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Electrospray Ionization High-Field Asymmetric Waveform Ion Mobility Spectrometry−Mass Spectrometry

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High-field asymmetric waveform ion mobility spectrometry (FAIMS) is a new technique that separates gas-phase ions at atmospheric pressure (760 Torr) and room temperature. A FAIMS instrument acts as an ion filter and can be set to continuously transmit one type of ion. Despite the stringent requirement for a flow of clean, dry gas in the FAIMS analyzer region, a method of coupling electrospray to FAIMS has been developed. The identity of the electrospray ions separated by FAIMS was determined using mass spectrometry (FAIMS−MS). The theory of FAIMS is discussed, and electrospray FAIMS−MS spectra of several compounds in modes P1, P2, N1, and N2 are presented. Ions appearing in P1 and N1 modes tend to have mobilities that increase as a function of increasing electric field strength, whereas ions appearing in P2 and N2 modes tend to have mobilities that decrease. In general, low-mass ions are focused in P1 and N1 modes, whereas larger ions (e.g., proteins) are focused in P2 and N2 modes. Short-chain peptides, $(Gly)_n$ where $n = 1-6$, are shown to cross over from P1 mode into P2 mode as the chain length increases. The removal of the low-mass solvent cluster ions, combined with a reduction of the background noise in electrospray FAIMS−MS, results in an improved signal-to-noise ratio for mass spectra of larger ions (e.g., cyctochrome *c*) when compared with conventional electrospray-MS. Preliminary results also suggest that various charge states of cytochrome *c* can be distinguished by FAIMS, implying that the ion mobility of these species at high electric field strength is sensitive to the structure of the protein ion. The linearity of response of electrospray FAIMS−MS was investigated using leucine enkephalin and shows the calibration curve to be linear for ~3 orders of magnitude.

Electrospray ionization, originally developed through the pioneering efforts of Dole,[1,2] Iribarne and Thomson,[3,4] and Fenn [5−7] has become a widely used and convenient means to generate atmospheric pressure, gas-phase ion populations of large hydrophilic compounds, including proteins. These ions are generally sampled into the vacuum system of a mass spectrometer; however, some advantages of ion analysis via high-pressure techniques, including ion mobility, have been realized.[8−14] Hill[12,13] reported the separation of charge states of proteins at atmospheric pressure in an electrospray high-resolution ion mobility spectrometer. These results confirm earlier observations that the charge states of protein ions have cross-sectional areas that are a function of charge and that the cross-sectional areas increase with increasing charge state.[14] This observation is presumably due to internal Coulombic repulsion forces that extend the conformation of an ion. Clemmer and Jarrold reported the separation of the gas-phase conformations of proteins using ion mobility, followed by quadrupole[15−17] or time-of-flight mass spectrometry.[18] Using a low-pressure (2−5 Torr) drift cell, Clemmer and Jarrold[16] were able to separate several conformers within individual charge states (e.g., +8) of cytochrome *c*.

High-field asymmetric waveform ion mobility spectrometry (FAIMS) [19−24] offers a new atmospheric pressure technique with

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which to study electrospray ions. This study describes the coupling of electrospray to FAIMS-E (electrometer detection) and to FAIMS−MS. Results collected for several analytes using these techniques are shown. As well, the potential for both analytical applications and fundamental studies of electrospray generated ions is discussed.

THEORY OF HIGH-FIELD ASYMMETRIC WAVEFORM ION MOBILITY SPECTROMETRY

FAIMS is a technique recently developed[19−24] to separate ions at atmospheric pressure (760 Torr), and room temperature. FAIMS can be compared with conventional ion mobility spectrometry (IMS) since both techniques are based on the motion of ions induced by electric fields at atmospheric pressure. A conventional IMS[25,26] is a chamber housing a series of metal plates to which incrementally decreasing (or increasing) dc voltages are applied (i.e., forming a drift tube). The electric field produced by this set of electrodes is designed to be as uniform as possible.[12,13] Ions are gated into the drift tube using a shutter grid assembly and are subsequently separated because of differences in their drift velocities. The ion drift velocity is proportional to the field strength at low electric fields (e.g., 200 V/cm). Thus, the ion mobility, K, which is determined from this experiment, is independent of the strength of the applied electric field.

At high electric fields (e.g., 10 000 V/cm), the ion drift velocity is no longer directly proportional to the applied field. K becomes dependent on the applied electric field[25,26] and is better represented by K_h, a nonconstant, high-field, mobility term. This dependence of K_h on the applied electric field has been the basis for the development of FAIMS, previously referred to as transverse field compensation ion mobility spectrometry[20] and field ion spectrometry.[20−22,27] The high-field behavior of K_h is compound-dependent, which makes it possible for FAIMS to separate ions on the basis of the difference in the mobility of an ion at high field relative to its mobility at low field. The change in mobility at high electric field appears to reflect the size of the ion, its interaction with the bath gas, and its structural rigidity.

The principles of operation of FAIMS have been described by Buryakov et al.[19] and are briefly summarized here. The mobility of a given ion under the influence of an electric field can be expressed by[19,25] $K_h(E) = K[1+f(E)]$, where $f(E)$ describes the functional dependence of ion mobility on the electric field. Of the virtually unlimited number of possibilities for $f(E)$, Figure 1 illustrates three changes in ion mobility, as a function of electric field, that we have observed. In this figure, the mobility of a type A ion increases with increasing electric field strength, a type C ion decreases, and a type B ion increases initially before decreasing at yet higher fields. The emphasis of this paper is on ions of types A and C, since, with few exceptions, ions we have studied can be classified into one of these two types.

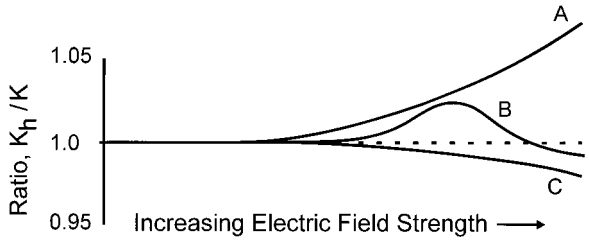

Figure 1. Hypothetical dependence of ion mobility on electric field strength for three different types of ions.

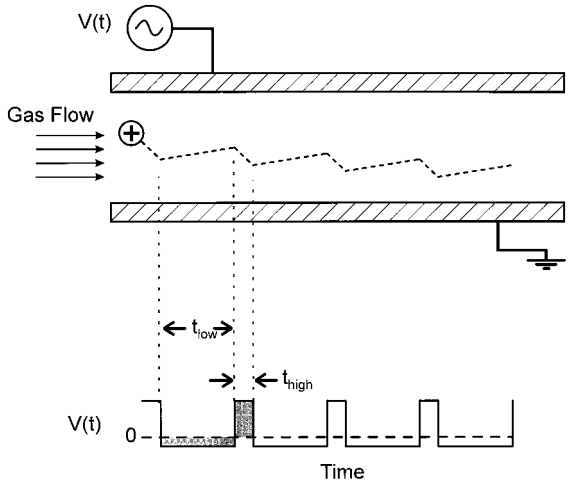

Figure 2. Illustration of the ion motion between two parallel plates during application of an electric potential shown as $V(t)$. The ion is transported horizontally by a gas flow (distance not to scale).

Consider an ion, for example type A in Figure 1, being carried by a gas stream between two parallel plates, as shown in Figure 2. The lower plate is maintained at ground potential while the upper plate has a (simplified) asymmetric waveform, described by $V(t)$, applied to it. The asymmetric waveform is composed of a high-voltage component, V_{high}, lasting for a short period of time, t_{high}, and a low voltage component, V_{low}, of opposite polarity, lasting a longer period of time, t_{low}. The waveform is synthesized such that the integrated voltage−time product (thus the field−time product) applied to the upper plate during a complete cycle of this waveform is zero (i.e., $V_{high}t_{high} + V_{low}t_{low} = 0$), for example, +2000 V for 10 μs followed by −1000 V for 20 μs. During the high-voltage portion of the waveform, the field will cause the ion to move with velocity $v_1 = K_hE_{high}$, where E_{high} is the applied electric field. The distance traveled will be $d_1 = v_1t_{high} = K_hE_{high}t_{high}$. During the longer duration, opposite-polarity, low-voltage portion of the waveform, the velocity of the ion will be $v_2 = KE_{low}$. Thus, the distance traveled is $d_2 = v_2t_{low} = KE_{low}t_{low}$. Since the asymmetric waveform ensures that $V_{high}t_{high} + V_{low}t_{low} = 0$, the field−time products $E_{high}t_{high}$ and $E_{low}t_{low}$ are equal in magnitude. If K_h and K are identical, d_1 and d_2 are equal, and the ion will be returned to its original position relative to the plates after one complete cycle of the waveform, just as would be expected if both portions of the waveform were low voltage. However, if E_{high} is sufficient to cause $K_h > K$, the ion will experience a net displacement from its original position relative to the plates because the distances d_1 and d_2 are not identical. This is illustrated

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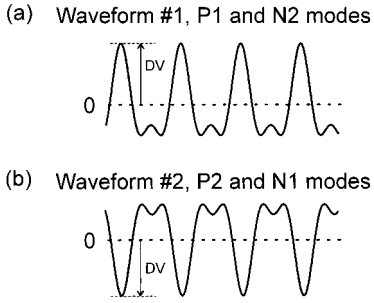

Figure 3. Asymmetric waveforms used in FAIMS. The maximum value of the waveform is called the dispersion voltage (DV).

by the dashed line in Figure 2, which shows the trajectory of a positive type A ion (Figure 1), when $K_h > K$. That is, a type A ion will travel further during the positive portion of the waveform ($d_1 > d_2$), and will migrate toward the lower plate. For similar reasons, positive type C ions migrate toward the upper plate. Positive type B ions migrate in a direction that depends on the magnitude of the field, since K_h is higher than K at moderate field, but becomes lower than K at very high field. Examples of type A and C ions will be shown in this study.

