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25. The range of inter- and intraassemblage diversity appears to be much greater among *Capsien supérieur* assemblages than has been recognized so far. We have attempted low-level statistical comparisons using published typological indices for approximately a dozen assemblages and only in a very few instances are strong similarities observable. Further work will be published shortly.
26. See P. Vermeersch, *L'Anthropologie* **79**, 215 (1975) for additional observations on variation in Capsian lithic assemblages.
27. For a discussion of the Neolithic of Capsian Tradition and other Neolithic assemblages in the Maghreb, see (*4*); A. Gilman, thesis, Harvard University (1973); and C. Roubet, *Le Gisement du Damous el Amar et sa place dans le Néolithique de Tradition Capsienne* (Travaux du Centre de Recherche Anthropologique, Prehistoriques et Ethnographiques, Alger, 1968).
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36. We have calculated the average accumulation rate at Aïn Misteheyia at 4.2 centimeters per 100 years of overall occupation; that is, net occupation plus vacancy. The rate from the bottom date to the middle date is about 10 centimeters per 100 years, and from the middle date to the top date about 2.5 centimeters per 100 years. This suggests an overall span of about 3500 years.
37. Identification of this material was provided by Dr. W. Stewart, Department of Botany, University of Alberta.
38. Correct identifications of this material were provided by Dr. C. S. Churcher, University of Toronto.
39. T. C. Losey, *Archaeol. Soc. Alberta Newsletter (Edmonton)*, **28** (1971), and personal communication.
40. Supported by the Wenner-Gren Foundation for Anthropological Research (1972) and the Canada Council (1973–74) through grants to D.L., whose participation was facilitated by a sabbatical leave from the University of Alberta. R. Ouahes, Direction of Scientific Research, Algiers, authorized this research, which was aided by the cooperation of M. Mammeri, Director of Centre de Recherches Anthropologiques Préhistoriques et Ethnographiques, Algiers, and his colleagues G. Aumassip, M. Couvert, C. Roubet, and the late F. Roubet. D.L. was responsible for overall direction and archeology, F.A.H. for local geomorphology and paleoecology as well as reconstruction of the subsistence regime, A.G. for modern and prehistoric faunal studies, and J.-L.B. for regional geology and geomorphology. We thank A. Close and C. Chippindale of Cambridge University and J. Elmendorf of George Washington University for their enthusiastic collaboration during the field season. Palynological analyses were supervised by T. Habgood of the University of Alberta and sedimentological analyses were run by the Soils Department, University of Alberta. Figures 1 to 5 and 8 were drawn by I. Wilson, Cartographic Services, Department of Geography, University of Alberta. D.L. takes this opportunity to publicly thank F.A.H., Close, Chippindale, and Elmendorf for remaining in the field and completing work under way when he and A.G. were forced to leave due to illness. We thank K. W. Butzer and J. Tixier for advice and encouragement.

Californium-252 Plasma Desorption Mass Spectroscopy

Nuclear particles are used to probe biomolecules.

R. D. Macfarlane and D. F. Torgerson

An impressive array of modern analytical instruments and techniques is being used with increasing efficiency and detail, to identify and characterize complex biomolecules. Among these is the mass spectrometer, which can serve for analytical measurements and can give structural information as well from fragmentation patterns. Mass spectroscopy has had limited application, however, because it requires volatilization and ionization of the sample. Molecules, such as hormones, enzymes, peptides, and nucleic acids, that play a role in biochemical pathways or regulation are generally complex and exhibit strong intermolecular interactions in aqueous solutions and in the solid state. Solid samples of these compounds generally have low vapor pressures, which means that too few ions can be formed to obtain a mass spectrum. The simplest method for increasing the vapor pressure is to heat the sample. Thermally labile molecules, however, decompose when subjected to elevated temperatures, so that this method is not applicable to these compounds. One approach to the solution of the problem is to increase the sensitivity of the mass spectrometer so that measurable ion currents can be detected even at extremely low vapor pressures. McIver *et al.* (*1*) have developed a trapped ion cyclotron resonance mass spectrometer that can detect vapor pressures down to 10^{-10} torr, a factor of 10,000 better than an electron impact mass spectrometer with direct sample introduction.

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Another approach is to enhance the rate of volatilization of the molecule while minimizing the rate of decomposition. Polar groups on the molecule, which produce the strong intermolecular interactions in the solid state that reduce volatility, can be modified chemically to nonpolar substituents, and the derivative will have a higher vapor pressure than the original molecule. This technique is widely used in mass spectroscopy and has the added attraction that the vaporized derivative can be analyzed by both gas chromatography and mass spectroscopy in a coupled mode (*2*). Beuhler *et al.* (*3*) showed that if a solid film of a peptide is deposited on a nonreacting surface such as Teflon, the vapor pressure of the peptide is effectively enhanced and vaporization can be effected at lower temperatures.

