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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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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.

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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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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).

2.3.2. Pretreatment of in-gel tryptic digests

For control experiments, in-gel tryptic digestion was split into three equal portions. One portion was dried by solvent evaporation and one portion was concentrated and desalted using μ-C18 Ziptip on an XCISE gel processor system (Shimadazu, Kyoto, Japan) after removing ACN by solvent evaporation. Meanwhile, the other portion was concentrated by dNDs. For Ziptip pretreatment approach, a μ-C18 Ziptip was activated according to the manufacturer's standard protocol, and then the peptides solution was allowed to flow through tip for 10 cycles. After five rinses with 0.1% aqueous TFA solution, the resin was slowly eluted five times with 1 μL of matrix solution, and then the eluate was transferred onto a MALDI plate for MS analysis.

2.4. Characterization of dNDs before and after tryptic BSA peptides adsorbed

dND particles before and after tryptic BSA peptides adsorbed were characterized by using Fourier transform infrared (FT-IR), respectively. FT-IR spectra were collected using a Nexus 470 FT-IR spectrometer (Nicolet, Madison, WI, USA). All samples were dehydrated before analysis. Absorbance spectra were acquired using 32 scans and an instrumental resolution of 4 cm^{-1}.

2.5. MALDI-TOF MS analysis

All MS experiments were performed on a 4700 Proteomics Analyzer (TOF/TOF) (Applied Biosystems, Framingham, MA, USA) equipped with a 355-nm Nd:YAG laser. The instrument was operated at an accelerating voltage of 20 kV. The laser energy was stabilized on a certain value, which was set slightly above the threshold to obtain good resolution and signal-to-noise ratio (S/N). All mass spectra were obtained in the positive-ion reflection mode with a mass range from 700 to 3200 Da and each spectrum was accumulated by 1000 laser shots typically with automatic mode. The instrument was calibrated with tryptic myoglobin peptides. For the identification of protein spots on 2-DE, five precursor ions with the highest intensity were automatically selected to produce tandem mass spectra. Mass accuracy was within 100 ppm. Mass spectral data were examined and processed using Data Explorer 4.0 software supplied by Applied Biosystems. GPS Explorer software (Applied Biosystems) with MASCOT (Matrix Science, London, UK) as a search engine was used to identify proteins. All proteins from rat kidney were identified using the combination of PMF and MS/MS against IPI Rat 3.25 database. The rat IPI database was downloaded from EBI (www.ebi.ac.uk/IPI/IPIhelp.html). The search parameters were set up as follows: enzyme was trypsin, the number of missed cleavage site was allowed up to 1, the variable modification was oxidation of methionine, the mass tolerance of precursor ions and fragments were 100 ppm and 0.5 Da, respectively.

3. Result and discussion

3.1. Adsorption of peptides on dNDs

The bare ND surface has proven to be very reactive towards adsorption of various kinds of small and larger molecules. The binding strength strongly depends on the surface groups of the particles, and the surface groups and structure of nanoscale diamond strongly depend on the production conditions. The abrasive diamond powder has a hydrogen-terminated surface. Due to the lack of polar interactions, such as, hydrogen bonding, hydrogen-terminated surfaces are much less likely to adsorb proteins or other biological molecules. To enhance the polar interactions with proteins or other biological molecules, hydrogen-terminated surface of ND are being treated with a carboxylation and oxidization procedure. There have been numerous reports on the non-covalent interaction between biomolecules and the carboxylized/oxidized ND surfaces [33–35]. However, the whole carboxylation and oxidization procedure is fussy and time-consuming. dND particles obtained by the destruction of bigger diamond crystals exhibit significantly different features. The extreme environment during the detonation produces a variety of functional groups on the surface of the particles, such as carboxyl, lactone, ketone, hydroxy, and some alkyl groups, which directly provides interplay of ionic, hydrogen bonding, hydrophilic and hydrophobic interactions between dNDs surface and proteins or other biological molecules. Therefore, dNDs can be directly applied as an adsorbent for enrichment of peptides or other biological molecules in dilute sample solution without a long-time carboxylation and oxidization procedure. Fig. S1 (Supporting information) presents the comparison of FT-IR spectrum of functional group on dNDs surface before and after tryptic peptides adsorption. After the physical adsorption of tryptic BSA peptides on dNDs, those functional groups of BSA, such as amide I (R–CONHR′, CO stretching) at

