Controlled Alternate Layer-by-Layer Assembly of Lectins and Glycopolymers Using QCM-D

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Supporting Information

ABSTRACT: Layer-by-layer (LBL) assembly of concanavalin A (Con A), peanut agglutinin (PNA) plant lectins, and well-defined synthetic glycopolymers via their biological affinities have been prepared using a quartz crystal microbalance with dissipation monitoring (QCM-D). We demonstrate the use of mannose/galactose glycopolymers as lectin binders due to their selective binding to Con A/PNA, respectively. A detailed analysis of the adsorption processes and the adsorbed layer are provided and tuning the composition of multilayers using a series of well-defined glycopolymers differing only in the pendant sugar ratio is discussed.

Lectin–carbohydrate interactions play key roles in a large number of important biological processes such as immune response, cell–cell communication, and pathogen infection. However, the affinities between most monosaccharides and their lectin receptors are usually weak. This can be overcome and dramatically enhanced by multivalent carbohydrate ligands through the “glycoside cluster effect”. However, the structural diversity and complexity of carbohydrates in nature has frustrated chemists to synthesize desired carbohydrates and to analyze lectin–carbohydrate interactions.

This difficulty gave rise to various carbohydrate mimics, especially glycopolymers, which are synthetic macromolecules with pendant carbohydrate moieties. As they can be both antagonists and agonists of many biological processes, glycopolymers have been applied in areas such as targeted drug delivery and macromolecular drugs. For example, because the first inhibitor of influenza hemagglutinin based on a glycopolymers was synthesized by Bovin and co-workers in 1990, glycopolymeric treatments of influenza viruses have been developed. Recently, various self-assembled structures of glycopolymers such as glyconanoparticles, carbon nanotubes, microarrays, multilayer films and capsules have emerged as novel carbohydrate-based biomaterials showing even more promising advantages over conventional application of glycopolymers. Sulfonated glycopolymer films on a silicon oxide surface exhibited improvements for in vitro blood compatibility. Hyperbranched poly(glycoacrylate) polymer films were supportive for human vein endothelial cell growth in culture.

LBL self-assembly has been significantly developed in recent years for the deposition of multilayer films from solutions onto solid surfaces. An advantage of LBL assembly is that it allows for a choice of various substrates and has very good control over the film thickness, composition, stability and surface functionality. LBL assembled films have been prepared previously via electrostatic forces of poly(cationic) and poly(anionic) materials, small molecule hydrogen bonding, or biological affinities such as avidin–biotin, lectin–carbohydrate, and antigen–antibody interactions.

In this current work, we make use of biological affinities to demonstrate in situ alternate LBL assembly of lectin and glycopolymers controlled by QCM-D, Figure 1. Well-defined

Figure 1. Schematic diagram of the LBL self-assembly by QCM-D using well-defined glycopolymers.

Received: September 12, 2011
Accepted: December 8, 2011

dx.doi.org/10.1021/mz200063r/ACS Macro Lett. 2012, 1, 180–183
glycopolymers were prepared to achieve fine-tuning of the composition of the resulting multilayer films and the deposition process was under real time control by using QCM-D. Two different ways were investigated to prepare the multilayer films in two different orders in which either lectins or glycopolymers were immobilized first onto the gold surface of the quartz crystal chip.

Both Con A and PNA were selected as the model lectins in this study. Con A, extracted from jack bean seeds, is a mannose-selective and well-studied homotetramer with four subunits (26.5 kDa each). PNA is a lectin isolated from peanuts as a 110 kDa tetramer composed of four identical subunits, which binds selectively to galactose. In all QCM-D experiments, HBS buffer (10 mM HEPES, 150 mM NaCl, pH 7.4) containing 1 mM Ca$^{2+}$, Mg$^{2+}$, and Mn$^{2+}$ was used as the presence of divalent metal ions and is essential for the activity of these lectins. The flow rate was set to 50 μL min$^{-1}$ to get efficient mass coverage over the quartz crystal surface and to reduce the time caused by the assays.