Heats of vaporization of biological molecules deposited on a solid surface are also reduced in the presence of a strong electric field gradient. This results in a "field desorption" of molecules and was first used by Beckey *et al.* (*4*) in mass spectroscopic studies of involatile biomolecules. A variation of this method was recently reported by Simons *et al.* (*5*), who were able to field-desorb molecular ions of sucrose and proline from liquid surfaces.

Volatilization with minimal decomposition is the objective in mass spectroscopic studies of biomolecules. The first step in the thermal decomposition of a molecule adsorbed on a surface is unimolecular fragmentation, which takes place when excitation to an unstable vibrational state results in bond cleavage. The rate of decom-

position is determined by the time needed to deposit energy into the unstable vibrational mode and by the vibrational period. If the rate of surface vaporization is greater than the rate of decomposition, there will be little interference from decomposition in the mass measurement. Beuhler *et al.* (*3*) showed that at temperatures above 400°K, vaporization is the dominant process. With heating at a rate of 12° per second, samples of peptides were volatilized with considerably less decomposition than when they were heated gradually.

It is clear from these studies that the ideal mode of heating is one which is extremely rapid and which produces high-temperature bursts that are so short that there is not enough time for the molecule to absorb energy into unstable vibrational modes. Laser volatilization has been shown by Mumma and Vastola (*6*) to be feasible for mass spectroscopic studies, but its applicability has not yet been demonstrated for thermally labile biomolecules.

A more intense heating source in terms of power density is a high-energy heavy ion. Ions heavier than Kr with energies in excess of 100 Mev can penetrate thin films (up to 10 μm thick) and deposit large quantities of energy in a small area in times on the order of 10^{-12} second. The radioactive nuclide ^{252}Cf is a convenient source of such heavy ions, which are produced in the process of spontaneous fission. Each fission fragment produces pulsed localized heating that is ideal for the vaporization of involatile biomolecules without decomposing them (*7*).

Obtaining mass spectroscopic information for a biomolecule depends on success in volatilizing the sample. The method used to produce an ion from a neutral molecule in the vapor phase depends on the kind of information being sought. Ionization by electron impact is the established mode, but new methods, such as chemical ionization, have evolved in which quasi-molecular ions are formed by ion-molecule reactions in the ion source (*8*). The most commonly employed reaction involves proton transfer from an acidic reactant ion such as NH_4^+ or t-$C_4H_9^+$. Several peptides were studied recently by Beuhler *et al.* (*9*), who volatilized them by rapid heating and ionized them with NH_4^+. The acidity of the protonating ion determines the amount of internal excitation imparted to the quasi-molecular ion, so that varying degrees of fragmentation can be produced and structural information can be obtained; for example, the technique can be used for peptide sequencing.

Ionization and volatilization are coupled in one process in field desorption (*4*). Very little of the energy goes into internal excitation, and the degree of fragmentation is relatively small. Laser heating also produces simultaneous volatilization and ionization (*6*). Ultrarapid heating by ^{252}Cf fission fragments results in volatilization and the formation of quasi-molecular cations and anions. In this article we examine this new method, which we call ^{252}Cf plasma desorption, in detail, and discuss its applications to the mass spectroscopy of involatile, thermally labile molecules of biological interest.

^{252}Cf Plasma Desorption

The ^{252}Cf plasma desorption technique utilizes energetic fission fragments from the decay of ^{252}Cf to volatilize and ionize a solid sample. The ^{252}Cf nucleus decays with a half-life of 2.6 years, 3 percent of the decay taking place by spontaneous fission and the remaining 97 percent by emission of alpha particles (*10*). The decay is random in time and fission fragments are emitted in all directions. Each fission event produces two fragments traveling in almost exactly opposite directions, the small departure from colinearity being caused by neutron emission. The spontaneous fission of ^{252}Cf is asymmetric, producing two nuclei with unequal masses and energies. Thus, a typical pair of fission fragments is $^{142}Ba^{+18}$ and $^{106}Tc^{+22}$, which have kinetic energies of about 79 and 104 Mev, respectively (*11*). Approximately 40 different pairs of fragments can result from spontaneous fission of ^{252}Cf.

A schematic of the ^{252}Cf plasma desorption mass spectrometer is shown in Fig. 1. To obtain a mass spectrum, a sample is dissolved in an appropriate solvent and the solution is deposited over an area of 1 cm^2 on a Ni foil 1×10^{-3} mm thick. The sample foil is then precisely aligned with a ^{252}Cf fission fragment source, and an elec-

