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Peanut agglutinin and β-cyclodextrin functionalized polymer monolith: Microextraction of IgG galactosylation coupled with online MS detection

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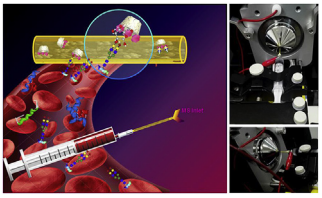
HIGHLIGHTS

- Poly(HEMA-EDMA-PNA-β-CD) monolith integrated with β-CD and PNA was prepared.
- The monolithic column was coupled with online Orbitrap MS.
- The functionalized monolith has high preconcentration abilities for IgG galactosylation glycopeptides.

GRAPHICAL ABSTRACT



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ABSTRACT

A facile online method coupling polymer monolithic microextraction (PMME) with mass spectrometry (MS) was developed for the detection of Immunoglobulin G (IgG) galactosylation glycopeptides. A peanut agglutinin-β-cyclodextrin (PNA-β-CD) functionalized poly(hydroxyethyl methylacrylate-ethyleneglycol dimethacrylate) monolith was designed *via* a click reaction. Thanking to the specificity of PNA-β-CD for the targets, the material exhibited enhanced enrichment selectivity and extraction efficiency for IgG galactosylation glycopeptides. Under optimal conditions, the developed method gave a linear range of 0.005–5 pmol for IgG glycopeptides with the regression coefficient greater than 0.9990, and the detection limit of IgG galactosylation glycopeptides as low as 0.5 fmol was achieved. The PMME-MS method was applied to IgG galactosylation glycopeptides in real samples including human serum and acute myelogenous leukemia (AML) cell lysate. A series of unique IgG galactosylation glycopeptides were captured by the monolith in the complex samples, indicating satisfactory enrichment ability for IgG galactosylation glycopeptides. The quick and integrated online PMME-MS method exhibited high selectivity for IgG galactosylation, demonstrating its perspectives on the development and broad applications of MS in studying galactosylation proteins regulated biological processes.

1. Introduction

Immunoglobulin G (IgG), a class of therapeutic antibodies glycoprotein, is characterized among various physiological and pathogenic conditions [1]. Quantitative analysis of IgG glycopeptides is of great importance, in which the determination by mass spectrometry (MS) has attracted increasing attention. Direct analysis of IgG glycopeptides at proteome or cellular level is confronted with great challenges without a proper pretreatment step in complex samples. In this respect, it is of great importance to develop resultful materials possessing highly efficient and selective enrichment capacity for IgG glycopeptides. In the field of

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pretreatment of IgG glycopeptides, β-cyclodextrin (β-CD) and its derivatives have been regarded as effective enrichment materials because they are not only favorably biocompatible, but also easy to be modified for diverse purporses [2–4]. Up to date, several β-CD-based materials have been reported and used for the enrichment of IgG glycopeptides, such as β-CD modified gold surfaces [5], hydroxyapatite nanoparticles β-CD complex [6], and β-CD polypseudorotaxane hydrogels [7].

Recently, IgG galactosylation was reported to be a novel candidate biomarker for further screening of pan-cancer according to Gu et al.'s research [8]. Thus, evaluation of them in complex biological samples are of vital significance for early-stage cancer detection and cancer screening in multiple cancer types. However, a literature survey revealed that the analysis of IgG galactosylation has never been reported. Peanut agglutinin (PNA) has strong affinity with IgG galactosylation [9,10], which opens a gate for the selective specificity of the latter. There are amino groups in PNA, making it possible to be connected with other compounds containing functional groups, *e.g.*, carboxyl group [11]. It can be expected that efficient selective enrichment of IgG galactosylation glycopeptides may be achieved taking the synergistic advantages of the specificity of PNA and affinity effect of β-CD.

MS has been proved to be powerful for glycoprotein analysis, but it is almost impossible to directly identify glycoproteins without the preparation and pretreatment of samples. Compared with commonly reported offline manner, online mode of combining pretreatment step with MS has apparent advantages of automation operation, time saving, and high efficiency. During the past years, various preconcentration methods have been focused on MS-online pretreatment, such as solid-phase extraction (SPE) [12], magnetic solid-phase microextraction (MSPE) [13], and solid-phase microextraction (SPME) [14,15]. In 2014, an in-tube SPME method coupled with online MS detection was developed, in which monolithic material incorporated with single-wall carbon nanotubes was employed and exhibited satisfactory sensitivity and reproducibility for triazine herbicides. Monolithic materials, especially polymer monoliths, have been extensively used in the field of preconcentration because of their advantages of simple preparation, favorable biocompatibility, and good control of porosity [16].

