Desorption electrospray ionization-mass spectrometry (DESI-MS) was evaluated for the detection of proteins ranging in molecular mass from 12 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-MS parameters optimized for protein detection included solvent flow rate, temperature of heated capillary, 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 mass: 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 mass proteins such as ovalbumin (44.4 kDa) and bovine serum albumin (66.4 kDa) yielded mass spectra of low signal-to-noise ratio, 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.

Since the development of soft ionization sources such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), mass spectrometry (MS) has become an important tool in the biosciences due to its information-rich and accurate data. 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 techniques allowed the analysis of samples in their native environment, that is, without pretreatment 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), direct analysis in real time (DART), desorption atmospheric pressure chemical ionization (DAPCI), electrospray-assisted laser desorption/ionization (ELDI), atmospheric solids analysis probe (ASAP), and jet desorption ionization. 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, pharmaceutical tablets, and plant tissues.
fruits, intact biological tissue, enzyme–substrate complexes, metabolites in urine, polymers, and thin-layer chromatography plates. 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 preparation.

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 gene. 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.

In this study, investigations were conducted on the various parameters that affect the DESI-MS analysis of proteins. Detection limits (DLs) were determined in the DESI-MS analysis of proteins with molecular weights (MWs) ranging from 12 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 poly(methyl methacrylate) (Plexiglas) and poly(tetrafluoroethylene) (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 used. 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 ~200 ng/mm² for most analyses, unless otherwise specified.

Bacteriophage MS2. The host bacterium, Escherichia 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 elsewhere. Briefly, E. coli was cultured in 5 mL of tryptose soy broth (TSB; BD Biosciences, San Jose, CA) at 37 °C overnight (16–18 h) and then collected by centrifugation at 2000 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 preincubated for 30 min 37 °C. After the 30-min preincubation, 25 mL of TSB was added to the mixture of E. coli and MS2 and the resultant mixture incubated for an additional 3 h at 37 °C. The stock suspensions of MS2 were prepared by centrifuging the E. coli–MS2 cultures at 3000 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) at 3000 rpm. The titer of the MS2 solution was ~2 × 10¹⁰ plaque-forming units/mL. A 5-μL aliquot of MS2 solution was deposited with a pipet 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 home-built 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 ~4 cm upstream from the nebulizer device. The spraying solvent consisted of 50% methanol in water with 0.1% acetic acid (v/v). The MS mass range was set to 150–2000 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). Digital filtering of the bacteriophage mass spectrum was performed using a five-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 surface. A minimum of three 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 ~5 standard deviations) was considered as the detection limit (DL) for the protein sample.
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 DESIMS for the detection of high molecular weight proteins was evaluated with a series of protein standards that range in molecular mass from 12 to 66 kDa using the optimal DESI-MS parameters established in separate experiments (see Supporting Information). 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 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 (three-dimensional quadrupole ion trap mass analyzer) and MALDI-MS (time-of-flight mass analyzer). However, proteins above 25 kDa, 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 DLs of the protein standards. For protein samples in the range of 12–18 kDa, DLs 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 μg/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 μg/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, DLs listed in Table 1, when compared on a per mole base, span over a 3 order of magnitude range. It is worth noting that DLs 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 three-dimensional quadrupole linear ion trap mass spectrometer (Finnigan LCQ Classic) and its sensitivity is lower than that for a two-dimensional (linear) quadrupole ion trap mass spectrometer (e.g., Finnigan LTQ or Applied Biosystems Q-Trap systems) used by other investigators. However, the observed trend of increasing DLs 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 system. The decrease in S/N with increasing protein MW may also contribute to the observed decrease in mass accuracy for proteins above 18 kDa.

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), (E) chymotrypsinogen A (>100 ng/mm²), and (F) BSA (>4000 ng/mm²). Proteins were deposited on a Plexiglas surface.

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 DESI-MS analysis with a solvent consisting of 70% formic acid in acetonitrile 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 13 851, 0.6% difference). This demonstrates the ability of DESI-MS to rapidly analyze semicrude biological samples for protein molecular weight determinations, offering a viable alternative to MALDI-MS analysis of low MW proteins.

**CONCLUSIONS**

Results presented demonstrate that DESI-MS is a viable approach for the analysis of intact proteins up to 18 kDa. The analysis is rapid with a total analysis time under 10 min including sample preparation and offers an alternative approach to MALDI-TOFMS measurements, where a matrix compound is required. At molecular masses above 25 kDa (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 points to a possible limited efficiency of sample desorption or ion transfer to the MS system in the current design of the DESI source. The recently reported hybrid ionization technique involving MALDI, ELDI, and ESI termed matrix-assisted laser desorption electrospray ionization (MALDE-
SI\(^{36}\) should yield insight into the factors limiting the sensitive detection of high molecular weight proteins by DESI-MS 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.

**ACKNOWLEDGMENT**

The authors acknowledge support of this work by the Agricultural Research Service, United States Department of Agriculture (USDA Grant 448800), the University of Wyoming Research Office, and the National Institute of Health/National Center for Research Resources (Grant RR-16474). The authors also acknowledge the assistance of Dr. Zhaojie Zhang of the University of Wyoming Microscopy Core Facility.

**SUPPORTING INFORMATION AVAILABLE**

A description of optimized DESI-MS parameters and supplementary experimental results. This material is available free of charge via the Internet at http://pubs.acs.org

Received for review December 28, 2006. Accepted February 26, 2007.

AC062451T