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Infrared Laser Ablation Sample Transfer for MALDI and Electrospray

Sung-Gun Park, Kermit King Murray

Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA

Abstract

We have used an infrared laser to ablate materials under ambient conditions that were captured in solvent droplets. The droplets were either deposited on a MALDI target for off-line analysis by MALDI time-of-flight mass spectrometry or flow-injected into a nanoelectrospray source of an ion trap mass spectrometer. An infrared optical parametric oscillator (OPO) laser system at 2.94 μm wavelength and approximately 1 mJ pulse energy was focused onto samples for ablation at atmospheric pressure. The ablated material was captured in a solvent droplet 1–2 mm in diameter that was suspended from a silica capillary a few millimeters above the sample target. Once the sample was transferred to the droplet by ablation, the droplet was deposited on a MALDI target. A saturated matrix solution was added to the deposited sample, or in some cases, the suspended capture droplet contained the matrix. Peptide and protein standards were used to assess the effects of the number of IR laser ablation shots, sample to droplet distance, capture droplet size, droplet solvent, and laser pulse energy. Droplet collected samples were also injected into a nanoelectrospray source of an ion trap mass spectrometer with a 500 nL injection loop. It is estimated that pmol quantities of material were transferred to the droplet with an efficiency of approximately 1%. The direct analysis of biological fluids for off-line MALDI and electrospray was demonstrated with blood, milk, and egg. The implications of this IR ablation sample transfer approach for ambient imaging are discussed.

Key words: MALDI, Electrospray, Infrared, Ablation, Ambient

Introduction

Ambient mass spectrometry is the direct analysis of materials in their native environment with ions typically created outside the mass spectrometer [1]. With ambient methods, sample preparation is minimal and, in some cases, unnecessary. Due to its capabilities for fast direct analysis, ambient mass spectrometry has important applications in forensics, environmental analysis, and medical diagnostics [2–4]. The rapid growth of ambient mass spectrometry has been led by the two techniques of desorption electrospray ionization (DESI) [5] and direct analysis in real time (DART) [6], which form ions by directing charged droplets or ions, respectively, at the sample of interest. In DESI, charged droplets from an electrospray ionization source bombard the sample. The droplets collect on the sample, solvent solublizes some components, and secondary droplets containing the sample are removed, assisted by the spray nebulizing gas. With DART, the sample is bombarded by metastable ions from a glow discharge that interacts with volatile species from the sample to form ions.

Lasers can be used to remove material from a sample and deliver it to the electrospray or other source for ionization, eliminating the need for charged droplet or metastable ion

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Correspondence to: Kermit K.Murray; *e-mail:* kkmurray@lsu.edu

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interaction with the surface. Laser ablation sample transfer to an electrospray source can be accomplished either with or without a matrix in analogy with laser desorption ionization (LDI; without a matrix) and matrix-assisted laser desorption ionization (MALDI; with a matrix). The laser-plus-electrospray method was first demonstrated in 2005 and used a 337 nm laser and no matrix [7]. With this approach, the UV laser is directed at the surface and ions are created when the plume of desorbing material interacts with the electrospray. The authors called this technique electrospray assisted laser desorption ionization (ELDI). A second variant of laser desorption combined with ESI used a 337 nm UV laser and a MALDI matrix to aid in desorption, but not necessarily in ionization. This technique was called matrix-assisted laser desorption electrospray ionization (MALDESI) [8]. An approach using a non-resonant femtosecond laser, called laser electrospray mass spectrometry (LEMS), has recently been reported [9] and demonstrated for spatially resolved ambient mass spectrometry [10].

Ablation and ionization can be decoupled if the material is captured and subsequently ionized. The transfer of material with a laser can be done with negligible degradation of the biological material. For example, double-stranded DNA molecules up to 1000 base pairs can be ablated and captured on a target with no fragmentation.[11, 12], and ablated proteins can be used to create thin films that retain the activity of the component biological molecules [13]. Recently, Huang et al. used a near-IR (1064 nm) pulsed laser to desorb and ablate material that was captured in a droplet. The droplet was deposited on a target and mixed with matrix for a standard MALDI analysis. Proteins could be transferred this way with little to no fragmentation [14]. Ovchinnikova *et al.* used a similar droplet capture approach coupled to an electrospray mass spectrometer [15]. A 532 nm visible laser was used to ablate proteins and other molecules that were captured in a solvent droplet and flow injected into the electrospray source with no fragmentation observed in the mass spectra.

Lasers operating in the mid-IR wavelength region near 3000 nm wavelength have been used for MALDI [16], but applications have been limited due to the large quantity of material ejected that is not compatible with commercial MALDI instruments operating at vacuum and with high extraction fields [17, 18]. In contrast, the greater material removal of IR lasers is an advantage for ambient ionization techniques such as MALDESI and the related LAESI technique [19–21]. The volumetric energy deposition with a mid-IR laser is comparable to that of UV lasers, but the depth of laser penetration is an order of magnitude larger [22]. The greater penetration depth of IR lasers can be an advantage in removing deeply embedded materials such as biomolecules in polyacrylimide gels [23, 24] and in tissue samples [25]. Another advantage of mid-IR lasers for ablation of material is that the characteristics of the ablation plume can be widely varied simply by changing the IR laser wavelength in a wavelength tunable system [26, 27]. This tunability of the ablation plume characteristics gives a greater level of control in the quantity of material removed.

We have used a mid-IR OPO laser system to ablate biomolecules from samples under ambient conditions. The ejected material was captured in a solvent droplet that was held a few millimeters above the sample surface. The solvent droplet was then deposited on a MALDI target and analyzed in a MALDI TOF mass spectrometer or was flow-injected into an electrospray mass spectrometer. Angiotensin II was used to investigate the effects of the number of laser shots, distance between the droplet and target, droplet size, and droplet solvent. Proteins were tested with both MALDI and ESI analysis to investigate the ability of the IR laser to remove materials without fragmentation. Various real world samples were used to assess the ability of the IR laser to ablate biomolecules from complex mixtures. These implications of these results for ambient imaging of tissue samples with subsequent MALDI or ESI mass spectrometry are assessed.

