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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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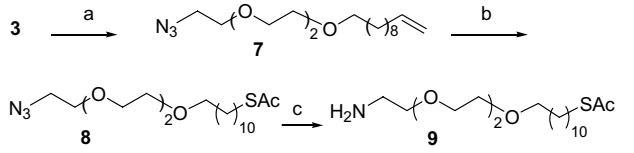
surfaces for the specific interaction with soluble P- and L-selectin.[20] Chaikof et al. synthesized biotin chain-terminated glycopolymers through cyanoxyl-mediated free-radical polymerization for surface glycoengineering.[21] In this research, our interest is to combine the self-assembly strategy with 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%.

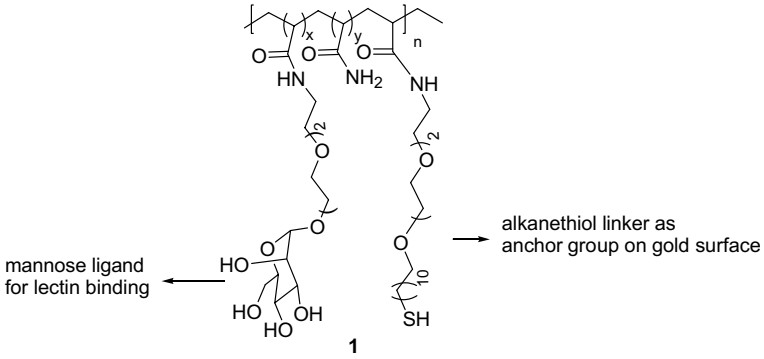
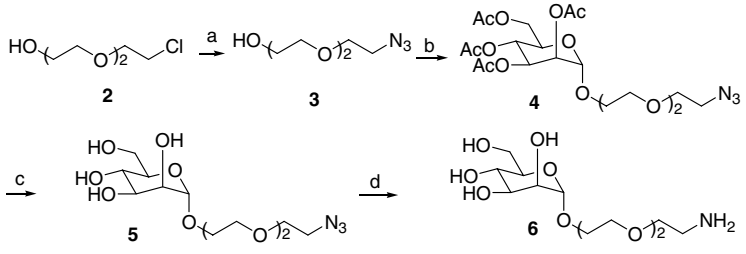
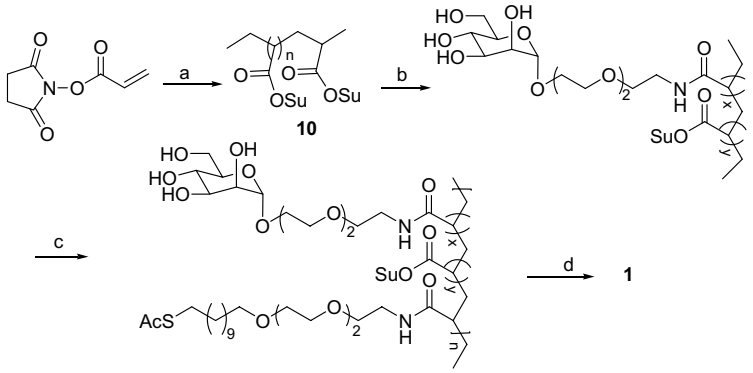


Figure 1. Glycopolymer pendant with mannose and alkanethiol linker.



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%.



Scheme 3. Reagents and conditions: (a) AIBN, benzene, 60 °C; (b) compound **6,** Et_3N, DMF, rt, 24 h; 65 °C, 6 h; rt, 24 h; (c) compound **8**, Et_3N, DMF, rt, 24 h; 65 °C, 6 h; rt, 24 h; (d) $NH_3{\cdot}H_2O$, rt, 24 h.

obtained by polymerization of *N*-(acryloyloxy) succinimide through the radical polymerization initiated by AIBN. The preactivated polymer was characterized following the literature.[26] The molecular weight of this preactivated polymer was determined by gel filtration chromatography after its complete hydrolysis to poly (acrylic acid) sodium salt. The average molecular weight of the hydrolyzed polymer was $M_w = 252$ kDa with a relatively narrow molecular weight distribution ($(M_w/M_N) = 1.5$). The degree of polymerization is $\sim 1.8 \times 10^3$. This preactivated polymer (590 μmol (moles of unit)) was then transformed into the multifunctional polymer by substituting with amine-containing functionalities. The functional polymer was prepared by a stepwise substitution of the active ester groups, first, by addition of mannose monomer (192 μmol, 0.32 equiv), second, by reaction with alkanethiol anchor groups (236 μmol, 0.4 equiv), and last, by quenching with aqueous ammonia. The resulting polymer thus incorporates two different features: carbohydrate ligands and surface anchor group. The actual composition of polymers was measured by ^{1}H NMR, [27] which was determined by the signal from methylene protons linked with thiol (2.54 ppm, CH_2) on the alkanethiol linker, the anomeric proton of mannose (4.83 ppm), as well as that of the polymer backbone methine (2.26 ppm, CH), to give the ratio of mannose unit, alkanethiol unit, and acrylamide unit to be 1:1:1.

