

Tutorial

An Introduction to Mass Spectrometry Based Proteomics

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Tutorial based mainly on some extracts of an article:

'An introduction to mass spectrometry based proteomics - Detection and characterization of gonadotropins and related molecules' by Andrew T Kicman, Mark C Parkin and Ray K Iles, Molecular and Cellular Endocrinology 2007; Vol 260-262;212-227.

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1. Introduction

One of the most powerful modern analytical techniques available to the laboratory analyst is mass spectrometry (MS). Over the last decade, **MS based proteomics** has rapidly become the analytical method of choice for the identification and characterization of proteins, being especially useful for assessing post-translational modifications. This tutorial will attempt to introduce some of the fundamental aspects of mass spectrometry. Most of this tutorial has been taken, with permission, from parts of a review (Kicman, Parkin and Iles, 2006).

1.1 What are the principal components of a mass spectrometer?

MS allows compounds to be identified by the gas phase production of ions and their subsequent separation and detection based on their mass to charge ratio (m/z), conferring a very high level of specificity with sensitivity. The principal components of a typical mass spectrometer are the *ion source*, the *mass analyzer* and the *detector*.

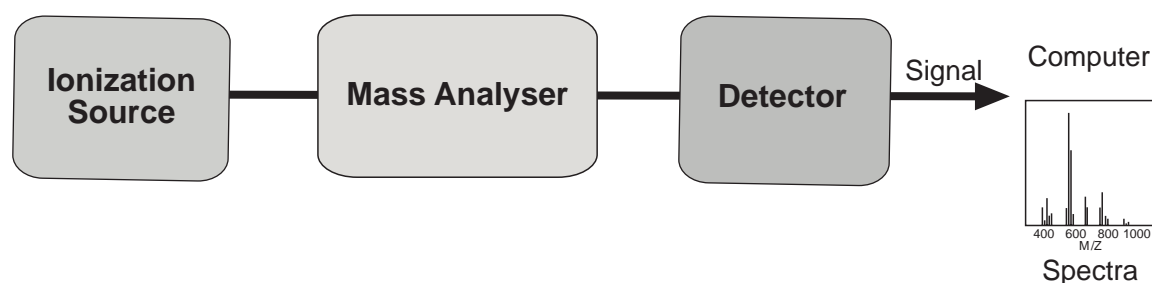


Figure 1. *The principle components of a mass spectrometer consisting of the ionization source followed by the mass analyzer and detector. A computer is used to generate mass spectra from the detector signal.*

Mass spectrometry relies on molecules being converted into **ions** in the **gas-phase**.

Large polar biomolecules, such as proteins and peptides, are involatile, and to overcome this problem two ionization techniques were developed: **Electrospray Ionization (ESI)** and **Matrix-Assisted Laser Desorption Ionization (MALDI)**.

Both these approaches are known as *soft ionization techniques*, allowing molecules to remain relatively **intact** during the ionization processes.

Ions produced by the above processes are subjected to separation according to their mass to charge ratio (m/z) in the **mass analyzer**. Both MALDI and ESI ion sources can be interfaced to different types of mass analyzers, some of the more common used in bioanalysis being **quadrupoles**, **ion-traps** and **time of flight instruments**. The type of mass analyzer chosen will dictate the type and quality of experimental data obtained with respect to mass accuracy, mass resolution, mass range and sensitivity. Each mass analyzer has inherent advantages/disadvantages with respect to these instrumental parameters.

Ions emerging from the mass analyzer are **detected** using electron multiplier or array detectors and the amount of electrical current that is subsequently generated for data processing will depend on the abundance of ions hitting the detector at that time. In all mass spectrometers other than Fourier Transform-Ion Cyclotron Resonance instruments, the ions are detected after mass analysis by converting the detector-surface collision energy of the ions into emitted ions, electrons, or photons that are then sensed with various light or charge detectors.

2. Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)

2.1 Introduction

One of the most common interfaces is between a MALDI source and a time-of-flight (TOF) mass analyzer. As singly charged ions are predominately formed with MALDI and because TOF analyzers have a large working mass range (~ 100 Da to

>250,000 Da), this combination offers a rapid and uncomplicated approach to the mass assignment of intact proteins. Singly charged molecular ions predominate, making data processing and interpretation of spectra straightforward. For example, this soft ionization process clearly shows the molecular ion (M+H)⁺ of recombinant human growth hormone (somatotropin) as the dominant peak in the spectrum, with the observed m/z species also including adduct ions such as those of sodium and potassium.

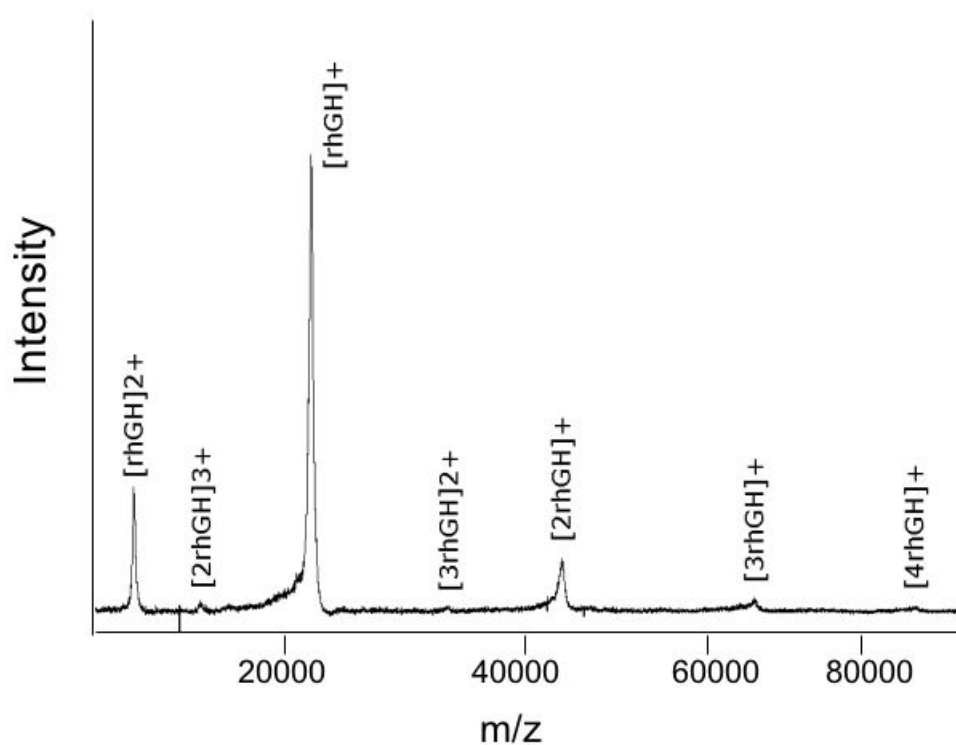


Figure 2. MALDI-TOF mass spectrum of 12 pmol of purified recombinant growth hormone (rhGH) using sinapinic acid matrix. . Figure reproduced with kind permission from Dr Hendrik Neubert, PhD Thesis - An Investigation into the enhancement of matrix-assisted laser desorption/ionisation - mass spectrometry by modification of surface chemistries. University of London 2002.

