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Desorption Electrospray Ionization-Mass Spectrometry (DESI-MS) of Proteins

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Abstract

Desorption electrospray ionization-mass spectrometry (DESI-MS) was evaluated for the detection of proteins ranging in molecular weight from 12 KDa to 66 KDa. Proteins were uniformly deposited on a solid surface without pretreatment and analyzed with a DESI source coupled to a quadrupole ion trap mass spectrometer. DESI parameters optimized for protein detection included solvent flow rate, temperature of heated capillary tube, incident and reflection angle, sheath gas pressure, and ESI voltage. Detection limits were obtained for all protein standards and they were found to decrease with decreasing protein molecular weight: for cytochrome-C (12.3 KDa) and lysozyme (14.3 KDa) a detection limit of 4 ng/mm² was obtained; for apomyoglobin (16.9 KDa) 20 ng/mm²; for β -lactoglobulin B (18.2 KDa) 50 ng/mm²; and for chymotrypsinogen-A (25.6 KDa) 100 ng/mm². The DESI-MS analysis of higher molecular weight proteins such as ovalbumin (44.4 KDa) and bovine serum albumin (66.4 KDa) yielded mass spectra of low signal-to-noise ratio (S/N), making their detection and molecular weight determination difficult. In this study, DESI-MS proved to be a rapid and robust method for accurate MW determination for proteins up to 17 KDa under ambient conditions. Finally, we demonstrated the DESI-MS detection of the bacteriophage MS² capsid protein from crude samples with minimal sample preparation.

INTRODUCTION

Since the development of soft ionization sources like electrospray ionization (ESI)¹ and matrix-assisted laser desorption ionization (MALDI)² mass spectrometry (MS) has become an important tool in the biosciences due to its information rich and accurate data³. However, extensive sample preparation is often required for these techniques and as such they are not suitable for the direct analysis of samples under ambient conditions.

The introduction of ambient MS techniques⁴ allowed the analysis of samples in their native environment, that is without pre-treatment of samples, such as dissolving sample molecules in appropriate solvents (*e.g.*, ESI) or solid matrices (*e.g.*, MALDI). To date, a number of ambient ionization methods for MS analysis have been introduced and include desorption electrospray ionization (DESI)⁵, direct analysis in real time (DART)⁶, desorption atmospheric pressure chemical ionization (DAPCI)^{7, 8}, electrospray-assisted laser

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Supplementary Material Available: A description of optimized DESI-MS parameters and supplementary experimental results are available as Supporting Information. Current ordering information is found on any masthead page.

desorption/ionization (ELDI)⁹, atmospheric solids analysis probe (ASAP)¹⁰, and jet desorption ionization (JeDI)¹¹. All these techniques have shown that ambient MS can be used as a rapid analysis tool with little to no sample preparation.

Since its introduction, DESI-MS has been applied to various forensic analyses^{5, 12–17}; pharmaceutical tablets^{5, 18–23}; plant tissues²⁴; fruits⁵; intact biological tissue²⁵; enzyme-substrate complexes²⁶; metabolites in urine^{27, 28}; polymers²⁹; and thin layer chromatography plates³⁰. Due to similarities in ionization mechanism, DESI-MS yields mass spectra similar to those obtained by ESI-MS, and its most attractive feature is its short total processing time since samples can be analyzed with minimal or no sample preparation⁴.

The analysis of intact proteins is an important component of proteomic research as it yields information about post-translational modifications of proteins and the ability to detect other post-translational events leading to multiple products derived from a single gene³¹. Application of DESI-MS as an analysis tool for protein molecules in the field of proteomics is ideal due to its simplicity, speed of analysis for high throughput applications, and analytical specificity. However, very limited attention has been placed on the detection of high molecular weight proteins (>16 KDa) with DESI-MS^{5, 32}.

In this study, investigations were conducted on the various parameters that affect the DESI-MS analysis of proteins. Detection limits were determined in the DESI-MS analysis of proteins with molecular weights (MWs) ranging from 12 KDa to 66 KDa. In addition, the analysis of a crude biological sample under ambient conditions is demonstrated by the detection of the capsid protein of the bacteriophage MS2 with DESI-MS.