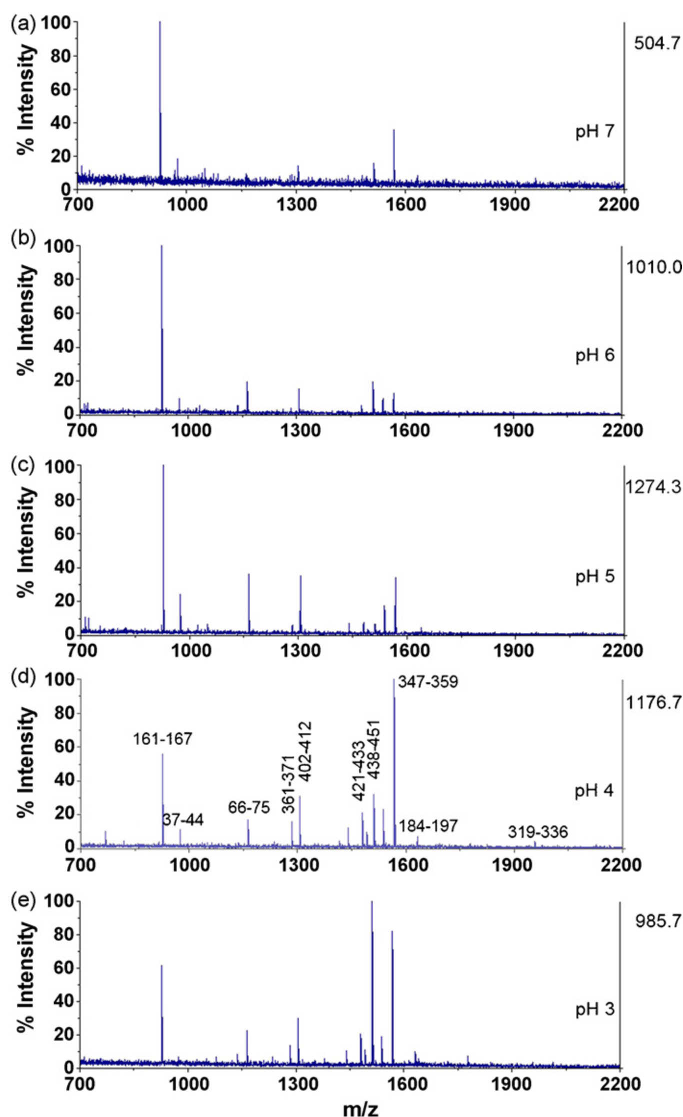

Fig. 1. MALDI-TOF MS of tryptic BSA peptides concentrated by the carboxylated/oxidized dNDs in dilute sample solution with different pH value (a) pH = 7, (b) pH = 6, (c) pH = 5, (d) pH = 4, and (e) pH = 3. Each spectrum was obtained for 200 μL of tryptic BSA peptides (1 fmol μL^{-1}) enriched by 10 μg of the carboxylated/oxidized dNDs. The labeled peaks are known tryptic BSA peptides.

1643 cm^{-1} and the amide II (R–NHR′, NH deformation, N–H bending and C–N stretching) at 3282 and 1536 cm^{-1}, were observed in the FT-IR spectrum of tryptic peptides adsorbing dND particles (Fig. S1(b)).

