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Off-line hyphenation of boronate affinity monolith-based extraction with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for efficient analysis of glycoproteins/glycopeptides

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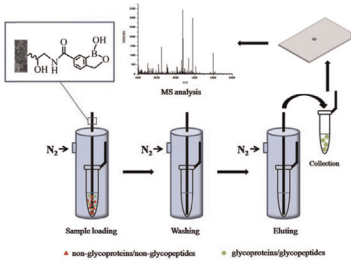
HIGHLIGHTS

- An off-line hyphenation of boronate affinity extraction with MALDI-TOF MS was established.
- It allowed for direct extraction of glycoproteins/glycopeptides from biosamples without pH adjustment.
- It showed the best performance for the analysis of glycopeptides from tryptic digest.
- Trace intact glycoproteins from human saliva were successfully identified.

GRAPHICAL ABSTRACT



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ABSTRACT

Boronate affinity materials have attracted increasing attentions as sample enrichment platforms for glycoproteomic analysis in recent years. However, most of the boronate affinity materials that have already employed for proteomic analysis are suffering from apparent disadvantages, such as alkaline pH for binding, weak affinity, and relatively poor selectivity. Benzoboroxoles are a unique class of boronic acids which have showed excellent binding properties for the recognition of cis-diol-containing compounds. Recently, a 3-carboxy-benzoboroxole-functionalized monolithic column had been reported and it had exhibited the best selectivity and affinity as well as the lowest binding pH among all reported boronate affinity monolithic columns. In this study, an off-line hyphenation of this boronate affinity monolithic column-based extraction with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was developed and the powerfulness of this hyphenated approach in the analysis of glycoproteins and glycopeptides in complex samples was investigated. The approach was first applied to the analysis of glycopeptides in the tryptic digest of horseradish peroxidase (HRP). Totally 22 glycopeptides were identified. To the best of our knowledge, this is the best performance among all the boronic acid-functionalized materials. We further employed this approach to the analysis of intact proteins in human saliva. Totally 6 intact glycoproteins were successfully identified. As comparison, when the samples were analyzed without extraction, only a few glycopeptides were identified from the tryptic digest of HRP while no glycoproteins were found from the saliva samples.

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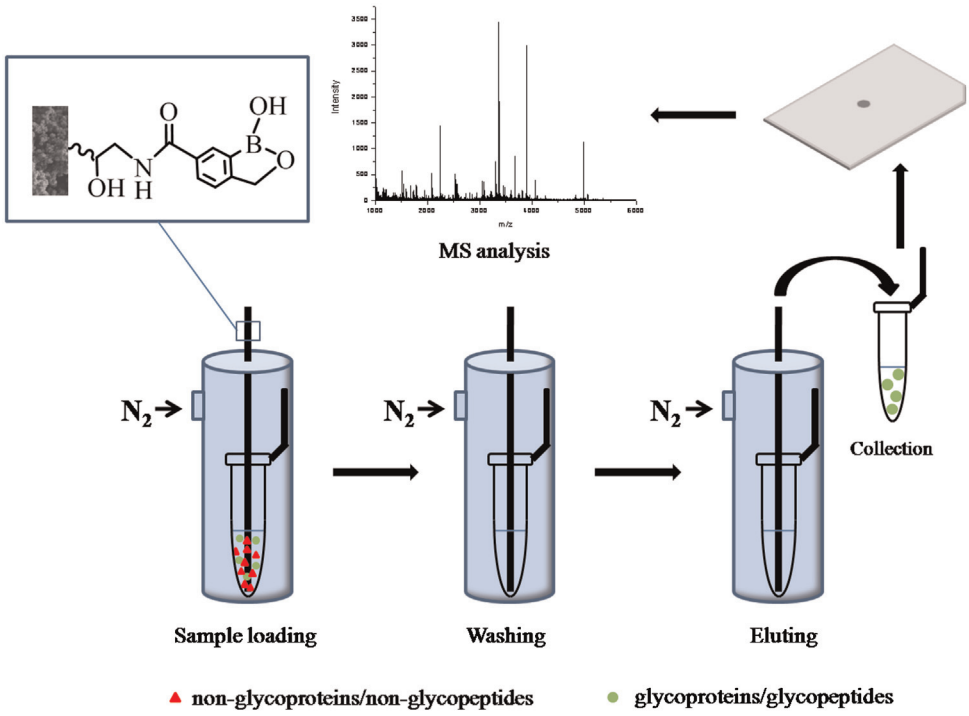

Fig. 1. Schematic of the off-line hyphenation of boronate affinity monolithic column-based extraction with MALDI-TOF MS.