EXPERIMENTAL SECTION

Materials

High performance liquid chromatography grade methanol, acetonitrile and deionized water were purchased from Burdick & Jackson (Morristown, NJ) and glacial acetic and formic acids were purchased from EMD Chemicals (Gibbstown, NJ). All protein standards were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. Glass slides were purchased from Fisher Scientific (Pittsburgh, PA), and polymethylmethacrylate (PMMA, Plexiglas[®]) and polytetrafluoroethylene (PTFE, Teflon[®]) were from Professional Plastics (Fullerton, CA).

Sample preparation

Protein standards—Cytochrome-C (12.3 KDa), lysozyme (14.3 KDa), apomyoglobin (16.9 KDa), β -lactoglobulin B (18.2 KDa), chymotrypsinogen A (25.6 KDa), chicken albumin (44.4 KDa), and bovine serum albumin (BSA; 66.4 KDa) were used directly without further purification. Each lyophilized protein was dissolved in deionized water, deposited onto the solid surface (*e.g.*, Plexiglas[®], Teflon[®], or glass slide), and then air-dried. To produce an evenly deposited layer of protein on the DESI probe surface, a nebulizer sample deposition system was used³³. This setup allowed for the thickness and uniformity of the protein on the DESI probe to be accurately and reproducibly controlled. The concentrations of protein deposited on the surfaces were approximately 200 ng/mm² for most analyses, unless otherwise specified.

Bacteriophage MS2—The host bacterium, *Escherichia coli* (*E. coli*, C-3000 strain, ATCC 15597), and bacteriophage MS2 (MS2, ATCC 15597-B1) were purchased from American Type Culture Collection (ATCC, Manassas, VA). The culturing method for these

microorganisms was adapted from elsewhere³⁴. Briefly, *E. coli* was cultured in 5 mL of trypticase soy broth (TSB, BD Biosciences, San Jose, CA) at 37 °C overnight (16 – 18 h), and then collected by centrifugation at 2,000 rpm for 15 min. In order to grow MS2, a 20 µL aliquot of MS2 stock suspension was added to the cell sediment and then pre-incubated for 30 min 37°C. After the 30 min pre-incubation, 25 mL of TSB was added to the mixture of *E. coli* and MS2 and incubated for additional 3 h at 37 °C. The stock suspensions of MS2 were prepared by centrifuging the *E. coli*-MS2 cultures at 3,000 rpm for 20 min. Crude bacteriophage MS2 was purified prior to DESI-MS analysis in order to remove media components and other cellular debris by passing the *E. coli*-MS2 culture supernatant through a 100 KDa molecular weight cutoff spin column (Vivaspin 20, Sartorius AG, Germany) at 3,000 rpm. The titers of the MS2 solution was approximately 2×10^{12} plaque forming units (PFU)/mL. A 5 µL aliquot of MS2 solution was deposited with a pipette onto the DESI-probe for analysis. In order to dissociate the MS2 capsid protein from its single stranded RNA, a DESI spraying solvent consisting of 70 % formic acid and 30 % acetonitrile (v/v) was used.

