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Alkanethiol containing glycopolymers: A tool for the detection of lectin binding

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Abstract—Glycopolymers are useful macromolecules with a non-carbohydrate backbone for presenting saccharides in multivalent form. Here, glycopolymers containing mannose and alkanethiol linker were synthesized through substituting preactivated poly [*N*-(acryloyloxy) succinimide] (pNAS) with amine-containing monomer. With the obtained glycopolymers, a glycosurface was generated on the gold surface of quartz crystal microbalance (QCM) through self-assembled strategy by the use of alkanethiol functional group. Furthermore, the resulting glycosurface was used to detect the binding of mannose specific lectin concanavalin A (Con A).

Many bacteria have lectins (proteins with carbohydrate-binding domains) present on their cell surfaces. These proteins, termed microbial lectins or adhesins,[1] play an important role in the initial stages of infection by mediating the interaction of pathogens with host cell surface glycoconjugates such as glycoprotein, glycolipid, and polysaccharide.[2] The studies of protein–carbohydrate interaction have been challenged by the complexity and heterogeneity of cell surfaces, the inherent structure complexity of carbohydrates, and the typically weak affinities of the binding. In the biological context, this limitation has been overcome by multivalent interactions, i.e., simultaneous contact between the clustered carbohydrates on cell surface and protein receptors that contain multiple carbohydrate recognition domains (CRDs).[3] It has been reported that the multivalent forms of synthetic ligands, either polymers or dendrimers, often have amplified inhibitory effects over the monovalent counterparts. The design and synthesis of polymers with pendant saccharide residues (glycopolymers) have been motivated in part by the recognition that glycopolymers may enhance the multivalent protein–carbohydrate interactions.[4–8]

Glycosurface, in which different carbohydrates are bound non-covalently or bonded covalently on solid surfaces, has been under active development to study protein carbohydrate interaction in recent years. As a popular tool for generating the glycosurface, self-assembled monolayers (SAMs) scaffold has appeared to be one of the most promising model systems for systematic study of the multivalent interaction.[9,10] Moreover, SAMs have been well applied to surface sensitive real-time, label-free analysis methods. Previous results from our laboratories have demonstrated that the combination of carbohydrate SAMs and the non-labeled transducer, i.e., quartz crystal microbalance can be successfully applied to elucidating the binding of carbohydrates with lectins or antibodies.[11,12] Among several classes of SAMs,[13,14] self-assembled monolayers of alkanethiolates on gold currently hold to be the best model system[15–17] since these monolayers form spontaneously by adsorption of alkanethiols from their solutions onto clean gold surfaces.

Recently functional glycopolymers have been synthesized with surface anchoring groups located along the polymer backbone to generate glycosurface with potential utility in bio- and immunochemical assays[18,19] as well as biocapture analysis. Kiesling and coworkers prepared the end-functionalized 3,6-disulfogalactose polymers by ring-opening metathesis polymerization (ROMP). These materials were immobilized onto the

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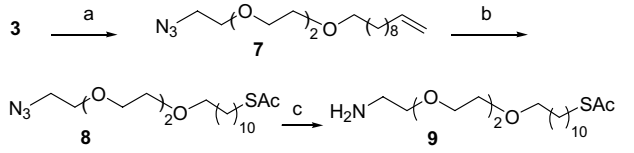
surfaces for the specific interaction with soluble P- and L-selectin.[20] Chaikof et al. synthesized biotin chain-terminated glycopolymers through cyanoxyl-mediated free-radical polymerization for surface glycoengineering.[21] In this research, our interest is to combine the self-assembly strategy with the specially designed functional glycopolymers (Fig. 1) to engineer self-organized, densely packed glycopolymeric recognition layers which could be chemo-adsorbed onto gold surface of QCM and generate multivalent binding cavity. The obtained glycosurface was verified by the specific recognition with a lectin.

A representative glycopolymer **1** with pendant functional groups is designed as shown in Figure 1, in which mannose units serve as ligands for the binding of lectin Con A and the alkanethiol linkers serve as anchor groups that can be self-assembled and covalently adsorbed on the gold surface. Alkanethiols terminated in oligo(ethylene glycol) moieties can effectively resist the non-specific adsorption of proteins[22–24] and the non-specific adhesion of mammalian cells.

