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Laser Electrospray Mass Spectrometry Minimizes Ion Suppression Facilitating Quantitative Mass Spectral Response for Multicomponent Mixtures of Proteins

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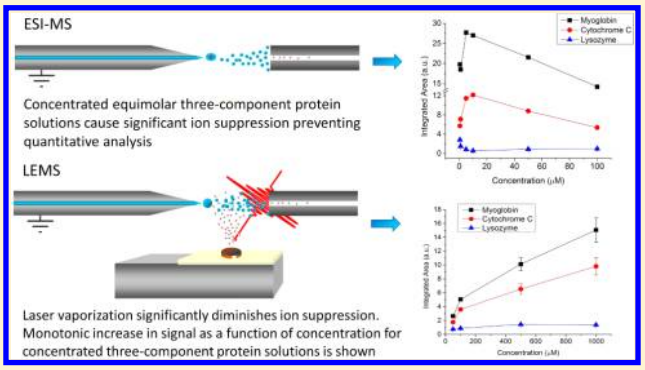
Supporting Information

ABSTRACT: A comparison of the mass spectral response for myoglobin, cytochrome c, and lysozyme is presented for laser electrospray mass spectrometry (LEMS) and electrospray ionization−mass spectrometry (ESI−MS). Analysis of multicomponent protein solutions using nonresonant femtosecond (fs) laser vaporization with electrospray postionization mass spectrometry exhibited significantly reduced ion suppression effects in comparison with conventional ESI analysis, enabling quantitative measurements over 4 orders of magnitude in concentration. No significant charge reduction was observed in the LEMS experiment while the ESI measurement revealed charge reduction for myoglobin and cytochrome c as a function of increasing protein concentration. Conventional ESI−MS of each analyte from a multicomponent solution reveals that the ion signal detected for myoglobin and cytochrome c reaches a plateau and then begins to decrease with increasing protein concentration preventing quantitative analysis. The ESI mass spectral response for lysozyme from the mixture initially decreased, before increasing, with increasing multicomponent solution concentration.



INTRODUCTION

Electrospray ionization−mass spectrometry (ESI−MS)[1] is a powerful technique for analyzing samples ranging from small molecules[2,3] to large protein mixtures.[4,5] Electrospray ionization has been extensively used for biological mass spectrometry of intact protein molecular ions; however, the method suffers from ion suppression, a phenomenon that can compromise interpretation of mass spectral data.[6,7] Ion suppression results when multiple analytes compete for available charge in the electrospray droplet leading to a selective response, for example, of one protein over another.[8,9] This causes the measured mass spectral ion abundance to decrease with increasing protein concentration. Ion suppression is a major concern for quantitative ESI−MS and has been investigated in an attempt to enable analysis of multicomponent solutions.[10−13]

Ion suppression is an undesirable phenomenon observed in ESI−MS measurements of multicomponent mixtures[5,14,15] and begins with the uneven nature of droplet fission events. Previous work has shown that progeny droplets carry away a small fraction of mass with an incommensurately high amount of charge.[16] Thus, analytes with high surface activity will disproportionately concentrate in the highly charged offspring droplets. The suppression causes a discrepancy between the ion intensity detected and the relative concentration of the analyte in solution. In an attempt to explain this phenomenon, Enke et al. developed the excess charge model which considers the electrospray droplet as two separate phases, the charge containing surface layer and the neutral droplet interior.[17] Analytes with hydrophobic character will partition to the excess charge containing surface layer and acquire the majority of charge. More hydrophilic analytes will remain in the droplet interior and have a lower probability to acquire charge.

Investigations of ion suppression in ESI−MS have been performed in an attempt to analyze concentrations where extreme charge deficient conditions are induced.[5,18] McLuckey et al.[5] show that the sum of available charge sites, which is the product of the protein concentration and the average charge in the protein's charge state distribution, enables prediction of the concentration where ion suppression will occur for a given protein, suggesting that surface partitioning is not a key factor for determining signal response. However, an additional study highlighted the suppression effects of the α-globin and β-globin subunits of hemoglobin.[18] Under both charge excess and deficient conditions, the α:β peak intensity ratios remained above unity, suggesting that surface partitioning and availability of charge were not the only factors responsible for the higher instrumental response of the α-globin subunit. Declustering the proteins by increasing the cone voltage led to the anticipated

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α:β ratio, suggesting that desolvation is also an important factor in ion suppression.