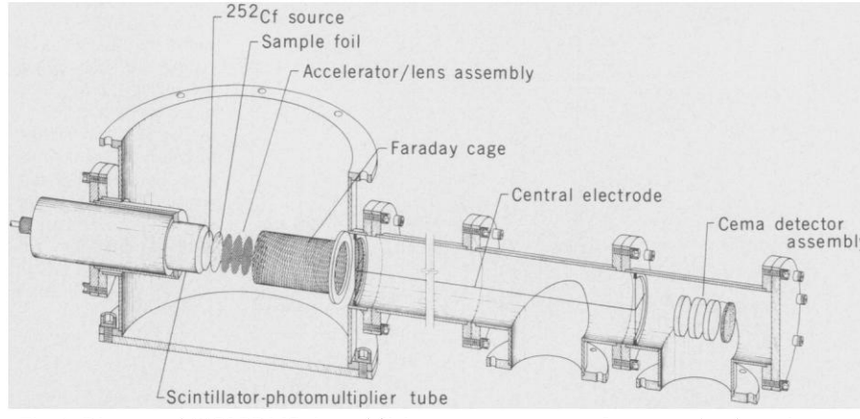

Fig. 1. Diagram of ^{252}Cf-PDMS time-of-flight mass spectrometer. Ions emerging from the sample foil are accelerated through the grid assembly and into a field-free region shielded by a Faraday cage. The main section of the flight tube contains an axial electrostatic field to increase ion transmission. The configuration shown here has an 8-m-long flight tube. Measurements were also made with a 1.5-m-long flight tube.

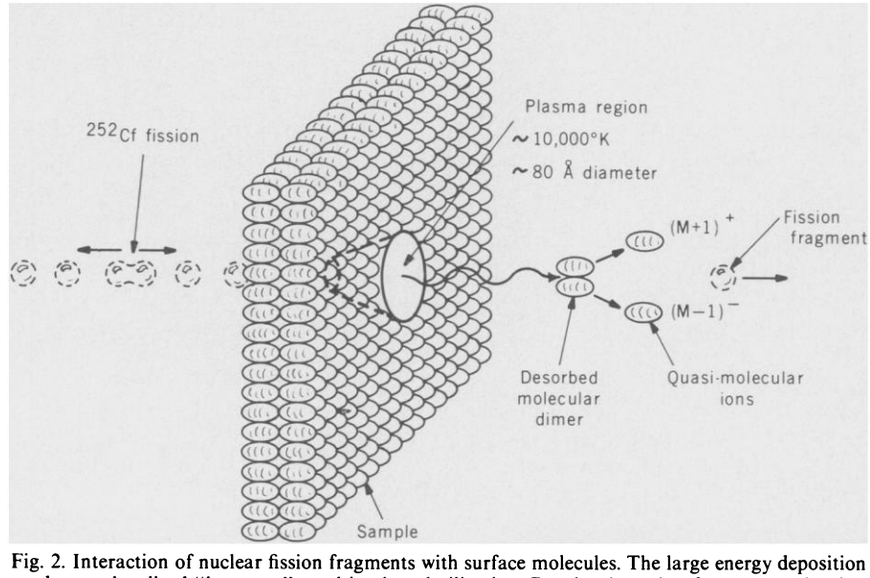

Fig. 2. Interaction of nuclear fission fragments with surface molecules. The large energy deposition produces a localized "hot spot," resulting in volatilization. Results show that for some molecules, such as amino acids, ion-pair formation takes place by proton transfer within a desorbed dimer.

trostatic grid system is used to accelerate ions into an 8-m-long time-of-flight tube. In this configuration, each fission fragment passing through the sample foil and producing ionization has a complementary fragment traveling in the opposite direction, which can be detected and used as a time zero marker. The fragments are precisely correlated, within 10^{-9} second. Once an ion is formed it is rapidly accelerated to a known kinetic energy by the electrostatic grid assembly into the time-of-flight tube. To minimize solid angle losses, a wire 1×10^{-3} mm in diameter is centered on the axis of the tube and is maintained at a small potential to produce a radial electric field. Ions entering the tube with small angular divergence are thus captured into a stable spiral orbit about the wire with no effect on their translational velocities. Single ions are detected by using three aligned channel plate electron multipliers having a total gain of 10^9. The time difference between an initial fission event and the arrival of an ion at the electron multiplier assembly can be measured with a precision time digitizer to better than 1×10^{-12} second. By measuring the time of flight, t, ion masses can be calculated to first order from the relation $M = 2E(t/s)^2$, where E is the kinetic energy and s is the flight path.

