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Gas Phase Stabilization of Noncovalent Protein Complexes Formed by Electrospray Ionization

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The use of gas phase additives to stabilize noncovalent protein complexes in electrospray ionization mass spectrometry (ES-MS) is demonstrated for two protein−ligand interactions, an enzyme−small molecule inhibitor complex, and a protein−disaccharide complex. It is shown that the introduction of gas phase imidazole into the ES ion source effectively protects gas phase protein−ligand complexes against in-source dissociation. The stabilizing effect of imidazole vapor is comparable to that observed upon addition of imidazole to the ES solution. The introduction of sulfur hexafluoride, at high partial pressure, into the source region also effectively suppresses in-source dissociation of protein complexes. It is proposed that evaporative cooling is the primary mechanism responsible for the stabilizing effects observed for the gas phase additives.

The direct electrospray ionization mass spectrometry (ES-MS) assay has emerged as a valuable addition to the arsenal of analytical tools available for detecting specific protein−ligand interactions in solution and quantifying their thermodynamic properties.[1−3] The assay is based on the direct detection of free and ligand-bound protein ions by ES-MS. The association constant (K_a) for a given protein−ligand complex (PL) is determined from the ratio (R) of the total abundance (Ab) of bound and unbound protein ions (e.g., PL^{n+}, P^{n+}) measured in the gas phase by ES-MS for solutions of known initial concentrations of protein ($[P]_o$) and ligand ($[L]_o$), eqs 1 and 2.

$$K_a = \frac{R}{[L]_o - \frac{R}{1+R}[P]_o} \quad (1)$$

$$\frac{[PL]_{eq}}{[P]_{eq}} = \frac{\sum_n Ab(PL^{n+})}{\sum_n Ab(P^{n+})} = R \quad (2)$$

The assay has a number of attractive features, most notably its simplicity and specificity. There is no requirement for the labeling or immobilization of the protein or the ligand of interest, making the assay very versatile. Furthermore, it can provide direct insight into binding stoichiometry and can easily be used to study multiple binding equilibria simultaneously. The assay is also very rapid and the measurements can normally be completed in less than 1 min. When performed using nanoflow ES (nanoES), the ES-MS assay affords high sensitivity, normally consuming picomoles or less of analyte per analysis. Additionally, when carried out using a temperature-controlled ES device, it is also possible to estimate the protein−ligand binding enthalpy and entropy from the temperature dependence of the measured K_a values.[4,5]

The ES-MS assay has been used to quantify a variety of protein−ligand interactions with binding constants in the 10^3−10^8 M^{-1} range[1−3] and, in many instances, the measured affinities agree with values obtained by more established biophysical methods, such as isothermal titration calorimetry (ITC), surface plasmon resonance (SPR), and nuclear magnetic resonance (NMR) spectroscopy. Despite these many successful examples, there remain challenges to the general implementation of the ES-MS assay, among these are problems associated with the dissociation of the complexes in the gas phase. Gas phase dissociation during MS analysis, commonly referred to as in-source dissociation, artificially reduces the relative abundance of the ligand-bound protein ions and, consequently, leads to artificially low binding constants.[3] In the extreme case, in-source dissociation leads to false negatives, wherein no ligand-bound protein ions are detected by ES-MS.[6] The importance of in-source dissociation in the ES-MS measurements of protein−ligand complexes depends on the gas phase stability of the complex, which is influenced by the nature of the specific interactions in solution, and the configuration of the MS and the extent of collisional heating that ions are subjected to in the ES source. Complexes stabilized in solution predominantly by weak nonpolar interactions generally exhibit low gas phase stabilities.[7] However, the gas phase stabilities of protein−ligand complexes do not always parallel the solution binding affinities. For example Loo and co-workers recently demonstrated that the α-synuclein-spermine complex,

