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A lectin array-based methodology for the analysis of protein glycosylation

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Abstract

Glycosylation is the most versatile and one of the most abundant protein modifications. It has a structural role as well as diverse functional roles in many specific biological functions, including cancer development, viral and bacterial infections, and autoimmunity. The diverse roles of glycosylation in biological processes are rapidly growing areas of research, however, Glycobiology research is limited by the lack of a technology for rapid analysis of glycan composition of glycoproteins. Currently used methods for glycoanalysis are complex, typically requiring high levels of expertise and days to provide answers, and are not readily available to all researcher.

We have developed a lectin array-based method, Qproteome™ GlycoArray kits, for rapid analysis of glycosylation profiles of glycoproteins. Glycoanalysis is performed on intact glycoproteins, requiring only 4–6 h for most analysis types. The method, demonstrated in this manuscript by several examples, is based on binding of an intact glycoprotein to the arrayed lectins, resulting in a characteristic fingerprint that is highly sensitive to changes in the protein's glycan composition. The large number of lectins, each with its specific recognition pattern, ensures high sensitivity to changes in the glycosylation pattern. A set of proprietary algorithms automatically interpret the fingerprint signals to provide a comprehensive glycan profile output.

Keywords: Glycosylation; Glycoanalysis; Lectins; Protein arrays; Proteomics; Glycomics

1. Introduction

Glycosylation is the most versatile and one of the most abundant co- and posttranslational modifications of proteins [1]. Glycosylation has a structural role, increasing the protein stability, protecting the protein from proteolysis, and improving the protein solubility. In addition, glycosylation plays important functional roles in many specific biological functions, including immune defense, fertilization, viral replication, parasitic infection, cell growth, inflammation, and cell–cell adhesion. Glycosylation sites on glycoproteins commonly display microheterogeneity as they can be occupied by ensembles of structurally related oligosaccharides. *In vivo*, proteins are glycosylated by the actions of a series of glycosidases and glycosyltransferases resulting in a variety of glycan structures that may vary according to the physiological status of the individual [2]. In *ex vivo* systems, different cell lines and different fermentation conditions can produce significantly different glycosylation patterns [3–5].

Glycosylation is not template-driven, and is currently impossible to predict. Glycoanalysis is a relatively complicated process due to the complexity of the glycans and due to their biophysical properties. Current glycoanalytical methods include mainly chromatography and mass spectrometry-based methods, but also ^{1}H-NMR spectroscopy is applied. These

Abbreviations: Asn, Aspargine; CHO, Chinese hamster ovary; Cy3, Cyanine 3 bihexanoic acid dye; Gal, Galactose; GalNAc, *N*-acetyl galactoseamine; Glc, Glucose; GlcNAc, *N*-acetyl glucoseamine; HPLC, High performance liquid chromatography; Man, Mannose; Neu5Ac, *N*-Acetylneuraminic acid; MS, Mass spectrometry; N, Aspargine; O, Oxygen; PSA, Prostate specific antigen; rHuEPO, Recombinant human erythropoietin; RNase B, Ribonuclease B; *S*, Sulfate; Ser, Serine; THp, Tamm Horsfall glycoprotein.

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methodologies usually require significant labor and expertise in sample preparation and analysis of results. Most methods involve removal of the glycans from the protein [6–8]. Glycoanalysis of complex glycoproteins is therefore often time consuming, and may demand combination of several methods and high expertise [7].

Lectins are a family of carbohydrate-recognizing proteins that are classified into a number of specificity groups based on the monosaccharides and/or disaccharides for which they exhibit the highest affinity [9–11]. They are often not affected by other features of the glycan in which these epitopes reside [12], and therefore demonstrate broad specificities towards

