Applications for Glycobiology

Summary

Label-free detection of a carbohydrate array using specific binding to lectins is demonstrated and compared to conventional fluorescence detection.

Background

50% or more of proteins carry post-translational carbohydrate modifications, or glycans, that mediate their activities. Glycans are involved in normal recognition, adherence, motility, and signaling processes, particularly in the immune system. In infectious disease, host glycans serve as recognition sites for pathogenic viruses and bacteria, and in turn pathogen glycans serve as antigens for their recognition by (or evasion of) the host immune system. Carbohydrate-protein interactions thus play a central role in homeostasis and disease, making glycobiology, the study of carbohydrates and the proteins and lipids that bind them, a prominent area for exploration in the post-genomics era. A microarray of carbohydrates may be used to probe and profile the specificity of a diverse range of glycan binding proteins, including C-type lectins, siglecs, galectins, anti-carbohydrate antibodies, lectins from plants and microbes, and intact viruses. LFIRE™ enables direct glycan binding assays in a single step, providing multiplexed endpoint and kinetic data key to understanding the specificity and avidity of the interactions, without requiring labeling of the often-sensitive glycan-binding structures.

Method and Protocol

In conjunction with a major research institute collaborator, a carbohydrate library on a standard glass slide microarray substrate was probed with lectins, plant proteins that bind specifically to carbohydrates, and LFIRE™ data was compared to fluorescent endpoint methods. Wheat Germ Agglutinin (WGA) a 36kD protein, binds N-acetylglucosamine and chitibiose, structures common to many serum and membrane glycoproteins. Bacterial cell walls, chitin, cartilage, and glycolipids also bind WGA. Sambucus Nigra Lectin (SNA), a 150kD protein isolated from elderberry bark, binds to sialic acid attached to terminal galactose in α-2,6 and α-2,3 linkage. SNA is a binding analogue of influenza hemagglutinin, which recognizes host species' target cells by the α-2,6 and α-2,3 sialic acids on their cell surfaces.

For the single-step LFIRE™ assay, lectins were incubated at 8μg/ml for 30 minutes, taking real-time data. For fluorescence readings, a six-step process was used, requiring incubation with biotinylated lectins, subsequent staining with 500ng/ml Alexa555 fluorescent streptavidin, two wash cycles, drying, and finally a fluorescent scan.

Results

Concordance was 98.3% for an array of 128 spots including carbohydrates and controls. The correlation coefficient of mean spot intensity was 0.91 despite saturating signals in the fluorescent scan. Real-time LFIRE™ data confirms characteristic kinetic curves for the lectins, information not available using the labeled assay. All assays were performed on standard glass microarray substrates; LFIRE™ substrates provide 40-fold signal amplification.

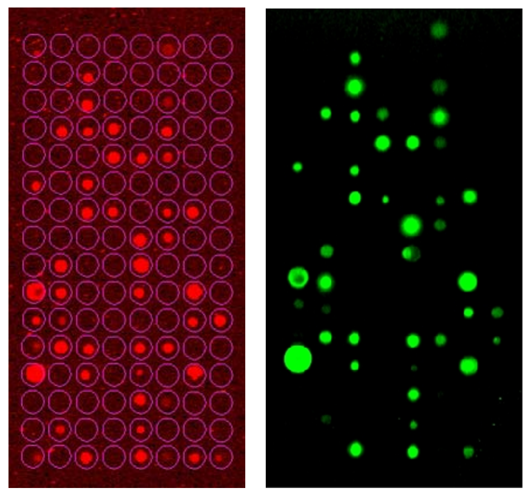

Fig. 1 a) Fluorescent scan of glycan array. b) False-colored LFIRE image at 30 minutes incubation. Activity concordance 98.3%, intensity correlation coefficient 0.91.

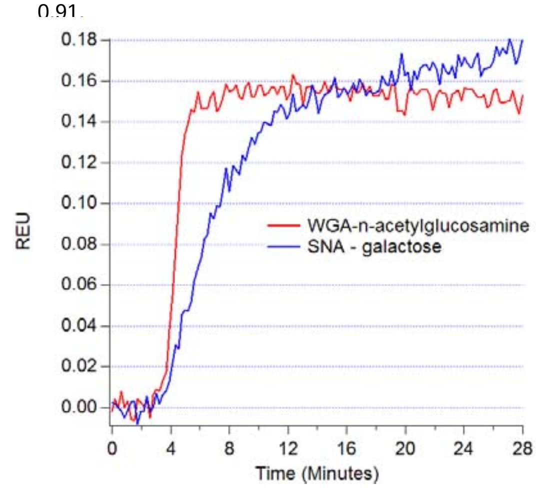

Fig. 2 WGA and SNA lectins binding to sugars in real-time. WGA and SNA differ in sugar specificity and the time constant of binding, producing characteristic curves.

LFIRE™ Applications Brief
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