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Plasma desorption mass spectrometry of large biomolecules*

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Abstract: Two mass spectrometric techniques, plasma desorption mass spectrometry (PDMS) and fast atom bombardment mass spectrometry (FABMS), are now extensively used for characterisation of large biomolecules. Mass spectra of protein molecules up to 34 kDa have been observed and mass spectrometry has been demonstrated to be an effective analytical tool for protein chemistry and biotechnology. Also carbohydrates and oligonucleotides can be analysed by mass spectrometry, but further improvements of the techniques are needed.

Keywords: *Plasma desorption mass spectrometry; proteins; biotechnology; sequence determination.*

Introduction

In the past 5 years two mass spectrometric techniques have found widespread application for the analysis of large biomolecules, namely plasma desorption mass spectrometry (PDMS), introduced as early as 1974 by Macfarlane *et al.* [1], and fast atom bombardment mass spectrometry (FABMS), introduced by Barber *et al.* in 1982 [2]. The first mass spectra of a protein molecule, insulin, was obtained by PDMS in 1982 [3] and later the same year by FABMS [4, 5], opening a new field of application for mass spectrometry.

FABMS quickly became adopted by many laboratories because it could be adapted easily to existing magnetic sector and quadrupole mass spectrometers. This resulted in the development of a new generation of instruments with high mass range. Such instruments, unfortunately, are expensive (0.5–1 million dollars) and require skilled operators. Molecular ions of molecules up to 24 kDa have been demonstrated [6], but as yet they are rarely used for analysis of molecules with molecular weights beyond 6000.

The development of PDMS was much slower because instrumentation only recently became commercially available (Bio-Ion Nordic AB, Box 15045, Uppsala, Sweden). In spite of this, the technique has demonstrated great potential as an analytical tool in the biochemical and the biotechnological laboratory. The instrument is relatively cheap and its operation does not require skilled personnel. The present paper therefore, will concentrate on a description of PDMS and its applications to the analysis of large

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biomolecules. For more information on FABMS of large biomolecules the reader is referred to recent reviews [7, 22].

Principles of PDMS

The instrument

The plasma desorption mass spectrometer is a time-of-flight instrument, i.e. the mass of a given ion is measured as a function of its flight time through a field-free flight tube under high vacuum (Fig. 1).

The sample is placed as a solid layer on a thin (0.5 μm) aluminised polyester foil. Desorption and ionisation of the sample molecules are effected by impact of high energy (80–100 MeV) particles obtained by spontaneous fission of the ^{252}Cf isotope. A source of such fission fragments is placed just behind the sample foil. Upon each fission event a number of neutral molecules and ions are desorbed from the surface. The latter are accelerated through 10–20 kV and allowed to drift through a field-free region to the stop detector. The flight times of the ions are measured by the time-to-digital converter (TDC), the start time being triggered by the arrival of the opposite fission fragment at the start detector (see Fig. 1). As each fission event only results in the formation of a few ions, it is necessary to accumulate a large number of spectra (5×10^5–10^6) to obtain sufficiently high ion statistics. With a fission rate of 1000–2000 fissions/s this corresponds to 4–17 min accumulation time.

Sample preparation

In PDMS, as in all desorption ionisation techniques, the purity of the sample is of the utmost importance for obtaining good spectra. Low molecular weight compounds and especially alkali metal salts, often reduce the molecular ion yields considerably and may entirely quench the production of molecular ions. A complete removal of salts from biological samples is very difficult, and methods have therefore been developed which allow the final purification to be carried out in the sample preparation step.

The most successful method to date is based on the binding of the sample molecule from an aqueous solution on to a thin film of nitrocellulose deposited on the aluminium foil followed by removal of the low molecular weight contaminants by washing with ultra pure water or dilute acids [8]. This method has been very successful for analysis of molecules which bind well to nitrocellulose, such as for example most peptides and proteins with molecular weights above 2000. Smaller peptides and other types of molecule which do not bind so well to nitrocellulose can be deposited from a small volume of solvent (2–10 μl) onto a spinning nitrocellulose covered target [9]. This results in the precipitation of the sample molecules on the nitrocellulose, whereas the low molecular weight compounds move to the periphery. If this does not achieve the required purity the spinning surface may be washed with small volumes (5–50 μl) of solvent without excessive removal of sample.

