Lectin Binding on MDCK Cells and Glycan Microarrays

Nadeen Allah Werdi, Taryn Lucas and Kamil Godula

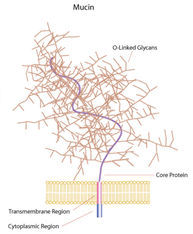
The Department of Chemistry & Biochemistry, University of California San Diego, 9500 Gilman drive, La Jolla, California

Abstract

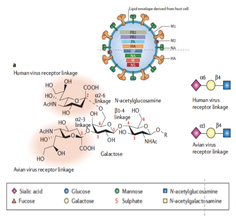
The influenza virus is known as a disease of the respiratory tract. It binds to mucosal glycans through hemagglutinin (HA) protein on its viral coat to enter an organism and start the infection. The receptor for HA is sialic acid (N-acetylneuraminic acid) which is a sugar that decorates glycoconjugates, like mucins, extending from the cell surface. In this study, we used lectins to probe a cell line that is commonly used to model influenza infection. We determined the equilibrium binding concentration of SNA to be 5.39 nM, RCA to be 2.89 nM, PHA-E to be 0.340 nM, MAL II to be 1.45 nM. We also constructed a microarray of mucin mimetics containing various valencies and densities of 3' and 6' sialyllactose and lactose. The array was incubated with SNA and MAL II in the hopes of generating a binding curve for comparison with the cell study. We were unable to obtain the curve but we did see that lectin specificity was maintained in the synthetic system which provides evidence that viral specificity will also be maintained. A future goal of this project is to incubate virus on the array to determine how receptor presentation influences binding.

The Role of Glycans in the Influenza Virus

The cell surface is covered with glycans that provide essential functions for development and survival of the organism. The sugars, commonly attached to proteins and lipids as glycoconjugates, can be involved in maintaining structural integrity, as well as being used for recognition and signaling events. Mucins are a type of glycoprotein that extends from the cell surface. They can also be secreted into the extracellular where they make up the mucosal barrier that serves to hydrate and protect underlying cells. The protein backbone of mucins contains regions rich in serine and threonine, From which sugar chains can be attached. Many of the chains are terminated with sialic acid residues that can be recognized by lectins which are carbohydrate binding proteins that bind to specific sugar linkages.



For example, the influenza virus contains a protein called hemagglutinin (HA) that acts as a lectin to bind to sialic acid residues present on the glycoconjugates, like mucin glycoproteins extending from the cell surface. Once the virus finds its sugar receptor and binds, it can then enter the cell to initiate infection. The HA of the influenza virus, which originates in birds, starts with a specificity for α2-3 linked sialic acid, but in humans, many mucins glycan chains are terminated with sialic acid that is linked in an α2-6 conformation. As such, avian HA must adapt to successfully infect human cells.



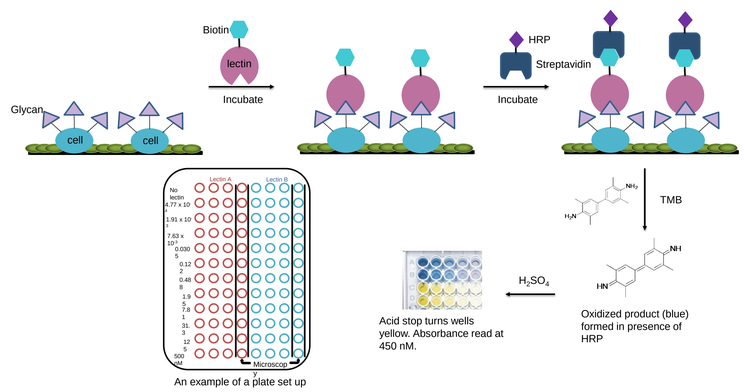
Madin-Darby Canine Kidney (MDCK) cells are often used to model influenza viral infection because they have high susceptibility to infection with various influenza virus strains. In this study, we incubated lectins containing known carbohydrate recognition domains (CRDs) with MDCK cells to determine their equilibrium binding concentrations (EC_{50}). Because lectins are specific for certain sugar linkages, this experiment can provide information on the type of glycans present on the cell surface.