The adsorption capacity of dNDs for peptides was evaluated by measuring the UV absorption value of peptides concentration before and after the addition of dNDs into tryptic BSA peptides. Fig. S2 (Supporting information) shows the adsorption isotherm of tryptic BSA peptides on dNDs. The isotherm was reached the saturated equilibrium after 0.725 pmol μL^{-1}. Attributed to the small particle size and a much larger surface area-to-mass ratio of dNDs, the adsorption of tryptic BSA peptides to dNDs saturated at 130 mg/g, which could be comparable to that of the carboxylized/oxidized ND with the same particle size (5 nm, abrasive diamond powder) [41]. Therefore, dNDs has the potential to be good adsorbent for the enrichment of peptides in dilute sample solution.

3.2. pH dependence adsorption on dNDs

pH dependence of the carboxylated/oxidized ND (100 nm, abrasive diamond powder) pretreatment approach has been discussed by Han et al. [35]. If the pH value of solution more basic than the p*I* value of the peptides, the peptides would not be efficiently adsorbed onto the carboxylated/oxidized ND particles attributed to the decreasing of ionic interactions between peptides and the carboxylated/oxidized ND particles. Then this kind of adsorbent can be applied favorably in the situation where special peptides/proteins are desired. On the other hand, this very property would severely limit the general application of the carboxylated/oxidized ND. The pH dependence of carboxylated/oxidized dNDs for peptides was studied. Mass spectra in Fig. 1 demonstrate the varying peptides adsorption behavior of the carboxylated/oxidized dNDs from tryptic BSA peptides at different pH values. As reducing the buffered pH from alkaline to acidic, the mass spectra reflected increased sequence coverage. Attributed to the increasing of ionic interactions between peptides and carboxylated/oxidized dND particles, the enrichment efficiency of dNDs at acidic values shows more than that at alkaline conditions. For example, those peptides (such as fragments 421–433, 402–412 and 347–359) with a p*I* value of 6.0, 5.3 and 4.4 were ineffectively enriched by the carboxylated/oxidized dNDs when the pH value of the solution was 6 or more. The fragment from 361 to 371 with a theoretical p*I* value of 6.7 was absent from the mass spectrum when the solution pH for the carboxylated/oxidized dNDs enrichment was adjusted from 6 to 7. This fragment could be obviously observed only when the

