Published in final edited form as:

Anal Chem. 2010 January 1; 82(1): 32–35. doi:10.1021/ac902133n.

Differential Ion Mobility Separations of Peptides with Resolving Power Exceeding 50

Alexandre A. Shvartsburg, Keqi Tang, and Richard D. Smith

Biological Sciences Division, Pacific Northwest National Laboratory, P.O. Box 999, Richland, WA 99352

Abstract

Differential ion mobility spectrometry (IMS) or field asymmetric waveform IMS (FAIMS) sorts gas-phase ions by mobility differences with respect to the electric field intensity. A major emerging FAIMS application is the fractionation of proteolytic digests. Using a planar FAIMS unit with helium/nitrogen mixtures, we have increased FAIMS resolving powers for peptide analyses from the prior maximum of ~20 – 30 to ~50 – 70. The resolution improved nearly 3-fold, allowing, in particular, separation of previously unresolved conformers.

Introduction

Ion mobility spectrometry (IMS) allows one to separate, characterize, or identify ions based on a property of their gas-phase transport in electric fields.¹ Conventional IMS (including the drift tube and traveling-wave modes) separates ions by mobility (K), generally at modest field intensity (E) where K is nearly independent of E . At sufficiently high E , the value of K for any ion deviates from its limit at $E = 0$, up or down depending on the ion-gas pair and temperature.² This is exploited in differential IMS³ to sort ions by the difference between the mobility at high and low E . As this difference is elicited using a periodic asymmetric electric field, the method is also called “field asymmetric waveform IMS” (FAIMS). In practice, the field is established in a gap between two electrodes, through which ions are carried by a gas flow, by applying a voltage waveform to one electrode.³ Ions oscillate in this field and drift toward an electrode where they are neutralized. For a given species, a fixed “compensation field” (E_C) created by “compensation voltage” (CV) applied to one electrode can offset that drift and thus prevent ion loss.³ Scanning the CV produces a spectrum of species entering the gap.

A key metrics of any separation is the resolving power (R) - the ratio of separation parameter (here E_C) to the peak width at half maximum. While the resolution of two species is proportional to the difference of E_C values, and thus depends on the analyte, it scales with R . Thus R describes the relative resolution of two instruments. The resolving power of FAIMS has been limited, with most systems providing $R \sim 10 - 15$ for typical analytes^{3–8} - an order of magnitude lower than ~100 – 150 reached in conventional IMS.¹ However, the $K(E)$ derivative and thus E_C are correlated with the ion mass much weaker than the absolute mobility, and isomers or isobars are often distinguished by FAIMS better than by conventional IMS.^{3,4,7} This permitted FAIMS to compete with conventional IMS for coupling to mass spectrometry (MS) and several FAIMS/MS systems were commercialized. Still, improving the resolution of FAIMS would substantially increase its utility, especially in analyses of complex mixtures such as proteolytic digests.^{9–11}

The FAIMS performance is dictated by the gap shape. In curved (cylindrical or spherical) geometries, a non-uniform field across the gap pushes ions to the gap median or electrodes, depending on the $K(E)$ profile and waveform polarity.^{3,12–14} For a subset of species, such

focusing reduces ion losses on the electrodes due to diffusion and Coulomb repulsion. However, ions remain stable and pass the gap over a range of E_C , which restricts the resolution^{14,15} regardless of the separation time (t). The focusing strengthens for steeper $K(E)$ curves that normally yield larger absolute E_C , and peaks usually broaden with increasing E_C . As the result, the resolving power is often nearly constant over wide E_C ranges. For example (Fig. 1), the peak widths for peptide ions are roughly proportional^{5,7} to E_C , and R is limited to ~ 15 and (on average) drops slightly for higher charge states (z) that statistically exhibit greater $|E_C|$ values.