The glycopolymers (Scheme 1) were synthesized via a combination of copper(I)-mediated living radical polymerization and copper-catalyzed azide–alkyne cycloaddition (CuAAC) click reaction following a common and versatile general procedure. The mannose-galactose glycopolymers (P1–P5) were prepared using the same polymeric backbone framework, and their compositions and molecular weights are shown in Table 1. The disulfide mannose glycopolymer (P6) was polymerized using a disulfide functionalized initiator (Supporting Information).

First, it is desirable to immobilize Con A onto the Au chip surface. The binding of Con A to bare gold was found not to be stable and the lectin was washed off slowly with HBS buffer. In order to circumvent this the Au chip was chemically modified with 11-mercaptoundecanoic acid (MUA), followed by 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide (EDC) hydrochloride and N-hydroxysuccinimide (NHS; Supporting Information). Con A was then bound to the modified surface via nucleophilic substitution with lysine, present on the surface. In this case, the QCM-D data showed that Con A had indeed attached to the modified quartz crystal surface and was not removed with extensive washing with HBS buffer, Figure 2 (left).

The resonating frequency of the Au coated chip decreased when Con A buffer solution passed over the surface. After it had reached a plateau, it was necessary to rinse the surface with HBS buffer so as to exclude the bulk effect from changes in composition and viscosity of the injected Con A solution until a further plateau was reached. Ethanolamine hydrochloride (1M, pH = 8.5) was used to block unreacted NHS groups to prevent their reacting with the glycopolymers. Subsequently, the mannose containing glycopolymer P1 in HBS solution was passed through the system. As the pendant mannose interacted with immobilized Con A, P1 on the surface was stable when washed by HBS buffer. The mass of the materials deposited on the quartz crystal surface over time was estimated by both Sauerbrey’s equation and by Voigt modeling, Figure 2 (right).

An alternative approach involves binding the glycopolymers to the gold coated quartz crystal. As the mannose-containing glycopolymer, P1, did not remain bound to the chip, it was replaced with the disulfide-containing mannose glycopolymer P6. In this case, the disulfide bond resulted in adsorption, and P6 remained bound and stable on the surface when washed with HBS buffer for several hours (Supporting Information). Thus, bilayer assemblies were constructed by adsorption of the disulfide mannose glycopolymer P6 directly onto the Au chip surface and then passing Con A buffer solution over the QCM-D chip, Figure 3 (left). Con A remained on the surface as the second layer when washed with HBS buffer, indicating that Con A interacted with glycopolymer P6. Two concentrations of

![Figure 2](image2.png)

**Figure 2.** QCM-D plot (left) and estimated mass (right) of materials deposited on the modified Au chip surface over time: (A) HBS buffer; (B) Con A in HBS buffer (0.5 mg mL$^{-1}$); (C) ethanolamine HCl in HBS buffer (1 M, pH 8.5); (D) P1 in HBS buffer (0.5 mg mL$^{-1}$).

![Figure 3](image3.png)

**Figure 3.** QCM-D plot (left) and D-f plot (right) of the self-assembly between P6 and Con A: (A) HBS buffer; (B) P6 in HBS buffer (0.5 mg mL$^{-1}$); (C) Con A in HBS buffer (0.5 mg mL$^{-1}$).

### Table 1. Composition of Mannose-galactose Glycopolymers

<table>
<thead>
<tr>
<th>Glycopolymer</th>
<th>Man. (%)</th>
<th>Gal. (%)</th>
<th>$M_n$ (kDa)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>100</td>
<td>0</td>
<td>22.1</td>
<td>1.31</td>
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<tr>
<td>P2</td>
<td>75</td>
<td>25</td>
<td>22.5</td>
<td>1.34</td>
</tr>
<tr>
<td>P3</td>
<td>50</td>
<td>50</td>
<td>22.2</td>
<td>1.33</td>
</tr>
<tr>
<td>P4</td>
<td>25</td>
<td>75</td>
<td>22.1</td>
<td>1.31</td>
</tr>
<tr>
<td>P5</td>
<td>0</td>
<td>100</td>
<td>22.1</td>
<td>1.29</td>
</tr>
<tr>
<td>P6</td>
<td>100</td>
<td>0</td>
<td>35.7</td>
<td>1.84</td>
</tr>
</tbody>
</table>
Con A buffer solution were investigated. The change in frequency of the chip caused by Con A in buffer solution (0.1 mg mL\(^{-1}\)), \(\Delta f = -108\) Hz, while that caused by using a higher concentration of Con A (0.5 mg mL\(^{-1}\)), \(\Delta f = -185\) Hz. The mass and thickness of the two layers can be tuned by changing the concentration of P6 and Con A. In this case, the factors that affect the lectin–carbohydrate interaction, such as concentration, pH value, temperature, ionic strength, and so on, can be investigated systematically due to the advantages of QCM-D.

