Californium-252 Plasma Desorption with Fourier Transform Mass Spectrometry

Sir: Plasma desorption (PD), such as that induced by the 100-MeV fission products of ^{252}Cf, is a particularly promising ionization method for large molecules (*1–5*), yielding molecular ion species even from trypsin, molecular weight 23 463. Further, with trypsin using nitrocellulose as the substrate, $(M + 3H)^{3+}$ is the most abundant molecular ion species and $(M + 6H)^{6+}$ is measurable (*4*), which greatly extends the mass values (m) observable for instruments with an upper m/z limit (z = number of charges). However, a 50-μCi ^{252}Cf source only produces 55 000 fissions s^{-1}, yielding ion currents that are generally much too low for scanning instruments. Until recently PD has only been used with time-of-flight (TOF) mass spectrometers of relatively poor resolution (~1000) and poor capabilities for tandem mass spectrometry (*2, 6*). An instrument with unusual capabilities for these, as well as for simultaneous ion detection over a wide mass range, is the Fourier transform (FT) mass spectrometer (*7–13*). With FTMS, Hunt (*14*) has measured $(M + H)^+$ ions of cytochrome *c*, molecular weight 12 384, ionized in an exterior fast-atom-bombardment source. A landmark PD/FTMS experiment on a single sample has been reported by Tabet and co-workers (*15*) showing abundant $(M + K)^+$ ions at m/z 594 from leu-enkephalin. However, from more recent PD/FTMS experiments Hercules et al. (*16, 17*) obtain spectra dominated by anomalous peaks, with the spectrum of the larger peptide gramicidin S (molecular weight 1141) showing only fragment ions such as m/z 994 (*16*). Here we describe techniques for obtaining PD/FT mass spectra for a variety of compounds with abundant molecular ion species of masses as high as 2016 (alamethicin).

EXPERIMENTAL SECTION

A prototype Nicolet FTMS-2000 instrument was used, measuring spectra in the source side of the dual ion cell when the pressure is reduced to 10^{-8} torr after sample introduction; the pressure is $\sim 2 \times 10^{-9}$ torr after another hour. A 50-μCi ^{252}Cf source, 3 mm diameter, was recessed in the end of the direct probe (Figure 1), with the sample on the opposite side of a 2-μm aluminized Mylar foil placed 2 mm away from the Cf source, inserted to be 5 mm from the ion source entrance. The potential on the probe tip could be separately varied; best results for positive ions were obtained with +5 V on the tip and cell trapping plates. For a typical measurement cycle ions were allowed to collect in the cell for 1 or 2 min, followed by a conventional 65-ms ion excite/detect sequence. In normal FTMS operation a large positive and negative voltage pulse is applied to the trapping plates for 1 ms at the start of each cycle, removing the ions from the cell by destroying the axial trapping well. However, with ^{252}Cf ionization it is generally preferable to operate without this ion quenching before the measurement cycle. Samples were either deposited on Mylar, that had been electrosprayed with nitrocellulose, by evaporation from aqueous solution followed by rinsing with a 0.1% trifluoroacetic acid solution in deionized water (*4*), or dissolved in $MeOH/H_2O$ with an equimolar amount of glutathione (*5*) and electrosprayed onto the Mylar. For the latter with gramicidin D the best results were obtained by spraying larger drops that coalesced to some extent ("electrosplotch"). By use of broad-band detection, the resolution routinely achieved was over 10 000 (full width at half height) at m/z 133 (Cs^+) and 1700 at m/z 1141.7 for the $(M + H)^+$ gramicidin S ion. For the latter, excitation of only ions above m/z 439 (bandwidth of 100 kHz) increased the resolution to 5300 (Figure 2, inset).

Strict precautions must be observed in the handling of californium-252, as previously described (*1–5*). Samples were loaded on the probe tip behind a 20-cm paraffin barrier for neutron screening. Permanent mounting of the ^{252}Cf on the ion cell entrance is in test.