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which has a solution binding constant of 10^3 M^{-1}, shows enhanced stability in the gas phase due to a strengthened charge−charge interaction in vacuo.[8] In contrast, other protein−ligand complexes that are stabilized predominantly by ionic interactions in solution exhibit low gas phase stabilities and are prone to in-source dissociation.[6,9,10] One notable example is the complex of the serine protease trypsin (Tryp) and its competitive inhibitor benzamidine (Bnz). The (Tryp + Bnz) complex has binding constants (at pH 7 and 25 °C) of 2.0×10^4 and 4.5×10^4 M^{-1} that have been reported.[6] In solution, the (Tryp + Bnz) complex is believed to be stabilized predominantly by an ionic interaction, wherein the positively charged amidinium group interacts with the deprotonated carboxylic group of Asp189.[11] Based on differences in the gas phase acidity and basicity values (for the side chain of aspartic acid and benzamidine, respectively), it can be shown that the ionic interactions stabilizing the (Tryp + Bnz) complex in solution are thermodynamically unstable in the gas phase.[10] Consequently, desolvation is expected to result in the neutralization of the ionized groups and, possibly, lead to the formation of one or two stabilizing hydrogen bonds.[10] In the absence of strong stabilizing interactions (or many weak interactions), the protonated ions of the (Tryp + Bnz) complex formed by ES are relatively unstable and prone to dissociation in the ion source. As a result, it has proven difficult to avoid dissociating part or all of the complex ions during ES-MS analysis.[6,10]

Recently, our laboratory demonstrated that in-source dissociation of protein−ligand complexes in ES-MS analysis can be minimized by the addition of the small molecule imidazole, at relatively high concentrations (≥10 mM), to the ES solution. For example, in the case of the (Tryp + Bnz) complex, the binding constant determined in the presence of imidazole by direct ES-MS measurement was shown to be in good agreement with the literature values, thereby suggesting that in-source dissociation was significantly reduced or eliminated.[10] While the nature of the stabilizing effect(s) of imidazole has not been conclusively established, it may arise from enhanced evaporative cooling of the protein−ligand complex ions in the ion source. The enhanced cooling was proposed to arise from the loss of multiple imidazole adducts, bound nonspecifically to the protein ions through neutral or ionic hydrogen bonds, in the ion source. The enhanced evaporative cooling achieved through the loss of the imidazole adducts serves to offset the collisional heating that ions generally experience in the ES ion source. At the lower internal energies, the gaseous protein−ligand complex ions are sufficiently stable (kinetically) to survive until detection.

The goal of the present study was to investigate an alternative strategy for stabilizing noncovalent biological complexes formed by ES. Specifically, the benefit of introducing a stabilizing gas phase additive to the ES source was evaluated. Two different approaches to implementing this stabilization strategy were considered: one involving the addition of imidazole vapor and the second involving the addition of the relatively heavy and polarizable sulfur hexafluoride, SF_6. SF_6 has been previously used in ES-MS as an electron scavenger to prevent electrical discharge and, thereby, stabilize the electrospray using aqueous solutions.[12−16] However, to our knowledge, the utility of SF_6 for stabilizing noncovalent biological complexes in ES-MS has not been previously reported. Here, we demonstrate that both SF_6 and imidazole vapor protect noncovalent protein−ligand complexes from in-source dissociation in ES-MS analysis and offer stabilizing effects similar to those observed using imidazole as a solution additive. The utility of this general approach to stabilize biological complexes in ES-MS analysis is illustrated quantitatively for the Tryp−Bnz interaction, as well as a protein−disaccharide complex.

