The observation of quasi-molecular ions from a tiger snake venom component (M_r 13309) using ^{252}Cf-plasma desorption mass spectrometry

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A method involving fast heavy-ion bombardment of a solid sample called ^{252}Cf-plasma desorption mass spectrometry has been used to study a non-enzymatic, non-toxic phospholipase homolog from Australian tiger snake (*Notechis scutatus*) venom. The protein consists of 119 amino acids in a single polypeptide chain cross-linked by 7 disulfide bridges. The isotopically averaged molecular mass as determined by protein sequence analysis is 13309 atomic mass units (amu). The mass distributions were studied by means of time-of-flight measurements. Quasi-molecular ions associated to the molecule and its dimer were observed. The mass of the quasi-molecular ion corresponding to the molecule was determined to be 13285 ± 25 amu.

Molecular mass determination *Mass spectrometry, of protein* *Plasma desorption mass spectrometry*

1. INTRODUCTION

During the past decade considerable efforts have been made in the field of mass spectrometry with the aim to produce molecular ions of large, thermally labile biomolecules. Particle-induced desorption techniques have been particularly successful in this respect. A new mass spectrometric method was introduced in 1974 called plasma desorption mass spectrometry (PDMS) intended to solve this problem [1]. In this method, fission fragments from a ^{252}Cf-source are used to bombard a solid sample in order to desorb and ionize molecules. The mass of the desorbed ions is determined by means of the time-of-flight technique (TOF). Of the many impressive results reported in [2] the most recent one is the detection of a fully protected oligonucleotide of 6275 atomic mass units (amu) and the corresponding dimer [2]. They were also able to deduce the base sequence of oligonucleotides by analyzing spectra of negative ions [3–5].

In collaboration with McNeal and Macfarlane in [2] we were able to observe quasi-molecular ions of bovine insulin (M_r 5733) for the first time [6]. In that study an accelerator beam of $^{127}I^{20+}$ was used instead of fission fragments. With the same technique we have also recorded spectra of a curariform protein neurotoxin with M_r 7821 [7]. Later we have shown that ^{252}Cf-PDMS can be used to yield equally good spectra as these two molecules [8].

Benninghoven and others have used low energy (keV) ions in SIMS-type studies and have been able to investigate many labile compounds such as amino acids and nucleotides [9,10]. A recent development of that method is the use of a liquid matrix bombarded by a beam of neutrons [11]. With this technique, called fast atom bombardment (FAB), bovine insulin and the sweet proteins monellin I and II have been successfully studied [12].

The protein studied here is a non-enzymatic, non-toxic phospholipase homolog from Australian tiger snake (*Notechis scutatus*) venom, called II-1

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[13,14]. It received attention due to the fact that it was structurally closely related to phospholipases with significant toxicity, blocking the signals from nerve to muscle [15–18]. The main structural difference appeared to rest with the substitution of an otherwise conserved glycine residue for serine [14]. Thus it was suggested to be an example of how a replacement of one amino acid can totally abolish functional activity.

2. MATERIALS AND METHODS

The Australian tiger snake venom component II-1 was purified by gel filtration and ion-exchange chromatography [13]. It was dissolved in trifluoroacetic acid and electrosprayed onto a thin aluminum foil (500 $\mu g/cm^2$) [19]. Since a large amount of sample was available, no attempts were made to reduce the quantity sprayed, which was about 200 μg spread over 1.5 cm^2. The sample film was mounted in the ion source of a ^{252}Cf time-of-flight mass spectrometer (TOF-MS) with a field-free flight path of 16 cm. The fundamental features of the spectrometer and the experimental technique have been described in [20]; a detailed description of the setup used in this study will appear soon [21]. Here only a brief, general outline of the apparatus will be given.

The acceleration voltage was maintained at 17 kV. The fast, primary ions, i.e., fission fragments from the ^{252}Cf-source, hit the sample and positive ions desorbed from the surface were accelerated through a Ni-grid on earth potential. In a fission disintegration two fission fragments are ejected almost co-linearly. Each time a fission fragment passes through the sample, the associated fission fragment ejected in the opposite direction is detected in a start detector. The start detector in this experiment was a thin, electron-emitting aluminum foil and two channel electron multiplier plates (Varian) coupled in tandem. The start rate was 1500 fission fragments/s. After acceleration, the ions drift in a field-free region to the end of the flight tube where they are detected in two-channel electron multiplier plates in an arrangement similar to that for the start detector.

The time measurements were performed with a time-to-digital converter (TDC), a multi-stop time-measuring electronic unit with $\pm$ 1 ns resolution. In each event the TDC was activated by the signal from the start detector and able to register flight times for up to 32 ions. The output from the TDC was fed into an Apple II^+ microcomputer which collected and displayed the TOF spectrum. After only a few minutes it was possible to see the quasi-molecular ion peak growing on the monitor. To obtain good statistics the spectrum shown was recorded for 5 h and then transferred to a VAX 11/780 for further analysis. The time spectrum was mass calibrated by use of the H^+ and Na^+ peaks.

3. RESULTS AND DISCUSSION

The smooth, exponentially decaying background, characteristic of ion-induced desorption time-of-flight spectra is subtracted in the spectrum shown in fig.1. The part of the time-spectrum displayed corresponds to a m/z region 8000–60000. The isotopically averaged mass of the tiger snake venom component II-1 as deduced from protein sequence analysis is 13309 amu. The conspicuous peak in the spectrum corresponds to m/z 13285 $\pm$ 25 and this is, to our knowledge, the largest biomolecule ion detected by means of a mass spectrometric method so far.

