# Velocity distributions of intact high mass polypeptide molecule ions produced by matrix assisted laser desorption

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The velocity distributions of polypeptide molecule ions (molecular mass 1000–15600 u) produced by matrix assisted ultraviolet laser desorption were measured using a modified time-of-flight mass spectrometer. The data demonstrate that polypeptide molecule ions produced by matrix assisted laser desorption at the ion production threshold irradiance have similar velocity distributions, with an average velocity of approximately 750 m/s. This result has important implications for the design of mass spectrometers that use the effect to generate polypeptide molecule ions and for the theoretical treatment of the laser desorption process. A jet expansion model for the desorption of high mass polymers is proposed to explain the results.

## 1. Introduction

The discovery by Karas and Hillenkamp [1] that massive polypeptide molecules could be placed into the gas phase and ionized by laser irradiation in the presence of a large excess of a "matrix" (nicotinic acid) has extended the mass range and sensitivity of the mass spectrometric analysis of proteins. Subsequently, it has been discovered that these peptide ions could be generated using other types of matrix materials [2]. The effect has been observed using a variety of pulse lasers [3–5] and it has been used in the ion sources of different types of mass spectrometers [1,2,6–8]. This method of ionization has rapidly become an accepted part of mass spectrometric research.

An understanding of how such large polypeptide molecules can be volatilized with little or no fragmentation has not been so rapid. Karas, Hillenkamp and co-workers [1,9] have proposed a model in which the absorption of the laser light leads to the ejection of large particles of matrix material containing the analyte polypeptide. These large particles then lose all of their matrix molecules by evaporation, leaving the polypeptide in the gas phase. Vertes et al. [10,11] have proposed a model of homogeneous sample heating followed by bulk sample evaporation that can lead to intact, relatively cool polypeptide molecules. This model assumes an inefficient vibrational energy coupling between the laser-excited matrix molecules and the polypeptide, allowing the matrix molecules to become temporarily much hotter than the polypeptide.

There is a very limited amount of experimental data characterizing the phenomena associated with matrix assisted laser desorption. Experimental studies have attempted to determine the intrinsic velocity distributions of ions produced by the effect. Matrix ions [7] and those of the protein insulin [12,13] have been studied. The studies of insulin indirectly measure the velocity distribution of protein ions after they have been extracted by a high electric field ($(1-4)\times10^6$ V/m) at the sample surface in the ion source of a time-of-flight mass spectrometer. Using this type of ion source, the contributions to the final velocity distribution caused by the intrinsic velocity distribution of the ion production process cannot be readily separated from those caused by ion extraction effects.

This paper reports the velocity distribution of three different polypeptide molecule ions, without any complications caused by extraction fields. These measurements indicate that all polypeptide molecule ions produced by matrix assisted laser desorption at the ion production threshold irradiance have a similar velocity distribution, with an average velocity of

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750 m/s. This result has important implications for the design of mass spectrometers that use the effect to generate polypeptide molecule ions and for the theoretical treatment of matrix assisted laser desorption.

## 2. Experimental

The instrument used to measure the velocity distributions is shown in fig. 1. This instrument differs from a previous description [2,12] by the addition of two electrodes to create an einzel lens. The einzel lens was necessary to focus ions onto the detector at the ion acceleration voltage (15 kV) employed in this study. It was empirically determined that $V_l = 9.0$ kV was optimum for the acceleration voltage conditions used in the following experiments.

All polypeptide samples were prepared following a method previously described [14]. The sample loaded into the mass spectrometer consisted of 10 pmol of polypeptide mixed with 50 nmol of matrix (3,5-dimethoxy-4-hydroxycinnamic acid, sinapic acid).

In order to measure the velocity distribution of the ions produced by matrix assisted laser desorption, two experiments were performed. The first experiment measured the transit time of a particular ion species through the time-of-flight mass spectrometer with a high extraction field in the ion source. The second experiment measured the transit time of the same ion species, without any initial extraction field, that is with a field-free drift region prior to the high voltage acceleration field. These two measurements allowed the calculation of the ion velocity distribution intrinsic to the ion production/sublimation process by calculating the velocity of ions crossing the field-free region used in the second experiment.