EXPERIMENTAL SECTION

Proteins and Ligands. Bovine pancreas trypsin, Tryp (MW 23 332 Da), and benzamidine hydrochloride (Bnz-HCl), were purchased from Sigma-Aldrich (St. Louis MO) and used without further purification. Imidazole was purchased from Hampton Research (Aliso Viejo, CA) or Sigma-Aldrich Canada (Oakville, Canada), and sulfur hexafluoride (SF_6) (99.99% purity) and Xenon (Xe) (99.999% Purity) were purchased from Airgas (Lakewood, CA) or Praxair (Edmonton, Canada). The disaccharide (Abe-(2-O-CH_3-Man)) was donated by D. R. Bundle (University of Alberta). The carbohydrate-binding antibody single chain fragment, scFv (MW 26 539 Da), was produced using recombinant technology.[17] The scFv was concentrated and dialyzed against deionized water using microconcentrators (Millipore Corp., Bedford, MA) with a molecular weight cutoff of 10 kDa and lyophilized. The scFv was weighed immediately after removing it from the lyophilizer, dissolved in a known volume of aqueous 50 mM ammonium acetate and stored at −20 °C until used. Lysozyme (MW 14 300 Da), which was used as a reference protein (P_{ref}) for some of the ES-MS binding measurements,[18] was purchased from Sigma-Aldrich Canada and used without further purification. The ES solutions were prepared from aqueous stock solutions of protein and ligand with known concentrations. Unless otherwise indicated, aqueous ammonium acetate was added to the ES solution to yield a final buffer concentration of 10 mM (pH 7). For experiments performed at Amgen (TOF and QTOF experiments), scFv was buffer exchanged into 50 mM ammonium acetate using a 10 kDa molecular weight cut off Vivaspin ultrafiltration concentrator (Sigma-Aldrich, St. Louis, MO); the final concentration of protein samples was estimated by measuring absorbance at 280 nm using a Nano Drop-1000 spectrophotometer (Thermo Scientific, Wilmington, DE).

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Mass Spectrometry. Mass spectra were measured using three different instruments, an Apex II 9.4 T Fourier-transform ion cyclotron resonance (FTICR) MS (Bruker, Billerica, MA), an ES-time-of-flight (TOF) MS (LCT Premiere. Waters Inc., Milford, MA), and a hybrid ion mobility quadrupole time-of-flight (QTOF) MS (Synapt, Waters Inc., Milford MA). A brief overview of the instrumental and experimental conditions used in this study is provided below.

FTICR Mass Spectrometry. NanoES was performed using borosilicate tubes (1.0 mm o.d., 0.68 mm i.d.), pulled to ~5 μm o.d. at one end using a P-2000 micropipet puller (Sutter Instruments, Novato, CA). The electric field required to spray the solution in positive ion mode was established by applying a voltage of ~800 V to a platinum wire inserted inside the glass tip. The solution flow rate was typically ~20 nL/min. Where indicated, SF_6 was introduced into the ion source, at flow rates of 3 or 5 L min^{-1}, using the custom built device shown in Figure S1; see the Supporting Information. The gaseous ions produced by nanoES were introduced into the mass spectrometer through a stainless steel capillary (i.d. 0.43 mm) maintained at an external temperature of 66 °C. The gas flow rate into the instrument was measured to be ~0.6 L min^{-1}. The ions sampled by the capillary (50 V) were transmitted through a skimmer (0 V) and accumulated electrodynamically in an rf hexapole. Ions were then ejected from the hexapole and accelerated (−2700 V) into the superconducting magnet, decelerated, and introduced into the ion cell. The trapping plates of the cell were maintained at a constant potential of 1.4 V throughout the experiment. The typical base pressure for the instrument was $\sim 5 \times 10^{-10}$ mbar. Data acquisition was controlled by an SGI R5000 computer running the Bruker Daltonics XMASS software, version 5.0. Mass spectra were obtained using standard experimental sequences with chirp broadband excitation. The time domain signal, consisting of the sum of 50−100 transients containing 128 K data points per transient, was subjected to one zero-fill prior to Fourier transformation.

TOF Mass Spectrometry. Positive ion nanoES mass spectra were acquired on two time-of-flight (TOF) instruments, a hybrid ion mobility quadrupole time-of-flight MS (Synapt, Waters Inc., Milford, MA) equipped with a nanoES source using metal coated borosilicate glass capillaries (nanoflow probe tips, long thin walled, Waters Corporation), and an ES-TOF MS (LCT Premiere, Waters Inc., Milford, MA) equipped with a chip based nanoES robot (Advion Triversa, Ithaca, NY). Solution flow rates of ~75 nL/min were used for all experiments. The source temperature was 45 °C, and the pressure of the vacuum/backing region of the TOF MS and QTOF MS were ~2.4 and ~5.0 mbar, respectively. Mass spectra were acquired from m/z 1000−5000 every 1 s. All data processing was performed using Igor Pro (WaveMetrics Inc., Lake Oswego, OR). Nitrogen, xenon, or sulfur hexafluoride were introduced as the cone/desolvation gas through the sheath cone assembly. The cone gas flow rate was set to 0.35 L min^{-1} in the MassLynx instrument control software (uncalibrated mass flow controller). When changing cone gases, the system was flushed with the new gas for at least 20 min prior to data acquisition. In order to introduce imidazole vapor into the source region of the QTOF instrument, the baffle in the nanoES source, which typically functions to sequentially sample the analyte and reference spray channel independently, was removed. Analyte ions were generated and introduced to the mass spectrometer with the analyte inlet in the usual way. Imidazole (1 mM solution) was introduced through the reference channel of the nanoES source using a modified probe held at ground potential with a sheath of heated nitrogen gas and a syringe pump at a flow rate of 0.5 μL min^{-1}.[19]

