Published in final edited form as:

Bioconjug Chem. 2011 April 20; 22(4): 546–550. doi:10.1021/bc100579d.

Supramolecular Complexing of Membrane Siglec CD22 Mediated by a Polyvalent Heterobifunctional Ligand that Templates on IgM

Lina Cui[†], Pavel I. Kitov[†], Gladys C. Completo[‡], James C. Paulson[‡], and David R. Bundle^{†,*}

[†] Department of Chemistry, Alberta Ingenuity Centre for Carbohydrate Science, University of Alberta, Edmonton, Alberta, Canada

[‡] Department of Chemical Physiology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, California 92037, U. S. A

Abstract

We report the synthesis and *in vitro* evaluation of a multivalent homing device, a polymer which contains pre-ordered pendant groups with dual specificity, a trisaccharide moiety, which is specific for the siglec CD22, and an antibody specific hapten, nitrophenol. The device efficiently attracts anti-hapten IgM to the surface of human lymphoma B cells as well as to CD22-conjugated magnetic beads by mediating formation of a ternary complex on the surface of the target.

Directing neutralizing proteins such as antibodies to pathogenic agents using heterobifunctional ligands that contain binding fragments for both receptors has emerged as a promising therapy against cancer,^{1–5} viral or bacterial infection,^{6–16} and Alzheimer's disease.¹⁷ Surface bound receptors and oligomeric proteins that possess multiple unidirectional binding sites, can bind multiple copies of heterobifunctional ligands, allowing the other head groups to attract their cognate proteins to form supramolecular ternary complexes.¹⁸ Due to saving of binding entropy, the efficiency of the ligand binding to its target can be enhanced by several orders of magnitude, while the templating protein can perform additional effector function.^{8, 14–15}

Our efforts are directed to application of supramolecular templating to cancer immunotherapy. CD22, a member of the siglec family of glycan-binding proteins, is expressed on the B cell surface as a key regulator of signaling.¹⁹ and has been identified as a target for immunotherapy (B cell depletion therapy) to treat B cell lymphomas and autoimmune diseases.²⁰ However, targeting CD22 using its glycan ligands is challenging due to the low intrinsic affinity that CD22 exhibits for its native ligands (NeuAc α (2–6)Gal β , $K_d \approx 0.2$ mM). Additionally, CD22 is glycosylated with the self ligand and these *cis* interactions mask the ligand binding sites.^{21–22}

Previously, we reported heterobifunctional ligands, which comprise CD22 ligand, NeuAc α (2–6)Gal β (1–4)GlcNAc, modified at C-9 position of neuraminic acid with biphenyl carbonylamido (BPC) substituent, which is known to increase affinity for CD22 by factor of 100.²¹ The heterodimer of this ligand with a nitrophenol (NP) moiety was able to overcome receptor-masking *cis* interactions and dock anti-NP antibodies to B cell.²³ We sought to develop an efficient way to construct polyvalent heterobifunctional ligands and to evaluate

*Correspondence should be addressed to Prof. David Bundle, Department of Chemistry, Alberta Ingenuity Centre for Carbohydrate Science, University of Alberta, Edmonton, Alberta, Canada. Phone: (+1) 780 492 8808., dave.bundle@ualberta.ca.

Supporting Information Available: Details of experimental procedures are available free of charge *via* the Internet at <http://pubs.acs.org>.

the influence of polyvalency on the ligand-mediated assembly of supramolecular immune complexes on cell membrane (Figure 1).

We have previously found that the supramolecular templating effect is not observed when the two binding functionalities are independently distributed on a polymer scaffold.¹⁶ In order to mediate formation of ternary complexes the pendant ligands must be arranged as heterobifunctional units on the polyvalent scaffold; hence, we designed a homing device comprising 5 major components: a multivalent scaffold, polyacrylamide (PAA); a spacer, triethyleneglycol derivative; a bifurcation unit, selectively protected lysine; and 2 specific ligands, trisaccharide and nitrophenol.

Compound **1** was assembled by sequential elaboration of polyacrylamide (Scheme 1). First, a spacer was introduced by trans-amination of PAA. To the resulting polymeric amine an orthogonally protected glycine derivative (Boc and Fmoc) was attached to provide a bifurcation fragment, which permits sequential installation of two different ligands. Removal of Fmoc protection followed by acylation of the liberated amine with the NHS ester of 4-hydroxy-3-nitrophenylacetic acid yielded polymer **6**. After removal of Boc the second amine was capped as propargyloxycarbonyl **7**. The trisaccharide ligand **8** was synthesized chemo-enzymatically as previously reported²⁴ and incorporated into the polymer *via* copper (I)-catalyzed Huisgen cyclization to give the desired polyvalent heterobifunctional ligand **1**.

