Point of VIEW

ACS chemical biology

Fluorescent Glycan Derivatives: Their Use for Natural Glycan Microarrays

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ABSTRACT Glycan microarrays have become powerful tools in the investigation of biological systems because they enable fast, quantitative, and simultaneous analysis of glycan−protein interactions with small quantities of samples. One hurdle in the construction of glycan microarrays is to obtain a diverse set of glycans for immobilization on surfaces. An article in this issue of *ACS Chemical Biology* describes the microscale preparation of fluorescently labeled reactive glycans in a cyclic form of the reducing sugar, from natural sources, that can be utilized directly to create natural glycan microarrays for use in functional glycomics.

Glycans, present in biological systems in the form of glycoconjugates such as glycolipids and glycoproteins, play key roles in a variety of physiological and pathological processes through their interactions with proteins (*1*, *2*). As a consequence, understanding recognition events that take place between proteins and glycans is of great importance in the postgenomic era. Glycan microarrays, which are composed of diverse glycans densely and orderly immobilized on solid surfaces, have received considerable attention as high-throughput analytical tools for evaluating glycan−protein interactions (*3*). This microarray technology has the advantage that a number of glycan−protein interactions can be probed simultaneously by using small quantities of carbohydrate samples.

The utility of glycan microarrays is largely dependent on the availability of a diverse range of glycans. However, it is often a difficult task to prepare glycans because of their complex structures and associated synthetic difficulties. In this issue of *ACS Chemical Biology*, Song *et al.* (*4*) describe recent progress they have made in studies of the microscale preparation of fluorescently labeled reactive glycans in their cyclic reducing sugar forms and the application of these substances to the construction of glycan microarrays.

Glycan microarrays are typically prepared through either covalent or noncovalent immobilization of sugars on properly modified surfaces. Carbohydrates employed in the immobilization processes are usually prepared by using chemical and/or enzymatic methods. One chemical method to generate glycan probes involves reductive amination reactions between free sugars and suitable amine-containing, bifunctional linkers (*5*). This process is simple, often quantitative, and sufficiently benign, thus allowing its application to labile sugars, such as sialyl acids and sulfates. However, this procedure leads to the production of glycan conjugate products, in which the reducing sugar moieties possess acyclic structures that sometimes affect protein binding properties. To overcome this problem, methods have been developed to generate glycan conjugates that have the reducing sugar groups in their cyclic forms. This approach is exemplified by the reactions of free glycans with *N*-methylaminooxy-containing bifunctional linkers, which lead to production of predominantly cyclic adducts (*6*). Preparation of various glycans by one-step or multistep procedures is a hurdle in the creation of glycan microarrays containing diverse glycans. To circumvent the need for modified sugars, an approach to construct glycan microarrays through direct immobilization of free carbohydrates on aminooxy- or hydrazide-modified surfaces has been exploited (*7*). Although the simplicity of this approach enables its use by practitioners that do not have expertise in synthetic organic chemistry, this method does not introduce a fluorescent group, which can be used to isolate and characterize small quantities of glycans from natural sources.

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Published online September 18, 2009

10.1021/cb9002078 CCC: $40.75

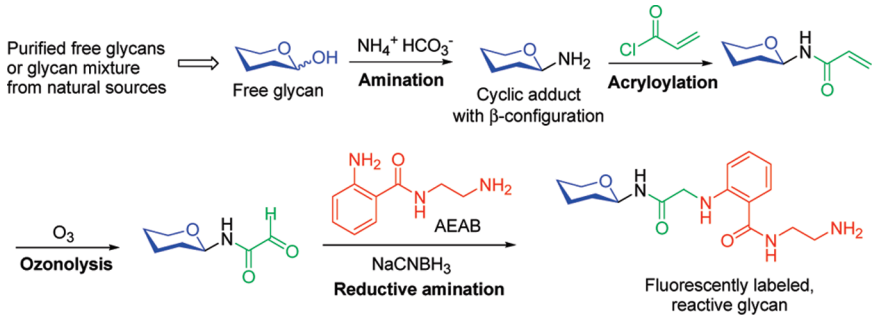

Figure 1. Derivatization procedure to produce fluorescently labeled, reactive glycans. The derivatized glycans are routinely prepared on a microscale by using initial glycosylamine formation followed by sequential *N*-acryloylation, ozonolysis, and reductive amination with AEAB.

