# $^{252}$Cf PLASMA DESORPTION FOURIER TRANSFORM MASS SPECTROMETRY OF INVOLATILE COMPOUNDS

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(Received 24 November 1986)

## ABSTRACT

A 50 $\mu$Ci $^{252}$Cf source in a Fourier transform mass spectrometer produces structurally characteristic spectra for compounds of molecular weights up to 2000. As found for plasma desorption ionization with time-of-flight instruments, use of the substrate, nitrocellulose, and the adduct, glutathione, greatly increase sensitivities. Ion collection times of 1–2 min and the elimination of the cell ion quenching event dramatically increase the sensitivity as well as the relative abundance of the molecular ion.

## INTRODUCTION

Fourier transform mass spectrometry (FTMS) [1] has proven itself as an important technique for the study of large, involatile biomolecules [2,3]. Its capabilities for tandem mass spectrometry (MS/MS) [4,5] and high resolution coupled with its multichannel measurement advantage are attractive assets for high mass analysis [6]. Many ionization techniques for large molecules have been successfully used with FTMS, such as laser desorption (LD) [5,7] and cesium ion desorption (Cs$^+$ SIMS) [8,9]. As recently reported by Hunt et al., an external fast atom bombardment (FAB) source coupled to an FTMS instrument with a 7T magnet produced a molecular ion for cytochrome C, M.W. 12384 [10].

Another promising desorption/ionization technique for biomolecules is plasma desorption (PD), usually induced by the 100 MeV fission fragments of $^{252}$Cf [11]. Not only has PD produced molecular ion species from trypsin, M.W. 23463, but multiply charged trypsin species up to $(M + 6H)^{6+}$ are measurable when nitrocellulose is used as the sample substrate [12]. This

greatly extends the observable mass value for instruments with an upper mass/charge ($m/z$) limit. Until recently, PD has only been interfaced to time-of-flight (TOF) mass spectrometers of relatively low resolution with no capability for MS/MS. Laboratory-sized $^{252}$Cf sources ($\leq 100 \ \mu$Ci) produce ion fluxes that are generally much too low for scanning instruments. An obvious marriage with extremely high potential is the PD/FTMS system. Tabet et al. first reported the $m/z$ 594 (M + K)$^+$ ion for leucine enkephalin from such an experiment [13]. Viswanadham et al. have also reported observing organic ions of relatively low molecular weight [14]. Our initial results from PD/FTMS are presented in a preliminary correspondence [15]. This paper shows in further detail how PD/FTMS yields abundant molecular ion species and structurally significant fragment ions from a variety of peptides and biological molecules, with ions detected up to $m/z$ 2000.

## EXPERIMENTAL

A 3 mm diameter 50 $\mu$Ci $^{252}$Cf source [26] with a thin Ni foil cover to prevent self-sputtering was recessed in a stainless steel holder and attached to the end of a 2.2 cm diameter direct probe as shown in Figure 1(a). Samples are placed on the front side of a piece of 2 $\mu$m thick aluminized mylar foil and secured 2 mm directly over the source. Fission fragments bombard the back of the foil over an area of 0.5 cm$^2$. The entire unit is inserted less than 5 mm from the entrance of the source cell of a prototype Nicolet FTMS-2000 instrument [27] with a 2.86 T superconducting magnet. Spectra are measured in the source side of the dual ion cell when the operating pressure was reduced to 10$^{-8}$ torr after sample introduction; in another hour, pressures approach 10$^{-9}$ torr. Best results are obtained with the cell trapping potential at +5 V for positive ion detection and -5 V for negative ions. The sample probe tip potential can be separately varied by a metal spring clip shown in the figure; optimum conditions were achieved with the probe at the cell potential. Ions are allowed to collect in the cell typically for 1-2 min followed by a 6 ms rf excitation pulse and an ion detection time of 65 ms for each measurement cycle. The cell quench pulse at the beginning of each cycle is turned off. Using a broad-band detection mode with a high-frequency limit of 500 kHz, which corresponds to $m/z \sim 88$ for a low-mass limit, the typical mass resolution without optimization at $m/z$ 1000 is greater than 1200 (FWHH definition) with a mass measurement accuracy of 0.1%. Decreasing the bandwidth to detect only ions above $m/z$ 1000 achieved a resolution of over 10 000 for the $m/z$ 1164 (M + Na)$^+$ ions from gramicidin S. Figure 1(b) shows the sample probe modified to effect front-side desorption and secondary ion emission orthogonal to the magnetic axis.