If a type A ion is migrating away from the upper plate, a constant negative dc voltage, called the "compensation voltage" (CV), can be applied to this plate to reverse or "compensate" for the offset drift. Thus, the ion will not travel toward either plate. If the ions derived from two compounds respond differently to the applied high electric field, the ratio of K_h to K is different for each compound. Consequently, the magnitude of the CV necessary to prevent ion drift toward either plate will also be different for each compound. Under conditions in which the CV is appropriate for transmission of one compound, the other will drift toward one of the plates and be lost. Hence, the FAIMS instrument is an ion filter, capable of selective transmission of only those ions with the appropriate ratio of K_h to K. To separate a mixture of ions, the CV can be scanned to yield a compensation voltage spectrum (CV spectrum).

The concept described above was first shown by Buryakov et al. [19] using flat plates. Later, Carnahan et al. improved the design by replacing the flat plates with concentric cylinders.[27,28] This modification has several advantages including higher sensitivity than the flat plate configuration. This instrument has been introduced by Mine Safety Appliances Co. for trace gas analysis and is called a "Field Ion Spectrometer" (FIS©). This instrument, with electrometer-based ion sensing, is referred to as FAIMS-E in this study. The FAIMS-E is capable of separating compounds having the same low-field mobility constant.[20] A major limitation with this recent technology[20,21,27] was that several of the peaks appearing in FAIMS-E CV spectra could not be unambiguously identified. In earlier reports,[22,23] we described a FAIMS interface to a mass spectrometer (FAIMS−MS) which allowed for peak identification.

The evaluation of the performance of FAIMS-E and FAIMS−MS revealed several unexpected results.[23] Figure 3 shows two asymmetric waveforms that are used in FAIMS, where waveform 2 is simply the result of reversing the polarity of waveform 1. The maximum value of the waveform, called the dispersion voltage (DV), is indicated on the figure. It was expected that a reversal of the polarity of the asymmetric waveform would result in the reversal of the polarity of the CV necessary to pass an ion through the FAIMS analyzer. Experimentally, much lower transmission of an ion, if any, was observed when the polarity of the CV was reversed.[23] This result created the need to consider the asymmetric waveform polarity as two special cases that can be used with either positive (P) or negative (N) ions. The waveform with positive DV (Figure 3a) yields spectra of types P1 and N2, whereas the reversed polarity waveform (Figure 3b) yields P2 and N1 type spectra.

Another unexpected result[23] was that the sensitivity of ion transmission increased substantially when DV was increased. This result was noted in earlier discussions of FAIMS.[20−22,27] The FAIMS instrument was expected to act as an ion "filter", with the capability of selectively transmitting one type of ion out of a mixture. With no voltages applied to the FAIMS analyzer, the optimum transmission for every ion was expected, albeit without any separation. The conditions designed to yield ion separation were expected to decrease ion transmission because of increased ion loss due to collisions with the walls during application of the high-voltage periods of the waveform (i.e., effectively narrowing the width of the analyzer region). Contrary to this prediction, the ion current increased as the DV was increased.[23] The unusual observations noted above were the result of atmospheric pressure ion focusing that occurs in the cylindrical FAIMS analyzer.[22−24] The physics behind this mechanism have been described in detail elsewhere.[24] Briefly, ions that are focused in modes P1 and N1 tend to have mobilities that increase with increasing electric field (type A, Figure 1), while the ions that are focused in modes P2 and N2 tend to have ion mobilities that decrease with increasing electric field (type C, Figure 1). Type B ions (Figure 1) may appear in different spectra under different conditions of electric field strength.

EXPERIMENTAL SECTION

Figure 4 shows the two FAIMS instruments that were used in this study. Figure 4a shows a three-dimensional view of FAIMS with electrometer-based detection (FAIMS-E), while Figure 4b shows a cross-sectional view of a FAIMS instrument with mass spectrometry-based detection (FAIMS−MS). Electrospray ionization was used in both FAIMS-E and FAIMS−MS studies, in place of the UV lamp or ^{63}Ni source used with FIS instruments. The electrospray needle and associated liquid delivery system were constructed by threading a 30 cm piece of fused-silica capillary (50 μm i.d., 180 μm o.d.) through a 5 cm long stainless steel capillary (200 μm i.d., 430 μm o.d). The fused silica protruded ~1 mm beyond the end of the stainless steel. This stainless steel capillary, in turn, protruded ~5 mm beyond the end of a larger stainless steel capillary (500 μm i.d., 1.6 mm o.d.) that was used for structural support and application of the high voltage. Solutions were pumped to the electrospray needle tip by a Harvard Apparatus model 22 syringe pump, at a flow rate of 1 μL/min. For generating positive ions, the needle was held at +2200 V, giving an electrospray current of ~0.03 μA. For negative ion generation, the needle was held at −2000 V, giving an electrospray current of about −0.05 μA.

The FAIMS ion filter was composed of two short inner cylinders, which were axially aligned and positioned 5 mm apart,

(28) Carnahan, B. L.; Tarassov, A. S. U.S. Patent 5420424, 1995.

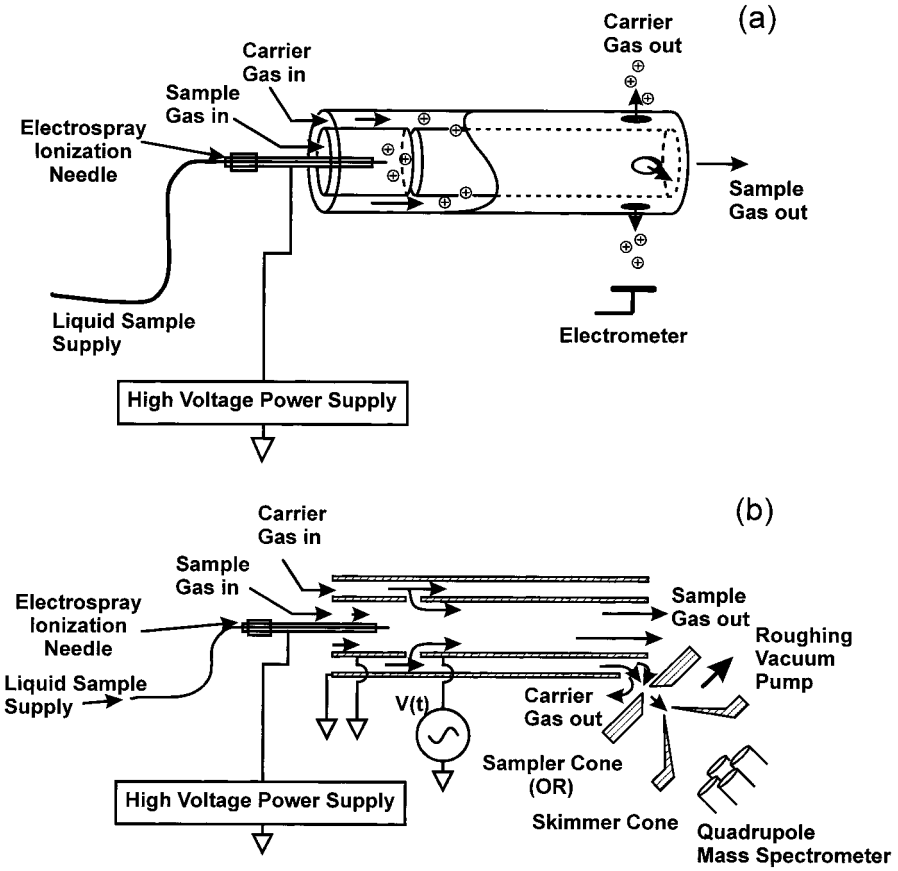

Figure 4. Schematic of the two electrospray FAIMS instruments used in this study. (a) three-dimensional view of FAIMS-E; (b) cross-sectional view of the FAIMS−MS.

and a long outer cylinder that surrounded the two inner cylinders. The inner cylinders (12 mm i.d., 14 mm o.d.), were about 30 and 90 mm long, respectively, while the outer cylinder (18 mm i.d., 20 mm o.d.) was ∼125 mm long. The outer cylinder and the shorter inner cylinder of the FAIMS instrument were held at the same electrical potential. The longer inner cylinder had the high-frequency (210 kHz), high-voltage (up to 4950 V_{p-p}), asymmetric waveform (Figure 3) applied to it, thereby establishing the electric field between the inner and outer tubes. In addition to this high-frequency waveform, the CV was also applied to the long inner cylinder. The electrospray needle was placed on the center axis of the shorter inner cylinder, terminating ∼5 mm short of the gap between the two inner cylinders. The electrospray ions were driven radially outward by the electric field to the analyzer region through the 5 mm gap between the two inner cylinders.

