High-resolution tandem mass spectrometry of large biomolecules
(electrospray ionization/polypeptide sequencing)

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ABSTRACT Unit-resolution mass spectra have been obtained for peptides as large as 17 kDa, providing information on impurities and adduct ions, as well as accurate molecular weight values. Electrospray ionization produces many multiply-charged species of the same mass; isotopic peak resolution provides direct charge state assignment from the unit mass spacing of the isotopes. This is of special value when the spectrum also has many masses, such as from precursor ion dissociation or impurities. Mass measuring errors not only are concomitantly lower (<0.1 Da) than when the isotopic peaks are unresolved but also are independent of variations in 13C/12C natural isotopic abundances. Also, larger errors are avoided that occur when the measured peak envelope includes impurity or adduct ions. This also benefits tandem mass spectrometry; dissociation of peptide ions as large as 8.5 kDa yields fragment masses consistent (<0.1 Da) with their amino acid sequences.

Electrospray ionization (ESI) (1–23) has revolutionized large-molecule mass spectrometry (MS), forming biomolecule ions of masses (m) up to 200 kDa (15) that carry a large number of charges (z) and measuring their m/z values with accuracies of 0.01–0.1%. We have shown (23) that Fourier-transform mass spectrometry (FTMS) (21–26) can be used to measure simultaneously all such ions as large as 17 kDa with 50,000–80,000 resolving power and, by using an internal standard, with <0.1 Da (<6 ppm) mass errors. As reported here, this enhanced high-resolution capability is also valuable for characterizing impurities (6–8) adducts (9–11) and, by tandem mass spectrometry (MS/MS) (3, 7, 8, 12–15), amino acid sequences.

EXPERIMENTAL PROCEDURES

Radio frequency-only quadrupole lenses and five stages of differential pumping are used to transport the ions electrosprayed at ≈10–10 torr to the FTMS ion-measurement cell at <10–8 torr in a 2.8-T magnet. A pulse of N2 gas is admitted to the ion cell to cool the ions translationally before measurement; other experimental details were as given (21–23).

RESULTS AND DISCUSSION

All ions of m/z > 400 from equine cytochrome c are recorded (broadband, 256,000 data) in 1.7 s (Fig. 1; 3 fmol admitted). Although only the m/z values are measured, the mass scale is obvious from the m = 1.0034 spacing of the isotopic peaks; for (M + 16H)16+ of Fig. 1, there are eight peaks between m/z, 773.0 and 773.5. The monoisotopic molecular weight (mMr, excluding the mass of the added protons) is 12,352.32, and the measured value (weighted average of isotopic peak values; external standard: gramicidin S, mMr = 1141) is 12,352.20 (12,358.22 for the most abundant isotopic peak). Note that a 5-per-mil shift in the 13C/12C ratio would change the isotopically averaged molecular weight (centroid of the unresolved isotopic peaks) (1–18) by 0.3 Da but would only affect the abundances of the isotopic peaks, not their masses. These experimental procedures have not produced, to date, high-resolution ESI spectra of molecules as large as albumin (66 kDa), although its spectrum (Fig. 2) was improved (22).

By using a quadrupole mass spectrometer (6, 7), the ESI mass spectra of bovine ubiquitin, mMr, 8559.62, showed peaks of measured masses 114.7 Da (6) and 115.1 Da (7) below those of the (M + nH)n+ ubiquitin peaks; this was postulated (7) to result from an impurity missing the two carboxyl-terminal glycines, which would lower the mass by 114.04 Da. The ESI/FTMS mass spectrum of the same sample (7) (Fig. 3) shows an mMr of 8559.45 and peaks presumably from the same impurity at a mMr of 8445.43 (Δm = 114.02). Additional anomalous peaks not resolved in the quadrupole spectra (6, 7) give an average mMr of 8623.39 (Δm = 63.94); replacing a valine by a tyrosine would increase the molecular weight by 63.99. As noted (23), identifying which isotopic peak is the most abundant is compromised by poor abundance accuracy for single scans; the peaks contain only a few ions, and only about three data points are measured per peak in this broadband scan.

