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Mass spectra of proteins and other biomolecules recorded using a handheld instrument

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Dedicated to Bob Cotter in appreciation of his remarkable contributions to analytical instrumentation.

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ABSTRACT

Proteins (myoglobin and cytochrome *C*), peptides (bradykinin and melittin), alkaloids in complex plant materials, and mixtures of phospholipids from a bacterial extract all give characteristic electrospray mass spectra using a handheld tandem mass spectrometer, Mini 10. The mass/charge range of the 10 kg Mini 10 was extended to m/z 2000 using resonant ion ejection at low frequency allowing analysis of proteins with molecular weights up to 17,000 Da. Fragmentation of peptides and proteins was observed.

Biomolecule characterization, including molecular weight, sequence and conformational information, is provided by mass spectrometers of various types [1–4]. It is possible to foresee mass spectrometric capabilities being extending to clinical diagnostics and personalized medicine, where in situ near-real-time analysis will be highly desirable. Such a development will require small and lightweight but powerful instruments. Miniature mass spectrometers capable of ionizing condensed-phase samples, as described here, represent one possible solution.

Building on a decade of work on small mass spectrometers [5], we recently developed a 10 kg handheld mass spectrometer [6] based on an ion trap mass analyzer [7,8] and fitted it with an electrospray ion (ESI) source [9,10]. Lab-scale ESI mass spectrometers have multiple stages of differential pumping to allow ions to be transferred from the ESI source to the mass analyzer under vacuum. By contrast, we use neither ion optical components nor differential pumping when interfacing an ESI ion source to a mini mass spectrometer; we simply use a 10 cm stainless steel capillary tube of 127 μm ID to transfer the ions directly into the vacuum manifold of the 10 kg instrument (Fig. 1a) (see Supplementary Information for a description of ion losses in the capillary). The limited pumping capacity of the miniature instrument results in a manifold pressure of 20 mTorr, which is several orders of magnitude higher than that used in lab-scale mass spectrometers. Nevertheless, using the pressure-tolerant rectilinear ion trap (RIT) [11] mass analyzer, we are able to record mass spectra at this pressure. Adequate analytical performance was achieved using the residual air rather than adding helium, as bath gas [5,6].

Complex biological mixtures are readily characterized using the miniature mass spectrometer. The spectrum of an extract of the seed of conium maculatum (poison hemlock) displays peaks for

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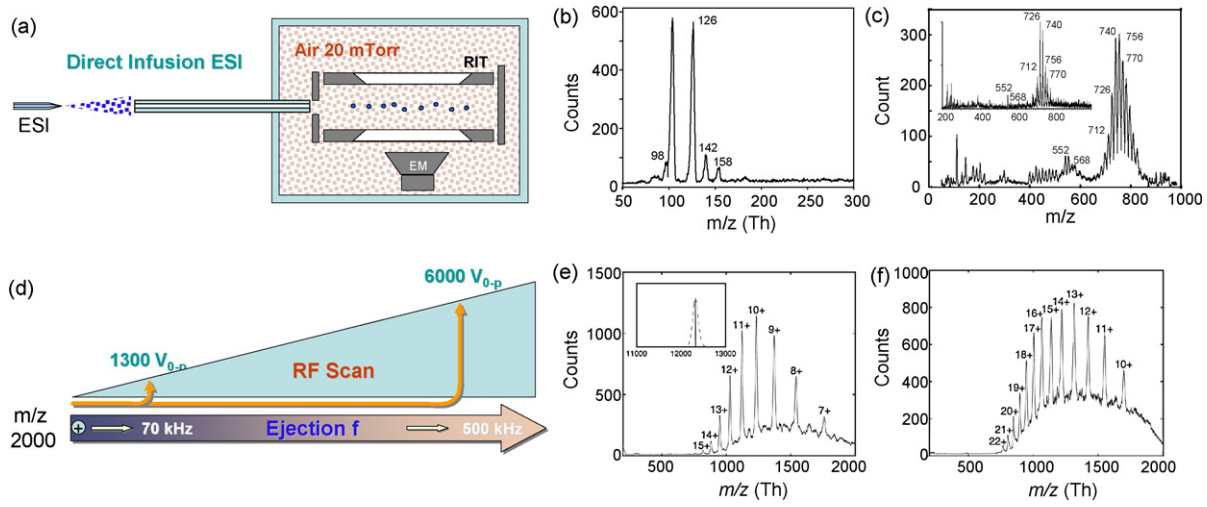