Nitrocellulose is compatible with most solvents used for dissolving large biomolecules, and even proteins which can only be dissolved using denaturing agents such as 8 M urea or 6 M guanidinium hydrochloride can be applied from such solutions, if the surface is subsequently washed extensively. Another advantage of the binding to nitrocellulose is that it is possible to carry out enzymatic or chemical reactions *in situ*, as pointed out by Chait and Field [10]. As will be shown, PDMS is essentially non-destructive. Thus a number of successive reactions can be carried out on the compound and spectra can then

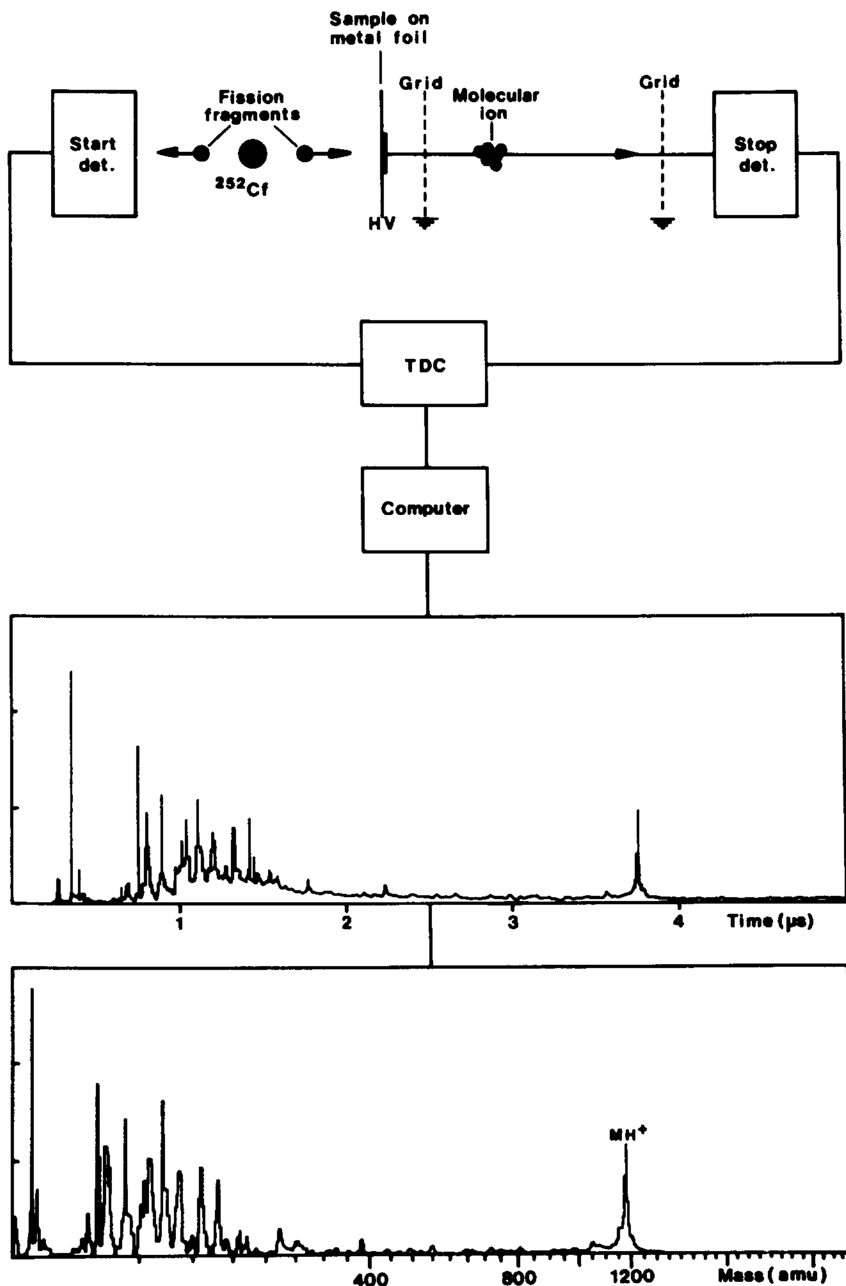

Figure 1

be recorded after each reaction. This increases the amount of information which can be obtained from a given amount of material.

