Investigations of Matrix Isolated, (UV) Laser Induced Polymer Sublimation Using a Time-of-Flight Mass Spectrometer

R.C. Beavis and B.T. Chait

Department of Mass Spectrometry
and Gas Phase Ion Chemistry
The Rockefeller University
1230 York Avenue
New York, NY 10021

Introduction

The matrix isolated, laser induced polymer sublimation (also referred to as matrix assisted laser desorption) process for the production of gas phase protein ions was originally described phenomenologically by Karas and Hillenkamp [1]. A macromolecule of interest, *e.g.* a protein, was dissolved in a solution containing a large molar excess of nicotinic acid. This solution was dried to form a deposit on a metal substrate. The substrate was then placed into the ion source of a time-of-flight mass spectrometer and irradiated by a pulsed UV laser. The laser light was absorbed by the nicotinic acid "matrix" and then, by some process involving ablation of the matrix, protein molecules were expelled from the surface with one to three positive charges per peptide chain. Subsequent studies have demonstrated that a range of matrix molecules can produce the macromolecule sublimation effect [2], wavelengths other than 266 nm can be employed [3] and that the originally broad peaks demonstrated by Karas and Hillenkamp were caused by photochemically generated reactions that resulted in the addition of a variable number of matrix molecules to the protein [4]. Both positive and negative ions have been observed from this process [3,5]. Recently, it has been demonstrated that infra-red laser wavelengths can be used to produce a similar effect [6].

In this paper we will describe a series of observations made in our laboratory concerning the laser induced polymer sublimation process, using (*trans*)3,5-dimethoxy-4-hydroxy-cinnamic acid [530-59-6], a naturally occurring material, which is commonly referred to as sinapic acid (from the Latin *sinapis*, meaning mustard), sinapinic acid and 5-methoxy-ferulic acid. This particular matrix was chosen because it has proven to be the most reliable and generally useful of the over one hundred matrix materials tested in our laboratory [7,8]. We will also describe an ion current detector that

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has proved to be useful in our instrument, consisting of a microchannel plate followed by a conventional gridded, discrete dynode electron multiplier.

Instrumental

Mass Spectrometer

The instrument used for all of the measurements described has been discussed in detail previously [2,7]. The frequency tripled output of our neodymium:yttrium aluminium garnet laser (λ = 354.5 nm) was used throughout. The laser irradiance employed to obtain the spectra shown was approximately 1 MW/cm^2.

Ion Detection

Figure 1 shows the geometry of two types of ion current detectors that have been tested in the Rockefeller instrument. The first type of detector is a conventional multichannel plate array (chevron plate orientation), with which our initial observation were made [2-4]. This detector suffers from a limit in the total ion current that can be measured from one laser pulse.

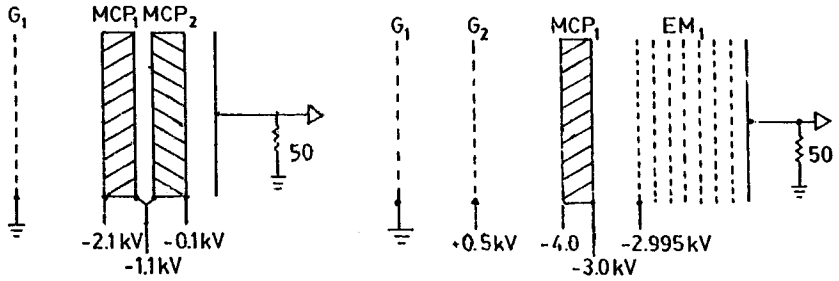

Figure 1 Two different detector arrays. MCP - multichannel plate electron multiplier; EM - discrete dynode gridded electron multiplier; G - 95% transmission electroformed nickel grid.

Because any channel of the multiplier is effectively deadened by a single ion cascade event for a time long compared to an ion's time-of-flight, each channel can only be used once during the recording of the transient current produced by a laser pulse. This problem is particularly acute in the second multichannel plate, which can have more than one channel affected by an individual incoming ion. This deadening of channels results in a subtle decrease in the effective gain of the detector for ions with long flight times.