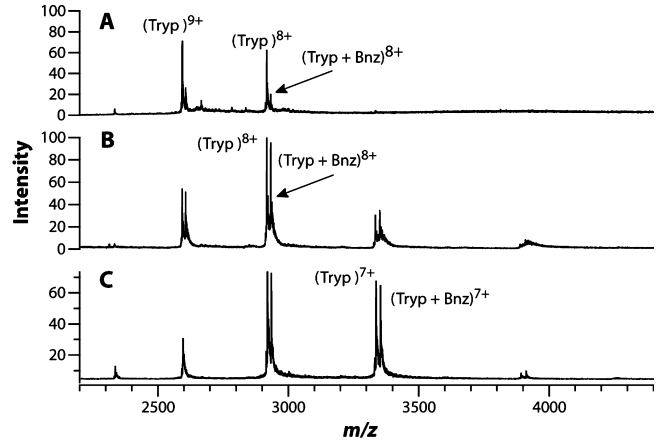

Figure 1. ES mass spectra acquired using a TOF MS for an aqueous solution (a) of 12 μM trypsin (Tryp) and 53 μM benzamidine (Bnz) in 10 mM ammonium acetate, (b) upon addition of 9 mM imidazole to the solution, and (c) upon introduction of gas phase imidazole into the ion source region.

RESULTS AND DISCUSSION

Stabilizing Effect of Gas Phase Imidazole. An ES mass spectrum acquired with the QTOF MS instrument for a solution of 12 μM Tryp and 53 μM Bnz in 10 mM aqueous ammonium acetate solution is shown in Figure 1a. The predominant ions observed in the spectrum correspond to protonated Tryp and the 1:1 complex with Bnz, i.e., $Tryp^{n+}$, and the $(Tryp + Bnz)^{n+}$, at $n = 8, 9$. Notably, the ratio of ligand-bound to free Tryp ions (R) determined from the mass spectrum is 0.32. The expected value, based on the initial concentrations of Tryp and Bnz and a K_a of 2×10^4 M^{-1}, is 0.94. The lower R value is attributed to extensive gas phase dissociation of the $(Tryp + Bnz)^{n+}$ ions, consistent with the findings of previous ES-MS studies.[6,10] It was recently demonstrated that using imidazole as a solution additive can stabilize the (Tryp + Bnz) complex in ES-MS measurements.[10] This stabilizing effect is illustrated in Figure 1b, which shows an ES mass spectrum, measured with the QTOF, for the same solution as described above but with the addition of 9 mM imidazole. The presence of imidazole resulted in a broadening of the charge state distribution, with ions at $n = 6-9$ detected. Notably, the addition of imidazole to the solution had a pronounced effect on the relative abundance of the $(Tryp + Bnz)^{n+}$ ions, and the measured R value of 0.95 is in excellent agreement with the expected value.