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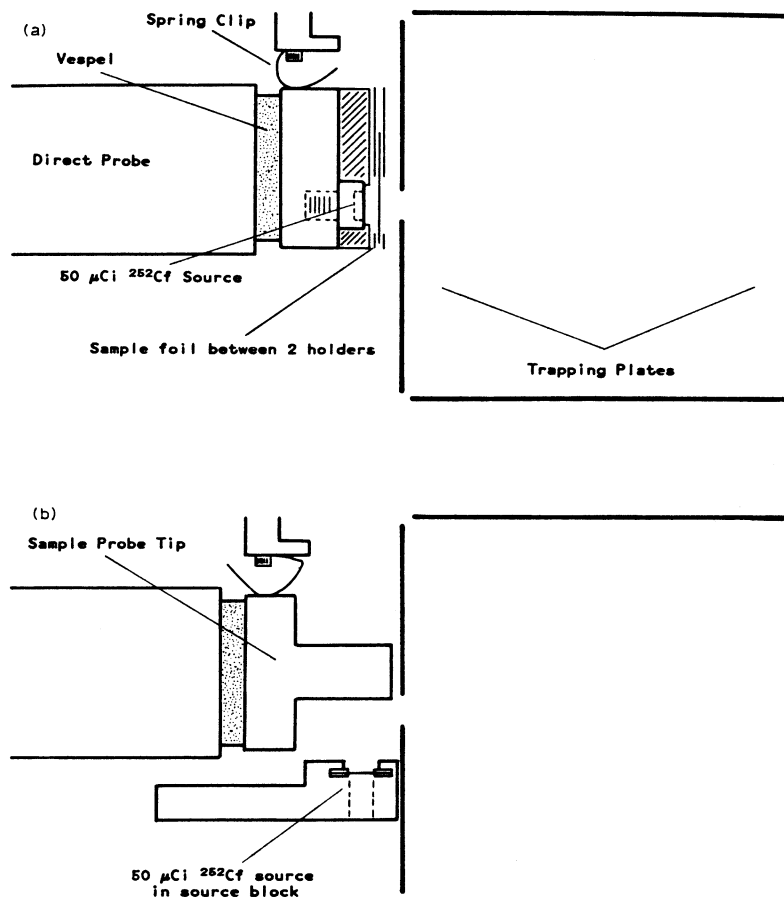be modified to effect
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Fig. 1. Source/sample configuration for (a) back-side desorption and (b) front-side desorption next to source cell.

Samples are deposited on the foil by two methods. A layer of nitrocellulose (60 $\mu$g cm$^{-2}$) was formed by electrospraying from an acetone solution; on to this is placed the sample dissolved in 0.1% trifluoroacetic acid (TFA) in deionized $H_2O$ [12]. The sample foil is then rinsed with 0.1% TFA/$H_2O$. Alternatively, samples mixed with an equimolar amount of glutathione [16] are dissolved in methanol/$H_2O$ and electrosprayed directly onto the aluminized mylar.

RESULTS AND DISCUSSION

The potential applied to the source and cell has a large effect on the ion signal. Using the probe tip at ground potential, no ions can be detected. By

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applying the cell potential to the probe, total ion signals are negligible below 3 V, increasing to be relatively constant between 5 and 10 V. However, the relative abundance of the molecular ion decreases steadily beyond 5 V. Application of large positive and negative voltage to the cell trapping plates before each measurement cycle clears all the ions from the cell, thereby starting each cycle with newly generated ions. Elimination of this quench pulse substantially increases sensitivity; for 30 two-minute ion collections followed by ion detection for gramicidin S (M.W. 1141) on nitrocellulose, the $(M + H)^+$ peak shows nearly an order of magnitude higher signal-to-noise ratio with the quench pulse off than with the quench on. Furthermore, the ratio of the $[M + H]^+$ to the abundance of the $m/z$ 197 fragment peak is also 10 times higher for the quench-off mode. For gramicidin D (M.W. 1881), the molecular ion adduct relative abundance is less than 1% with the quench pulse; $m/z$ 1904 $(M + Na)^+$ becomes the base peak in the spectrum by not ejecting the sample ions from the cell during measurement cycles, suggesting that a substantial number of molecular ion species survive at least 2 min, to be detected more than once, and/or that chemical ionization of intact neutrals constantly occurs.