A number of less intense multiply charged (multimeric) m/z species are also observed, which are often characteristic of proteins analyzed by MALDI. Whether these multiply charged species are just artifacts of the ionization process or a result of biological aggregation of molecules at high concentrations is a subject for debate.

Sample work-up to investigate whether intact molecules are present is relatively simple, usually being limited to rapid desalting of samples, e.g. by mini-reversed phase (C₁₈) solid-liquid extraction, the 'ZipTip®' approach, or centrifugal ultrafiltration, followed by preparing the protein/matrix mixture.

For identification of proteins, which employs enzymic digestion to cleave complex protein structures for confirmation by MS ('peptide mass mapping' -see section 4), each protein in a mixture will need to be separated prior to digestion and to this purpose gel electrophoresis is traditionally the technique of choice. In the future, with automation problems being overcome, gel electrophoresis separation may be superseded by LC, the effluent being directly deposited onto the MALDI target and then mixed with a suitable matrix.

MALDI-TOF MS with peptide mass mapping can be a powerful and cost-effective approach for protein identification. The instruments are generally easy to operate and the skill in optimizing the protein/matrix mix for analytes of interest can be acquired expeditiously.

2.2. What is the principle of MALDI?

In the late 1980s MALDI was introduced by two key workers, Koichi Tanaka and Franz Hillenkamp following a number of years research into the use of lasers for the ionization and analysis of biomolecules. The principle of MALDI is based on depositing non-volatile analyte mixed with a large excess of small organic material known as the matrix in an aqueous or organic solvent.. This dilutes and isolates the analyte preventing clusters or aggregates too large to desorb. A few microlitres is

applied to a MALDI target plate and then allowed to evaporate resulting in a bed of co-crystallized matrix/analyte mix.

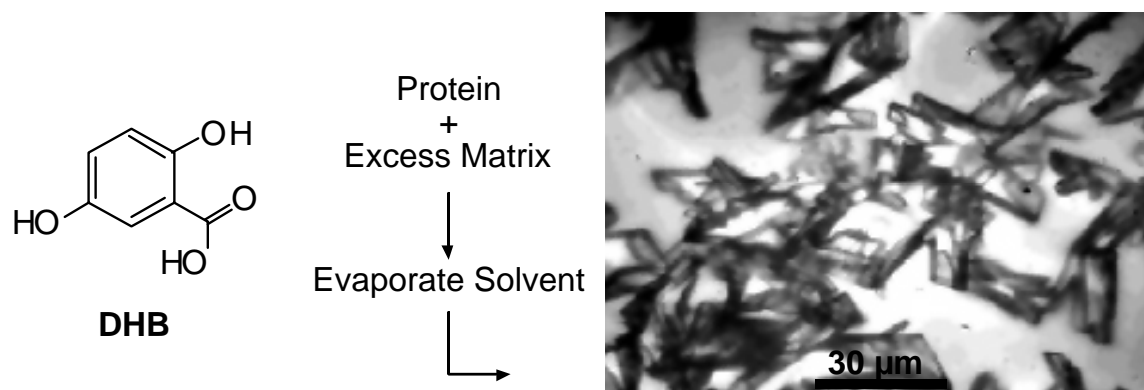


Figure 3. Scanning electron microscope image of sample matrix crystals formed on a MALDI plate. The proteins of interest are mixed with an excess of a suitable matrix (such as DHB) in the presence of a suitable solvent. The solvent is allowed to evaporate forming a bed or co-crystallized matrix/analyte mix as shown by the image on the right.

Typical matrix materials are small, highly conjugated organic acids such as commonly used 2,5-dihydroxybenzoic acid (DHB). The target plate is positioned in the high vacuum source region of mass spectrometer and is irradiated with a pulsed laser beam (usually 337 nm). This enables the matrix to strongly absorb energy in the ultraviolet region at the laser wavelength. The matrix, being present in a greater concentration than the analyte, absorbs most of this energy in the form of heat. This means that analytes such as proteins remain intact. A dense gas cloud is formed by sublimation of the matrix and rapid expansion of matrix and analyte into the gas phase. This cloud heads supersonically towards the instrument's vacuum and the energy is transferred from matrix to analyte. During this transitional stage analytes are protonated (or deprotonated) by a process believed to be the direct result of collisions between the neutral analyte and excited matrix ions. Desorption occurs by proton transfer between the photoexcited matrix and analyte:

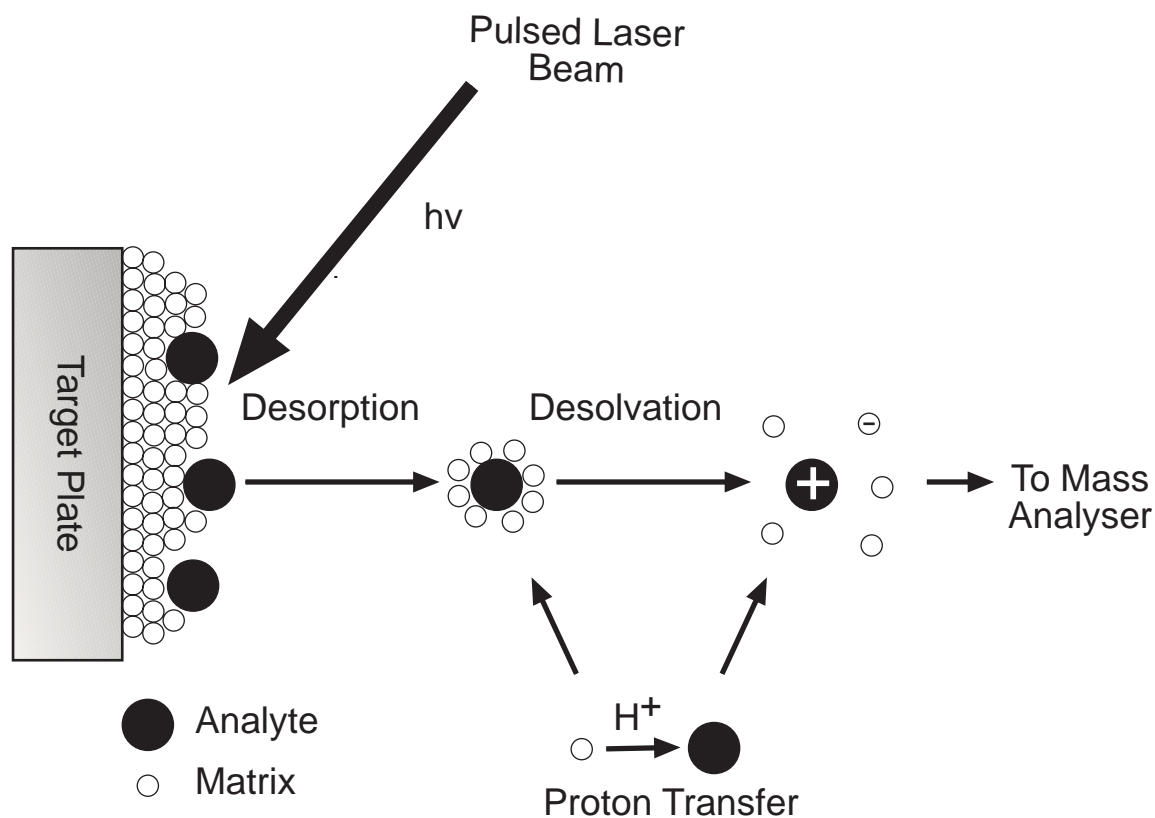


Figure 4. Ionization of analytes by MALDI. The co-crystal of matrix and sample is targeted by a laser causing a desorption process followed by desolvation and then introduction into the mass analyzer. Figure adapted from de Hoffman and Stroobant (Hoffmann et al. 1996), reproduced with permission from John Wiley and Sons Ltd, © 2003.

