Fourier-Transform Mass Spectrometry of Large Molecules by Electrospray Ionization

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Fourier-transform mass spectrometry of large molecules by electrospray ionization

(tandem mass spectrometry/peptide sequencing)

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ABSTRACT **The multiply charged ions produced by electrospray ionization of peptides of molecular masses up to 29 kDa have been successfully introduced into a Fourier transform mass spectrometer of unique capabilities for tandem mass spectrometry, large ion dissociation, and resolution. Electrospray ionization places an unusually high number of charges on a peptide yielding mass/charge (m/z) values of 600–1500; in this range at normal operating pressures ($\approx 10^{-9}$ torr; 1 torr = 133.3 Pa) Fourier-transform mass spectrometry resolving power is >100,000. Although only 10^{-7} torr pressure has been obtained with the initial interface, the resulting resolving power of 5000 makes possible the resolution of isotopic peaks of multiply charged ions. Mass measuring accuracies of a few daltons for molecular masses up to 17 kDa have also been achieved.**

Of the ionization methods that have revolutionized the applicability of mass spectrometry to large molecules, electrospray (1–10) has the unusual advantage of producing ions with by far the highest relative number of charges. This was first demonstrated by Fenn and coworkers (4–6), based on the pioneering work of Dole and coworkers (3), with very recent exciting applications to proteins and nucleotides with molecular masses up to 130 kDa by Smith, Loo, and coworkers (7, 8), and with highly accurate mass measurements by the Fenn (6), Smith (8), and Henion (9, 10) groups. For even the largest proteins, molecular ions with mass/charge (m/z) values of 600–1400 can be obtained with minimal fragmentation, making it possible to use a quadrupole mass spectrometer with correspondingly small upper mass limits (4–10). In considering alternative instrumentation, Fourier-transform mass spectrometry (FTMS) exhibits resolution that is uniquely high, although inversely proportional to the m/z value, with resolving power values of >100,000 reported for this m/z range (11–13). Increasingly large molecules require an exponential increase in the amount of information necessary for structural characterization; here, the extra data dimensions of tandem mass spectrometry (MS/MS, MS^n) are extremely promising (14, 15), especially for sequencing linear molecules (16–19). FTMS is also uniquely qualified for MS^n, as it can perform mass analyses sequentially in time with a single mass analyzer, not in space requiring multiple analyzers as with magnetic sector and quadrupole instruments. Furthermore, its multichannel detection of normal mass spectra over a wide mass range [e.g., 100–16,000 (20)] and the Hadamard method for multichannel detection of MS/MS spectra (ref. 21; E.R.W., S. Y. Loh, and F.W.M., unpublished work) promise greatly enhanced signal/noise ratios compared to instruments that must scan the second mass analyzer over such a large mass range for each selected primary ion. On the other hand, the pressure requirement for high-resolution FTMS is much more stringent than for most other mass spectrometers ($\approx 10^{-9}$ torr vs. $\approx 10^{-6}$ torr; 1 torr = 133.3 Pa), an obvious disadvantage for the atmospheric-pressure electrospray process. This paper reports successful FTMS spectra by using electrospray ionization for peptides and proteins of molecular masses up to 29 kDa, although ion source pressures of only 10^{-7} torr have been achieved with this preliminary system.

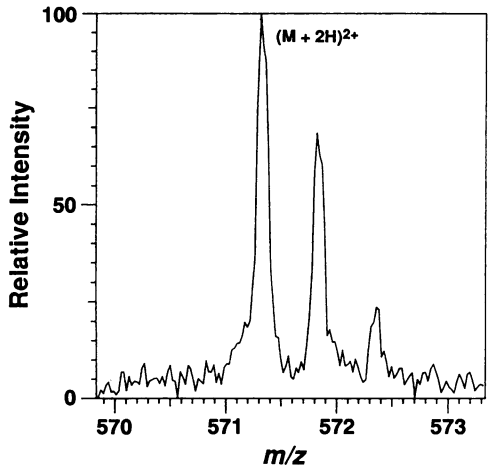

FIG. 1. Major mass spectral peaks from the electrospray ionization of gramicidin S.