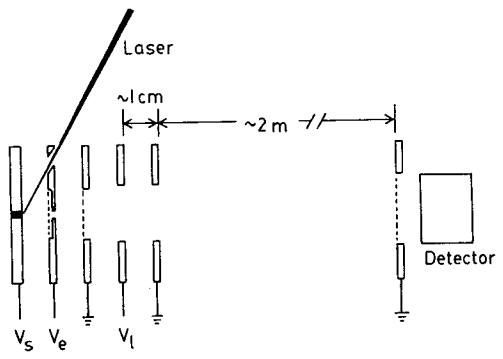


Fig. 1. The time-of-flight mass spectrometer used in the experiments.

The first experiment was carried out by operating the mass spectrometer in its normal mode, i.e. with a high voltage extraction field in the ion source. An electric field of $3.8 \times 10^6$ V/m was produced in the region between the first and second electrode (see fig. 1, $V_s = 15$ kV; $V_e = 0$ V). The laser (a Lumonics HY400 Nd:YAG, operated in the $Q$-switched mode at 2.5 Hz, frequency tripled to $\lambda = 354$ nm) was focused onto the sample using an $f = 30$ cm lens, resulting in a laser fluence at the sample of 10–20 mJ/cm$^2$. After each laser shot, the detector transient signal was recorded with a Lecroy TR8828D transient recorder (200 MHz). The results of up to two hundred consecutive laser shots were added together to improve ion counting statistics. The resulting time-of-flight spectrum accurately determined the transit time of the ion species of interest.

The second experiment was performed on the same sample. The ion source electrode voltages were changed so that the extraction field between the first and second electrode was zero ($V_s = V_e = 15$ kV), by attaching a conductor between these two electrodes. A time-of-flight spectrum was acquired under these conditions in the same manner as in the high-extraction-field case. The molecule ion signals were delayed (compared to the high-field case) by the time it took the ions to drift across the field free region between the two electrodes and exit the aperture in the second electrode ($r = 1.0$ mm), a distance of 7.3 mm. By taking the time difference between the molecule ion signal in the high-extraction-field case and the zero-extraction-field case, the velocity of the ions produced by the matrix assisted laser desorption effect was calculated.

## 3. Results

An example of the time-of-flight spectra produced by the high-extraction-field experiment is shown in fig. 2. The polypeptide used was bovine superoxide dismutase ($m = 15590$ u). The spectrum shows intense singly and doubly charged species, character-

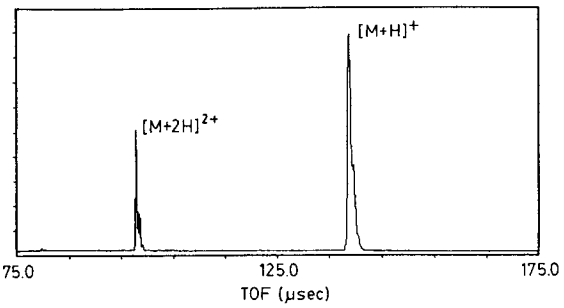


Fig. 2. The time-of-flight spectrum of bovine superoxide dismutase ($m=15590$ u), taken in the high-extraction-field mode ($V_s=15$ kV, $V_e=0$ V).