DESI-MS system and Optimization

A homebuilt DESI source was interfaced with a Finnigan LCQ classic™ quadrupole ion trap mass spectrometer (Thermo Electron, San Jose, CA), and operated in the positive ion mode. The nebulizer component of the DESI source was constructed using a stainless-steel (SS) tee union (Swagelok, Solon, OH). Inside the SS union, the liquid capillary consisted of a 75 µm *i.d.* and 375 µm *o.d.* fused silica capillary (Polymicro Technologies, Phoenix, AZ) fitted inside a nitrogen sheath gas PEEK™ tubing (UpChurch Scientific, Oak Harbor, WA) with dimensions of 500 µm *i.d.*, 1,588 µm *o.d.*, and extending 3 mm from SS union. The DESI spraying tip was moved using an x-y-z translation stage (Newport, Irvine, CA). The ESI high voltage, optimized at 6 KV, was applied to the spraying solvent through a SS tube union located about 4 cm upstream from the nebulizing device. The spraying solvent consisted of 50 % methanol in water with 0.1 % acetic acid (v/v). The MS mass range was set to 150 – 2,000 *u* and spectra were collected for 1 min in spectral average mode. Measured DESI mass spectra were analyzed using the deconvolution module in BioWorksBrowser™ (ver. 3.0) program (Thermo Electron, San Jose, CA). Digital filtering of bacteriophage mass spectrum was performed using a 5-point boxcar smoothing built-in function of the Origin™ software package (ver 7.5, OriginLab Corp., Northampton, MA). The mass spectrometer was tuned by spraying cytochrome C in DESI solvent onto a blank probe surface³⁰. A minimum of 3 trials were conducted per experiment. The amount of protein present on the probe surface that showed three times higher than the peak-to-peak noise level of the baseline (peak-to-peak noise is approx. 5 standard deviations) was considered as the detection limit (DL) for the protein sample³⁵. Detailed experimental results on the optimization of DESI-MS for the detection of proteins are provided in the Supporting Information.

RESULTS AND DISCUSSION

Detection capability of DESI-MS with high molecular weight proteins

The capability of DESI-MS for the detection of high molecular weight proteins was evaluated with a series of protein standards that range in molecular weights from 12 KDa to 66 KDa using the optimal DESI-MS parameters established from the above experiments. Figure 1 illustrates DESI-mass spectra along with their corresponding deconvoluted mass for a series of protein standards. Overall, DESI-mass spectra of proteins ranging from 12 KDa to 25 KDa showed well defined deconvoluted masses with S/N well above 5 and mass accuracies in the range of those obtained by either ESI-MS (3-dimensional quadrupole ion trap mass analyzer) and MALDI-MS (Time-of-Flight mass analyzer). However, proteins

above 25KDa such as ovalbumin (44.4 KDa, data not shown) and BSA (66.4 KDa), generated poorly defined DESI-mass spectra with S/N near or below 3 and correspondingly poor mass accuracies (Table 1).

Tests were also conducted to determine DESI-MS detection limits (DL) of the protein standards. For protein samples in the range of 12KDa to 18 KDa, DL's varied from 4 to 50 ng/mm² of DESI probe surface. Overall, as the molecular weight of the protein tested increased, its DL also increased (Table 1). For instance, a DL of 4 ng/mm² was obtained for cytochrome-C and lysozyme, but increased to 20 ng/mm² for apomyoglobin, 50 ng/mm² for β -lactoglobulin B, and 100 ng/mm² for chymotrypsinogen A. Moreover, even as the surface concentrations of ovalbumin and BSA were increased up to 80 μ g/mm², the S/N of spectra for these proteins did not improve above 3, and no significant multiply-charged peaks were detected at concentrations below 4 μ g/mm² (data not shown). This result is a significant finding as it indicates that for proteins above 25 KDa signal sensitivity may depend on sample desorption more than on sample availability. Finally, DL's listed in Table 1, when compared on a per mole base, span over a 3 order of magnitude range. It is worth noting that DL's for cytochrome-C, lysozyme, and apomyoglobin obtained in this study are about 100–250 times higher than those obtained by other investigators, and we attribute this difference to the type of mass spectrometer utilized in each investigation: Our current study made use of a 3-dimensional quadrupole linear ion trap mass spectrometer (Finnigan LCQ Classic™) and its sensitivity is lower than those for a 2-dimensional (linear) quadrupole ion trap mass spectrometer (*e.g.*, Finnigan LTQ® or Applied Biosystems Q-Trap® systems) used by other investigators⁴. However, the *observed trend* of increasing DL's with increasing protein molecular weight should be independent of the mass analyzer. This finding should spur more investigations at optimizing the design of the DESI probe and ion guides for efficient desorption and ion transfer into the MS system³². The decrease in S/N with increasing protein MW may also contribute to the decrease in mass accuracy for proteins above 18KDa.

