Improved Fourier-Transform Ion-Cyclotron-Resonance Mass Spectrometry of Large Biomolecules

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Initial results from a Fourier-transform mass spectrometer with a 6.2 Tesla magnet using electrospray ionization show substantial improvements in resolution, mass accuracy, mass range, signal/noise, and tandem mass spectrometry capabilities compared to our earlier 2.8 T instrument that demonstrated the first unit resolution mass spectra of molecules as large as myoglobin (17 kDa). The new instrument exhibits greater than 10^6 and 10^5 resolving power for 8.6 and 29 kDa, respectively, proteins. Using an internal standard, the mass measuring error for myoglobin is less than 1 ppm. Nozzle-skimmer dissociation during electrospray of carbonic anhydrase (29 kDa) has yielded 38 fragment ions for which both mass and charge are identifiable; of these, 21 have been assigned to expected oligopeptide fragments. (*J Am Soc Mass Spectrom* 1993, 4, 190–192)

Two of the most exciting recent advances in mass spectrometry have been ionization methods for large molecules [1–3] and tandem mass spectrometry for sequencing linear molecules such as those of biological importance [4–6]. The effective exploitation of these, especially in combination, requires unusual instrumentation improvements in resolution, mass accuracy, sensitivity, and speed. Recently we have reported that Fourier-transform (ion-cyclotron-resonance) mass spectrometry (FTMS) offers promising advances in each of these areas [7–9]. Electrospray ionization (ESI) mass spectra of proteins as large as myoglobin (17 kDa) were reported with resolving powers of 70,000 but this was reduced to below unit resolution for larger molecules [7, 8]. Such unit resolution capability solves a serious restriction of assigning mass (m) values from the mass to charge ratio (m/z) values for the multiply charged ions produced by ESI; the $^{12}\text{C}/^{13}\text{C}$ isotope peaks must exhibit unit mass spacing, so that the number of them in a single mass to charge ratio unit must represent the number of charges, z . This is especially valuable for tandem mass spectra containing ions of many mass values and/or of few z values. With frequency calibration, mass measuring accuracies of 20–50 ppm were achieved; this was reduced to 5 ppm for myoglobin measured with an internal standard. This resolution could be achieved for all masses over the range 500–17,000, measuring

these simultaneously in broadband mode after admitting only a few femtomoles of sample. Other similar achievements with ESI/FTMS have been reported recently [10–12].

We report here improvements in ESI/FTMS performance made possible by instrument modifications including a heated metal capillary on the electrospray interface [13, 14], an open analyzer cell [15] of cylindrical geometry, a higher field magnet, use of accumulated trapping [16, 17], a faster pumping system, and a higher capacity data system.

Experimental

The 2.8 T magnet of the previous instrument [7–9] was replaced with a 6.2 T magnet of Extrel FTMS, Millipore Corp. The ion and diffusion pumps on either end of the magnet were replaced by Cryo-Torr model 8 cryopumps (1500 L/s) from CTI-Cryogenics. An all-copper open ion analyzer cell [15] of cylindrical geometry was constructed and installed in place of the original stainless-steel closed cell. The electrospray source was modified to include a resistively heated metal capillary [13, 14] with no counter current gas flow for desolvation. Ion measurement employed chirp excitation and an Extrel Odyssey Data Station that acquired and processed 1M data points per spectrum (256K in the previous system) [7, 8]. This allows measuring transients for 3 s at a bandwidth of 150 kHz, corresponding to a lower mass limit of m/z 600. Heterodyne techniques were employed to acquire for longer peri-

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ods for ultrahigh resolution measurements. Ions were injected and accumulated in the trapped ion cell for 0.5 to 2.0 s [16, 17], while simultaneously increasing the analyzer pressure to 5×10^{-6} torr with nitrogen introduced through a pulsed valve to enhance trapping efficiency.

Results and Discussion

A previous electrospray interface [7–9] delivered 1–3 pA of ion current to the analyzer cell, yielding spectra of signal/noise of approximately 10/1. The modified electrospray system, improved pumping, higher ion injection energies, and pulsed-gas-assisted accumulated trapping have resulted in 30–150 pA delivered to the analyzer cell, giving spectral signal/noise even greater than 100/1. The ion accumulation process yields a larger trapped ion population at the expense of greater sample consumption per ion acquisition event, with 0.25–2.5 pmol of sample admitted for each spectrum.

