Fast Atom Bombardment Mass Spectrometry of Bovine Insulin and Other Large Peptides

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The observation of protonated molecular species from bovine insulin and adrenocorticotropic hormone is reported, together with sequence information for melittin, obtained by fast atom bombardment mass spectrometry.

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 *ca.* 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 6980.[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. Using an 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] (2845 u), and less well resolved $(M + H)^+$ species from glucagon (3841 u) and the oxidized B chain of bovine insulin (3494 u). Here we report measurements on adrenocorticotropic hormone (ACTH) (4538 u) and complete bovine insulin (5730 u) which form part of a project designed to delineate the useful mass range of current magnetic mass spectrometers.

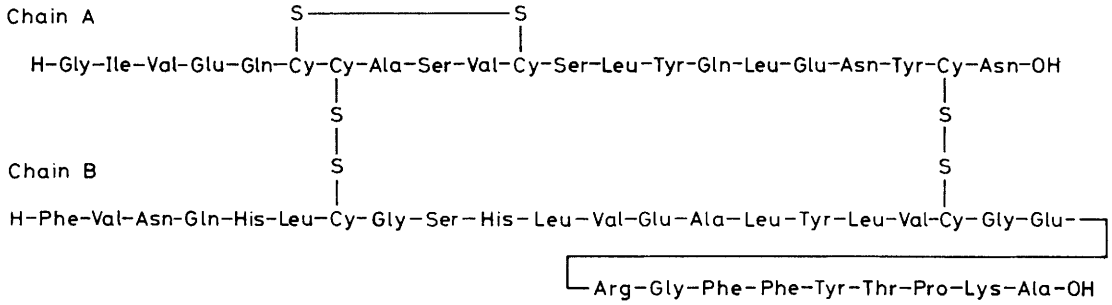
Measurements were made using a V.G. Analytical MM ZAB-HF mass spectrometer fitted with a standard FAB ion source. At an ion energy of 8 keV, this instrument will transmit ions up to m/z 3300 u, an upper mass limit determined by the maximum magnetic flux attainable ($m/z \propto H^2/V$). The atom source was operated using 8 keV xenon atoms, at plasma discharge currents of 1—2 mA. Samples were mounted on a stainless steel support and were examined in the ion source at room temperature. Insulin and ACTH were dissolved in α-monothioglycerol (10 nmol μl^{-1}) with the addition of 1% glacial acetic acid in the latter case. For melittin, which contained some of its *N*-formyl derivative, we used a 4:1 mixture of α-monothioglycerol and tetraethyleneglycol containing 1% of glacial acetic acid. This latter solvent system gives more intense spectra of melittin than does the α-monothioglycerol–acetic acid system.

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 2845 u and an ion energy of 8 keV to be focused. We have previously reported molecular weight confirmations[5] for peptides up to 3000 u and complete sequence information[6] up to molecular weights of 2000 u. The latter ability depends on the detection of fragmentation processes giving ions which frequently will be present at intensities which are an order of magnitude below that of the molecular species. Consequently the ability to observe these is related to the sensitivity of the system. The determination of a complete sequence from the FAB mass spectrum of a peptide which gives a protonated molecular species at a relatively low (*ca.* 5:1) sample: background ratio at a reduced ion energy would appear to be unlikely. In contrast, for a pepide of molecular weight 3000 u, which can be examined at full sensitivity (8 keV), it would appear feasible to obtain the complete amino-acid sequence. This point can be illustrated by melittin (**1**) for which the sequence, with the exception of residues 16—22 has been determined by the observation of the acylium ions ($-NH-CHR-\overset{+}{C}O$) in the positive ion FAB mass spectrum.

H-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-
1
Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH_2
16 22