Gas connections to both FAIMS instruments are shown in Figure 4. Compressed air (for negative electrospray ionization) or nitrogen gas (for positive electrospray ionization) was passed through a charcoal/molecular sieve gas purification cylinder and introduced into the FAIMS device. In general, gas was introduced through the Carrier In (C_{in}) and/or Sample In (S_{in}) ports, and exited via the Carrier Out (C_{out}) and/or Sample Out (S_{out}) ports. All four flows could be adjusted ($C_{in} + S_{in} = C_{out} + S_{out}$). In this study, $S_{in}$was plugged and the gas was introduced entirely through C_{in} at a flow rate of 3 (FAIMS-E) or 6 L/min (FAIMS−MS). The gas exited through S_{out} at 1 L/min (both instruments) and through C_{out} at 2 (FAIMS-E) or 5 L/min (FAIMS−MS). A fraction of C_{in} was directed radially inward through the 5-mm gap between the inner cylinders, and acted as a curtain gas. While the ions formed by electrospray were driven radially outward through the gap by the electric field, the curtain gas prevented neutrals from entering the annular analyzer region. This portion of C_{in}, along with the neutrals, exited the FAIMS device via the S_{out} port.

The gas stream carried the electrospray ions along the length of the annular space between the outer cylinder and the long inner cylinder. If the combination of DV and CV was appropriate for ion transmission, four openings (each ∼2 mm in diameter) near the end of the outer cylinder allowed the gas to carry ions to the electrometer (FAIMS-E). In the FAIMS−MS, there was only one opening that allowed the ions to travel from the FAIMS analyzer into the orifice of the mass spectrometer. For either instrument, ion abundance was measured as a function of CV and displayed as a "CV spectrum".

For FAIMS−MS experiments, a custom-built interface was constructed for a PE Sciex API 300 triple-quadrupole mass spectrometer. Ions separated via FAIMS were transferred to the vacuum chamber of the mass spectrometer through a "sampler cone" placed at the end of the FAIMS at a 45° angle relative to the axis of the FAIMS cylinders, as is shown schematically in Figure 4b. The diameter of the orifice in the sampler cone is ∼250 μm. The sampler cone was electrically insulated so that a separate voltage (OR) could be applied to it. A voltage was also applied to the entire FAIMS unit (V_{FAIMS}) for the purpose of enhancing the sensitivity of the FAIMS−MS. For the electrospray FAIMS−MS analysis of proteins in positive (negative) mode, V_{FAIMS} and OR were both set to ∼50 (−50) V unless otherwise indicated. When

looking at smaller species (i.e., m/z < 1000), V_{FAIMS} and OR were both set to about 15 (−15) V. The skimmer cone of the API 300 remained at ground potential for all of the experiments described herein. The small ring electrode located behind the orifice of the conventional API 300 interface was not incorporated into the new design, resulting in some loss of sensitivity, most notably for low m/z ions.

Ion-selected CV spectra (IS-CV spectra) were obtained by scanning the CV applied to the FAIMS, while monitoring a single m/z value. "Total ion current" CV spectra (TIC-CV spectra) show the sum of the signal for all detected ions in a given m/z range as CV was scanned. If the total ion current was low, the resolution of the mass spectrometer was lowered to produce so-called "low-resolution TIC-CV spectra". Mass spectra collected at fixed values of DV and CV revealed the identity of any ions transmitted through the FAIMS under those conditions**.** When the identity of an ion was ambiguous based on its m/z value alone, MS/MS experiments were carried out.

RESULTS AND DISCUSSION

The FAIMS instrument can be operated in four modes: P1, P2, N1, and N2. As noted earlier, P and N describe the ion polarity, while 1 and 2 are indicative of the instrumental conditions (Figure 3). These modes have been studied using both FAIMS-E and FAIMS−MS, with the emphasis in this study being on FAIMS−MS.

P1 Mode. For the collection of spectra in P1 mode, the asymmetric waveform is of positive polarity (Figure 3a). Figure 5 illustrates the P1 mode electrospray FAIMS−MS spectra of a 50:50:0.1 methanol/water/acetic acid (v/v/v) solution. Figure 5a shows a TIC-CV spectrum obtained by monitoring the ions from m/z 30 to 300 during a CV scan. Only the negative portion of the CV scan is shown because ion transmission in the FAIMS is significantly reduced due to defocusing at positive CV values.[24] Mass spectra, collected with the FAIMS operating continuously at CV values of −8.1, −6.6, and −4.3 V, are shown in Figure 5b−d, respectively. Unlike a conventional IMS, the FAIMS analyzer acts like a filter and transmits a continuous ion beam consisting only of the ions being transmitted at a single CV. These ions are easily mass analyzed by the quadrupole mass spectrometer using slow scans and, if necessary, averaging of multiple scans. The spectra in Figure 5b−d each represent an average of 10 repeat scanning acquisitions lasting 5 s and covering m/z 30−300. No ions with m/z 300−3000 were observed in a separate experiment using the same solution. The ions that dominated the electrospray FAIMS−MS spectrum of the methanol/water/acetic acid solution in P1 mode included sodium-containing ions such as $(CH_3OH)Na^+$ (m/z 55), shown in Figure 5b, and a protonated methanol dimer (m/z 65), Figure 5c. Other species appearing as low-intensity peaks in the TIC-CV spectrum were also present. For example, at CV = −4.3 V, the dominant ion is m/z 83, as shown in Figure 5d. This ion has several plausible structures and illustrates a situation where MS/MS was used to determine the identity of the ion. MS/MS of m/z 83 produced a CID product ion at m/z 23, suggesting that m/z 83 was $(CH_3COOH)Na^+$.

Figure 6 shows the electrospray FAIMS−MS spectra of a solution of 10 μM CsCl in 50:50:0.1 methanol/water/acetic acid (v/v/v). The upper trace is the TIC-CV spectrum (m/z 30−300),

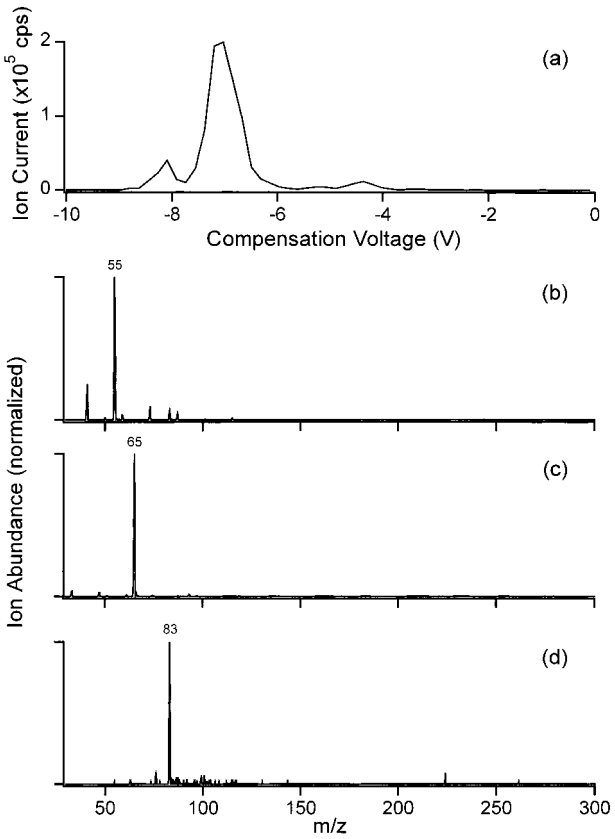

Figure 5. CV scan with MS detection and corresponding mass spectra collected at fixed CV values for a 50:50:0.1 methanol/water/acetic acid (v/v/v) solution. P1 mode, (a) TIC-CV spectrum (m/z 30−300). Mass spectra were collected at (b) CV = −8.1 V, (c) CV = −6.6 V, and (d) CV = −4.3 V. DV = 2200 V.

while the lower traces are the mass spectra of the three major peaks in Figure 6a. Figure 6b shows the mass spectrum collected at CV = −8.1 V. Several solvent−Na^+ adducts were identified, including $(CH_3OH)Na^+$ (m/z 55), $(CH_3OH)_2Na^+$ (m/z 87), $(CH_3OH)(H_2O)Na^+$ (m/z 73), $(H_2O)Na^+$ (m/z 41), and $(H_2O)_2Na^+$ (m/z 59). Figure 6c shows that the major peak occurring at CV = −6.6 V was due primarily to methanol clusters, $(CH_3OH)_2H^+$ (m/z 65) and $(CH_3OH)_3H^+$ (m/z 97). The peak at CV = −4.9 V was from Cs present in solution as shown in Figure 6d, where the peaks in the mass spectrum were identified as Cs^+ (m/z 133), $Cs^+(H_2O)$ (m/z 151), and $Cs^+(CH_3OH)$ (m/z 165). Because of the expansion/declustering process that occurred when ions were transmitted into the mass spectrometer, the degree of solvation of the ions in the mass spectrum cannot be assumed to reflect the degree of solvation of those ions being transmitted through the FAIMS device. The adjustment of various mass spectrometer interface voltages can affect the apparent degree of solvation. The changes in the degree of solvation are illustrated by comparing the mass spectra of the background ions in Figure 6a and b with those in Figure 5a and b, respectively. Much gentler conditions in the interface region of the mass spectrometer were used in collecting the mass spectra in Figure 6, and as a result, more solvent-cluster peaks are observed than in Figure 5. Nevertheless, there is no doubt that the ions transmitted through the FAIMS device at CV −8.1, −6.6, and −4.9 V contained sodiated solvent ions, protonated methanol ions, and Cs ions, respectively.