Fig. 1. (a) Configuration of the handheld Mini 10 mass spectrometer for bioanalysis. ESI mass spectra of (b) conium maculatum (poison hemlock) seed, showing the presence of γ-coniceine as the active alkaloid ingredient at *m*/*z* 126 and *N*-methyl coniine and *N*-methyl pseudoconhydrine at *m*/*z* 142 and 158, respectively, and (c) *E. coli* K-12 bacterial lipid extract showing numerous phospholipids. The inset of (c) shows the same extract run on a commercial LTQ. The lipids were identified mainly as phosphoethanolamines (PE). (d) High mass/charge range achieved using low amplitude RF voltages by ejecting ions at low frequency. Ions of *m*/*z* 2000 normally ejected at 6000 V are ejected at 1300 V using resonant ejection. This method is used to record ESI spectra of (e) cytochrome *C* ejected at 70 kHz and (f) myoglobin ejected at 80 kHz. The inset of (e) shows the charge deconvoluted spectrum (i.e., the pure mass spectrum rather than mass/charge spectrum); the peak width is due to natural isotopic contributions.

all the major alkaloids [12] (Fig. 1b), including γ-coniceine, *N*-methyl coniine and *N*-methyl pseudoconhydrine (*m*/*z* 126, 142 and 158, respectively). The spectrum of a lipid extract of *E. coli* shows phosphatidylethanolamine at *m*/*z* 712, 726, 740, 752, 766 and 780 (Fig. 1c) and compares well with data obtained using a commercial instrument.

The handheld mass spectrometer has a low power RF driving circuit (1300 V_{max}) which provides a mass/charge range up to *m*/*z* 550. Examination of biomolecules such as peptides and proteins requires a mass/charge range above *m*/*z* 2000. To extend the mass range, ions were resonantly ejected at lower frequency (Fig. 1d) using a supplementary ac signal. Using a resonance ejection frequency of 70 kHz, a scan range as high as *m*/*z* 2000 can be accessed. Fig. 1e shows the mass spectrum of cytochrome *C*, a 12.3 kDa protein, recorded in this way. The spectrum is of a quality that allows a fully automated charge state deconvolution by the ZSCORE algorithm [13], as seen in the insert in the figure (the inner curve in the insert indicates the peak width broadening due to the isotopic distribution, as calculated using IsoPro software [14]). Fig. 1f shows the mass spectrum of myoglobin, a protein with molecular weight 17.1 kDa, recorded at a resonance ejection frequency of 80 kHz. Note that the high signal (apparently background, but see below) around the peaks for myoglobin is possibly due to the different charge states lying relatively close together.

Fig. 2 shows the mass spectra of three peptides, bradykinin (1059 Da), KGAILKGAILR (1139 Da) and melittin (2843 Da), recorded at ejection frequencies of 120, 140 and 120 kHz, respectively. Apart from these peptides, spectra were recorded for the glycopeptide vancomycin (1449 Da) and the polypeptide hormone insulin (5808 Da,) and showed characteristic multiply charged ions (+1 and +2 for vancomycin and +1, +2 and +3 for insulin, see Supplementary Material). In each spectrum, the main peaks associated with the different charge states of the intact molecules are easily identified. In addition, there occur a number of other signals due to fragment ions. This was confirmed using a commercial benchtop ion trap mass spectrometer to record MS/MS, MS^3 and MS^4 spectra. The same peaks occur as fragments from collision-induced dissociation of the multiply charged molecular ions (inserts in Fig. 2). A large number of the peaks in the full scan spectra are reproduced in the MS/MS product ion spectra, not just with respect to their mass/charge values but also their mutual relative intensities.