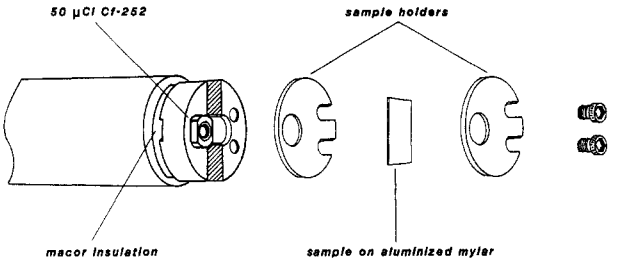

Figure 1. Exploded view of the end of the modified sample probe.

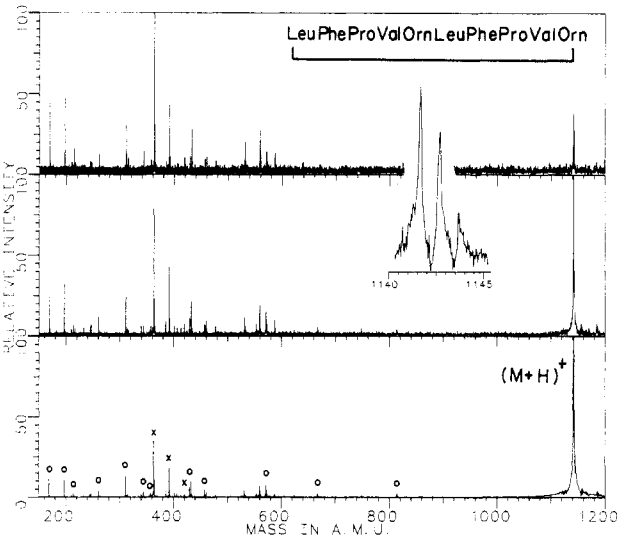

Figure 2. ^{252}Cf PD-FTMS positive ion spectra of gramicidin S on nitrocellulose with (top to bottom) 5, 30, and 300 measurement cycles of 2-min ion collection times: (O) sequence peaks, (X) background peaks; inset, narrow (100 kHz) bandwidth detection, 300 measurement cycles, nonapodized.

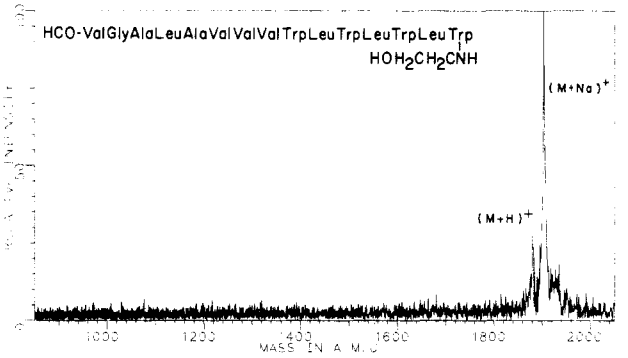

Figure 3. Positive ion spectrum of gramicidin D, 1:1 with glutathione, "electrosplotched" on Mylar, 150 1-min measurement cycles.

RESULTS AND DISCUSSION

By use of PD/FTMS the abundance of molecular ion species of molecules larger than leu-enkephalin (*15*) can be increased dramatically (Figures 2, 3, and 5) with a combination of special techniques. As observed in the pioneering time-of-flight PD experiments, desorbing the sample from nitrocellulose (*4*) or mixed with glutathione (*5*) substantially in-

