Plasma Desorption Mass Spectrometry of Peptides and Proteins

PETER ROEPSTORFF

Department of Molecular Biology, Odense University, DK 5230 Odense M, Denmark

Received March 30, 1989 (Revised Manuscript Received August 16, 1989)

Introduction

The application of mass spectrometry to the structure determination of peptides and proteins has been considered a major challenge to mass spectrometrists for many years, but due to the requirements for volatile samples and limited mass range and sensitivity, mass spectrometric techniques were not competitive with "wet" techniques. They only gained widespread acceptance to the solution of specific problems such as sequencing peptides with blocked N-termini and determination of the structure and position of unusual or modified amino acid residues.

A major breakthrough was achieved by the introduction of plasma desorption mass spectrometry (PDMS) by Macfarlane and co-workers in 1974.[1] With this technique were obtained mass spectra of gramicidin (MW 1881)[2] and β-endorphin (MW 3464)[3] and the first mass spectrum of a protein, insulin (MW 5730), in 1982.[4] Insulin spectra were also independently obtained with another technique,[5,6] fast atom bombardment mass spectrometry (FABMS), introduced by Barber et al. in 1981.[7]

FABMS was quickly adapted in many mass spectrometric laboratories because it could be used readily on existing magnetic sector and quadrupole mass spectrometers, and it is today a well-established technique for analysis of peptides and other nonvolatile compounds of biological origin. In contrast, the use of PDMS is still limited to rather few laboratories because commercial instrumentation only recently became available.[8] The fundamental studies related to the desorption ionization process in PDMS were reviewed in this journal in 1982,[9] and more recent reviews were published in 1985[10] and 1988.[11] This Account focuses on the utilization of PDMS in peptide and protein studies, which is probably one of the most promising fields of application.

Principles and Instrumentation

In all particle-induced-desorption methods, a sample in the solid or liquid state is desorbed by bombardment with a beam of neutrals or ions with energies in the kiloelectronvolt to megaelectronvolt range. In plasma desorption mass spectrometry, a low flux of primary ions in the 100-MeV energy range obtained by spontaneous fission of californium-252 is used.

The principle of the ^{252}Cf plasma desorption time-of-flight mass spectrometer is shown in Figure 1. The sample is deposited on a thin 0.5–1-μm-thick aluminum or aluminized polyester foil. A 10-μCi ^{252}Cf source is positioned just behind the sample foil. One of the two collinear fission fragments hits the start detector and triggers the time measurement, whereas the other penetrates the sample and causes desorption of a number of secondary ions. These ions are accelerated between the sample foil at 10–20 kV potential and a grid at ground potential and allowed to drift through the field-free flight tube to the stop detector. The flight times are measured by the time to digital converter (TDC). As each fission event only results in the recording of a few ions, it is necessary to accumulate a large number of spectra. In our laboratory, the standard is 5×10^5 corresponding to an accumulation time of 3–8 min, depending on the age of the californium source. When weak signals are analyzed, the number of accumulations is increased to $(2–10) \times 10^6$ in order to obtain better ion statistics. The flight time T is correlated to the mass to charge ratio (m/z) by the equation $T = a(m/z)^{1/2} + b$ where a and b are constants. Thus mass calibration can be performed if mass and flight time of two peaks in a spectrum are known. Traditionally the peaks for H^+ and Na^+, which are nearly always present in the spectra, are used. With the nitrocellulose sample preparation procedure (see below), the Na^+ peak is often of too low abundance and NO^+ is used instead.

Sample Preparation

In PDMS, sample quality and sample preparation are of the utmost importance for good results. The traditional method for sample preparation in PDMS was the electrospray procedure[12] (Figure 2). With this procedure, molecular ions have been observed from a number of high molecular weight samples (reviewed in ref 10

Peter Roepstorff was born in Copenhagen in 1942. He received a "Diplome d'Etudes Supérieures" in Spectroscopy from the University of Marseille in 1965 and graduated in Chemistry from the Technical University of Denmark in 1966. He was a research associate at the Danish Institute of Protein Chemistry until 1974, when he joined the Department of Molecular Biology at the Odense University as assistant professor and, since 1975, as associate professor. His primary research interest is protein structure and the occurrence of posttranslational modifications in proteins. Mass spectrometry has been the primary tool in these investigations.

(1) Torgerson, D. F.; Skowronski, R. P.; Macfarlane, R. D. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 616.
(2) Macfarlane, R. D.; Thorgerson, D. F. *Science* **1976**, *191*, 920.
(3) Macfarlane, R. D.; Uemura, D.; Ueda, K.; Hirata, Y. *J. Am. Chem. Soc.* **1980**, *102*, 875.
(4) Håkansson, P.; Kamensky, I.; Sundquist, B.; Fohlman, J.; Peterson, P.; McNeal, C. J.; Macfarlane, R. D. *J. Am. Chem. Soc.* **1982**, *104*, 2948.
(5) Dell, A.; Morris, M. R. *Biochem. Biophys. Res. Commun.* **1982**, *106*, 1456.
(6) Barber, M.; Bordoli, R. S.; Elliot, G. J.; Sedgewick, R. D.; Tyler, A. N.; Green, B. N. *J. Chem. Soc., Chem. Commun.* **1986**, 936.
(7) Barber, M.; Bordoli, R. S.; Sedgewick, R. D.; Tyler, A. N. *J. Chem. Soc., Chem. Commun.* **1981**, 325.
(8) BioIon, Nordic A.B., P.O. Box 15045, S-750 15, Uppsala, Sweden.
(9) Macfarlane, R. D. *Acc. Chem. Res.* **1982**, *15*, 268.
(10) Sundqvist, B.; Macfarlane, R. D. *Mass Spectrom. Rev.* **1985**, *4*, 421.6.
(11) Cotter, R. J. *Anal. Chem.* **1988**, *60*, 781A.
(12) McNeal, C. J.; Macfarlane, R. D.; Thurston, E. L. *Anal. Chem.* **1979**, *51*, 2036.

