Recognition Specificity of Neoglycopolymers Prepared by Ring-Opening Metathesis Polymerization

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Protein−saccharide interactions facilitate fundamental cell−cell recognition events in processes as diverse as host−pathogen interactions, fertilization, development, and the mounting of an immune response.[1] Despite their importance in cell recognition, individual protein−carbohydrate interactions are generally weak (i.e., with $K_a = 10^3-10^4$ M^{-1}).[2] Additionally, carbohydrate-binding proteins often exhibit broad recognition specificity because they generally bind a group of related saccharide structures.[3] To enhance the strength of cell surface binding, Nature often assembles multiple protein−saccharide complexes to provide the necessary avidity.[2,4] In biological systems, both affinity and specificity are critical components of cell−cell recognition. Proteins that mediate cell adhesion must select their biologically-relevant ligand in an environment containing other sugars for which they have some affinity (Figure 1). For example, influenza viral hemagglutinins bind preferentially to cells displaying either 2,3- or 2,6-linked sialic acid residues, depending on the viral strain.[5] If multivalent interactions contribute to the avidity of biological recognition processes, what is their effect on specificity?

We are investigating the functional properties of a new class of polyvalent carbohydrate ligands, substances prepared by ring-opening metathesis polymerization (ROMP).[6,7] We demonstrated that such saccharide-substituted polymers act as polyvalent ligands for the mannose/glucose-binding protein concanavalin A.[6] Moreover, these materials function as high-avidity ligands in cell agglutination assays. To examine the effect of multivalent binding on the specificity of protein−carbohydrate interactions, we synthesized several carbohydrate-bearing polymers and tested them for their ability to bind to concanavalin A. The inhibitory properties of the polyvalent ligands were compared with those of the corresponding monosaccharides to ascertain the effects of multivalent presentation on affinity and specificity.

Concanavalin A can engage in mono- and multivalent binding, rendering the lectin well suited for investigating structure/function relationships of both ligand classes.[8] Concanavalin A recognizes α-glucose- and α-mannose-containing saccharides, with the *manno* configuration at C2 preferred.[9] The protein causes agglutination of red blood cells through interaction with cell surface glycoproteins. Multiple concanavalin A−saccharide complexes are required for hemagglutination, but agglutination can be inhibited by either monovalent or polyvalent glucose and mannose derivatives.[6,8,10]

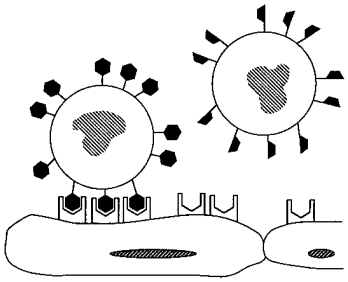

Figure 1. Multivalent protein−carbohydrate interactions may enhance the strength and specificity of cell−cell binding in an environment containing other ligands.

To probe the intrinsic affinity[13] and specificity of concanavalin A, pairs of monovalent ligands of differing affinities for the protein were selected. For example, methyl α-D-mannopyranoside binds with 4-fold-higher affinity than does methyl α-D-glucopyranoside.[8,11] The specificity of interaction of concanavalin A with α-*C*-allyl glycosides is diminished; the free energies of binding for α-*C*-allyl glucoside **1** (−4.7 kcal/mol) and α-*C*-allyl mannoside **2** (−4.9 kcal/mol) differ by only 0.2 kcal/mol.[12] Similarly, the difference between the binding free energies of α-*O*-ethyl mannopyranoside **6** and α-*O*-ethyl glucopyranoside **5** is 0.8 kcal/mol, but the binding free energies of the related α-*C*-propyl mannoside **4** and α-*C*-propyl glucoside **3** are indistinguishable.[12] Thus, concanavalin A binds both *O*- and *C*-glycosides of the α-configuration, but higher diastereoselectivity is observed in the *O*-glycoside series.

To elucidate the effect of multivalent presentation on the functional affinity[13] of these ligands, we determined the inhibitory properties of mannose- and glucose-derived neoglycopolymers for concanavalin A-facilitated agglutination. Polyvalent *C*- and *O*-glycoside derivatives of the *gluco* or *manno* configuration were generated via an aqueous ROMP (Figures 2 and 3). The corresponding bicyclic oxanorbornene derivatives[6] were polymerized to afford glucose- and mannose-substituted *C*-glycoside neoglycopolymers **9** and **10**. To decrease the amount of residual metal in the resulting carbohydrate-substituted materials, the polymerization was effected using a catalyst solution derived from mixing the oxanorbornene substrate and $RuCl_3$ in water.[14,15] Application of this general strategy afforded the corresponding α-*O*-glucoside and α-*O*-mannoside polymers **11** and **12**. For each polymer, the backbone was produced as a 1:1 ratio of cis and trans alkene isomers. The four resulting neoglycopolymers were subjected to gel filtration chromatography. By comparison of their migratory aptitudes with those of dextran standards, a relative molecular mass (M_r) of approximately 10^6 was obtained for each.

Neoglycopolymers **9**−**12** were tested for their specificity as multivalent ligands for the glucose/mannose binding lectin con-

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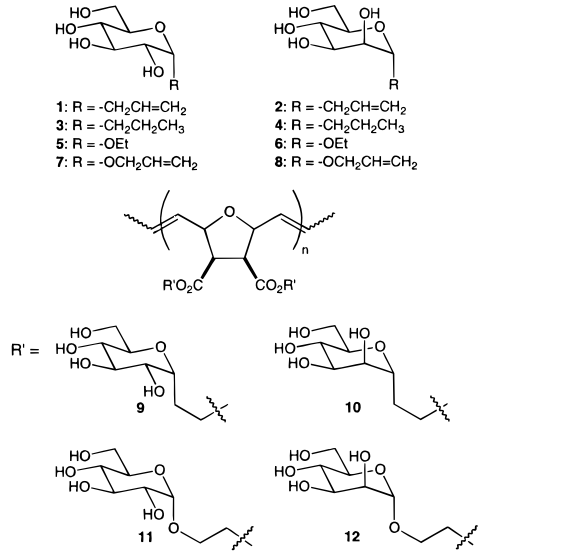

Figure 2. Monovalent and polyvalent inhibitors of concanavalin A-mediated cell agglutination.