It has been proposed that the stabilizing effect of dissolved imidazole in ES-MS analysis of protein−ligand complexes arises from enhanced evaporative cooling of the protein ions, wherein the dissociation of nonspecifically bound imidazole molecules (formed during the ES process) cools the complex in the ion

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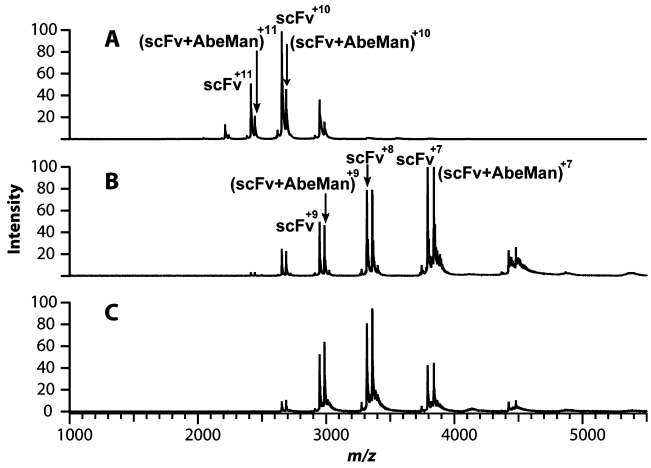

Figure 2. ES mass spectra acquired using a QTOF MS for an aqueous solution (a) of 7 μM scFv and 10 μM Abe(2-O-CH_3-Man) in 10 mM ammonium acetate, (b) upon addition of 9 mM imidazole to the solution, and (c) upon introduction of gas phase imidazole into the ion source region.

source and, thereby, reduces the rate of gas phase dissociation.[10] If stabilization is indeed due to enhanced evaporative cooling then a similar effect should be achieved when ES is performed in an environment containing a high partial pressure of imidazole. The gaseous imidazole molecules would be expected to solvate the gas phase protein ions in the high pressure region of the ion source. Subsequent desolvation of the protein ions will offset the effect of collisional heating and, thereby, stabilize the protein complexes. Figure 1c shows the ES mass spectrum of a solution of 12 μM Tryp and 53 μM Bnz with gas phase imidazole introduced into the source region of the TOF instrument. The spectrum is qualitatively very similar to that obtained with imidazole added directly to the ES solution (Figure 1b), although there is slightly more charge stripping occurring with imidazole introduced directly into the gas phase, presumably due to a higher partial pressure of imidazole in the gas phase. The relative abundance of the (Tryp + Bnz) complex determined from the mass spectrum shown in Figure 1c is 0.81. This value is in good agreement with the expected relative abundance in solution. Notably, the mass spectra shown in Figure 1 all contain the same number of averaged scans; the signal-to-noise ratios of the base peak in each spectrum are within 10% of each other. This demonstrates that there is minimal loss in sensitivity using imidazole as either a solution or gas phase additive.

To demonstrate that the stabilizing effect of imidazole vapor on labile complexes is general, ES-MS measurements were performed with the carbohydrate-binding antibody single chain fragment (scFv) which specifically binds the disaccharide Abe-(2-O-CH_3-Man). The binding constant for this interaction at neutral pH and 25 °C has been determined by ITC to be $(1.4 \pm 0.1) \times 10^5$ M^{-1}.[23] As discussed in detail elsewhere, protonated protein−carbohydrate complexes, particularly those involving small carbohydrate ligands, such as mono-, di-, or trisaccharides, are relatively labile in the gas phase and prone to in-source dissociation during ES-MS analysis.[3] Shown in Figure 2a is an illustrative ES QTOF mass spectrum acquired for an aqueous solution of 7 μM scFv and 10 μM Abe(2-O-CH_3-Man) in 10 mM ammonium acetate. The relative abundance of the (scFv + Abe(2-O-CH_3-Man)) complex (R = 0.39) is significantly smaller than the theoretical value (0.93). With the addition of 9 mM imidazole to the ES solution (Figure 2b), the observed charge states are shifted from 9−11 to 6−9. Similar charge stripping is also observed if imidazole vapor is introduced into the source region of the TOF (Figure 2c). The ions corresponding to the ligand-bound scFv complex also increase in intensity and R values of 0.92 and 1.12 are obtained, with addition of solution and gas phase imidazole, respectively. These values are in good agreement with the expected value suggesting that, in the absence of imidazole, a significant fraction of the (scFv + Abe(2-O-CH_3-Man))$^{n+}$ ions undergo in-source dissociation. The (scFv + Abe(2-O-CH_3-Man)) complex undergoes more extensive charge stripping compared to the Tryp/Benz complex. Because higher charge states are more susceptible to collisional dissociation, this charge stripping may play a role in stabilizing the complex. Since imidazole vapor was found to effectively protect both the (Tryp + Bnz) and (scFv + Abe(2-O-CH_3-Man)) complexes, despite differences in the extent of charge stripping, it is unlikely that the reduction in charge state represents the dominant mechanism of complex stabilization. Additionally, as shown below, SF_6 provides similar complex stabilization, without charge stripping the ions. For the 7+ and 6+ charge states, additional small intensity peaks are observed corresponding to imidazole adducts on the scFv^{n+} and (scFv + Abe(2-O-CH_3-Man))$^{n+}$ ions. The direct observation of these imidazole adducts provides additional evidence that imidazole molecules can "solvate" the gas phase protein ions in the high pressure region of the ES ion source. These adducts appear to be very weakly bound as they readily dissociate if the skimmer voltage is increased.