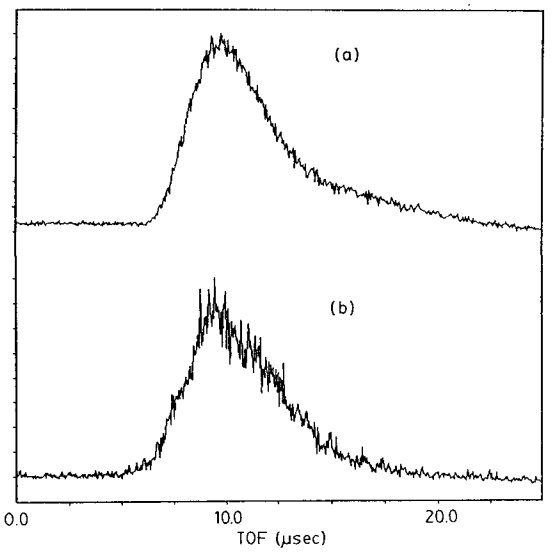


Fig. 3. The time-of-flight spectra of (a) porcine insulin and (b) superoxide dismutase taken in the zero-extraction-field mode ($V_s=V_e=15$ kV). See text for an explanation of the time scale.

istic of matrix assisted laser desorption [1,3,4]. Because of the limited dynamic range of the transient recorder (8 bits), it was not possible to record the signal corresponding to the matrix ions (sinapic acid, $m=224$ u) and the protein ions simultaneously using the same amplification. Therefore, the low mass region of fig. 2 has been omitted.

Fig. 3 shows two time-of-flight distributions obtained for polypeptides in the zero-extraction-field experiment, displayed with $t=0$ corresponding to the ion flight times measured in the high-extraction-field experiment.

Figs. 4a–4d are the velocity distributions (with the maximum intensity normalized to unity) calculated from flight time distributions such as those in fig. 3, for four different molecule ion species: sinapic acid (the matrix); $m=224$ u; angiotensin, $m=1030$ u; porcine insulin, $m=5730$ u; and bovine superoxide dismutase, $m=15590$ u. The most probable velocity for each distribution is indicated in the figure. A line was drawn through each distribution to guide the eye. The intensity is a direct measure of the probability of a particular species of ion having the corresponding velocity in the direction normal to the sample's surface. The speed resolution of the apparatus is approximately 1% (the error bars have been omitted for clarity). The distribution shown for sinapic acid is actually a composite of signals from two ion species that occur at equal intensities in the mass spectrum of sinapic acid: (1) the intact protonated molecule $[M+H]^+$; and (2) a fragment caused by the loss of water $[M-18]^+$. Because the difference between the transit times of these two species is small, they cannot be resolved in the zero-extraction-field experiment.

Fig. 5 shows translation energy distributions corresponding to the velocity distributions in fig. 4. It is important to note that the abscissa of fig. 5 is on a logarithmic scale and ranges over three orders of magnitude. The lines shown were drawn to guide the eye, not to imply a fit to theory.

## 4. Discussion

The data in figs. 4 and 5 indicate that matrix assisted laser desorption produces intact gas phase polypeptide molecule ions that all travel at the same velocity, regardless of their molecular mass. The translational energy distributions produced by this process are mass dependent and scale linearly with the molecular mass. There is a difference between the most probable velocities of polypeptide and matrix ions, however. The matrix ions move at an appreciably higher speed than the polypeptides.

The mass dependence of the product ion translational energy distribution has important consequences for the design of analytical time-of-flight mass spectrometers that utilize laser desorption ion sources. The mass resolution of a linear time-of-flight

