Effective Enrichment of Glycopeptides in Drop-HILIC Approach Using iSPE®-HILIC Material

Kathirvel Alagesan[1], Wen Jiang[2], and Daniel Kolarich[1],
[1]Institute for Glycomics, Griffith University, Southport, QLD, Australia, [2]HILICON AB

Glycoproteomics aims at the concomitant identification of not only the composition of the glycan but also the sites of glycosylation and the determination of the protein attached with glycans. However, glycopeptide analysis is challenging because their microheterogeneity results in the reduced concentration of each individual glycopeptide molecule compared to unmodified peptides, even if they are obtained from the very same digest sample (1). Also, glycopeptides exhibit poor ionization efficiency compared to their nonglycosylated counterparts (2). Therefore, it is essential to perform selective and efficient glycopeptide enrichment by solid-phase extraction (SPE) prior to detection and identification by mass spectrometry (MS) analysis (3–10). Hydrophilic interaction liquid chromatography (HILIC) SPE has been extensively applied in the past decade because of its low bias towards different glycan types (9,10). In contrast to normal-phase liquid chromatography, the HILIC retention mechanism is mainly based on the hydrophilic partitioning of the analyte to the enriched superficial water layer surrounding the surface of the polar stationary phase (11). Ionic interaction and hydrogen bonding may also be involved in the separation depending on the sample properties and the character of HILIC stationary phase. Here, we demonstrate the glycopeptide enrichment efficiency of iSPE®-HILIC material for glycoproteomics applications using a well characterized glycoprotein standard.

Experimental

Sample Preparation and HILIC SPE Enrichment: Three micrograms of standard glycoprotein (human IgG) were subjected to trypsin digestion. Glycopeptides were enriched using 25 mg iSPE®-HILIC material (50 µm, 60 Å, HILICON) by a drop-HILIC

