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MATRIX ASSISTED UV LASER DESORPTION OF LARGE BIOMOLECULES.

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Ultraviolet (UV) laser desorption time-of-flight (TOF) mass spectrometry has undergone a revolution in the past two years allowing it to be used for the analysis of picomole quantities of proteins. This revolution has been achieved by the adoption of a new strategy in laser desorption sample preparation: the use of volatile UV absorbing matrices mixed with the sample molecules. A pulsed laser incident on the sample surface apparently causes a sudden phase transition in a small volume of the matrix/sample mixture, sweeping the involatile sample molecules embedded in the matrix into the gas phase and ionizing them.

The strategy has been employed in two different ways. Tanaka et al [1] reported in 1988 that protein molecules embedded in a UV transparent glycerol matrix could be desorbed and ionized if a finely divided metal powder was added to the mixture. These metal particles (with a diameter << 337 nm, the wavelength of the nitrogen laser used) absorb the laser radiation and are rapidly heated , causing explosive effects in the matrix. By a judicious choice of particle concentration and laser irradiance, intact pseudomolecular ions of large proteins could be detected. The mass spectrometer used for these studies was a TOF instrument with an ion mirror to correct for an initial energy spread in the ions. The mass resolution obtained for protein pseudomolecular ions was $m/\Delta m \equiv 50$ (FWHM definition), even though the mass resolution of the instrument was much higher.

Karas and Hillenkamp [2] in 1988 reported that protein molecules embedded in a UV absorbing solid matrix could be desorbed intact. In this case, the matrix absorbs the laser radiation (Nd-YAG laser, λ = 266 nm) and the volume irradiated rapidly gains sufficient energy to unload from the surface. The matrix material used was 3-pyridinecarboxylic acid (nicotinic acid), a water soluble compound that could be mixed with the protein sample in an aqueous solution, and the solution dried to form a matrix/sample solid mixture. These deposits were examined using a LAMMA laser microprobe TOF instrument with an ion mirror. The mass resolution obtained for protein molecules was $m/\Delta m \equiv 50$ (FWHM), although the actual resolution of the instrument was approximately 600 (FWHM).

These two results suggest that something fundamental in the desorption process is causing much wider peaks for protein molecules desorbed in this indirect fashion. Possible explanations include: 1) ions are being ejected with such a high initial energy that the ion mirror cannot compensate for it; 2) ions are being released from the surface over a much longer time period than the duration of the laser pulse; or 3) ion

signals are composed of many unresolved peaks, caused by the formation of adduct ions, either with the matrix or with other impurities in the sample. The first possibility includes the effects produced by the sudden charge separation that occurs at the surface after the laser pulse: very large numbers of ions leaving the dielectric surface of the matrix should produce surface charging. It would also include Coulomb explosion effects, caused by the rapid production of a high space charge density just over the surface of the sample.

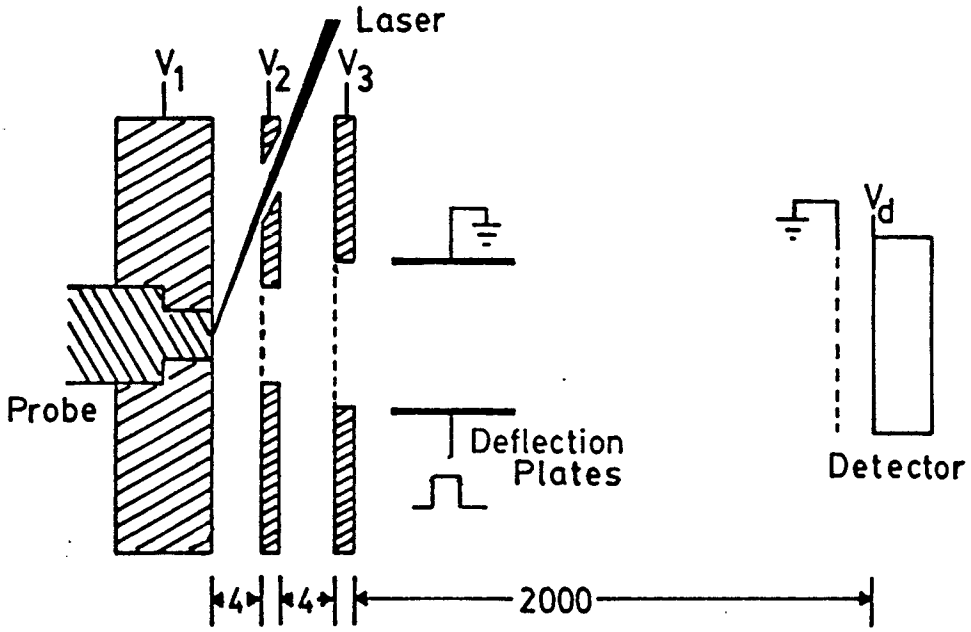

Fig. 1 A schematic diagram of the TOF mass spectrometer used. V_3 is normally held at ground and V_1 and V_2 used to accelerate ions formed by the laser.

