MATRIX ASSISTED UV LASER DESORPTION OF BIOLOGICALLY INTERESTING MOLECULES.

Brian T. Chait and Ronald C. Beavis

The Rockefeller University, New York, NY, USA 10021.

Recent developments in the volatilization of large molecules using matrix assisted ultraviolet (UV) laser desorption have made it possible to produce intact quasimolecular ions from materials with molecular weights in excess of 100,000 using nicotinic acid matrices [1]. We have constructed a linear time-of-flight (TOF) mass spectrometer to be used for both basic research and rapid mass determinations of high mass molecules. A detailed description of the instrument is given in reference [2]. Our results demonstrate that mass resolutions of $m/\Delta m \cong 500$ (FWHM definition) can be obtained for small proteins ($m/z < 20,000$), with sensitivities better than 1 picomole and mass range in excess of 100,000 u. Our results also indicate that the actual dimensions of the laser focus are not a critical factor in producing high mass ions. Our irradiation area of 0.1 mm x 0.3 mm produced sufficient numbers of ions so that detector saturation (tandem multichannel plates, 25 mm dia.) was a significant problem.

A survey of possible matrix materials has shown that several different types of compounds produce very similar results to those obtained from the original matrix material 3-pyridinecarboxylic acid (nicotinic acid). Materials were selected for trial on the following criteria: 1) strong UV absorption at 266 nm (the wavelength of our frequency quadrupled Nd-YAG laser); 2) water solubility (so that they could be co-dissolved with proteins); and 3) volatility (volatile/sublimable solids were chosen). Four matrix materials (in addition to nicotinic acid) were found to be useful. These were : 2-pyrazinecarboxylic acid (pyrazinoic acid); 3-methoxy, 4-hydroxybenzoic acid (vanillic acid); thymine; and thiourea. Surprisingly, the nicotinic acid isomers picolinic acid and isonicotinic acid did not work well as matrices, even though their UV absorption properties were very similar to nicotinic acid. Presumably the detailed interaction of the matrix molecules with the protein molecules and with each other is changed by the changing the position of the carboxylic acid group with respect to the nitrogen in the pyridine ring. The much less volatile and relatively insoluble dicarboxylic acid analogues of nicotinic acid did not work as matrices. Analogues of thiourea that did not have strong UV absorptions at 266 nm,

urea and guanidine hydrochloride, were also ineffective as matrices.

Another important finding is that matrix assisted UV laser desorption produces both positive and negative quasimolecular ions, with the preferred intact species being singly charged. Figure 1 shows the complete positive and negative TOF mass spectra of insulin (MW = 5733.5 u). Both singly and doubly charged species are seen in both spectra, but the singly charged species is more abundant. This pattern has held true for all of the proteins and peptides so far examined (in excess of fifty different compounds).

Figure 2 is a detail of Fig. 1 (a), showing the positive, singly charged quasimolecular ion region. Fig. 2 clearly demonstrates the presence of several different adduct species. The most intense peak is the protonated insulin molecule ion. The second most intense peak is an adduct with a mass 80 u higher than the molecular weight. This ion can be explained by the addition of the matrix molecule's ring system (pyrazine, MW = 80 u) to the protein molecule. Pyrazine is an expected decarboxylation photochemical product formed by UV irradiation of the matrix (pyrazinoic acid, MW = 124 u). This type of adduct ion (i.e. the matrix molecule minus CO_2H) is a feature common to all of the acidic matrices used. It is most significantly a major contribution to peak broadening for high mass proteins, where the resolution is not sufficient to separate the protonated and matrix adduct ion peaks.

The sensitivity of the technique is typically 1 picomole or less. Figure 3 shows the positive ion mass spectrum of *E. coli* β-galactosidase (MW = 116,336) showing the singly, doubly and triply charged quasimolecular ion species, obtained from 0.7 picomoles of protein using pyrazinoic acid as the matrix. The area of the sample probe is 3 mm^2 and less than 5% of this area was irradiated to produce this spectrum. Total sample probe loadings of 0.1 picomoles of insulin produce spectra with the protonated molecular ion as the base peak. More than 20 peptides, proteins and lipoproteins were examined, and each one produced strong quasimolecular ion signals from ≅ 1 picomole of applied protein.

1. M. Karas and F. Hillenkamp, Anal. Chem. 60, 2299 (1988).
2. R.C. Beavis and B.T. Chait, Rapid Commun. Mass Spectrom. 3, 233 (1989).