Ion suppression of multicomponent protein mixtures has been investigated using several laser-based techniques, including matrix-assisted laser desorption ionization (MALDI),[19,20] electrospray-assisted laser desorption ionization (ELDI),[21] laser ablation electrospray ionization (LAESI)[22] and matrix-assisted laser desorption ionization electrospray ionization (MALDESI).[23] Suppression of ladder peptides was observed in MALDI experiments when a hydrophobic matrix was employed.[20] ELDI and MALDESI investigations of various proteins and biological fluids attribute inconsistencies in the data to ion suppression effects, without quantitative assessment. A LAESI investigation of verapamil and reserpine exhibited quantitative response over 4 orders of magnitude linear dynamic range and no ion suppression effects.[22] However, subsequent LAESI investigations of tissue revealed significant matrix and suppression effects requiring mathematical modeling to provide quantitative measurements.[24,25] A comparison of ESI and ELDI measurements of protein mixtures revealed a significant decrease in ion suppression effects using laser desorption, suggesting that ELDI provides a more accurate measurement of the solution's composition.[21,26] ELDI measured ion signal for insulin, cytochrome c, lysozyme and myoglobin from solution with added matrix. Quantitative measurements were obtained with myoglobin, a known ion suppression agent. When subjected to conventional ESI–MS analysis, measurements revealed that myoglobin dominates the spectrum, and no measurable ion signal was detected from either insulin or cytochrome c.

The development of laser electrospray mass spectrometry (LEMS) has enabled rapid analysis of proteins with no sample preparation, and in particular, no matrix application. LEMS combines nonresonant femtosecond laser vaporization with electrospray ionization mass spectrometry to introduce nonvolatile samples into the mass spectrometer. Previous LEMS investigations of proteins and complex biological solutions include human blood,[27,28] raw egg yolk,[27] and milk.[28] Linear mass spectral response for hen egg lysozyme was observed as a function of concentration ranging from 10^{-3} to 10^{-6} M.[27] Additional LEMS investigations revealed that solution phase protein structure was preserved when subjected to intense, nonresonant ultrafast laser vaporization into the electrospray droplets.[29] LEMS analysis has also been conducted on a variety of analytes such as pharmaceuticals, lipids,[27,30] narcotics,[31] dipeptides,[32] explosives,[33,34] black powders[35] and plant tissue organ type and phenotype.[36,37]

In this study, equimolar mixtures of myoglobin, cytochrome c, and lysozyme were analyzed with ESI–MS and LEMS. The mass spectral responses of multicomponent protein solutions were measured as a function of concentration. The response for conventional electrospray is compared to that for laser electrospray mass spectrometry revealing significantly reduced ion suppression effects in the case of fs laser vaporization of proteins into the electrospray stream. The charge state distributions are measured as a function of protein concentration to provide insight into the signal response factors and charge suppression mechanisms for conventional electrospray and LEMS analysis.

EXPERIMENTAL SECTION

Materials. Myoglobin and cytochrome c were purchased from Sigma-Aldrich (St. Louis, MO) and lysozyme was purchased from USB Corporation (Cleveland, OH). Stock solutions were prepared by dissolving the protein in HPLC grade water (Fisher Scientific, Pittsburgh, PA) in plastic containers at a concentration of 2×10^{-3} M.

Sample Preparation. The stock protein solutions were diluted to 0.5, 1, 5, 10, 50, and 100 μM with methanol (EMD Chemicals, Gibbstown, NJ) to make electrospray solutions comprised of 1% acetic acid, 1:1 (v/v) water/methanol. All protein mixtures were composed of equimolar concentrations similar to the individual solutions. All solutions were sonicated prior to the addition of protein.

Protein Analysis using LEMS. The stock protein solutions were diluted to 50, 100, 500, and 1000 μM with HPLC grade water. Prior to vaporization, 10 μL aliquots of solution were deposited using a micropipet over an area of 150 mm^2 on a stainless steel plate. Each mass spectrum was obtained from a fresh aliquot of solution by translating the sample stage. Ten mass spectra were obtained for each concentration. Methanol and acid were not added to the LEMS sample solution to facilitate vaporization of native protein. The electrospray solution for postionization is comprised of 1:1 (v/v) water/methanol with 1% acetic acid.