Lectins were also incubated with a microarray printed with known sugar linkages to visualize specificity. Glycan microarrays are a high throughput tool to determine binding preferences of proteins to sugar residues. The Godula lab utilizes click chemistry to immobilize mucin mimetic polymers terminated with an azide functional group to a cyclooctyne-coated glass slide. Employing mimetic polymers offers the advantage of being able to define the sugar structures present on the array, a feat that is not possible when using natural mucins that are heterogeneous due to the many modifications that are possible. Our glycopolymers can be synthesized to contain various amounts of sialic acid and can be printed on the array at different concentrations. By incubating the slide with lectins at various concentrations, binding data similar to the EC_{50} from the MDCK cell study can be generated. The lectins used in this study were SNA and MAL II that bind to the receptors for both human and avian adapted viruses, respectively. The ultimate goal is to incubate virus on the arrays to determine how receptor presentation affects viral binding. Before that can be done, it is important to ensure that the synthetic system behaves similarly to what is seen biologically. Lectin binding can achieve that goal because if their specificity is maintained, it provides an indication that viral specificity also will be.

Table of Lectin Binding

Lectins	Source	Carbon Binding Domain
SNA *Sambucus nigra*	Elderberry bark	Neu5Acα2-6Gal(NAc)
RCA *Ricinus communis Agglutinin, RCA_{120}*	Castor Bean Seeds	Galβ1-4GlcNAcβ1
MALII *Maackia amurensis*	Maackia tree seeds	Neu5Acα2-3Galβ1-4GlcNAcβ1
PHA-E *Phaseolus Vulgaris Erythroagglutinin*	Red kidney beans	Complex N-glycans terminated with galactose

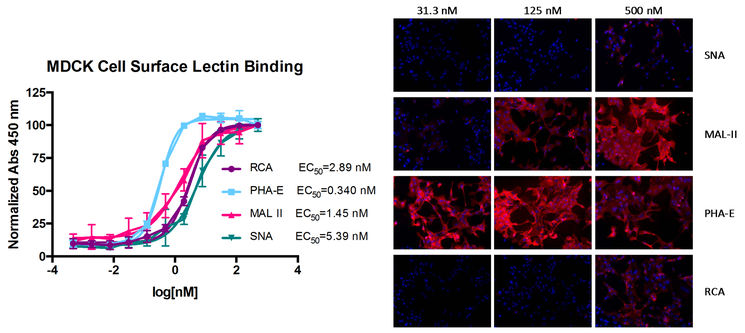
Enzyme Linked Lectin Assay (ELLA) Procedure



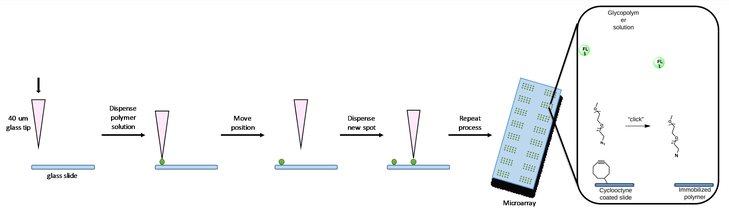
An example of a plate set up

To start the procedure, the media was removed and the cells were fixed with paraformaldehyde (PFA). The wells were blocked with 2% BSA and 0.5% Tween in PBS for one hour. Then, biotinylated lectins were added to the wells at the indicated concentrations and allowed to incubate with the cells for an additional hour. After multiple washing steps, streptavidin-HRP enzyme was added to each well. The TMB ELISA substrate was added after washing. This step caused the solution in the wells to turn blue in color due to the oxidation of TMB by HRP. Following substantial color change, H_2SO_4 was added to denature HRP and stop the oxidation reaction. Lastly, the plate absorbance was measured at 450 nm. The indicated lanes on the plate were saved for microscopy images. As such, instead of HRP, streptavidin-Cy5 and Hoechst stain were added and incubated for one hour. Images were taken at magnification of 20X.

