NEW APPROACH TO THE MASS SPECTROSCOPY OF NON-VOLATILE COMPOUNDS

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Summary

A new method has been developed to obtain mass spectra of non-volatile compounds from the solid state at room temperature. Volatilization and chemical ionization of the sample are effected using a ^{252}Cf fission fragment source. Mass spectra are measured using a new time-of-flight mass spectrometer featuring single ion counting and fast electronics. We present positive and negative ion mass spectra for the amino acids arginine and cystine which are characterized by intense quasi-molecular ions.

In this communication we introduce a new concept for the mass spectroscopy of biological molecules which obviates the necessity for chemical derivitization and leads to intense quasi-molecular ions. Many compounds of biological interest are difficult to volatilize directly and are usually derivatized in order to increase volatility for ion production by electron impact in the vapor phase. This frequently leads to thermal fragmentation which renders a complicated mass spectrum often devoid of an intense parent ion peak. If the parent ion intensity is weak or non-existent, identification must be made primarily on the basis of the fragmentation pattern which may give an ambiguous result. Arginine and cystine are two amino acids which are difficult to characterize by conventional mass spectrometry for this reason. This has been a long-standing problem in the sequencing of proteins using mass spectrometry.

We present spectra for arginine and cystine which do not produce parent ions even when derivatized in electron impact or chemical ionization mass spectroscopy (1,2). Our technique produces spectra comparable to those obtained using field desorption methods with some important differences (3).

Method

Amino acid samples, obtained from Sigma Chemical Company, were dissolved in H_2O and nebulized onto a 1 x 10^{-3} mm thick Ni foil, producing a solid film of ~200 g/cm^2 thickness. Samples were introduced into the mass spectrometer and aligned with a ^{252}Cf fission fragment source as shown in Figure 1. Geometries were chosen such that ionization created in the

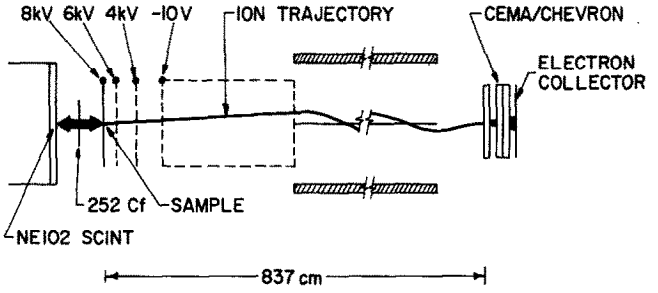

Figure 1 Schematic of the time-of-flight fission fragment source mass spectrometer.

sample by a fission fragment is in coincidence with the complementary fragment detected in an NE102 scintillation counter. The pulse from the scintillation counter is used to establish "time zero" in the time-of-flight measurement.

When the fission fragment passes through the thin Ni foil, a thermal spike is created which vaporizes quantities of mobile impurity ions (principally H^+, Na^+, and H^-). These secondary ions then react with sample molecules in the region of the thermal spike to produce quasi-molecular ions which are immediately accelerated through a gridded lens system and an 8 kv potential into an 8 m long time-of-flight tube. To reduce solid angle losses, the drift tube is an electrostatic particle guide consisting of a wire held at ±10 v potential centered on the axis of the tube. The resulting radial field maintains the ions in a stable spiral orbit about the wire until detected at the end of the drift tube. A CEMA windowless electron

multiplier array is used to detect the ions giving the "stop pulse." The time interval between the "start" pulse derived from the fission fragment and the "stop" pulse derived from the detection of an ion is measured using constant fraction discrimination and an EG + G TDC-100 time digitizer to generate fast logic signals. With a fixed acceleration potential, the time-of-flight uniquely determines the mass to charge ratio.

Data were stored in a 4096 memory array and were transferred to magnetic tape for further analysis. Further details of the system are to be published elsewhere.

Results and Discussion

Positive ion mass spectra for arginine and cystine are shown in

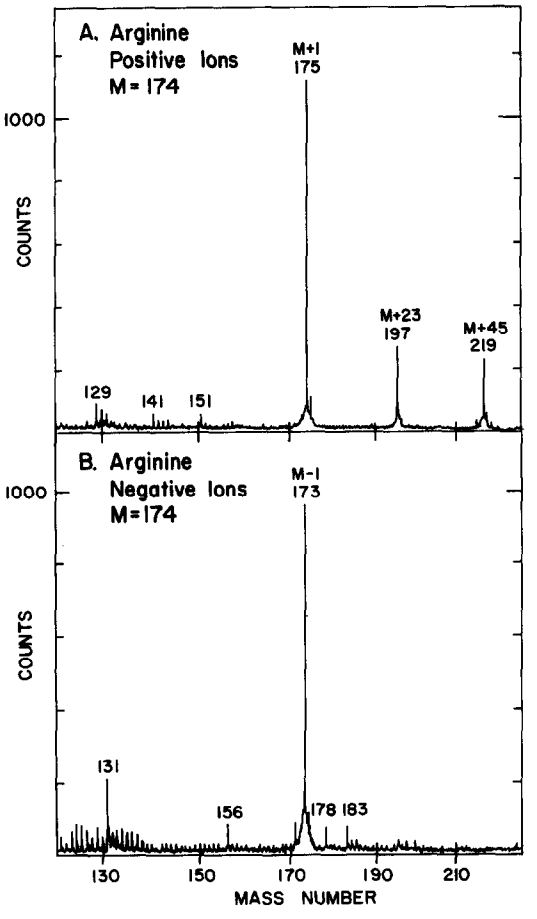

Figure 2 Positive and negative ion mass spectra of arginine.