To evaluate the ability of the polyvalent ligand **1** to drive complex formation between IgM and CD22 on B cells, human lymphoma cells (BJAB) were incubated with anti-NP IgM at fixed concentration (10 µg/mL), and polyvalent ligand **1**, its unimeric analogue **2**, then stained with FITC-labeled anti-IgM antibody, and analyzed by flow cytometry. As shown in Figure 3 only in the presence of ligand **1** (at all tested concentrations higher than 0.04 µg/mL) anti-NP IgM was associated with B cells. In comparison, only background fluorescence was observed for the unimeric ligand **2**. In separate experiments, the polyvalent LacNAc control (compound **3**), lacking the terminal neuraminic acid moiety, showed no binding to the BJAB cells (not shown). This is consistent with our previous observations that although the longer linker between the ligand and antigen (NP) reduces the ability of the heterobivalent ligand to mediate formation of a ternary complex between two multivalent proteins due to the loss of conformational entropy,²³ but still allows templating on anti-NP-IgM when presented on a polymeric scaffold.¹⁵ If confirmed by further examples, this effect may have important implications for the design of multivalent heterobifunctional homing devices.

Complex formation between IgM and CD22 on B cells was concentration dependent for polyvalent ligand **1**, and as expected was described by a bell shape curve (Figure 3b). The binding peaked at 2.5 µg/mL of ligand **1**, which became inhibitory at higher concentrations, presumably due to saturation of one or both proteins by the ligand. Such dose dependence is typical for cross-linked complexes and was previously observed by us²³ and others.^{25, 26}

To eliminate cell related effects such as ligand-mediated endocytosis and lateral mobility of CD22 in the cell membrane, we also evaluated complex formation using magnetic beads with immobilized CD22 (Figure 4). Anti-NP IgM and magnetic bead were incubated with ligand **1** at various concentrations at both 37 °C and 4 °C, and the beads were treated with FITC-anti-IgM antibody and analyzed using flow cytometry. The results agreed well with the experiments performed on B cells. Complex formation was a dose-dependent process at both temperatures, and more IgM were associated with magnetic beads with the increase of the concentration of ligand **1** until a plateau was reached. Neither the polyvalent control lacking the CD22 ligand (compound **3**), or the monovalent compound **2** (not shown)

supported binding of the anti-NP antibody to beads. The apparent K_d values were found to be 0.12 $\mu\text{g/mL}$ (0.024 nM per pendant ligand) and 0.17 $\mu\text{g/mL}$ (0.034 nM per pendant ligand) at 37 °C and 4 °C respectively. This represents a 5000-fold (at 37 °C) and 200-fold (at 4 °C) higher binding affinity over the best univalent ligand identified previously.²³

More interestingly, the polymeric heterobifunctional ligand exhibited significantly positive binding entropy with little temperature dependency (very similar K_d 's at different temperatures) (Figure 4). This is in sharp contrast with previously examined unimeric ligands, which showed 10 to 20-fold decrease in affinity when temperature increased from 4 °C to 37 °C due to faster dissociation at higher temperature.²³ Considering mainly entropic nature of the multivalency effect, which reduces entropy loss upon complex formation (smaller value of ΔS term in binding energy), the weak dependence of the apparent binding constants on temperature for multivalent ligands is expected. Binding of carbohydrate ligands to lectins are often accompanied by large favorable enthalpy change (ΔH), which is partially offset by almost equally large unfavorable entropy term ($-T\Delta S$) resulting in low net change in free energy.²⁷ Multivalent presentation leads to decrease in absolute value of both thermodynamic terms per pendant ligand with entropy term changing faster.²⁸ It has also been shown that ΔS can even change the sign and become positive for polymeric ligands.²⁹ In accord with higher avidity, increase in net binding of IgM to the beads was observed at 37 °C, which can be attributed to reduced rate of dissociation and more IgM retaining after washing steps as discussed previously in our report on unimeric heterobifunctional ligands.²³

In conclusion, we report a polymer-based heterobifunctional ligand as a potent homing device that redirects antibodies to cell-surface receptors that are over-expressed on lymphoma B cells. These data suggest new applications of polymeric drugs in targeted cancer therapy that together with other properties, such as enhanced permeability and retention (EPR) effect and prolonged retention in the circulatory system, render them attractive as potential therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Ryan T. McKay (NANUC, Edmonton) for his help in characterization of compound **1** using 800 MHz NMR, Dr. Norihito Kawasaki for his kind assistance in preparation of the manuscript. This work was supported by Alberta Ingenuity Centers Program, and NIH grants GM60938 and AI16165).