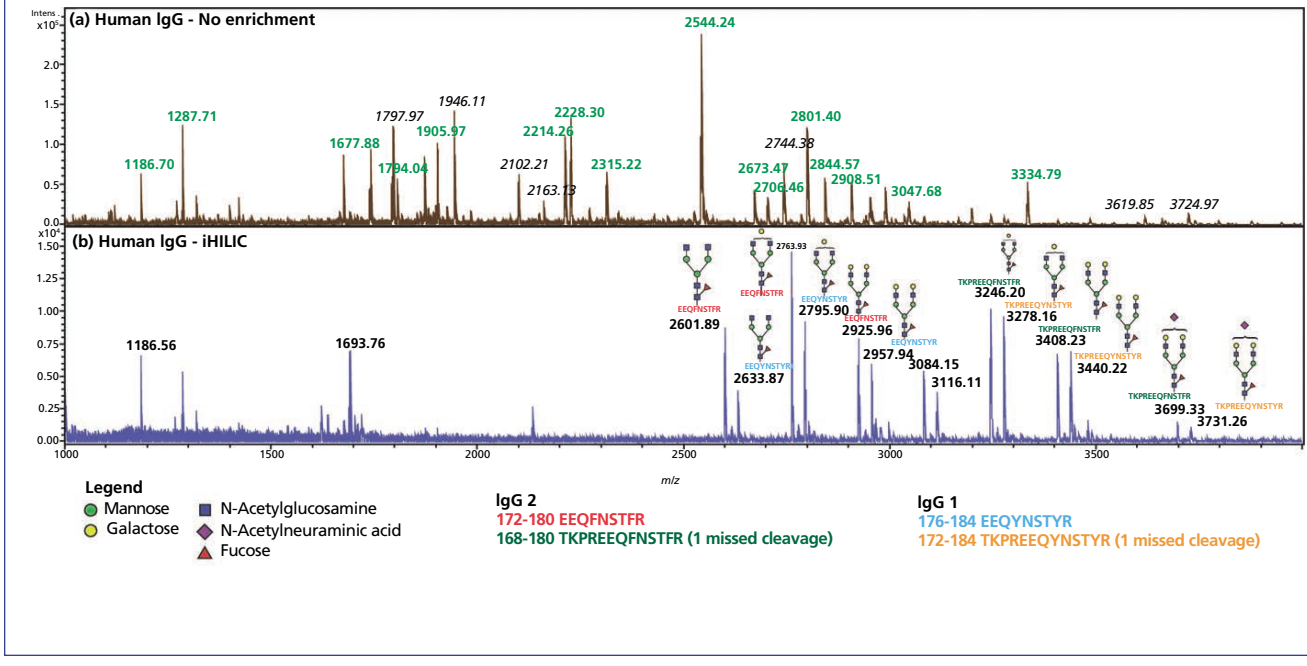

Figure 1: MALDI-TOF-MS spectra of tryptic IgG (glyco)peptides co-crystallized with DHB and analyzed in reflectron-positive ion mode. (a) No enrichment; *m/z* values highlighted in green correspond to the tryptic peptides derived from Human IgG 1-4 subclasses. (b) Enrichment using iSPE®-HILIC material; the annotated exemplary glycopeptides with the most likely structures in the same sample after enrichment. The lists of (glyco) peptides are available on request.

approach as described earlier (9). The dried samples, which contained about an aliquot of 30 ng enriched glycopeptides in each sample, were reconstituted with 100 µL of 0.1% TFA and used for the MALDI-TOF-MS analyses. Equal volumes of sample and matrix (20 mg/mL 2,5-dihydroxybenzoic acid [DHB] in 30% acetonitrile/0.1% TFA) were spotted onto a MTP 384 target plate ground steel (Bruker Daltonics).

MS System: MALDI-TOF-MS analysis was performed on a spectrometer equipped with Smartbeam™ 3D laser optics running at 5000 Hz and controlled by FlexControl 4.0 software (Bruker Daltonics). MS analysis was performed in reflector-positive mode, and the spectra were acquired within the mass range of *m/z* 1000 to 4000. In total, 20,000 shots were accumulated per spot, baseline corrected, and smoothed using Gauss algorithm with *m/z* 0.2 width and 1 cycle.

Results and Conclusion

The efficiency and selectivity of the iSPE®-HILIC material for glycopeptide enrichment were evaluated using a human Immunoglobulin G (IgG) mix. The tryptic (glyco)peptides mixture was analyzed using MALDI-TOF-MS before and after HILIC enrichment. As shown in Figure 1(a) with non-enriched samples, no signals corresponding to the IgG glycopeptides could be detected. Nevertheless, after glycopeptide enrichment using iSPE®-HILIC material, a total of 14 individual glycopeptides were identified from 30 ng of glycoprotein by MALDI-TOF-MS, shown in Figure 1(b). Next to these glycopeptides, a few unmodified polar peptides (in the lower *m/z* region as shown in Figure 1[b]) were also coenriched, but they did not interfere with glycopeptide detection. Considering the fact that HILIC enriches glycopeptides based on their hydrophilicity, it does not come as a surprise other hydrophilic compounds are coenriched (10). Such an unavoidable coenrichment of smaller hydrophilic peptides usually does not interfere with the ionization and detection of the target glycopeptides in the higher *m/z* range. For detailed information on the detected peptides, please contact us to retrieve the list of identified peptides corresponding to the different IgG subclass by MALDI-TOF-MS analysis in the nonenriched sample and the list of tryptic IgG Fc N-glycopeptides detected in the samples after iSPE®-HILIC enrichment.

In summary, iSPE®-HILIC offers a robust, reliable, and easily implemented solution for effective glycopeptides enrichment in glycoproteomics. In combination with optimized sample digestion protocols (for example, salt removal), iSPE®-HILIC-based drop-HILIC glycopeptides enrichment opens a wide range of opportunities for site-specific and in-depth glycoproteomics research.

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HILICON AB
Tvistevägen 48, SE-90736 Umeå, Sweden
Tel.: +46 (90) 193469
E-mail: info@hilicon.com
Website: www.hilicon.com