EXPERIMENTAL

The Virginia FTMS instrument (19, 22, 23) has a 7 T magnet and a 2.8 × 2.8 × 7.8 cm ion cell, with ion introduction through 14, 25, and 86 cm rf-only (1.25, 0.87, 0.87 MHz) quadrupoles, each with an HV-202-6C 680 liters/s cryogenic pump. Electrospray (and "electrosplotch") ionization used gravity feed through a 90° bent syringe tip (500-μl Hamilton 700 series) with 30-gauge needle, 3- to 12-kV potential relative to the nozzle and skimmer at ground. Solutions of peptide in CH_3OH, with sufficient H_2O/AcOH for dissolution were added at 17 μl/min with heated counter-flow CO_2 as a drying gas, and with pumping before the 90° skimmer entrance to the first quadrupole by a 38 liters/s (1 torr) Sogevac SV280 rotary pump. A Nicolet FTMS-2000 data system was used to average several hundred scans for the spectra of larger molecules. The ribonuclease samples were supplied by H. A. Scheraga (Cornell University); other sam-

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Abbreviations: FTMS, Fourier-transform mass spectrometry; MS/MS, tandem mass spectrometry.

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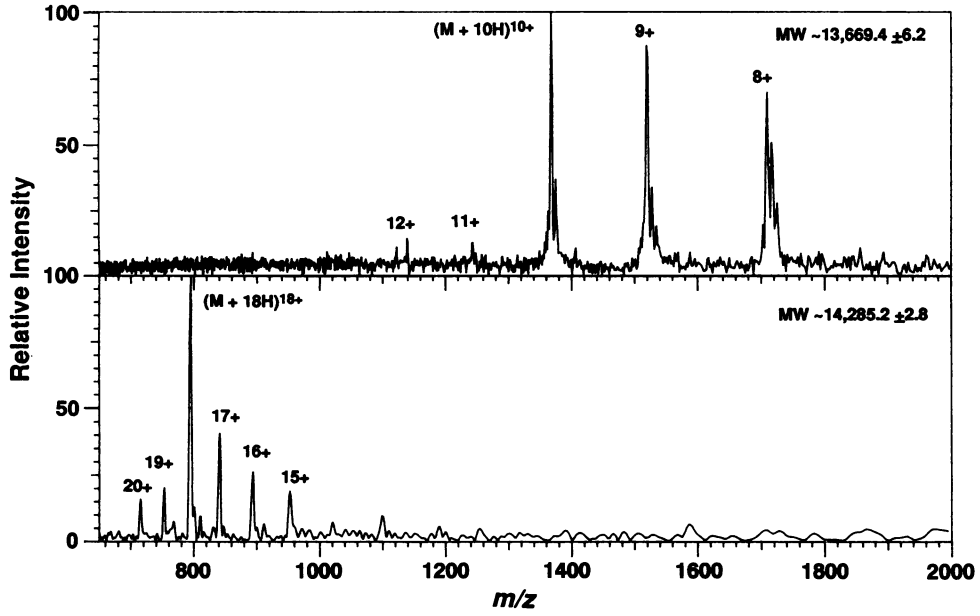

FIG. 2. FT mass spectra from the electrospray ionization of RNase A (*Upper*) and derivatized with methyl aminoethanethiolsulfonate (*Lower*) (24).

ples were from Sigma and were used without further purification.

RESULTS AND DISCUSSION

FTMS measurement of electrospray-produced ions of high molecular weight compounds has been achieved for a variety of peptides and proteins. The cyclic decapeptide gramicidin S gives dominant peaks at m/z 571.3, 571.8, and 572.3 (Fig. 1), representing the isotopic variants of the doubly charged ion $(M + 2H)^{2+}$. Although under the best vacuum conditions achieved ($\approx 10^{-7}$ torr) to date the resolving power was only 5000, this is sufficient to determine the number of charges on at least $(M + 5H)^{5+}$ ions, which will show five resolved isotopic peaks per m/z unit.

Bovine insulin, average molecular weight 5733.6, produces large $(M + 4H)^{4+}$ and $(M + 5H)^{5+}$ peaks, giving a calculated average molecular weight of 5733.5 ± 0.8 SD using the algorithm of Fenn and coworkers (6). Equine cytochrome *c*, average molecular weight 12,360, provides a spectrum relatively similar to that reported previously by Fenn and coworkers (4) and by Loo *et al.* (8). In keeping with the accuracy shown by these authors, our measured molecular weight utilizing seven molecular ions of different charges is 12,357 ± 0.9 SD. Similarly, the spectra of RNase A and its $(-SSCH_2CH_2NH_2)_8$ derivative (Fig. 2) and its reduced form (molecular weights 13,682, 14,291, and 13,690) indicate values of 13,669 ± 6.2, 14,285 ± 2.7, and 13,679 ± 7.3. The RNase A data show a relatively low effective proton affinity (even lower with reduced CO_2 flow rate), with m/z values above the upper m/z limit of most commercial quadrupoles. Derivatization nearly doubles the number of charges (Fig. 2 *Lower*) and reduction increases them by ≈50%, consistent with the higher basicity and the exposure of additional basic