Experimental

The pulsed laser ablation sample preparation station (Figure 1) consisted of a 3 mL syringe mounted on a manually controlled xyz stage (model 461 Newport; Irvine, CA, USA) above a sample target that could be irradiated by an infrared laser. The syringe was connected to a Luer taper adaptor union that was connected to a 360 µm o.d. and 50 µm i.d. coated fused silica capillary. The capillary was 6 cm long and was held several millimeters above a stainless steel sample target. The syringe was operated manually and droplets of various sizes could be

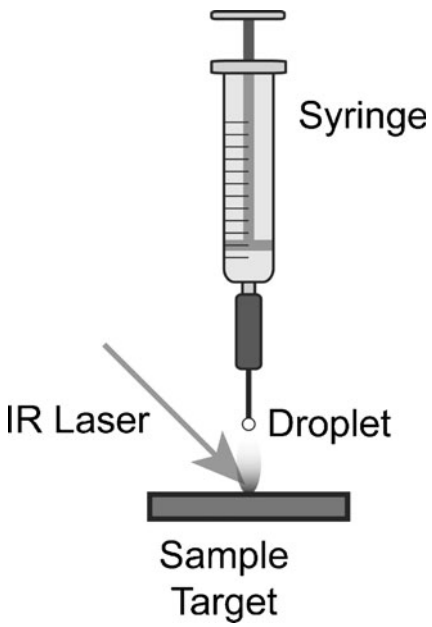

Figure 1. Schematic of the laser ablation sample transfer collection system

produced; a video camera and macro lens was used to observe the droplet.

A wavelength tunable pulsed infrared optical parametric oscillator (OPOTEK, Carlsbad, CA, USA) was used to ablate the sample from the metal target. The wavelength was set at 2.94 μm to overlap with the OH stretch absorption of the analyte [28] and the repetition rate was 2 Hz. The laser was directed at the target at a 45° angle and was focused onto the target with a 250 mm focal length lens and the spot size of the laser beam at the capillary tip was approximately 200 μm×300 μm as determined with laser burn paper. The maximum laser energy was 1.75 mJ with no attenuation. The pulse width is 5 ns corresponding to a maximum fluence of 3 J/cm^2 and an irradiance of 500 MW/cm^2. The laser was attenuated using laser control software to reduce the energy to lower values; no other optical elements were used for attenuation.

Samples for ablation were deposited on a stainless steel target from a 10 μL volume of 1:1 (vol/vol) mixture of acetonitrile and 0.1% TFA, unless otherwise indicated, and were irradiated while some solvent remained. The solution in the syringe was either a pure solvent or a saturated matrix solution. The sample target was placed below the syringe and capillary and the distance between the target and droplet (measured between the target and bottom of the droplet) and the droplet diameter was manually adjusted. The laser irradiated the target for a given number of shots, typically between 60 and 300. After the ablation of the sample and collection in the droplet, the syringe was removed from the xyz stage and the droplet was deposited on a MALDI target. For samples collected in a pure solvent, 2 μL of saturated matrix solution was added to the target; for samples collected in matrix solution, no additional matrix solution was added to the target.

MALDI mass spectra were obtained on a commercial MALDI-TOF/TOF mass spectrometer (Ultraflextreme; Bruker Daltonics, Billerica, MA, USA). The mass spectrometer was operated in reflectron mode for peptides and linear mode for proteins. For electrospray analysis, a quadrupole ion trap mass spectrometer (M8000; Hitachi, Japan) was modified to replace the original electrospray source with a pulled nanospray tip. Tips were prepared by cutting a small length of 360 μm× 50 μm capillary with a binder clip suspended at the bottom end and heating the middle with a flame [29]. No nebulizing gas was used for the electrospray. After laser ablation sample transfer to the liquid droplet, the droplet solution was delivered to a 500 nL injection loop (model 7410; Rheodyne, Cotati, CA, USA) connected to the nanoelectrospray source. Ion trap mass spectra were acquired at two microscans in positive-ion mode. Ions were accumulated for 100 ms in the trap and each mass spectrum was averaged for 2 min. The skimmer cone was held at 40 V and heated to a temperature of 180 °C.

The peptide and protein standards angiotensin II, bovine insulin, and equine cytochrome *c*, reagent trifluoroacetic acid (TFA), and matrix compounds α-cyano-4-hydroxycinnamic acid (CCA) and 4-hydroxy-3-methoxycinnamic acid (ferulic acid) were obtained from Sigma-Aldrich (St. Louis. MO, USA) and used without further purification. HPLC grade acetonitrile (ACN) was purchased from Fisher Scientific (Pittsburgh, PA, USA) and house ultrapure water (18 MΩ cm, Barnstead E-pure System; Dubuque, IA, USA) was used. Analyte solutions were prepared by dissolving angiotensin II, bovine insulin and cytochrome *c* in 1:1 (vol/vol) mixture of acetonitrile and 0.1% TFA to a concentration of 1 mM. A saturated matrix solution for the peptide angiotensin II was prepared by dissolving 50 mg/mL of CCA in 1:1 (vol/vol) mixture of acetonitrile and 0.1% aqueous TFA and the solution for proteins and biological fluids (blood, milk, and egg white) was prepared by dissolving 35 mg/mL of ferulic acid in 1:1 (vol/vol) mixture of acetonitrile and 0.1% aqueous TFA. Chicken eggs and bovine milk were purchased from a local market. The egg whites were separated from the egg yolks for analysis. Ten μL of the egg white or the bovine milk were deposited onto a stainless steel target for collecting materials without any sample pretreatment. Undiluted human blood (10 μL) was obtained from a volunteer and transferred to a stainless steel target with a micropipette.

