupta, S.; Kuzelka, J.; Singh, P.; Lewis, W. G.; Manchester, M.; Finn, M. G. *Bioconjugate Chem.* **2005**, *16*, 1572−1579. (b) Prasuhn, D. E., Jr.; Yeh, R. M.; Obenaus, A.; Manchester, M.; Finn, M. G. *Chem. Commun.* **2007**, 1269−1271.
(10) Wang, Q.; Lin, T.; Tang, L.; Johnson, J. E.; Finn, M. G. *Angew. Chem., Int. Ed.* **2002**, *41*, 459−462.
(11) Kozlovska, T. M.; Cielens, I.; Dreilinna, D.; Dislers, A.; Baumanis, V.; Ose, V.; Pumpens, P. *Gene* **1993**, *137*, 133−137.
(12) (a) Lin, T.; Chen, Z.; Usha, R.; Stauffacher, C. V.; Dai, J.-B.; Schmidt, T.; Johnson, J. E. *Virology* **1999**, *265*, 20−34. (b) Liljas, L.; Golmohammadi, R. *Structure* **1996**, *4*, 543−554.
(13) Wang, Q.; Kaltgrad, E.; Lin, T.; Johnson, J. E.; Finn, M. G. *Chem. Biol.* **2002**, *9*, 805−811.
(14) Strable, E.; Prasuhn, D. E., Jr.; Udit, A. K.; Brown, S.; Link, A. J.; Ngo, J. T.; Lander, G.; Quispe, J.; Potter, C. S.; Carragher, B.; Tirrell, D. A.; Finn, M. G. *Bioconjugate Chem.* **2008**, in press.
(15) See Supporting Information.
(16) Turton, K.; Natesh, R.; Thiyagarajan, N.; Chaddock, J. A.; Acharya, K. R. *Glycobiology* **2004**, *14*, 923−929.

JA077801N