Taking the fact that active ester polymers react fast and quantitatively with amines to form the corresponding polyacrylamides, it opens the possibility to obtain multifunctional polymer.[25] First, mannose monomer and alkanethiol monomer were prepared, respectively. Scheme 1 shows the synthesis of mannose monomer **6**. Commercially available 2-[2-(2-chloro-ethoxy)-ethoxy]ethanol **2** was chosen as a spacer building block. Transformation of chloride group of**2** to azide was accomplished in DMF at 90 °C and provided 2-[2-(2-azido-ethoxy)-ethoxy]ethanol **3** in good yield. Glycosylation of **3** with peracetylated mannose activated by Lewis acid BF_3 gave compound **4** in 75% yield. The desired α configuration was confirmed by the coupling constant of anomeric proton ($J_{1,2}$ = 1.5 Hz). The glycoside **4** was deacetylated quantitatively by the Zemplen method to give **5**. Hydrogenation of compound **5** provided mannose monomer **6** in 80% yield.

Scheme 2 illustrates the preparation of the amine-terminated alkanethiol monomer **9**. 2-[2-(2-Azido-ethoxy)-ethoxy]ethanol **3** was deprotonated in 50% NaOH, then reacted with 11-bromoundec-1-ene to yield 11-(azidoundecyl)triethylene glycol **7** in 60% yield. The introduction of thioacetate was achieved by addition of thiolacetic acid to the olefin group of **7** using the procedure described by Whitesides[22] to give **8** in 80% yield. The desired alkanethiol monomer **9** was achieved after the azido group of **8** was converted to primary amine by employing the Staudinger reaction in the yield of 95%.

The functional glycopolymer was synthesized from the precursor preactivated poly [*N*-(acryloyloxy) succinimide] (pNAS) as shown in Scheme 3. pNAS **10** was



Scheme 2. Reagents and conditions: (a) 11-bromoundec-1-ene, 50% NaOH, 100 °C, 60%; (b) AcSH, AIBN, MeOH, hv (pyrex), 80%; (c) Ph_3P, THF, H_2O, 60 °C, 95%.

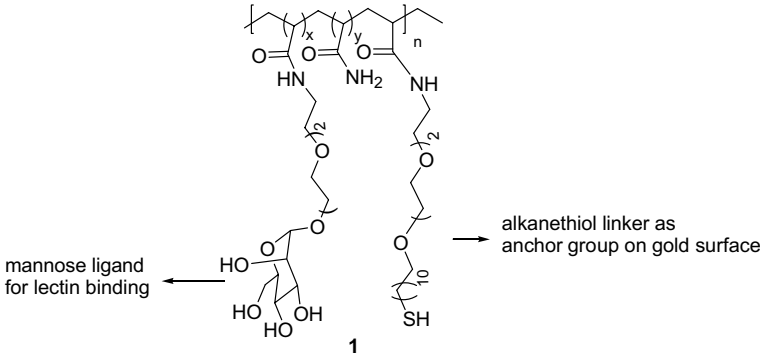
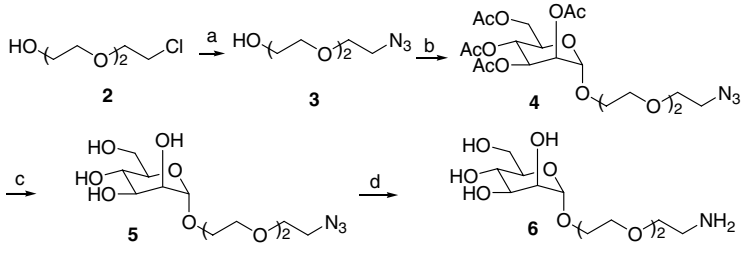


Figure 1. Glycopolymer pendant with mannose and alkanethiol linker.



Scheme 1. Reagents and conditions: (a) NaN_3, DMF, 90 °C, 90%; (b) $BF_3{\cdot}Et_2O$, peraacetylated mannose, CH_2Cl_2, rt, 75%; (c) NaOMe, MeOH, rt, quantitative; (d) H_2 (50 psi), Pd/C, 80%.