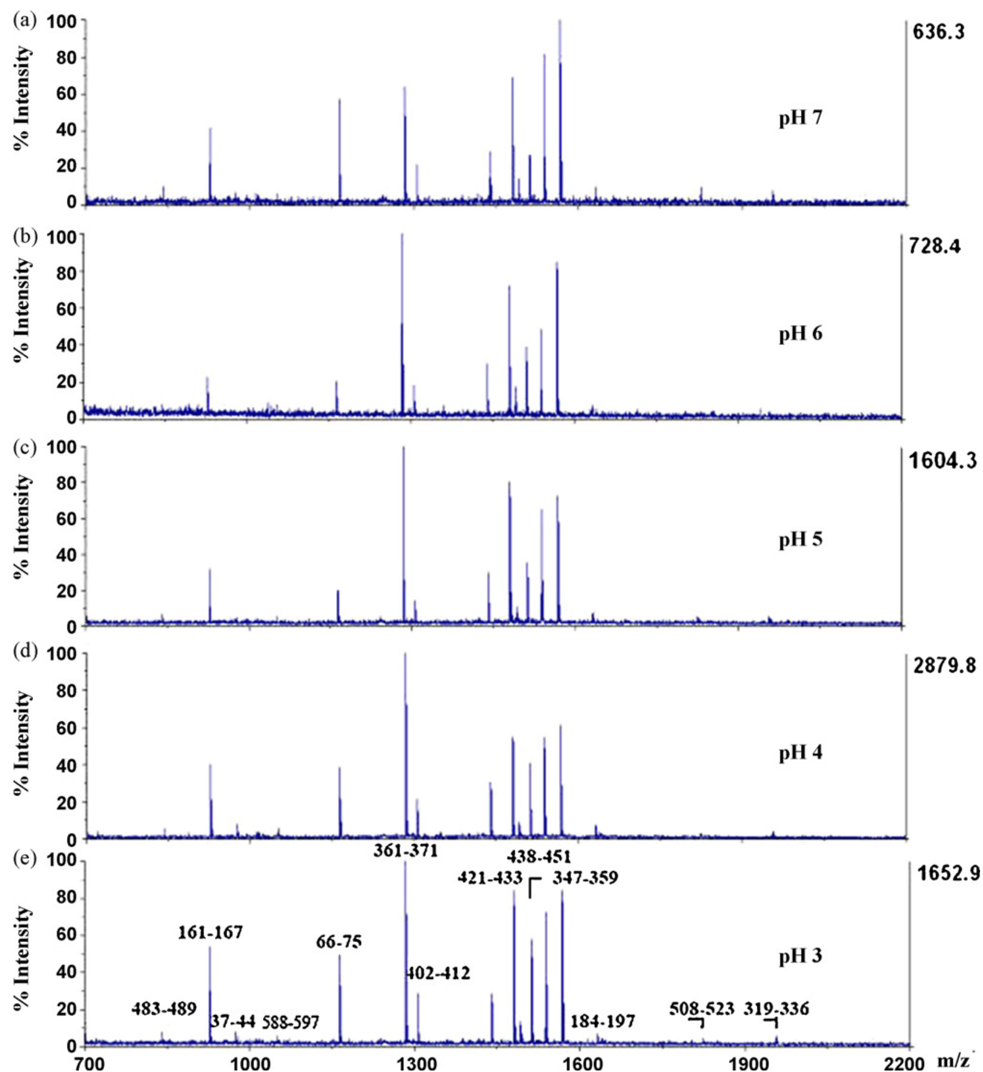

Fig. 2. MALDI-TOF MS of tryptic BSA peptides concentrated by dNDs pretreatment approach in dilute sample solution with different pH value: (a) pH = 7, (b) pH = 6, (c) pH = 5, (d) pH = 4, and (e) pH = 3. Each spectrum was obtained for 200 μL of tryptic BSA peptides (1 fmol μL^{-1}) enriched by 10 μg of dND particles. The labeled peaks are known tryptic BSA peptides.

solution of pH was adjusted to less than 5. Therefore, the carboxylated/oxidized ND/dND particles were unsuitable for the general enrichment of peptides in dilute sample solution within a wide pH range.

The pH dependence adsorption on dNDs was also studied. The same amount of tryptic BSA peptides was enriched by dNDs at different pH values and analyzed by MALDI-TOF MS. In Fig. 2, though the enrichment efficiency of peptides slightly changed when the pH value of the solution decreased from 7 to 3, peptides could also be enriched by dNDs in each pH value regardless of its theoretical p*I* value. For example, these peptides (such as fragments 421–433, 402–412 and 347–359) with a p*I* value of 6.0, 5.3 and 4.4 were effectively enriched by dNDs when the pH of the solution was 6 or more. Because there are a multiple functional groups on dNDs surface, such as carboxyl, lactone, ketone, hydroxy, and some alkyl groups, peptides were adsorbed on dNDs through a multitude of interactions, ionic interaction, hydrogen bonding, Van der Waals interaction, hydrophilic and hydrophobic interaction. Though the ionic interactions between peptides and the carboxyl groups of dNDs decreased with the varying pH, peptides could be stably adsorbed on dNDs with other interactions. Therefore, peptides can be adsorbed by dNDs in a dilute solution within a wide pH range.