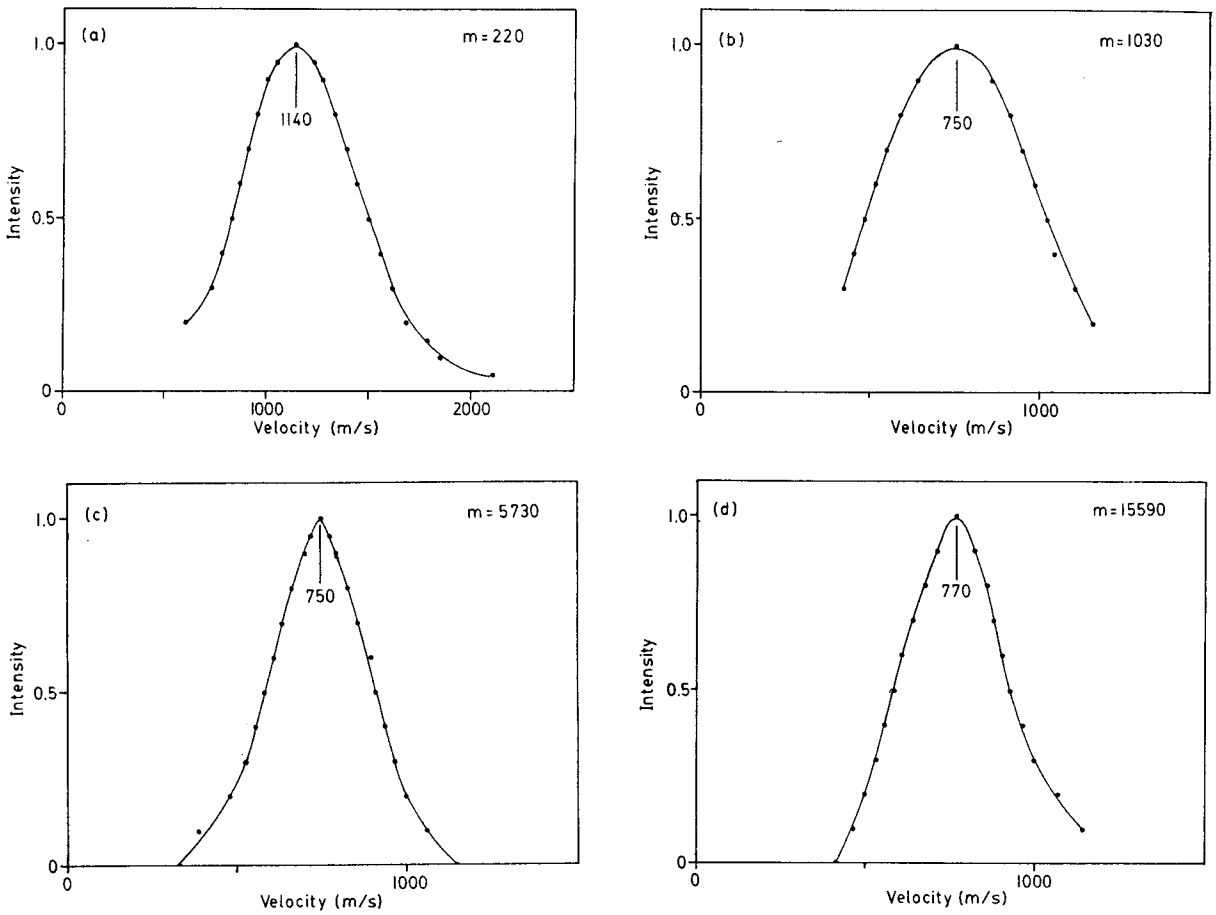


Fig. 4. The experimental velocity distributions of (a) sinapic acid, (b) angiotensin II, (c) porcine insulin, and (d) bovine superoxide dismutase.

mass spectrometer is inversely proportional to the absolute width of the kinetic energy distribution of an ion species. The data presented in fig. 5 indicate that the resolution of these instruments will decrease with increasing molecular mass. Time-of-flight mass spectrometers that use an electrostatic mirror to compensate for time spreads caused by the kinetic energy distribution of the ions (referred to as "reflectrons") only correct for time spreads produced in the field-free drift region of the mass spectrometer. Ion source transit time differences are not compensated for in this arrangement. When the intrinsic ion velocity is a significant fraction of the ion's average velocity in the ion source, significant uncompensated time spreads can be produced that limit the mass resolution of the instrument. This effect can be minimized by decreasing the amount of time that an ion spends in the ion source, either by increasing the ion source voltage or by decreasing the dimensions of the ion source in the direction of ion acceleration.