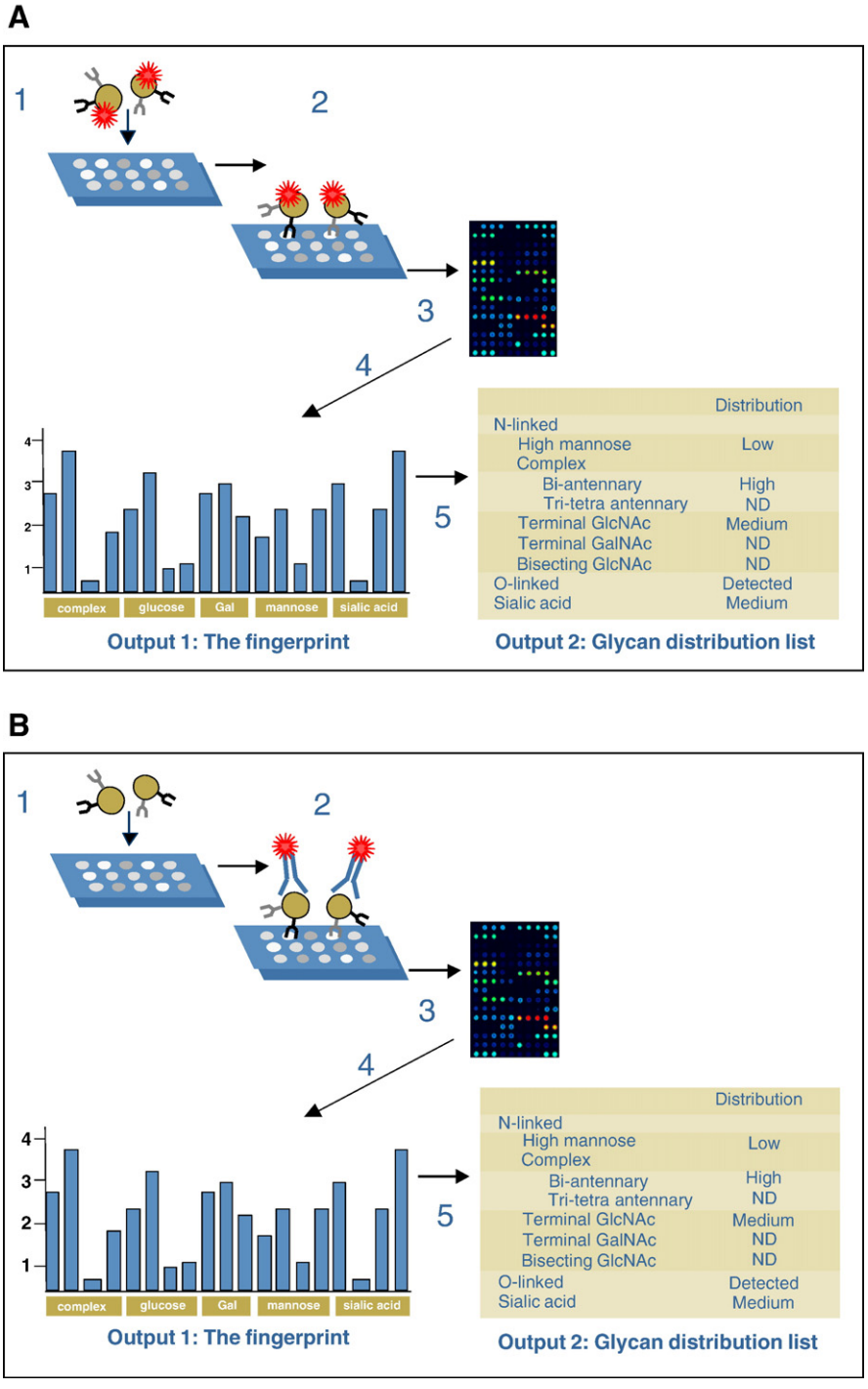

Fig. 1. Schematic representation of the Qproteome™ GlycoArray glycoanalysis process. A. Glycoanalysis process of pre-labeled sample; B. Glycoanalysis process of unlabeled sample. Process steps are: 1). Intact glycoprotein sample is incubated with the lectin array. The sample can be either pre-labeled with a fluorescent dye (A) or unlabeled (B); 2). The glycoprotein binds the array through its glycans, and binding is detected either by a direct fluorescence labeling of the sample prior to incubation with the array (A) or by applying a fluorescently-labeled antibody probe to unlabeled sample (B); 3). Array is scanned; 4). Fluorescent signals are presented as a histogram (the fingerprint), each bar represents a lectin. Lectins are grouped according to their specificity; 5). Proprietary algorithms are used to interpret the fingerprint signals into a glycan distribution list.