Plasma desorption spectrum of proteins

The plasma desorption spectrum of a sample of porcine insulin prepared in the manner outlined previously is shown in Fig. 2. The spectrum consists of a rather intense continuum

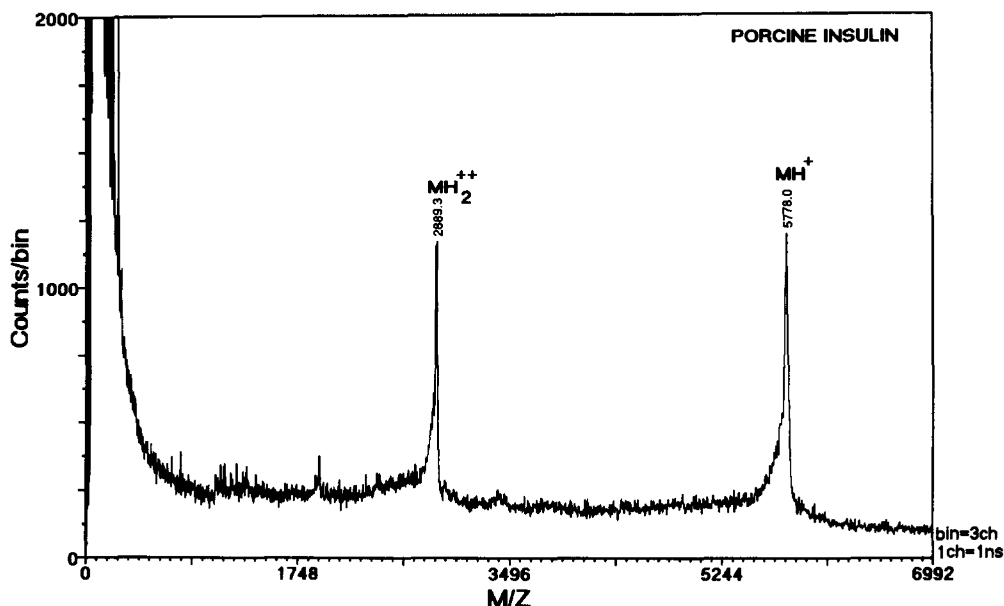

Figure 2

above which the two peaks are seen. These peaks represent the protonated molecular ion (MH^+) and the doubly protonated molecular ion ($MH_2{}^{2+}$). A weak triply charged molecular ion is also seen. Such spectra are characteristic for most proteins. The continuum consists of a mixture of fragment ions, metastable ions and background ions from the matrix. The abundance of the multiply charged ions increases with the molecular weight of the sample and above 13–14 kDa, the singly charged molecular ion is weak or absent. Interestingly this is not the case in FABMS. A number of protein molecules in the 10–30 kDa range have been analysed successfully by PDMS [11–16] with porcine pepsin (molecular weight determined as 34,630) being the largest protein molecule for which molecular ions have been observed [17].

The resolution of the instrument is low due to the lack of energy focusing. As a result, the peak width at half height, is approximately 0.2% of the mass of the ion. For large molecules, this corresponds approximately to the width of the cluster of ions observed due to the natural content of stable isotopes, mainly ^{13}C. At the base of the peak a further broadening is often observed due to ions which have survived acceleration but undergone metastable decomposition in the flight tube. Such decompositions result in ions and neutral fragments which are detected with the same flight time as the stable ion, but with larger energy distributions. The mass is calculated as the centroid of the sharp component of the complex peak shape. The measured mass thus determined represents the isotopically averaged mass. The precision of the mass determination obviously depends on the peak shape, i.e. both the intensity and the proportion between stable and metastable contribution. For peaks like MH^+ in Fig. 2, it is usually 0.02% of the mass or better. In general the precision is better than 0.1%. The sensitivity of the method is 1–10 pmol of protein.

