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MATRIX-ASSISTED LASER DESORPTION AND IONIZATION OF BIOMOLECULES

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Recent developments in the volatilization and ionization of large molecules using matrix-assisted ultraviolet laser desorption have made it possible to produce intact protonated molecule ions from proteins with molecular masses ranging from a few thousand to greater than 100,000 mass units[1].

In the paper presented at the workshop, we described the construction and performance of a linear time-of-flight mass spectrometer with improved performance for the measurement of proteins and other biomolecules. Details on this instrument and its performance can be found in references 2-5. The specifications of the present instrument are summarized below:

mass range	1 to greater than 300,000 u
resolution	300 - 500 Full width half maximum
mass accuracy	0.01% for resolved peaks
sensitivity	< 1 pmol
universality	Spectra have been obtained from more than 200 different proteins including heavily glycosylated proteins and membrane proteins (see example given in Fig. 1).

During experiments designed to elucidate the detailed role of the matrix and its effect upon the mass spectrum, we discovered a number of new matrix materials[2-5] with improved

properties compared with the nicotinic acid, which was the matrix used by the originators of the matrix-assisted laser desorption technique[1]. One class of these new matrix materials - the cinnamic acid derivatives sinapic acid, ferulic acid, and caffeic acid - was found to have especially favourable properties:

(i) Spectra obtained from nicotinic acid were found to exhibit ion peaks showing adduction to the protein of a large number of photochemically generated products from the matrix[2]. This adduction caused a considerable lowering of the quality of the spectra and the information that could be extracted therefrom. The cinnamic acid derivatives produced spectra exhibiting much lower levels of photochemically generated adduction products and were therefore of much higher quality[3,5].

(ii) The spectra from nicotinic acid were obtained with laser irradiation from a frequency quadrupoled Nd(YAG) laser giving a wavelength of 266 nm. Because the cinnamic acid derivatives absorb strongly at longer wavelengths (to greater than 350 nm)[4], it is also possible to obtain spectra with a much less expensive, low power nitrogen laser.

(iii) The cinnamic acid derivatives showed an unprecedented ability to produce high quality spectra from complex mixtures of peptides and proteins in the face of large concentrations of non-proteinaceous impurities such as salts, buffers, and lipids[6]. It has even proved possible to obtain informative mass spectra from unpurified or partially purified biological fluids[6,7].

A number of other fundamental features of matrix-assisted laser desorption were described. These include:

(a) The finding that a significant proportion of the fully accelerated intact protein ions appear to undergo metastable decomposition in the flight-tube of the mass spectrometer[8], indicating that the proteins are vibrationally excited during the desorption/ ionization and ion acceleration process.

(b) The finding that the initial velocity of polypeptide ions (prior to ion acceleration by externally applied electric fields) is the same (750 m/s) for polypeptide ions ranging in mass from 1,000 to 16,000 u[9], indicating that the polypeptide ions are entrained in a supersonic expansion of the matrix molecules after the laser ablation event.

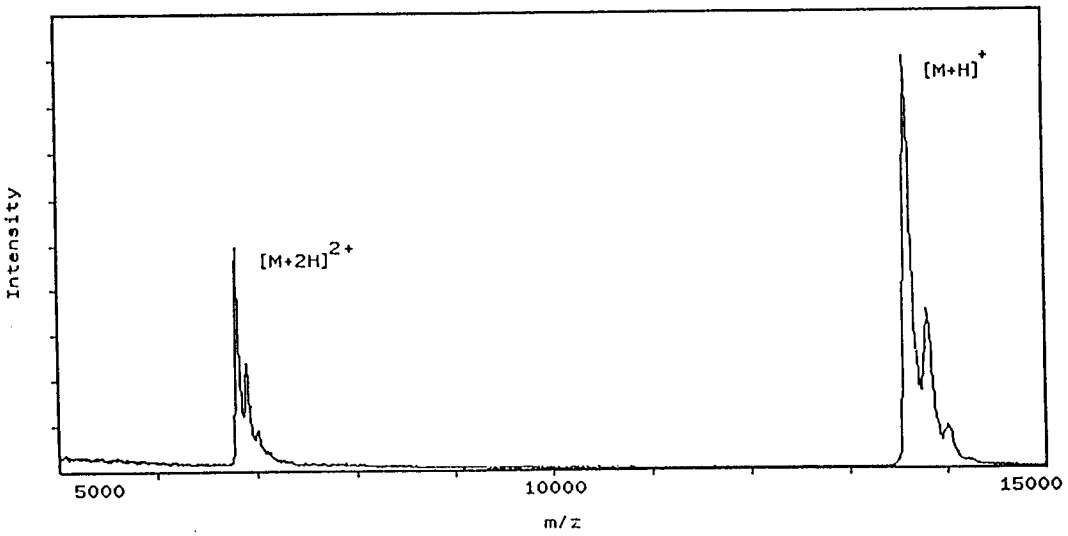

Fig. 1. Positive matrix-assisted laser desorption mass spectrum of phospholipase A_2 from the venom of *Crotalus attrox*. 1 pmol of protein inserted into the mass spectrometer .
Measured molecular mass = 13,582
Calculated molecular mass = 13,581.