The optimal ion collection times are typically between 1 and 2 min, with longer times better for larger ions. $Cs^+$ and $Cs^{2+}$ ions from CsI can be detected with 10 s collection times, while reasonable spectra from gramicidin S can be obtained with ion collection times of 30 s. Repeated measurement cycles up to a total time of $\sim$ 14 h predictably improve the signal-to-noise ratio but also enhance the relative abundance of the molecular ion; an increase of over 60% for the $(M + H)^+$ species of the peptide bradykinin is observed (2 min ion collection times) for 30 measurement cycles versus 15 cycles. Figure 2 is the spectrum of renin substrate tetradecapeptide after 14 h of data collection. A spectrum collected immediately afterwards from the same sample shows a large peak for the fragment ion at $m/z$ 1643 that may be due to sample decomposition from fission fragment exposure; this peak is observed in the FAB spectrum [17].

As previously observed by the TOF/PD experiments [12,16], the use of nitrocellulose or glutathione significantly increases the abundance of the molecular ion species. Background peaks separated by 28 mass units as labeled in Fig. 3, similar in appearance to those recently described in TOF experiments [18], persist independent of sample preparation but are reduced by desorbing samples from nitrocellulose. (These peaks are present even without the introduction of a sample foil. Running samples of mylar, human fingerprints, and pump oil did not conclusively identify the source of the background. However, with a new $^{252}$Cf source, exact mass measurement and other evidence indicates $m/z$ 391 is $(M + H)^+$ from dioctyl phthalate.) Figure 3 compares the spectra obtained with both sample preparation

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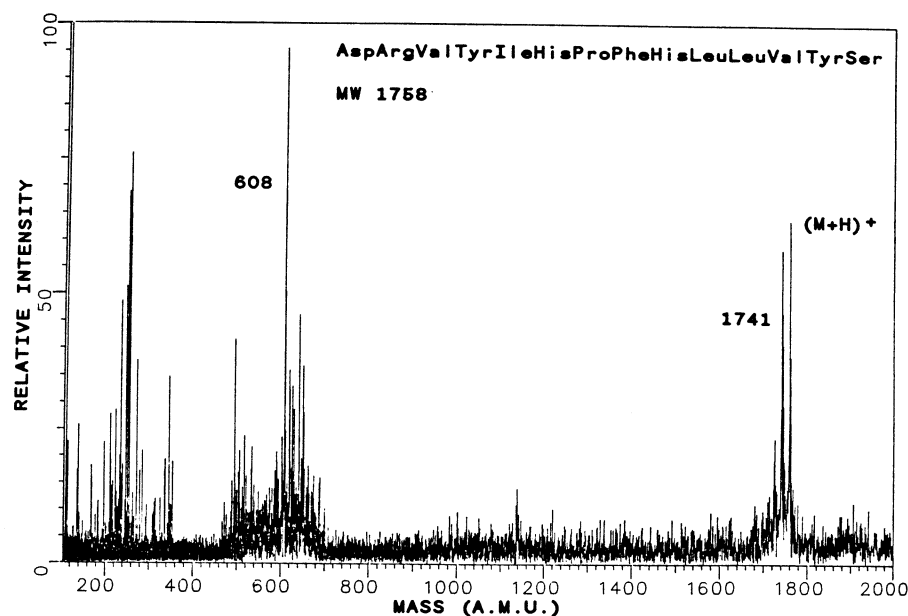


Fig. 2. $^{252}$Cf PD/FTMS positive ion spectrum of renin substrate tetradecapeptide on nitrocellulose; 420 two-minute measurement cycles.