Over the past six months, our group has investigated the solid phase matrix laser desorption technique, using a modified version of the Karas and Hillenkamp experiment. The TOF mass spectrometer used is shown in Figure 1 [3]. The laser used was a Lumonics HY-400 Nd-YAG laser, with harmonic generation and selection attachments. The laser was operated in the Q-switched mode, with internal triggering to produce a pulsed output (10 nsec duration) running at 1 - 5 Hz. Part of the output was split off using a silica beam splitter and detected by a phototransistor to give the "start" pulse for the TOF measurement electronics. The intensity of the laser beam incident on the surface was varied with a Newport 935-5 variable attenuator (2 - 27 dB attenuation, < 20 microradian beam deviation over the attenuation range). The laser beam was focussed onto a spot 0.1 x 0.3 mm (at the sample probe tip) using a 25 cm focal length plano-convex fused silica lens, mounted outside of the mass spectrometer's vacuum system. The incidence angle of the laser at the sample was 70 degrees. Typical irradiances used were 0.5 - 1 MW/cm^2.

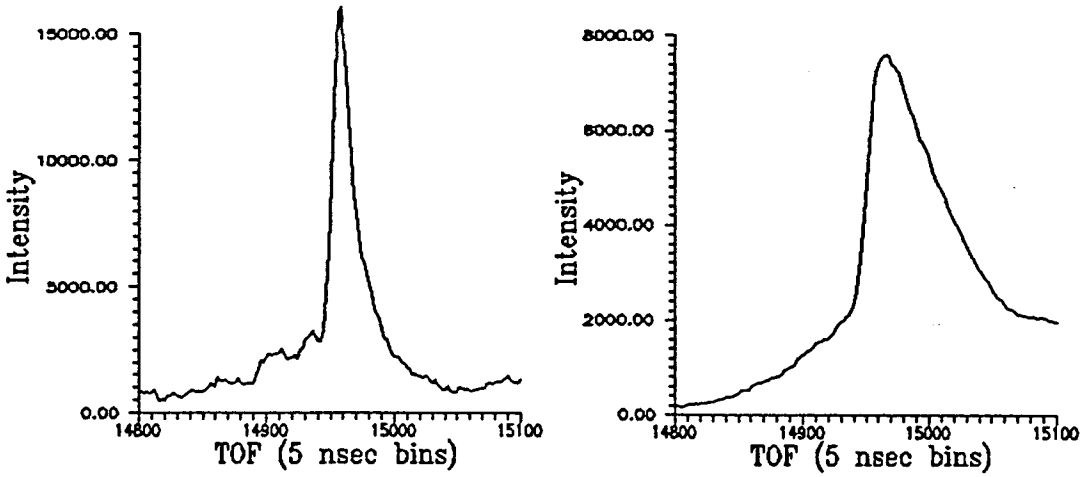

Fig. 2 The protonated molecule ion of insulin: (a) at threshold irradiance; and (b) and twice the irradiance of (a). Conditions: 5 picomoles loaded in 0.5 µl of 10 g/l vanillic acid solution; V_1 = + 20 kV, V_2 = + 15 kV and V_d = - 2.0 kV; 50 shots averaged; displayed bin size = 10 nsec.

The precise positioning of the optical components was not critical in this particular design. The distance from the lens to the sample could be varied over 5 cm without affecting the quality of the mass spectra obtained. The most important factor was the ability to control the irradiance of the laser finely and reproducibly. Figure 2 shows the pseudomolecular ion region of mass spectra of insulin with (a) near threshold irradiance and (b) twice the irradiance of (a). Both spectra were obtained from the same sample. The FWHM mass resolutions are: (a) $m/\Delta m$ = 450; and (b) $m/\Delta m$ = 110. These results demonstrate a more general finding: increasing the irradiance increases the width of the protonated molecular ion peak and changes the value of the centroid to higher times. Such effects are consistent with sample charging during ion formation/extraction. An additional component of the peak width could be caused by a Coulomb explosion near the surface.

