Journal of Chromatography B, 877 (2009) 3631–3637

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Pretreatment of low-abundance peptides on detonation nanodiamond for direct analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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ARTICLE INFO

Article history:
Received 2 December 2008
Accepted 2 September 2009
Available online 6 September 2009

Keywords:
Detonation nanodiamond
Sample pretreatment
Low-abundance peptides
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

ABSTRACT

Detonation nanodiamond (dND) was firstly employed as adsorbent for pretreatment of peptides in dilute/contaminated sample solution. Detonation nanodiamond showed high efficiency for isolating and enriching peptides in a wide pH range. Remarkably, good tolerance capability toward salts and detergents could be achieved by using dNDs. Due to the inherent specificities of dNDs, dND-bound peptides could be directly analyzed by MALDI-TOF MS, so as to avoid the elution step and reduce sample loss. This pretreatment method also exhibited a better performance for protein identification compared to solvent evaporation and Ziptip pretreatment approach.

1. Introduction

With the development of proteome research, one of the most attractive contemporary endeavors is the mapping of proteins and establishing their linkages to normal and pathological conditions. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is one of the most frequently used MS techniques for protein identification, because of its inherent simplicity, low sample consumption and high sensitivity [1,2]. However, the identification of trace amounts of proteins for complex biological samples encounters some difficulties, such as the isolation and concentration of peptides before MS analysis [3,4]. Conventional pretreatment techniques, such as pre-concentration by the solvent evaporation, inevitably result in sample loss, mainly due to the adsorption of sample onto the surface of container. Besides the time-consuming process, an additional drawback of these conventional techniques is the simultaneous concentration of buffer components (e.g., salts or detergents) or other contaminants in solution. Therefore, a simple and wide applicable peptides pretreatment technique that could avoid excessive sample loss and concentration analyte simultaneously is still needed in MALDI-TOF MS analysis.

During the last two decades, a variety of sample pretreatment techniques have been developed. Among them, efficient solid-phase extraction (SPE) and pre-column techniques were popular owing to their high recovery and good reproducibility [5,6]. There are some available commercial products for the concentration and purification of peptides/protein using reverse phase supports such as C4, C8 or C18 chromatographic phase. To enhance enriching efficiency and practicability of these techniques, surface-modified substrates with either non-specific or specific affinity have been developed [7,8], such as silicon micro-extraction chips (for peptides) [9], C8, C18 or C60 functionalized magnetic beads (for peptides in serum) [10–12], zeolite nanocrystals (for peptides) [13], functionalized nano-CdS (for peptides) [14], $CaCO_3$, ZnO_2, SnO_2 or TiO_2–poly(methyl methacrylate) nanoparticles (for peptides) [15,16], MCM-41 porous nanoparticles (for endogenous peptides) [17] and mesoporous silica particles (for peptides in plasma) [18], polymeric beads (for peptides/proteins) [19], porous glass beads (for protein) [20], multi-walled carbon nanotubes (for protein) [21], Au magnetic particle-based probes (for charged protein) [22], etc. Meanwhile, some specific absorbents have been developed for the post-modified peptides/proteins, such as fullerene derivatives (for N-terminal sulfonated peptides and low-mass serum peptides) [23,24], iminodiacetic acid (IDA)–Cu^{2+} carbon nanotubes (for serum proteins and specific peptides containing histidine) [25], metal oxide nanoparticles and their coated magnetic beads (for

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1570-0232/$ – see front matter.
doi:10.1016/j.jchromb.2009.09.004

phosphorylated peptides) [26,27], boronic acid modified magnetic beads or porous material (for glycosylated peptides) [28,29], and so on.

Nanoscale diamond (ND), a carbon derivative nanomaterial, has a great potential for biological applications, due to their inertness, smallness, surface structure, chemical stability, biological compatibility, non-toxicity and the unbleachable fluorescence from nitrogen vacancy centers [30–32]. High adsorption capacity of nanoparticles makes ND a good candidate for effective concentration and immobilization of proteins. Chang et al. have evaluated the carboxylated/oxidized ND (100 nm, abrasive diamond powder) as an exceptional platform for protein adsorption. The amount of cytochrome C adsorbed onto the 100 nm diamonds saturated at 99 mg/g [33]. And proteolytic digestion of adsorbed proteins can be performed directly on the diamond particles [34]. However, the pH dependence of the carboxylated/oxidized ND (100 nm, abrasive diamond powder) pretreatment approach limited its general application [35]. Compared with ND synthesized by other methods (such as abrasive action, chemical vapor deposition (CVD), etc.), nanodiamond prepared by detonation (dND) shows unique physical and chemical properties, such as small size of diamond nucleus, chemically active surface area, stability towards corrosive media, etc. [36]. Because the surface of dND is covered with a variety of functional groups including carboxyl, lactone, ketone, hydroxy, and some alkyl groups, which may be directly provide interplay of ionic, hydrogen bonding, hydrophilic and hydrophobic interactions with peptides without further carboxylation and oxidization procedure.

