Applications of sustained off-resonance irradiation (SORI) and quadrupolar excitation axialization (QEA) for the characterization of biomolecules by Fourier-transform mass spectrometry (FTMS)

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Introduction
Electrospray ionization (ESI) [1] and matrix-assisted laser desorption ionization (MALDI) [2] have become increasingly important techniques for ion generation in MS. In conjunction with a Fourier-transform MS (FTMS), they enable one to obtain accurate mass information, ultra-high mass resolution data and structural information through multiple-stage MS (MS²) experiments. The recent introduction of sustained off-resonance irradiation (SORI) [3,4] and quadrupolar excitation axialization (QEA) [5–7] has further enhanced the performance of FTMS. The examples given here show the advantages of using SORI and QEA for analysis of biomolecules.

A Bruker Spectrospin BioAPEX™ 47e, with a differentially pumped external ion source and a 4.7 T superconducting magnet, was used for all experiments. The vacuum system, shown in

Abbreviations used: ESI, electrospray ionization; MALDI, matrix-assisted laser desorption/ionization; FTMS, Fourier-transform mass spectrometry; QEA, quadrupolar excitation axialization; SORI, sustained off-resonance irradiation; CID, collision-induced dissociation; MS², multiple-stage mass spectrometry; S/N, signal-to-noise ratio.

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Figure 1, consists of an analyser region with a radio-frequency-corrected Infinity™ Cell, a transfer region and a source region with a series of cylindrically symmetrical electrostatic lenses for extracting and focusing the ion beam. The analyser, transfer and source regions are pumped by 800 l/s, 400 l/s and 800 l/s cryopumps respectively. In addition, the ESI source is differentially pumped by two 500 l/min mechanical pumps and has two conical skimmers to provide a pressure differential of 10\(^{-12}\) between the needle exit and the detection region. The differential pumping stages with flow restrictions provide the ultra-high vacuum, 10\(^{-9}\) mbar, in the analyser that makes it possible to obtain high-resolution spectra with excellent mass accuracy.

The ions are extracted from the source region, accelerated and focused to pass through the flow restrictions. The ion beam is decelerated and guided through the fringing field of the superconducting magnet, then trapped in the Infinity™ Cell using Sidekick™, Bruker’s patented ion accumulation scheme [8]. Ion accumulation times range from 10 to 100 ms with complete spectra recorded at rates of 0.1–10 Hz, depending on the required resolution. The BioAPEX™ 47e provides ESI and MALDI mass spectra with mass resolution greater than 500,000 with acquisition times of the order of a few seconds.

The determination of molecular structure by MS requires that the molecule be ionized and undergo structurally informative fragmentations. Fragmentation may be induced by the ionization process itself or by some other means of excitation. Often the optimum methods of ion formation do not impart sufficient energy to cause appreciable fragmentation. This is particularly true for the ionization techniques, such as ESI and MALDI, that are routinely used in biological MS. Consequently, it is often desirable to perform collision-induced dissociation (CID) in order to obtain more detailed structural information [9,10]. In many cases, biochemical problem-solving by MS relies on CID of the analyte to provide information on key sequence or structural features. To maximize the amount of useful information obtained, it is desirable to have high precursor-to-product ion conversion efficiencies. This is possible by combining the efficient dissociation technique of SORI–CID [3,4] with the high-performance characteristics of the BioAPEX™ (FTMS).

Traditionally, FTMS–CID experiments have incorporated a pulsed on-resonance excitation of the precursor ion concomitantly with a pressure burst of collision gas. On average, this produces only single energetic collisions between the precursor ion and the collision gas. The result is potentially low conversion efficiencies of pre-
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Figure 2
NanoSPRAY™–FTMS spectrum of myoglobin tryptic digest showing the structural information that can be obtained by SORI–CID experiments

Myoglobin tryptic digest 200 fmol/ul 4 scans isolation of B04

T2. Sequence = VEADIACHQEVLR, +2

Myoglobin tryptic digest 200 fmol/ul, CID of B04
400 fmol Total Sample Loaded. 25 fmol Consumed

The addition of QEA to the external-ion-source BioAPEX™ FTMS greatly enhances performance by providing selective isolation, effective ion ‘cooling’, sensitivity enhancement for off-axis energetic ions and the ability to effectively remeasure ions. In many cases, ions are generated with kinetic energies that cause them to be trapped in the cell with different cyclotron/magnetron radii. Consequently, during excitation detection many ions collide with the cell walls, are neutralized and lost. During

Figure 3
MALDI–FTMS spectrum of bovine insulin B-chain, demonstrating how QEA™ is used to enhance sensitivity

MALDI–FTMS of bovine insulin B-chain
Single shot, S/N = 6:1

×4

25 remeasurements of the above ion packet
Single shot, S/N = 32:1
allowing more sensitive detection of all product ions. The QEA™ pulse is applied over a given mass range and leads to sensitivity enhancement of all fragment ions within that preselected range. Ions outside the applied QEA™ field are simply lost due to scattering collisions with the thermalizing gas. For MALDI–FTMS, the advantage of QEA™ arises from the ability to remeasure MALDI-generated ions. This leads to increased sensitivity and lower detection limits.