Single protonated molecular ions are dominant in MALDI ionization as the analyte typically accepts a proton. Non-volatile biological macromolecules, such as glycoprotein hormones, are readily ionized by MALDI and interpretation of spectra is relatively simple mainly due to the presence of singly charged ions.

2.3. How does a 'Time-of-Flight' mass analyzer work?

One of the simplest mass analyzers used in mass spectrometry is the time-of-flight (TOF) mass analyzer, often interfaced with MALDI ionization source. TOF analysis is also extremely rapid and offers high resolution and sensitivity making the instrument a popular choice in techniques such as proteomics.

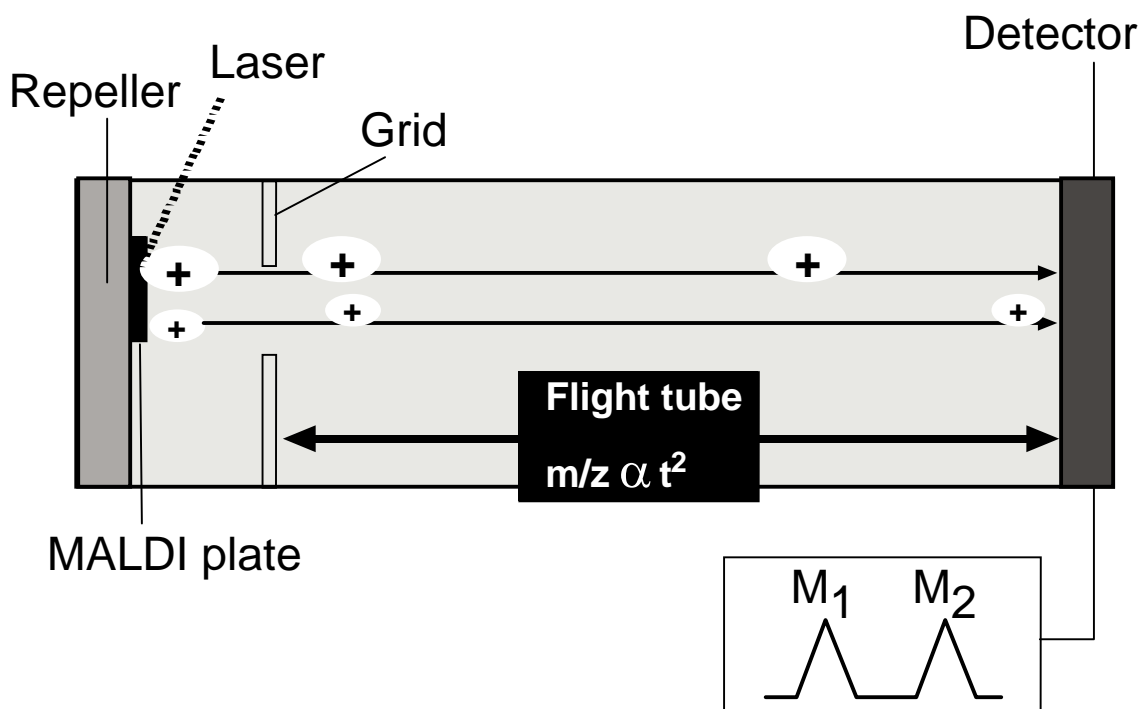


Figure 5. The Time of Flight (TOF) mass analyzer. Ions will separate according to their velocity along the flight tube from the MALDI plate towards the detector.

After production of ions by MALDI a fixed potential difference (typically 20 to 30 kV) accelerates all the ions into a field-free drift tube (the analyzer) where time-of-flight separation occurs. As all the ions are accelerated with the same potential then they all have the same kinetic energy.

The TOF analyzer works by measuring the time required for ions generated in the source to fly through the analyzer and strike the detector at the other side. The principle is based on an ion of mass m leaving the ionization source with a charge z and accelerating potential V , thus having energy zV equal to the kinetic energy of the ion:

$$K = zV = \frac{mv^2}{2} \quad (\text{Equation 1})$$

If the time taken, t , for the ion to fly the distance d of the flight tube at velocity v is given by:

$$t = \frac{d}{v} \quad (\text{Equation 2})$$

Substituting (2) into (1) gives:

$$t^2 = \frac{m}{z} \left(\frac{d^2}{2V} \right)$$

The terms in parentheses (related to a fixed distance and accelerating potential) remain constant thus m/z can be determined from t^2 . As all the ions are accelerated with the same potential then they all have the same kinetic energy. Singly charged ions with a larger mass will travel with a lower velocity down the tube compared to those with a smaller mass, i.e. they separate according to their velocities. It is this simple feature of TOF analyzers that it is conducive to their large mass range, making it ideal for coupling with MALDI for the analysis of large intact biomolecules such as proteins.

3. Electrospray Ionization-Quadrupole MS

3.1. Principle of Electrospray Ionization (ESI)

Electrospray ionization (ESI) represented a significant breakthrough in biological analysis, so much so that John B Fenn shared the 2002 Nobel Prize in chemistry for the development of methods for identification and structure analyses of biological macromolecules (the other Laureates included Koichi Tanaka for utilizing MALDI and Kurt Wüthrich for adopting nuclear magnetic resonance spectroscopy).

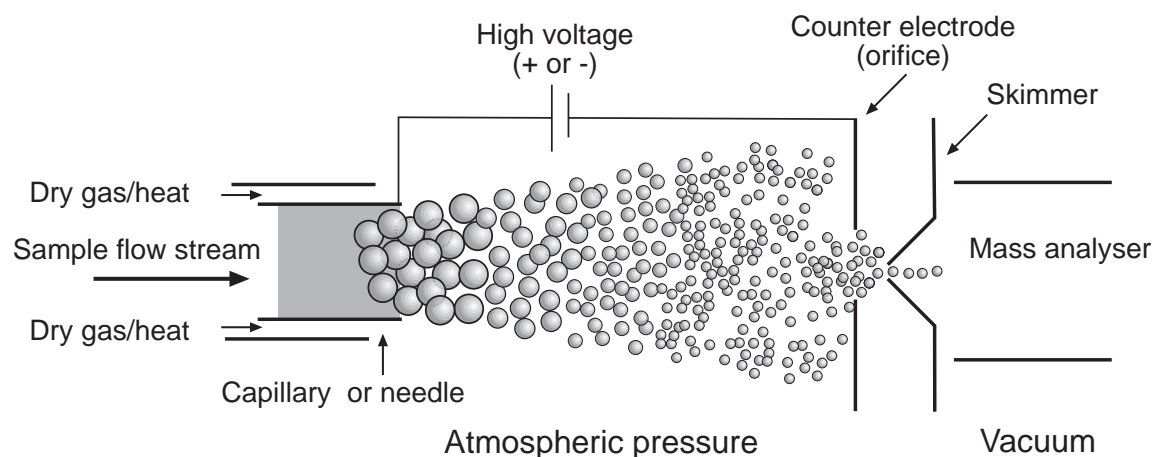


Figure 6. *Electrospray ionization process. A fine mist of analyte is formed from the sample stream as droplets are formed from the Taylor cone. The charged droplets desolvate by a Coulombic explosion and ions free from solvent can then enter the mass spectrometer via an intermediate vacuum region.*

