Mass and Charge Assignment for Electrospray Ions by Cation Adduction

Michael W. Senko, Steven C. Beu, and Fred W. McLafferty

Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, New York, USA

The assignment of the mass (m) value from the m/z value for ions with a multiple number of charges (z) in electrospray mass spectra usually utilizes multiple peaks of the same m but different z values, or unit-mass-separated isotopic peaks of the same z value from high resolution spectra. The latter approach is also feasible with much less resolving power using adduct ions of much higher mass separation. The application of this to mixture spectra containing many masses, such as spectra from tandem mass spectrometry (MS/MS) ion dissociation, does not appear to have been pointed out previously. Thus, replacing two protons by one Cu^{2+} ion increases the mass by 61.5 Da, with this shift providing a mass scale for assignment of m and z from this pair of m/z values. The more common Na^+ adduct peaks provide a 22.0 Da separation, of utility for 1000 resolving power only below approximately 10 kDa. Further, collisional dissociation lowers the degree of Cu^{2+} adduction in the resulting sequence-specific fragment ions much less than that of the corresponding Na^+ adducts, making the Cu^{2+} adducts far more useful for m and z determination in MS/MS studies. (*J Am Soc Mass Spectrom* 1993, 4, 828–830)

Electrospray ionization (ESI) to produce ions with a multiple number of charges (z) has extended the capabilities of mass spectrometry for large biomolecules by allowing measurement in the limited m/z range of most instruments [1]. However, the ESI benefit of multiple charging is also a complication in that mass (m) determination from a peak's m/z value requires that z be assigned. For spectra with few m values and multiple z values, this is usually possible from the spacing between peaks of the same m , but different z , using simple algorithms [2–4]. However, for mixtures yielding many m values, especially those from ion dissociation [5, 6], identification of same m ions of multiple z values is equivocal and often not possible. In the pioneering tandem mass spectrometry study of Smith and co-workers [6] on the dissociation of $(M + 18\text{H})^{18+}$ of the hemoglobin β^A chain (146 amino acids), only one of the 13 fragment masses identified from the known sequence could have been identified from a multiplicity of peaks of different z and the same m . Assignments can be made from a single z value if the $^{12}\text{C}/^{13}\text{C}$ isotopic peaks can be resolved, as their spacing provides a unit-mass scale [7]. However, sufficient resolution for large ions (> 10 kDa) is only obtainable presently using the Fourier-transform instrument [8–12].

Such reference spacing to provide a mass scale measured at less than unit-mass resolution can also be obtained from adduct peaks, for which many types have already been described for both positive and negative ions in the ESI literature [13–16]. Apparently the only method proposed for single- m -single- z value peaks was that of McLuckey et al. [17] using ion-molecule clustering and stripping reactions such as $(\text{insulin} + n\text{H})^{n+}$ plus 1,6-diaminohexane. A disadvantage of this method is that it requires either an ion-trapping or triple-quadrupole instrument to perform the ion-molecule reaction. This note describes the similar utility of copper adducts formed simply in the ESI feed solution by adding millimolar cupric acetate to the sample (or passing acidic sample solutions through copper/brass fittings in the ESI system [12]); this yields spectra with adduct peaks spaced at 61.52 Da intervals, requiring resolving power 1/62 of that utilizing isotopic spacing. Chemical derivatization can also provide such a mass scale [18], but this can require knowledge of the sample structure and can greatly increase sample consumption.

Experimental

Electrospray ions are generated from 20 μM protein solutions with 2% acetic acid and 76% MeOH by volume, with 0.5 mM cupric acetate added to provide the mass scale. Spectra were collected using a Fourier-transform mass spectrometer, described separately [12].

Address correspondence to: Prof. Fred W. McLafferty, Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, NY 14853-1301.

Single-scan low-resolution spectra are obtained using 4 K data points at a bandwidth of 200 kHz. For confirmation of charge assignments, high resolution spectra were collected at the same bandwidth using 256 K data points.

Results and Discussion

The most common instruments used for ESI cation spectra can achieve resolving powers of 500–1000. For an internal mass scale consistent with these capabilities, additional peaks can be formed by cation impurities replacing H^+ in the ion [1, 13–16]. For example, the ESI spectrum of recombinant thioredoxin (11.7 kDa) shows $[M + (10 - n)H + nNa]^{10+}$ peaks for $n = 0-7$ separated by 22.0 Da [9]. However, for the unresolved isotopic peak cluster representing each value of n , the width at half height is 13.2 Da at a resolving power of 1000, so that this sodium ion mass scale would be marginal for the most common ESI mass spectrometers. A larger mass separation obviously will reduce the required resolving power, and the same mass spectrum at high resolution showed a peak approximately 61 Da higher, identified as due to the adventitious presence of Cu^{2+} [12]; replacing two protons by one copper should increase m by 61.52 Da for the isotopically unresolved peak ($[^{63}Cu]:[^{65}Cu] = 100:45$). Cu -adduct peaks allowing direct z assignment have also been observed in the spectra of mellitin, ubiquitin, cytochrome *c*, hemoglobin, myoglobin, and carbonic anhydrase using the brass-fitting ESI apparatus [12].

Addition of NaCl at concentrations less than 0.1 mM to sample solutions of ubiquitin (8.6 kDa) showed extensive adduction, forming species with up to five Na^+ attached. At 1000 resolving power the separate charge-state peaks are discernible. However, applying a nozzle-skimmer potential high enough to cause dissociation of covalent bonds [19] lowered the degree of Na^+ adduction to the molecular ions, and no Na^+ adducts were observed on any fragment ions. Addition of KCl at concentrations above 1 mM gave almost no K^+ adducts to either the molecular ions or fragments.