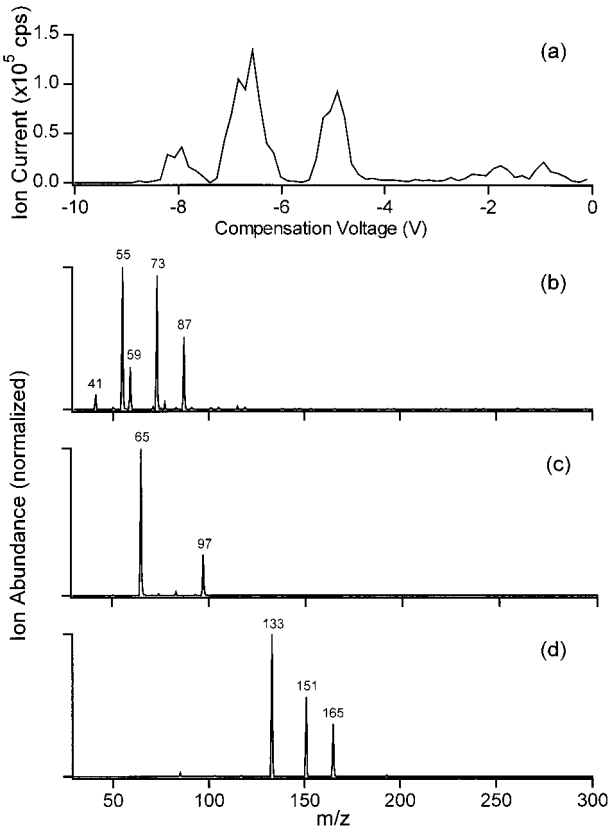

Figure 6. CV scan with MS detection and corresponding mass spectra collected at fixed CV values for 10 μM CsCl in a 50:50:0.1 methanol/water/acetic acid (v/v/v) solution. P1 mode, (a) TIC-CV spectrum (*m*/*z* 30−300). Mass spectra were collected at (b) CV = −8.1 V, (c) CV = −6.6 V, and (d) CV = −4.9 V. DV = 2200 V.

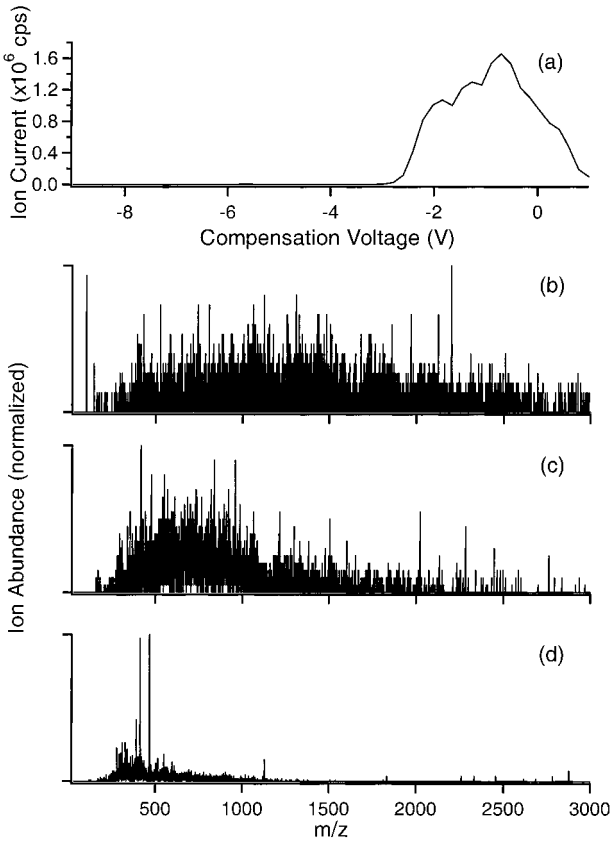

Figure 7. P2 mode electrospray FAIMS−MS spectra of a 50:50:0.1 methanol/water/acetic acid (v/v/v) solution. (a) Low-resolution TIC-CV spectrum (*m*/*z* 30−3000). Mass spectra were collected at (b) CV = −2.0 V, (c) CV = −1.5 V, and (d) CV = −0.5 V. DV = −2200 V, V_{FAIMS} = 49 V, and OR = 49 V.

P2 Mode. Collection of spectra in P2 mode requires the asymmetric waveform to have negative polarity (Figure 3b). Figure 7 illustrates the P2 mode electrospray FAIMS−MS spectra of the same solution analyzed in Figure 5, i.e., 50:50:0.1 methanol/water/acetic acid (v/v/v). Figure 7a is a low-resolution TIC-CV spectrum (*m*/*z* 30−3000) collected from CV = −9 to +1 V and Figure 7b−d are the mass spectra collected at CV values of −2.0, −1.5, and −0.5 V, respectively. By comparison with P1 mode studies of the same solution (Figure 5), the presence of signal across a wider *m*/*z* range indicates that P2 mode is suitable for transmission of much larger ions than P1 mode. Mass spectra acquired at CV values of −2.0 and −1.5 V (Figure 7b and c) lacked well-defined ions; the continuum of ions is believed to result from the formation of complex cluster ions during the electrospray process. These complex ions formed a wide, apparently continuous, band of background ions, as shown in Figure 7b−d. The apparent maximum ion current shifted to higher *m*/*z* as the CV voltage became more negative, ranging from about *m*/*z* 400 in Figure 7d to *m*/*z* 1200 in Figure 7b. In addition to the continuum background of cluster ions, the mass spectra at low CV usually included some well-defined, but unidentified ions, as shown in Figure 7d. These ions appear to originate from solvent impurities or other contaminants.

A FAIMS−MS TIC-CV spectrum (*m*/*z* 30−3000) of 5 μM equine cytochrome *c* in 50:49:1 methanol/water/acetic acid (v/v/v) is shown in Figure 8. The mass spectrum obtained at the maximum ion transmission in the TIC-CV spectrum (i.e., CV = −3.8 V) is shown in Figure 8b. The mass spectrum collected under these conditions was similar to cytochrome *c* spectra collected with a conventional arrangement of electrospray MS, except that the low-mass solvent-derived cluster ions were absent. These ions, which were lost to the walls of the FAIMS analyzer in P2 mode, were detected by operating the FAIMS in P1 mode (not shown). A mass spectrum was also collected at CV = −2.6 V and is shown in Figure 8c. The slight difference in charge-state distribution between the mass spectra in Figure 8 indicates that the FAIMS analyzer is capable of some discrimination between the multiply charged ions of cytochrome *c*. Recent results show that the FAIMS can separate different conformers of a protein ion.[29] In addition, the continuum background in Figure 8b and c is reduced compared with electrospray MS analysis (i.e., without FAIMS) of the same solution. The signal-to-noise ratio (S/N) of the major charge states using electrospray FAIMS−MS was improved by a factor of between 2 and 4 with respect to the conventional electrospray MS arrangement. The improvement in S/N increased further when the CV was optimized for a particular charge state.

Relationship between P1 and P2 Modes. A mixture of short-chain glycine (Gly, G)-based peptides ($(Gly)_n$ for n = 1−6),

(29) Purves, R. W.; Guevremont, R.; Barnett, D., unpublished results.