3.3. Pretreatment of peptides in dilute/contaminated sample solution

The advantage of applying dNDs for the concentration of peptides from dilute/contaminated sample solution is demonstrated by tryptic BSA peptides. Fig. 3a shows the mass spectrum from 1 μL of 1 fmol μL^{-1} tryptic BSA peptides. No meaningful signals could be observed unless the same sample was concentrated by some pretreatment approaches. After pre-concentration using dNDs, the signal intensities of peptides were increased in the mass spectrum (Fig. 3b). And the peptides enriched by dNDs covered more than 30% of BSA sequence. Even when compared with solvent evaporation approach (Fig. 3c), dNDs pretreatment approach showed better enrichment efficiency. For example, the signal-to-noise (S/N) ratios of three peptides obtained by dNDs pretreatment approach were increased from 70.8 (*m*/*z*: 1163.65), 99.16 (*m*/*z*: 1479.82), and 121.5 (*m*/*z*: 1567.77) to 626.4, 842.3, and 1094.7, respectively. It is noted that the enhancement in detection sensitivity by dNDs pretreatment approach is more than 8-fold compared with that of solvent evaporation approach, which may be explained by the following reasons: firstly, the dNDs-bound peptides could be directly analyzed by MALDI-TOF MS, so as to avoid the elution step and reduce sample loss; secondly, as mentioned previously, dNDs layer had the potential to be a MALDI support for enhancing the ionization efficiencies of peptides [37]; moreover, peptides would be adsorbed onto container surface in the procedure of solvent evaporation approach.

Generally, real biological samples always contain salts, or other contaminants, and can hardly result in good mass spectra in the presence of these contaminants. Therefore, a desalting or accessional step is necessary after conventional pretreatment processes. In the presence of 100 mM NH_4HCO_3, a mass spectrum with high quality of 1 μL of tryptic BSA peptides (10 fmol μL^{-1}) can hardly be obtained (Fig. 4a). However, with dNDs pretreatment approach, a total of 25 peptides could be assigned to BSA with the sequence coverage of 45%, even in the presence of 100 mM NH_4HCO_3 (Fig. 4b). Unfortunately, no peak was observed after the same amount of sample solution pretreated by solvent evaporation, due to the co-concentration of salts and peptides (Fig. 4c). Salts, as reported in previous papers, improve the enrichment efficiencies of some hydrophobic compounds for both SPE and micro-SPE owing to the increased ionic strength of aqueous phase, which in turn precipitates hydrophobic compounds out of solution into the solid phase [42]. Furthermore, salts increase the surface tension of an aqueous solution, thereby increasing the hydrophobic force [43]. Therefore, in the presence of salt, the ionic interactions and hydrophilic interactions between peptides and dNDs surface were damaged, and then increased the adsorption of hydrophobic peptides on dNDs surface. For example, the hydrophobic peptide (*m*/*z* 1567.7) with a grand average of hydropathicity (GRAVY) value of −0.08 exhibited higher enrichment efficiency than other peptides. However, it did not affect the successful identification of protein. Besides NH_4HCO_3, three more salts (NaCl, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$) were applied in dNDs pretreatment approach to find out whether it is capable in various salt contaminates (Supporting information, Figure S3). From this figure, peptides in the presence of different salts were successfully adsorbed by dNDs. It can be safe to draw the conclusion that dNDs has good salt tolerance capability.

