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Young Scientist Feature

Mass spectrometric analysis of the *N*-glycoproteome in statin-treated liver cells with two lectin-independent chemical enrichment methods

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This paper is dedicated to Professor Terry McMahon on the occasion of his 70th birthday.

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ABSTRACT

Protein *N*-glycosylation is essential for mammalian cell survival and is well-known to be involved in many biological processes. Aberrant glycosylation is directly related to human disease including cancer and infectious diseases. Global analysis of protein *N*-glycosylation will allow a better understanding of protein functions and cellular activities. Mass spectrometry (MS)-based proteomics provides a unique opportunity to site-specifically characterize protein glycosylation on a large scale. Due to the complexity of biological samples, effective enrichment methods are critical prior to MS analysis. Here, we compared two lectin-independent methods to enrich glycopeptides for the global analysis of protein *N*-glycosylation by MS. The first boronic acid-based enrichment (BA) method benefits from the universal and reversible interactions between boronic acid and sugars; the other method utilizes metabolic labeling and click chemistry (MC) to incorporate a chemical handle into glycoproteins for future affinity enrichment. We comprehensively compared the performance of the two methods in the identification and quantification of glycoproteins in statin-treated liver cells. Based on the current results, the BA method is more universal in enriching glycopeptides, while with the MC method, cell surface glycoproteins were highly enriched, and the quantification results appear to be more dynamic because only the newly-synthesized glycoproteins were analyzed. In addition, we normalized the glycosylation site ratios by the corresponding parent protein ratios to reflect the real modification changes. In combination with MS-based proteomics, effective enrichment methods will vertically advance protein glycosylation research.

1. Introduction

Protein glycosylation is critical in determining protein folding, trafficking, stability and activity [1,2]. Among multiple types of protein glycosylation, N- and O-linked glycosylation are the two major types [3,4]. N-linked glycosylation occurs on the side chain of the asparagine residue and often has an N-X-S/T/C (X stands for any amino acid residues other than proline) [5,6], while O-linked glycosylation is on the side chains of serine and threonine residues [7–9]. *N*-glycosylation typically begins with the synthesis of the dolichol-linked precursor oligosaccharide ($GlcNAc_2Man_9Glc_3$), followed by *en bloc* transfer of the precursor oligosaccharide to newly synthesized peptides in the endoplasmic reticulum (ER) [10,11]. Due to its importance in biological systems [12–16], *N*-glycosylation has also brought extensive attention for its role in human disease, such as Alzheimer's disease (AD), cancer, and infectious diseases [13,17,18].

With the development of mass spectrometry (MS) instrumentation and computation techniques, current MS-based proteomics is very powerful in analyzing protein modifications, including glycosylation, in complex biological samples [19–32]. Due to the low abundance of many glycoproteins, sub-stoichiometry of protein glycosylation, and the complexity of biological samples, it is imperative to enrich glycoproteins prior to MS analysis [28,33–37]. Conventional lectin-based enrichment methods have been extensively used [38,39]. However, due to the binding specificity of lectin, no single or several types of lectin can cover all glycoproteins that have highly diverse glycans in human cells.

In recent years, several very elegant methods have been developed and tremendously advanced the glycoproteomics field [6,28,29,40–45]. In this work, we systematically compared two lectin-independent chemical methods to enrich and analyze glycoproteins in human cells: one based on boronic acid and *cis*-diol interactions [33,46–48] and the other benefited from metabolic labeling and click reaction [49–51]. For the first method, we utilized the universal and reversible interactions between boronic acid and sugar molecules. Boronic acid and *cis*-diols can form reversible covalent bonds in basic solutions, and conversely, the bonds are prone to cleavage under acidic conditions. The reversible nature of this bond ensures that glycopeptides can be effectively

