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Enhancing performance of liquid sample desorption electrospray ionization mass spectrometry using trap and capillary columns

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ABSTRACT

Desorption electrospray ionization mass spectrometry (DESI-MS) is a recent and important advance in the field that has extensive applications in surface analysis of solid samples but has also been extended to analysis of liquid samples. The liquid sample DESI typically employs a piece of fused silica capillary to transfer liquid sample for ionization. In this study, we present the improvement of liquid sample DESI-MS by replacing the sample transfer silica capillary with a trap column filled with chromatographic stationary phase materials (e.g., C4, C18). This type of trap column/liquid sample DESI can be used for trace analysis of organics and biomolecules such as proteins/peptides (in nM concentration) in high salt content matrices. Furthermore, when the sample transfer capillary is modified with enzyme covalently bound on its inside capillary wall, fast digestion (<6 min) of proteins such as phosphoproteins can be achieved and the online digested proteins can be directly ionized using DESI with high sensitivity. The latter is ascribed to the freedom to select favorable spray solvent for the DESI analysis. Our data show that liquid sample DESI-MS with a modified sample transfer capillary has significantly expanded utility in bioanalysis.

1. Introduction

Desorption electrospray ionization (DESI), as a representative ambient ionization technique, was introduced in 2004 by Cooks and co-workers as a milestone in the field of mass spectrometry (MS)[1]. This technique is used as a rapid analytical tool to provide efficient desorption and ionization of target compounds for mass spectrometric characterization. It has become very successful in the fast analysis of a variety of analytes including pharmaceuticals [2,3], metabolites [4,5], drugs of abuse [6,7], explosives [8,9], chemical warfare agents [10], and even intact tissues [11]. Traditional DESI addresses solid samples deposited onto a surface. In our and other's laboratories, DESI was extended for the analysis of liquid samples [12–16]. Analyte ionization by liquid sample DESI occurs via the interaction of the liquid sample with charged droplets generated by the DESI spray and the resulting ions are collected and analyzed by a mass spectrometer. It is useful for in directly analyzing samples including large proteins/protein complexes from their native environments [12,17–22]. Furthermore, it is possible to use liquid sample DESI-MS for studying fast reaction kinetics with submillisecond time resolution [23] or coupling with liquid chromatography (LC) [24–27], microfluidics [13], and microextraction [28]. The combination of DESI with electrochemistry (EC) [29] has shown that liquid DESI-MS can be used to capture transient intermediates [30–32]. The coupled EC/DESI-MS (i.e., the combination of EC with DESI-MS) is also useful for the structural analysis of disulfide bond-containing proteins in both top-down [33] or bottom-up MS approaches [24,34] and for probing protein 3D-structures and protein–protein interactions in combination with cross-linking chemistry [35].

In liquid sample DESI experiments, a fused silica capillary is employed to transfer liquid sample for ionization. The delivery of liquid sample using the silica capillary overcomes an issue that the liquid sample could be blown away by the nebulization gas of DESI if it is directly deposited on a surface, therefore allowing the continuous introduction and ionization of sample to gain continuous signal. In this study, we attempted to further enhance

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the analytical performance of liquid DESI via replacing the sample transfer capillary with a trap column filled with C4/C18 material and modifying the sample capillary with enzyme (e.g., trypsin) bound on its inner wall. The former modification would allow sample desalting and enrichment using the trap column, while the latter enables fast on-column protein digestion. Our experimental results show that these improvements enable liquid DESI-MS to analyze trace amount of analytes in complicated matrices and examine proteins via an accelerated shotgun MS approach.

2. Materials and methods

2.1. Materials

C4 and C18 trap columns (1.5 cm in length, 1 mm ID) were purchased from Optimize Techonologies. Acetic acid (HOAc), formic acid (FA), sodium hydroxide, hydrochloric acid, phosphate buffer (sodium dihydogen phosphte/sodium monohydrogenphosphate), flunitrazepam, tris buffer, sodium cyanoborohydride, glutaraldehyde solution, ammonium hydrogen difluoride ($(NH_4F)HF$), (3-aminopropyl)trimethoxysilane (APTES), trypsin from porcine pancreas, Gly-Phe-Ser, Tyr-Tyr-Tyr, angiotensin II, insulin from bovine pancreas, ubiquitin from bovine erythrocyte, β-casein from bovine milk and myoglobin from horse heart were all purchased from Sigma Aldrich (St. Louis, MO, USA). Fused silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA). Methanol, ethanol and acetonitrile (HPLC grade) were from Fisher Scientific (Fair Lawn, NJ). De-ionized water used for sample preparation was obtained using a Nanopure Diamond Barnstead purification system (Barnstead International, Dubuque, IA). A propane microflame torch and Diet Pepsi were purchased from a local Walmart store.