0001-4842/89/0122-0421$01.50/0

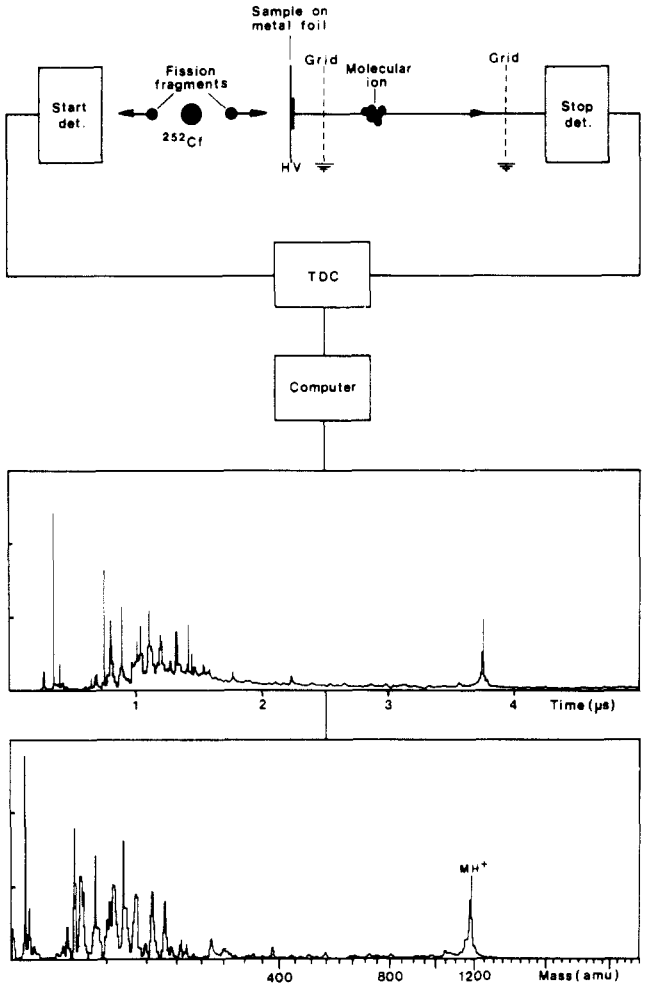

Figure 1. Principle of the plasma desorption mass spectrometer. (Reproduced by courtesy of BioIon AB, Uppsala.)

and 13). Many of the samples studied were 10–15-kDa peptides and proteins,[14] and a single example was above 20 kDa, trypsin (MW 23 463).[15] (Da ≡ dalton.) However, many samples failed with the electrospray technique. The problem seemed related to the content of low molecular weight compounds and mainly alkali-metal ions, which are known to reduce the spectrum yields dramatically.

Rather early, Macfarlane suggested that an alternative sample preparation procedure might involve adsorption of the sample on a suitable matrix. One of the first matrices described was Naphion, an ion-exchange material.[16] Two alternative matrices were later introduced. Macfarlane et al. studied polypropylene and poly(ethylene terephthalate),[17] and Jonsson et al. studied nitrocellulose.[18] Especially the latter, with which it was possible to remove salts by washing the surface (Figure 2), showed dramatically improved molecular ion yields and increased abundance of multiply charged molecular ions. Similar although less dramatically improved results were obtained by adding an excess of reduced glutathione to the sample prior to sample application by electrospray.[19] The nitrocellulose technique has since been improved, and sensitivities in the low picomolar range have been obtained by application of the sample solution on a spinning nitrocellulose target (Figure 2).[20]

Figure 3 shows molecular ion yields as a function of the insulin amount applied as obtained by different sample application techniques. Comparable molecular ion yields are obtained with the two different nitrocellulose techniques (cf. Figure 2), but the best sensitivity is obtained by application on a spinning target. The maximum molecular ion yield for the spray techniques is 3 times less than that obtained by adsorption to nitrocellulose and requires 3–4 orders of magnitude more sample. The reasons for the improved results with the nitrocellulose matrix are poorly understood, probably because several independent factors are involved, but also because the fundamental mechanism for desorption of large biomolecules from a solid layer is still unclear.

From Figure 3 it is clear that the maximum yields are obtained with very thin sample layers, probably monolayers, on the nitrocellulose. In the case of larger amounts of protein applied by the spin technique and the spray techniques, multilayers are formed, whereas adsorption to nitrocellulose followed by washing only leaves a monolayer adsorbed (Figure 2). If the binding of the protein to nitrocellulose is considerably weaker than the binding to other protein molecules, the best yields should be obtained when the protein molecules exposed to the surface are bound only to the nitrocellulose and do not interact with other protein molecules. Several experiments support this hypothesis. Chait has demonstrated that virtually all molecules were decomposed before arriving at the detector when insulin was electrosprayed, whereas 90% of the molecules survived when desorbed from nitrocellulose.[21] A reduced fragmentation is also observed for the peptide antibiotic nisin, which upon electrospraying yields several distinct fragment ions but only MH_2^{2+} and MH^+ when desorbed from nitrocellulose.[22] If the sample layer is increased or if sodium salts are added to the nitrocellulose-bound sample, fragmentation is again observed.[23] Finally, experiments with primary ions of different energy from a tandem accelerator show that substantially less energy is needed to desorb insulin from a nitrocellulose surface than from insulin multilayers formed by electrospray.[24,25]