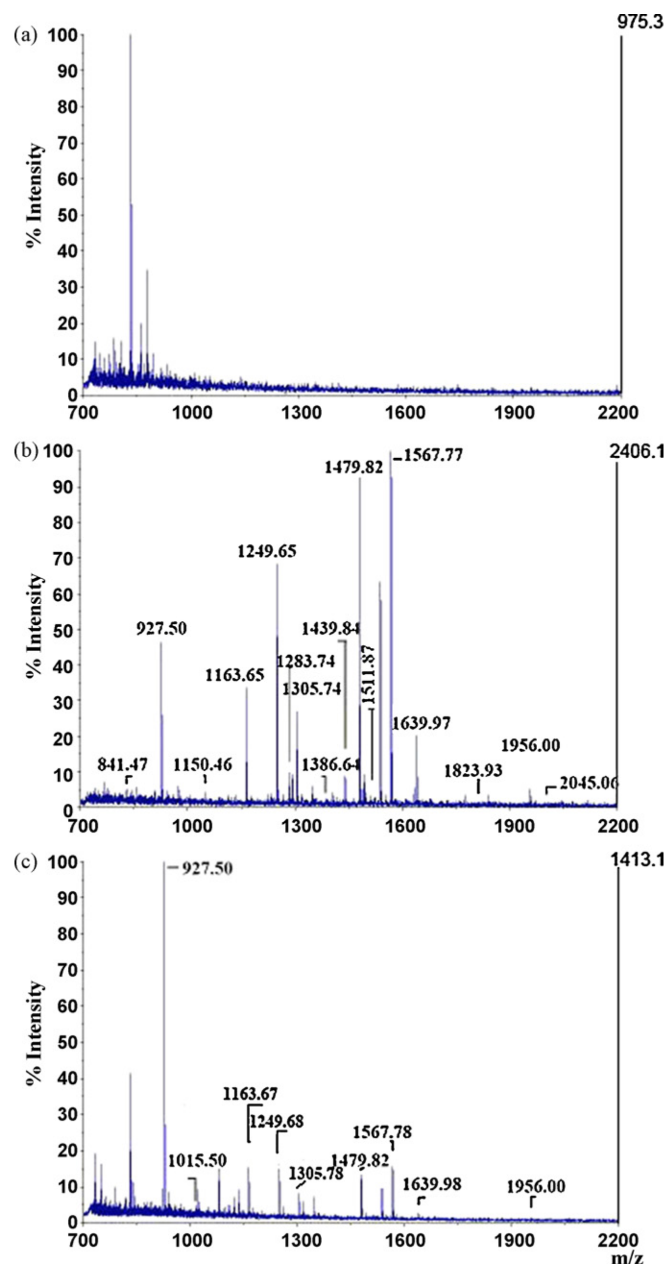

Fig. 3. MALDI-TOF MS of 1 fmol μL^{-1} tryptic BSA peptides (250 μL) (a) directly analyzed without pretreatment, (b) pretreated by 10 μg of dNDs, and (c) concentrated by solvent evaporation. The labeled peaks were assigned to tryptic BSA peptides.