For ubiquitin (8.6 kDa, Figure 1), a resolving power (RP) of greater than 10^6 and signal/noise greater than 80:1 is achieved with heterodyne mode data acquisition. For carbonic anhydrase (29 kDa, Figure 2), RP = 75,000 was achieved for an average of nine charge states, 27+ to 35+. For large molecules such as albumin (66 kDa), isotopic peak resolution has not yet been achieved. This may be due to the higher charge density on the larger ions and/or difficulty in acquiring and maintaining sufficiently quiescent ion populations.

The improved signal/noise also improves the relative abundance accuracy of isotopic peaks (see Figures 1 and 2). As noted previously [7, 8], correct identification of the isotopic peak composition is necessary to avoid errors of one (or more) full mass unit(s) in

Figure 1. Single scan ESI spectrum of the 11+ charge state of ubiquitin RP greater than 10^6 acquired in heterodyne mode, observation time 19 s. Inset: theoretical isotope distribution, RP = 2×10^5 .

Figure 2. Single scan ESI spectrum of carbonic anhydrase acquired in broadband mode. Each of the isotopic envelopes for the charge states 27+ to 35+ have been normalized to zero charge and averaged. Inset: theoretical isotope distribution, RP = 10^5 .

molecular weight determination. Illustrating this, Biemann and co-workers [18] have pointed out a one mass unit error in the predicted molecular weight of myoglobin because amino acid 122, originally identified as asparagine, should be aspartic acid; using the erroneous prediction, our original FTMS measurement of this molecular weight [7] was in error by a full mass unit, even though the value in the tenths decimal place was correct. Computer techniques for more accurate assignment of such isotopic-peak elemental compositions will be reported separately [19].

The improved resolution also gives improved mass measuring accuracy. For carbonic anhydrase (see Figure 2), the mass of the most abundant isotopic peak ($^{13}\text{C}_{17}$) using external-standard frequency calibration with ubiquitin is 29,023.2, while the calculated value is 29,023.7, a 17-ppm difference. Repeating the earlier measurement of the molecular weight of myoglobin by matching its $(M + 18\text{H})^{18+}$ ions against the $(M + 13\text{H})^{13+}$ ions of chicken cytochrome *c* [7] gave the spectrum of the overlapping isotopic envelopes of Figure 3. One peak represents contributions from both charge states of very similar mass to charge ratio values calculated to be 942.564 and 942.562, respectively. This 2-ppm agreement is reflected in the peak's apparent resolving power of 106,000 (half height width, 0.16 Da), comparable to the average RP values for five of the cytochrome *c* peaks and seven of the myoglobin peaks of 96,000 and 109,000, respectively. Even greater mass accuracy can be achieved using all 17 designated peaks of Figure 3. The average mass to charge ratio differences of the isotopic peaks are 0.05584 ± 0.00103 for myoglobin and 0.07723 ± 0.00105 for cytochrome *c*, versus the expected values of $1.0034/18 = 0.05574$ and $1.0034/13 = 0.07718$, respectively; these are errors of much less than 1 ppm of the molecular mass. Using the mass difference between the unknown and calibrant isotopic peaks m/z 942.506 and 942.486 gives an

Figure 3. Single scan ESI spectrum of the 13+ charge state of cytochrome *c* (circles) and the 18+ charge state of myoglobin (squares) acquired in broadband mode. Measured mass to charge ratio differences of the isotopic peaks are indicated by values on the bars.

error of 2.5 mDa (0.15 ppm; 16,946.985 Da theory versus 16,946.983 found for $^{13}\text{C}_6$) in measuring the myoglobin mass. Such milli-Dalton accuracy has been exploited for decades for structural characterization at lower masses; 1-ppm accuracy at 17 kDa (17 mDa) is sufficient to distinguish isobaric glutamine and lysine, whose residue masses are 128.058 and 128.095, respectively ($\Delta m = 37$ mDa).

For tandem mass spectrometry, photodissociation or collisionally activated dissociation in the analyzer itself shows increasingly poor trapping efficiency as the precursor ion mass increases. Although photodissociation of gramicidin S (1.1 kDa) produces eight identifiable sequence fragment ions, that of ubiquitin, 8.6 kDa, produces only five such ions. Nozzle-skimmer dissociation [20] during the electrospray process is more effective, producing nine identifiable sequence ions from ubiquitin. Even the nozzle-skimmer dissociation of carbonic anhydrase (29.0 kDa) yielded 38 fragment ions for which both mass and charge are identifiable from the separated isotope peaks; of these, to date 21 have been assigned to oligopeptide fragments (masses 2200–15,300) expected from the known structure. Obviously, the improved resolution is critical to these assignments; note that the approximately 20 peaks of Figure 2 represent less than 0.1% of the mass range recorded.

A more extensive description of the instrument and these applications is in preparation.

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