Laser Vaporization and Ionization. A Ti:sapphire laser oscillator (KM Laboratories, Boulder, CO) seeded a regenerative amplifier (Coherent, Inc., Santa Clara, CA) to create a 2.5 mJ pulse centered at 800 nm with a duration of 70 fs, operating at 10 Hz to couple with the electrospray ion source. The laser was focused to a spot size of ~400 μm in diameter using a 16.9 cm focal length lens, with an incident angle of 45° with respect to the sample. The intensity of the laser at the substrate was approximately 2×10^{13} W/cm^2. The steel sample plate was biased to −2.0 kV to compensate for the distortion of the electric field between the capillary and the needle caused by the sample stage. The area sampled was 6.4 mm below and 3 mm in front of the electrospray needle. The sample was ejected in a direction perpendicular to the electrospray plume, where capture and ionization occurred. An ESI solvent background mass spectrum was acquired before vaporization of each sample set to allow background subtraction of solvent peaks. Negative features in the background-subtracted mass spectra result from charge competition between the vaporized analytes altering the solvent ion distribution. Positive peaks not labeled in the blank-subtracted mass spectra are solvent-related features.

Mass Spectrometry. The mass spectrometer used in this experiment has been previously described.[32] The system combines an intense femtosecond laser for the nonresonant transfer of analyte into the gas phase with an electrospray ion source for capture and ionization of the vaporized material. The electrospray ionization source (Analytica of Branford, Branford, CT) was configured for positive ions. The ESI source consists of an electrospray needle and dielectric capillary with a metal shroud and a 500 μm inlet aperture. The electrospray needle was maintained at ground while the inlet capillary was biased to −4.5 kV to analyze positive ions. The ESI needle was 6.4 mm above, and parallel to, the sample stage and approximately 6.4 mm in front of the capillary entrance. The electrospray solvent mixture was pumped through the needle at a flow rate of 3 μL/min as set by the syringe pump (Harvard Apparatus, Holliston, MA). The ESI plume was dried by countercurrent nitrogen gas at 180 °C before entering the inlet capillary. Upon exiting the dielectric capillary, the ions traverse two hexapole ion guides to enter the pulsed extraction time-of-flight analyzer. The first hexapole operates in a trapping mode, where the positive ions were collected at 10 Hz. After exiting the first hexapole, the ions

were transferred to the pulsed extraction region by a second hexapole, operating in continuous mode. Ions were injected orthogonally into the linear time-of-flight analyzer and extracted via two high voltage pulsers (Directed Energy Inc., Fort Collins, CO, and Quantum Technology Inc., Lake Mary, FL) triggered 180 μs after the ions exit the first hexapole. The positive ions were then detected, and the resulting mass spectra were averaged using a digital oscilloscope for 50 laser shots (5 s) for LEMS analysis and for 5 s for conventional ESI–MS analysis.

Safety Considerations. Appropriate laser eye protection was worn by all lab personnel.

RESULTS AND DISCUSSION

Electrospray Ionization of Equimolar Protein Mixtures. The signal response for multicomponent mixtures is known to depend on solution conditions and instrumental settings.[5] To study the effect of ion suppression on the ESI process, equimolar solutions of varying concentrations were prepared containing myoglobin, cytochrome c, and lysozyme. The ESI solutions were made under denaturing conditions using 1% acetic acid in a 1:1 water/methanol mixture by volume. The width of the charge state distributions of the protein ions in the ESI mass spectra (Figure 1) suggest that apomyoglobin (mw 16958, $[M + 26H]^{26+}$ to $[M + 10H]^{10+}$), cytochrome c (mw 12382, $[M + 18H]^{18+}$ to $[M + 9H]^{9+}$) and lysozyme (mw 14333, $[M + 10H]^{10+}$ to $[M + 8H]^{8+}$) were in the denatured state. At low concentrations, 0.5 μM, the most intense molecular ions for the three charge state distributions are $[M + 20H]^{20+}$, $[M + 14H]^{14+}$ and $[M + 9H]^{9+}$ for myoglobin, cytochrome c, and lysozyme, respectively. As the concentration increases to 100 μM, the distribution shifts to lower charge states, as seen in the shift of the most intense molecular ions to $[M + 17H]^{17+}$ for myoglobin and to $[M + 13H]^{13+}$ for cytochrome c. While the most intense ion remains the 9+ charge state for lysozyme, the 8+ state increases in relative intensity at the expense of the 10+ state. The formation of lower charge states at higher protein concentrations has been observed previously using electrospray mass spectrometry and is due to the limited available charge in the droplet surface layer with increasing protein concentration.[5,38,39] The shift in the charge state distribution to lower charge states and the significant suppression of cytochrome c and lysozyme charge states observed in this investigation are in agreement with the previous studies.