Applications of plasma desorption mass spectrometry

As can be seen from Fig. 2, the PDMS of peptides and proteins generally gives only

molecular weight information. Whilst this does not give structural sequencing, the molecular weight is sufficiently precise to distinguish between proteins differing in size by only a single amino acid residue, and in favourable circumstances even a single amino acid substitution can be detected. Such precision is not obtainable with conventional techniques such as gel electrophoresis and gel permeation chromatography.

Applications in biotechnology

PDMS has been successfully used for the characterisation of proteins produced by DNA-recombinant techniques, such as human proinsulin, human growth hormone [14] and interferons [16]. Characterisation of variant proteins produced by site-specific mutagenesis, so called protein engineering, is another growing field of application. A molecular weight determination is often sufficient to demonstrate that the desired modification is introduced, as illustrated for a mutant of insulin (Fig. 3). If this information is not sufficiently precise, or if verification of the exact modification site is needed, mass spectrometric peptide mapping can be applied [14, 16]. This technique requires the digestion of the protein with an appropriate enzyme, followed by analysis of the resulting mixture by PDMS. This can be carried out either externally and the mixture applied as a sample to the mass spectrometer, or by *in situ* experiments on the nitrocellulose bound protein. By taking advantage of enzyme specificities, it is possible to map substitutions which only result in a change of one atomic mass unit, for example a Glu/Gln mutation [16].

Application in protein sequence determination

PDMS has found considerable use in protein sequencing [14, 16]. The first step is determination of the molecular weight of the intact protein which, combined with amino

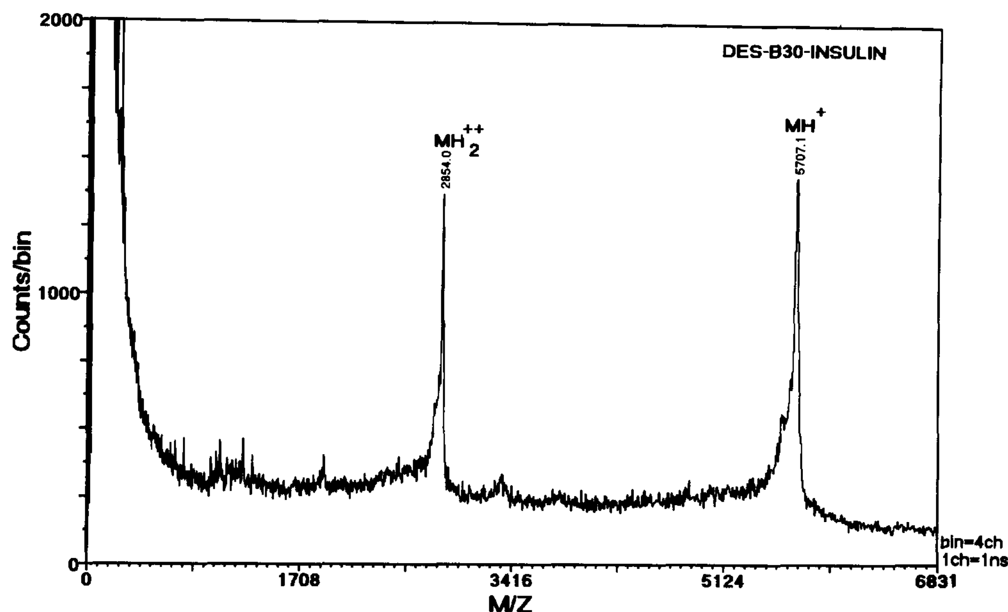

Figure 3

acid analysis data, often discloses the presence of prosthetic groups introduced by post-translational modifications. Later the molecular weight determined serves as confirmation of the proposed structure. The next step is enzymatic digestion to obtain peptides of a size suitable for sequence determination with an automatic sequenator. The time course of such digestions is easily monitored by PDMS after application of small aliquots of the digestion mixture on nitrocellulose targets. The resulting mass spectra allow rapid optimisation of digestion conditions [14]. Such experiments often allow alignment of the peptides in the protein because of the kinetics of the hydrolysis rates of the different cleavage sites.