Results and Discussion

An important issue in laser ablation sample transfer to solvent droplets or other collection devices is whether biomolecules can be transferred without fragmentation. Another issue is the efficiency of sample transfer: whether a sufficient quantity of material can be transferred to achieve a mass spectrometry or other analysis. Our initial studies involved the use of mid-infrared laser ablation to transfer pure peptides and proteins for MALDI analysis to assess the ability to transfer these molecules without fragmentation using mid-IR wavelengths. Next, the ability of the mid-IR laser to manipulate complex biological samples was assessed. These studies were then extended to electrospray mass spectrometry for IR laser ablation droplet capture and nanospray flow-injection analysis.

IR Laser Ablation Sample Transfer for MALDI

MALDI mass spectra of the peptide angiotensin II prepared by pulsed infrared laser ablation droplet capture are shown in Figure 2. A 10 μL volume of a 1 mM solution of angiotensin II in a 1:1 (vol/vol) solution of ACN and 0.1% TFA was deposited on a stainless steel target that was held 3 mm below a solvent droplet. The solvent in the droplet was also a 1:1 (vol/vol) solution of ACN and 0.1% TFA and was approximately 1.5 mm in diameter. Material was ablated from the target at 2.94 μm and 1 mJ energy and captured in the solvent droplet then deposited on a standard MALDI target. The 2.94 μm wavelength was chosen because it has been used most often in mid-IR ablation [16] and also because this wavelength is near the peak of the OH stretch absorption of water and other solvents and the strong absorption leads to the vigorous evolution of plume material and the production of large quantities of particulate [26, 27].

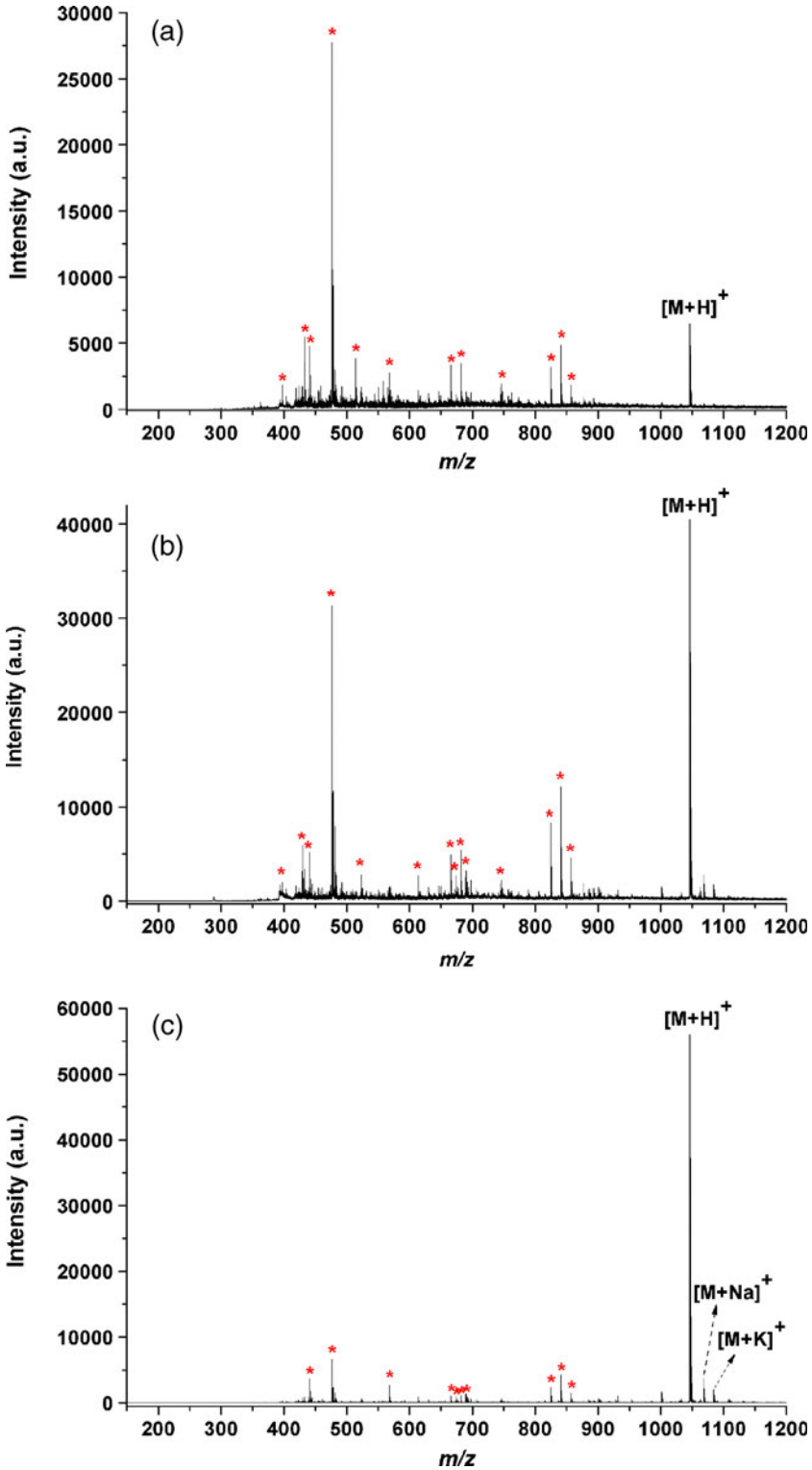

Figure 2. MALDI mass spectra of the peptide angiotensin II with sample delivery by ambient infrared laser ablation sample transfer using different numbers of laser shots: **(a)** 60 laser shots, **(b)** 180 laser shots, **(c)** 300 laser shots. Matrix peaks are indicated with asterisks