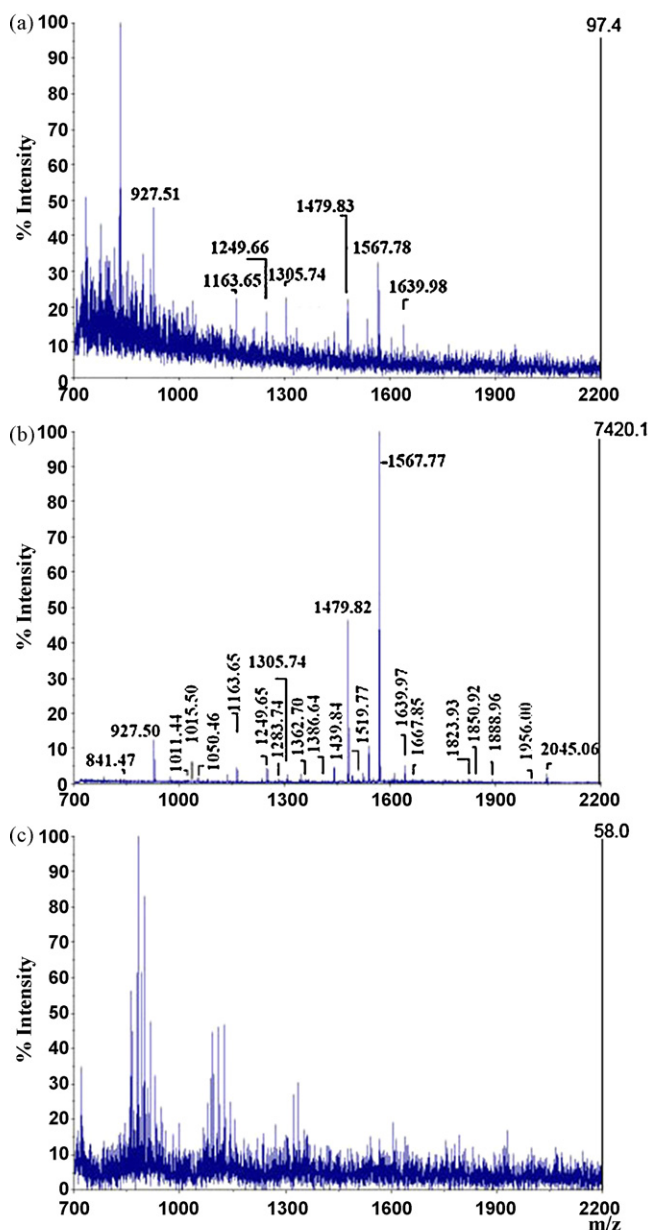

Fig. 4. MALDI-TOF MS of 10 fmol μL^{-1} tryptic BSA peptides (250 μL) in the presence of 100 mM NH_4HCO_3 (a) directly analyzed without any pretreatment, (b) pretreated by dNDs (10 μg), and (c) concentrated by solvent evaporation. The labeled peaks were assigned to tryptic BSA peptides.

As less soluble proteins become more and more of interest to those wishing to conduct proteomic experiments, the use of detergents in protein extraction or isolation is becoming more important. However, the introduction of detergents produces a significant decrease in signal intensity for MALDI MS analysis of peptides/proteins [44,45]. Therefore, the detergents must be eliminated before MS analysis. To investigate the detergent tolerance capability of dNDs, tryptic BSA peptides (10 fmol μL^{-1}) in the presence of 0.5% SDS was applied to analysis. To remove the contaminants, dNDs-bound peptides were additionally rinsed with deionized water (0.5 mL in each rinse) before MALDI-TOF MS analysis. At the same time a commercial μ-C18 Ziptip was used as a comparison. No signal could be observed in the mass spectrum (Fig. 5a). However, after pretreatment with dNDs, mass spectrum with high quality could be obtained (Fig. 5b). It is also noticed that peptides could be adsorbed by dNDs without discrimination in the presence of SDS. To the contrary, there were no peptides detected by MS when the same sample was concentrated by solvent evaporation, due to the co-concentration of SDS and peptides (Fig. 5c). Furthermore, in the mass spectrum of tryptic BSA peptides pre-