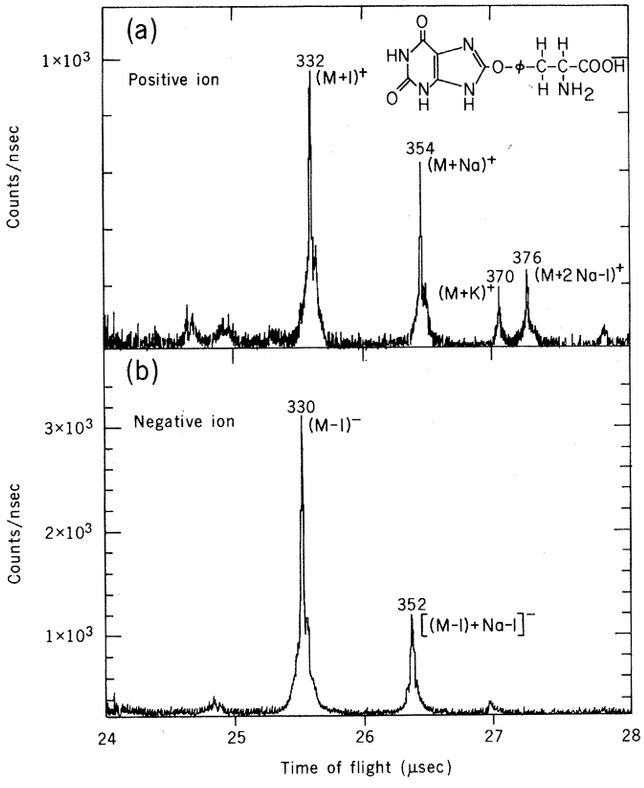

Fig. 3. Mass spectra of xanthine-tyrosine in the mass region of the quasi-molecular ions. Spectra were recorded for 15 minutes with a 2-μg sample and a fission intensity of 5000 sec^{-1}. The 1.5-m-long flight tube was used. The positive ion spectrum (a) shows the effect of the presence of alkali metal ion impurities. The negative ion spectrum (b) gives a strong $(M - 1)^-$ peak, indicating that this molecule can produce ion pairs. Previous attempts to obtain a mass spectrum by derivative formation and chemical ionization gave ambiguous results.

Each penetration of a fission fragment into the sample constitutes a measurement where a few, several, or no ions are detected. By continuing to expose the sample to the fission source, the measurement is automatically repeated several times a second, depending on the ^{252}Cf source strength. A mass spectrum is developed by accumulating the results for each fission event either in core memory or event by event on magnetic tape. Subsequent analysis is performed by an off-line computer.

As ionization originates on a potential plane defined by the sample foil, the acceleration field and path length are the same for all ions. This design therefore obviates some of the traditional objections to time-of-flight mass spectroscopy. With an 8-m flight path, masses can be measured to better than 0.5×10^{-3} atomic mass unit (amu) (*12*). As the resolution is proportional to the time of flight, shorter flight paths yield lower resolution. However, a shorter path length has the advantage of higher transmission and therefore greater sensitivity. Thus, for work requiring greater sensitivity, a 1.5-m drift tube with no focusing properties is substituted for the 8-m electrostatic particle guide, the rest of the system remaining unchanged. Ions are directed into the tube by using a two-dimensional spatial adjustment device on which the ion source–accelerator assembly is

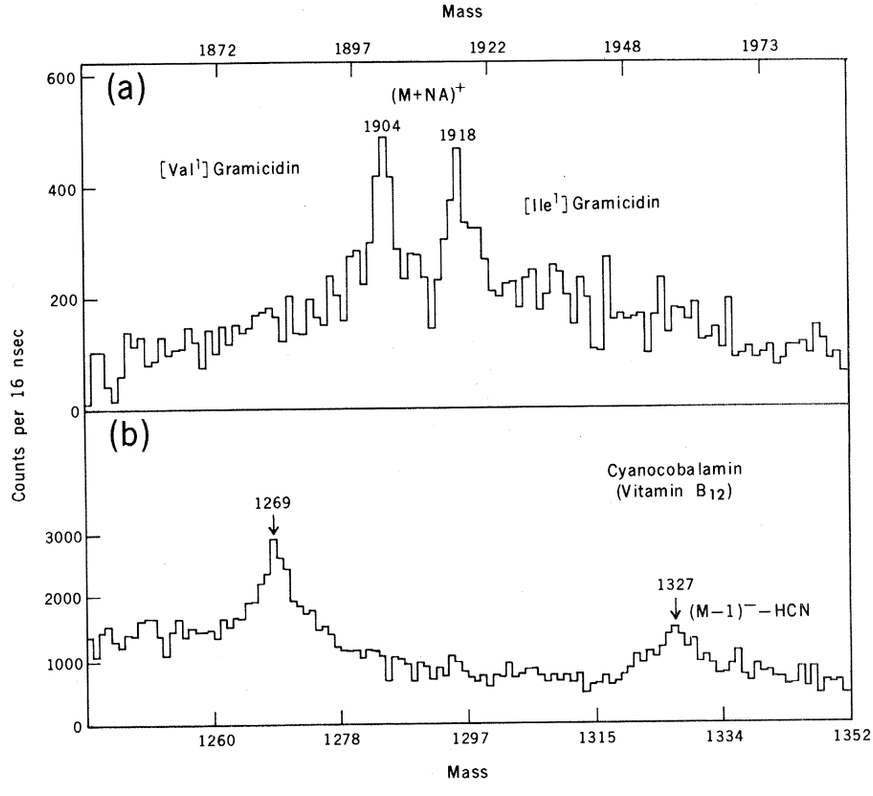

Fig. 4. Partial mass spectra of (a) gramicidin A, positive ion, and (b) cyanocobalamin, negative ion. Spectra were recorded for 2 hours with the 1.5-m flight path, 2-μg samples, and a fission intensity of 5000 sec^{-1}. Only the region near the quasi-molecular ions is shown. There is an extensive fragmentation pattern at lower masses. These results show that large quasi-molecular ions can be produced by this method. The counts observed between peaks are due to random events, which are uncorrelated with pulses from the start detector. Fluctuations in the spectrum are due to statistical effects.

mounted. This configuration allows the resolution of adjacent masses up about mass 1000 and can be used for routine analytical work.