In order to minimize this problem, the second detector was constructed and tested. In this detector a Hamamatsu gridded electron multiplier (R2362) replaced the second multichannel plate of the first array. The wide dynamic range of EM_1 has allowed the use of much higher ion currents, without detector paralysis. A detector consisting of the gridded electron

multiplier gave unsatisfactory performance because the total gain (or secondary electron collection efficiency) was sufficiently low to make the detector insensitive to ions at the laser irradiance threshold for ion production. Therefore, higher laser irradiances were necessary to produce observable signals, resulting in signal broadening.

One feature of the hybrid detector shown in Figure 1 is a sensitivity to ion and electron induced feedback between the various elements. The voltage on grid G_2 and the small voltage between the back face of MCP_1 and the first grid of EM_1 were both necessary to eliminate unwanted peak tailing caused by these feedback effects.

Materials

The matrix material used ((*trans*)-3,5-dimethoxy-4-hydroxycinnamic acid) was obtained from Aldrich Chemical Co. (catalogue # D13,460-0, lot # 07201TV). The proteins (bovine pancreatic insulin and horse muscle skeletal apomyoglobin) were obtained from Sigma Chemical Co..

Results and Discussion

Ion Production

Experiments were performed to determine the relative contributions of production/extraction times and initial axial energy distributions to the observed peak width of singly charged protein ion signals produced by matrix assisted laser induced sublimation. Two small proteins (bovine pancreatic insulin and horse skeletal muscle apomyoglobin) were examined. These experiments were performed by varying the length of the field-free drift region of the linear time-of-flight instrument used. The normal length of the drift region is 200 cm. Both a longer (400 cm) and shorter (40 cm) drift region were used. When the 400 cm drift region was employed, the initial axial kinetic energy spread of the ions produced by the laser ablation event is the dominant cause of ion peak widths. The maximum initial kinetic energy spread calculated from mass spectra obtained with the long tube was 40 - 50 eV. In the alternative case, the 40 cm drift length, the width of the ion signal is dominated by the initial ion production time. This measurement suggests a typical ion formation and extraction time of approximately 50 nanoseconds. Each of these measurements assumes that the width of the ion signal was caused by instrumental effects only and were not caused by unresolved signals due to oxidation of the protein or cation ion adduct formation. In the case of bovine insulin, sufficient instrumental resolution was obtained to directly rule out alkali metal attachments, but the possibility still exists that photochemical changes induced by the laser pulse could account for part of the peak width.

Both of these measurements have significant implications for the ion formation process. The rather large initial kinetic energy spread indicates that there is either a large transfer of momentum from the matrix to the protein molecule without a very large transfer of vibrational energy or the initial ion extraction process occurs over an extended volume of space. The

observation of a production time that is significantly longer than the laser pulse (10 nanoseconds) suggests that either ions are produced from the surface for much longer than the laser is on, or that the ions produced by the laser are not immediately accelerated from the surface, but encounter some delay time before they are free to accelerate.

Metastable ion decays

The unimolecular decay of ions in the field-free drift region of time-of-flight mass spectrometers has been frequently observed [9,10] and is generally referred to as a metastable ion decay. Such decays can be readily observed using energy selection grids at the end of the field free region. A common scheme for such grids is shown in Figure 2. If an ion undergoes a

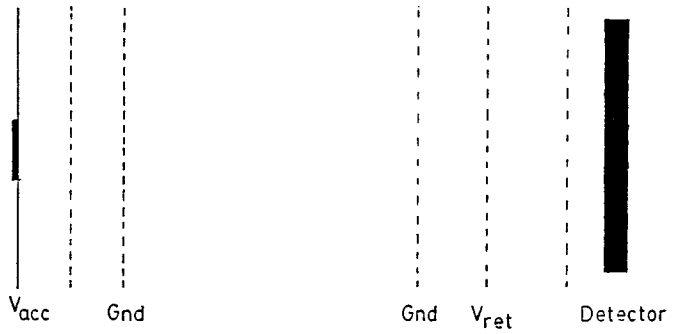

Figure 2 The acceleration/deceleration scheme used to detect metastable decays in the field free region of the mass spectrometer.