There are several contributions to the error given. One is the uncertainty in the mass calibration. Another contribution is the determination of the centroid, especially as there is a broader peak which interferes. The background subtraction also influences the precision. The details of the technique and the precision in the mass determinations will be discussed elsewhere [21]. The data suggest that we observe protonated quasi-molecular ions, $(M + H)^+$, an assumption which is supported by recent precise ($\pm$ 1 amu) TOF-measurements on other proteins; e.g., bovine insulin (M_r 5733), for which the $(M + H)^+$ ion seems to be the dominating quasi-molecular ion [21].

A characteristic feature of the fast heavy-ion-induced mass spectra, namely the abundance of cluster ions, is also illustrated in the present spectrum. The broad distribution corresponding to dimer ions and also a small peak in the mass region of the trimer can be seen in fig.1. The broad distributions, in particular the distributions of the polymers, are mainly due to metastable decay; i.e., decay of an ion into two fragments before detection. If the ion decays in the field-free region the centroids will still give the correct mass. It should

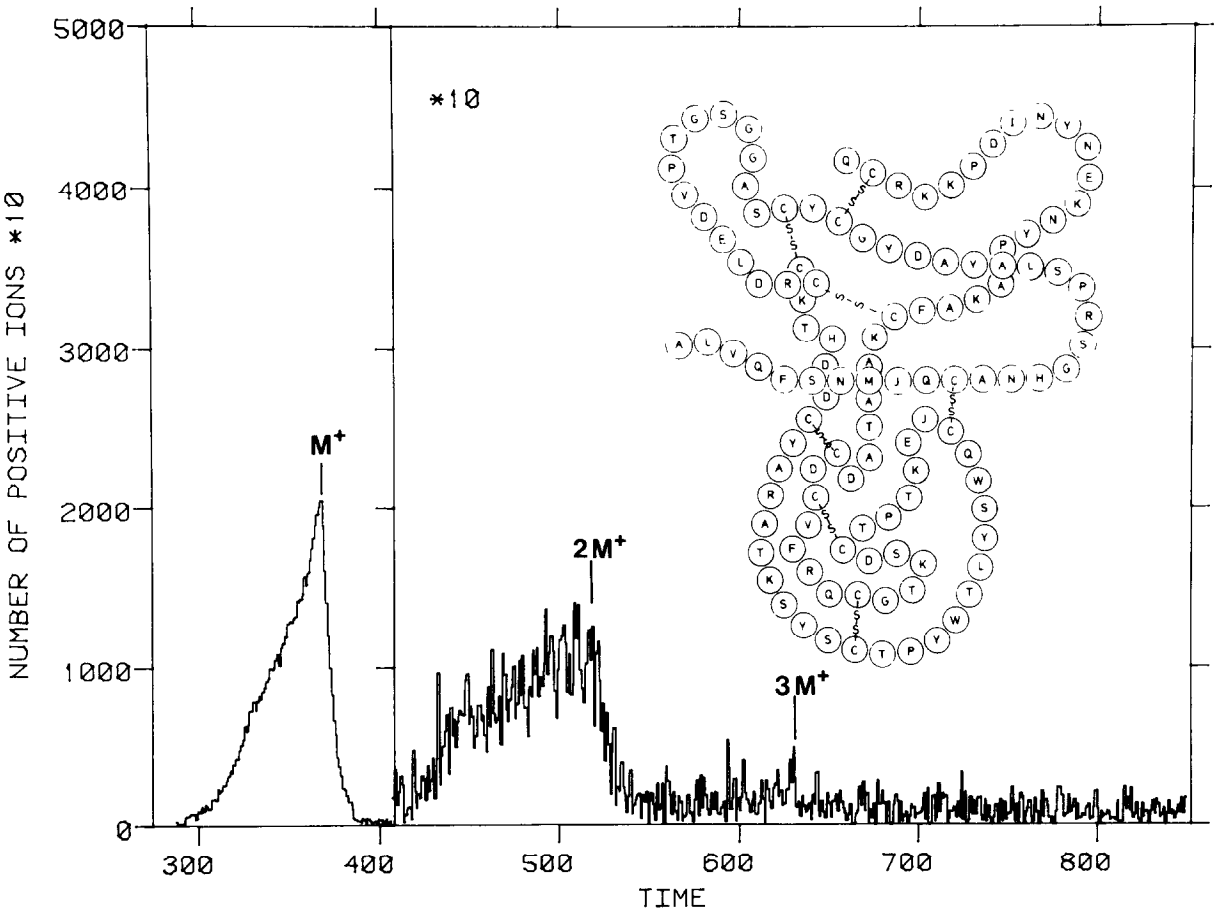

Fig.1. Part of the time-of-flight spectrum of *Notechis scutatus* II-1 by ^{252}Cf-PDMS. Insert shows the covalent structure [14]. The disulfide pairing is based upon [25].

also be pointed out that the contributions from isotopic broadening of the peaks is of the order of 10 mass units; i.e., a much smaller effect than the widths observed here.

This study indicates new exciting possibilities to observe and determine the mass of large molecules like proteins with masses in the range 10000–20000 amu. It is possible to improve the TOF technique such that the precision will be of the order of a few mass units. Possible applications of the method apart from molecular mass determinations (e.g., pro-analysis for sequencing work) may be to study modifications. II-1 is one example of a protein with a suggested critical replacement of a single amino acid residue leading to a dramatic functional change. Other examples are the hemoglobinopathies [22], post-translational modifications like phosphorylation and laboratory chemical modifications. A very recent and interesting case is the discovery that a bladder carcinoma cell line differs from a normal cell population only by one single base change invoking replacement of glycine for valine [23,24]. Given a few μg pure sample, the molecular mass can already be determined to an accuracy considerably better than one amino acid unit, within a few hours with the present technique. An additional advantage of PDMS is that most of the sample can be recovered after the mass-measurement.

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