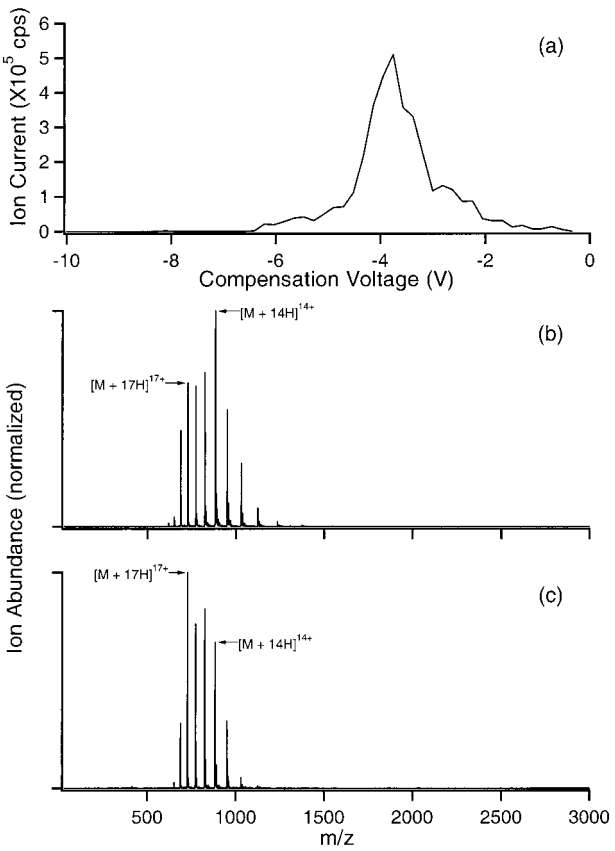

Figure 8. P2 mode electrospray FAIMS−MS spectra of 5 μM equine cytochrome *c* in a 50:49:1 methanol/water/acetic acid (v/v/v) solution. (a) TIC-CV spectrum (*m*/*z* 30−3000). Mass spectra were collected at (b) CV = −3.8 V and (c) CV = −2.6 V. DV = −3000 V.

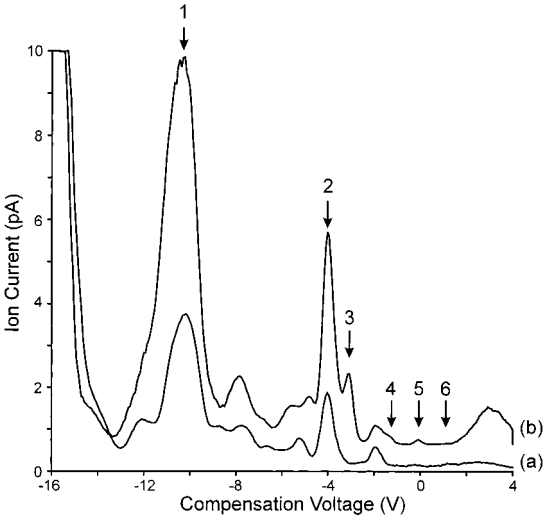

Figure 9. P1 mode electrospray FAIMS-E CV spectra (electrometer-based detection) of a mixture of short-chain glycine-based peptides ((Gly)$_n$ for $n = 1-6$) each at a concentration of 20 μM in a 50:50:0.1 methanol/water/acetic acid (v/v/v) solution. (a) CV spectrum of a blank solution; (b) CV spectrum of the mixture. DV = 3000 V. The labeling of the peaks was carried out based on electrospray FAIMS−MS data (see text).

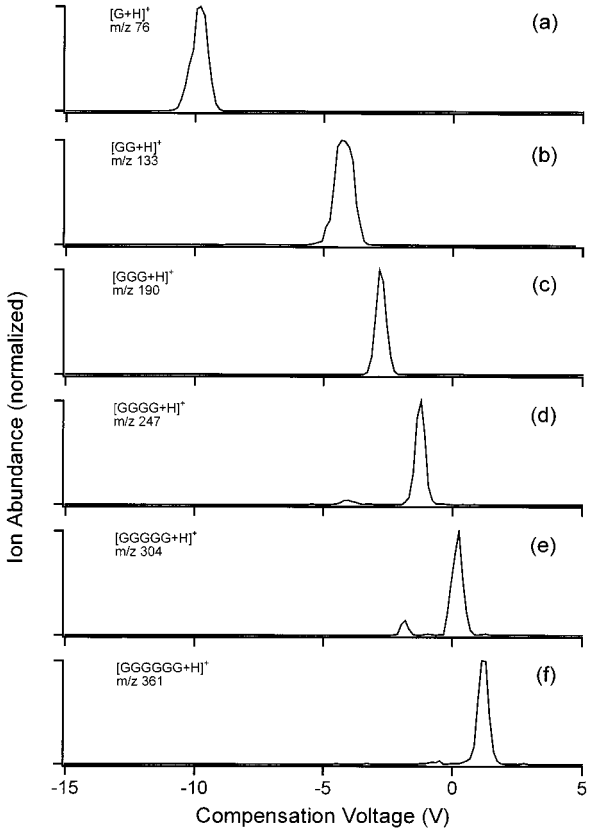

Figure 10. P1 mode single ion monitoring CV scans (IS−CV spectra) of a mixture of short-chain glycine (Gly, G)-based peptides ((Gly)$_n$ for $n = 1-6$) each at a concentration of 20 μM in a 50:50:0.1 methanol/water/acetic acid (v/v/v) solution: (a) (Gly)$_1$, (b) (Gly)$_2$, (c) (Gly)$_3$, (d) (Gly)$_4$, (e) (Gly)$_5$, and (f) (Gly)$_6$. DV = 3000 V.

each at a concentration of 20 μM in 50:50:0.1 methanol/water/acetic acid (v/v/v), was analyzed using FAIMS. This analysis is summarized in Figures 9−14 and serves to illustrate the relationship between P1 and P2 modes.

Figure 9, trace a, represents the electrospray FAIMS-E CV spectrum for a blank solution (50:50:0.1 methanol/water/acetic acid (v/v/v)). Trace b on this figure is the CV spectrum for a sample containing the mixture of six peptides, (Gly)$_n$, where $n = 1-6$. Electrospray is an inherently noisy ion source, consequently, there are several peaks in the background CV spectrum. Determining the CV of the six peptides by comparing trace b with trace a is, at best, difficult, and demonstrates some of the problems in using electrometer-based detection. Note that higher concentrations could be used, but may further complicate the CV spectrum due to cluster ion formation.[30]

Consequently, the location of each peptide in the CV spectrum was determined by mass spectrometry (Figure 10) and is noted on Figure 9. The CV at which each compound was transmitted through the FAIMS analyzer decreased with increasing peptide length. However, the CV increment between the glycine peaks does not appear to follow a pattern. The signal intensity of this series of compounds also decreased with increasing peptide chain length. (Gly)$_6$ could not be easily detected in P1 mode since its optimum CV is positive. This means that in P1 mode, (Gly)$_6$ traveled through the FAIMS analyzer in a defocusing "virtual" electric field[24] rather than a focusing field that was felt by the short-chain peptides up to (Gly)$_4$. In other words, the behavior of (Gly)$_6$ was characteristic of an ion transmitted in P2 mode, having a decreasing ion mobility as a function of increasing electric field

(30) Guevremont, R.; Purves, R. W. *J. Am. Soc. Mass Spectrom.*, in press.

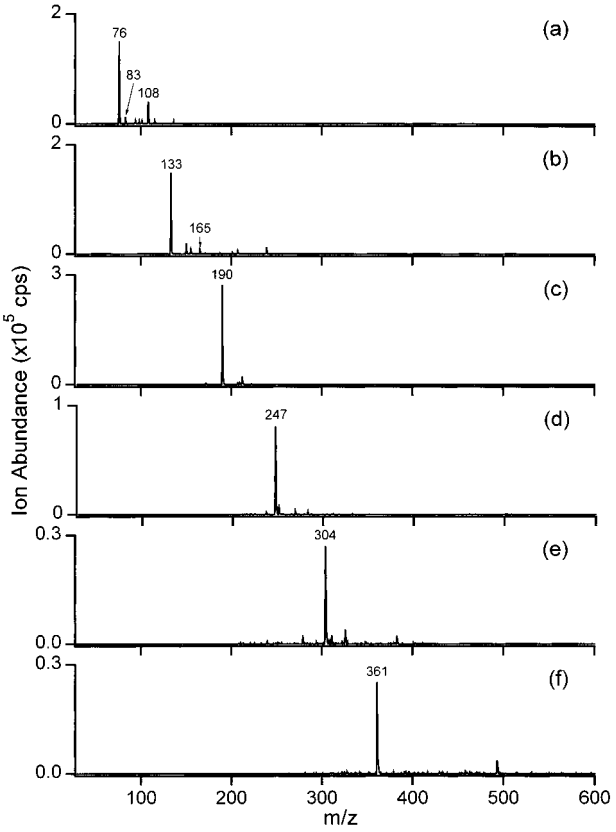

Figure 11. Mass spectra collected at several CV values for a mixture of short-chain glycine-based peptides ($(Gly)_n$ for $n = 1-6$) each at a concentration of 20 μM in a 50:50:0.1 methanol/water/acetic acid (v/v/v) solution. P1 mode, (a) $(Gly)_1$, CV = −9.8 V, (b) $(Gly)_2$, CV = −4.3 V, (c) $(Gly)_3$, CV = −2.8 V, (d) $(Gly)_4$, CV = −1.2 V, (e) $(Gly)_5$, CV = +0.2 V, and (f) $(Gly)_6$ CV = +1.2 V. DV = 3000 V.