Pulsed gas cooling allows such a broadband high-resolution FTMS spectrum to be measured every 2 min; summing 10 spectra of recombinant thioredoxin (mMr, 11,667.06, Calbiochem; Fig. 4) lowered the resolution but improved isotopic abundance measurements and retained mass accuracy (measured mMr, 11,667.19). Peak groups at higher masses can be seen in the reported (6, 12) quadrupole/ESI mass spectra, but these were not assigned, even (6) with deconvolution techniques that combine peaks of the same mass but of different charge states (19, 20). Higher resolution (Fig. 4) shows that most of these peaks arise from other ions of the same charge spaced at intervals of ≈22 Da, consistent with substitution of H+ by Na+. However, some peak groups, such as those including the (M + 7H + 3Na)10+ isotopic peaks (Fig. 4, lower middle spectrum), contain extra

Abbreviations: ESI, electrospray ionization; MS, mass spectrometry; MS/MS, tandem MS; FTMS, Fourier-transform MS; CAD, collisionally activated dissociation; mMr, monoisotopic molecular weight.

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For C3 vs. C4 photosynthetic pathways, the 13C/12C ratio can vary by >25 per mil (66C: C4 = 22 to ~35; C3 = 8 to ~16) (27), corresponding to a 0.025 × 0.011 = 0.028% change in the carbon mass of the molecule.

Another published quadrupole/ESI spectrum of ubiquitin shows peaks that could arise from this source (18).
Fig. 1. ESI mass spectrum of equine cytochrome c (3 fmol of sample consumed, a 1000-s ion cooling, and a single scan of all ions above m/z 400 at an average resolving power of 63,000).

Fig. 2. ESI mass spectrum of porcine albumin (single scan, 64,000 data; transient lifetime, <4 ms).

Fig. 3. (Top) ESI mass spectrum of ubiquitin (sum of 10 scans, 64,000 data). (Center) Regions expanded to show the presence of impurities. (Bottom) MS/MS fragment ions from collisionally activated dissociation of (M + 10H)10+ and from placing 200 V between the nozzle and skimmer of the ESI source.
lower-mass peaks; these are consistent with the presence of an impurity whose molecular weight is 61 Da higher.

By using MS/MS (28–39), multiply-charged ESI peptide ions have been dissociated to form some product ions consistent with known sequences for large peptides, even albumin (66 kDa) and IgG (150 kDa) (3, 7, 8, 12–15). For unknowns, however, this multiplicity of isotopically unresolved fragment peaks makes mass assignment from the \( m/z \) value seriously ambiguous, as for each mass two or more corresponding peaks of different charge values must be identified. Collisionally activated dissociation (CAD) (3, 7, 8, 12–15, 28–35) of the (M + 10H)\(^{10+}\) peaks of ubiquitin (Fig. 3) gave isotopic peaks whose \( m/z \) spacing clearly shows their \( z \) values. This gives monoisotopic fragment masses (mM) excluding the mass of the added protons) of 2096.14 and 2725.46; the \( Y_3^{18} \) and \( Y_3^{24} \) fragment ions (30) have calculated mM values of 2096.19 and 2725.50. Dissociating all of the incoming ions by a 300-V potential difference between the nozzle and skimmer (2, 12–14) produced ions with a measured mM value of 6527.44, with the \( Y_3^{38} \) fragment (30) calculated to be 6527.49.

Attempts were also made to measure MS/MS spectra of larger ions. Molecular ions of cytochrome c, ribonuclease, myoglobin, and albumin were dissociated in the ion cell, but to date no appreciable quantities of fragment ions could be measured. [After CAD of the anti-RNA IgG immunoglobulin, only ions corresponding to the 24-kDa light chain, not the 51-kDa heavy chain, could be observed (13).] Dissociation methods utilized included CAD, 200–400-V nozzle–skimmer potential, 193-nm laser photodissociation (35, 36), and surface-induced dissociation with the ion cell at −50 or −100 V (37–39), attempting to collect these ions with pulsed gas cooling and/or electrostatic trapping. For solving these problems, our new 6.3-T instrument should have much higher capabilities.

Sequence information is routinely obtained from large peptides by degrading them enzymatically or chemically to form oligopeptide (<3 kDa) mixtures that are then characterized by MS/MS (30, 40, 41). With ESI/FTMS, this original degradation could be carried out instead in the mass spectrometer with this high-mass accuracy; pairs of fragment ions whose mass sum corresponds to that of the precursor should thus represent different parts of the precursor. From each part a further pair would be produced, continuing until each fragment ion is small enough for its spectrum to provide the sequence of that part. Ordering the parts could make possible reconstruction of the original molecular sequence, providing a complementary tool for characterizing large biomolecules.

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