(13) Roepstorff, P.; Sundquist, B. In *Biochemical Applications of Mass Spectrometry*; Gaskell, S., Ed.; J. Wiley and Sons Ltd.: Chichester, 1986; p 269.
(14) Sundqvist, B.; Håkansson, P.; Kamensky, I.; Kjellberg, J.; Salehpour, M.; Widdiyasekera, S.; Fohlman, J.; Peterson, P. A.; Roepstorff, P. *Biomed. Mass Spectrom.* **1984**, *11*, 242.
(15) Sundqvist, B.; Roepstorff, P.; Fohlman, J.; Medin, A.; Håkansson, P.; Kamensky, I.; Lindberg, M.; Salehpour, M.; Säve, G. *Science* **1984**, *226*, 696.
(16) Jordan, E. A.; Macfarlane, R. D.; Martin, C. R.; McNeal, C. J. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *53*, 345.
(17) Macfarlane, R. D.; McNeal, C. J.; Martin, C. R. *Anal. Chem.* **1986**, *58*, 1091.
(18) Jonsson, G. A.; Hedin, A. B.; Håkansson, P. L.; Sundquist, B. U. R.; Säve, G.; Roepstorff, P.; Nielsen, P. F.; Johansson, K. E.; Kamensky, I.; Lindberg, M. S. L. *Anal. Chem.* **1986**, *58*, 1084.
(19) Alai, M.; Demirev, P.; Fenselau, C.; Cotter, R. J. *Anal. Chem.* **1986**, *58*, 1303.
(20) Nielsen, P. F.; Klarskov, K.; Højrup, P.; Roepstorff, P. *Biomed. Environ. Mass Spectrom.* **1988**, *17*, 355.
(21) Chait, B. *Int. J. Mass Spectrom. Ion Processes* **1987**, *78*, 237.
(22) Roepstorff, P.; Nielsen, P. F.; Kamensky, I.; Craig, A. G.; Self, R. *Biomed. Environ. Mass Spectrom.* **1988**, *15*, 305.
(23) Nielsen, P. F.; Roepstorff, P. *Biomed. Environ. Mass Spectrom.* **1988**, *17*, 137.
(24) Hedin, A.; Håkansson, P.; Sundqvist, B.; Jonson, R. E. *Phys. Rev. B* **1985**, *31*, 178.
(25) Roepstorff, P.; Nielsen, P. F.; Sundquist, B. U. R.; Håkansson, P.; Jonsson, G. *Int. J. Mass Spectrom. Ion Processes* **1987**, *78*, 229.

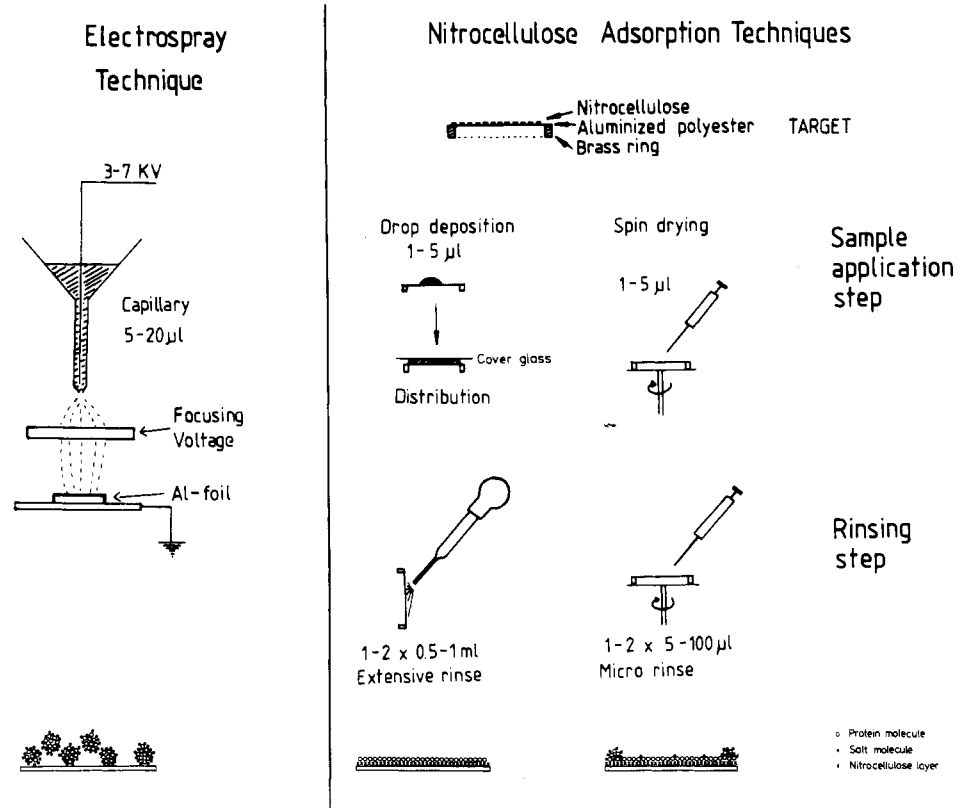

Figure 2. Sample preparation techniques for plasma desorption mass spectrometry. (Adapted from ref 32 with permission from J. Wiley & Sons.)