The idea of electrospray was based on the industrial process of a liquid aerosol generated by electrostatic charging to spray paint cars and metals. This technique has been around for almost a century but was first adopted as an ionization method by Dole et al in the late 1960's (Dole et al. 1968). However, it was Fenn et al that demonstrated its application to the ionization of biological macromolecules for mass spectrometry (Fenn et al. 1989). The process of electrospray ionization works by forcing a liquid sample stream through a needle or capillary into an ionization source. A potential difference (either positive or negative) between the outlet of the sample stream and the inlet (or orifice) of the mass analyzer is applied. The liquid inside the needle becomes unstable as it tries to retain more and more charge. At the tip of the needle the droplet can no longer hold more charge and assumes a conical shape referred to as the *Taylor cone* which results in a fine jet that, in turn, blows apart into a plume of tiny, highly charged droplets. The droplets are repelled from the needle towards the sampling cone on the counter electrode at the orifice of the instrument. As the charged droplets traverse the space between the needle tip and the cone, solvent evaporation occurs causing them to diminish in size. This desolvation process

is further assisted by a warm flow of nitrogen known as the drying gas, which passes across the front of the ionization source. As the solvent evaporation occurs, the droplet shrinks until it reaches the point that the surface tension can no longer sustain the charge, known as the *Rayleigh limit*. At this point a *Coulombic explosion* occurs and the droplet is ripped apart producing smaller droplets. The whole process is repeated until eventually charged sample ions, free from solvent, are released from the droplets. Some of the ions pass through the sampling cone into an intermediate vacuum region and from there through a small aperture into the analyzer of the mass spectrometer, which is held under high vacuum. These charged analyte molecules can be singly or multiply charged.. This is a very soft method of ionization as very little residual energy is retained by the analyte upon ionization.

Large biomolecules such as proteins contain many basic groups that can be polyprotonated when subjected to positive electrospray ionization. Due to multiple charging, quadrupole mass analyzers that have an upper m/z limit around 2000 can therefore filter very large molecules, e.g. the mass of bovine serum albumin has a molecular weight of 66,430 but with 44 charges (protons) attached gives an m/z of 1510.8, an m/z value well within the working mass range (calculation: $m/z = (MW + nH^+)/n$ where n = the integer number of charges on the ions and $H = 1.008$ Da).

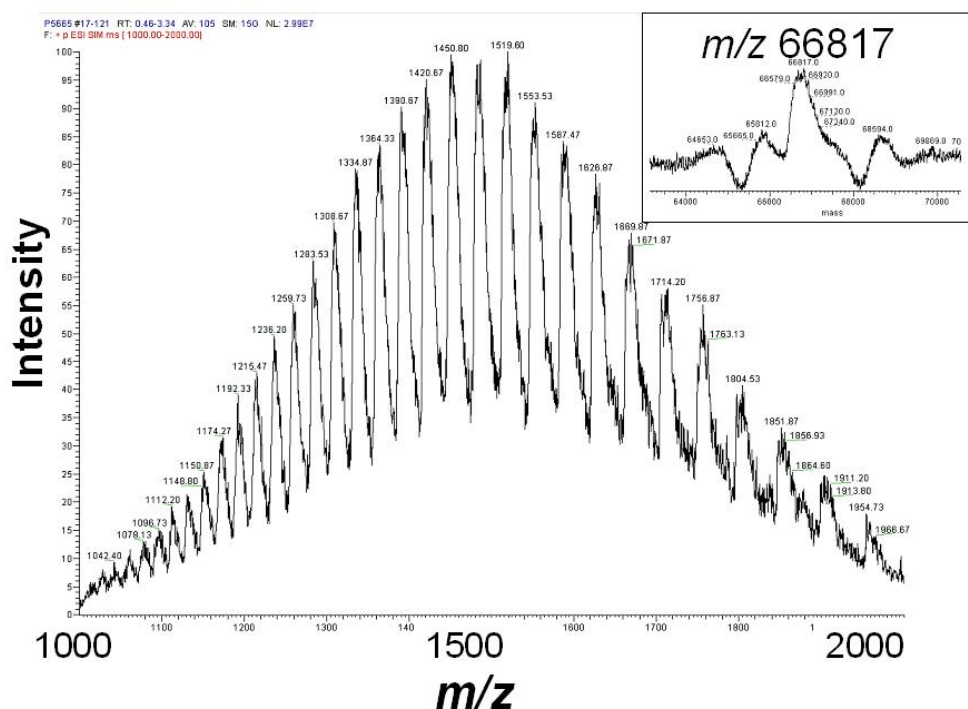


Figure 7. Spectrum of bovine serum albumin resulting from electrospray ionization (positive mode) displaying the characteristic envelope of peaks with a typically bell-shaped distribution, each peak representing a different charge state. The inset shows the deconvoluted spectrum representing the mass of the singly charged species.

There is a variability in analyte protonation which is dependent on the primary sequence, base spacing, and conformation of the protein molecules within the droplets that undergo Coulombic explosion. The resultant spectrum is characterized by an envelope of peaks, typically bell-shaped in distribution, each peak representing a different charge state of the analyte, e.g. as observed for bovine serum albumin. A process known as deconvolution can determine the molecular weight of a large multiply charged species. Deconvolution involves transforming the multiply charged spectrum into a singly charged reconstructed spectrum. In theory this process is straightforward and can be achieved by simple simultaneous equations. However, in real life situations ESI spectra of multiply charged species can be complicated, as such most commercial MS instrumentation have software complete with deconvolution algorithms making the process that much easier.

3.2. Quadrupole Mass Analyzer - an analyzer that ‘filters’ ions

The quadrupole mass analyzer consists of four mutually parallel circular metal rods such that the electric field between them is hyperbolic (quadrupolar). A dynamic electrical field is created by applying a simultaneous radio frequency (r.f) which is 180 degrees out of phase on the opposite pairs of rods (this means that the shape of one waveform is the inverted equivalent of the other), each pair of opposing rods also being supplied with direct current (d.c.) of equal magnitude but opposite polarities with respect to ground.