In planar gaps, a uniform field induces no focusing and only one species with certain E_C can be in equilibrium. Then R increases with continued separation (in principle, infinitely), scaling¹⁶ as $t^{1/2}$. The absence of focusing also excludes discrimination against ions with near-flat $K(E)$ that are focused poorly. In simulations,¹⁵ the planar gaps provide the optimum resolution/sensitivity balance - the maximum R at any set transmission efficiency (at least, for moderate ion currents). Unlike with curved gaps, the peak width is independent of E_C and thus the resolving power increases for species with greater $|E_C|$ values. Guided by those calculations, we engineered a planar FAIMS device that, for peptides, achieved $R \sim 25 - 30$ using the N_2 gas.¹⁵

With any gap shape, the resolution also depends on the gas composition and often benefits from the use of He/N_2 mixtures, largely because of the non-Blanc phenomena that raise the $|E_C|$ values.¹⁷ Employing a planar FAIMS analyzer and 1:1 He/N_2 gas, here we demonstrate peptide separations with the resolving power up to 70.

Instrumentation

We used the previously described planar FAIMS unit¹⁵ with the gap width adjusted to 1.88 mm. A commercial Selectra FAIMS system (Ionalytics/Thermo Fisher) delivered the bisinusoidal asymmetric waveform with the frequency of 750 kHz and amplitude ("dispersion voltage", DV) of 4.0 kV, corresponding to the field of 21 kV/cm or 85 Td, and purified He/N_2 mixtures with 0 – 50% He (v/v) at the rate of 1.7 L/min. This leads to the calculated separation time of 0.2 s, which is close to that for commercial cylindrical analyzers. The FAIMS unit was attached to an LCQ ion trap MS (Thermo Fisher), spaced 2 mm from the capillary inlet. As ion desolvation after FAIMS is redundant,¹⁵ the capillary was kept at 50 °C. The ions were generated by electrospray ionization (ESI) from 1 μM solutions of peptides in 50:49:1 water/methanol/acetic acid infused to an emitter at 0.2 $\mu L/min$. The emitter was biased at 1.8 kV above the FAIMS curtain plate voltage of 1 kV referenced to ground. The MS analyses were performed in the full scan regime, with long accumulation times (~ 1 s) to explore the possible peptide charge reduction after FAIMS filtering.

Results

Bradykinin (Bk, 1060 Da) is a common model peptide in MS and FAIMS research.^{15,18,19} ESI typically generates protonated Bk with $z = 2$ (dominant) and 3. For $z = 2$, FAIMS in N_2 showed two partly resolved conformers with the cylindrical¹⁸ device and three using the planar one (Ref. [15] and Fig. 2). Raising the He fraction from 0 to 50% increases the E_C values for all features by 1.9 – 2.0 times, from 34 – 39 to 65 – 79 V/cm (Fig. 2). As the shifts for different conformers are slightly disproportional, the separation width (between the "bookend" peaks) expands by 2.5-fold, from 5.3 to 13.5 V/cm. Further, the peaks narrow by $\sim 10 - 40\%$: the peak width in planar FAIMS scales^{3,16} (approximately) as $K^{-1/2}$, and the mobility of any ion increases at growing He concentration. Overall, the resolving power increases by 2.3 – 2.8 times, reaching $\sim 50 - 60$, and the peak capacity of isomeric separation improves by 3.2 times - from 3 in N_2 to 10 in He/N_2 . This resolution gain allows baseline separation of the three

known features (b, c, d) and identification of a new conformer (g) previously merged with peaks b and c.

Multiply-charged ions may reduce charge in the FAIMS/MS interface, producing peaks in the FAIMS spectra for lower z at the E_C of same species with higher z . A signature of this effect is the coincidence of some peaks found for lower and higher z , over a range of gas compositions and/or DV values.²⁰ Charge reduction of peptides would proceed via the proton stripping in collisions with neutral contaminants in the gas,²⁰ perhaps arising from the ESI solvent vapor. Thus, to deduce if any peaks seen for $z = 2$ may be the charge-reduced artifacts, we should inspect the spectra for $z = 3$. At any He fraction, Bk (3+) exhibit (Fig. 2) a single peak far from the major features for (2+), which upholds their identification as Bk (2+) conformers. The ledge (a) at lower E_C that was assigned¹⁸ as a minor (2+) conformer may indeed arise from the charge reduction of (3+). Regardless, its effect on the data for (2+) is marginal because the intensity of (3+) was just ~1% that of (2+).