The Cu^{2+} adduct mass scale was also tested with an ESI spectrum from the nozzle-skimmer dissociation of ubiquitin; unusual stability has been reported for Cu^{2+} adducts of small peptides [13–15]. Figure 1 shows a portion of the spectrum containing the $(M + 10H)^{10+}$ and $(M + 11H)^{11+}$ molecular ions and the y_{58}^{8+} fragment ion at a resolving power of approximately 1000. Adding 0.5 mM cupric acetate to the sample solution and rerunning under the same ESI conditions gave the Figure 2 spectrum. The group of peaks starting at m/z 858 shows spacings of 6.12 m/z units, clearly indicating a charge state of 10^+ ($61.52/6.12 = 10.05$); note that replacing only one H^+ by one Cu^+ would yield a value nearly as accurate ($62.53/6.12 = 10.22$). The y_{58} fragment ion also shows a surviving adduct peak shifted by 7.89 m/z units, providing an assignment of

Figure 1. Partial ESI Fourier-transform mass spectrum of ubiquitin with a nozzle-skimmer potential of 180 V.

$z = 8$ ($61.52/7.89 = 7.8$). Although formation of the y_{58} fragment ion involves only a 25% mass loss from the molecular ion, its ability to retain Cu^{2+} ions has obviously been reduced several fold. Less abundant fragment ions such as y_{24}^{4+} and y_{18}^{3+} were visible in the Figure 2 spectrum, but with signal/noise ≤ 10 , so that their copper adduct peaks were not discernible. The addition of Cu^{2+} to the sample solution changes the relative abundances of the y_{58}^{7+} from 37, 100, and 37, respectively, to 63, 100, and 26, indicating that the loss of charge accompanies Cu^{2+} loss, consistent with a unimolecular dissociation; displacement of Cu^{2+} by H^+ ions in the high pressure collision region would not lower the charge state.

The use of other heavy adduct ions should be investigated to find those effective at lower concentrations or that suffer less loss during fragmentation. Multidentate binding of different ions causing specific fragmentation could even be useful for structural characteri-

Figure 2. Mass spectrum under the same conditions as Figure 1 except with 0.5 mM $Cu(OAc)_2$ added to the sample solution.

zation [20]. Although cation adduction provides an alternative method for mass and charge assignment, this requires a separate spectral measurement to avoid the reduced signal/noise of the structurally indicative peaks caused by the addition of the mass-scale adduct peaks. Resolved isotopic peaks still provide the most convenient mass scale for assignment of m/z values, effective for low abundance ions and requiring no sample modification.

Acknowledgments

We gratefully acknowledge the help of P. B. O'Connor, J. P. Quinn, and F. M. Wampler, and the generous financial support of the National Institutes of Health (Grant GM16609) and the Gavlin Foundation.

References

1. Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64-71.
2. Mann, M.; Meng, C. K.; Fenn, J. B. *Anal. Chem.* **1989**, *61*, 1702-1708.
3. Covey, T. R.; Bonner, R. F.; Shushan, B. I.; Henion, J. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 249-256.
4. Reinhold, B. B.; Reinhold, V. N. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 207-215.
5. Loo, J. A.; Udseth, H. R.; Smith, R. D. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 207-210.
6. Light-Wahl, K. J.; Loo, J. A.; Edmunds, C. G.; Smith, R. D.; Witkowska, H. E.; Shackleton, C. H. L.; Wu, C.-S. C. *Biol. Mass Spectrom.* **1993**, *22*, 112-120.
7. Henry, K. D.; McLafferty, F. W. *Org. Mass Spectrom.* **1990**, *25*, 490-492.
8. Henry, K. D.; Quinn, J. P.; McLafferty, F. W. *J. Am. Chem. Soc.* **1991**, *113*, 5447-5449.
9. Loo, J. A.; Quinn, J. P.; Ryu, S. I.; Henry, K. D.; Senko, M. W.; McLafferty, F. W. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 286-289.
10. Beu, S. C.; Senko, M. W.; Quinn, J. P.; McLafferty, F. W. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 190-192.
11. Suckau, D.; Shi, Y.; Beu, S. C.; Senko, M. W.; Quinn, J. P.; Wampler, F. M. III; McLafferty, F. W. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 790-793.
12. Beu, S. C.; Senko, M. W.; Quinn, J. P.; Wampler, F. M.; McLafferty, F. W. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 557-565.
13. Hutchens, T. W.; Nelson, R. W.; Allen, M. H.; Li, C. M.; Yip, T. *Biol. Mass Spectrom.* **1992**, *21*, 151-159.
14. Hutchens, T. W.; Allen, M. H. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 469-473.
15. Allen, M. H.; Hutchens, T. W. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 308-312.
16. Loo, J. A.; Ogorzalek Loo, R. R.; Light, K. J.; Edmunds, C. G.; Smith, R. D. *Anal. Chem.* **1992**, *64*, 81-88.
17. McLuckey, S. A.; Clish, G. L.; Van Berkel, G. J. *Anal. Chem.* **1991**, *63*, 1971-1978.
18. Loo, J. A.; Edmunds, C. G.; Smith, R. D. *Anal. Chem.* **1993**, *65*, 425-438.
19. Smith, R. D.; Loo, J. A.; Barinaga, C. J.; Edmunds, C. G.; Udseth, H. R. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 53-65.
20. Speir, P.; Gorman, G. S.; Amster, I. J. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 106-110.