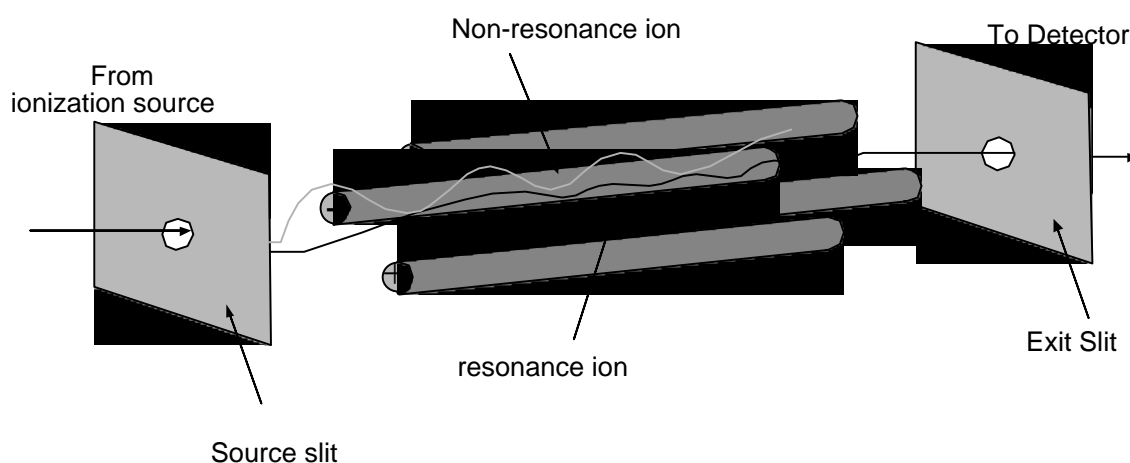


Figure 8. A schematic of a quadrupole mass filter. Resonant ions will travel down through the poles towards the detector. Non-resonant ions will strike the poles and become neutralized.

The electrical field created in the mass analyzer acts as a filter in that it only allows ions of certain masses (‘resonant ions’) to pass down the gap between the four rods, i.e. the quadrupole is a mass-to-charge *filter*. By controlling the ratio of the r.f. to d.c. voltages, a *narrow band* filter can be produced allowing a ions of one m/z value, to take a stable trajectory between the rods to the detector, a simple analogy being too that of a corkscrew-like path. Ions with different mass to charge ratios (non-resonant

ions) will collide with the rods and become neutralized. By ramping (increasing) the d.c. and r.f. voltages, stable trajectories for ions of different m/z values pass through to the detector, see Figure 8. This whole process is extremely rapid and typically after less than $1/6^{\text{th}}$ of a second, ions over a wide m/z range may be analyzed or *scanned*. The mass range of the analyzer is governed by the size of rods, the distance between the rods and the frequency of the radio wave, producing an upper limit between m/z 2000 to 4000 (depending on the instrument manufacturer). If an increase in sensitivity (lower limit of detection) is required, selected ions can be filtered to allow the detector to integrate signals of relevant ions for a longer time.

Single quadrupole mass spectrometers have now been superseded by triple-quadrupole mass analyzers, allowing tandem MS capabilities. The second quadrupole is used as a collision cell to fragment the precursor ions filtered by the first quadrupole (Q1) into product ions to be filtered by the third quadrupole (Q3). A scan of all the ions yields a full scan spectrum that confers specificity, a 'fingerprint' of the analyte that can be of immense value with the MS/MS of proteins.

3.3. Ion Trap Analyzers - an alternative approach

A quadrupole ion trap is a three-dimensional analogue of the linear quadrupole mass analyzer, consisting of a ring electrode sandwiched between two end-capped electrodes.

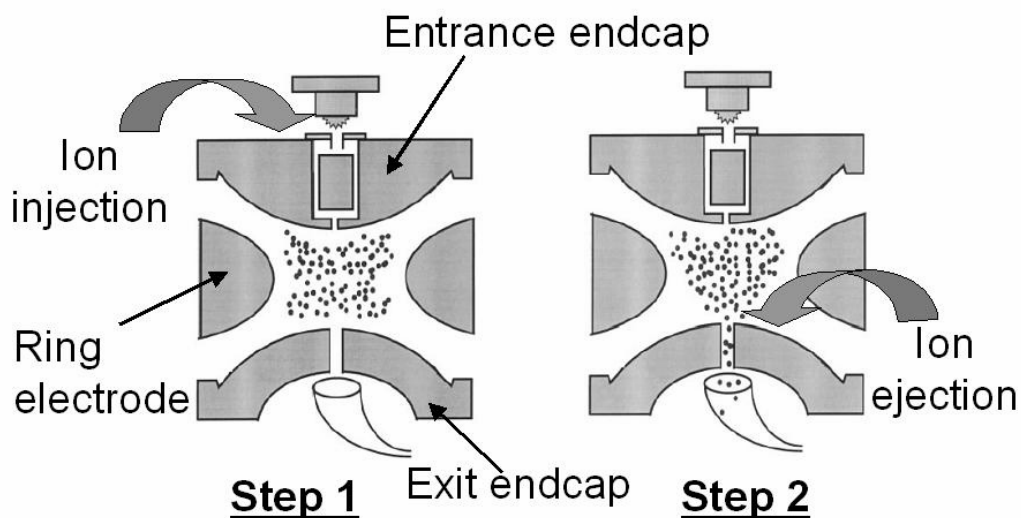


Figure 9. Schematic of an ion trap quadrupole showing an overview of MS-in-time. *Step 1: a trapping r.f. amplitude is applied for 0-30 ms during which ions are formed from sample molecules and stored. Step 2: an r.f. amplitude is ramped over the period 30-85 ms during which mass-selective ion ejection and mass analysis occurs. Adapted from March (March 1997), reproduced with permission from John Wiley and Sons Ltd, © 1997.*

Holes in the end caps allow ion injection into the analyzer from an external source and ion ejection from the analyzer to the detector. It is called a trap because it enables ions to be trapped in an electric field by applying an r.f voltage to the ring electrode (when the source is positive electrospray ionization, a small negative direct current offset is also applied to all three electrodes). Ions formed from sample molecules, such as by ESI, are *stored* for a finite time (1 to 30 ms), the ions being trapped in a Lissajous ‘figure of 8’ trajectory. Increasing the RF potential amplitude destabilizes the ion trajectories and ejects ions sequentially from the ion-trap region through the end cap electrodes. The ions can be ejected and detected according to their m/z ratio to yield a full scan mass spectrum. A small amount of ‘damping gas’ (Helium) improves resolution and also enables multiple MS experiments (MSⁿ) experiments.

A very simple example of data to illustrate a multiple MS experiment is from analysis of a tryptic fragment of the female pregnancy hormone, hCG (β T1 +2).

