

Simple Novel Assays in Glycobiology

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Abstract

In response to an invitation from this journal, I am providing this mini-review of recent work from the Oppenheimer lab. Over the past 4 decades we have developed many assays that help us examine the role of specific glycans in cellular interactions. Recently we have used two model systems for this work, the sea urchin embryo and yeast (*Saccharomyces cerevisiae*). We have developed two assays using the sea urchin embryo. One involves a microplate method where living sea urchin embryos are incubated with specific glycans or glycosidases. A specific set of cellular interactions, development of the primitive gut...archenteron, is examined over time in the presence and absence of the sugars or enzymes in living embryos. L-rhamnose and polyglucans have been identified as playing a role in mediating these cellular interactions. The second assay involves microdissection of the primitive gut away from the blastocoel roof to which it adheres.

Using independently characterized glycosidases, we showed that polyglucans appear to mediate this cellular interaction. In the second system using yeast, we examine yeast disaggregation from lectin-derivatized agarose beads in the presence and absence of specific glycans using a quantitative, kinetic graphic profile assay.

We found that D-melezitose was the best adhesion inhibitor and may be therapeutically useful in anti-adhesion venues of pathogen binding to cells and in cancer cell binding.

Keywords: Glycobiology anti-adhesion assays; Sea urchin archenteron; Microplate assay; Microdissection assay; Yeast lectin-derivatized bead assay; L-rhamnose; Polyglucans

The Sea Urchin, NIH Designated Model System

Microplate assay

We have used the sea urchin embryo in our glycobiology research for over 4 decades. The sea urchin has been designated by NIH as a model system for understanding mechanisms of importance in human health and disease [1]. Because of its transparency and simplicity, the sea urchin is well suited for research in glycobiology. We have developed new assays using the sea urchin embryo to learn about the roles of specific glycans in mediating cellular interactions [2-5]. The first assay involves culturing sea urchin embryos in 96 well microplates with and without glycans or glycosidases over time and counting the different morphologies of the developing gut (archenteron) in tens of thousands of living embryos [2,4]. The developing gut is a simple model for investigating cellular interactions. Because molecules easily enter the interior of sea urchin embryos [6], we are able to precisely quantify the effects of the added reagents on specific cellular interactions. Using these methods we have developed statistically evaluated, quantitative, kinetic graphic profiles of the effects of glycans and glycosidases on the development of the sea urchin archenteron, a model set of cellular interactions, and found that L-rhamnose and polyglucans appear to be involved in these cellular interactions. Of importance is that we independently characterize enzymes used in our studies and test for unit activity and contaminants.

Assay scheme: Grow sea urchin embryos to 24 hrs or 32 hrs post fertilization in normal artificial sea water. In 96 well microplates distribute embryos in wells containing no additions (controls) or containing specific concentrations of glycans or purified and independently characterized glycosidase in normal artificial sea water or low calcium (1.5 mM calcium) artificial sea water that speeds entry of reagents into the embryo interior without microinjection. Incubate embryos to 48 hrs post fertilization. Fix living, swimming embryos with 10% formaldehyde. Photograph all wells. Record percentages of embryos with complete archenterons (primitive guts), incomplete unattached archenterons, non-invaginated archenterons,

exogastrulated archenterons, dead embryos. Graph all experimentals and controls with standard error bars showing percentage of each morphology versus reagent concentration. Do t-tests comparing experimentals with controls. Thousands of embryos are assessed in this manner.

Microdissection assay

In order to directly identify the roles of specific glycans in mediating a specific cellular interaction in the sea urchin model, we microdissected the developing gut (archenteron) from the blastocoel roof to what it specifically attaches [3]. Then we assess whether or not the gut will attach to the blastocoel roof in the presence or absence of independently characterized contaminant-free glycosidases. In this way we are able to provide convincing evidence for a role of specific glycans in mediating a specific model cellular interaction in a model system [3]. In these experiments, using independently characterized glycosidases, we have found a role for polyglucans in this cellular interaction.

Assay scheme: Microdissect formaldehyde fixed 48 hr sea urchin embryos, with fine insect pins, separating archenteron from blastocoel roof. Treat the dissected "pieces" with and without (controls) specific unit concentrations of purified and independently characterized glycosidases for 1 min to 12 hrs. On glass microscope slides observe if the pieces will or will not adhere. This is a direct assay to determine if specific glycosidases influence adhesion of the archenteron to the blastocoel roof, a model cellular interaction of interest to investigators for over a century.

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The yeast model

Lectins have seen a re-emergence in their usefulness as therapeutic agents. They also function in the attachment of pathogens to cells and can cause cell clumping, such as in biofilms and cancer cells that helps cancer cells escape the body's defenses [7-10]. We use yeast (*Saccharomyces cerevisiae*) and lectin-derivatized agarose beads to identify glycans that can dissociate yeast from the beads as a model system for identifying glycans that can prevent binding of pathogens to cells [5]. This is done quantitatively by counting the numbers of yeast that remain bound to the lectin beads over time in hundreds of individual experiments in the presence and absence of specific glycans [5]. In this way glycans are identified that may be clinically useful in anti-adhesion venues.

In these studies we test all glycans at several concentrations over time using graphic, kinetic profiles and found that the sugar D-melezitose was the best inhibitor of yeast binding to lectin-derivatized agarose beads, making it a good candidate for therapeutic usefulness in various anti-adhesion venues.

Assay scheme: Yeast (*Saccharomyces cerevisiae*) are incubated with agarose derivatized lectin beads in distilled water. Single beads with attached yeast are isolated in droplets on glass microscope slides. Droplets with or without specific concentrations of glycans are stirred and numbers of yeast remaining bound at 10 minute intervals over a 60 min time course are compared with initial counts. In this way, the percentage of initial yeast remaining bound with and without reagents is assessed. Results of mean percentage of initial yeast bound are graphed against time for controls and each reagent concentration with standard error bars. T-tests are conducted to compare control and experimental results. Thousands of beads with bound yeast are assessed using this assay. In this way, a concentration-dependent kinetic profile of the effectiveness of each glycan is established.

Over the last century, many assays for identifying the role of glycans in biological functions from simple hemagglutination to microarrays and physical chemistry studies have been developed [11-17]. None however are as simple and nearly cost-free as those described here.

Some assays compared

Microplate (this review):

- A. Thousands of samples quickly assessed
- B. Highly quantitative
- C. Accurate
- D. Measures indirect effects
- E. Precise kinetic profiles generated
- F. Inexpensive

Microdissection (this review):

- A. Smaller numbers of samples
- B. Very direct effects measured
- C. Accurate
- D. Inexpensive

Yeast/Bead (this review):

- A. Highly quantitative

B. Accurate

C. Inexpensive

D. Precise kinetic profiles generated

Microarray:

A. Complex

B. Hundreds of reagents quickly assessed

C. Accurate

D. Expensive

Hemagglutination:

A. Limited usefulness

B. Inexpensive

Physical Chemistry Assays:

A. Highly Complex

B. Usually small sample number

C. Accurate

D. Expensive

Conclusion

This mini-review provides some insights into simple, inexpensive, quantitative, kinetic assays in model systems that are novel and may help lead to identifying glycans that may be clinically useful.

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