Cummings and his co-workers (*4*) developed a microscale derivatization procedure of free glycans to produce fluorescently labeled reactive glycans that contain reducing sugar moieties in their cyclic forms. A method for microscale preparation of fluorescently tagged glycans is incredibly valuable since it facilitates both the isolation of modified glycans from natural origins and the derivatization of sugars containing primary amine functionality so that they can be utilized directly in the construction of microarrays. In this approach, natural glycans are generated by enzymatic or chemical cleavage reactions of glycoproteins or glycolipids. For example, glycans attached to asparagine residues of glycoproteins/glycopeptides are produced by PNGase F or Endo F catalyzed cleavage processes that liberate whole glycans or truncated glycans without one GlcNAc on the reducing end, respectively (*8*). In addition, glycans attached to asparagine (*N*-glycans) and serine/threonine residues (*O*-glycans) in glycoproteins are generated by treatment with hydrazine (*9*). The glycans obtained by using these methods react with ammonium carbonate to produce glycosylamines with β-configuration (Figure 1). The glycosylamines are then converted to aldehyde-containing glycosylamides by sequential acryloylation and ozonolysis. Reductive amination reactions of the aldehyde-containing glycans with a fluorophore-containing bifunctional linker, *N*-aminoethyl 2-aminobenzamide (AEAB), affords fluorescently labeled glycans with primary amine groups that can be separated by using multidimensional chromatography. Importantly, the fluorescently tagged glycans obtained in this manner have sugar moieties that exist in their cyclic β-pyranose forms. Moreover, derivatization of glycans can be accomplished on a microscale using routine laboratory protocols. Finally, the purified tagged glycans are immobilized on the *N*-hydroxysuccinimide (NHS)-activated surface to create glycan microarrays (Figure 2). Owing to the fact that they derive from natural sources and have structures that resemble closely those of natural glycans, the glycans generated by this procedure are highly useful in probing biological issues.

To demonstrate the usefulness of the fluorescent derivatization process, a variety of fluorescently tagged glycans were generated from both purified free glycans and mixtures of glycans present in human milk after chromatographic purification. Microarrays were fabricated from the derivatized glycans and employed in the analysis of glycan interactions with lectins or antibodies. The positive results coming from this investigation clearly show that the approach has practical applicability.

The elegant study by Song et al. (*4*) represents a significant step forward in developing glycan microarrays composed of natural glycan derivatives that have high utility in functional glycomics. The implications of the findings are manifold. First, owing to the simplicity of the microscale derivatization procedure and the diversity of glycans found in nature, a variety of derivatized glycans in cyclic forms of the reducing sugar can be produced. In addition, implementation of this methodology should enable the prepa-

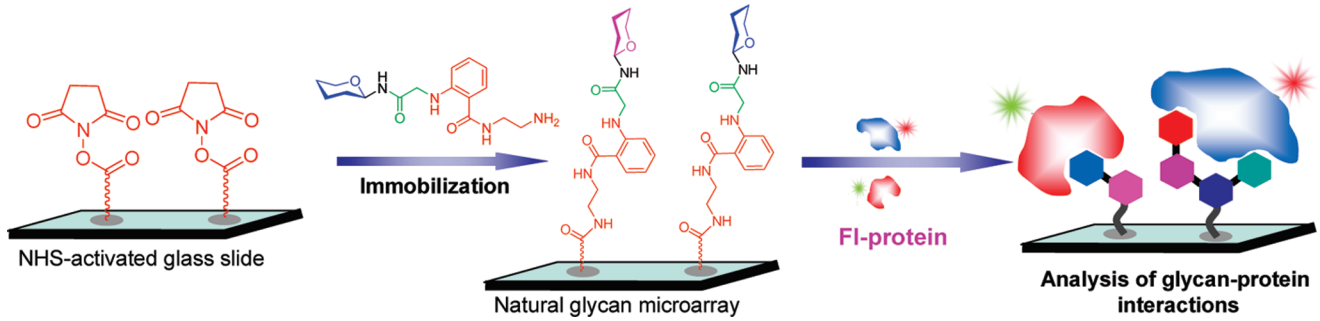

Figure 2. Construction of natural glycan microarrays by immobilizing fluorescently labeled, reactive glycans on NHS-activated surfaces and applications to the analysis of glycan–protein interactions.

ration of derivatives of complex oligosaccharides from natural origin. As a result, it should be possible to assess a wider range of biologically relevant glycan−protein interactions by using microarrays containing these glycans. Finally, the presence of fluorescent tags linked to glycans facilitates their chromatographic isolation from natural sources and the characterization of the immobilized glycans on microarrays. Overall, the availability of a large number of glycans should enhance the power of glycan microarray based technologies in the assessment of glycan−protein recognition events.

While this effort established an exciting new direction for the development of glycan microarrays, it has led to issues that need to be addressed in future investigations. For example, the route used to generate fluorescently tagged glycans containing reducing sugars in their cyclic forms involves several steps and, as a result, might hinder reproducibility. As a consequence, the development of shorter derivatization sequences would enhance the generality of the method. Another issue arises from the fact that derivatization of natural glycans by using this procedure affords *N*-glycosylated derivatives but not *O*-glycosylated counterparts. Since the nature of the linkage at the anomeric position of the reducing sugar could potentially influence protein binding, the development of a method to prepare both *N*- and *O*-glycosylated derivatives from natural sources would offer greater versatility. If these issues are addressed in future, the glycan microarray methodology would have enhanced utility.

Acknowledgment: Work in the authors' laboratory is supported by grants from the NRL and WCU programs (KOSEF/MEST).

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