The results for the (Tryp + Bnz) and (scFv + Abe(2-O-CH_3-Man)) complexes demonstrate that a high background pressure of imidazole in the ES ion source offers similar protection to labile noncovalent protein complexes as dissolved imidazole. These results also represent further evidence that the stabilization of the protein complexes in ES-MS by dissolved imidazole is, at least in part, a gas phase effect. It is also interesting to note that similar results, demonstrating the stabilizing effect of a gas phase additive, were observed upon introduction of diethylamine to the ion source (data not shown). We are currently investigating the nature of the gas phase interactions formed between additives such as imidazole and proteins and their kinetic and thermodynamics properties.

Stabilizing Effect of SF_6. As noted above, the stabilizing effect of imidazole, whether it is introduced in solution or in the gas phase, likely arises from the dissociation of nonspecific intermolecular interactions between imidazole molecules and the protein ions. However, due to the higher mass of imidazole, compared to that of N_2 or air, which are normally used as the cone/desolvation gas, it is also possible that collisional (translational) cooling of the protein ions in the source contributes to the stabilizing effect observed with gas phase imidazole.[20−22] To

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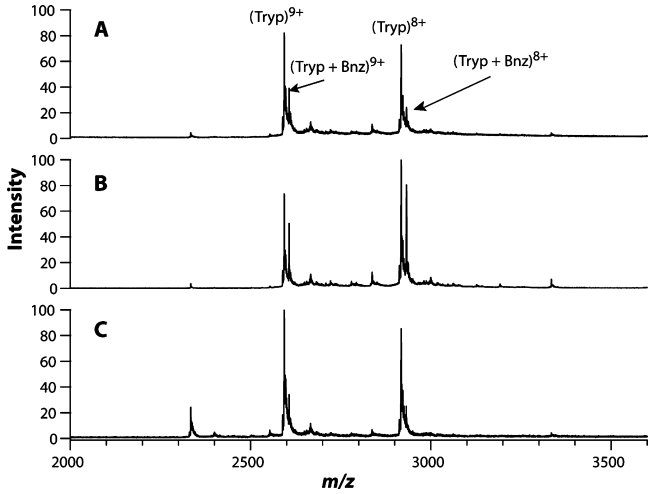

Figure 3. ES mass spectra acquired using a QTOF MS for an aqueous solution of 12 μM trypsin (Tryp) and 53 μM benzamidine (Bnz) in 10 mM ammonium acetate using (a) N_2, (b) SF_6, or (c) Xe as the cone gas.

investigate this possibility, ES-MS measurements were performed on the solution of Tryp and Bnz using three different cone/desolvation gases. Shown in Figure 3a–c are mass spectra acquired under identical conditions but using N_2 (Figure 3a), SF_6 (Figure 3b), and Xe (Figure 3c) as the cone gas. Notably, similar R values, ~0.2, were determined using either N_2 or Xe. However, a significantly larger R value, 0.69, was measured using SF_6. These results clearly demonstrate that SF_6 can effectively reduce the extent of in-source dissociation of the (Tryp + Bnz)$^{n+}$ ions, although to a slightly lesser extent than was observed with dissolved or gas phase imidazole. Since the atomic/molecular weights of Xe and SF_6 are similar, the present results suggest that the stabilizing effect of SF_6 is not due simply to more efficient collisional cooling with a heavy bath gas and that intermolecular interactions also play a role. Experiments to confirm this hypothesis are currently underway in our laboratories.