(**1**) Melittin



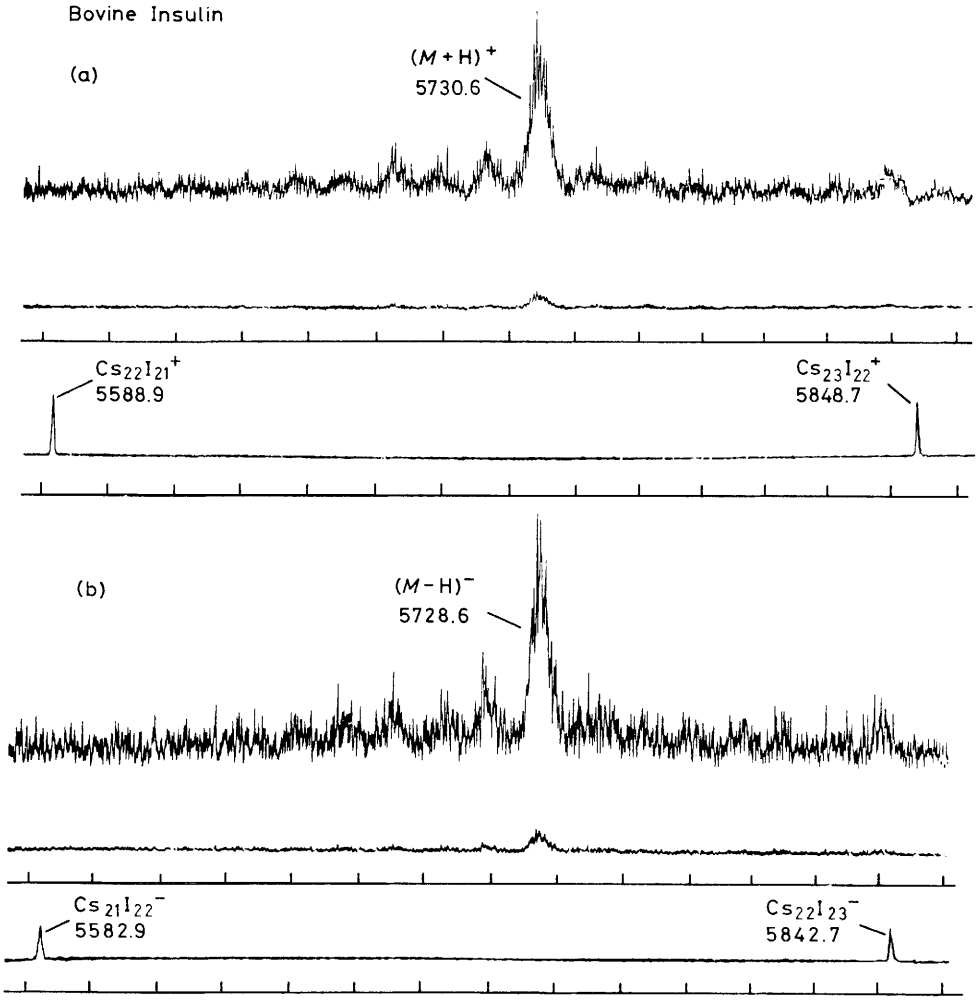


Figure 1. (a) Protonated molecular species in the positive ion FAB mass spectrum of bovine insulin, and (b) deprotonated molecular species in the negative ion FAB mass spectrum of bovine insulin. For bovine insulin, $^{12}C_{254}{}^{1}H_{377}{}^{14}N_{65}{}^{16}O_{75}{}^{32}S_{6}$, $M = 5729.6$ u.

We have now found it possible to use the FAB ion source to study molecules with even higher molecular weights. The protonated molecular species in the positive ion FAB mass spectrum of a sample of synthetic ACTH, were obtained at an ion energy of 5 keV but with consecutive mass numbers only resolved to 50% (R.P. = 3000). The protonated molecule, measured as m/z 4539.5 u is only a minor component in the isotopic distribution and the major component occurs 2 mass numbers higher. The measured molecular weight is in good agreement with that calculated for the known composition of this 39-unit peptide, $^{12}C_{207}{}^{1}H_{308}{}^{14}N_{56}{}^{16}O_{58}{}^{32}S$, $M = 4538.3$ u. A further extension of the mass scale is illustrated in Figure 1 where the protonated [Figure 1(a)], and the deprotonated molecular species [Figure 1(b)], obtained from the complete and underivatised molecule of bovine insulin are reproduced. The spectra were acquired at an ion energy of only 4 keV, at which the mass scale is extended to 6000 u, and the mass calibration was achieved by comparison with the FAB mass spectrum of solid CsI which produces cluster ions of the type $Cs(CsI)_n^+$ and $(CsI)_nI^-$. The mass resolution is less than 4000 but nevertheless consecutive masses can still be distinguished. The limited dynamic range makes identification of the protonated molecule, expected at m/z 5730.6 u, difficult to distinguish unambiguously from the background; however, the cluster of isotopic peaks observed is not inconsistent with the published structure of this molecule.[7] These FAB mass spectra represent the result of a careful balance between the sensitivity and resolving power and the sample: background intensity ratio is 10:1, from which we project that little sequence information is likely to be deduced from the rest of the spectrum acquired under these conditions. Some sequence information will be available from those fragment ions with masses below 3000 u which are present in partial mass spectra obtained at full sensitivity. An alternative strategy is, however, available for insulin which involves separate examinations by FAB mass spectrometry of the oxidised forms of the constituent A and B chains, where the disulphide bridges have been oxidised to sulphonic acids.[8]

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