References

1. Carlson CB, Mowery P, Owen RM, Dykhuizen EC, Kiessling LL. Selective tumor cell targeting using low-affinity, multivalent interactions. *ACS Chem Biol*. 2007; 2:119–127. [PubMed: 17291050]
2. Owen RM, Carlson CB, Xu JW, Mowery P, Fasella E, Kiessling LL. Bifunctional ligands that target cells displaying the $\alpha_v\beta_3$ integrin. *ChemBioChem*. 2007; 8:68–82. [PubMed: 17154219]
3. Murelli RP, Zhang AX, Michel J, Jorgensen WL, Spiegel DA. Chemical control over immune recognition: a class of antibody-recruiting small molecules that target prostate cancer. *J Amer Chem Soc*. 2009; 131:17090–17092. [PubMed: 19888723]
4. Popkov M, Gonzalez B, Sinha SC, Barbas CF. Instant immunity through chemically programmable vaccination and covalent self-assembly. *Proc Natl Acad Sci U S A*. 2009; 106:4378–4383. [PubMed: 19255430]

5. Lu YJ, Segal E, Leamon CP, Low PS. Folate receptor-targeted immunotherapy of cancer: mechanism and therapeutic potential. *Adv Drug Deliv Rev.* 2004; 56:1161–1176. [PubMed: 15094213]
6. Bertozzi CR, Bednarski MD. Antibody targeting to bacterial-cells using receptor- specific ligands. *J Amer Chem Soc.* 1992; 114:2242–2245.
7. Bertozzi CR, Bednarski MD. A receptor-mediated immune-response using synthetic glycoconjugates. *J Amer Chem Soc.* 1992; 114:5543–5546.
8. Liu JY, Zhang ZS, Tan XJ, Hol WGJ, Verlinde C, Fan EK. Protein heterodimerization through ligand-bridged multivalent pre-organization: Enhancing ligand binding toward both protein targets. *J Amer Chem Soc.* 2005; 127:2044–2045. [PubMed: 15713072]
9. Krishnamurthy VM, Quinton LJ, Estroff LA, Metallo SJ, Isaacs JM, Mizgerd JP, Whitesides GM. Promotion of opsonization by antibodies and phagocytosis of Gram-positive bacteria by a bifunctional polyacrylamide. *Biomaterials.* 2006; 27:3663–3674. [PubMed: 16527349]
10. Shokat KM, Schultz PG. Redirecting the immune-response – ligand-mediated immunogenicity. *J Amer Chem Soc.* 1991; 113:1861–1862.
11. Naicker KP, Li HG, Heredia A, Song HJ, Wang LX. Design and synthesis of alpha Gal-conjugated peptide T20 as novel antiviral agent for HIV-immunotargeting. *Org Biomol Chem.* 2004; 2:660–664. [PubMed: 14985805]
12. Perdomo MF, Levi M, Sallberg M, Vahlne A. Neutralization of HIV-1 by redirection of natural antibodies. *Proc Natl Acad Sci U S A.* 2008; 105:12515–12520. [PubMed: 18719129]
13. Parker CG, Domaoal RA, Anderson KS, Spiegel DA. An antibody-recruiting small molecule that targets hiv gp120. *J Amer Chem Soc.* 2009; 131:16392–16394. [PubMed: 19839582]
14. Solomon D, Kitov PI, Paszkiewicz E, Grant GA, Sadowska JM, Bundle DR. Heterobifunctional multivalent inhibitor-adaptor mediates specific aggregation between shiga toxin and a pentraxin. *Org Lett.* 2005; 7:4369–4372. [PubMed: 16178535]
15. Kitov PI, Lipinski T, Paszkiewicz E, Solomon D, Sadowska JM, Grant GA, Mulvey GL, Kitova EN, Klassen JS, Armstrong GD, Bundle DR. An entropically efficient supramolecular inhibition strategy for Shiga toxins. *Angew Chem Int Ed Engl.* 2008; 47:672–676. [PubMed: 18098248]
16. Kitov PI, Mulvey GL, Griener TP, Lipinski T, Solomon D, Paszkiewicz E, Jacobson JM, Sadowska JM, Suzuki M, Yamamura KI, Armstrong GD, Bundle DR. In vivo supramolecular templating enhances the activity of multivalent ligands: A potential therapeutic against the *Escherichia coli* O157 AB (5) toxins. *Proc Natl Acad Sci U S A.* 2008; 105:16837–16842. [PubMed: 18955695]
17. Gestwicki JE, Crabtree GR, Graef IA. Harnessing chaperones to generate small-molecule inhibitors of amyloid beta aggregation. *Science.* 2004; 306:865–869. [PubMed: 15514157]
18. Kitov PI, Sadowska JM, Mulvey G, Armstrong GD, Ling H, Pannu NS, Read RJ, Bundle DR. Shiga-like toxins are neutralized by tailored multivalent carbohydrate ligands. *Nature.* 2000; 403:669–672. [PubMed: 10688205]
19. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. *Nat Rev Immunol.* 2007; 7:255–266. [PubMed: 17380156]
20. Browning JL. B cells move to centre stage: novel opportunities for autoimmune disease treatment. *Nat Rev Drug Discov.* 2006; 5:564–576. [PubMed: 16816838]
21. Collins BE, Blixt O, Han SF, Duong B, Li HY, Nathan JK, Bovin N, Paulson JC. High-affinity ligand probes of CD22 overcome the threshold set by cis ligands for binding, endocytosis, and killing of B cells. *J Immunol.* 2006; 177:2994–3003. [PubMed: 16920935]
22. Han S, Collins BE, Bengtson P, Paulson JC. Homomultimeric complexes of CD22 in B cells revealed by protein-glycan cross-linking. *Nat Chem Biol.* 2005; 1:93–97. [PubMed: 16408005]
23. O'Reilly MK, Collins BE, Han S, Liao L, Rillaan C, Kitov PI, Bundle DR, Paulson JC. Bifunctional CD22 ligands use multimeric immunoglobulins as protein scaffolds in assembly of immune complexes on B cells. *J Amer Chem Soc.* 2008; 130:7736–7745. [PubMed: 18505252]
24. Kaltgrad E, O'Reilly MK, Liao LA, Han SF, Paulson JC, Finn MG. On-virus construction of polyvalent glycan ligands for cell-surface receptors. *J Amer Chem Soc.* 2008; 130:4578–4579. [PubMed: 18341338]