Similar results were also obtained for the (scFv + Abe(2-O-CH_3-Man)) complex as illustrated in Figure 4a,b which shows QTOF mass spectra acquired using N_2 (Figure 4a) and SF_6 (Figure 4b) as the cone gas (gas flow rate of 0.35 L min^{-1}). The introduction of SF_6 resulted in a marked increase in the relative abundance of the protein disaccharide complex; the relative abundance of the (scFv + Abe(2-O-CH_3-Man)) complex determined from the mass spectrum shown in Figure 4b is 0.84, in good agreement with the theoretical value.

The Waters TOF mass spectrometers used in these studies are equipped with "z-spray" ionization sources in which the skimmer orifice is orthogonal to the nanospray and analyte ions follow a flattened Z shape. In order to investigate if SF_6 provides the same stabilizing effect in instruments equipped with a conventional "line of sight" ES inlet system, experiments were also performed on a Bruker ApexII FTICR MS. Shown in Figure 4c is an illustrative ES FTICR mass spectrum of 10 μM scFv and 10 μM Abe(2-O-CH_3-Man) in 10 mM ammonium acetate acquired using a hexapole accumulation time of 4 s. To test the stabilizing effect of SF_6 on the FTICR instrument,

(23) Bundle, D. R. unpublished results.

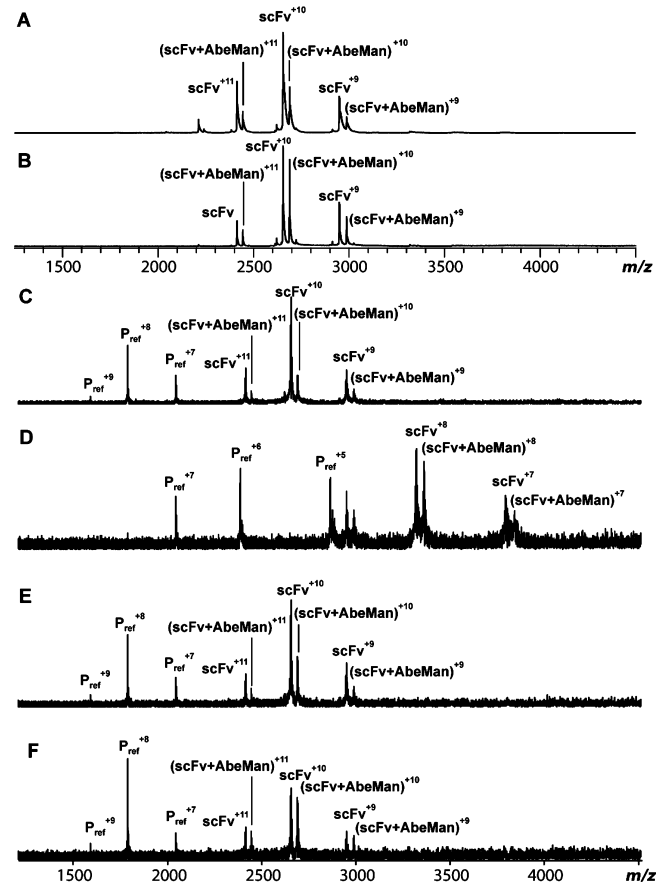

Figure 4. ES mass spectra acquired by (a and b) QTOF MS for an aqueous solution of 7 μM scFv and 10 μM Abe(2-O-CH_3-Man) in 10 mM ammonium acetate using (a) N_2 and (b) SF_6 as the cone gas (0.35 L min^{-1} flow rate). (c–f) FTICR mass spectra for an aqueous solution (c) of 10 μM scFv and 10 μM Abe(2-O-CH_3-Man) in 10 mM ammonium acetate, (d) upon addition of 9 mM imidazole to the solution, (e) using SF_6 at flow rate of 3 L min^{-1}, and (f) using SF_6 at flow rate of 5 L min^{-1}. For spectra c–f, 5 μM lysozyme (P_{ref}) was added to the solution as a reference protein to monitor for nonspecific protein–ligand complex formation during the ES process.