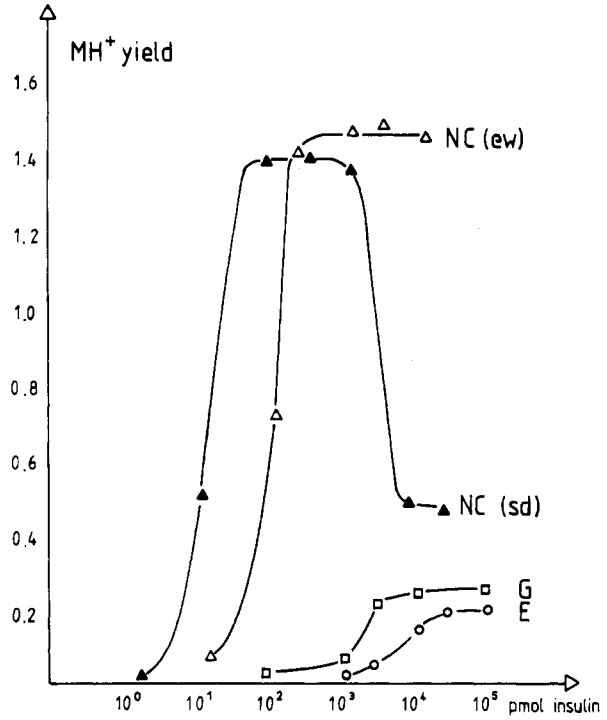

Figure 3. Molecular ion yield of insulin (expressed as number of molecular ions formed per 100 primary ions) as a function of the sample amount applied by different sample preparation techniques: ▲, spin drying on nitrocellulose (NC(sd)); △, adsorption on nitrocellulose followed by extensive wash (NC(ew)); □, electrospray with glutathione (G); ○, electrospray (E). (Adapted from ref 32 with permission from J. Wiley & Sons.)

Reactions on the Nitrocellulose Surface

In plasma desorption mass spectrometry, the low flux of primary ions leaves most of the sample intact even after long exposure to primary ions. As suggested by Chait et al.,[26,27] it is therefore possible after recording a spectrum to perform chemical or enzymatic reactions on the remaining nitrocellulose-bound sample. The principle is illustrated in Figure 4. After the spectrum of a modified insulin is recorded, Figure 4A, the target is removed from the mass spectrometer and a drop of an aqueous solution of dithiothreitol (DTT) is added, followed by a short incubation and removal of excess solvent and reagent by spin drying. DTT reduces the disulfide bonds between the A and B chains of insulin and the intrachain disulfide bond in the A chain. The spectrum now shows molecular ions for the A and B chains, Figure 4B. At this point, the sample is again removed and a solution of *Staphylococcus aureus* protease (SAP) added. This enzyme cleaves at the carboxy side of glutamic acid residues, resulting in two A-chain and three B-chain peptides. Upon reinsertion, the B-chain peptides are easily seen, whereas certain A-chain peptides are suppressed (Figure 4C) (suppression effects in peptide mixtures will be described later). This methodology has been applied successfully for the analysis of a number of insulin and mini proinsulin derivatives prepared by site-directed mutageneses.[28] Similar in situ reactions have been used for C-terminal sequence determination of peptides,[27,29-31] for confirmation of the tentative identifi-

(26) Chait, B. T.; Field, F. H. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 420.
(27) Chait, B. T.; Chaudhary, T.; Field, F. H. In *Methods in Protein Sequence Analysis 1986*; Walsh, K. A., Ed.; Human Press: New York, 1987; p 483.
(28) Nielsen, P. F.; Roepstorff, P.; Clausen, I. G.; Jensen, E. B.; Jonassen, I.; Svendsen, A.; Balschmidt, P.; Hansen, F. B. *Protein Engineering* **1989**, *2*, 449.
(29) Craig, A. G.; Engstrøm, Å.; Bennich, H.; Kamensky, I. *Biomed. Environ. Mass Spectrom.* **1987**, *14*, 669.
(30) Klarskov, K.; Breddam, K.; Roepstorff, P. *Anal. Biochem.* **1989**, *180*, 28.

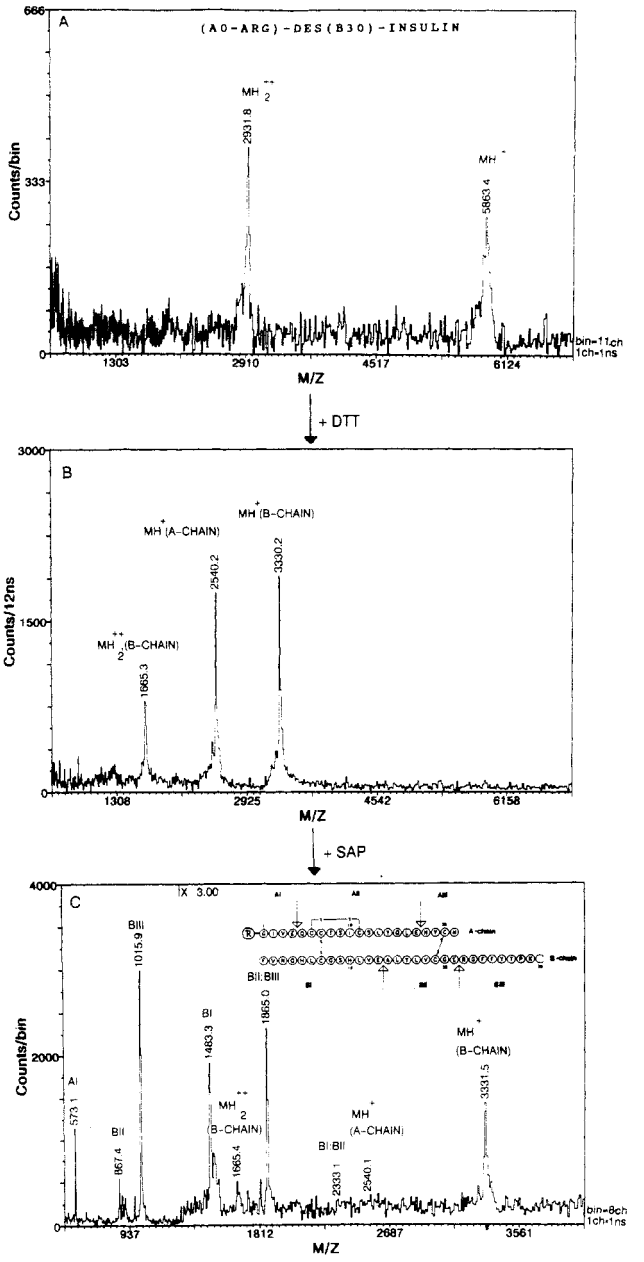

Figure 4. Example of the application of successive in situ reactions on a modified insulin sample (A0-Arg, des(B30) insulin): A, the intact molecule; B, after in situ reduction of A with dithiothreitol (DTT); C, after in situ digestion of B with *S. aureus* protease (SAP).