A typical ^{252}Cf fission fragment has an atomic charge of 20^+ and a radius of < 1 Å. When it passes through a film 1 μm thick composed of C, H, and O, it deposits about 10 Mev through Coulomb interactions on a time scale of 10^{-13} second. In terms of power, this corresponds to 16 watts. If the effective area heated by the fission fragment is ~ 5000 Å^2 (*13*), each fission fragment is essentially a microprobe energy source yielding power densities of the order of 10^{13} watt/cm^2. Some of the characteristics of the ^{252}Cf fission fragment volatilization and ionization process are summarized in Fig. 2.

The combination of high power densities and short deposition times is the essential characteristic of ^{252}Cf plasma desorption mass spectroscopy (PDMS). Rapid heating with fission fragments apparently occurs on a time scale in which large amounts of energy are not coupled into vibrational motion. Most of the energy is available as translation motion, leading to desorption from the surface. Vaporization occurs before the molecule has time to decompose.

An estimate of the temperature of a species desorbed with fission fragments can be obtained by measuring the line broadening in the mass spectrum as a function of the square root of the mass. The temperature produces a mass-dependent spread in the velocity distribution of the ions before they are accelerated in the electric field. After correction for instrumental effects, the slope of the plot yields an ion temperature of 10^4 °K.

Ionization proceeds mainly through ion-molecule reactions or ion-pair formation. Positive and negative ions are produced with the same intensity. Many molecules, such as amino acids, small peptides, and nucleotides, that are zwitterionic in aqueous media form quasi-molecular ion pairs $(M + 1)^+$ and $(M - 1)^-$. Results of experiments in which labile hydrogen atoms on these molecules are replaced with deuterium atoms indicate that the principal mode of ion formation involves intermolecular proton transfer within a vaporized dimer or loose-association complex:

$$\begin{pmatrix} M \\ + \\ M \end{pmatrix}_{\text{solid}} \xrightarrow[\text{fragment}]{\text{fission}} (M + 1)^+_{\text{vapor}} + (M - 1)^-_{\text{vapor}}$$

Once the quasi-molecular ions are formed, they may fragment, depending on their inherent stability and internal excitation. The positive ion spectrum generally contains an $(M + 1)^+$ peak plus a fragmentation pattern produced by the loss of small molecules like CO_2 and HCOOH from the $(M + 1)^+$ ion and from bond cleavage. The fragmentation patterns are similar to those obtained by chemical ionization with the *t*-butyl ion. The negative ion spectra generally indicate that $(M - 1)^-$ quasi-molecular ions, when they are produced, are formed with considerably less internal excitation. The fragmentation spectrum is much weaker for negative ions, but the pattern generally correlates with the positive ion spectrum. The information obtained from both of the spectra has been useful for deducing molecular structure.

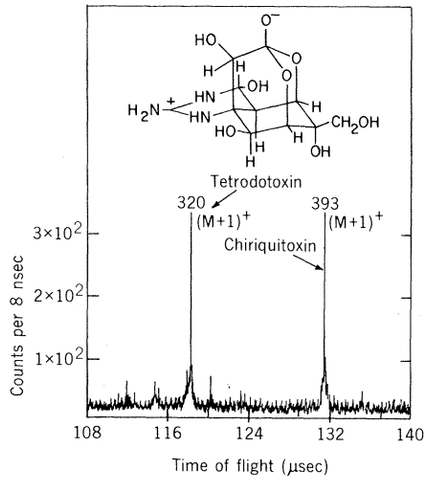

Fig. 5. Mass spectrum of tetrodotoxin and chiriquitoxin recorded with the 8-m flight tube. Counting time was 2 hours with a fission intensity of 500 sec^{-1} and a 100-μg sample prepared by solvent evaporation. The structure of chiriquitoxin is unknown, but is probably related to tetrodotoxin. This is the first determination of the molecular weight of chiriquitoxin.

Only a small fraction of the sample is consumed in an experiment. If 10^6 fission fragments are used to generate the mass spectrum and each fission fragment probes an area of 5000 Å^2, only 10^{-6} of a 1-cm^2 sample is used in the measurement. Most of the sample remains unchanged and can be recovered.

The sensitivity of the method, in terms of the minimum amount of material that can be used to obtain meaningful mass spectra, depends on the method of sample preparation. Plasma desorption is a surface phenomenon and the yield is proportional to the area of surface actually covered by sample molecules. Solvent evaporation produces nonuniform samples and up to 100 μg/cm^2 must be deposited to adequately cover the surface. We recently employed the electrospray technique to produce thin, uniform molecular films, which give comparable intensities with much smaller amounts of material (*14*).