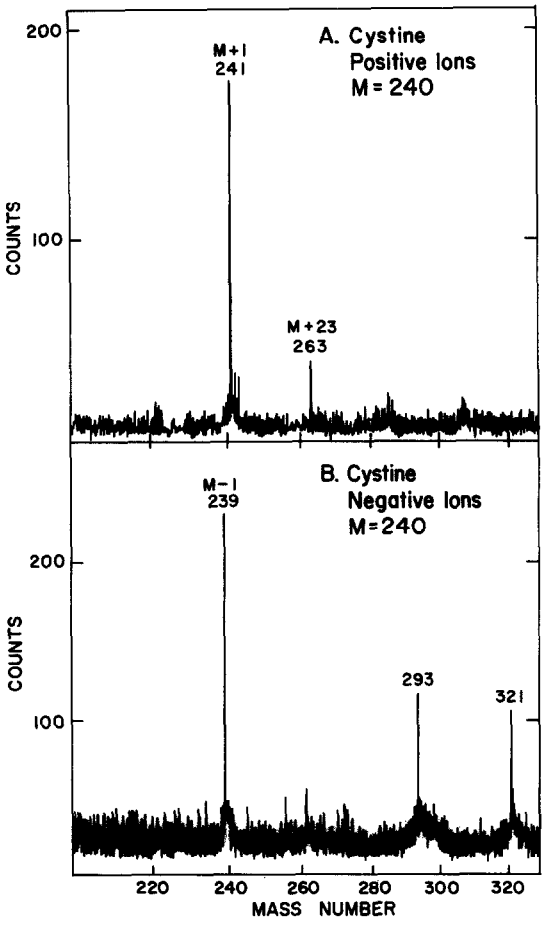

Figure 3 Positive and negative ion mass spectra of cystine.

Figures 2 and 3, respectively. The salient features are readily interpretable as the spectra are characterized by a large $(M + 1)^+$ quasi-molecular ion. In the absence of the sample, the fission fragments create large quantities of H^+ ions in the Ni foil which are highly mobile. We believe that the $(M + 1)^+$ ion is formed via H^+ attachment to basic sites on the amino acids.

The ion Na^+ is also created in abundance and gives rise to the $(M + 23)^+$ peak. An $(M + 45)^+$ ion is prominent in the arginine spectrum but constitutes a weak peak in the cystine data. The $(M + 45)^+$ peak appears to vary with the Na^+ concentration, leading to the conclusion that it consists

of two Na^+ ions attached to the parent with the loss of one H^+. One possibility for the formation of this ion is double Na^+ attachment with the simultaneous loss of one H^+. As the thermal spike creates a dense sea of Na^+ ions, we suspect that this is the most plausible explanation.

At masses below the quasi-molecular ion, peaks are observed which arise from impurities on the Ni foil as well as from primary coulomb interactions between the fission fragments and sample. In addition, small peaks can be identified corresponding to the loss of NH_3, CO_2, COOH, and $COOH_2$ from $(M + 1)^+$ in the arginine spectrum, but these are at least an order of magnitude reduced in intensity relative to the quasi-molecular ion. Thus, the $(M + 1)^+$ peak represents an unambiguous signature for the presence of these two amino acids.

Neither electron-impact nor chemical ionization methods yield molecular or quasi-molecular ions of arginine and cystine. Field desorption techniques lead to $(M + 1)^+$ quasi-molecular ions, with the most intense peak for arginine corresponding to the loss of NH_3, and for cystine to the loss of $COOH_2$ (3). Neither of these peaks is prominent in our spectra. Peaks corresponding to the loss of H_2O from the molecular or quasi-molecular ion are not observed in this work, in agreement with the conclusion that H_2O loss is a thermal effect occurring in the sample inlet system prior to electron impact or chemical ionization (3).

A further dimension contributing to the analytical and structural use of the present technique is the observation of negative ion peaks of comparable intensity to the positive ions. The most prominent feature of the negative ion spectra of arginine and cystine (Figures 2 and 3) is the presence of the $(M - 1)^-$ ion. Negative ion spectra recorded with a bare Ni foil are dominated by an intense H^- peak. We therefore believe that the $(M - 1)^-$ ions arise via proton abstraction from the parent ion by the strongly basic H^-. Proton abstraction may prove to be a new method for negative ion mass spectroscopy of a wide class of biological compounds.

At lower masses, peaks corresponding to foil impurities and direct fission fragment/sample interactions are observed as in the positive ion spectra. In addition, we observe peaks at mass 293 and 321 in the cystine spectra which have not yet been identified.

Conclusions

We have demonstrated that the non-volatile and thermally unstable amino acids arginine and cystine can be studied in the solid state and at room temperature using a new time-of-flight mass spectroscopy technique. Both negative and positive ion spectra are relatively easy to interpret, being dominated by quasi-molecular ions in the vicinity of the parent mass.

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References:

1. Junk, G. and Svec, J. (1963) J. Am. Chem. Soc. 85, 839-845.
2. Leclercq, P. A. and Desiderio, D.M. (1973) Org. Mass Spec. 7, 515-533.
3. Winkler, H.U. and Beckey, H.D. (1972) Org. Mass Spec. 6, 655-660.