cation of peptides on the basis of their molecular weight,[31,32] to locate modification sites by reactions that chemically modify proteins,[32] to find errors in peptide synthesis,[33] and to assign disulfide bridges in proteins.[34,35]

Mixture Analysis

It is well-known that suppression of certain components limits the utility of FABMS for peptide mixture analysis and that this is mainly caused by differences in hydrophobicity of the components.[36] Suppression is also observed in PDMS. Thus PD analysis of mixtures of peptides produced by tryptic digestion of interleukin 2 and human growth hormone did not show all the expected peptides,[34] and the same was the case for peptide maps produced by in situ digestion of insulins and mini proinsulins with *S. aureus* protease.[28] Generally, however, peptide maps obtained by PDMS seem more complete than those obtained by FABMS.[36]

The suppression effect in PDMS has been demonstrated to be related to the net charge on the peptides, i.e., peptides with a potential positive net charge are predominant in the positive-ion spectra, whereas peptides with a potential negative net charge are mainly observed in the negative-ion spectra.[37] Thus, the positive- and negative-ion spectra are complementary for the analysis of mixtures. Interestingly, the peptide with net negative charge, which is completely suppressed in a positive spectrum of a mixture, may, when analyzed alone, give a high positive-ion yield. It therefore seems that the amount of protons is a limiting factor and that the peptide with the highest proton affinity is the "winner".

Another factor in suppression may be competitive binding to the surface. As the surface has a limited binding capacity,[26] this is especially possible if peptide mixtures are added in amounts that exceed the binding capacity of the surface or if the surface, when in situ reactions are performed, is already saturated with starting material.

Applications of PDMS to Peptide and Protein Chemistry

Most applications of PDMS in protein chemistry take advantage of the ease of producing molecular ions. The number of applications is rapidly expanding. Therefore, in this survey only examples of types of applications are described with a few references to illustrate the potential of PDMS in protein chemistry.

Molecular Weight Determination of Proteins. One of the first measurements performed to characterize a protein is determination of the molecular weight. The classical methods are gel permeation chromatography and SDS (sodium dodecyl sulfate) or gradient gel electrophoresis. However, such measurements are susceptible to large errors, and the molecular weight determination is influenced by properties of the molecule that are irrelevant to the molecular weight, such as shape of the molecule or hydrophobicity. In our laboratory, PDMS has now replaced these methods for all proteins with a molecular weight accessible to PDMS analysis. The hitherto largest protein for which the molecular weight has been determined by PDMS is porcine pepsin (MW 34 630).[38] It is our experience that most proteins up to 20 kDa and a fair number of proteins between 20 and 35 kDa can be analyzed by PDMS. The reasons for failures are still unclear, but may be related to both sample purity and inherent structural properties of the molecules. The precision of molecular weight determination by PDMS depends

(31) Klarskov, K.; Højrup, P.; Andersen, S. O.; Roepstorff, P. *Biochem. J.* **1989**, *262*, 923.
(32) Roepstorff, P.; Nielsen, P. F.; Klarskov, K.; Højrup, P. In *The Analysis of Peptides and Proteins by Mass Spectrometry*; McNeal, C. J., Ed.; J. Wiley and Sons: Chichester, 1988; p 55.
(33) Chait, B. T. *Ibid.*; p 21.
(34) Tsarbopoulus, A.; Becker, G. W.; Occolowitz, J. L.; Jardine, J. *Anal. Biochem.* **1988**, *171*, 113.
(35) Højrup, P. Personal communication.
(36) Kleough, T. In *The Analysis of Peptides and Proteins by Mass Spectrometry*; McNeal, C. J., Ed.; J. Wiley & Sons: Chichester, 1988; p 115.
(37) Nielsen, P. F.; Roepstorff, P. *Biomed. Environ. Mass Spectrom.* **1989**, *18*, 131.
(38) Craig, A. G.; Engström, Å.; Bennick, H.; Kaminsky, I. Presented at the 35th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, Colorado, May 1987.