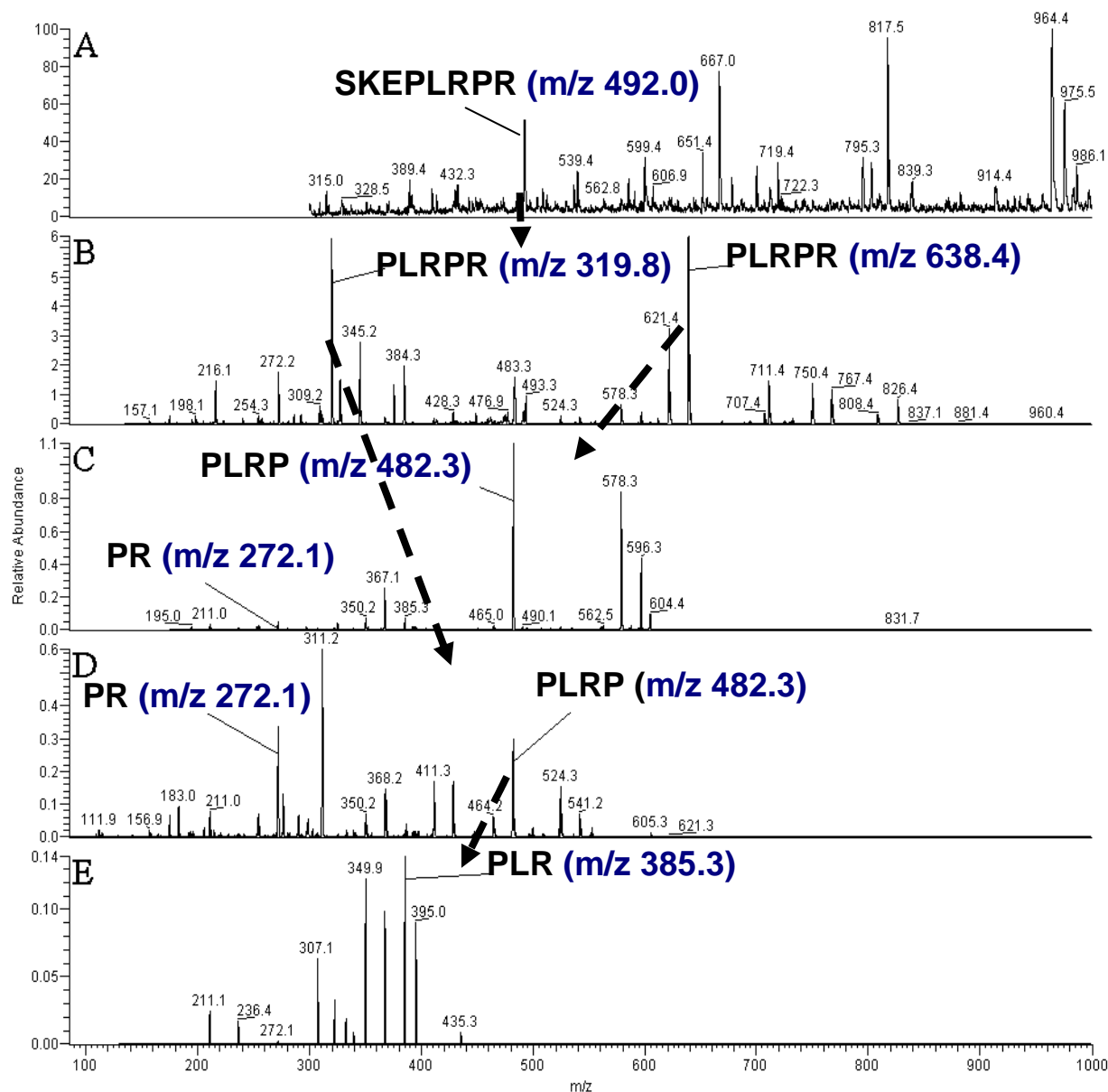


Figure 10. Employing a quadrupole ion trap to perform a multiple MS experiment to demonstrate its capability for sequence analysis. The example is that of tryptic fragment of hCG β T1 +2 (calculated mass of protonated molecule is 983.2). The m/z and corresponding sequence of selected precursor and product ions are displayed in spectra A (MS), B (MS²), C and D (MS³) and E (MS⁴).

Multiple MS experiments have been proven to be helpful in the characterization of carbohydrate moieties on glycoproteins. The application of multiple MS for the glyco-fingerprinting of gonadotropin-related molecules would seem to be extremely useful.

Mass spectrometers incorporating ion traps are usually far more sensitive than linear quadrupole analyzers for providing full scan spectra; recently ‘linear ion traps’ have become commercially available which have even greater sensitivity, and these too have been applied for proteomic purposes. On the other hand, unlike traps, linear quadrupoles can be operated to filter selected ions, which confers a much lower limit of detection for ions of interest. If quantification is important at low concentrations of analyte, triple-quadrupole mass spectrometers are usually the instruments of choice because of their favorable ion statistics.

4. Proteomic Methods – Protein Identification

Protein identification using mass-spectrometry involves using one of two established methodologies these are *peptide mass mapping* (or *fingerprinting*) and *peptide sequencing*. Both techniques use a proteolytic enzyme (typically trypsin) to specifically cleave the proteins into peptide fragments of a suitable length (mass) for mass spectrometric analysis.

Peptide mass mapping involves an initial separation of the proteins by gel electrophoresis (one or two-dimensional) followed by an *in situ* protein digest.

Protein spots on the gel are visualized and quantitated by staining and then excised for subsequent digestion followed by mass spectral analysis by MALDI-TOF. Observed accurate mass values acquired by the TOF analyzer provide a “fingerprint”. This

peptide fingerprint can be compared to a predicted list of peptides derived from the choice of protease used and their corresponding masses for all proteins in a database. The classic example of this would be the cleavage of cytochrome *c* with trypsin, an enzyme that cleaves the peptide bond on the carboxy terminal side of arginine and lysine (except when adjacent to proline). With a sufficient number of peptide masses a match is usually found. Generally 5 or more accurate masses are required to identify a protein with enough confidence and 3 or 4 for a more ambiguous match. Two-dimensional electrophoresis (2DE) is hampered by some limitations, these are mainly attributed to its limited dynamic range. Low abundant proteins, low and high mass proteins, membrane proteins and proteins with extreme isoelectric points all present a challenge for 2DE.

For MALDI-MS analysis, typically the individual protein spots from the gel are treated with a protease and each subsequent digest is spotted individually onto the MALDI plate. In this fashion the method can be considered as being performed *off-line*. The operator can visualize the MALDI plate and sample/matrix crystals can be picked out and targeted by the laser. A number of crystals from one protein digest can be hit maximizing the number of ions obtained from the sample spot. This has the effect of increasing the probability of detecting some peptide ions that may have their signal suppressed by the presence of more readily ionizable peptides in the sample crystal. This process can then be repeated for each individual protein digest spotted onto the MALDI plate.

In ESI, as the technique inherently involves a continuous stream of liquid then sample introduction to the mass spectrometer is performed *on-line*. Each individual digest from the protein spot excised from the gel (possibly containing many peptides from

the original protein) could be infused as a liquid sample stream directly into the instrument, however in practice this is rarely done. Separation of individual peptides from the digest can be achieved by using liquid chromatography thus limiting the effects of readily ionizable peptides suppressing the signal from peptides that are not so readily ionizable ('ion suppression'). It could be argued that chromatographic separation prior to sample deposition on MALDI plates would also be beneficial and indeed this process is being introduced into MS proteomics (Chen et al. 2005; Bodnar et al. 2003). However, it involves the use of careful automation for the collection of the chromatographic eluent together with a subsequent increase in the number of sample spots required for analysis and hence increasing the workload. Peptide sequencing involves the production of fragment ion spectra by tandem MS (or by sequential MS experiments using an ion trap). Peptides are collided with gas molecules inside the mass spectrometer causing fragmentation along the polypeptide backbone. This usually results in the formation of two fragments, one containing the N-terminus and the other the C-terminus. N-terminal fragments are known as b-ions and are numbered starting from the n-terminus. C-terminal fragments are known as y-ions and are numbered starting from the C-terminus.