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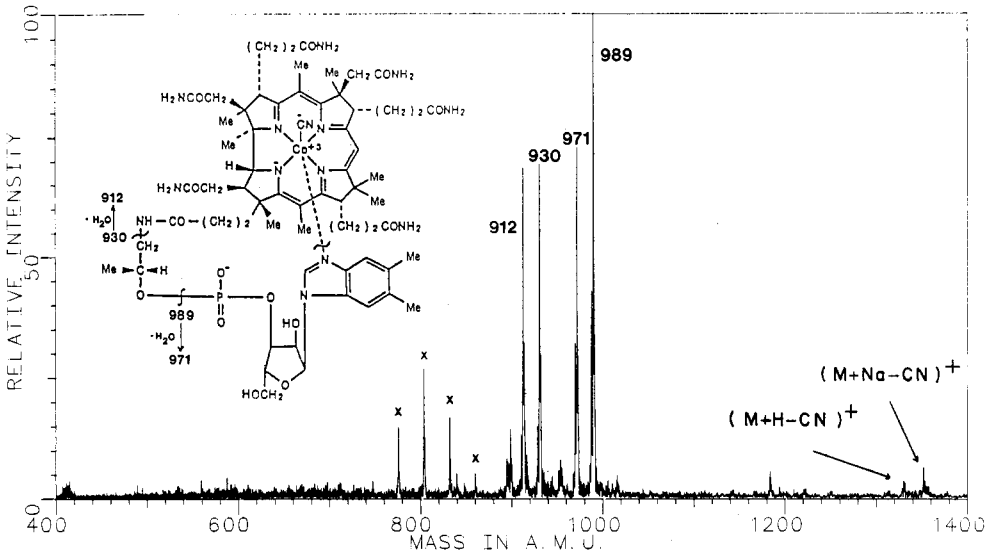

Figure 4. Positive ion spectrum of vitamin B_{12} on nitrocellulose, 524 2-min measurement cycles: X, background peaks.

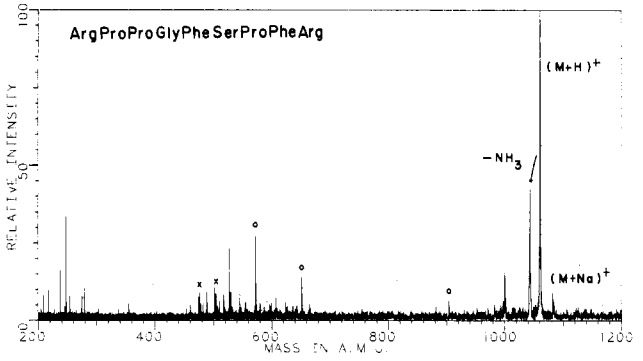

Figure 5. Positive ion spectrum of bradykinin on nitrocellulose, 90 1.5-min scans: (O) sequence peaks, (X) background peaks.

creases the $(M + H)^+$ abundance and reduces background peaks in PD/FTMS. Secondly, a relatively high positive potential on the probe tip and trapping plates is necessary to detect positive ions. Total ion signals are negligible below 3 V, increasing to be relatively constant for 5–10 V, but the abundance of molecular ion species decreases above 5 V. No advantage was observed for different voltage values on the probe and trapping plates. Thirdly, sensitivity is also increased substantially by not ejecting ions from the cell during measurement cycles; for 30 2-min ion collections followed by measurement of gramicidin S on nitrocellulose, $(M + H)^+$ with "quench-off" shows signal/noise nearly an order of magnitude greater than that of "quench-on". This indicates ion lifetimes much longer than the collection time, encouraging with respect to PD/TOF evidence (*2*) of a substantial proportion of short-lived ($<10^{-3}$ s) ions. The optimal time for ion collection between measurements was 1 or 2 min, with longer times better for larger ions. Repeated measurement cycles, at least up to 14 h, continue to improve signal/noise and enhance the relative abundance of $(M + H)^+$ ions (Figure 2). In the case of renin substrate tetradecapeptide, the fragment ion at m/z 1643 increased dramatically vs. $(M + H)^+$ after 14 h of irradiation, possibly due to decomposition.

Although the molecular ion species m/z 1881 and 1904 from gramicidin D (Figure 3) and 2002 and 2016 $(M + K)^+$ from alamethicin (a mixture) are easily discernible under these conditions, unfortunately this was not true for the larger molecules studied. Polyethylene glycol 2000 (average molecular weight 2000) yields only fragment ions of m/z <800. Samples on nitrocellulose were measured by conventional PD techniques (*1–5*) on the Rockefeller University time-of-flight instrument (*2*) and the next day on the Cornell FTMS instrument; a sample of bovine insulin that gave a large $(M + H)^+$ (m/z 5735) by PD/TOF was not clearly above background after 20 h of PD/FTMS measurement. Relative TOF/FTMS signal strengths on the same samples and TOF performance data (*1–5*) indicate that our current FTMS ion trapping efficiencies are not greater than 1/1000. Similarly, our PD/FTMS spectrum of CsI shows Cs_2I^+ as the largest cluster detectable, compared to $Cs_{63}I_{62}^+$ in our 11-keV Cs^+ desorption/FTMS spectrum of CsI (*18*). Current experiments for front (*4*) as well as back-side desorption at various angles to the magnetic field direction so far have not yielded significant improvements to ion trapping.