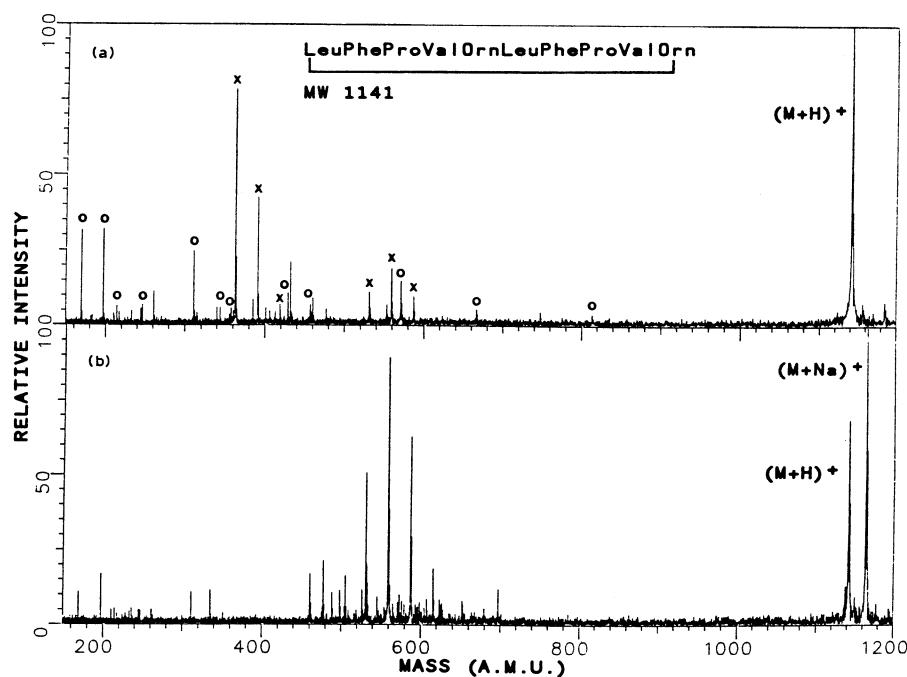Fig. 3. Positive ion spectra of gramicidin S (a) on nitrocellulose and (b) electrosprayed with glutathione; 30 two-minute measurement cycles. O, sequence peaks, ×, background peaks. Ions between $m/z$ 360 and 420 were ejected for bottom spectrum.
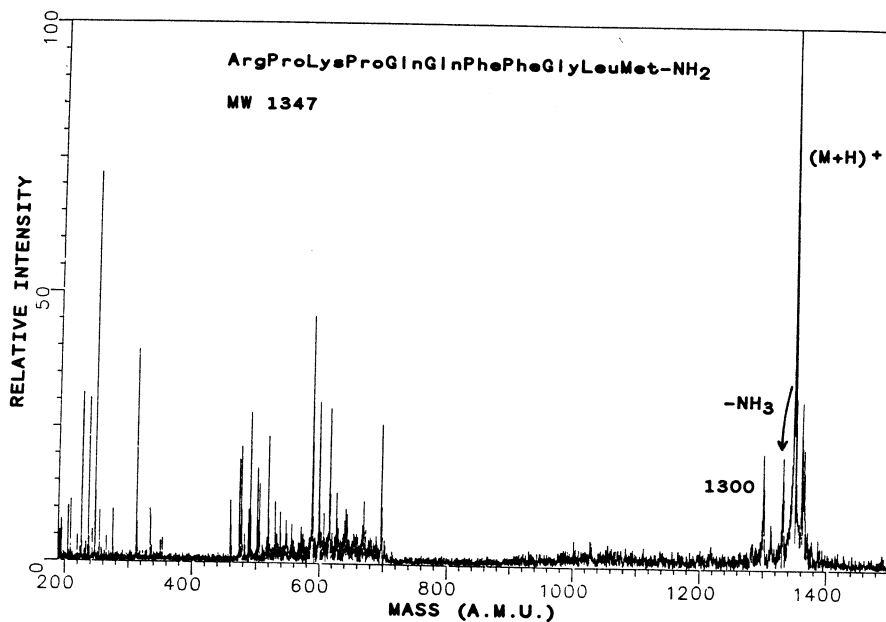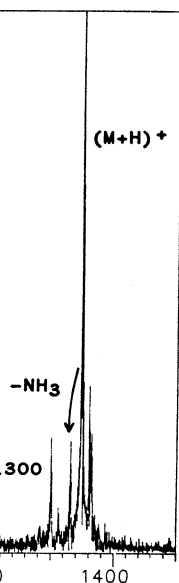
Fig. 4. Positive ion spectrum of substance P on nitrocellulose; 16.9 h of data collection (two-minute ion collection times).

methods for gramicidin S. Rinsing the sample foil effectively rids the substrate of sodium ions; however, contrary to the TOF experience [12], adding appropriate cations to the sample does not affect the sensitivity. Adding or rinsing a gramicidin S sample on nitrocellulose with an equimolar aqueous solution of LiCl, NaCl, and KBr produces relatively abundant cationized molecular adduct ions, with the $(M + Li)^+$ the most abundant. Previously described experiments using much lower energy 11 keV $Cs^+$ primary ions for desorption found the sodium-attached species to dominate [8]. Addition of an alkali earth metal, Ca, and Cd also form the corresponding $(M + metal)^+$ complex.