The electrospray technique has also made it possible to begin to study the method quantitatively. The sensitivity limit depends on the molecule studied; for the amino acids it is on the order of 1 ng/cm^2 with a strong ^{252}Cf fission source. Knowing the fission fragment flux through the sample and the sample thickness, we can measure cross sections for the production of quasi-molecular ions and characteristic fragments from the mass spectrum. It is possible, for example, to quantitatively compare the yields of quasi-molecular ions from arginine with those from γ-glutathione or adenosine monophosphate. This is useful for understanding the relationship between molecular structure and quasi-molecular ion formation and for measuring the improvement of ion yields obtained by using derivatives or modifying the sample composition. With good control of the sample preparation, it is possible to obtain reproducible mass spectra, because the irradiation conditions at the target do not change.

Because of the way the data are obtained, ^{252}Cf mass spectra differ in some features from the mass spectra that are usually reported. The mass scale is nonlinear, as in all time-of-flight mass spectra, since the time of flight is proportional to $M^{1/2}$. The mass range or time window is preset for the experiment and can vary considerably, depending on the mass region to be studied. For example, the spectra shown in Fig. 3, taken with a 1.5-m flight tube, were recorded in a 24- to 28-μsec time window to detail peaks in the mass range 300 to 400; 4096 time interval channels were used, each channel being 1 nsec wide. The entire spectrum, including the fragmentation pattern, could be recorded in a time window of 0 to 32 μsec by using 4096 time interval channels, each channel set to be 8 nsec wide.

The intensity scale in the mass spectrum gives the number of events detected in the experiment in a particular time channel. If the time channel is 1 nsec wide, the intensity has units of counts per nanosecond and the spectrum will contain 4096 data points, the limit of our memory core storage. The total number of events recorded in a given time channel is proportional to the duration of the measurement. This varies from a few minutes to hours depending on the rate of ion production. For a sample with a particularly low ion yield it took 12 hours to accumulate 100 events that were due to the quasi-molecular ion.

Results and Applications

We studied classes of biomolecules for which mass spectra had been obtained by conventional methods in order to compare

ion yields and fragmentation patterns. Amino acids, including arginine and cystine (*7*), form $(M + 1)^+$ and $(M - 1)^-$ ions in good yield. Fragmentation patterns in the positive ion spectrum are very close to those observed in *t*-butyl ion chemical ionization (*15*). The $(M - 1)^-$ ion dominates the negative ion spectrum. Alkali metal ions present in the sample readily attach to the amino acids, forming $(M + Na)^+$ or $(M + K)^+$ ions with a corresponding loss in $(M + 1)^+$ intensity. The $(M - 1)^-$ intensity is not appreciably changed, which suggests that ion-pair formation by H^+ transfer still dominates, with exchange occurring between the labile H atoms and alkali metal ions.

Dipeptides and tripeptides also produce $(M + 1)^+$ and $(M - 1)^-$ ions. Fragmentation patterns in the positive and negative ion spectra contain N-terminal imine ions and C-terminal ions. The amino acid sequence of two isobaric tripeptides, alanylleucylglycine (Ala-Leu-Gly) and glycylleucylalanine (Gly-Leu-Ala), could be deduced from the fragmentation pattern data. Peptides also readily form $(M + Na)^+$ quasi-molecular ions, and there is some evidence, from a comparison of N-imine ion intensities, that these quasi-molecular ions do not fragment as much as the $(M + H)^+$ ions.

Higher-order peptides also produce quasi-molecular ions plus extensive fragmentation patterns in the positive and negative ion spectra. We have not yet used the fragmentation data to sequence these peptides because a high mass resolution analysis is required to obtain reliable sequence information. An important hexapeptide, γ-glutathione, gives $(M + 1)^+$, $(M + Na)^+$, and $(M - 1)^-$ ions in good yield. Quasi-molecular ions from the reduced form are also present in the spectrum. The largest peptide that has been studied is gramicidin A, a 15-residue peptide, which is blocked in the natural state at the N-terminals by a formyl group and at the C-terminals by a hydroxyethylamide group (*16*). Two variants of gramicidin A differ only in the final N-terminal residue (L-valine in one form and L-isoleucine in the other) and have molecular weights of 1881 and 1895, respectively. Figure 4a shows a partial mass spectrum of a mixture of the two variants in the region of the quasi-molecular ions. The $(M + Na)^+$ ions were observed in this case, possibly because the Na^+ impurity level was relatively high. No $(M - 1)^-$ ions were observed, which indicates that this ion is unstable toward fragmentation, or that ion-pair formation by proton transfer is not a probable process for this molecule.

The possibility of detecting quasi-molecular ions of large peptides suggests a variation of the approach that has been used to sequence peptides by enzymatic degradation coupled with mass spectroscopy. It may be possible to detect not only the individual residues that are removed in the degradation but also the remaining oligopeptides to provide a check on the identification of the residues removed. The fragmentation pattern, by itself, might give the same information, but this will require a detailed analysis of high-resolution data.