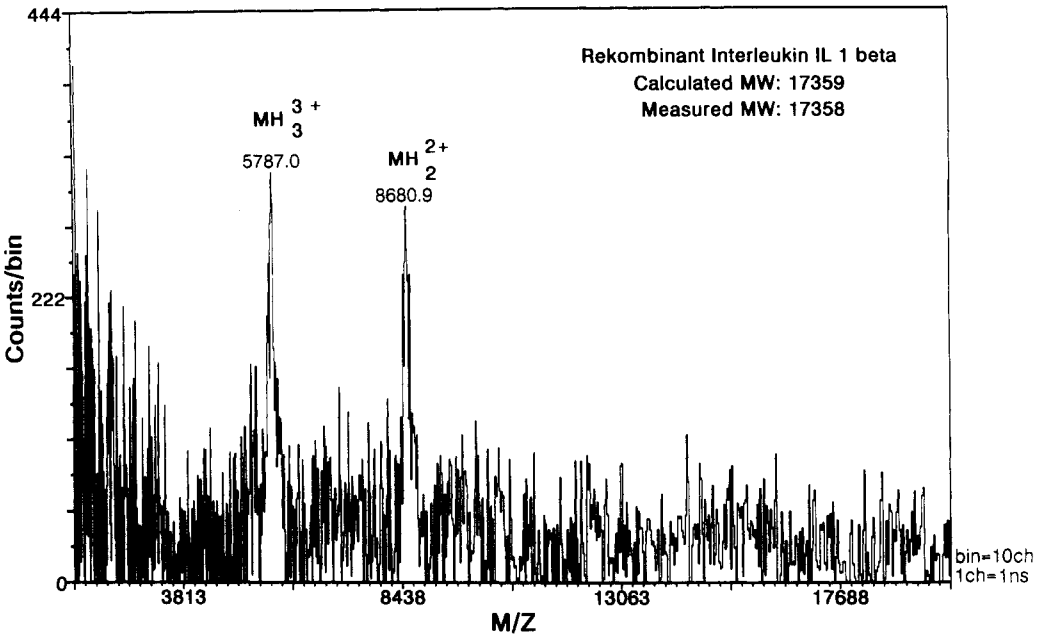

Figure 5. Plasma desorption spectrum of biosynthetic interleukin 1. The measured molecular weight is the average of that determined from the doubly and triply charged molecular ions.

on the peak shape and the ion statistics. It is usually better than 0.05% of the mass below mass 5000 and about 0.1% at higher masses.

The first example of a practical application of mass spectrometric molecular weight determination of a protein beyond 10 kDa was in the structure determination of a locust cuticle protein.[39] A discrepancy between the molecular weight (21 600) estimated by SDS gel electrophoresis and that based on the determined sequence (15 323) was solved in favor of the latter, as the molecular weight after adsorption to nitrocellulose was measured by PDMS to 15 329 ± 50.

Since then, several proteins from the locust cuticle have been analyzed by PDMS in our laboratory (ref 31 and 32 and unpublished results). A number of insulin-like growth factors (IGFs) in the molecular weight range 7400–7800 were isolated from human Cohn fraction IV and could, on the basis of their molecular weight, be classified as belonging to the IGF I or IGF II family.[40] A modified β_2-microglobulin from a lung cancer patient[32] was demonstrated to contain an in-chain cleavage resulting in a molecule consisting of two peptides chains linked with one disulfide bond. Due to fragmentation of this bond, the mass spectrum allowed exact positioning of the cleavage point and identification of an excised amino acid residue.

Analysis of Proteins Produced by DNA-Recombinant Techniques. In the production of proteins by DNA-recombinant techniques, it is important to identify the protein and to ensure that desired posttranslational modifications have taken place and that undesired posttranslational modifications are absent. Molecular weight determination by PDMS has been found very useful for this purpose. The molecular weigths of biosynthetic proinsulin (MW 9388.6) and human growth hormone (MW 22 125) were determined as 9388 ± 5 and 22 122 ± 20 by PDMS. In the former, a small ion at m/z 6240 indicated the presence of a dimer of proinsulin, this ion being the triply charged ion of the dimer. In the latter, the molecular weight determination also showed that an N-terminal methionine as intended was removed.[41] Interleukin 1 was synthesized as a precursor containing a leader sequence that was four amino acid residues long. After removal of the leader sequence, the plasma desorption spectrum shown in Figure 5 was obtained. The molecular weight obtained confirmed removal of the leader sequence and identified the protein expected.

An alternative approach to analysis of DNA-recombinant proteins is based on chemical or enzymatical cleavage of the protein followed by analysis of the mixture of peptides by PDMS (PD mapping) either directly or after partial purification.[34]

In protein engineering, i.e., production of variants of proteins by site-directed mutagenesis or by chemical modification, it is often necessary to identify the precise location of the modification site. For such analysis, PD mapping and especially the concept of gradually obtaining more detailed information by successive reactions on the nitrocellulose-bound sample is an excellent choice. The general concept[41] follows the outline described in Figure 4. It has been applied to analysis of a number of insulin and proinsulin variants produced by site-directed mutagenesis.[28] Even glutamic acid to glutamine mutations resulting in a 1-amu mass difference, which is on the limit of the mass precision obtained by PDMS, could be ascertained from the peptide map, taking advantage of the specificity of the enzymes applied for cleavage of the proteins. An alternative for such analysis, which also allows identification of partial deamidation, is based on counting the number of carboxyl groups by PDMS analysis before and after methyl-esterification of all carboxyl groups in the peptide and protein.[42]

(39) Roepstorff, P.; Højrup, P.; Sundqvist, B. U. R.; Jonsson, G.; Håkansson, P.; Andersen, S. O.; Johansson, K. E. *Biomed. Environ. Mass Spectrom.* **1986**, *13*, 689.

(40) Van den Brande, J. L.; Hogerbrugge, M.; Beyreuther, K.; Roepstorff, P.; Jansen, J.; Van Bull-Offers, S. C. *Acta Endocrinol.* Submitted.

(41) Roepstorff, P.; Nielsen, P. F.; Klarskov, K.; Højrup, P. *Biomed. Environ. Mass Spectrom.* **1988**, *16*, 9.

(42) Højrup, P.; Knudsen, J.; Olson, J. M.; Ødum, L.; Roepstorff, P. *Adv. Mass Spectrom.* **1989**, *11*, 1388.

Processing of Precursor Proteins. In nature, processing of large precursor proteins is an important pathway in the production of biologically active peptides and proteins. Such processes are often tissue-specific, and the processing consists of both enzymatic cleavage of the precursor molecules to smaller peptides and posttranslational modification, e.g., acylation, amidation, sulfatation, or glycosylation. The sequence of many such precursor proteins has been obtained from the cDNA, but this information does not allow prediction of the final products and the processing pathway.

