Modulating Glycan Surface Density Via a "Click" Conjugation Strategy

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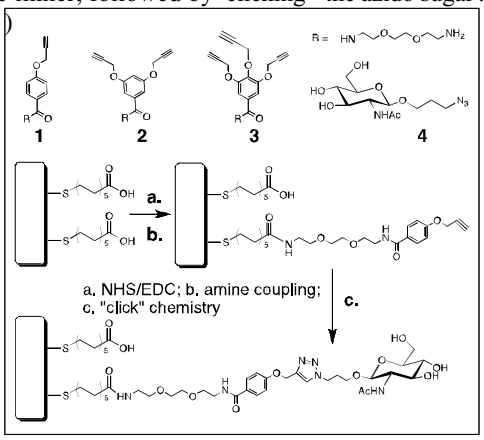
Statement of Purpose: Carbohydrate microarrays and biosensors are powerful tools for the study of carbohydrate-mediated biological processes, including host-pathogen interactions, cell adhesion and signaling. Glycans displayed on the solid surface of the array/biosensor elicit selective recognition by carbohydrate-binding proteins (lectins and adhesins) which are frequently presented on viral, bacterial, and mamlian cell surfaces. Carbohydrate-lectin interactions often depend on the dense presentation and multivalency of glycans "clusters". Recognition of the role played by multivalency on glycan array performance is growing among the glycomics community , but the biointerface of most glycan arrays lack accurate control of surface density. This complicates the interpretation of binding results. "Click" chemistry [1] provides a reliable and stoichiometric means of conjugating bioactive molecules, afforing reliable access to discrete structures. Herein, we demostrate that glycan surface density can be modulated using a "click" chemistry linking strategy permitting the tuning of glycan multivalency on array/biosensor surfaces.

Methods: Mono- (**1**), di- (**2**), and tri-functionalized (**3**) linkers bearing propargyl and N-acetyl group and glucosamine propyl azide (azidoGlcNAc **4, Figure 1**) were synthesized as previously described.[2] A Biacore™ gold chip was immersed in 11-mercaptoundecanoic acid ethanolic solution to construct carboxyl-terminated self-assembled monolayers. The individual flow channels (FCs) on the chip were activated by Biacore amine coupling, and injected with **1**, **2**, and **3**, respectively. The resulting FCs were injected with a "click" reaction mixture of glycan **4**, $CuSO_4$, and sodium ascorbate. Following conjugation , the "clicked" sensor chip was blocked with BSA-Tween (BSA-T) to eliminate non-specific protein fouling of the surface . The plant lectin, wheat-germ agglutinin (WGA) , with concentration varying from 10 nM to 4 μM were flowed through the FCs and regenerated by glycine solution. SPR sensorgrams were obtained and subtracted from bulk refractive index changes.

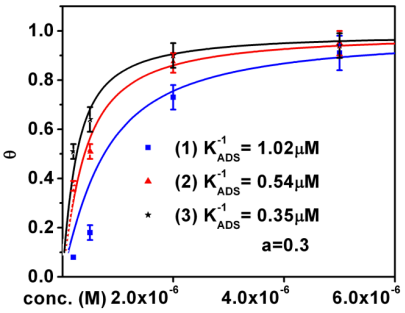
Results: WGA is inhibited by N-acetylglucosamine (GlcNAc) and its (β1→4) oligomers, and is, therefore, widely used to selectively recognize surface bearing GlcNAc headgroups. At neutral pH, WGA is a dimmer with an isoelectric point of 8.7 ± 0.3. As a basic protein, WGA would be attracted by a negatively charged surface. We observed that this nonspecific binding onto the "clicked" chip surface could be remarkably reduced by prior blocking with BSA-T. When WGA was flowed over the FCs, the SPR response increased due to specific WGA-GlcNAc binding. The SPR response returned to a baseline following a glycine rinse . The adsorption isotherms for the binding of WGA to the three 'clicked" FCs starting from **1**, **2**, **3** were obtained by plotting the relative WGA surface coverage as a function of WGA solution concentration. The solid lines in Fig. 2 are the fitting curves using a Frumlin isotherm model. [3]

Fig. 1 Structures of mono-, di-, and tri-functional linkers, and GlaNAc propyl azide for glycan surface modification. Surface conjugation is achieved via conjugation of the alkyne linker, followed by "clicking" the azido sugar.



The dissociation coefficients K_{ADS}^{-1} [4] for WGA binding to the mono- di and tri- "clicked" surfaces containing **1**, **2**, and **3** were estimated to be 1.0, 0.5 , and 0.3 μM, respectively. When compared to the millimolar minimal concentration of free GlcNAc required to inhibit agglutinin binding [5], the lectin/glycan binding on our "clicked" surface was enhanced by a factor of 10^3 to 10^4.

Fig. 2 Relative WGA surface coverage as a function of WGA solution concentration on the "clicked" surfaces.



Conclusions: We have successfully modulated the glycan surface density through a "click" conjugation strategy on a biosensor surface. This approach allows us to tune the glycan "clustering" effects and multivalency at the glycan array biointerface, which is currently being explored by surface analytical tools and molecular simulation.

References: [1] Hartmuth C.K. Angrew. Chem. Int. Ed. 2001;40;2004-2021. [2] Collman J.P. Eur. J. Org. Chem. 2006;2707-2714. [3] Smith E.A. J. Am. Chem. Soc. 2003;125;6140-6148. [4] Dhayal M. Langmuir 2009;25;2181-2187. [5] Monsigny M. Eur. J. Biochem. 1979; 98; 39-45.