1. Introduction

Protein glycosylation, as one of the most popular post-translational modifications of proteins, plays various essential roles in many biological processes, such as molecular recognition, inter- and intra-cell signaling, immune response, sperm–egg interaction, and regulation of development [1,2]. Because of their biological significance and application prospect, glycoproteins and glycopeptides have been an important subproteome in proteomics research. Unfortunately, the direct analysis of glycoproteins and glycopeptides from real samples has been still rather difficult because glycoproteins and glycopeptides of significant importance in biosamples such as serum, saliva, tears and cell lysate often exist in very low abundance [3]. As a result, the first step of glycoproteomics analysis is the enrichment of glycoproteins and glycopeptides from the biosamples [4].

Glycoproteins and glycopeptides can be enriched by various approaches, including solid-phase hydrazide capture [5], chemo-enzymatic approach [6], hydrophilic interaction chromatography (HILIC) [7–9] and lectin affinity chromatography [10–13]. Recently, boronate affinity chromatography (BAC) [14–30] has been becoming a promising technique for the selective extraction of glycoproteins and glycopeptides. A variety of boronate affinity materials (predominantly in microbeads [14–17], nanoparticles [18–24], mesoporous materials [25–27] and monolith format [28–30]) have been used for the extraction of glycoproteins and glycopeptides. As compared with the above competing methods, BAC provides several significant advantages: (1) broad-spectrum selectivity, one boronic-acid ligand can capture different kinds of glycans (in contrast, one lectin can capture only a limited number of glycan type); (2) covalent reaction, which favors the selectivity since nonspecific interactions can be suppressed or even eliminated by choosing appropriate conditions; (3) pH-controlled capture/release process, which makes the procedure very straightforward; (4) fast desorption kinetics, which ensures low analyte carryover; and (5) moderate acidic eluting condition, which is very compatible with mass spectrometry (MS) [31]. However, most of the boronate affinity materials that have already employed for

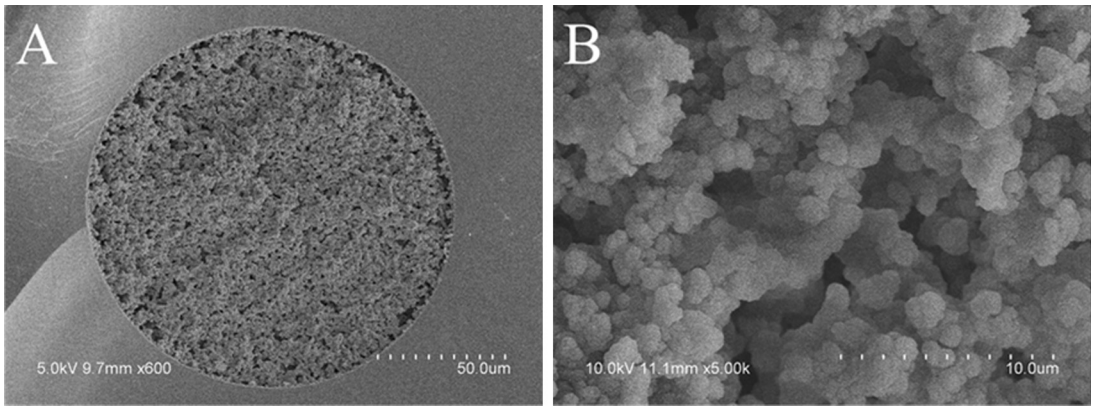

Fig. 2. The SEM images of cross-section of the 3-carboxy-benzoboroxole-modified monolithic capillary. (A) 600×; (B) 5000×.