Lectin Binding of MDCK Cells

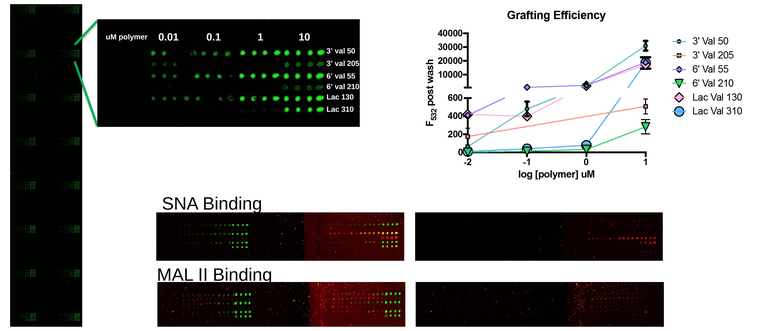


Microarray Printing Procedure



Mucin mimetic glycopolymers with varying sugar valencies and linkage types were printed on a glass slide. The polymers were labeled with a fluorophore so that their concentration could be obtained. They were also terminated with an azide functional group for immobilization onto a cyclooctyne-coated glass slide. The first step was to dissolve the polymers in a printing buffer (2.5% glycerol and 0.1% BSA in PBS) at the desired concentrations. Then a microcontact printer was used to spot four replicates of the glycopolymer solutions into arrays on the slide. A total of sixteen arrays were constructed. The resulting slide was stored at 4°C overnight to allow the click reaction to occur. The following day, excess polymer was removed through washing. The remaining polymer could be visualized using the fluorescent scanner. Biotinylated lectins were also incubated with the slide for one hour. Their binding was visualized through the additional incubation of streptavidin-Cy5 conjugate.

Glycan Microarray Images/Data



Discussion and Conclusions

The equilibrium concentration (EC_{50}) provides a measure of binding affinity. In the MDCK cell study we used the absorbance results from the ELLA to determine the EC_{50} of each lectin to the cell surface. Lower EC_{50} values correspond to greater binding. HRP is responsible for the colorimetric change seen in the assay. Because of the strong biotin-streptavidin interaction, higher absorbance values indicate greater lectin binding. The amount of HRP present depends on the amount of biotinylated lectin bound to the sugars on the cell surface. The data shows that PHA-E has the lowest EC_{50}, implying that it has the highest affinity for the cell surface. This feature can be seen in the microscopy images where lectin binding is observed for PHA-E at a concentration of 31.3 nM while minimal binding of the other lectins is seen. SNA appears to have the lowest affinity because it has the highest EC_{50} and minimal binding at the highest concentration in the microscopy images.

We also used microarray technology to print 16 arrays of 6 mucin mimetic polymers (a lower and higher valency of 3' and 6' sialyllactose and a lower and higher valency of lactose) at 4 different concentrations each. We then constructed a plot of grafting efficiency which indicated that more polymer grafted to the slide as it was printed at increasing concentrations. The plot shows that the 3' sialyllactose with a valency of 50 grafted the best, followed closely by the 6' with a valency of 55 and both lactose polymers. These polymers also appear the brightest in the microarray image. The higher valency sialyllactose polymers did not graft as well, shown by their decreased fluorescence in the image of the array as well as the quantification of fluorescence in the grafting efficiency plot. It is difficult to make a comparison between the lactose and sialyllactose polymers because their valencies are vastly different, but it appears that increasing the valency of lactose does not have as great of an effect on grafting since the high and low valency lactose polymers reach the same fluorescence intensity at the 10 uM printing concentration. The sialyllactose polymers may decrease in grafting efficiency when printed at higher valencies because of increased charge repulsions of the carboxylate moiety of these residues when present in greater amounts.

We then attempted to construct a binding curve for the microarray with the intentions of comparing these results to the ones obtained from the MDCK cell study. We focused on SNA and MAL II for the binding assay because, like influenza HA, they are specific to sialic acid receptors containing the α2-6 and the α2-3 linkages, respectively. However, we were unable to construct a binding curve because binding of the lectins to the array did not occur at enough concentrations of lectin. However, we were able to see the specificity of each of the protein. The glycan microarray images after lectin incubation show that SNA is more specific to the α2-6 linkage because no binding is seen to the α2-3 linkage. MAL II appears to be less specific than SNA but binds to the α2-3 linkage to a greater extent.

Since we know that the specificity of lectins are maintained in this system, a future goal of this project will be to incubate virus onto the arrays to elucidate how receptor presentation can affect viral binding.

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