In a linear TOF mass spectrometer, peaks can be widened by kinetic energy spreads produced during desorption by effects such as sample charging. The relationship between mass resolution and an initial kinetic energy spread (ΔE) is simply: $m/\Delta m = E/\Delta E$; where E is the acceleration voltage of the mass spectrometer (V_1). Fig. 3 shows the relationship between mass resolution and acceleration energy for the insulin protonated molecule. Increasing the V_1 above $\cong$ 20 kV does not seem to have a significant effect on the resolution. This result suggests that there are other contributions to the peak width, in addition to an initial energy spread.

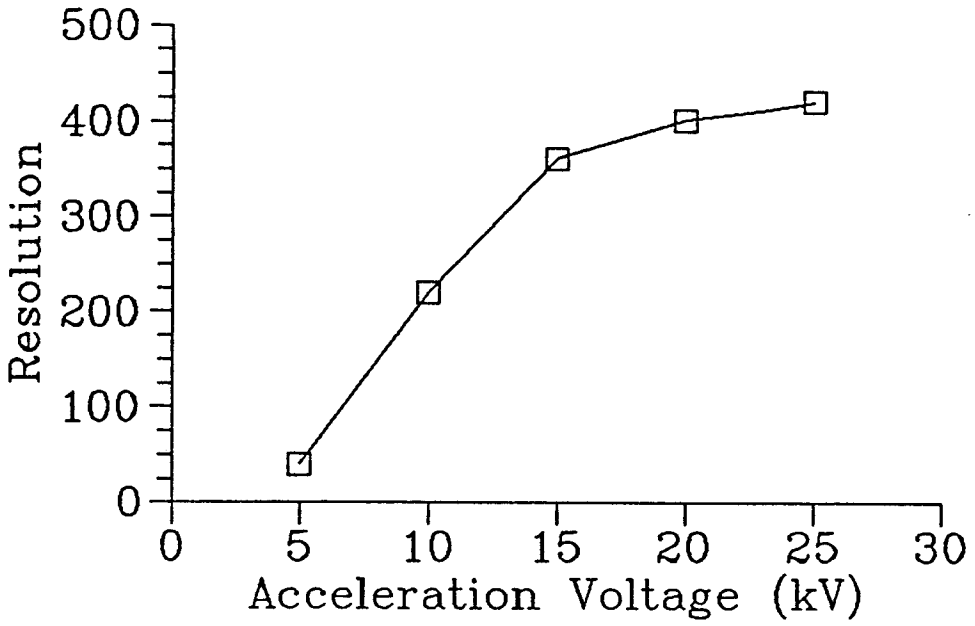

Fig. 3 The measured mass resolution (FWHM) of the insulin quasimolecular ion peak as a function of V_1.

The recent observation of intense matrix related quasimolecular adduct ions [3] has made increasing the mass resolution of the technique important for the confident assignment of molecular weights of high mass ions. These adduct ions are caused by the addition of either the intact matrix molecule or a photochemical fragment (most frequently the loss of CO_2 from a carboxyl group) to the protein. The presence of adduct ions produces significant peak broadening in the quasimolecular ion region if they cannot be resolved. They also shift the TOF centroid of the unresolved peak to higher times, resulting in incorrect mass assignments. The same types of adduct ions occur in both positive and negative ion spectra.

An example of adduct formation in the negative ion mode is shown in Figs. 4 and 5. Fig. 4 is the negative ion mass spectrum of 1 picomole of porcine pepsin (MW = 34,504 u) mixed with a vanillic acid (VA) matrix. The low mass matrix related ions in the spectrum have been suppressed using the deflection plates in Fig. 1, to prevent detector gain losses caused by high integrated signal intensities. The quasimolecular ion peak in Fig. 4 corresponds to a mass resolution of $m/\Delta m = 60$ (FWHM) and is the sum of the $(M-H)^-$ and the matrix adduct ion $(M+VA-H)^-$. Fig. 5 is an expansion of the quasimolecular ion region with decreased time binning, showing that the two peaks can be resolved, corresponding to a mass resolution of $\cong 300$ (FWHM).