A 2 μL volume of saturated CCA matrix in a 1:1 (vol/vol) solution of ACN and 0.1% TFA was deposited with the droplet. MALDI mass spectra were obtained in reflectron mode and were the sum of 500 UV laser shots. Figure 2a was obtained from 60 infrared laser ablation shots, Figure 2b from 180 laser ablation shots, and Figure 2c from 300 laser

ablation shots. In Figure 2a, the large peaks (indicated with asterisks) in the low mass region of the mass spectrum correspond to matrix, matrix cluster, and matrix fragment ions typical for this matrix [30–32]. At 180 IR laser shots, the protonated angiotensin II ion is the most intense peak and, at 300 shots, the protonated molecule peak is the most intense peak with only a few other less intense peaks in the mass spectrum.

Optimization of the ablation setup included the number of laser shots, distance between the droplet and sample, droplet diameter, solvent, and laser energy. These results are presented in the Supplemental Information. A plot of the signal intensity (peak height) as a function of the number of laser shots show that the signal increases rapidly between 60 and 120 laser shots and levels off somewhat after several-hundred laser shots (Figure S1). This is likely due to sample removal by ablation at the 1 mJ laser energy. The distance between the droplet and the target was varied from 1 to 5 mm, and it was found that the closest distance gave the greatest signal intensity (Figure S2). This is consistent with the relatively large radial divergence observed in IR laser ablation plumes [27, 33, 34]. Additionally, the stopping distance for a plume of ablated material at atmospheric pressure is several millimeters, and target to droplet distances larger than this may prevent particles from reaching the droplet. A relatively large droplet size is favored even though this leads to a dilution of the sample in a greater solvent volume. The signal is greatest for the largest droplet size (2 mm diameter; Figure S3). The signal increased by a factor of five when the droplet volume increased by eight times, suggesting that the larger droplet is captures more material from the expanding plume. The laser energy was varied from 0.5 to 1.75 mJ per pulse and higher pulse energies give the greatest signal, but it does not increase as rapidly above 1 mJ (Figure S4). This effect has been observed previously and is attributed to shielding of the target by the dense plume formed at higher energies [26]. No additional peptide fragmentation was observed at the higher pulse energies.

Different solvents were tested in the capture droplet: water, 0.1% TFA in water, ACN, 0.1 % TFA in acetonitrile, 1:1 (vol/vol) ACN and water, and 1:1 (vol/vol) ACN and 0.1% TFA in water (Figure S5). The best solvent is pure water, which is most likely due to the higher solubility of the peptide in this solvent. The drawback to the pure water solvent is the longer time that is required for solvent evaporation when the droplet is deposited on the MALDI target. An ACN and 0.1% TFA solvent that also contained the matrix was found to produce mass spectra comparable to those where the matrix was added separately. A difficulty with the matrix in capture droplet approach is that matrix crystals tend to form in the capture droplet as the solvent evaporates.

It was found that proteins could also be transferred to the droplet by IR laser ablation without fragmentation. Mass spectra of bovine insulin and cytochrome *c* are shown in Figure 3. The proteins were deposited on the ablation target from 1 mM solutions in 1:1 (vol/vol) ACN and 0.1% TFA and 1.5 mJ laser energy to transfer the material from the target to the droplet. Ferulic acid matrix was added with the laser ablation capture droplet to the MALDI target. Other conditions were a 2 mm capture droplet, 1 mm target to droplet spacing, and 60 laser shot collection.

Several samples were used to test to the ability of laser ablation to analyze complex mixtures of biological molecules; mass spectra are shown in Figure 4. In all cases, a total of 60 laser shots at 1.5 mJ energy was used to transfer the material from the target into a 2 mm diameter droplet suspended 1 mm above the target. A ferulic acid matrix was used. A mass spectrum from an analysis of whole human blood is shown in Figure 4a. A droplet of blood from a volunteer was deposited on a metal target and irradiated with the IR laser and the laser-transferred material was analyzed by UV MALDI. Two sets of peaks corresponding to the hemoglobin α- and β-chains without heme were observed and are indicated in Figure 4a. A signal corresponding to heme that was more than 30 times stronger than the a- and b-chain peaks was observed at *m/z* 617 (not shown). Heme loss and α- and β-chains fragmentation is typically observed in MALDI analysis of whole blood [35]. A MALDI mass spectrum of undiluted whole blood showed a broad unresolved feature between 500 and 4000 *m/z* (data not shown). A mass spectrum obtained from whole milk is shown in Figure 4b. Several proteins were observed: proteopeptone pp81, γ_3-casein/ γ_2-casein/ γ_1-casein, α-lactalbumin, β-lactalbumin B and A, and α_{S1}-casein/β-casein. The assignment of the peaks is based on previously reported spectra reported for MALDI analysis of milk [36, 37]; the spectrum in Figure 4b is similar to that reported previously for diluted milk. A MALDI mass spectrum of a direct deposit of milk was dominated by the α-lactalbumin peak (data not shown), suggesting that there may be signal suppression effect in the direct MALDI analysis that is avoided by the laser ablation and droplet capture approach. Egg white from a fresh egg with laser ablation sample treatment led to the mass spectrum shown in Figure 4c. Both singly and doubly protonated lysozymes were observed with the latter significantly less intense. The mass spectrum is similar to direct MALDI of egg white (data not shown).