To quantify ion suppression in multicomponent mixtures, each protein's mass response was plotted as a function of concentration as shown in Figure 2. The charge states of each protein were identified and integrated using the Cutter software package[40] and the integrated intensity was plotted with respect to the protein's concentration in the equimolar solution. The integrated intensity of myoglobin slightly decreases from 0.5 to 1 μM and then increases for concentrations between 1 and 5 μM. Above 5 μM, saturation and a subsequent decrease in signal intensity is observed as a function of increasing concentration. Cytochrome c shows a linear correlation between measured ion abundance and concentration over an order of magnitude (500 nM to 5 μM). However, further increase in protein concentration results in a marked decrease in the measured ion abundance indicating the onset of charge deficient conditions in the electrospray droplet. The saturation and subsequent decrease in signal intensity has been observed previously.[5] In addition, the elevated baseline observed in the 0.5 and 1 μM ESI multicomponent mass spectra introduces

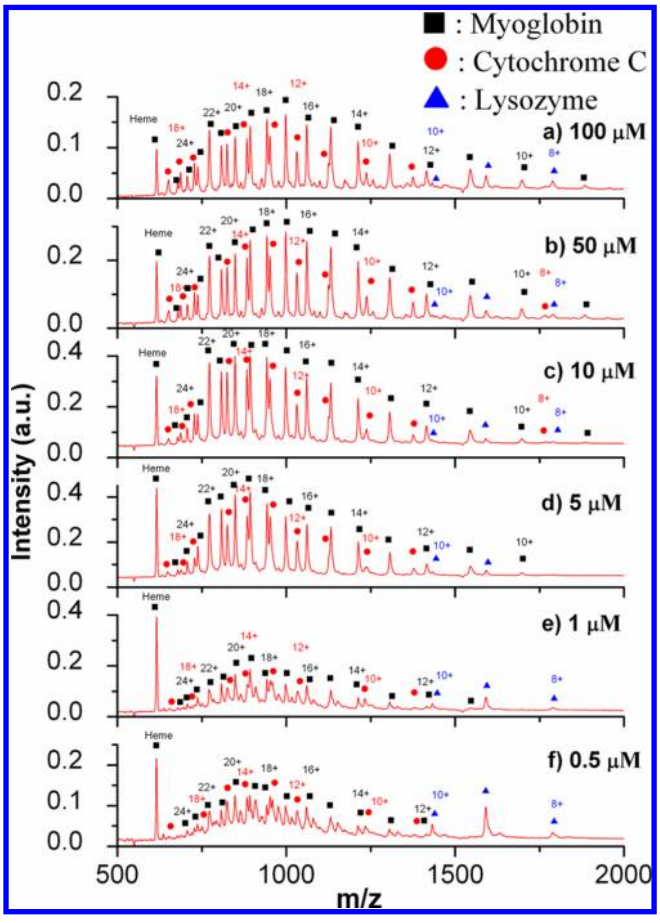

Figure 1. Positive ion ESI mass spectra of different equimolar myoglobin, cytochrome c and lysozyme solution concentrations: (a) 100, (b) 50, (c) 10, (d) 5, (e) 1 and (f) 0.5 μM. The protein mixtures were electrosprayed in water/methanol (1:1 v/v) solutions with acetic acid (1% v/v).