Another feature of the peptide and protein spectra in Figs. 1e and f and 2, apparent upon comparison with similar data from a full-size instrument, is the absence of peaks of highly charged ions in the low *m*/*z* region. Fig. 3 compares the ESI mass spectra of ubiquitin, an 8.6 kDa protein, recorded using the Mini 10 (at 80 kHz resonance ejection) and the LTQ instrument fitted with the same electrospray ion source. Using the LTQ (mass/charge limit *m*/*z* 2000), the 5+ ion is barely present while the 13+ peak at *m*/*z* 660 is clearly present. By contrast, using the miniature instrument, the 5+ ion is the most abundant and the charge state ranges from +10 down to +4. A similar shift in charge states between the two instruments is observed for all proteins and peptides investigated.

The mass resolution in the miniature instruments is modest (ca. 100) at the high pressure used, although unit resolution is available at lower pressures. For the melittin spectrum recorded at 120 kHz, the width of the 4+ peak at *m*/*z* 712 is 7 and the 2+ peak at *m*/*z* 1423 is 14. The Cytochrome *C* spectrum, recorded at 70 kHz, displays peak widths of 13 for the 13+ peak at *m*/*z* 949 and 15 for the 8+ peak at *m*/*z* 1542. In the spectrum of cocaine a peak width of 3 is observed for the protonated molecule at *m*/*z* 304 with a optimal resonance ejection frequency at 350 kHz, while the peak width goes down to about 1 when the pressure is reduced to about 1 mTorr. Two operational factors responsible for the low resolution are the relatively high pressure (20 mTorr) and the low frequencies used for resonance ejection.

The high degree of fragmentation of peptides, as well as the high background in the protein spectra, is likely attributable to a continuum of fragment ions. However, in the case of other small molecules, no fragmentation is observed in the spectra, unless deliberately induced using CID in an MS/MS spectrum. This is a remarkable contrast, especially since peptide bonds are strong and typically require higher energies to cleave than most other chemical bonds. A single explanation accounts for both the shift in the charge states between the two types of instruments and the difference in the degree of fragmentation. The atmospheric pressure interfaces are very different: multiple differential pumping stages are used in lab-scale instruments but the ions are transferred from 760 to 20 mTorr on Mini 10 with a single stage. The ions trapped in the RIT