IR Laser Ablation Sample Transfer for Electrospray

Electrospray mass spectrometry was accomplished with samples that were infrared laser ablated from a stainless steel target at 2.94 μm wavelength and captured in a 2 mm droplet held 1 mm above the target. Mass spectra of pure peptides and proteins are shown in Figure 5. A nano-electrospray mass spectrum of angiotensin II prepared by infrared laser ablation sample transfer is shown in Figure 5a. A 10 μL volume of a 1 mM solution of angiotensin II in 7:3 (vol/vol) ACN and 0.1% TFA was deposited on the target.

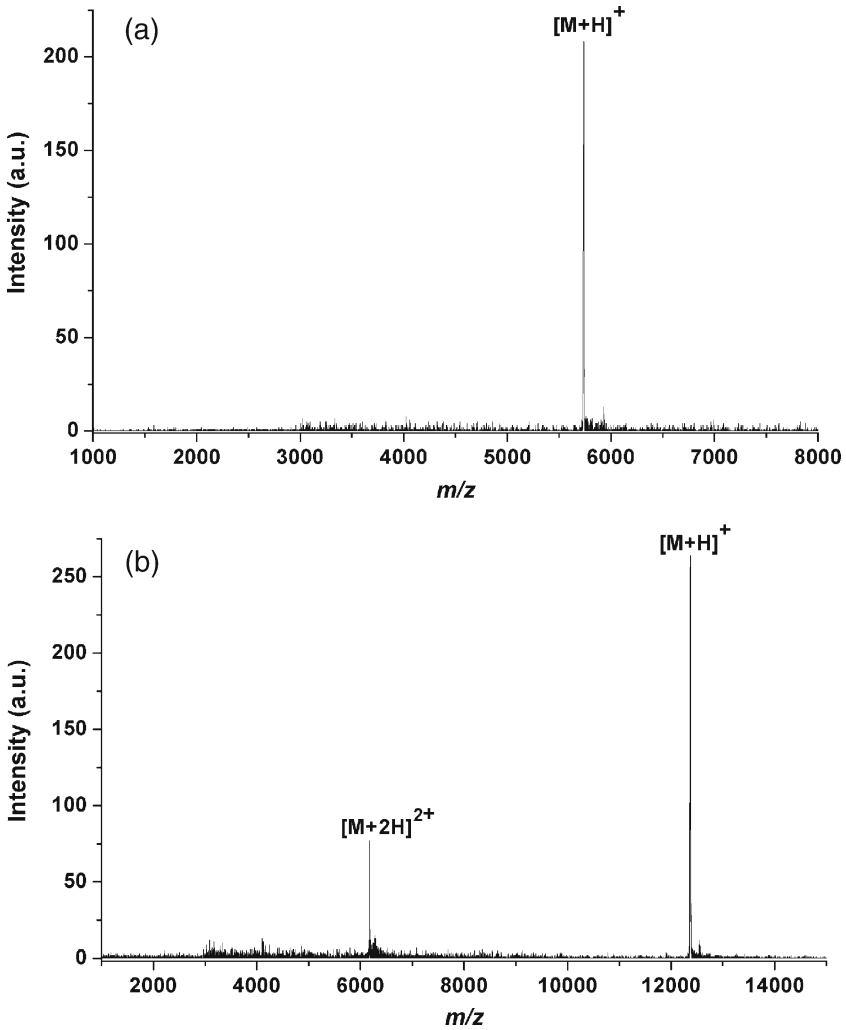

Figure 3. Infrared laser ablation sample transfer MALDI mass spectra of **(a)** bovine insulin and **(b)** cytochrome *c*

The sample was ablated by 300 infrared laser ablation shots at 2.94 μm and 1.5 mJ energy. The droplet was manually withdrawn into a 10 μL syringe and delivered to a 500 nL injection loop for delivery to the electrospray. The peptide signal is half as intense as a 500 nL flow injection of a 10 μM solution of peptide, suggesting that the concentration of the peptide in the droplet approximately 5 μM. For a 2 mm droplet, this corresponds to 40 pmol of peptide transferred to the droplet. Under similar IR laser ablation conditions, it was found that approximately 4000 glycerol particles averaging 1 μm in diameter are ablated from a metal target per laser shot, and that these particles accounted for a significant fraction of the ablated material [38]. If a similar quantity of solid peptide is ablated in the droplet capture experiments, the transfer of 40 pmol to the droplet would require a 1% capture efficiency.

Nanoelectrospray mass spectra of proteins transferred from target to droplet by IR laser ablation are shown in Figure 5b (bovine insulin) and 5c (cytochrome *c*). The capture conditions were identical to those for the peptide. The mass spectrum of bovine insulin shows multiply-charged ions from $[M+7\ H]^{7+}$ to $[M+4\ H]^{4+}$ and multiply-charged cytochrome *c* ions from $[M+20\ H]^{20+}$ to $[M+9\ H]^{9+}$ are observed. No dimers or trimers are observed in both cases. The mass spectrum from each droplet was similar to those obtained from the direct injection of a 10 μM solutions of bovine insulin and cytochrome *c* into a 500 nL injection loop (data not shown). The similarity of the apparent transfer efficiency suggests that there is not a significant mass discrimination effect in the laser ablation transfer efficiency.