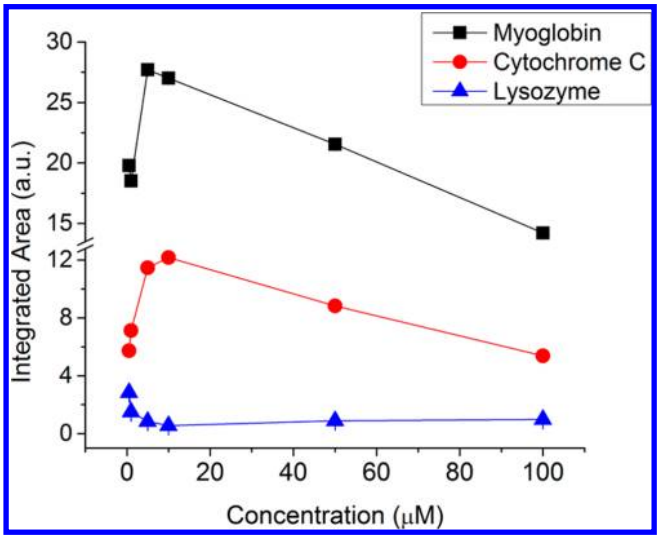

Figure 2. Integrated area of protein charge states as a function of solution concentration for ES–MS equimolar measurements of myoglobin, cytochrome c and lysozyme.

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additional uncertainty to the integrated intensity of myoglobin and cytochrome c. This elevated baseline could be responsible for the slight decrease in the response of myoglobin 0.5 to 1 μM. In contrast, the measured lysozyme ion abundance shows an initial anticorrelation with increasing lysozyme concentration. At 5 μM, ion suppression nearly eliminates the lysozyme signal at m/z 1789.8 $[M + 8H]^{8+}$. As the concentration increases and the charge state distribution of the proteins shift to higher mass to charge ratios (m/z), the lysozyme ion intensity increases slightly. These observations suggest that ion suppression effects have a significant influence on the ESI−MS measurements for the three proteins and will impair quantitative analysis of multicomponent protein solutions using conventional ESI−MS.

The resolution of the mass spectrometer and changing charge state distributions for myoglobin and cytochrome c create challenges for quantification. In particular, the molecular ions of myoglobin $[M + 22H]^{22+}$ at m/z 772.8 and cytochrome c $[M + 16H]^{16+}$ at 773.5 overlap in the ESI mass spectra. To approximate the contribution of each to the unresolved peak at m/z 772.5, we fit the protein charge state distribution to a Gaussian distribution, as shown in Figures S2 and S3 (Supporting Information). The fraction of myoglobin is given by the amplitude of the Gaussian at m/z 772.5 divided by the measured peak height of the mass spectral feature at m/z 772.5. The remainder is attributed to cytochrome c. Repeating this procedure using the cytochrome c Gaussian fit results in two estimates for the myoglobin and cytochrome c fractions as a function of concentration. These agree within 25% under charge deficient ESI conditions and 8% under charge surplus LEMS conditions. Taking the average of the contribution ratios for myoglobin and cytochrome c provides the approximate contribution presented in Table S1 (Supporting Information). The contribution of cytochrome c to the unresolved peak at m/z 772.5 was determined for the various concentrations. The ratio of the integrated m/z 772.5 peak belonging to cytochrome c was estimated to be 45% for 0.5 μM, 25% for 1 and 5 μM, 35% for 10 and 50 μM and 43% for 100 μM. The m/z 772.5 molecular ion is at the lower end of the myoglobin charge state distribution and its contribution changes as the collective solution concentration increases. The myoglobin $[M + 22H]^{22+}$ ion contribution was estimated to be 55% for 0.5 μM, 75% for 1 and 5 μM, 65% for 10 and 50 μM and 57% for 100 μM. Note that attribution of the unresolved peak (m/z 772.5) exclusively to myoglobin or cytochrome c produced minimal change in quantitative analysis of the protein concentrations shown in Figures 2 and 4.