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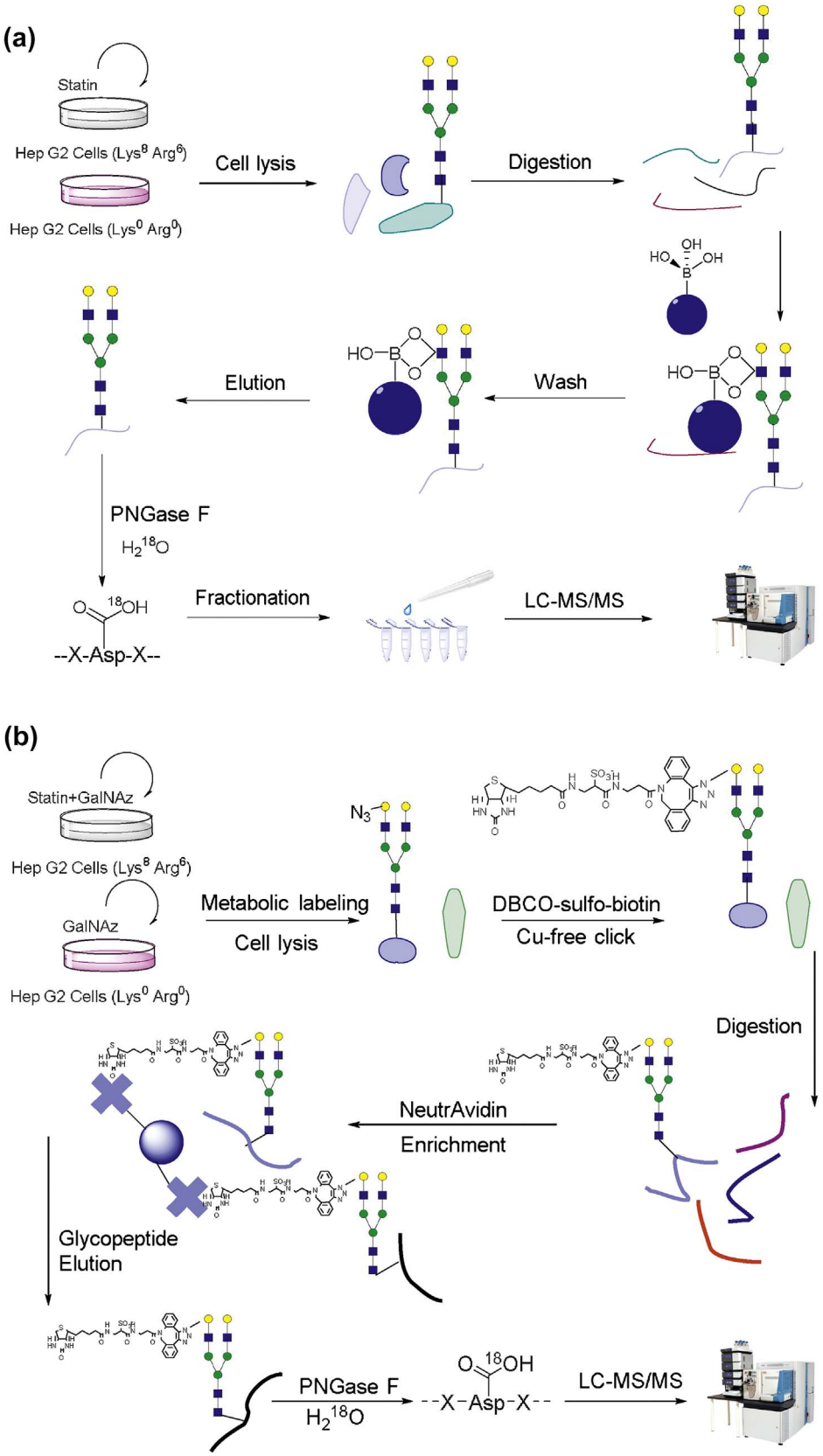

Fig. 1. Experimental schemes of the (a) BA and (b) MC experiments. In the BA experiment, cells were treated with 15 μM atorvastatin for 24 hours before harvest. After cell lysis, proteins were reduced, alkylated, and digested. Enrichment was performed at the peptide level with boronic acid-conjugated magnetic beads. Enriched glycopeptides were deglycosylated, fractionated, and subjected to LC–MS/MS analysis. In the MC experiment, 100 μM $Ac_4GalNAz$ was added into both heavy and light cells, and heavy cells were treated with atorvastatin. After cell lysis, the lysate was incubated with 100 μM DBCO-sulfo-biotin, followed by protein reduction, alkylation, and digestion. Enrichment was performed at the peptide level with NeutrAvidin agarose beads.