The type of instrument that may be most affected by the energy distributions in fig. 5 is the Fourier-transform ion-cyclotron resonance mass spectrometer [15]. In this device, ions that are produced by a desorption method are trapped by small electric potentials (typically $\approx 1$ V). The results shown in fig. 5 suggest that peptides with masses as low as 1000 u will be inefficiently trapped by such a low potential barrier. Larger peptides, i.e. those of the greatest interest to researchers currently, will not be trapped at

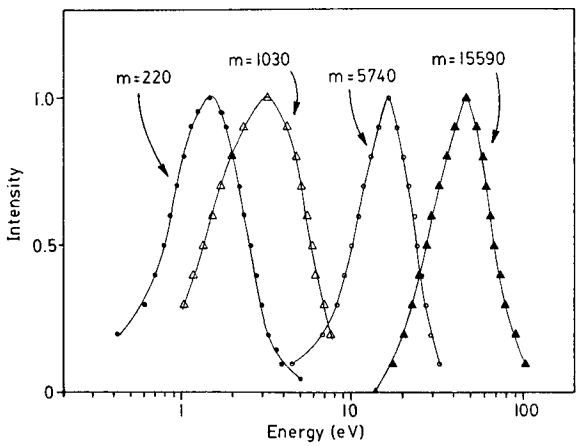


Fig. 5. The experimental energy distributions corresponding to the velocity distributions in fig. 4.

all. The observation that there appears to be an upper mass limit for biopolymers produced by matrix assisted laser desorption in such instruments [8] may be caused by this phenomenon. Increasing the ion trapping potential should have the effect of increasing the accessible molecular mass range.

The velocity distributions shown in fig. 4 eliminate from consideration any model that would give rise to a Boltzmann distribution of product molecule velocities. Purely Boltzmann velocity distributions have been observed in some experiments involving pulsed UV laser desorption of polymer fragment ions [16]. The distributions shown in fig. 5 demonstrate that a thermal equilibrium of translational energies has not been established between the matrix and polypeptide molecules.

One reasonable explanation for the observed velocity distributions is a model based on the formation of a supersonic jet expansion produced by laser irradiation. This type of model has been proposed for the laser ablation of polymers under certain experimental conditions [17–19], the ablation of large molecules in water ice by rapid asymmetric laser heating [20], and matrix assisted laser desorption [12]. In this model, a layer of the matrix is excited by the laser light, causing a rapid phase transition of the matrix molecules from a solid to a high-pressure fluid. This fluid is then free to expand adiabatically into the vacuum, forming a supersonic jet, which carries large molecules contained within the expanding jet into the gas phase. The large molecules would be accelerated by the expanding matrix gas, resulting in a uniform velocity distribution for all molecules entrained in the jet.

Such a hypothesis does not explain the difference in velocity between the matrix ions and the polypeptide ions. This difference can be explained by "straggling" of the large molecule ions, i.e. they do not achieve the same velocity as the matrix ions because of their greater mass and different collision cross sections compared to the matrix molecules. Linear polymer molecules, e.g. peptides, are unusual in that the ratio of the gas phase geometrical cross section of a molecule to its mass is, at least to first order, independent of the length of the polymer chain. Each additional polymer unit added to a molecule will increase the mass and geometrical cross section of the molecule in a linear fashion, leading to the independence of the cross section to mass ratio. Therefore, it is expected that the velocity distributions of all polypeptides driven off a surface by an adiabatic, supersonic expansion should be the same.

The complicated nature of the interaction of a jet with flexible, linear molecules make this hypothesis difficult to model. Long linear molecules can adopt orientations that make their collision cross sections in the direction of fluid flow very small compared to their overall area. A simple physical model of this process is a ribbon of paper being blown by a wind. The ribbon, when blown by a strong wind, spends most of its time oriented so that its overall cross section in the direction of air flow is minimized. The ribbon is blown along by the wind, but at a lower velocity than the average velocity of the fluid. Because polypeptide molecules are topologically similar, they may all behave in a similar manner when blown off a surface by a supersonic jet.

## 5. Conclusions

The data presented in this paper demonstrate that for the matrix assisted laser desorption of polypeptides, the intrinsic velocity distribution of ejected biopolymer ions is independent of their molecular mass. The most probable value of the kinetic energy for these ions scales linearly with mass leading to rel-

atively large product ion kinetic energy for high mass molecules.

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