ES-MS measurements were performed in the presence of SF_6, which was introduced into the source region at flow rates of 3 or 5 L min^{-1} using the device shown in Figure S1 (see the Supporting Information). Lysozyme, which does not specifically bind to the solution components, was added to the solution as a reference protein (P_{ref}) to monitor nonspecific carbohydrate–protein binding during the ES process.[18] From the illustrative mass spectra shown in Figure 4e,f, it can be seen that the introduction of SF_6 resulted in a measurable increase in the relative abundance of complex. Notably, the stabilizing effect increases with increasing flow rate, with an R value of 0.78 achieved at 5 L min^{-1}, in excellent agreement with the theoretical value of 0.79. This value is, within the precision of the measurements, identical to the value obtained in the presence of dissolved imidazole (Figure 4d). As shown previously, collisional heating of the gaseous ions is most significant during accumulation in the rf hexapole of the FTICR MS used for the present measurements.[3] Shown in Figure 5 is a plot of R values measured using a single nanoES tip for the same solution of scFv and Abe(2-O-CH_3-Man) in the absence and presence of SF_6. Notably, in the absence of SF_6, the complex is nearly

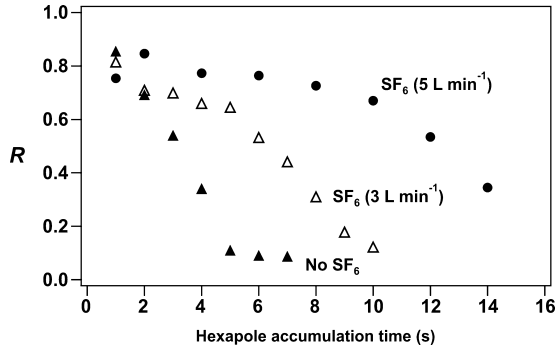

Figure 5. Influence of hexapole accumulation time on the *R* value determined from ES mass spectra acquired by FTICR MS for an aqueous solution of 10 μM scFv and 10 μM Abe(2-O-CH_3-Man) in 10 mM ammonium acetate.

completely dissociated at accumulation times greater than 4 s. However, in the presence of SF_6, ions of the complex can be detected using significantly longer accumulation times. For example, at a SF_6 flow rate of 5 L min^{-1}, the complex is detected at accumulation times as long as 14 s.

The stabilizing effect of SF_6 was also tested on the FTICR for ES-MS analysis of the (Tryp + Bnz) complex. Importantly, the introduction of SF_6 at high partial pressures (~5 L min^{-1}) led to a ~50% increase in the measured *R* value (from 0.21 (no SF_6) to 0.31 (with SF_6)). The stabilizing effect of SF_6 for the (Tryp + Bnz) complex is not as dramatic as observed on the QTOF instrument. This is presumably due to the low kinetic stabilities of the $(Tryp + Bnz)^{n+}$ ions and the significantly longer time scale of the FTICR MS experiment (seconds) compared to the TOF experiment (milliseconds). These results nevertheless confirm the stabilizing effect of SF_6 with the FTICR MS.

Taken together, the results obtained for the (Tryp + Bnz) and the (scFv + Abe(2-O-CH_3-Man)) complexes demonstrate that a high background pressure of SF_6 can serve to stabilize labile noncovalent protein complexes in ES-MS analysis. Furthermore, this method is shown to be compatible with two very different MS instrument configurations.

CONCLUSIONS

The results of the present study demonstrate that gas phase additives, such as imidazole or SF_6, when introduced into the ion source at high partial pressures can stabilize noncovalent protein−ligand complexes in ES-MS. The effectiveness of the gas phase additives, which was demonstrated for an enzyme−small molecule inhibitor complex and a protein−disaccharide complex, was found to be comparable to that achieved using imidazole as a solution additive. The main advantage of gas phase additive approach, compared to the use of a solution additive, is that it will not lead to changes in protein−ligand interactions due to changes in solution composition. This technique should be relatively easy to implement on a variety of MS instruments, and it has great potential to simplify the application of ES-MS for the characterization of weakly bound protein−ligand complexes.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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