The objective of this research is the set-up of an integrated strategy for the analysis of IgG galactosylation. A polymer monolith incorporated β-CD and PNA was designed and used for online polymer monolithic microextraction (PMME) [17] of IgG galactosylation glycopeptides. Orbitrap Fusion Tribrid MS was used in this work because it can employ a full scan mode with higher resolutions, provide higher resolution, and give more qualitative information with preferable analysis accuracy, selectivity, and sensitivity when compared with time of flight–MS (TOF MS) [18–20]. Potential factors in the PMME process which might affect the extraction efficiency were investigated in detail. Under the optimized experimental conditions, the developed method gave low detection limits and high repeatabilities with only about 30 min needed for an entire analysis process. The combination of PMME with MS provided a rapid and sensitive method for the determination of trace IgG galactosylation in human serum and acute myelogenous leukemia (AML) samples with satisfactory detection sensitivities.

2. Experimental

2.1. Reagents and instrumentation

Hydroxyethyl methylacrylate (HEMA, 99%), ethyleneglycol dimethacrylate (EDMA, 99%) γ-methacryloxypropyl trimethoxysilane (γ-MAPS), PNA, human serum IgG (≥95%, essentially salt-free, lyophilized powder), bovine serum albumin (BSA), trypsin, N-hydroxysuccinimide (NHS), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich Company (St, Louis, MO, USA). 2,2-Azobis(isobutyronitrile) (AIBN), cyclohexanol, dodecanol, ethylene diamine tetraacetic acid (EDTA), peptide-N-glycosidase (PNGase F) and β-CD were acquired from Aladdin Reagent Company (Shanghai, China). Sodium hydroxide (NaOH), hydrochloric acid (HCl), methanol (MeOH), acetonitrile (ACN), ethanol, acetone, thiourea, urea, para-toluensulfonyl chloride (*p*-TsCl), chloroacetic acid, formic acid (FA), and ammonium bicarbonate (NH_4HCO_3) were obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Water was purified by a Milli-Q system (Millipore, MA, USA). HEMA and EDMA were distilled under vacuum conditions before use. AIBN was purified by recrystallization from ethanol and dried under vacuum at room temperature prior to use. All the other reagents were used as received.

Fused-silica capillaries (530 μm i. d. × 690 μm o. d.) were purchased from Yongnian Optical Fiber Factory (Handan, China). A precise syringe pump (GT0785, USA) was used to push liquids through monoliths. A KQ-200KDE digital ultrasonic cleaner was obtained from Kunshan Ultrasonic Instruments Co., Ltd. (Jiangsu, China). An SC-3164 low speed centrifuge was from Anhui USTC Zonkia Scientific Instruments Co., Ltd., China. pH measurements of sample solutions were achieved by a pHS-3C digital pH meter (Shanghai Rex Instruments Factory, China).

2.2. Construction and characterization of poly(HEMA-EDMA-PNA-β-CD) monolith

Firstly, PNA-β-CD was prepared from β-CD, thiourea, chloroacetic acid, and PNA. The prepared PNA-β-CD was characterized using Fourier transformed infrared spectra (FT-IR) and thermogravimetric (TG) determinations (Fig. S1). Then the freshly synthesized PNA-β-CD materials were dispersed in the polymerization mixture and copolymerized with the monomer (HEMA) *via* a click reaction. Prior to the preparation of monolithic columns, the inner wall of fused-silica capillaries (530 μm i. d. × 40 cm) were vinylized to enable covalent attachment. The inner wall was pretreated and rinsed with 1.0 mol L^{-1} NaOH for 30 min, water for 10 min, 1.0 mol L^{-1} HCl for 30 min, water for another 10 min, and acetone for 10 min, successively. Then a solution of γ-MAPS/acetone (40%, *v*/*v*) was employed to modify the inner wall at 55 °C for 14 h as described in our previous study [21]. As shown in Scheme 1, the polymerization solution consisting HEMA (480 mg), EDMA (320 mg), cyclohexanol (160 mg), dodecanol (1040 mg), AIBN (1% with respect to monomer, *w*/*w*), and PNA-β-CD with different amounts was purged with N_2 for 10 min, ultrasonicated for 15 min to remove the dissolved O_2, and then inlet into the as-pretreated capillary. Subsequently, the capillary was sealed by silicon rubbers at both ends and heated to 60 °C in a water bath for 24 h. Finally, the obtained monolith was washed with MeOH to remove the unreacted component and porogenic solvent overnight before use.

2.3. Characterization

Scanning electron microscope (SEM) images were acquired on an S4800 ESEM microscope (Hitachi, Japan). Transmission electron microscope (TEM) images were recorded on an H600 electron microscope (Hitachi, Japan) with an accelerating voltage of 100 kV. Samples were immobilized on copper grids covered with carbon. The samples were diluted with ethanol and the specimens were dried at room temperature before determinations. TG curves were carried out on a Q500 thermal gravimetric analyzer (TA Instruments Inc., USA) at the heating rate of 10 °C min^{-1} from room