Adding appropriate cations to the gramicidin S before sample preparation did not increase the sensitivity but did produce relatively abundant $(M + A)^+$ ions (A = Li, Na, K, or Ca). On addition of (or rinsing with) a solution containing equimolar amounts of LiCl, NaCl, and KCl, $(M + Li)^+$ is the most abundant $(M + A)^+$ peak; in similar experiments using lower energy (~11 keV) primary ions for desorption (*19*, *20*), $(M + Na)^+$ ions are the most abundant. Fragment peaks from gramicidins S and D and vitamin B_{12} (Figures 2–4) qualitatively resemble those from 11 keV Cs^+ ion desorption (*20*), as well as from ^{252}Cf PD/TOF (*1–5*), consistent with previous observations (*21*). However, the same bradykinin sample run by TOF and FTMS (Figure 5) shows a much more abundant $(M + 2H)^{2+}$ ion in the TOF spectrum. Unusual (and highly variable) background peaks (indicated by X in Figures 2, 4, and 5, with 1251 as the highest mass observed) do not apear to arise from skin lipids, as found (*22*) in recent PD/TOF experiments. A new ^{252}Cf source produces a large m/z 391 peak whose exact mass is consistent with that of dioctyl phthalate, while other background is consistent with alkyl phthalates, yielding $(M_n + A)^+$, where n = 1–3 and A = H, Na, or K.

The resolution of 5300 at m/z 1141.7, although greater than that of reported TOF spectra of larger molecules, is far poorer than that of 150000 reported for a single-scan FTMS spectrum of gramicidin S using laser desorption (*11*). Much of this resolution loss could be due to frequency misalignments in coadding the repeated transient measurements recorded over a 10-h period.

Preliminary experiments indicate that using primary ion beams of much lower current, but of much higher energy, can have advantages in the study of radiation mechanisms. Poly(butene-1 sulfone), an electron beam resist, when irradiated with 11-keV Cs^+ ions in the FTMS shows positive ions

indicative of telomeric products (*23*) that are inconsistent with a conventional depolymerization mechanism (*24*). The same sample with ^{252}Cf PD yields no positive ions, but its negative ion spectrum (peaks to m/z 2159) appears to represent the primary ionic products, RSO_2^-, of the radiation degradation, suggesting that much of the Cs^+ positive ion spectrum results from secondary ionization of neutral products of the radiation degradation (*23*).

CONCLUSIONS

These preliminary results extend those of Tabet (*15*) in indicating that this unique ionization technique can be used with FTMS. To utilize the unusual ^{252}Cf PD capabilities for ionization of high molecular weight compounds, the present FTMS trapping efficiency of $<10^{-3}$ must be improved. Better methods for combining repeated spectral measurements could give resolution approaching the unusual FTMS capabilities demonstrated with higher intensity ionization methods. Although the much longer lifetimes required by FTMS than by TOF can reduce sensitivity, this make possible a variety of ion dissociation (e.g., by 193 nm photons) (*25*, *26*) and reaction techniques for MS/MS, for which FTMS is far better suited than is TOF (*2*, *6*).

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Registry No. ^{252}Cf, 13981-17-4; bradykinin, 58-82-2; gramicidin S, 113-73-5; vitamin B_{12}, 68-19-9; poly(butene-1-sulfone), 25104-10-3.

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AIDS FOR ANALYTICAL CHEMISTS

Comparison of Sample Loops Constructed of Several Different Materials for Gas Chromatographic Analysis of Parts-per-Billion-Level Organic Gas Mixtures

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The ability to accurately quantify very low concentration levels of volatile toxic organic compounds is of great importance in ambient air and source emission measurements. For this reason, 27 gaseous organic compounds at parts-per-billion

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