For many of the spectra, the fragmentation pattern appears to be fairly similar to those obtained by SIMS, consistent with the literature [19]. Vitamin $B_{12}$ and gramicidins S and D spectra from $^{252}$Cf PD/FTMS are qualitatively similar to those from $Cs^+$ SIMS/FTMS [15]. However, for substance P (M.W. 1347), very few of the sequence peaks present in the SIMS/FTMS spectrum are found in the PD spectrum (Fig. 4). Both cases show a peak for the loss of $NH_3$, but the $m/z$ 1300 peak possibly from $HSCH_3$ loss is not found in the $Cs^+$ SIMS spectrum. Comparing two different instruments, a sample of bradykinin was also run on the Rockefeller University TOF mass spectrometer, producing a much more abundant $(M + 2H)^{2+}$ ion. Alamethicin, a peptide antibiotic, analyzed by PD/FTMS
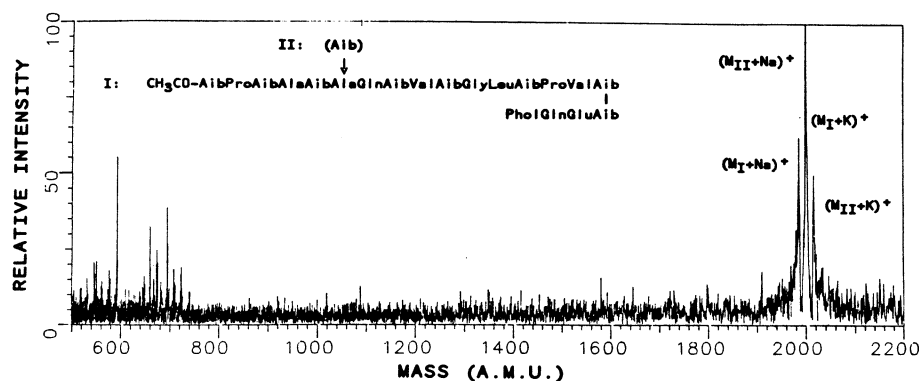
Fig. 5. Positive ion spectrum of alamethicin electrosprayed with glutathione and KBr; 956 one-minute measurement cycles.

revealed large peaks at $m/z$ 1986 and 2000, corresponding to $(M + Na)^+$ for the mixture of alamethicins I and II. The spectrum by PD/TOF contains many more fragment ions [20]. Figure 5 shows the FTMS spectrum with the addition of potassium to the sample. Such spectral differences could be due in substantial part to the large time scale differences between the two techniques, minutes for PD/FTMS and microseconds for PD/TOF. Chait and Field have shown that a large proportion of molecular ions in this mass range formed by fission fragment ionization undergo unimolecular fragmentation during their flight in a TOF–MS [21]. Any metastable ions dissociating in the FT analysis cell during the 1–2 min ion collection time should appear as fragment ions in the spectrum.

For most of the samples analyzed, sensitivity for negative ions is relatively low, although the $(M - H)^-$ ion for bradykinin is observable, and structurally significant fragment ions are present for vitamin $B_{12}$. No positive ions were detected for a polymer positive resist used in electron beam microlithography, poly(butene-1 sulfone) (M.W. $\sim$ 500 000) but abundant negative ions beyond $m/z$ 2000 representing the ionic products $RSO_2^-$ were found [22]. The production of many electrons thought to accompany the passage of the fission fragment could give rise to negative ions through thermal capture by the products from fragmentation due to heavy particle bombardment.

Current work includes improvement of ion trapping efficiencies and MS/MS experiments. Comparison of TOF performance data and relative source and signal strengths indicate that our FTMS trapping efficiency is less than 1/1000. Samples of a synthetic peptide composed of 32 amino acid residues (M.W. $\sim$ 3576) and porcine insulin gave appreciable molecular ion signals on the Rockefeller TOF–MS but did not reveal an $(M + H)^+$ ion above background on the FTMS, even after several hours of data collection.

Mounting of the source inside the vacuum chamber and perpendicular to the magnetic field lines [Fig. 1(b)] allows for front- or back-side desorption through a sample foil; in initial experiments, the improvement in trapping efficiency is disappointing. This internally mounted $^{252}$Cf source is much more desirable in terms of personal safety. Ion dissociation techniques, such as electron impact excitation of ions from organics (EIEIO) [23], surface-induced dissociation [24], and photodissociation [25] are promising methods for PD/FTMS.

## CONCLUSION

The encouraging results shown here indicate that $^{252}$Cf plasma desorption can indeed be successfully used with a Fourier transform mass spectrometer for structural analysis of biological compounds. Unfortunately, to date, only masses up to around 2000 are easily detected. A sample of polyethylene glycol (PEG) with average weight 2000 yielded only sodium-attached fragment ions with $m/z < 800$, although the same sample showed molecular ions using SIMS/FTMS [8]. Obviously, ion trapping efficiencies must be improved, but the potential for PD tandem mass spectrometry is an exciting prospect.

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