PDMS can be used to study precursor protein processing. The basic approach is to perform a simple extraction of the tissue followed by separation of the components by reverse-phase high-performance liquid chromatography (RP-HPLC) and determination of the molecular weight of the components in each fraction by PDMS. The molecular weights determined can then be matched with the sequence of precursors known to be active in the tissue studied, and peptides possibly derived from these precursors can be located. The identity of the peptides can be confirmed by in situ reactions with endo- or exopeptidases or by a few steps of Edman degradation. Peptides accounting for the total sequence of the three known precursor molecules in bovine pituitary posterior lobe, propressophysin, prooxyphysin, and proopiomelanocortin could be identified.[43] Several of the peptides were posttranslationally modified or found in both the modified and nonmodified versions. Some of the peptides identified were known and others predicted but not yet isolated, whereas others were novel. The sensitivity of the techniques is sufficient to perform such an analysis on a single rat pituitary.[44] A similar concept was applied to analyze prosomatostatin processing in catfish pancreas. A number of processing products including several somatostatins, glucagon, glucagon-like peptides, insulin, and the proinsulin C-peptide were identified.[45]

Protein Sequence Analysis. Analysis by PDMS has been extensively used in our laboratory as a supplement to automatic Edman degradation in protein sequence determination. Based on the experience gained with a number of proteins,[31,46,47] a general strategy has been developed which takes advantage of molecular weight determination, mixture analysis, and in situ reactions.[48] PDMS is used at all stages of the sequencing process, i.e., molecular weight of the protein, monitoring of enzymatic digests, purity check, molecular weight determination and alignment of subpeptides, C-terminal sequence determination with carboxypeptidases, and localization of disulfide bridges.

The advantage of introducing PDMS in protein sequencing is that the overall sample consumption is reduced, the sequenator use is approximately halved and the costs are correspondingly reduced, and, not least, PDMS provides an independent method for confirmation of the results.

State of the Art and Future Trends

Plasma desorption mass spectrometry is in the present state of the art an excellent tool in protein chemistry. The simplicity and relatively low cost of the instrument, compared to, for example, a high mass FAB mass spectrometer, allows it to be an integrated element in the protein chemistry laboratory. The nitrocellulose matrix has drastically improved the practical utility of the technique because it is directly compatible with most solvents used in protein chemistry, allows in situ removal of low molecular weight contaminants and salts, and increases the sensitivity to an adequate level.

PDMS has two major drawbacks compared to FABMS. One is the lower precision in mass determination, which for PDMS may vary between 0.01 and 0.2% of the mass, depending on the sample amount and the quality of the spectrum. In our general protein studies, we have found the mass precision sufficient for most purposes, and although we have access to a high mass FAB sector instrument, it is very rare that it has been necessary to supplement the PDMS data with FABMS. It is, however, possible to improve the mass precision by approximately 1 order of magnitude by replacing the simple straight flight tube with a reflectron.[49,50] The other drawback is that the simple time-of-flight system, especially in combination with the nitrocellulose matrix, gives very little structurally meaningful fragmentation and that is not possible to enhance such information with collision activation and MS/MS analysis as in FABMS. The possibility of performing in situ reactions on the nitrocellulose-bound sample to a certain degree represents an alternative but is not as informative. The reflectron also allows analysis of metastable decay and thus a sort of MS/MS analysis,[50] but an enhancement of the fragmentation is still missing. The advantages of PDMS compared to FABMS are mainly better sensitivity,[43] the simplicity of operation of the instrument, and the universality of the nitrocellulose matrix.

The question always asked is: "What is the upper mass limit for PDMS?" Although molecular ions for a 45-kDa protein molecule, ovalbumin, have been observed,[51] it seems, with the present state of the art, increasingly difficult to analyze proteins as their molecular weight passes beyond 15–20 kDa. It is possible that discovery of new matrix systems may break this barrier.

Two other mass spectrometric techniques seem at present more promising for analysis of very large protein molecules. With laser desorption mass spectrometry (LDMS), molecular ions beyond m/z 200 000 have recently been observed by using a nicotinic acid matrix,[52] and very high molecular ion yields have been obtained for a number of proteins in the 10–150-kDa range.[52,53] LDMS is, like PDMS, based on a time-of-

(43) Feistner, G. F.; Højrup, P.; Evans, D. J.; Barofsky, D. F.; Faull, K. F.; Roepstorff, P. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6013.
(44) Feistner, G. F.; Roepstorff, P.; Højrup, P.; Faull, K. F.; Evans, C. J.; Barofsky, D. F. *Adv. Mass Spectrom.* **1989**, *11*, 1380.
(45) Andrews, P. C.; Alai, M.; Cotter, R. S. *Anal. Biochem.* **198**, *174*, 23.
(46) Højrup, P.; Andersen, S. O.; Roepstorff, P. *Biochem. J.* **1986**, *236*, 713.
(47) Mikkelsen, J.; Højrup, P.; Nielsen, P. F.; Roepstorff, P.; Knudsen, J. *Biochem. J.* **1987**, *245*, 857.
(48) Roepstorff, P.; Klarskov, K.; Højrup, P. In *Methods in Protein Sequence Analysis 1988*; Wittmann-Liebold, B., Ed.; Springer-Verlag: Berlin, 1989; p 191.
(49) Della Negra, S.; LeBeyec, Y. *Int. J. Mass Spectrom. Ion Processes* **1984**, *61*, 21.
(50) Tang, X.; Beavis, R.; Ens, W.; LaFortune, F.; Schuler, B.; Standing, K. G. *Int. J. Mass Spectrom. Ion Processes* **1988**, *85*, 43.
(51) Jonsson, G.; Hedin, A.; Håkansson, P.; Sundqvist, B. U. R.; Bennick, H.; Roepstorff, P. *Rapid Commun. Mass Spectrom* **1989**, *3*, 190.
(52) Hillenkamp, F. *Adv. Mass Spectrom.* **1989**, *11*, 354.
(53) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299.

flight instrument, and it should in principle be possible to use laser and plasma desorption alternatively in the same instrument. With electrospray ionization of proteins, a series of peaks in the m/z 900–1200 range is observed. These peaks represent highly charged molecular ion species and can be used to calculate the corresponding molecular weight of the protein.[54] Proteins up to 60 kDa have been analyzed with this technique,[55] but the upper limit is not known.