In the following purification of the peptides mixtures by HPLC, verification of the purity and determination of the molecular weight of the peptides isolated can be rapidly carried out by PDMS. The molecular weight thus determined in combination with amino acid analysis data shows how many steps of Edman degradation are necessary for sequence determination of the peptide. It also serves as confirmation of the sequence assigned and may even resolve ambiguities encountered in identification of the amino acid residues during Edman degradation [18].

A further advantage of this strategy can be obtained where difficulties are found in using the Edman method to determine the last few C-terminal residues. *In situ* experiments using carboxypeptidases and monitoring digestion by PDMS can often give the missing information directly without further sacrifice of sample [16].

The final alignment of the peptides in the protein sequence is normally carried out by sequence determination of the peptides from a second digest with another enzyme. This step can often be replaced by a mass specrometric peptide map of the second digest and simple correlation of the molecular weights of the peptides with the sequence of the peptides from the first digest. If unambiguous results are not obtained directly, this can be further extended, for example by C-terminal sequence determination of the nitrocellulose bound peptides using carboxypeptidases.

Information about the position of disulphide bridges in the protein may likewise be obtained by mass spectrometric peptide mapping of an enzymatic digest of the protein prior to reduction of the disulphide bridges, followed by reduction directly on the sample. If the peptide maps thus obtained are too complex the peptides from the unreduced sample can be separated by HPLC, followed by analysis of the fractions by PDMS before and after reduction.

Analysis of oligonucleotides and carbohydrates

The experience in using PDMS for analysis of oligonucleotides and carbohydrates is much more limited than for peptides and proteins, and such compounds are in general considered difficult to analyse. Attempts to analyse these compounds in the underivatised state have normally resulted in low molecular ion yields and low quality spectra. Derivatised nucleotides containing up to seven bases have successfully been analysed by PDMS [19]. Similarly high quality spectra have been obtained for peracetylated oligosaccharides [20]. In both situations the spectra contained, in contrast with peptide spectra, fragment ions which allowed sequence determination. Glycopeptides have also been successfully analysed in a few cases by PDMS either as the t-Boc-derivatives [21], or as the peracetyl, methylesters [20]. On the other hand FABMS has been used successfully in these areas [7, 22]. It is apparent therefore that further studies are needed before it is possible to evaluate the full potential of PDMS for analysis of these compounds.

Discussion

As mentioned above, the type of data for PDMS can be obtained using FABMS. However, PDMS seems to have practical advantages for the analysis of molecules above 10 kDa. On the other hand the mass precision obtained by FABMS is better than that by PDMS, because high-resolution sector mass spectrometers are used. With such mass spectrometers, unit resolution up to approximately 10 kDa can be obtained, allowing recording of the fully resolved isotopic molecular envelope. This feature is only of importance in very specific cases because the molecular weight of unknown middle to high molecular weight compounds will always be determined as the isotopically averaged molecular weight, based on the centroid of the isotopic envelope.

The cost for establishing FABMS is about five times that of PDMS. Moreover, PDMS is instrumentally and operationally a much simpler technique. It would seem therefore, if only for these reasons, that in the future PDMS should find its place as a standard technique in the biochemical laboratory.

In conclusion, mass spectrometry is now a viable tool in protein chemistry, and widespread application can be foreseen in the forthcoming years. In oligonucleotide and carbohydrate chemistry, further studies are needed to demonstrate whether mass spectrometry can be of similar importance in future.

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