A larger peptide that yields more (3+) ions is substance P (SP, 1348 Da). As with Bk, shifting to 50% He increases the resolving power for (2+) and (3+) by ~2.5 times, to ~50 – 70 (Fig. 2). The spectrum for (3+) at 50% He comprises three features (a, b, c) spaced apart by ~3%, or close to the resolution limit. Hence the emergence of multiple (3+) conformers with increasing He fraction may reflect the improved resolution and/or the isomerization in FAIMS driven by the field heating that strengthens at higher He content.²¹ The minor peaks observed for (2+) at higher E_C coincide with those for (3+) at any He percentage and thus are most likely due to the charge reduction of (3+).

An even larger peptide, syntide 2 (St, 1508 Da), produces intense signal for $z = 2, 3$, and 4 (Fig. 2). Again, the resolving power for all 3 charge states increases at higher He content by ~2 – 3 times, reaching ~40 – 70 at 50% He. The overlap of the lesser peak for (3+) with the peak for (4+) and of the two lesser peaks for (2+) with the features for (3+) clearly identifies those secondary peaks as the charge-reduced products. Hence all three St species exhibit no conformational diversity, possibly except for a minor feature (b) for (3+).

Contrary to the common concept of $|E_C|$ for peptides increasing at higher charge states, the E_C values are lower for Bk (3+) than all Bk (2+) conformers, and for St (4+) compared to St (3+). Noting that the $|E_C|$ values for small proteins such as ubiquitin and cytochrome *c* decrease upon unfolding²² at higher z , we hypothesize the same trend for peptides. Indeed, conventional IMS measurements and molecular modeling indicate that Bk (2+) is compact, but Bk (3+) is not.²³ Larger peptides tend to resist unfolding at a given z better, hence it would not be surprising for SP (3+) and St (3+) to stay folded as suggested by their E_C values exceeding those for the corresponding (2+) ions. Then the decrease of E_C for St (4+) is perhaps due to unfolding upon further charging.

Conclusions

We have achieved FAIMS resolving powers of $R \sim 50 - 70$ in peptide analyses using a planar device with helium/nitrogen gas. These values are still lower than $R \sim 150$ demonstrated in drift tube (DT) IMS, but often allow better separation (i.e., higher peak capacity) for isomers/isobars because FAIMS is based on the differential mobility that is less correlated to the ion mass than the absolute mobility relevant to DTIMS. For example, four conformers of bradykinin (2+) were resolved by FAIMS here, versus two by DTIMS at $R \sim 110$ (and none at lower R).²⁴ Thus FAIMS/MS analyses of peptides using the present unit will have the peak capacity at least competitive with that of best existing DTIMS/MS systems. The increase of resolution in FAIMS by ~2.5 times reported here opens many new applications, including FAIMS/DTIMS

analyses^{7,22} with the projected 2-D peak capacity approaching that of 2-D liquid-phase methods.

Acknowledgments

We thank J. J. Dunyach (Thermo Fisher) for his help with instrumentation, Drs. D. Barnett and R. Guevremont for providing their measurements for peptides using cylindrical FAIMS, and Dr. R. Mabrouki for the peptide samples. Portions of this work were supported by Battelle and NIH NCRR (RR18522).