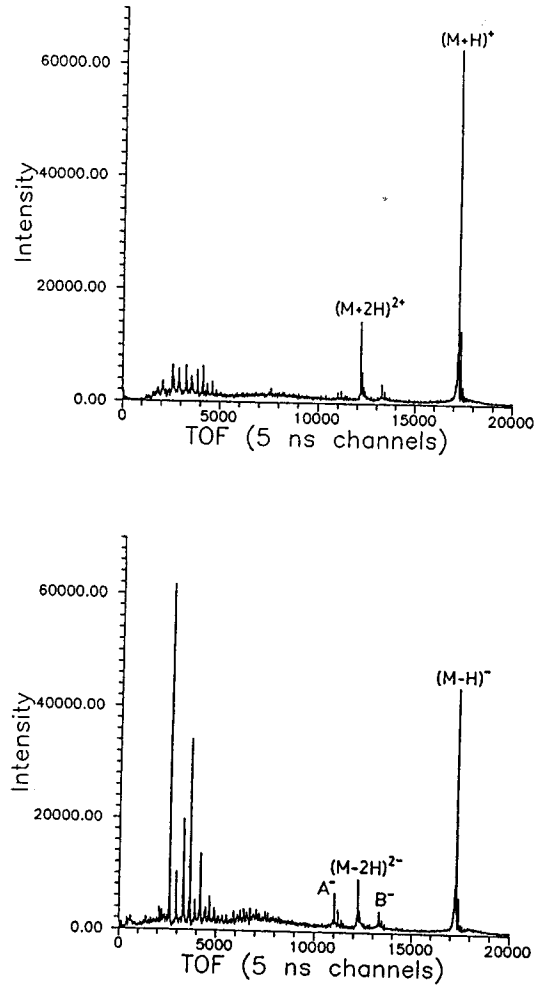

Fig. 1 The positive (a) and negative (b) LD TOF mass spectrum of insulin (MW = 5733.5). Conditions: 0.5 µl of protein/matrix solution loaded onto the probe (2 picomoles protein + 5 µg pyrazinoic acid); total acceleration voltage (w.r.t. the detector) = + 17 kV; 200 laser shots accumulated; data recorded in 5 ns channels and displayed in 10 ns bins

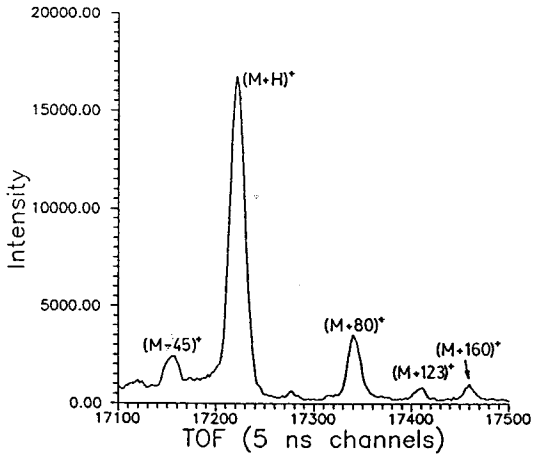

Fig. 2 The quasimolecular ion region of the LD TOF mass spectrum given in Fig. 1 (a). This spectrum shows ions caused by the addition of by-products from matrix photochemical reactions (see text).

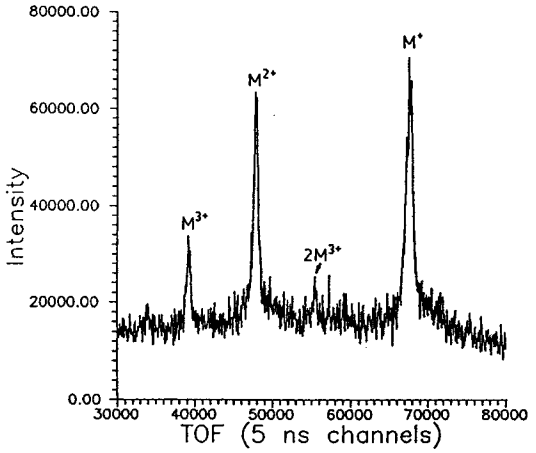

Fig. 3. The high mass portion of a LD TOF mass spectrum of *E. coli* β-galactosidase (MW = 116,336). Conditions: 0.5 µl of protein/matrix solution loaded onto the probe (0.7 picomoles protein + 5 µg pyrazinoic acid); total acceleration voltage (w.r.t. the detector) = + 22 kV; 200 shots accumulated; data recorded in 5 ns channels and displayed in 250 ns bins.