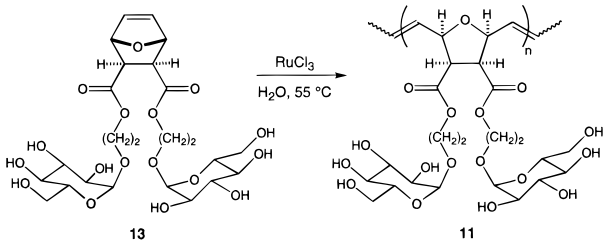

Figure 3. Active catalyst solution was generated by the reaction of $RuCl_3$ with the monomer (8 h, 55 °C). A solution of the catalyst was then transferred to an aqueous solution of the monomer. Polymerizations were complete within 2 h; typical yields: 60−80%.[15]

Table 1. Inhibition of Concanavalin A-Induced Hemagglutination

inhibitor	rel inhibitory dose	inhibiting dose[a] [carbohydrate residues], M
α-methyl glucopyranoside	1	5.0×10^{-2}
α-methyl mannopyranoside	0.25	1.3×10^{-2}
α-*C*-allyl glucopyranoside **1**	0.5	2.5×10^{-2}
α-*C*-allyl mannopyranoside **2**	0.5	2.5×10^{-2}
α-allyl glucopyranoside **7**	2	1.0×10^{-1}
α-allyl mannopyranoside **8**	0.5	2.5×10^{-2}
C-Glc neoglycopolymer **9**	0.001	5.0×10^{-5}
C-Man neoglycopolymer **10**	0.00001	5.0×10^{-7}
O-Glc neoglycopolymer **11**	0.04	2.0×10^{-3}
O-Man neoglycopolymer **12**	0.00025	1.3×10^{-5}
C-Fuc neoglycopolymer[b]	NI[c]	NI[c]

[a] Inhibiting doses were obtained by averaging the results of four independent experiments. The error associated with the dose determination is a factor of 2, as dictated by the 2-fold dilutions of the assay. [b] The synthesis of this polymer, which is similar to **9** and **10**, is described in ref 6. [c] No inhibition of concanavalin A was observed.

canavalin A. The activities of the polymers were assessed by comparing their ability to inhibit erythrocyte agglutination by concanavalin A.[16] The concentration of saccharide residues required for inhibition of four agglutinating doses of concanavalin A was determined for each substrate within a 2-fold dilution. In both the *C*-glycoside and *O*-glycoside series, multivalent carbohydrate ligands were compared to their monovalent analogs (Table 1).

We found that the polyvalent ligands display significant increases in functional affinity in all cases. This gain can be dramatic, as in the 50000-fold enhancement in inhibitory activity observed for the mannose-containing polymer **10** relative to the monovalent derivative **2**.[17] Overall, the *C*-glycoside polymers are more efficacious than are the *O*-glycoside derivatives, a result that may be due to increased hydrophobicity of the *C*-glycoside polymers relative to their *O*-glycoside counterparts.

The inhibition data show that the differences in binding affinity between mannose-containing polymers and glucose-containing polymers in the *O*-glycoside and *C*-glycoside series reflect the differences in the intrinsic binding affinity of the monovalent sugars. Specifically, the small difference in the relative free energies of binding to concanavalin A for α-*C*-allyl mannoside **2** and α-*C*-allyl glucoside **1** cannot be detected in an agglutination inhibition assay. In contrast, a sizable difference in potency exists between the corresponding polyvalent *C*-glycosides: the *C*-mannoside polymer **10** inhibits agglutination at a 100-fold-lower saccharide concentration than does the *C*-glucoside analog **9**. In the *O*-glycoside series, methyl α-D-mannopyranoside inhibits concanavalin A-promoted agglutination at a 4-fold-lower concentration than does the methyl α-D-glucopyranoside as would be expected from the free energies of binding. The polyvalent analogs of these compounds show even greater discrimination in their binding to concanavalin A. The *O*-mannoside polymer **12** inhibits agglutination at a 160-fold-lower concentration than does the *O*-glucoside derivative **11**. Thus, there is an amplification of monovalent binding selectivity, which is dependent on the intrinsic affinity of the various monomeric sugars.[18]

Our measured enhancement in recognition specificity for multivalent saccharides suggests an explanation for the specificity of cell recognition, which is often mediated by cell surface carbohydrate-binding proteins that exhibit weak affinity and broad specificity for the individual ligands. With multiple copies of these ligands and receptors presented on apposing cell surfaces, binding affinity and specificity can be enhanced as we have shown here. This type of recognition specificity may regulate many cell−cell interactions in nature. Viral hemagglutinins appear to show much greater specificity for 2,3-linked sialosides in cell-based assays than in a monomeric binding assay, and it has been proposed that the multivalency of the cell-based system could account for the difference.[5] Our results indicate that a subtle change in structure of an individual saccharide residue may have significant consequences when multiple copies are presented to a receptor.[4] Consequently, polyvalent saccharide derivatives may be useful in the selective targeting of specific carbohydrate binding proteins or cell types. The application of ROMP to the synthesis of such materials affords compounds that exhibit both high affinity and selectivity.

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Supporting Information Available: Experimental procedures and spectral data for the preparation of **9**−**12** and spectral and analytical data for their precursors (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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