(54) Yamashita, M.; Fenn, J. B. *J. Phys. Chem.* **1984**, *88*, 4451.
(55) Meng, C. K.; Mann, M.; Fenn, J. B. *Z. Phys. D* **1988**, *10*, 361.

In conclusion, PDMS is already a valuable tool for the protein chemist, and there seems to be room for improvements and for combining PDMS with other techniques and, thus, for increasing the practical applicability of mass spectrometry in the future.

Sincere gratitude is expressed to graduate students and postdoctoral collaborators whose names are cited in the references. A special thank-you to Professor B. Sundqvist, Uppsala University, who introduced me to the world of sputtering of large molecules. Support for our work has been provided by the Danish Technical Science and Natural Science Research Councils.

Coal Pyrolysis

LEON M. STOCK

Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439, and Department of Chemistry, The University of Chicago, Chicago, Illinois 60637

Received January 6, 1989 (Revised Manuscript Received September 7, 1989)

Introduction

Coals are composed of fossil plant materials that have been subjected to chemical and biochemical decay before burial and to pressure and heating after burial.[1] The components of the plants decompose at different rates depending on their chemical constitution and the environment in which they are deposited. The organic components transform into a collection of petrographically distinct materials known as macerals.[2] These substances, which occur in three distinct groups, the liptinites, the vitrinites, and the inertinites, are photogenetic organic entities or optically homogeneous aggregates of phytogenetic entities possessing distinctive chemical and physical properties.[3] The vitrinites, which are derived for the most part from the lignin in wood, are the principal constituents of most coals. Consequently, the vitrinite-rich coals have received a proportionally significant share of scientific and technological attention.

Although neither the macerals nor the coals have a chemical structure in the conventional sense of the term, several workers in the field have proposed working hypotheses that account for the spectroscopic information such as the solid state proton and carbon magnetic resonance spectral data[4] and for chemical observations such as the substances obtained in supercritical extraction experiments[5] or in carefully designed oxidation reactions.[6] A contemporary representation of an often-studied bituminous coal from the Illinois No. 6 seam is shown in Figure 1.[7]

Considerable technological progress in coal utilization has been made in recent years, with two new major gasification installations in operation in the United States. One at Coolwater, CA, produces gas turbine fuels for power generation;[8] the other, at Grand Forks, ND, produces methane.[9] It is now well appreciated in the technological community that the discovery of new, more economically feasible methods for the fuller utilization of coal in the United States for energy generation, transportation fuels, chemical feedstocks, and other related purposes will require a rather thorough appreciation of the chemical nature of its constituents and their patterns of reactivity. It is equally well appreciated in the scientific and engineering communities that the achievement of these objectives is an exceedingly challenging intellectual problem. Chemists who, generally speaking, are accustomed to carefully designed investigations of the behavior of pure substances in carefully controlled environments are challenged by the enormous complexity of coal chemistry.

Virtually all of the technologically important processes such as liquefaction, in which coal is converted into substances that may be recovered by distillation, and gasification, in which coal is converted into substances that are volatile, begin with thermal reactions that involve deep-seated molecular transformations. The thermal decomposition reactions of the coal macromolecules that occur during the earliest stages of

Leon M. Stock was born in Detroit, MI, in 1930. He received his B.S. in Chemistry from the University of Michigan in 1952. After two years in the U.S. Army, he continued his education at Purdue University, where he received his Ph.D. degree in 1959 under the direction of Herbert C. Brown. He is now Professor of Chemistry at The University of Chicago and Director of the Chemistry Division of Argonne National Laboratory. He received the H. H. Storch Award of the American Chemical Society for his work in coal chemistry in 1987.

(1) Given, P. H. *Coal Sci.* **1984**, *3*, 65.
(2) Stach, E.; Mackowsky, M.-Tu.; Teichmuller, M.; Taylor, G. H.; Chandra, D.; Teichmuller; R. *Stach's Textbooks of Coal Petrology*, 3rd ed.; Gebruder Borntraeger: Berlin, 1982.
(3) (a) Spackman, W. *Trans. N.Y. Acad. Sci.* **1958**, Ser. II, *20*, 411. (b) Given, P. H.; Dyrkacz, G. R. In *New Trends in Coal Science*; Yurum, Y., Ed.; Kluwer Academic Publishers: Dordrecht, 1988; p 53.
(4) Davidson, R. M. *Nuclear Magnetic Resonance Studies of Coal*; IEA Coal Research Publication: London, 1986.
(5) Mayo, F. R.; Krishen, N. A. *Fuel* **1978**, *57*, 405.
(6) Hayatsu, R.; Scott, R. G.; Winans, R. E. In *Oxidation in Organic Chemistry*; Trahanovsky, W. S., Ed.; Academic Press: New York, 1982; Part D, p 279.
(7) Shinn, J. H. *Fuel* **1984**, *63*, 1187.
(8) (a) Plumley, D. R. *J. Eng. Gas Turbines Power* **1985**, *107*, 856. (b) Plumley, D. R.; Adams, T. J. *Proc. Am. Power Conf.* **1985**, *47*, 230.
(9) Holt, N. A.; Gluckman, M. *Proc.—Refin. Dep., Am. Pet. Inst.* **1985**, *64*, 248.

0001-4842/89/0122-0427$01.50/0