Simple nucleosides and nucleotides all give $(M + 1)^+$ and $(M - 1)^-$ ions in good yield, without making derivatives. The fragmentation patterns include ions from the base and sugar moieties in the positive and negative spectra. A dinucleotide phosphate, guanylyl-(3′→5′)-adenosine (GpA), forms a good $(M - 1)^-$ quasi-molecular ion peak plus a fragmentation pattern that includes Gp^-, Ap^-, G^-, and A^-. No positive quasi-molecular ions were observed, possibly indicating instability toward fragmentation. We have also investigated some trinucleotides, but have not been able to detect quasi-molecular ions. Trinucleotides and oligonucleotides are important in the elucidation of the structures of nucleic acids, and there has been considerable work on their mass spectra (*17*). None of the methods developed to date has produced molecular ions of a trinucleotide. There are several possible reasons why ^{252}Cf-PDMS has not worked for these molecules, and perhaps by utilizing another degree of freedom, the sample composition, we may be able to solve the problem. For example, if the reason for lack of success is involatility, we might isolate the oligonucleotide in a matrix to enhance the volatility, or complex it to reduce intermolecular interactions. Or it may be that a volatilized trinucleotide dimer will not undergo proton transfer to form a free ion pair. In that case, if we deposit an amino acid layer over the sample it may be possible to produce volatilized trinucleotide–amino acid dimers, which might more readily split into a free ion pair. The point here is that it is possible to make use of the chemical properties of the sample molecules to affect what molecular ions are produced in the process.

Large organic complexes containing nucleotide groups can produce quasi-molecular ions. An example is cyanocobalamin (vitamin B_{12}), which has a molecular weight of 1335. Figure 4b shows a portion of the negative ion spectrum with mass peaks at 1327 [due to loss of HCN from the $(M - 1)^-$ quasi-molecular ion], another

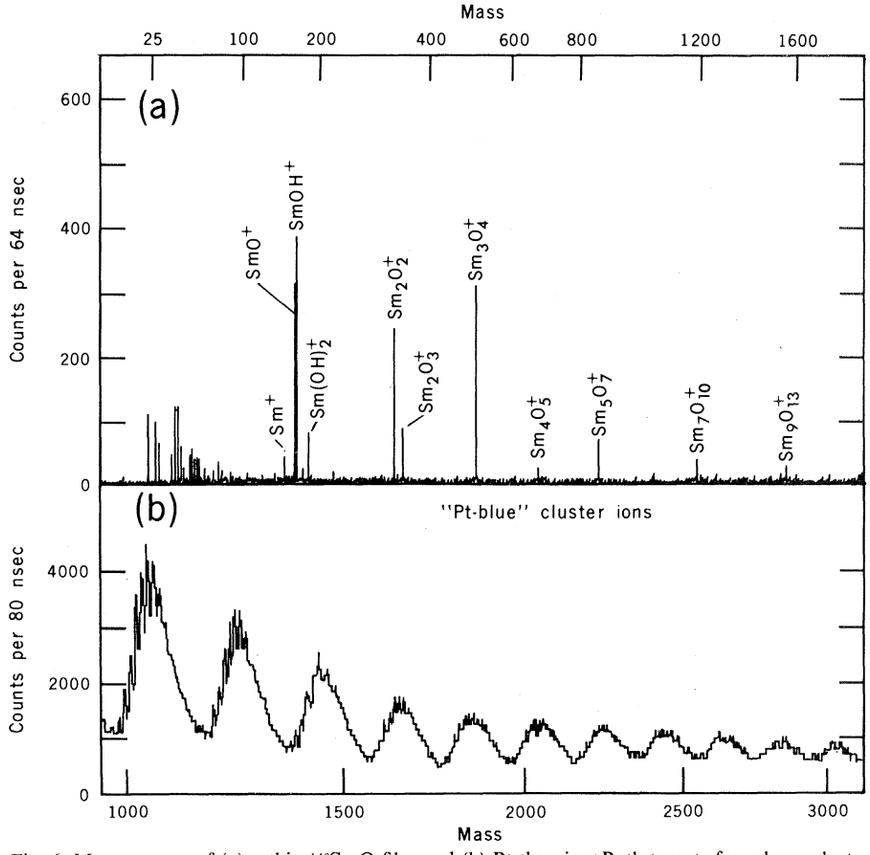

Fig. 6. Mass spectra of (a) a thin ^{148}SmO film and (b) Pt-thymine. Both targets form large cluster ions. The structure of Pt-thymine is unknown. The cluster ions are produced at least up to mass 3000. Each group with increasing mass corresponds to the addition of a Pt atom. The distribution of masses within each group probably represents different fragments of the thymine moiety.

group of unknown structure at 1269, and an intense fragmentation pattern below 1000. We show this as an example of the advantage of having both positive and negative mass spectra. This molecule does not give mass peaks above 1000 in the positive ion spectrum.