The anticorrelation of the lysozyme mass spectral intensity in the ESI−MS measurement with respect to the myoglobin and cytochrome c intensities as a function of concentration as shown in Figure 2 was surprising. The lysozyme integrated area initially decreases with increasing mixture concentration and reaches a minimum at 10 μM followed by a slight increase in integrated area at higher concentration. The much lower mass spectral response relative to cytochrome c and myoglobin suggests that lysozyme partitions least to the surface of the droplet. The decreasing intensity as a function of increasing concentration could also result from a combination of charge reduction and instrumental response, in particular, the two hexapole ion guides used in the mass spectrometer. The hexapoles were tuned to have higher transmission efficiency for low m/z ions (higher charge states) in comparison with higher m/z ions (lower charge states). We optimized the instrument's potentials to maximize the signal in the m/z range detected. Ion suppression effects, the spectrometer's mass range, and the shift of the charge state distribution to higher m/z (lower charge states) with increasing protein concentration could cause the initial decline of the lysozyme ion intensity with increasing concentration. Note that lysozyme has the highest m/z ions. In addition, the timing of the time-of-flight extraction plates was also optimized for low m/z, which further enhances the spectrometer for higher charge states. In ESI, the combination of low transmission efficiency for high m/z ions with limited excess charge distributed among an increasing quantity of protein results in less charge per protein providing lower cumulative signal response for higher m/z (lower charge states). The increase in lysozyme ion signal at higher equimolar mixture concentrations is likely due to charge reduction of the myoglobin and cytochrome c creating higher m/z features. The transfer of signal from these ions into the same mass range as lysozyme decreases suppression due to microchannel plate saturation. When the experiment was repeated with the hexapole ion guides tuned for lower charge states (higher m/z), lysozyme showed similar suppression effects as seen in Figure 2 for myoglobin and cytochrome c (Supporting Information Figure S1). Ion suppression was observed whether the spectrometer was tuned for low or high m/z ions.

The structure of a given protein will have an impact on mass spectral response. For example, the mass spectral response of solutions containing either myoglobin, cytochrome c or lysozyme reveal that lysozyme reaches charge deficient conditions at higher concentrations than the other two proteins (Supporting Information Figure S2). We attribute this to the fact that lysozyme contains four disulfide bonds that stabilize tertiary structure and prevent denaturation in comparison with myoglobin and cytochrome c which lack disulfide bonds. The folded structure of lysozyme reduces the number the accessible charge sites and the hydrophobic side chains remain buried reducing the tendency to partition to the droplet surface. In addition, the concentration of available charge sites is lowest for folded lysozyme in comparison with cytochrome c and myoglobin.[5]

The ESI−MS measurements suggest that ion suppression occurs around 1−5 μM, rendering any quantitative ESI−MS measurements of protein mixtures above this concentration unreliable. The sensitivity to mass spectral tuning suggests that charge reduction alone can significantly affect the ability to perform quantitative measurement in ESI−MS. This experiment suggests that caution must be exercised when attempting to perform quantitative measurements of multicomponent protein solutions using ESI−MS and that an alternative approach will be required for complex solutions.

Laser Electrospray Ionization Mass Spectrometry of Equimolar Protein Mixtures. Laser electrospray mass spectrometry was performed on an equimolar protein mixture of myoglobin, cytochrome c and lysozyme to compare with the conventional ESI−MS measurements. Figure 3 displays the LEMS mass spectra for the equimolar protein mixture as a function of concentration. The charge state distribution does not shift to higher m/z with increasing solution concentration in the LEMS measurements, unlike the conventional ESI−MS measurements. The most intense molecular ion peaks from 50 μM to 1 mM in the LEMS myoglobin, cytochrome c and lysozyme charge state distributions are detected at m/z 847.5 $[M + 22H]^{22+}$, m/z 824.8 $[M + 15H]^{15+}$ and m/z 1591.3 $[M + 9H]^{9+}$, respectively. Comparison of the ESI−MS and LEMS