Several samples were used to test infrared laser ablation sample transfer for the analysis of complex biological mixtures. Mass spectra of various biological fluids are shown in Figure 6. In all cases, a total of 300 laser shots at 2.94 μm wavelength and 1.5 mJ laser energy was used to transfer the sample materials from a metal target into a 2 mm diameter droplet suspended 1 mm above the metal target. A mass spectrum from whole human blood obtained using IR laser ablation sample transfer is shown in Figure 6a. A droplet of blood from a volunteer was deposited on a metal target and irradiated with the IR laser. The laser-transferred material was ionized with a nanoelectrospray source. In the

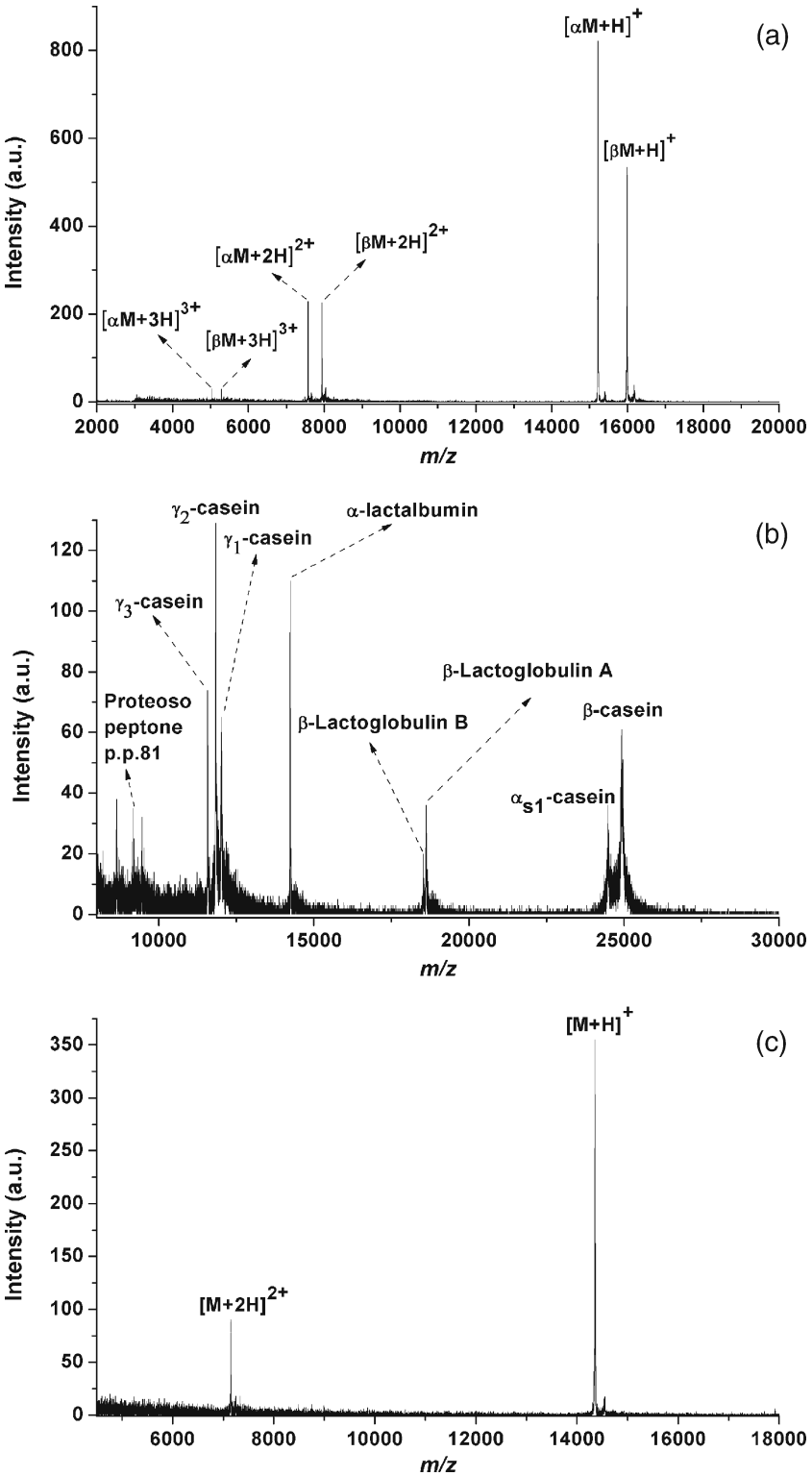

Figure 4. Infrared laser ablation sample transfer MALDI mass spectra of **(a)** human blood, **(b)** whole milk, and **(c)** egg white

mass spectrum, two sets of peaks corresponding to distributions of multiply-charged ions can be observed that correspond to the hemoglobin α- and β-chains without the heme group and charge states $[M+12\ H]^{12+}$ to $[M+23\ H]^{23+}$. The peak at *m/z* 616 corresponds to the free heme. Several other singly and multiply charged ions are also observed. On the basis of the published data [20, 39–42] and the Human Metabolome Database [43], the peaks at *m/z* 1125, 1069, and 1093 may result from α heme (+14), glycated α- (+15) and β-chains (+14),