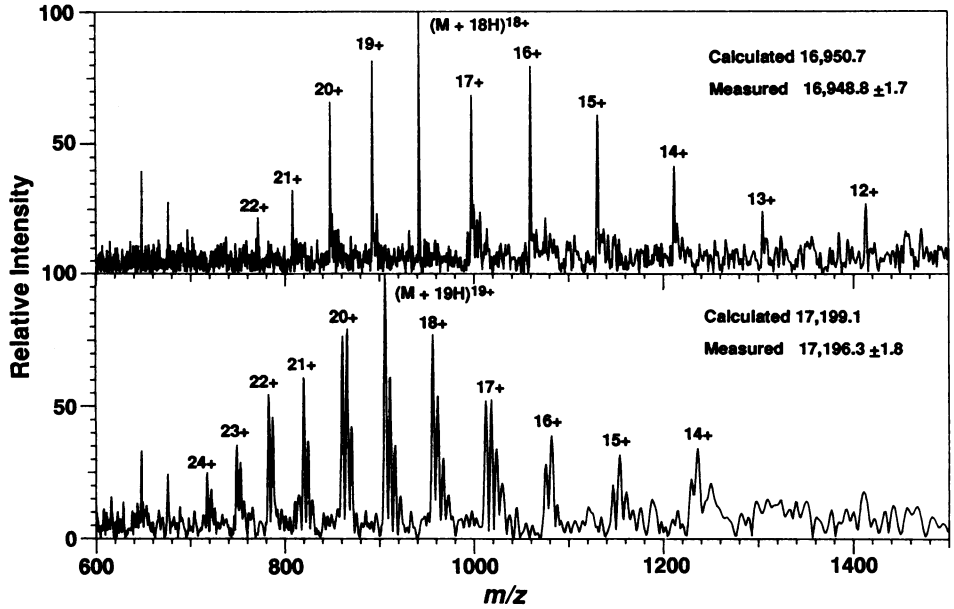

FIG. 3. FT mass spectra from the electrospray ionization of equine skeletal muscle myoglobin (*Upper*) and sperm whale skeletal muscle myoglobin (*Lower*).

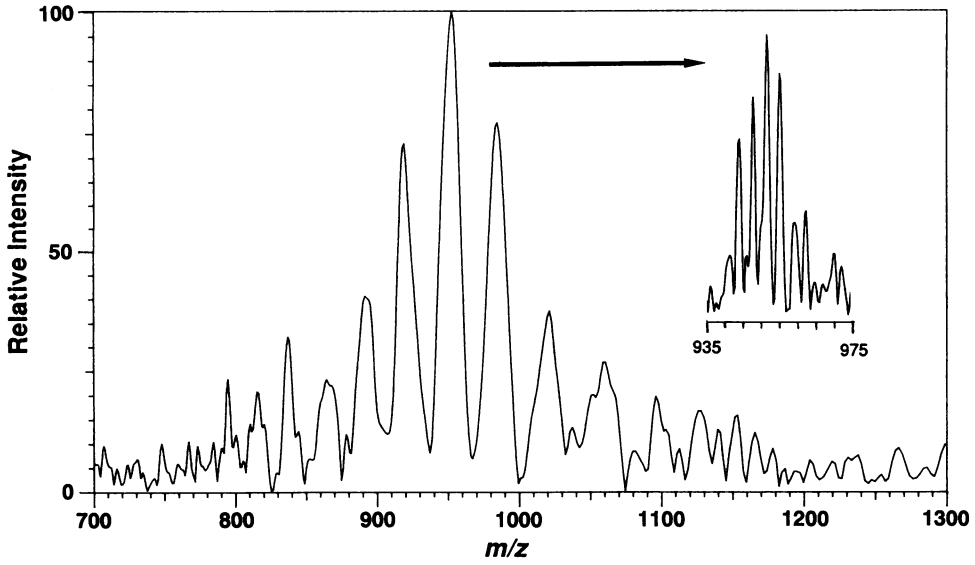

FIG. 4. FT mass spectrum from the electrospray ionization of carbonic anhydrase. FT of 16,000 data points. (*Inset*) Higher resolution from FT of 64,000 data points.

sites by unfolding. Two types of myoglobin (equine and sperm whale), of molecular weights 16,951 and 17,199, gave values of 16,949 ± 1.7 and 17,196 ± 1.8 (Fig. 3). These spectra were not run under identical experimental conditions, so the differences in degree of protonation do not necessarily reflect basicity differences. However, the sperm whale spectrum also shows peaks corresponding to values of 17,294 and 17,390, whose source we have not identified. In the spectrum of β-lactoglobulin B, Loo *et al.* (8) also observed similar peaks indicating values of 18,278, 18,372, 18,497, and 18,594; they have recently observed (personal communication) that these peaks are greatly reduced under conditions (increased nozzle/skimmer voltage bias) expected to reduce solvent clustering. Such peaks are also present in our spectrum of reduced RNase A and of carbonic anhydrase (Fig. 4), the largest molecule for which data were obtained. The molecular weight calculated from the spectrum of the latter is approximately that (≈29,022) found by Loo *et al.* (8). Again, more peaks are present for each charge; these were not removed by reverse-phase HPLC purification. MS/MS should aid in establishing the source(s) of these extra peaks.