2.2. Methods

Experiments were performed using a DECA LCQ ion trap mass spectrometer (Thermo Finnigan) and a quadrupole time-of-flight (Q-TOF; Waters) equipped with a commercial electrospray ionization (ESI) source and a homemade DESI source (described below). The voltage on the DESI sprayer was set at 5 kV. Scheme 1a shows the configuration for integrating a trap cartridge column with liquid sample DESI-MS. 5 mL of test sample in low concentration (either flunitrazepam in Diet Pepsi or proteins/peptides in phosphate buffer) is loaded into the trap column; the column can retain the sample and allow the sample matrix or buffer to be washed away with water. Then an organic-containing solvent is injected to elute the sample out, which can be monitored by DESI-MS with a spray solvent of $MeOH/H_2O$/1%HOAc aiming at the exit surface of the trap column. The nitrogen nebulization gas of DESI was set to 160 psi. For flunitrazepam sample desalting, a C18 trap column was first equilibrated with 1 mL water, and then 5 mL of 50 nM flunitrazepam in Diet Pepsi was infused to the trap column followed by washing with 1 mL of water. Elution was carried out use ACN. A similar process was used for desalting and enrichment of protein/peptide samples. A 200 μL wetting solution (ACN/water/TFA, 2/98/0.1%, by volume) was first introduced to the trap column to balance the C18/C4 trap column. Then 5 mL of 50 nM angiotensin II or 40 nM insulin in 0.1 M phosphate buffer was introduced to the trap column for trapping. 1 mL of water was injected to remove the phosphate buffer and organic phase (ACN/water, 65/35 by volume) was used to elute the peptide or protein out for DESI-MS analysis.

Scheme 1b displays the configuration for integrating a piece of enzyme-modified fused silica capillary with liquid sample DESI-MS. The enzyme trypsin is immobilized on the inner wall of the silica capillary (modification procedure described below). When protein is infused through the capillary, digestion can be achieved. The resulting peptides can be directly detected by DESI-MS. In this case, the protein sample is infused into the capillary column at 1 μL/min and the DESI spray solvent of ACN/H_2O/5%FA is injected at 1 μL/min. The DESI probe capillary tip is in contact with the tip of the modified sample capillary. When the digested protein sample exits from the capillary column, it merges with the DESI spray solvent. Because the DESI spray solvent is charged to 5 kV, the mixed sample solution is sprayed out. No sheath gas is used for the DESI spray. Because of the freedom to choose a favorable DESI spray solvent [17] regardless of the nature of the digested protein sample, high ionization efficiency can be obtained. Collision induced dissociation (CID) is used for ion structural confirmation in these experiments.

2.3. Trypsin-immobilized capillary column preparation

Trypsin is immobilized onto a silica capillary following a modified method from the literature [36]. A piece of fused silica capillary (360 μm OD, 250 μm ID, 12 cm in length) is first flushed with 10% NaOH at 5 μL/min for overnight and then flushed with 5 mL water at 500 μL/min. 5 mL of 0.5 M HCl is introduced into the capillary at 50 μL/min and then the capillary is flushed with 5 mL water at 500 μL/min for removing the impurities in the silica capillary column. An aqueous solution of saturated ammonium hydrogen difluoride is infused into the capillary at 20 μL/min for inner wall corrosion to increase the inner surface area of the capillary. 2 mL of APTES is mixed with 1 mL 0.25% ammonia, 20 mL ethanol and 1 mL water and then infused into the capillary at 5 μL/min for 10 h, to react with inner wall surface hydroxyl groups for introducing terminal amino groups. Then the capillary is flushed with glutardialdehyde (30 μL in 5 mL water) at 3 μL/min for 16 h at room temperature to effect the Schiff base reaction. Subsequently the capillary is washed with water and dried with argon. 4 mg/mL trypsin dissolved in water is injected at 3 μL/min into the capillary and covalently bound to the surface, again via the formation of Schiff base with the remaining aldehyde group of glutardialdehyde. Finally 5 mg/mL sodium cyanoborohydride in water is infused at 3 μL/min to the capillary for reducing the C=N double bond. The end of this capillary column is flame heated by the torch and pulled to form a tapered tip. The immobilized trypsin column is stored at 4 °C prior to use for both DESI-MS experiments and nano-ESI-MS experiments for comparison.

3. Results and discussion

3.1. Liquid DESI-MS using a trap cartridge column

First, we examined the performance of liquid DESI-MS when a trap cartridge column was used for sample introduction. Different samples such as drug-of-abuse in drink, and proteins/peptides in phosphate buffer were chosen as test samples.

3.1.1. Detection of drug of abuse in drink

Flunitrazepam (structure shown in Fig. 1b) is a potent sedative and powerful drug for the treatment of insomnia used around the world. At the same time, it is employed in some cases that involve drug facilitated sexual assaults (DFSA) and robberies [37]. It is used by predators because of its rapid onset of action (e.g., dosed in drink), hypnotic effect and its ability to cause anterograde amnesia. Because this drug has a very short lifetime and rapid clearance, the fast and sensitive detection of this drug is desired.

In our experiment, a low concentration of flunitrazepam (50 nM) in Diet Pepsi was first tested. In the acquired ESI-MS spectrum shown in Fig. 1a, no flunitrazepam was detected. Instead, aspartame and caffeine from Diet Pepsi were seen at m/z 295 and