Experimental
For analysis by ESI, the samples, from various suppliers, were used without further purification. Sample solutions of concentrations 1–10 pmol/µl in a methanol/water/acetonitrile mixture were prepared. The analyte in solution was introduced at near-atmospheric pressure through a needle held at earth potential and electrosprayed across a 3–5 kV potential. Solvent evaporation, required for the formation of macromolecular ions, was accomplished by means of a heated nitrogen countercurrent drying gas. Once formed, the ions traverse a gold-plated glass capillary and pass into the first skimmer region which typically has a pressure of 2 mbar. On passing through a 1 mm orifice into the second skimmer region, the ions experience a drop in pressure to $6 \times 10^{-2}$ mbar. Beyond the second skimmer, standard external ion source optics are used to focus the ions as they pass to the Infinity™ Cell where they are subsequently trapped. In order to ensure high mass resolution detection, the pressure in the analyser region is maintained in the $10^{-9}$ mbar range.

In addition to the conventional ESI source, use of the new NanoSPRAY™ technique [11] enables one to analyse complex mixtures of peptides, even from salt-containing buffers, with high sensitivity and low sample consumption. This method is ideally suited for analysing small amounts (subpicomolar) of crude digest products. A major advantage of the NanoSPRAY™ source is that it is far more efficient than a conventional ESI source since it uses a glass needle which has been drawn out to a fine point, typically 1–3 µm. Using this needle, small droplets are produced with much lower voltage differentials between the needle and the capillary entrance than in conventional ESI. Formation of smaller droplets leads to efficient ion evaporation and better desolvation of the ions, thus giving higher sensitivity and lower background noise. The high field strength at the small needle tip allows aqueous, organic or high-salt solutions to be sprayed.

For analysis by NanoSPRAY™, the conventional electrospray needle housing is removed and replaced by an assembly which supports the glass needle and allows micromanipulation of the needle position. A small-diameter 250 µm metal cap is used to cover the capillary entrance. No countercurrent drying gas is required and the capillary, held at 800 V, is maintained at room temperature. NanoSPRAY™ needles with external electrodeposited gold coatings were supplied by the European Molecular Biology Laboratory. The needle, containing 1–2 µl of sample solution, is positioned so that the tip is about 1.5 mm from the capillary entrance. These conditions give a flow rate of 25 nl/min so that 1 µl of sample lasts for 40 min, sufficient time to optimize conditions and record multiple spectra. A commercial sample of equine myoglobin tryptic digest was diluted with methanol/water (30:70, v/v) and 3% acetic acid to give a concentration of 200 fmol/µl and used without further purification. An internal calibration standard of angiotensin I, at a concentration of 200 fmol/l, was added to the sample.

For analysis by MALDI, the analyte was dissolved in a water/acetonitrile (70:30, v/v) and 0.1% trifluoroacetic acid mixture. The matrix used was a saturated solution of 2,5-dihydroxybenzoic acid. On the stainless-steel target, 1 µl of analyte was mixed with 1 µl of the matrix solution and allowed to air-dry. The crystals formed consisted of analyte molecules located in the host matrix crystalline structure. The sample holder was then placed into the inlet system and evacuated. A nitrogen laser (337 nm) was used for ion formation. The focal spot size of the laser on the target was 100 µm, giving a power density of $10^4$ W·cm$^{-2}$ at the crystal surface. In this way, ions are generated by a focused laser pulse, and by keeping the Infinity™ Cell open for a specific period, it is possible to trap ions of a desired mass range. The time-of-flight effect makes it possible to eliminate interference from abundant matrix ions, since these lower-mass ions typically arrive earlier than the analyte ions. Consequently, by adjusting the delay time, one is able to trap exclusively the heavier analyte ions.

Results and discussion
The peptides, produced by tryptic digestion of equine myoglobin, were subjected to analysis by...
NanoSPRAY™–FTMS because the stable signal produced is ideal for SORI–CID analysis. From a concentration of 10 pmol/μl and a sample consumption of 750 fmol, one can obtain the charge states of the product ions and accurate mass assignments. Figure 2 shows an example of SORI–CID of the myoglobin tryptic digest at a concentration of 200 fmoVp1, with a total sample consumption of 25 fmol. The top spectrum shows the isolation of the ion at m/z 804 and the lower trace shows the SORI–CID mass spectrum obtained. With such small sample amounts, there is some sacrifice in signal-to-noise (S/N), but many fragment ions can be identified and the resolution is still sufficient to assign charge states and accurate masses to the fragments.

Figure 3 shows an example of how QEATM is used to enhance MALDI–FTMS data. The upper trace shows a single-shot (detected after one QEA event) MALDI spectrum of the [M + H]⁺ ion of bovine insulin B-chain (S/N = 6). Twenty-five remeasurement cycles, using QEATM, of the same ion packet increases the S/N to 32. This shows how QEATM can be used to enhance both sensitivity and detection limits.

The examples given here demonstrate how SORI and QEATM techniques can be used to enhance FTMS data. In conjunction with the high sensitivity, mass resolution and mass accuracy, these methods make the BioAPEX™ FTMS an important tool for mass analysis and structural characterization of biomolecules.

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Use of HPLC with inductively coupled plasma mass spectrometry (ICP-MS) for trace element speciation studies in biological materials

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Introduction

Inductively coupled plasma mass spectrometry (ICP-MS) can be used for simultaneous multi-element and multi-isotope analysis in liquid sample matrices, and is therefore a powerful tool for trace element speciation studies when used on-line to monitor chromatographic protein separations. This paper describes briefly the principles of ICP-MS, its coupling to an HPLC system and the application of HPLC–ICP-MS to some zinc studies in biological samples.

ICP-MS

ICP-MS combines a high-temperature ion source (7000–8000 K) at atmospheric pressure with sensitive detection under high vacuum by a mass spectrometer. The resolution of quadrupole mass analysers is approximately one mass unit so that, in addition to multi-element detection, ICP-