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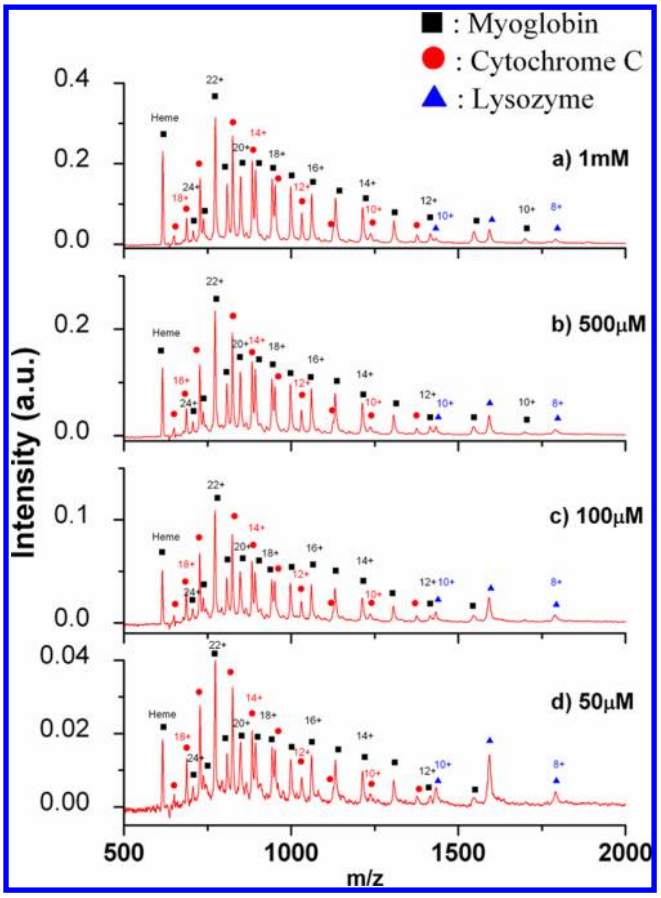

Figure 3. Positive ion LEMS mass spectra of different equimolar myoglobin, cytochrome c, and lysozyme solution concentrations: (a) 1 mM, (b) 500, (c) 100 and (d) 50 μM. The protein solutions were prepared in pure water and spotted on a metal sample plate in 10 μL aliquots. The electrospray consisted of water/methanol (1:1 v/v) and acetic acid (1% v/v).

charge state distributions as a function of concentration suggests that the droplet environments are different. The LEMS experiments reveal less charge reduction and significantly reduced ion suppression effects.

Figure 4 displays the integrated area of the LEMS mass spectra for each protein as a function of concentration in the equimolar solution. A monotonic increase in signal is observed for myoglobin, cytochrome c and lysozyme with increasing concentration. Like the ESI measurements, myoglobin dominates the mass spectral intensity, followed by cytochrome c and then lysozyme. The ratio of signal for myoglobin to cytochrome c is approximately 3:1, which is also similar to the ESI response. The LEMS measurement does not saturate and decrease as a function of increasing concentration, and the average and maximum charge state in the charge state distribution does not change as a function of concentration. In the data shown in Figure 3, the heme to myoglobin ratio remains relatively constant as a function of concentration, unlike the ESI–MS measurement where the heme to myoglobin ratio decreases by a factor of 3 with increasing concentration. Figure 1 shows that the heme is the largest feature in the mass spectrum at 0.5 mM and reduces significantly in relative intensity compared to the myoglobin features. Finally, the LEMS mass spectra exhibit little charge competition and suppression effects. These trends suggest that ion suppression effects are minimized in LEMS in comparison

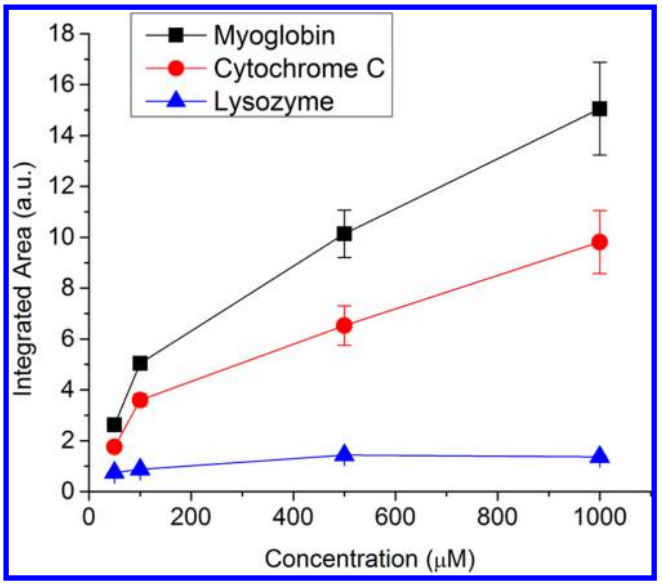

Figure 4. Integrated area of protein charge states as a function of solution concentration for LEMS equimolar measurements of myoglobin, cytochrome c and lysozyme.

with ESI–MS and that the LEMS measurements are quantitative.