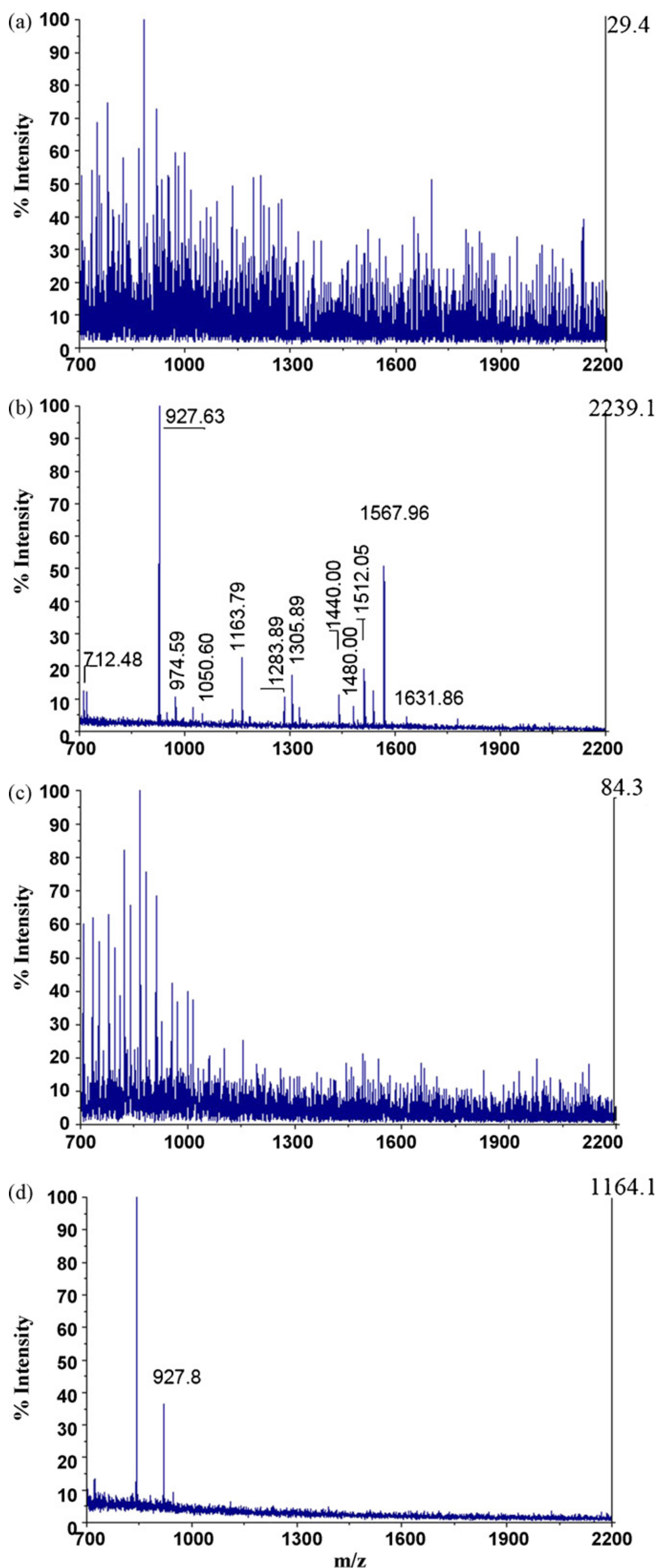

Fig. 5. MALDI-TOF MS of 10 fmol μL^{-1} tryptic BSA peptides (250 μL) in the presence of 0.5% SDS (a) directly analyzed without any pretreatment, (b) pretreated by dNDs (10 μg), (c) concentrated by solvent evaporation and (d) pretreated by a commercial μ-C18 Ziptip. The labeled peaks were assigned to tryptic BSA peptides.

treated by a commercial μ-C18 Ziptip (Fig. 5d), only one peptides could be observed, which was attributed to the hydrophobic interactions between SDS and C18 beads in Ziptip. Therefore, dNDs may be widely utilized for pretreatment of low-abundant peptides in dilute/contaminated sample solution in future.

3.4. Application to the identification of low-abundance protein

The proposed pretreatment approach has been proven to be effective in the identification of protein spots separated from 2-DE gel. Conventional pretreatment approaches such as solvent evaporation and μ-C18 Ziptip were used as comparison. Proteins extracted from rat kidney were separated by 2-DE, and 15 protein spots were randomly selected and numbered as shown in Fig. S4 (Supporting information). Table S1 presents data on the success rate of protein identification by three approaches. dNDs pretreatment approach exhibited better identification rate and sequence coverage for proteins than others approaches. For example, protein spots of low optical density (spots 1–8) could barely be identified by solvent evaporation due to the peptides adsorption onto the container surfaces and interference by co-concentrated contaminants. Compared with dNDs pretreatment approach, μ-C18 Ziptip exhibited lower fidelity for protein identification attributed to its lower recovery. Fig. S5a–c displays the comparison of mass spectra of spot 7 pretreated by dNDs pretreatment approach, μ-C18 Ziptip and solvent evaporation. dNDs pretreatment approach gave the higher signal intensity and sequence coverage, as well as the better recovery (Fig. S5a). Therefore, dNDs pretreatment approach has the potential in the application of proteome analysis.

4. Conclusion

In summary, the direct application of dNDs (3–10 nm) for pretreatment of peptides in dilute/contaminated sample solution has put forward for the first time and has successfully demonstrated its application to mass spectrometric identification of proteins separated by 2-DE gel. Due to the small particle size, the much larger surface area-to-mass ratio and the multiple surface functional groups, dNDs shows high enrichment efficiency and good salt/detergent tolerance capability for peptides. Meanwhile, dNDs can be successfully applied to adsorb peptides in a wide pH range of sample solution. Therefore, we believe that dNDs pretreatment approach has excellent potential for proteome analysis in future.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2009.09.004.

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