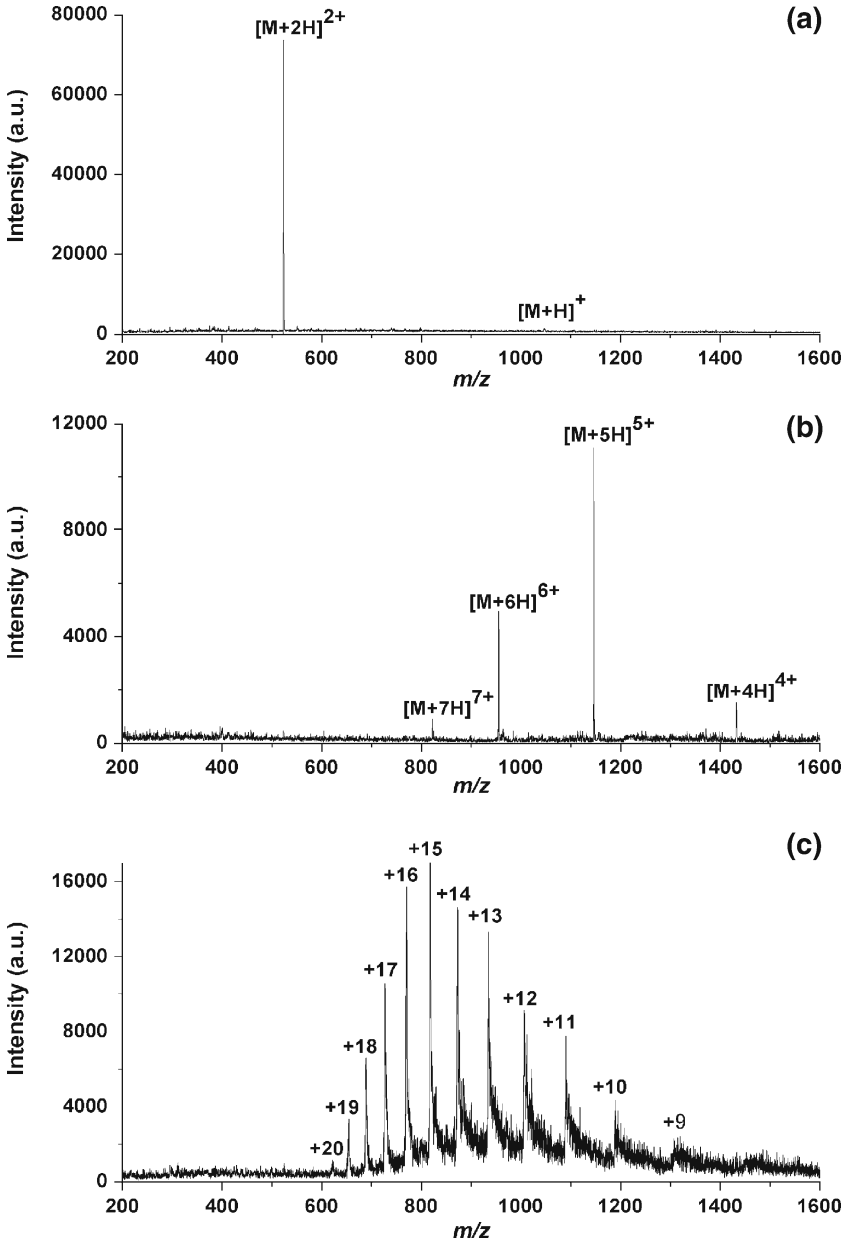

Figure 5. Nanoelectrospray mass spectra of **(a)** angiotensin II **(b)** bovine insulin, and **(c)** cytochrome *c* prepared by infrared laser ablation sample transfer

respectively. The peaks indicated with asterisks may originate from phosphocholine (PC); the peaks at *m/z* 413, 742, and 784 may correspond to protonated LysoPC (10:0), PC (15:0/18:3), and PC (14:1/22:2), respectively.

A mass spectrum obtained from whole milk is shown in Figure 6b. The abundant peaks correspond to the sodium and potassium adducts of lactose as well as the sodium and potassium adducts of the dimers of lactose. The assignment of the peaks is based on previously reported mass spectra [44–46]. The additional ion at *m/z* 533 (indicated with an asterisk) corresponds to a singly charged lipid ion or other component of the milk. The mass spectra of chicken egg yolk and egg white from a fresh egg are shown in Figure 6c and d, respectively. Figure 6c shows several peaks from phosphatidylcholine (PC) with different fatty acid compositions; PC 16:0/18:2 (*m/z* 758), PC 16:0/18:1(*m/z* 760), PC 16:0/20:4 (*m/z* 782), PC 18:0/18:2 (*m/z* 786), PC 18:0/20:4 (*m/z* 810), PC 18:0/22:6 (*m/z* 834). Sodium adducts phosphatidylcholine ions are also observed at *m/z* 780 and 808. The peak at *m/z* 496 is attributed to lysophosphatidyl-choline containing one palmitic acid substitution. The peaks at *m/z* 422 and 369 are attributed to 1,2-dilaurylpho-sphatidylcholine by loss of an acyl group and from cholesterol by the loss of water [19, 39, 47–49]. A mass spectrum obtained from egg white is shown in Figure 6d. Multiply-charged ions