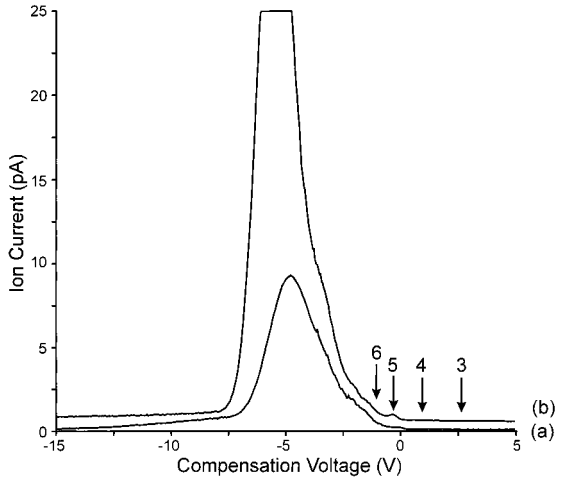

Figure 12. P2 mode electrospray FAIMS-E CV spectra (electrometer-based detection) of a mixture of short-chain glycine-based peptides ($(Gly)_n$ for $n = 1-6$) each at a concentration of 20 μM in a 50:50:0.1 methanol/water/acetic acid (v/v/v) solution. (a) CV spectrum of a blank solution; (b) CV spectrum of the mixture. DV = −3000 V. The labeling of the peaks was carried out based on electrospray FAIMS−MS data (see text).

strength (type C, Figure 1). Conversely, the shorter chain peptides had increasing ion mobilities with increases in electric field strength (type A, Figure 1). This increase in ion mobility was most pronounced for $(Gly)_1$, and successively less pronounced for

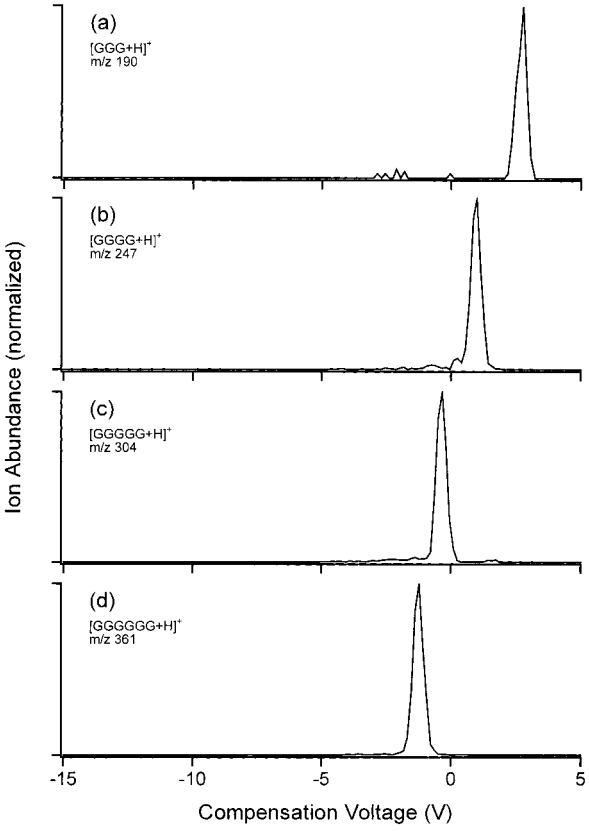

Figure 13. P2 mode single ion monitoring CV scans (IS−CV spectra) of a mixture of short-chain glycine (Gly, G)-based peptides ($(Gly)_n$ for $n = 1-6$) each at a concentration of 20 μM in a 50:50:0.1 methanol/water/acetic acid (v/v/v) solution: (a) $(Gly)_3$, (b) $(Gly)_4$, (c) $(Gly)_5$, and (d) $(Gly)_6$. DV = −3000 V.

$(Gly)_2$−$(Gly)_4$. The peptide $(Gly)_5$ experienced virtually no change in ion mobility at high electric field and was transmitted near CV = 0 V.

Illustrated in Figure 10 are the electrospray FAIMS−MS IS-CV spectra for the same solution used in Figure 9b. Mass spectra obtained at each of the peak maximums in Figure 10 are shown in Figure 11. Compared with electrometer-based detection, determinating the CV of the six peptides is greatly simplified. In Figure 11a, CV = −9.8 V, the two most abundant ions in the mass spectrum correspond to the $(Gly)_1$ species MH^+ (m/z 76) and $MH(CH_3OH)^+$ (m/z 108). Less abundant ions in the mass spectrum represented $((CH_3COOH))Na^+$ (m/z 83) and its H_2O and CH_3OH adduct peaks (m/z 101 and 115, respectively). These ions constitute the background peak in Figure 9a (CV ∼ −10 V) and are transmitted through the FAIMS device at the same CV as $(Gly)_1$.

$(Gly)_2$ also showed a methanol adduct peak, Figure 11b, while $(Gly)_3$−$(Gly)_6$, shown in Figure 11c−f, did not. Figures 10f and 11f show $(Gly)_6$ to be transmitted at positive CV, under defocusing conditions. The presence of this compound was not detected using FAIMS-E (Figure 9) because it was at low abundance relative to the complex ion clusters at positive CV values.

The P2 mode electrospray FAIMS-E scans of CV for the same blank and mixture of $(Gly)_n$ peptides discussed above are shown in Figure 12 as traces a and b, respectively. The peak at CV −5 V is the current resulting from the detection of complex ion clusters formed in the electrospray process.[30] The presence of

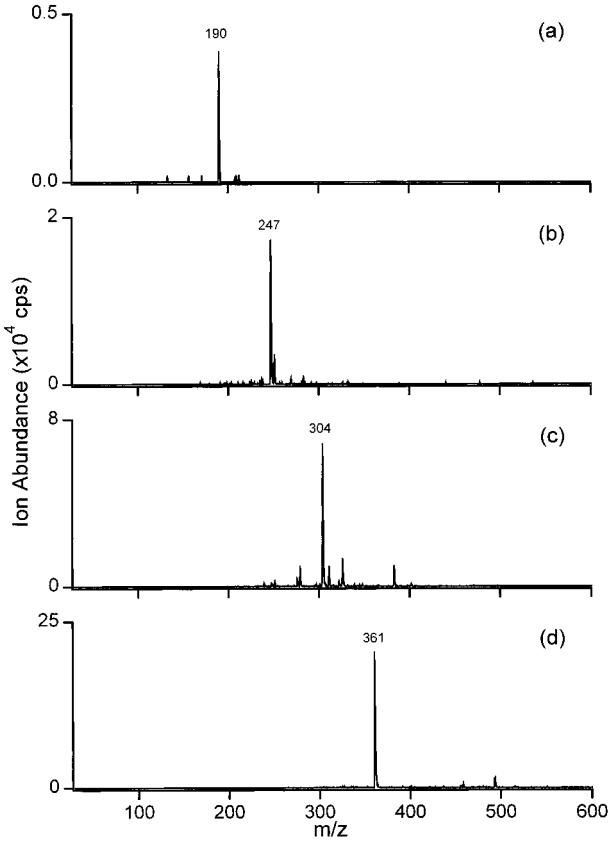

Figure 14. Mass spectra collected at several CV values for a mixture of short-chain glycine-based peptides ($(Gly)_n$ for $n = 1-6$) each at a concentration of 20 μM in a 50:50:0.1 methanol/water/acetic acid (v/v/v) solution: (a) $(Gly)_3$, CV = +2.7 V, (b) $(Gly)_4$, CV = +1.0 V, (c) $(Gly)_5$, CV = −0.3 V, and (d) $(Gly)_6$, CV = −1.2 V. DV = −3000 V.

