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FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF HUMAN PROINSULIN

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SUMMARY: The observation of protonated molecular species from human proinsulin obtained by fast atom bombardment mass spectrometry is reported.

Hitherto, the classical methods of ionizing organic compounds for mass spectrometric analysis have required sample volatilization before ionization, which has limited the attainable mass range to approximately 1500 u, for all but a few curiosities. Field desorption (1) and plasma desorption (2) have been used above this limit and the latter has been used to volatilize and ionize a derivatised oligonucleotide of molecular weight ca 7000 u (3). However, this low flux ion source has only been used successfully with a coincidence time-of-flight method of mass analysis. While there is no theoretical upper limit to the mass range of such a system, unit mass resolution has not been demonstrated above m/z 1000. In contrast the recently developed fast atom bombardment (FAB) ion source (4) has sufficient intensity to permit the use of conventional scanning organic mass analysers. These have higher mass resolution but a limited useful mass range which is traditionally defined as the range over which unit mass resolution is maintained.

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Using a FAB ion source in conjunction with a high performance magnetic sector mass spectrometer, we have demonstrated the ability to observe fully resolved mass spectra containing protonated molecular species $(M + H)^+$ from the bee-venom peptide melittin (5) (2846 u), and less well resolved $(M + H)^+$ species from glucagon (3482 u) and the oxidized B chain of bovine insulin (3495 u). We have also reported measurements on adrenocorticotropic hormone ACTH (4539 u) and complete bovine insulin (5731 u) (6) which form part of a project designed to delineate the useful mass range of current magnetic sector mass spectrometers.

However, we have now found it possible to use the FAB ion source to study molecules with even higher molecular weights. In this short communication we wish to report the detection of the unresolved molecular ion species of human proinsulin, which, to our knowledge, is the largest underivatised natural biological molecule which has been observed in mass spectrometry.

Measurements were made using a V.G. Analytical MM ZAB-HF mass spectrometer fitted with a standard FAB ion source. At an ion energy of 8 keV, this instrument will transmit ions up to m/z 3200 u, an upper mass limit determined by the maximum magnetic flux attainable ($m/z \propto H^2/V$). The atom source was operated using 8 keV xenon atoms, at plasma discharge currents of 1-2 mA. The data were acquired by magnetic scanning at a rate of 100 u min^{-1} with a span of 300 u. Data were recorded oscillographically using ultra-violet sensitive chart paper. The sample was mounted on a stainless steel support and was examined in the ion source at room temperature. The solvent system used was acidified glycerol, which gave a considerably enhanced spectral lifetime over other systems tried.

With current instrumentation, maximum sensitivity is invariably obtained at maximum ion energy, which unfortunately corresponds to a minimum mass range for a particular magnetic

field strength. Since the mass resolving power is strongly dependent on instrument transmission, there is always a trade off made between sensitivity and resolving power. This effect has already been illustrated with melittin (5) where the protonated molecular species were fully resolved only after improvements in ion source brightness and the use of a high field magnet which permitted ions with m/z 2846 u and an ion energy of 8 keV to be focused.

In order to acquire the data presented in this communication, the ion accelerating voltage of the mass spectrometer needed to be reduced to 2.7 keV. The concomitant reduction in sensitivity together with the low intrinsic ion current from the sample, required a reduction in resolution to increase the overall instrument transmission. Thus it is readily seen from the figure that the isotope distribution for the protonated molecular species $(M + H)^+$ is completely unresolved.

To obtain molecular weight information a fully resolved spectrum would ordinarily be acquired. At low masses the largest ion in the parent region would be assigned as the protonated molecular ion for the monoisotopic species, composed of the isotopes with the greatest abundance for the atoms making up the molecule. However, we have previously shown that even with molecular weights of as little as 3000 u this monoisotopic species ceases to be the largest peak in the parent isotope distribution. Indeed we have shown with insulin, that the monoisotopic $(M + H)^+$ species has an intensity of only ca 15% with respect to the largest peak in the parent isotope distribution. Consequently for proinsulin the situation is further exacerbated.

Thus, even if the parent ion region were fully resolved, identification of the monoisotopic $(M + H)^+$ ion would present, to say the least, great difficulties, without studying model compounds.

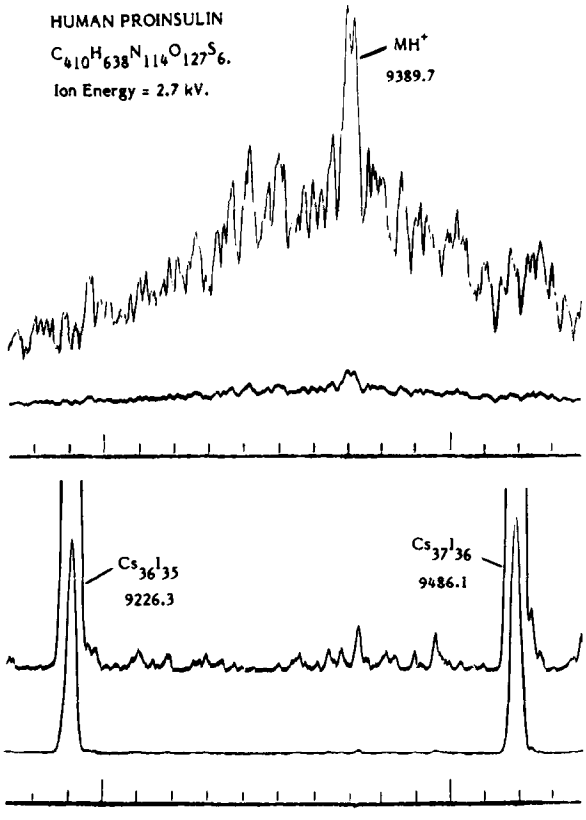

Figure 1

It is,however, possible to measure from the presented data a chemical molecular weight based on the chemical atomic weights of the elements comprising the molecule. This can be achieved by measuring the mass centroid of the unresolved parent ion region shown in the figure. We have, using the above method, measured the chemical molecular weight of protonated proinsulin to be 9390 u using caesium iodide as a reference. The theoretical chemical molecular weight of protonated proinsulin is 9389.7 u based on the empirical formula shown in figure 1.

These results not only demonstrate the ability of the fast atom bombardment ion source to volatilize and ionize molecules of this size and complexity, but show that meaningful molecular weight information may be deduced from unresolved data.

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