References

1. Eiceman, GA.; Karpas, Z. *Ion Mobility Spectrometry*. CRC Press; Boca Raton, FL: 2004.
2. McDaniel, EW.; Mason, EA. *Transport Properties of Ions in Gases*. Wiley; NY: 1988.
3. Shvartsburg, AA. *Differential Ion Mobility Spectrometry: Nonlinear Ion Transport and Fundamentals of FAIMS*. CRC Press; Boca Raton, FL: 2008.
4. Guevremont R, Barnett DA, Purves RW, Vandermeij J. *Anal Chem* 2000;72:4577–4584. [PubMed: 11028613]
5. Barnett DA, Ells B, Guevremont R, Purves RW. *J Am Soc Mass Spectrom* 2002;13:1282–1291. [PubMed: 12443018]
6. Guevremont R. *J Chromatogr A* 2004;1058:3–19. [PubMed: 15595648]
7. Tang K, Li F, Shvartsburg AA, Strittmatter EF, Smith RD. *Anal Chem* 2005;77:6381–6388. [PubMed: 16194103]
8. Barnett DA, Belford M, Dunyach JJ, Purves RW. *J Am Soc Mass Spectrom* 2007;18:1653–1663. [PubMed: 17662612]
9. Venne K, Bonneil E, Eng K, Thibault P. *Anal Chem* 2005;77:2176–2186. [PubMed: 15801752]
10. Canterbury JD, Yi XH, Hoopmann MR, MacCoss MJ. *Anal Chem* 2008;80:6888–6897. [PubMed: 18693747]
11. Xia YQ, Wu ST, Jemal M. *Anal Chem* 2008;80:7137–7143. [PubMed: 18652493]
12. Guevremont R, Purves RW. *Rev Sci Instrum* 1999;70:1370–1383.
13. Guevremont R, Thekkadath G, Hilton CK. *J Am Soc Mass Spectrom* 2005;16:948–956. [PubMed: 15907709]
14. Shvartsburg AA, Tang K, Smith RD. *J Am Soc Mass Spectrom* 2004;15:1487–1498. [PubMed: 15465362]
15. Shvartsburg AA, Li F, Tang K, Smith RD. *Anal Chem* 2006;78:3706–3714. [PubMed: 16737227]
16. Shvartsburg AA, Smith RD. *J Am Soc Mass Spectrom* 2007;18:1672–1681. [PubMed: 17723907]
17. Shvartsburg AA, Tang K, Smith RD. *Anal Chem* 2004;76:7366–7374. [PubMed: 15595881]
18. Purves RW, Barnett DA, Ells B, Guevremont R. *Rapid Commun Mass Spectrom* 2001;15:1453–1456. [PubMed: 11507759]
19. Mabrouki R, Kelly RT, Prior DC, Shvartsburg AA, Tang K, Smith RD. *J Am Soc Mass Spectrom* 2009;20:1768–1774. [PubMed: 19616967]
20. Purves RW, Barnett DA, Ells B, Guevremont R. *J Am Soc Mass Spectrom* 2001;12:894–901. [PubMed: 11506222]
21. Baker ES, Clowers BH, Li F, Tang K, Tolmachev AV, Prior DC, Belov ME, Smith RD. *J Am Soc Mass Spectrom* 2007;18:1176–1187. [PubMed: 17512752]
22. Shvartsburg AA, Li F, Tang K, Smith RD. *Anal Chem* 2006;78:3304–3315. [PubMed: 16689531]
23. Rodriguez CF, Orlova G, Guo Y, Li X, Siu CK, Hopkinson AC, Siu KWM. *J Phys Chem B* 2006;110:7528–7537. [PubMed: 16599534]
24. Kemper PR, Dupuis NF, Bowers MT. *Int J Mass Spectrom* 2009;287:46–57.

Fig. 1.

Resolution of peptides in cylindrical FAIMS using the 1:1 He/N₂ mixture and (as in this work) DV = 4 kV and gap width of ~2 mm, based on the measurements in [5, 7]: the peak width (a) and resolving power (b). Symbols are for 318 ions with $z = 1 - 5$ [5], the average R is 8.1 for 1+, 7.4 for 2+, and 7.2 for 3+. The line is the mean trend of the data from [7], where the peaks were slightly narrower as the resolution was maximized by translating the inner electrode [13]. The underlying data for [5] were provided by Dr. R. Guevremont (private communication).

Fig. 2.

Present FAIMS spectra for 3 peptides with $z = 2$ (blue), 3 (green), and 4 (red) using the He fraction varied from 0 to 50%, as marked. The widths (V/cm) are given for all well-separated sufficiently intense peaks, with the resolving power in parentheses.