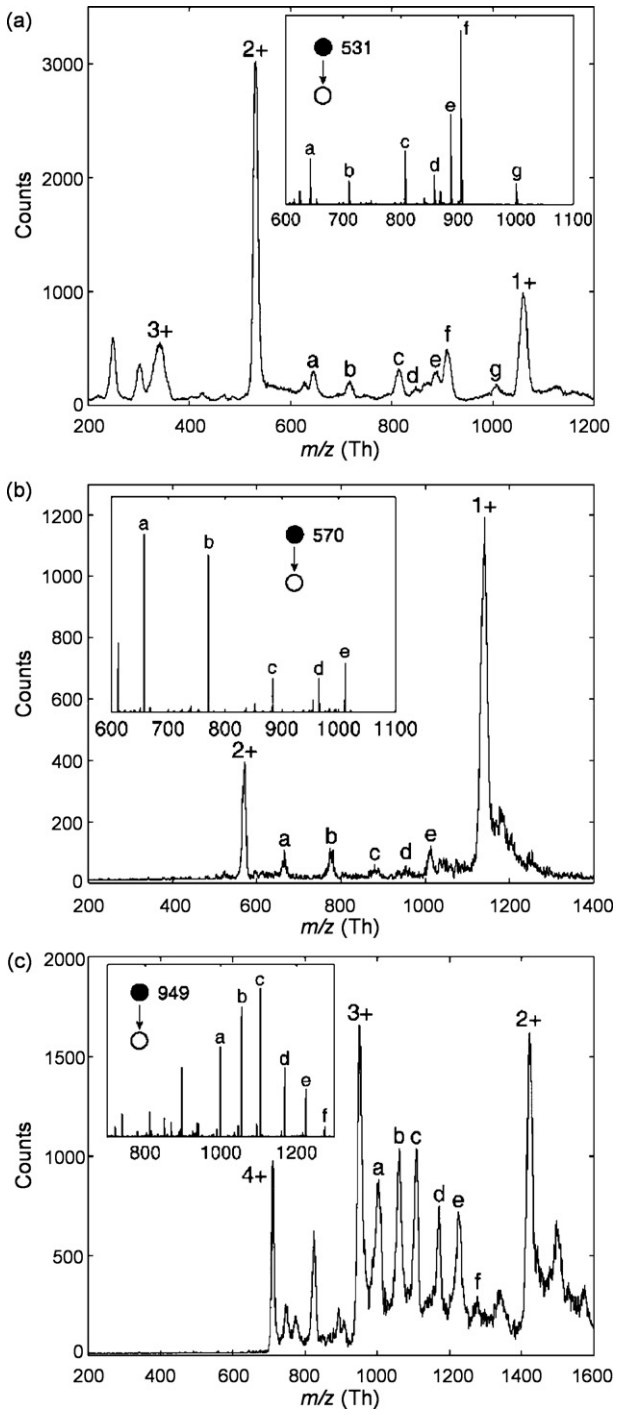

Fig. 2. Mass spectra of the peptides (a) bradykinin, (b) KGAILR and (c) melittin recorded using resonance ejection at 120, 140 and 120 kHz, respectively. The insert shows fragmentation patterns in MS/MS spectra recorded on the commercial LTQ.

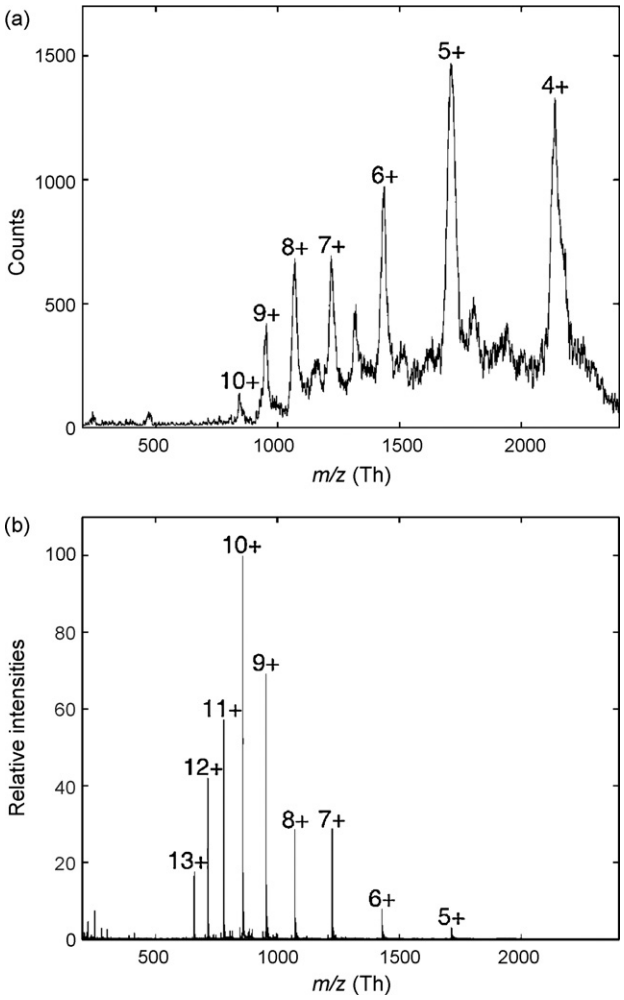

Fig. 3. Mass spectra of ubiquitin recorded using (a) handheld Mini 10 and (b) commercial LTQ, showing differences in charge states and degree of fragmentation.

are subjected to the gas flow through the capillary, which results in a high degree of fragmentation through CID. A recently developed discontinuous atmospheric pressure interface [15], which closes after the ion introduction, might help to minimize the fragmentation of the trapped ions. The difference in the atmospheric pressure interfaces is also the reason for the differences in desolvation, which is known to be high relevant to the observed charge state distributions for ions generated by ESI [16,17]. The new capabilities of recording the mass spectra of proteins and other biological samples using a miniature mass spectrometer have potential significance in biomedical research and clinical practice.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2008.04.022.

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