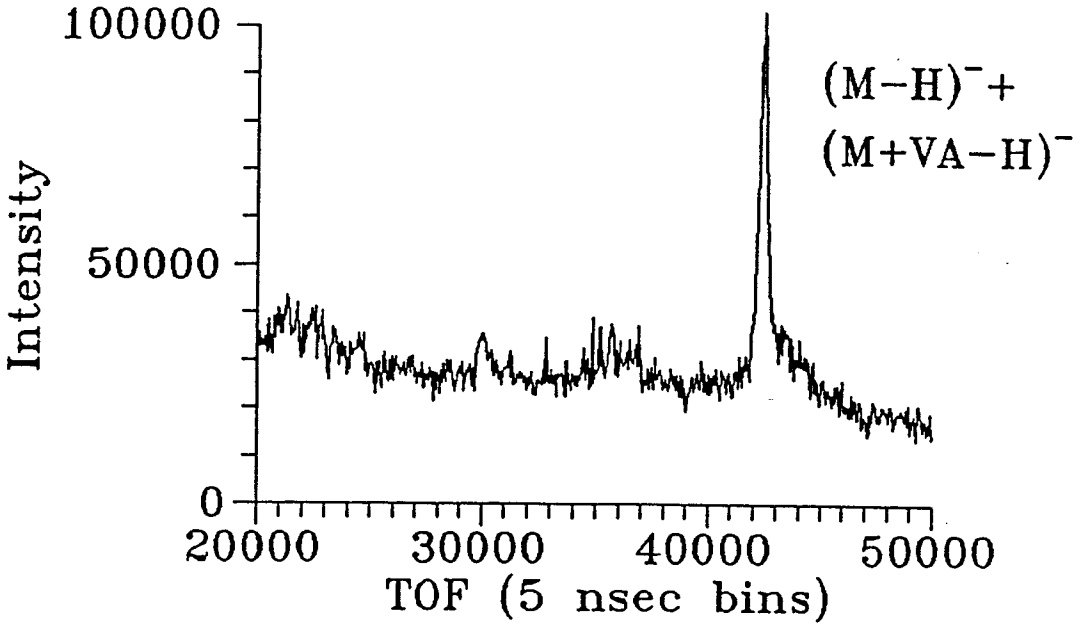

Fig. 4 The negative mass spectrum of porcine pepsin (MW = 34,500 u). Conditions: 1 picomole loaded in 0.25 µl of 10 g/l vanillic acid solution; V_1 = - 15 kV, V_2 = - 10 kV and V_d = - 2.1 kV; 250 shots averaged; displayed bin size = 250 nsec.

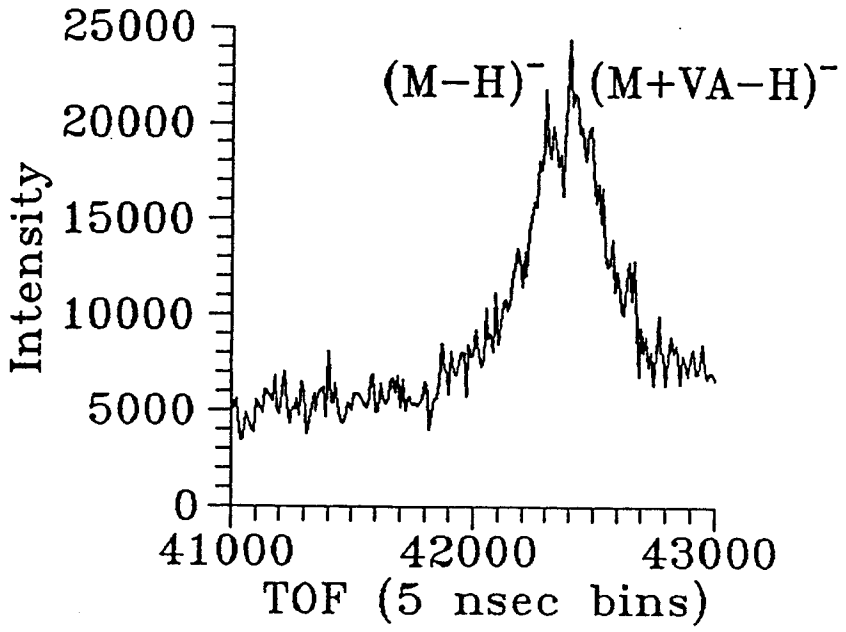

Fig. 5 The quasimolecular ion region of Fig.4. Conditions: the same as Fig. 4, except that the displayed bin size = 100 nsec.

Acknowledgements.

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3. R. C. Beavis and B. T. Chait, Rapid Commun. Mass Spectrom., in press.