25. Sulzer B, Perelson AS. Immunons revisited: binding of multivalent antigens to B cells. *Mol Immunol.* 1997; 34:63–74. [PubMed: 9182877]
26. Stone JD, Cochran JR, Stern LJ. T-cell activation by soluble MHC oligomers can be described by a two-parameter binding model. *Biophys J.* 2001; 81:2547–2557. [PubMed: 11606269]
27. Dam TK, Brewer CF. Thermodynamic studies of lectin-carbohydrate interactions by isothermal titration calorimetry. *Chem Rev.* 2002; 102:387–429. [PubMed: 11841248]
28. Dam TK, Roy R, Das SK, Oscarson S, Brewer CF. Binding of multivalent carbohydrates to concanavalin A and *dioclea grandiflora* lectin. *J Biol Chem.* 2000; 275:14223–14230. [PubMed: 10799500]
29. Otsuka I, Blanchard B, Borsali R, Imberty A, Kakuchi T. Enhancement of plant and bacterial lectin binding affinities by three-dimensional organized cluster glycosides constructed on helical poly(phenylacetylene) backbones. *ChemBioChem.* 2010; 11:2399–2408. [PubMed: 21053235]

Figure 1.
Assembly of immune complex of IgM and CD22 mediated by a polyvalent heterobifunctional ligand.

Figure 2. Structures of polyvalent heterobifunctional ligand **1**, its unimeric control ligand **2**, and its polyvalent LacNAc control ligand **3**.

Figure 4.

Avidity of complex formation mediated by ligand **1** and **3** was analyzed by titrating ligands in the presence of CD22 immobilized on magnetic beads and FITC labeled anti-NP IgM (5 μg/mL), and measuring bead fluorescence by flow cytometry. Mean channel fluorescence (MCF) is plotted against ligand concentration.

Scheme 1.
Synthesis of polyvalent heterobifunctional ligand **1**.