An amino acid conjugate, xanthine-tyrosine, is being studied by Stöhrer as a model for understanding protein–nucleic acid interactions. This molecule gives strong $(M + 1)^+$ and $(M - 1)^-$ peaks and also exhibits H^+-Na^+ exchange in the presence of Na^+ ions. The mass spectrometric problem in this study was to confirm the presence of xanthine-tyrosine ($M = 331$) in a sample that was prepared by chemical synthesis. The mass spectra in the region of the quasi-molecular ion are shown in Fig. 3. Mass peaks were observed at 332 in the positive ion spectrum and at 330 in the negative ion spectrum, suggesting ion-pair formation from a molecule with a molecular weight of 331. Additional peaks were observed at 354, 370, and 376 in the positive ion spectrum, which correspond to Na^+ and K^+ addition to the parent molecule and to H^+-Na^+ exchange. The peak at 352 in the negative ion spectrum, also due to H^+-Na^+ exchange, suggests that H^+-Na^+ exchange may take place before ion-pair formation. The presence of these alkali metal quasi-molecular ions in the spectrum provides an independent method for identifying the parent molecule. In addition to the peaks observed in Fig. 3, a fragmentation pattern is also present which can be related to loss of small molecules from the tyrosine moiety, cleavage of the tyrosine-xanthine bond, and splitting up of the xanthine ring. Another related amino acid conjugate, cystine-β-pseudouridine, also gives similar results.

Natural Products

A particularly interesting class of natural products is the naturally occurring toxins. A molecule that is biologically active generally contains reactive groups that give it its functionality. This same functionality can also give rise to strong intermolecular interactions and produce thermal instability. The mass spectroscopy of these compounds is difficult because of their low volatility and tendency to thermally decompose. An example is the well-known neurotoxin, tetrodotoxin, which has been studied extensively by Mosher *et al.* (*18*). The structure has been elucidated by chemical degradation and is shown in Fig. 5. The proliferation of polar groups on the ring system and the presence of the guanidine moiety are probably responsible for the low volatility, which has made it difficult to obtain a mass spectrum of the parent molecule even by field desorption. Figure 5 shows a mass spectrum of a sample containing tetrodotoxin and a new toxin, chiriquitoxin, which was isolated by Shindelman *et al.* (*19*) from the skin of the Panamanian frog, *Atelopus chiriquiensis*. This spectrum was recorded with an 8-m-long flight tube, so the resolution is considerably better than that of the spectra shown in Fig. 3, which were taken with a 1.5-m flight tube. The $(M + 1)^+$ ion is the major peak in the spectrum and most of the fragment ions are below mass 200. The negative ion spectrum shows mainly fragment ions but a weak $(M - 1)^-$ ion is present. One measure of the mass resolution of a system is the resolution of the ^{13}C satellite from the main peak. This does not show up well in Fig. 5 because the spectrum is compacted in time. We have repeated this measurement with a time grid of 1 nsec per channel and have obtained a spectrum showing a time-of-flight separation of 175 nsec between the $(M + 1)^+$ ion peak of tetrodotoxin and the ^{13}C satellite peak. The width of the $(M + 1)^+$ peak in this experiment was 22 nsec. Most of the line broadening is from the kinetic energy distribution of the ions before acceleration. The present system is capable of a mass resolution of better than 0.003 amu up to mass 500. Modifications in the method of measuring the time of flight will give considerably improved resolution, which is essential for elemental composition determination and for fragmentation pattern analysis.

Large Molecules

The positive result obtained with gramicidin A demonstrates that ^{252}Cf-PDMS is sensitive to molecular ions with molecular weights up to 2000. How much higher in mass it is possible to go with this method depends not only on the production of a molecular ion but also on its detection, since lower-velocity ions do not produce secondary electrons in the ion detector with good efficiency. The detection efficiency for large molecular ions has never been studied with channel plate multipliers. We obtained some information on this question by studying the PDMS spectra of thin ^{148}Sm oxide targets. This sample produces molecular cluster ions up to $Sm_9O_{13}^+$ which has a molecular weight of 1539. The spectrum is shown in Fig. 6a. This result also shows that there are other ways of producing ions in PDMS than by H^+ transfer. A sample of Pt-thymine (Pt-blue) (*20*) produces a periodic mass distribution of cluster ions up to mass 3000, as shown in Fig. 6b. The structure of these ions is not known, but the spectrum demonstrates that if ions up to mass 3000 are produced, they can be detected.

Summary

We have shown that ^{252}Cf-PDMS is capable of producing mass spectra of quasi-molecular ions for a wide variety of compounds, including amino acids, moderately large peptides, nucleotides, and natural products. Positive and negative ion mass spectra can be obtained, and in many cases quasi-molecular ions are observed in both. The method is nondestructive, as only a relatively few molecules are used and samples can be recovered after the measurement is made. Fragmentation patterns are obtained which can yield structure information. The present sensitivity of the method is at the nanogram level and there are possibilities for reducing this to picograms. The mass resolution is sufficient to give elemental identification up to mass 500. This may be extended to higher masses with improved time-of-flight techniques. There are indications that ^{252}Cf-PDMS may extend the mass range of molecules that can be studied to as high as 3000 or more.

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