The obtained polymer was tested as the biorecognition element via QCM transducer. QCM is a mass sensor and gives a response that characterizes the binding event between a sensing layer, which is immobilized on the surface of the QCM transducer, and the analyte to be detected. The synthetic functional glycopolymer was immobilized on the gold surface of QCM. mPEG-thiol (purchased from Nektar) was used as a blocking reagent to reduce the non-specific adsorption. If the immobilization is rigid, the resonant QCM frequency change depends upon the mass attached to the surface according to the Sauerbrey equation,[28] $\Delta f = -2\Delta m n f_0^2 / \left[A(\mu_q \rho_q)^{1/2}\right]$, where n is the overtone number, μ_q is the shear modulus of the quartz 2.947×10^{11} g/(cm s^2), and $\rho_q$is the density of the quartz (2.648 g/cm^3), and which assumes the foreign mass is strongly coupled to the resonator.[29]

Mannose can bind with a lectin, Con A,[30] which was isolated from the seeds of the jack bean. The mannose sensor specificity was examined using a negative control, Erythrina cristagalli lectin (ECL), a galactose-specific legume lectin.[31] As shown in Figure 2, negligible frequency change was observed for the addition of ECL. After exposure to ECL, Con A solution at different concentrations was consecutively added to the same sensor, and strong signals were observed (Fig. 2). This study demonstrates that the mannose-QCM sensor has high sensitivity and specificity for binding with the Con A even after exposure of the sensor surface in a complex matrix (i.e., ECL). Shown in Figure 2 inset, the polymannose immobilized on the gold QCM sensor shows much better sensitivity than the mono-mannose QCM sensor in our previous work. The higher sensitivity of polymannose suggests the presence of a multivalent interaction between Con A and mannose ligands.

To further examine the sensor specificity, fetal bovine serum (FBS) and ECL were added to the polymannose-QCM sensor in series (Fig. 3), either really small or no non-specific adsorption was detected. These

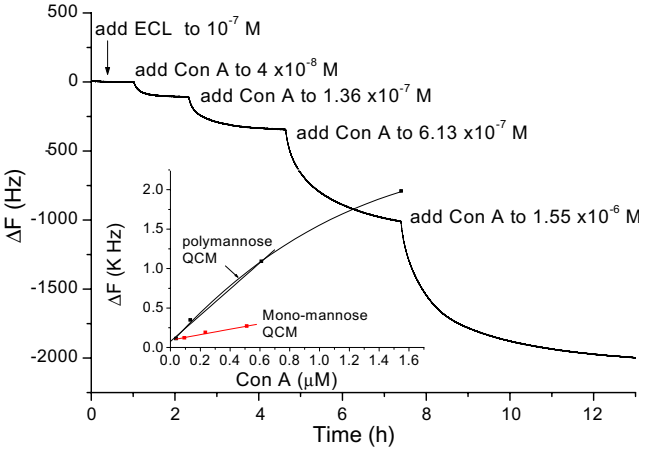


Figure 2. Frequency change versus time curve when ECL (100 nM) and different concentrations of Con A solutions were added sequentially to the polymannose QCM in 1.0 mL PBS buffer (pH 7.2) with 1 mM Mn^{2+} and 1 mM Ca^{2+}. Inset: calibration curve: frequency shift versus Con A concentration on Mono-mannose QCM surface (red curve); and polymannose QCM surface (black curve).

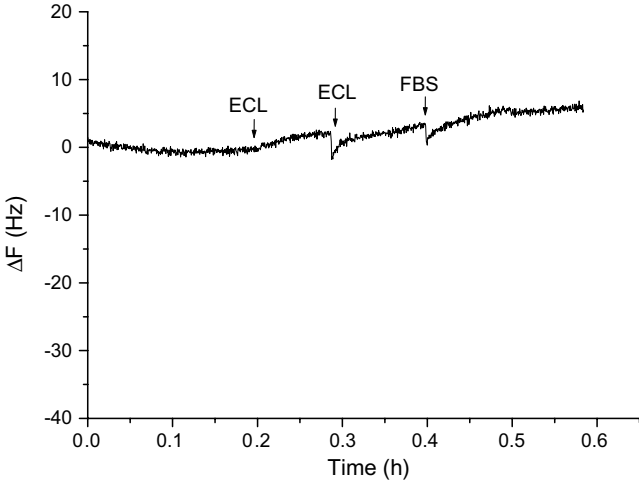


Figure 3. Frequency change versus time curve when ECL (200 nM) and FBS (5.2 μg/ml) were added sequentially to the mannose-QCM in 1.0 ml PBS buffer (pH 7.2) with 1 mM Mn^{2+} and 1 mM Ca^{2+}.

results indicated that the polymannose-QCM electrodes specifically detected Con A in the presence of contaminants.