DESI-MS of bacteriophage MS2 capsid protein—The ability of DESI-MS to analyze biological samples was tested with a sample of bacteriophage MS2 grown in *E. coli* bacteria host cells. Initial efforts at analyzing crude samples of the bacteriophage MS2 in the presence of *E. coli* host cells and growth media did not yield any useful signals. Because the sample ionization mechanism is very similar between ESI and DESI *after* sample pickup by the initial charged droplet, we believe that the complexity of the crude cell lysate sample caused extensive ion suppression during the DESI process. Subsequently, crude samples were treated with a 100 KDa molecular weight cutoff spin column followed by application of 70% formic acid (on probe) in order to dissociate the capsid protein from its single stranded RNA. DESI-MS analysis of the treated bacteriophage MS2 sample (Figure 2) yielded a mass spectrum and deconvoluted mass in agreement with its capsid protein molecular weight (capsid protein phage MS2 accession number: 9626313; MW 13851, 0.6 % difference). This demonstrates the ability of DESI-MS to rapidly analyze semi-crude biological samples for protein molecular weight determinations, offering a viable alternative to MALDI-MS analysis of proteins.

CONCLUSIONS

Results presented demonstrate that DESI-MS is a viable approach for the analysis of intact proteins up to 18KDa. The analysis is rapid with a total analysis time under 10 minutes including sample preparation and offers an alternative approach to MALDI-TOF-MS measurements, where a matrix compound is required. At molecular weights above 25KDa (for the LCQ classic™ MS system used in this work), however, the efficiency of the DESI process to desorb and ionize proteins decreases yielding spectra with lower S/N. The

observed trend of increasing protein DL with increasing molecular weight clearly points to a limited efficiency of sample desorption and/or ion transfer to the MS system in the current design of the DESI source. The recently reported hybrid ionization technique involving MALDI, ELDI⁹ and ESI termed Matrix-Assisted Laser Desorption Electrospray Ionization (MALDESI)³⁶ should yield insight into the factors limiting the sensitive detection of high molecular weight proteins by DESIMS in that in MALDESI neutral desorption is governed by a matrix-assisted laser desorption process and it is decoupled from the sample droplet pickup and ionization (ESI) steps.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

DESI-mass spectra and corresponding deconvoluted mass of intact proteins: (A) cytochrome C (>10 ng/mm²), (B) lysozyme (>10 ng/mm²), (C) apomyoglobin (>50 ng/mm²), (D) β -lactoglobulin B (>100 ng/mm²), and (E) chymotrypsinogen A (>100 ng/mm²), and (F) bovine serum albumin (>4000 ng/mm²). Proteins were deposited on a Plexiglas surface.

Figure 2.

DESI-mass spectrum and deconvoluted mass of bacteriophage MS2 sample. Signal detected corresponds to the MS2 capsid protein (actual MW 13851). Crude bacteriophage MS2 suspension was passed through a 100 KDa MW cutoff filter. DESI-mass spectrum was smoothed with a 5-point boxcar filter.

Table 1

Detection capability of DESI-MS with intact proteins

Protein*	Theoretical MW (Da)	Measured MW		Detection limit (protein in area exposed to spray) (ng/mm ²) [§]
		Average [§] (Da)	% error vs. theoretical MW	
Cytochrome-C	12,361.20	12,361.0	0.002	~ 4
Lysozyme	14,306.10	14,306.5	0.003	~ 4
Apomyoglobin	16,952.50	16,953.9	0.008	~ 20
β-Lactoglobulin B	18,278.20	18,297.7	0.106	~ 50
Chymotrypsinogen A	25,657.10	25,713.5	0.220	~ 100
Ovalbumin	44,401	44,689.3	0.649	~ 4,000 ^{§§}
Bovine serum albumin	66,431	66,720.6	0.436	~ 4,000 ^{§§}

* Each protein was dissolved in deionized water and was spray-deposited on the Plexiglas[®] and then air-dried

[§] Average of 5 replicate measurements

[§] Detection limit of each protein was determined as the amount of sample present in the probe surface exposed to the spray that showed three times higher than the peak-to-peak noise level of the baseline³⁵

^{§§} The smallest amounts that can be measured were noted instead of detection limits.