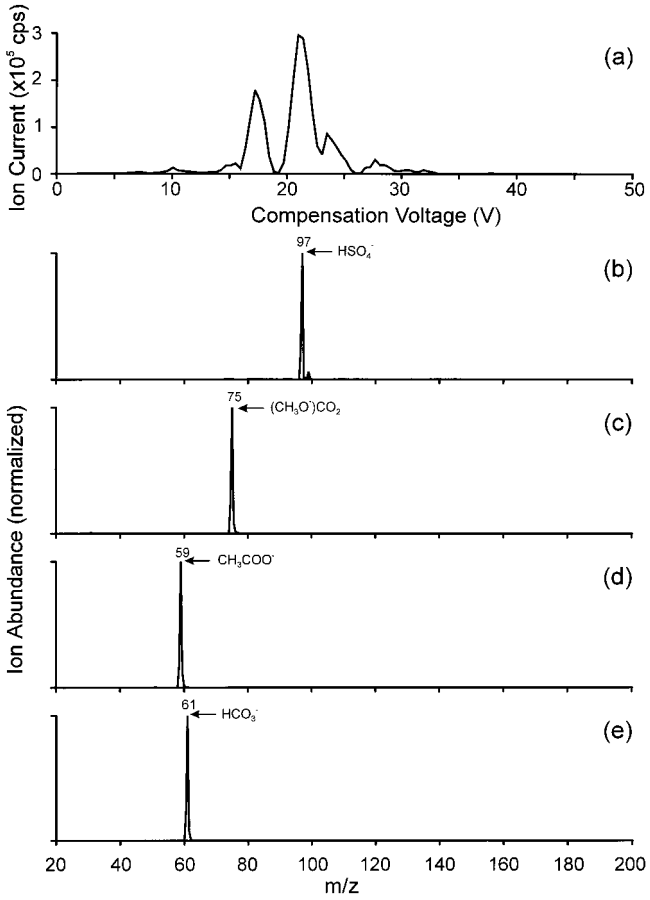

Figure 15. N1 mode electrospray FAIMS−MS spectra of 10 μM ammonium sulfate in a solution of 9:1 methanol/water (v/v). (a) TIC-CN spectrum (m/z 30−200). Mass spectra were collected at (b) CV = +17.2 V, (c) CV = +20.9 V, (d) CV = +23.6 V, and (e) CV = +27.6 V. DV = −3000 V, V_{FAIMS} = −45 V, and OR = −30 V.

glycine in the mixture appears to affect the complex ion cluster formation, resulting in the increase in signal intensity at CV −5 V over that of the blank. The CV values of transmission of $(Gly)_5$ and $(Gly)_6$, while not readily apparent using electrometer detection (due to the current produced by the background ion clusters), have been established by mass spectrometry and are noted on the figure. Single ion monitoring traces for $(Gly)_3-(Gly)_6$ are shown in Figure 13a−d, respectively. Due to the presence of a strong defocusing field, $(Gly)_1$ and $(Gly)_2$ were not detected; consequently, their traces are not shown. $(Gly)_3$ and $(Gly)_4$ were also defocused, but their appearance in the single ion traces at positive CV implies that the defocusing action for these species was weaker than it was for $(Gly)_1$ or $(Gly)_2$. Again, $(Gly)_5$ was observed near CV = 0 V and did not experience significant focusing or defocusing effects. Mass spectra acquired at the peak maximums in Figure 13 are shown in Figure 14. A comparison of the mass spectra shown in Figure 14 with those shown in Figure 11 illustrates the strength of the focusing/defocusing action. For example, on switching from P1 mode to P2 mode, the intensity of MH^+ for $(Gly)_6$ increased by ∼1 order of magnitude, whereas the intensity of MH^+ for $(Gly)_3$ decreased by almost 2 orders of magnitude.

The preceding electrospray FAIMS experiments in P1 and P2 modes serve to illustrate the relationship between the two modes. In P1 mode, those ions whose mobility increases with increasing electric field strength tend to be focused, whereas in P2 mode, ions whose mobility decreases with increasing electric field strength tend to be focused. The strength of the focusing and defocusing action decreases as the CV approaches zero. Compounds that are transmitted near CV = 0 V can appear in both P1 and P2 traces as long as the defocusing field is not sufficiently strong to drive the ions to the walls of the FAIMS analyzer. At a CV of zero, the transmission efficiency of an ion is the same as (or lower than) it would be in the complete absence of the high-voltage, high-frequency, asymmetric waveform.[24]

N1 Mode. CV spectra in N1 mode are collected with the asymmetric waveform in negative polarity (Figure 3b). Figure 15a shows the N1 mode electrospray FAIMS−MS TIC-CV spectrum (m/z 30−200) of a solution of 10 μM ammonium sulfate in 9:1 methanol/water (v/v). Parts b−e in Figure 15 are mass spectra collected at CV values of +17.2, +20.9, +23.6, and +27.6 V, respectively. At CV = +17.2 V, Figure 15b, the peak at m/z 97 corresponds to HSO_4^- being transmitted through the FAIMS analyzer. At CV = +20.9 V, Figure 15c, the peak at m/z 75 is consistent with the transmission of $(CH_3O^-)CO_2$, an ion routinely seen in background negative ion spectra where methanol has been used as a solvent.[31] The ions shown in Figure 15d and e appear to be CH_3COO^- (m/z 59) and HCO_3^- (m/z 61), respectively.

(31) Barnett, D. A.; Horlick, G. *J. Anal. At. Spectrom.* **1997**, *12*, 497−501.

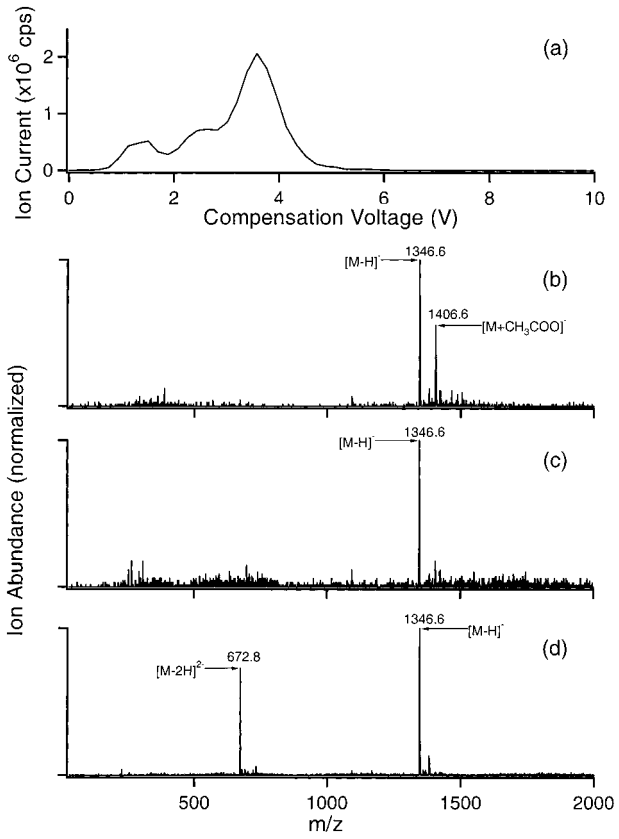

Figure 16. N2 mode electrospray FAIMS−MS spectra of 5 μM substance P and 0.2 mM ammonium acetate in a 9:1 methanol/water (v/v) mixture. (a) Low-resolution TIC-CV spectrum (*m*/*z* 30−2000). Mass spectra were collected at (b) CV = +1.2 V, (c) CV = +2.5 V, and (d) CV = +3.6 V. DV = 3000 V.

FAIMS−MS data for N1 mode have previously been shown using corona discharge ionization.[23] The HCO_3^- ion was observed in the corona discharge, along with nitrogen-containing ions, including NO_3^- and NO_2^-. Due to the small size of the chamber in which the electrospray ionization takes place within the FAIMS device, there is a tendency for corona discharge to occur. The onset of corona discharge corresponds to the appearance of the above ions in the negative ion FAIMS−MS spectra and the simultaneous decrease in signal intensities for electrospray ions. For experiments carried out using solutions with high percentages of methanol, corona discharge was not observed to be a severe problem.

N2 Mode. N2 mode CV spectra are collected with the asymmetric waveform in positive polarity (Figure 3a). Electrospray FAIMS−MS spectra in N2 mode for 5 μM substance P and 0.2 mM ammonium acetate in a 9:1 methanol/water (v/v) solution are shown in Figure 16. Figure 16a is a low-resolution TIC-CV spectrum (*m*/*z* 30−2000), and mass spectra of the three peaks appearing in this CV spectrum are shown in Figure 16b−d. At CV = +1.2 V, the $[M - H]^-$ ion of substance P was identified at *m*/*z* 1346.6 and an acetate adduct was observed at *m*/*z* 1406.6, as shown in Figure 16b. Figure 16c indicates that substance P is primarily responsible for the peak found at CV = +2.5 V. In the mass spectrum collected at CV = +3.6 V, shown in Figure 16d, both the singly and doubly charged (*m*/*z* 672.8) ions of substance P were identified.

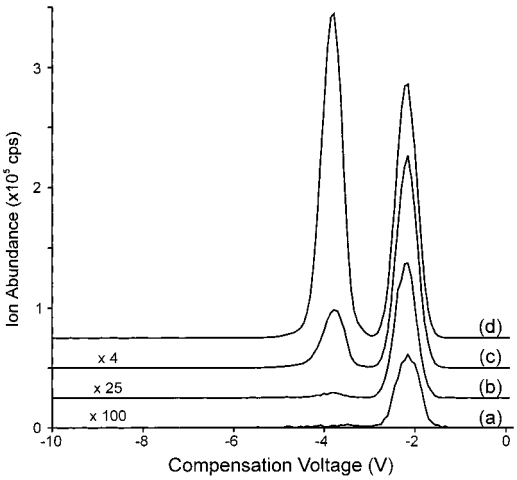

Figure 17. P2 mode electrospray FAIMS−MS, IS−CV spectra (*m*/*z* 556.5) of leucine enkephalin in a 50:50:0.1 methanol/water/acetic acid (v/v/v) solution collected at concentrations of (a) 25 nM, (b) 250 nM, (c) 2.5 μM, and (d) 25 μM. DV = −3000 V.