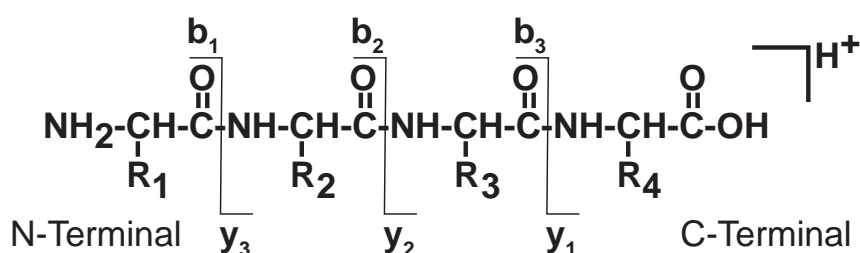


Figure 11. A schematic diagram showing fragment ion nomenclature for N-terminal b-ions and C-terminal y-ions.

Other smaller fragments are also usually formed by internal fragmentation however, but y and b-type ions usually predominate (Wysocki et al. 2000; Tsaprailis et al.

1999). The product ion spectra produced can be compared with available amino acid sequences obtained from a protein database to help identify the analyte of interest. Moreover, and very importantly, MS/MS can be particularly useful in carbohydrate characterization of glycopeptides. Both fingerprinting and sequencing are summarized by the schematic below:

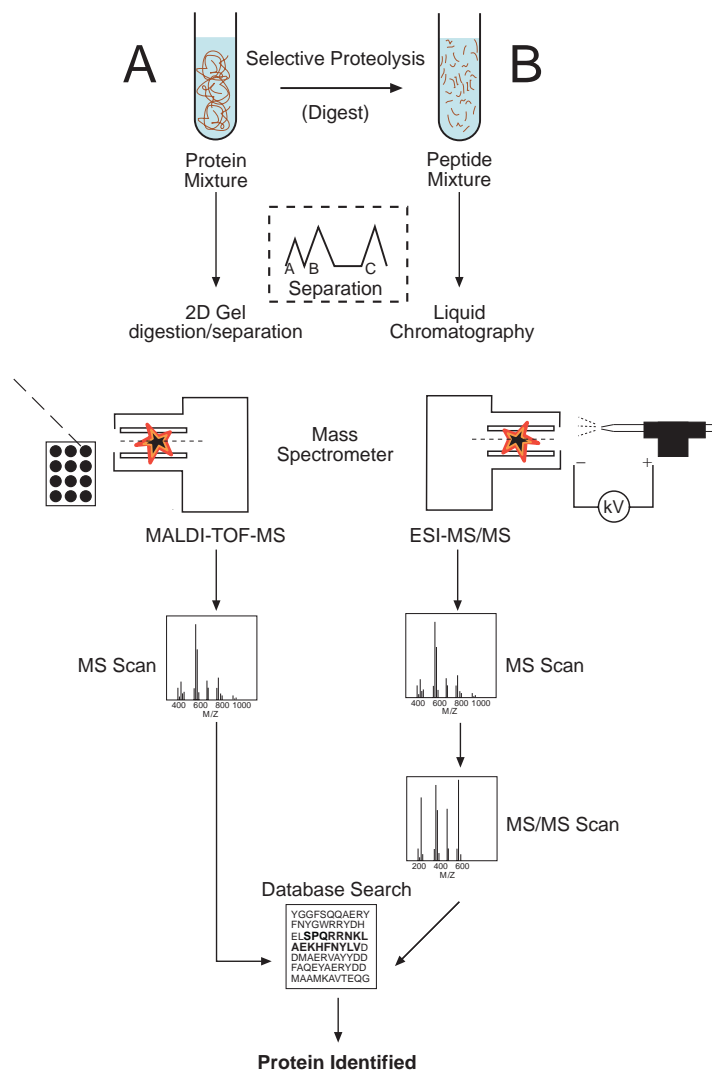


Figure 12. Peptide Mass Mapping (side A) and Peptide Sequencing (side B). Two common approaches for mass spectrometry based proteomic analysis.

5. A Recent Example of the Complementary Approach of MALDI-TOF and ESI-MS/MS

Identification of “hCG-Associated Fragments” in Pharmaceutical Preparations of the protein hormone human chorionic gonadotropin (hCG)”

A recent publication describing the identification of hCG-associated fragments serves as a useful example of the complementary approach of MALDI-TOF and ESI-MS/MS (Malatos et al. 2005). The urinary hCG preparation CG-10 (Sigma), was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), showing protein bands at 27, 19, and 10 kDa under non-reducing conditions. Under reducing conditions two bands were observed at 15 and 10 kDa. The results (as shown in Figure 13) indicated the presence of two polypeptides that could complex, accounting for the third band on the non-reducing SDS-PAGE.

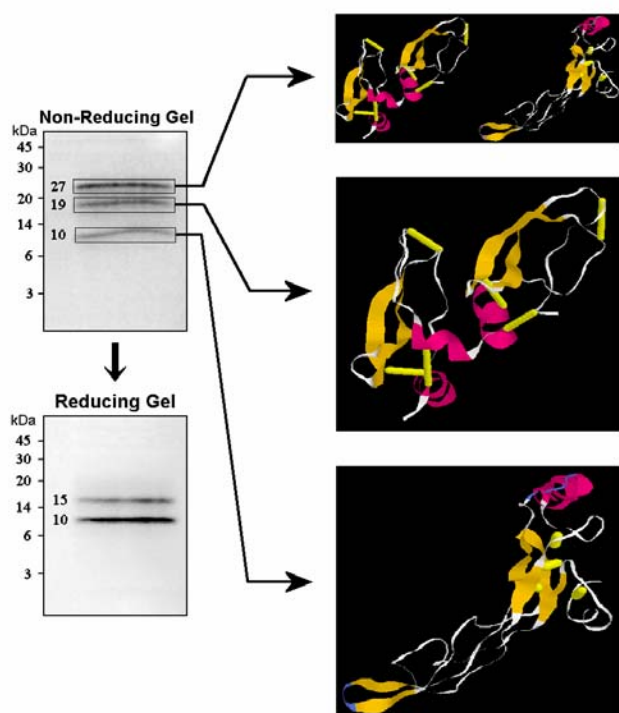


Figure 13. Molecular identification of common contaminants of clinical grade urinary hCG. Consisting of a ~27kD PAGE band that rapidly dissociated into two protein of ~10 and ~15-19kD on reducing and non-reducing PAGE. Part of figure from Malatos et al. 2005, reproduced with permission from The American Society for Biochemistry and Molecular Biology, Inc, © 2005.

Polypeptides were eluted from the individual gel bands and subjected to MALDI-TOF MS. Two large signals were observed at m/z 15,770.5 and at m/z 11,361 and subsequent peptide mass mapping, employing trypsin, identified sequences consistent with a section of placental transforming growth factor- β and bikunin. As the intact mass of pTGF- β has a mass of 12.5 kDa and that of intact bikunin is 25 to 26 kDa but the intact material observed was of lower mass it was reasoned that the polypeptides were metabolites (designated as m-pTGF- β and m-bik respectively). ESI-MS-MS of a tryptic fragment of m-pTGF β (peptide 'P5') yielded y and b ions consistent with the expected amino acid sequence (Figure 14).