The molecular weight measurements were based on a previous calibration with singly charged ions; note that all reported values are low. FTMS calibration is known to be affected by the number of ions in the source, so that use of an internal standard should improve mass accuracy if the resolution is sufficient to distinguish sample and standard peaks. The vacuum systems for ion introduction in both the Virginia and Cornell FTMS instruments are being modified with the expectation of improving resolution.

FTMS has unusual MS^n capabilities, in that its single mass analyzer can be used sequentially in time for repeated selection of one or more ions for their dissociation (or reaction) into product ions. These then can be mass analyzed for structural characterization, and the dissociation process can be repeated on these, and even on their products (MS^n), to obtain more detailed information on smaller parts of the molecule. The dissociation of FTMS-trapped ions by energetic species such as photons is also much more efficient than such dissociation of the fast ions of magnetic sector or quadrupole analyzers (11, 12). Fig. 5 shows the 193-nm laser photodissociation (refs. 22 and 23; E.R.W., J.J.P. Furlong, and F.W.M., unpublished work) of $(M + 2H)^{2+}$ ions from

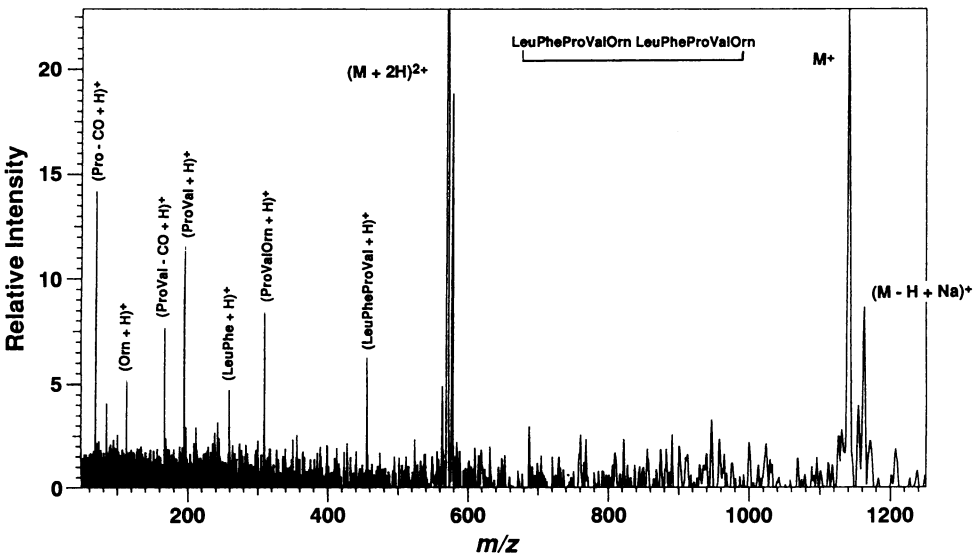

FIG. 5. Photodissociation of the ions from gramicidin S (Fig. 1) by using 193-nm photons from a pulsed excimer laser.

gramicidin S. The six labeled sequence peaks correspond to singly charged oligopeptide fragments found in the similar photodissociation of the singly charged ion. Surface-induced dissociation, a method pioneered by Cooks for magnetic sector and quadrupole mass spectrometers (25), also appears very effective for the 25- to 100-eV translational energy of FTMS ions (unpublished work). For MS/MS of $(M + nH)^{n+}$ of a specific mixture component, FTMS has the special sensitivity advantage that ions of many n values can be selected and dissociated simultaneously by the SWIFT technique (26). Collisionally activated dissociations (27) of multiply charged ions (28), including those from electrospray ionized proteins up to 17 kDa,[§] have been achieved recently but are poorly understood at this time because of their complexity; determining the number of charges in the spectra in Figs. 2–5 requires identifying peaks of different charges having the same mass. Unit mass resolution (Fig. 1) makes this unnecessary; with improved differential pumping FTMS resolving power of >>17,000 should be possible.

[§]Smith, R. D., Edmonds, C. G., Loo, J. A., Udseth, H. R. & Barinaga, C. J., Sixth Montreaux Symposium on Liquid Chromatography/Mass Spectrometry, July 21, 1989, Ithaca, NY, p. 30.

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