The quantitative nature of lysozyme measurements using LEMS is evident when compared to traditional electrospray. The LEMS measurements show a small increase in lysozyme ion intensity as a function of increasing solution concentration, with a plateau in signal from 500 μM to 1 mM. The constant lysozyme charge state distribution and increasing ion signal as a function of concentration suggests that ion suppression and charge reduction effects are significantly reduced in comparison with conventional ESI–MS. The LEMS measurements suggest that charge deficiency is not an issue in this set of proteins.

Another reason for the enhanced quantitative nature of LEMS vs ESI–MS is that the LEMS charge state distribution for a given protein is constant for multicomponent samples. This indicates that the analyte concentration in the droplet does not cause ion suppression effects for the LEMS measurement. The LEMS mass spectra also reveal that the myoglobin $[M + 22H]^{22+}$ and cytochrome c $[M + 16H]^{16+}$ molecular ions at m/z 772.5 overlap similar to conventional electrospray measurements. However, the deconvolution is straightforward for the LEMS measurement because ion suppression and charge state reduction effects are not as significant. Using the procedure described for the ESI measurement, the contribution of each protein to the 772.5 feature can be obtained. The contribution of myoglobin $[M + 22H]^{22+}$ to the detected ion signal was approximately 35% for 50, 100, 500, and 1000 μM, therefore making the contribution of cytochrome c $[M + 16H]^{16+}$ to be approximately 65%.

The mechanism by which LEMS provides quantitative data in comparison to conventional electrospray mass spectrometry remains an open question. Previous work shows the LEMS vaporization method results in approximately 1% capture efficiency of the sample material in the electrospray plume.[30] This may result in lower concentration of protein in the droplet and thus greater charge availability with respect to the analyte concentration. In addition, previous work suggests that femtosecond laser vaporization into the electrospray droplets prevents equilibration of analyte partitioning between droplet

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surface and interior that occurs during conventional electrospray.[29] We propose that the reduced time analytes spend in the electrospray droplet during the LEMS process prevents partitioning into the interior of the droplet. In that event, a more hydrophilic analyte, such as lysozyme, would remain on the surface instead of partitioning to the inner phase of the droplet, promoting charging and thus detection. Such nonequilibrium partitioning would reduce ion suppression effects resulting in a more representative mass spectral response for otherwise low-surface-partitioning analytes in concentrated multicomponent solutions. This experiment cannot confirm whether nonequilibrium partitioning is the mechanism for diminished ion suppression in the LEMS experiments as reduction of analyte concentration in the electrospray droplet may also contribute to diminished ion suppression effects.[41] The femtosecond laser vaporization mechanism results in a more universal analysis of all components in solution and enables detection of both high and low surface activity proteins.

CONCLUSION

The ability to make quantitative measurements of all components in a mixture is important for mass spectral analysis of complex systems. Ion suppression effects in the electrospray ionization mass spectrometry experiment (ESI−MS) restrict the ability to perform quantitative measurement. We have demonstrated that quantitative measurements can be performed for equimolar protein mixtures of myoglobin, cytochrome c, and lysozyme with laser electrospray mass spectrometry. The LEMS measurements as a function of concentration reveal no evidence for charge reduction with the result that charge suppression effects are significantly reduced in comparison to ESI−MS. The mechanism for the enhanced quantitative capability for LEMS may be due to both the nonequilibrium nature of the partitioning of the proteins in the droplet and the reduced protein concentration in the droplet due to the ∼1% pickup of the vaporized material. The enhanced quantitative ability of LEMS is valuable for the analysis of complex samples like tissue and forensic samples where matrix effects present significant challenges for most mass spectral methods.

ASSOCIATED CONTENT

Supporting Information

Supplemental figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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