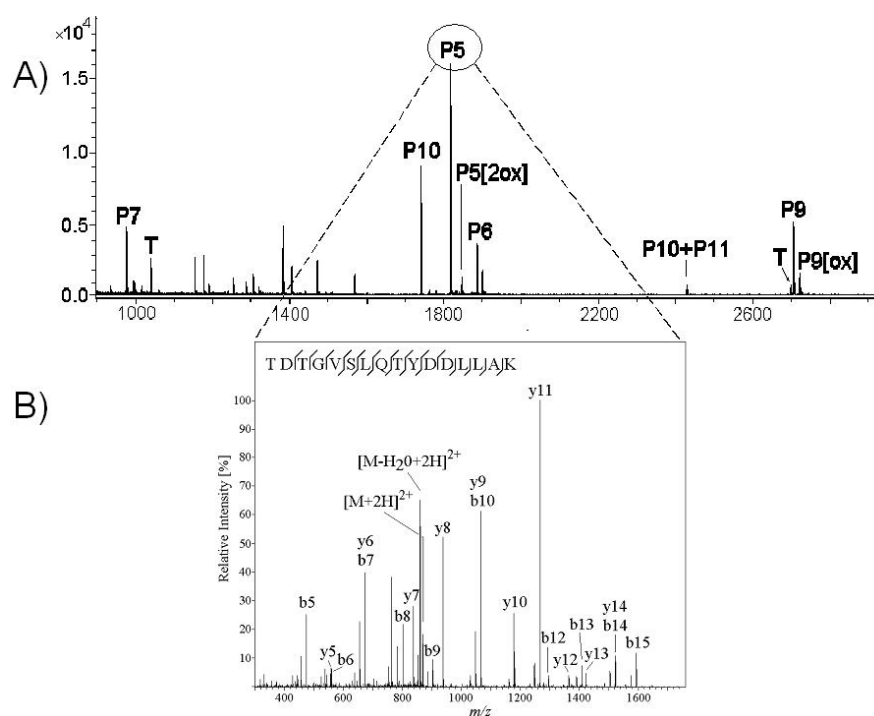


Figure 14. (A) MALDI-TOF MS spectrum of the tryptic digest of m-pTGF- β . P: m-pTGF- β fragments, ox: oxidation, T: trypsin autolysis fragments. The masses of the fragments were consistent with that of a part of the monomer of placental TGF, i.e. it is a metabolite and not the mature protein. (B) Nanospray ESI-MS/MS of m-pTGF- β tryptic peptide (P5) was selected for nESI-MS for further confirmation of peptide identity. The sequence at the top of the figure indicates the ion coverage for the peptide. Only the y and b ions are labelled in the spectrum. Figure adapted from Malatos et al (Malatos et al. 2005), reproduced with permission from The American Society for Biochemistry and Molecular Biology, Inc, © 2005.

A similar approach but using post-source decay MALDI-TOF MS yielded y and b ions consistent with that of a tryptic fragment of m-bik (B6).

Following identification of these metabolites, two pharmaceutical products of hCG (Pregnyl and Profasi), together with CG-10, were desalted using C-18 Zip-Tips and subjected to MALDI-TOF MS. In all three preparations a signal consistent with the presence of m-TGF β was observed (Figure 15) and there was some evidence to support the presence of a further degradation product of bikunin. Importantly, no other non-hCG-related protein species were observed in these preparations, large losses of analytes being unlikely due to the very simple sample preparation employed. Thorough investigations are now required to test the authors' supposition that these contaminant proteins in commercial hCG preparations are responsible for the anti-HIV associated Kaposi's sarcoma activity.

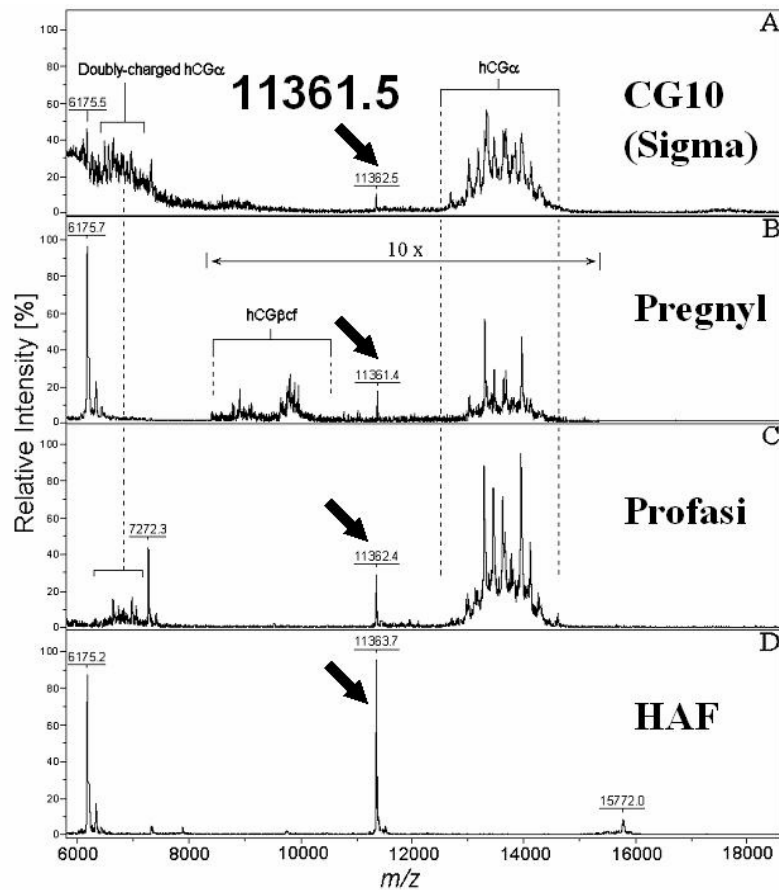


Figure 15. MALDI-TOF MS of different hCG preparations. A: CG-10 (Sigma), B: Pregnyl (Organon), C: Profasi (Serono), D: HAF preparation. The spectra focus on the mass region between 6 and 18 kDa. hCG β was observed in A, B, and C, but is omitted here for clarity. The mass region from 8.5 to 15 kDa in B is expanded 10x. Figure from Malatos et al (Malatos et al. 2005), reproduced with permission from The American Society for Biochemistry and Molecular Biology, Inc, © 2005.

6. Hybrid Mass Spectrometers

The capability of hybrid mass spectrometers, e.g. ESI-QTRAP and MALDI-TOF-TOF-MS, will further strengthen the advances and ease the characterization of complex proteins. Quadrupole-time of flight (Q-TOF) mass analyzers were one of the first types of hybrid instruments commercially available and their significance in proteomic analysis should be noted. The combination of a quadrupole and a TOF analyzer provides high sensitivity and excellent mass accuracy. This has distinct advantages in enhancing data for subsequent peptide mass mapping and thus represents a powerful new tool in mass spectrometry based proteomics. Although

beyond the scope of this introductory review to MS-based proteomics, the interested reader is referred to Chernushevich et al. (2001), who have written a very detailed tutorial on Q-TOF mass spectrometry.

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