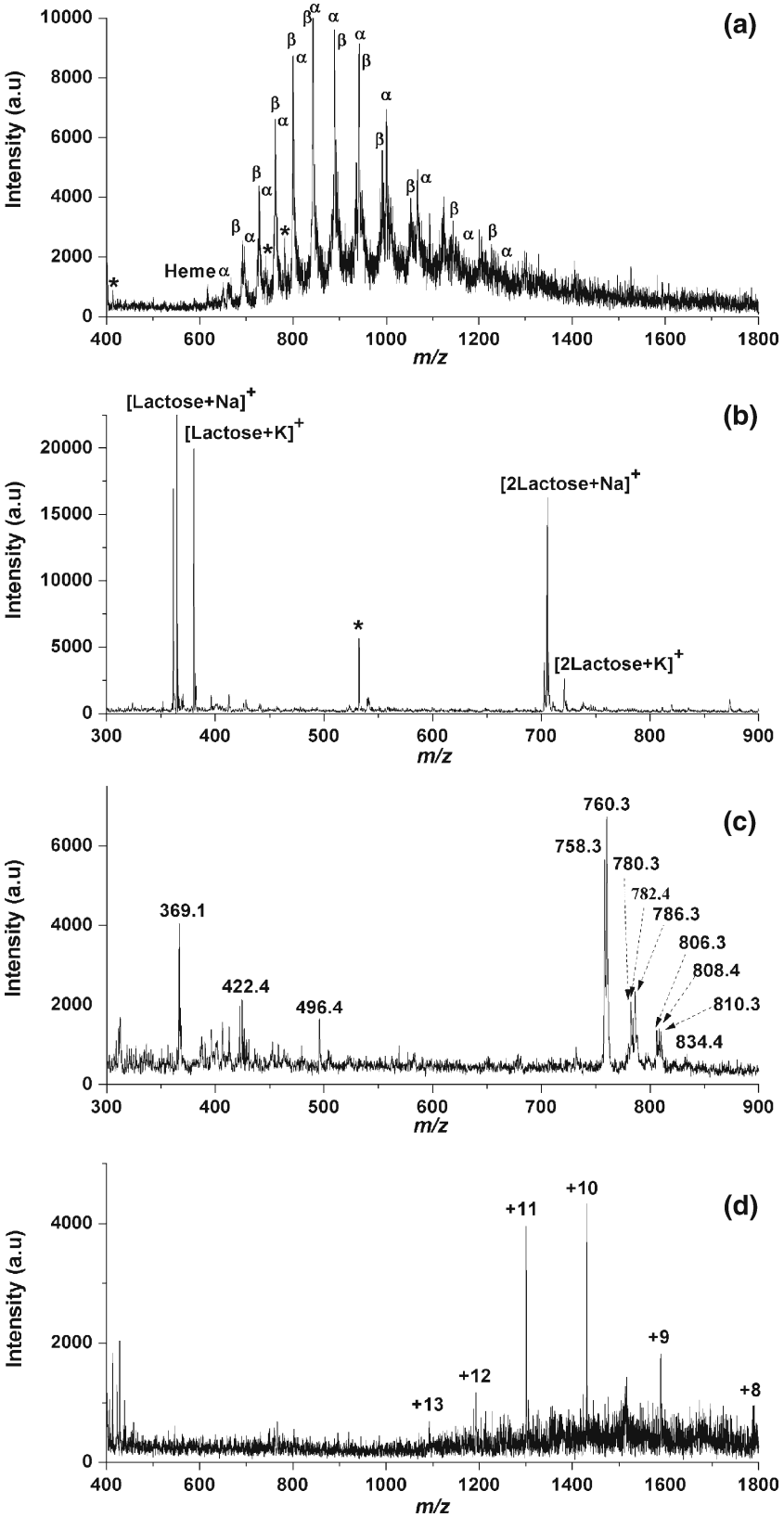

Figure 6. Nanoelectrospray mass spectra of **(a)** human blood, **(b)** whole milk, **(c)** egg yolk, and **(d)** egg white prepared by infrared laser ablation sample transfer

from lysozyme [50] ($[M+13\ H]^{13+}$ to $[M+8\ H]^{8+}$) and unidentified ions ($<m/z$ 500) are observed.

Conclusions

Laser ablation sample transfer has been demonstrated for ambient sampling and subsequent MALDI analysis using a commercial TOF mass spectrometer and electrospray analysis using an ion trap mass spectrometer. With the use of a mid-infrared laser operating in the 2.94 μm wavelength region, it was possible to ablate material from solid samples, capture the material in a solvent droplet, and create ions both by conventional MALDI and conventional electrospray approaches. Due to the nature of the expanding plume of material, it was found that the optimum conditions for sampling are a relatively large 2 mm solvent droplet held close to the sample (1 mm) so as to capture the largest quantity of sample. The efficiency of material capture in the droplet is estimated to be 1% with approximately 10 pmol estimated to be transferred to the droplet in a typical experiment. The best solvent for plume capture for MALDI is water, although an equal volume mixture of 0.1% TFA in water and acetonitrile resulted in faster evaporation when the droplet was placed on a MALDI target. More than 1 mJ IR laser energy could be used for laser ablation sample transfer without sample degradation observed for either peptides or proteins observed with either electrospray or MALDI. The laser fluence was 1000 times greater than that used for UV ablation/electrospray [15] and 10 times greater than for near IR ablation/MALDI [14] yet little to no fragmentation of biomolecules was observed. The infrared laser ablation sample transfer was used to analyze complex biological mixtures for MALDI and ESI analysis.

The mechanism of sample transfer is either through the ejection of free molecules or particulate. Previous studies suggest that a significant fraction of the material ejected by the IR laser is ablated as coarse particulate [27, 38]. Additional material is ejected as smaller particles and free molecules. All of these ejected materials could be captured by the suspended droplet and contribute to the resulting MALDI or ESI signal. Microscopy studies of material collected as thin films on solid targets could help to quantify the ratio of free to particulate material ejected from samples by laser ablation [51].

Laser ablation sample transfer opens up some new possibilities for ambient imaging both with MALDI and electrospray ionization. Laser desorption-based imaging mass spectrometry is currently done under vacuum using MALDI directly from tissue to which matrix has been added [52]. Drawbacks to this approach are the need to add matrix directly to the tissue and the requirement for analysis with the sample under vacuum. Ambient imaging using laser desorption coupled with electrospray ionization is currently under development through the techniques of ELDI, MALDESI, LEMS, and LAESI, and infrared lasers show great promise due to their ability to efficiently remove material [25]. Laser ablation sample transfer decouples the ablation of material from the ionization and the efficiency of material transfer is high. One possible adaptation of IR laser ablation transfer is to ablate material from a tissue sample for direct collection on a MALDI target. Scanning the IR ablation laser across the tissue will ablate biomolecules that are collected on a matrix coated or nanostructured matrix-free target. Transmission geometry IR laser ablation would allow the separation between the tissue and MALDI target to be reduced to less than a mm spacing and allow tight focusing of the ablation laser beam.

Coupling spatially resolved IR laser ablation of tissue with electrospray can be done either off-line or on-line. In the off-line mode, the sample is ablated into droplets that are delivered sequentially to the electrospray source. With the on-line approach, the laser scans across the tissue sample and ablates material into an exposed flowing stream of solvent that carries the ablated material to the electrospray source. The basic principle of this approach has been described [53] and demonstrated with UV laser ablation [15]. In our ongoing work, we are developing a microfluidic chip system that uses high pulse energy IR laser ablation sample transfer and flowing liquid stream capture for electrospray mass spectrometry imaging.

Acknowledgments

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