By obtaining the damping resistance through fitting the Butworth–vanDyek Circuit, we can study whether the modified layer shows viscoelastic properties and to verify whether the Sauerbrey equation is validate in our system. In the course of all experiments, the damping resistance was fluctuating within a range of $|R_q|/R_q \leqslant 1.5\,\%$. This result proved that the attached biofilms behave as a rigidly attached mass and the Sauerbrey equation is valid.

In order to obtain accurate affinity constant for the binding between Con A and mannose, we used experiment conditions in which mass transfer is very fast and is not the rate-limiting step by thoroughly stirring the solution using magnetic stir. Under this condition, the binding will be the rate-limiting step and the apparent binding affinity for Con A binding to the mannose QCM surface could be estimated by using the Langmuir adsorption model.[32] According to the model, the mass change at equilibrium was related to the original concentration of Con A.

$$\frac{[\text{Con A}]}{\Delta M} = \frac{[\text{Con A}]}{\Delta M_{\max}} + \frac{1}{\Delta M_{\max} K_A}$$

In this equation, $\Delta M_{\max}$ is the maximum amount that Con A can be bound. It is the characteristics of the sensor surface, which is equivalent to the binding of Con A when all the mannose is converted to Con A–mannose complex. ΔM is the measured binding amount at equilibrium and [Con A] is the original concentration of Con A.

The apparent binding constant K_A for the binding between Con A and mannose was estimated to be $(1.4 \pm 0.6) \times 10^6\ M^{-1}$ ($n = 3$), this data showed that the immobilization of glycopolymeric ligands yields a synthetic surface with the similar binding affinity as the surface displaying monovalent ligand shown in our previous work.[33] An analyte Con A has four identical binding sites at pH 7, when the first binding site bound to the ligand mannose, the second free Con A site is brought in close contact with another mannose ligand. The formation of the second binding will depend on the flexibility of the analyte, ligand, and the availability of the free ligand. Due to the tetrameric structure of Con A, there will be four sets of rate constants. The meaning of the four sets of rate constants and particular the second and subsequent ones is extremely difficult to measure when the ligand is immobilized on the surface. The apparent affinity constant obtained using Langmuir adsorption model may not be the best indication for the multivalency binding. A distinctive advantage of glycopolymer is that the ratios of mannose unit, alkanethiol unit to acrylamide can be further optimized to have high ligand presenting density as well as the size specificity. Both can be studied to increase the avidity of the multivalent carbohydrate and protein interaction. Our next goal is to prepare the polymeric ligand by increasing the ratio between the ligand and the surface anchor group so as to achieve the enhanced carbohydrate–lectin multivalent recognition.

In conclusion, we have demonstrated for the first time the design and synthesis of alkanethiol containing glycopolymer. Compared to other glycopolymer, the novelty of polymer presented here is based on its multifunctionality, as it incorporates carbohydrate ligands and alkanethiol surface anchor group. As an extension of this work, we envisage that synthesis of glycopolymer with optimal ratio of ligand to alkanethiol could furnish the multivalency enhancement of carbohydrate–lectin interactions and this work is currently in progress.

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27. Compound **1** ^{1}H NMR (400 MHz, D_2O): δ 4.83 (br, 1H), 3.89–3.90 (m, 1H), 3.82–3.83 (t, J = 4 Hz, 1H), 3.80 (br, 1H), 3.75–3.77 (d, J = 8 Hz, 1H), 3.73–3.74 (d, J = 4 Hz, 1H), 3.70–3.66 (m, 4H), 3.64 (br, 4H), 3.63 (br, 6H), 3.60–3.61 (m, 4H), 3.54–3.58 (t, J = 16 Hz, 6H), 3.41–3.45 (t, J = 16 Hz, 2H), 2.45–2.54 (m, 2H), 2.37–2.41 (m, 2H), 2.26–2.30 (m, 2H), 1.74–1.78 (m, 4H), 1.35 (br, 2H), 1.28 (br, 14H).
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