Herein, dNDs, as an adsorbent, is directly applied for the enrichment of peptides in dilute/contaminated sample solution. Peptides can be enriched by dNDs within a wide pH range. Moreover, due to their small particle size, inertness and optical transparency, dND-bound peptides can be directly spotted on a MALDI plate for mass analysis, thus avoiding the elution step and the possibility of sample loss. Furthermore, since dNDs layer has a potential as a MALDI support to enhance the signal intensity of peptides [37], the detection sensitivity of peptides bound on dNDs increased too. The dNDs-based pretreatment approach was also successfully applied in the two-dimensional gel electrophoresis (2-DE) MALDI-TOF MS proteomics workflow and exhibited a better performance for protein identification than other conventional pretreatment approaches, such as solvent evaporation and Ziptip pretreatment approach.

2. Experimental

2.1. Chemicals and materials

dND particles were purchased from Gansu Lingyun Nanomaterial Corp. (Lanzhou, China). The particles had spherical shape with an average diameter of 3–10 nm. Bovine serum albumin (BSA), horse heart myoglobin (Myo), α-cyano-4-hydroxycinnamic acid (α-CHCA) and ammonium bicarbonate (NH_4HCO_3) were purchased from Sigma–Aldrich (Mississauga, ON, Canada). Porcine trypsin was from Promega (Madison, WI, USA). Analytical grade acetonitrile (ACN) and trifluoroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany). μ-C18 Ziptip was obtained from Millipore (Bedford, MA, USA). Sodium dodecyl sulfate (SDS) and all other reagents for gel electrophoresis were from Bio-Rad Laboratories (Hercules, CA, USA). Water used for all experiments was purified using a Milli-Q Plus system (Millipore), with resistance ≥18.2 MΩ/cm.

2.2. Sample preparation

2.2.1. dNDs stock suspension

dND particles (10 mg) were ultrasonically dispersed in 1 mL of deionized water and stored at 4 °C for further use. To obtain the carboxylized/oxidized dND particles, dND particles were first pretreated in a 9:1 (v/v) mixture of concentrated H_2SO_4 and HNO_3 at room temperature for 1 day, subsequently in 0.1 M NaOH aqueous solution at 90 °C for 2 h, and finally in 0.1 M HCl aqueous solution at 90 °C for 2 h according to previous report [34]. The resulting carboxylized/oxidized dND particles were thoroughly washed with deionized water and separated by centrifugation at 16,400 rpm. The carboxylized/oxidized dNDs stock solution containing 10 mg of particles/mL was also prepared with deionized water and stored at 4 °C for further use.

2.2.2. Protein digestion

One milligram of BSA or Myo was dissolved in 1 mL of ammonium bicarbonate aqueous solution (25 mM) separately, denatured at 95 °C for 5 min and digested with modified trypsin (sequencing grade, Promega) (enzyme-to-protein ratio of 1:30, w/w) overnight at 37 °C. To stop the digestion, 1 μL of formic acid (96%) was added. All stock solutions were refrigerated at around 4 °C for further use.

2.2.3. In-gel digestion

Proteins extracted from rat kidney (250 μg) were separated by 2-DE, which was performed using non-linear pH 3–10 IPG strips (Amersham Pharmacia, Uppsala, Sweden). Isoelectric focusing electrophoresis was performed using an IPGphor apparatus (Amersham Pharmacia, Uppsala, Sweden). The second dimension gel was run in a Protean II system (Bio-Rad). The IPG strip was first rehydrated overnight at 20 °C and then focused over a voltage gradient of 250–8000 V for 56 kVh. The strip was then sequentially reduced, alkylated and embedded on the top of a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Finally, protein spots were visualized by silver staining according to the protocol described by Blum et al. [38]. Before in-gel digestion, these excised spots were treated according to previous work [39]. In short, the destained and dried gel was incubated with trypsin and digested for 12 h in 25 mM NH_4HCO_3 buffer (pH 8.0) at 37 °C. After in-gel digestion, peptides were extracted sequentially from gel [40].

2.3. Pretreatment of peptides in dilute/complicated sample solution

2.3.1. Pretreatment of trytic BSA peptides

An aliquot (1 μL) of dNDs suspension (10 mg mL^{-1}) was added into 250 μL of aqueous peptides solution. After 5 min incubation at room temperature with vortex, the suspension was centrifugated at 16,400 rpm for 5 min and then the supernatant was decanted. Finally, the dND particles was resuspended in 1 μL of matrix solution (4 mg mL^{-1} CHCA in 50% acetonitrile/0.1% trifluoroacetic acid) and deposited onto a MALDI plate for MS analysis. To investigate the pH dependence/independence of dND, the peptides stock solution was diluted by aqueous solution with different pH value, adjusted by 0.01% aqueous formic.

To investigate the adsorption capacity of dNDs for peptides, 10 μL of dNDs suspension (10 mg mL^{-1}) was added into 300 μL of aqueous tryptic BSA peptides at concentrations of 0.145–1.45 pmol μL^{-1} in a centrifuge tube. To ensure the equilibration of adsorption, peptides and dNDs suspension were thoroughly mixed with a shaker for 30 min, after which the mixture was centrifuged and the supernatant was collected. And then, Bradford reagent was added into the supernatant part mentioned above. At last, the amount of peptides adsorbed (mg/g) on dNDs was determined from changes in the peptides concentration before and after addition of dNDs suspension into the solution by a Shimadzu UV-2450 spectrophotometer (Shimadzu Corp., Kyoto, Japan).