Changing of the OR from −45 to −30 V altered the distribution of ions observed in Figure 16b, such that $[(M - H)(CH_3COOH)]^-$ and $[(M - H)(CH_3COOH)_2]^-$ were the two most intense peaks in the mass spectrum; however, it did not significantly affect the distribution of ions at CV = +3.6 V. This observation suggested that the separation of the substance P peak at CV = +1.2 V from the major peak at CV = +3.6 V was the result of some fraction of the substance P that was transmitted through the FAIMS analyzer as an acetate adduct.

Quantitative Analysis with Electrospray FAIMS−MS. Preliminary studies, using gas-phase introduction of ions into a FIS© with electrometer-based ion sensing, have indicated that response to ions is nonlinear.[27] The linearity of response of the electrospray FAIMS−MS system has been studied here using leucine enkephalin as the analyte. FAIMS−MS IS−CV spectra (*m*/*z* 556.5) collected in P2 mode for several concentrations of leucine enkephalin are shown in Figure 17. A single peak was observed at CV = −2.1 V for low-concentration solutions, as shown for a 25 nM solution in trace a of Figure 17. The peak was identified by FAIMS−MS to be the MH^+ ion of leucine enkephalin. At a concentration of 250 nM, the onset of a second peak appeared at CV = −3.6 V, as observed in trace b. Mass spectra collected for this peak indicated that it was also leucine enkephalin, presumably the CID product of the decomposition of higher molecular weight cluster ions that formed during electrospray ionization of solutions containing higher analyte concentrations. The existence of poorly defined aggregate ions formed by the electrospray process is easily confirmed by conventional ion mobility experiments, as shown by the appearance of very low mobility ions in IMS spectra of peptides[32] and proteins[9] at high concentrations. The onset of formation of such clusters often corresponds to a decrease in sensitivity for analyte monomer ions,[33,34] resulting in nonlinear electrospray-MS calibration curves at high concentration.

(32) Counterman, A. E.; Valentine, S. J.; Srebalus, C. A.; Henderson, S. C.; Hoaglund, C. S.; Clemmer, D. E. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 743−59.

(33) Zook, D. R.; Bruins, A. P. *Int. J. Mass Spectrom. Ion Processes* **1997**, *162*, 129−47.

(34) Enke, C. G. *Anal. Chem.* **1997**, *69*, 4885−93.

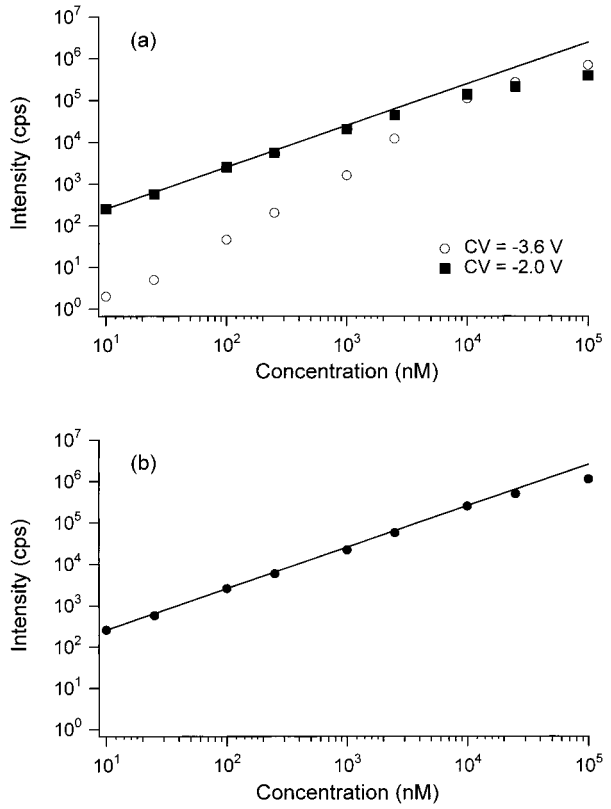

Figure 18. log−log plots of intensity vs concentration for leucine enkephalin. (a) Intensities of the peaks at CV = −2.1 V and CV = −3.6 V are considered separately. (b) Intensity of the peaks at CV = −2.1 V and CV = −3.6 V are added together. The slope of the line is 1.

Quantitation using electrospray FAIMS−MS may be expected to be more difficult than using a conventional electrospray-MS arrangement because of the separation capabilities of the FAIMS analyzer. The transmission, at different CV values, of the analyte monomer and the complex cluster ions, which can be broken down within the mass spectrometer, may result in more than one peak in the CV spectrum corresponding to the protonated molecular ion. By single ion monitoring at the m/z of the protonated molecular ion of the analyte during a CV scan, and adding together the peak heights of all detected ions, a calibration curve can be obtained. It is argued that this calibration curve is similar to that found by conventional electrospray-MS, where no separation of the analyte monomer ions and cluster ions occurs before entry into the mass spectrometer. The protonated molecular ions, whether originating from monomers or clusters, are detected as one peak. That is, the degree to which cluster ions will break down to give protonated molecular ions is governed by conditions in the mass spectrometer interface.

An electrospray FAIMS−MS calibration curve for leucine enkephalin is shown in Figure 18. The ion abundances of the leucine enkephalin peaks at CV = −2.1 V and CV = −3.6 V are plotted as a function of nanomolar concentration in Figure 18a. The response of MH^+ at CV = −2.1 V appeared to be linear below a concentration of ∼250 nM but deviated from linearity as the concentration was increased further. This onset of signal suppression occurred when the contribution from leucine enkephalin at CV = −3.6 V to the total current became significant. The calibration curve generated by summing the intensities of the peaks at CV = −2.1 V and CV = −3.6 V is shown in Figure 18b. It was assumed that the FAIMS−MS response to cluster ions of leucine enkephalin was approximately equal to the response of the MH^+ ion. The new calibration curve appeared to be linear for 3 orders of magnitude, over a concentration range of approximately 10 nM to 10 μM leucine enkephalin. This first attempt at quantitation using the electrospray FAIMS−MS technique showed that FAIMS transmission permits linear calibration. As well, because FAIMS−MS combines separation that is independent of m/z, with mass spectrometry, there is potential for a wider dynamic range for quantitation. Further investigations into the applications of electrospray FAIMS−MS are currently underway.

CONCLUSIONS

FAIMS is a relatively new technique for separating gas-phase ions at atmospheric pressure, based on changes in mobility at high electric fields relative to low electric fields. The FAIMS device behaves like an ion filter, capable of transmitting some fraction of a mixture of ions entering the device. Recently,[23] this system was coupled to a mass spectrometer to identify the (corona discharge) ions that were transmitted under various operating conditions and in this study has been extended to the coupling of electrospray to FAIMS−MS. Although the operation of a FAIMS instrument requires extremely clean and dry gas flows in the analyzer region, a method for introduction of electrospray ions has been developed. The flows of gas in the FAIMS instrument have been adjusted so that the ions must travel through a countercurrent of flowing gas prior to entering the FAIMS analyzer, thus minimizing the contamination in the FAIMS analyzer by the large quantity of solvent delivered by the electrospray needle.

The compensation voltage spectra, and the mass spectra corresponding to the major peaks observed, were evaluated for all four operating modes, i.e., P1, P2, N1, and N2. Positive and negative ions that have increasing mobility at high electric field strength tend to be transmitted, with focusing, through the FAIMS device in P1 and N1 modes, respectively. Ions with decreasing mobility at high electric field strength tend to be transmitted, with focusing, in P2 and N2 modes. For the limited number of analytes investigated, low-molecular-weight organic and inorganic ions have been transmitted, with focusing, in P1 and N1 modes, while higher molecular weight ions (e.g., proteins) have been transmitted, with focusing, in P2 and N2 modes. This permits a very rough, preliminary separation of ions into high- and low-molecular-weight fractions and, in some cases, such as with the collection of protein spectra in P2 mode, results in the simplification of mass spectra. This rough separation also seems to attenuate the transmission of the very high molecular weight cluster ions that are characteristic of electrospray ionization at elevated (μM) analyte concentrations, thereby minimizing the background continuum of ions normally observed in the electrospray spectra of proteins. As a consequence, signal-to-background noise levels for a mass spectrum of cytochrome *c* were improved using electrospray FAIMS−MS when compared with conventional electrospray-MS. Although the resolution of ion separation using FAIMS is low, preliminary results suggest that various charge states of cytochrome *c* can

be distinguished. This result implies that the ion mobility of these species at high electric field strength is sensitive to the structure of the protein ion.

The first quantitative study by electrospray-FAIMS−MS showed that the FAIMS transmission provided linear calibration for ∼3 orders of magnitude. The combination of FAIMS, which provides ion separation indeendent of m/z, and mass spectrometry appears to offer a new capability for sensitive detection and a wider dynamic range of quantitation than conventional mass spectrometry techniques.

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