A Simple and Versatile Method to Fabricate Glycan Arrays

Jing Shang, Fang Cheng, Daniel M. Ratner.
Department of Bioengineering, University of Washington.

Statement of Purpose:

Specific carbohydrate-macromolecule interactions govern many critical cellular functions, such as recognition, communication and adhesion.[1] Glycan arrays have become a powerful technology for studying carbohydrate interactions in a high-throughput manner.[2] However, reliable immobilization of carbohydrate on a solid support remains a significant challenge in achieving widespread adoption of the carbohydrate microarray paradigm. A variety of strategies have been developed to address array surface modification with carbohydrate. However, most involve multiple steps of glycan synthesis and surface functionalization, which is both time-consuming and complex. [3-6]

We report here a simple and versatile method for immobilizing unmodified, thiolated or aminated glycans as well as proteins and glycoproteins on gold surfaces. Specific interactions of these immobilized sugars with carbohydrate-binding proteins (lectins) were demonstrated using surface plasmon resonance imaging (SPRi). This immobilization technique simplifies the process of glycan array fabrication and surface modification of biomaterials. Moreover, it expands the range of glycans available for surface functionalization, facilitating continued progress in the study of glycan-dependent biological interactions.

Methods:

Gold chips coated with a self-assembled monolayer (SAM) containing hydroxyl end groups were treated with divinylsulfone (DVS) in alkaline buffer solution, activating the surface towards nucleophilic attack. After activation, solutions of free or derivatized sugars were spotted on the surface resulting in immobilization. Finally, the unreacted vinylsulfone was quenched with mercapto-oligoethylene glycol. SPRi was employed to detect the binding between immobilized sugars and specific lectins. Multiple lectins were screened on each gold chip by regenerating with urea solution between runs.

Results / Discussion:

SPRi demonstrated specific protein-carbohydrate binding, as would be expected for bioactive immobilized sugars. For example, Concanavalin-A (ConA) bound to mannose, glucose and maltose but not galactose or lactose. Ricin, a protein toxin, bound only to galactose and lactose. As shown in figure 1, specific glycan-lectin binding results in the change of refractive index and increases of image brightness on the corresponding spots. The sugar immobilization conditions, such as concentration, pH and incubation time, were optimized for maximal binding sensitivity.

In addition to hydroxyl groups, vinylsulfone reacts with amino and sulfhydryl groups. We demonstrated the wide range of biomolecules that can be immobilized using this method, immobilizing thiolated and amino-functionalized glycans, proteins and glycoproteins. The bioactivity of these sugars was established using SPRi to measure lectin binding.

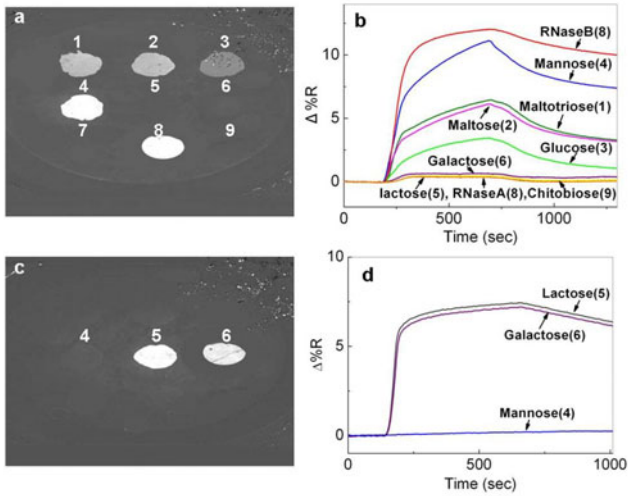

Figure 1. SPR images and sensorgrams of ConA (a,b) and ricin (c,d) binding to a glycan array fabricated from unmodified carbohydrates and proteins.

This array immobilization method effortlessly expands microarray research to include any biomolecule with nucleophilic hydroxyl, sulfhydryl or amino groups. Their interaction with binding partners such as lectins, antibodies, mammalian cells, pathogens and viruses can be studied using the label-free SPRi method. The facile fabrication and detection method will expedite the study of carbohydrate-mediated biomolecular interactions.

Conclusions:

A simple and versatile method to immobilize carbohydrates on gold surfaces has been developed. Using this method, glycan arrays were fabricated and characterized. The immobilized carbohydrates retained their biological activity, according to SPRi of their interactions with specific lectins. The combination of this efficient carbohydrate-immobilization strategy with label-free SPRi will make the glycan array more amenable to non-specialized labs and also advance research on carbohydrate-functionalized biomaterials.

References:

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