The structure and dynamic viscoelastic properties of the absorbed layers were obtained by using a dissipation versus frequency plot (D-f plot), Figure 3 (right). For adsorption of glycopolymer P6, the dissipations for both concentrations were very small and the D-f relations were approximately linear, which indicated that the two films formed by P6 were close to rigid. The adsorption of Con A was more complicated. The slope of the D-f plot for Con A increased in both cases, signaling more dissipation per added molecule during the adsorption process. This showed the binding of Con A to the P6 monolayer film was not only kinetically controlled, but also influenced by the transport limitations, such as the conformational rearrangement of Con A, trapped liquid in the layer, or even the interfacial processes. The influence on the layer of Con A with higher concentration (0.5 mg mL\(^{-1}\)) was larger than that of the lower concentration (0.1 mg mL\(^{-1}\)), and the absorbed film was less rigid.

Lectin–glycopolymer assemblies, starting with the attachment of the disulfide bonds in the polymeric backbones to quartz crystal surface provide a facile approach to multilayer bioactive films, Figure 4 (left). The glycopolymer P6 (0.5 mg mL\(^{-1}\), \(\Delta f = -23\) Hz) was attached to the surface, followed by Con A (0.5 mg mL\(^{-1}\), \(\Delta f = -187\) Hz), mannose–galactose glycopolymer P3 (0.5 mg mL\(^{-1}\), \(\Delta f = -15\) Hz) and PNA (0.5 mg mL\(^{-1}\), \(\Delta f = -63\) Hz) sequentially. The four layer alternate assembly was achieved using QCM-D as the mannose groups in P3 bound to Con A, while the galactose moieties interacted with PNA.

The slope of the D-f plot for P3 adsorption was higher than that for P6 adsorption, which was comparable to the slope for the lectin Con A and PNA adsorption, Figure 4 (right). This indicates that the absorbed P3 layer was less rigid than the P6 layer and the interactions between P3 and Con A, PNA, and P3 were viscoelastically similar. In addition, the part of the D-f plot for P3 binding was nearly linear, which indicated a monolayer was formed by P3 without any obvious conformational changes. This factor should be taken into consideration when preparing multilayer films.
first layer, which also exhibited signal amplification. The tuning of the composition of the multilayer films was also studied using different glycopolymers. The controlled LBL multilayer self-assembly of lectins and synthetic glycopolymers through their specific interactions controlled by QCM-D showed a facile way to prepare multilayer bioactive films. The future studies will focus on the binding kinetics of lectins and glycopolymers using QCM-D.

**ASSOCIATED CONTENT**

Supporting Information

Synthesis procedures and characterization of the glycopolymers, QCM-D experimental procedures, and complementary results. This material is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We thank EPSRC Dorothy Hodgkin Scholarship and the China Scholarship Council (Y.G.), Unilever, and EPSRC (S.S.), the EU Marie Curie Fellowship scheme (Proposal Number 235999 (WBAWBA); CRB) for funding. Thanks go to National University of Defense Technology for supporting Y.G.’s CSC Application. Some equipment used in this research was supported by the Innovative Uses for Advanced Materials in the Modern World (AM2), with support from Advantage West Midlands (AWM) and part funded by the European Regional Development Fund (ERDF). CRB is currently a Science City Research Fellow and D.M.H. is currently a Royal Society/Wolfson Fellow.

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