metastable decay after it has been fully accelerated in the ion source, the kinetic energy of each of the two daughter fragments (usually one charged and one neutral) must be smaller than the initial kinetic energy of the parent ion. The kinetic energy of a daughter is directly proportional to the fraction of the parent ion mass carried by the fragment. Therefore, a grid set at a potential intermediate between the full acceleration potential and ground will have three effects on the signals observed. It will: 1) increase the flight time of the intact parent ions; 2) increase the flight time of the charged daughters and will reflect daughters with masses such that:

$$m(daughter)/m(parent) > V_{ret}/V_{acc};$$

(see Figure 2 for definitions of V_{ret} and V_{acc}) and 3) have no effect on the flight time of the neutral daughters. Therefore, if a non-zero V_{ret} is used, peak splitting of an ion signal will be observed if metastable decay has taken place. A signal corresponding to the neutral

fragment of the decay will remain at the centroid of the original signal, because it is unaffected by the electric field caused by V_{ret}. A second family of peaks should occur at longer times, corresponding to slowed charged fragments of the decay. When $V_{ret} > V_{acc}$, then only the signal corresponding to the neutral fragment (ie. the signal located at the same centroid as the ion signal with $V_{ret} = 0$) will remain: a clear signature of a metastable decay.

Figure 3 shows the molecule ion region of the mass spectra obtained from porcine insulin using several different values of V_{ret}. These spectra and others obtained at a variety of combinations of different V_{acc} and V_{ret} voltages indicate that there was a significant amount of metastable decay of insulin ions in flight. The presence of an observable signal with $V_{ret} > 0$ at the same time-of-flight as with $V_{ret} = 0$ demonstrates that there must be metastable decay of ions into an ion/neutral daughter pair. The fact that

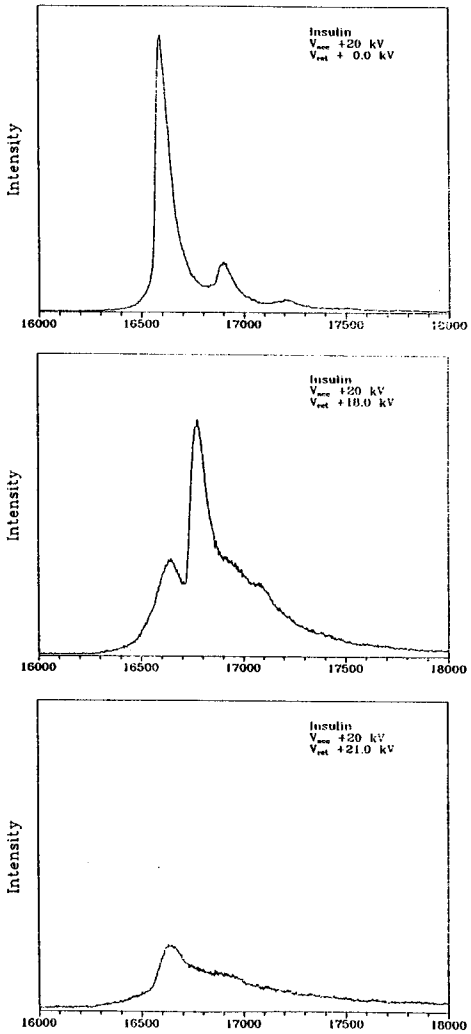

Figure 3 The molecular ion signal of porcine insulin, produced at different values for V_{ret}.

the ion formed by this decay has nearly the full kinetic energy of the parent indicates that the decay is mainly by the loss of a relatively small neutral molecule. This type of decay may be difficult to observe using an ion mirror energy correction apparatus, because it requires relatively high mass resolution.

The observation of metastable decay of ions produced by matrix isolated, laser induced polymer sublimation also has implications for the ablation/sublimation mechanism. Metastable decay indicates that the ion produced must be excited to some relatively high temperature during the process involved in the ablation, ionization and extraction of the ion from the surface. The laser wavelength used in these experiments was 354.5 nm and was therefore not absorbed by any of the chromaphores in the molecule itself. The excitation energy present in the protein ion must have come from the excited matrix molecules that do absorb at this wavelength or from collisional excitation during acceleration. This excitation suggests that the matrix molecules can transfer quite a bit of energy to the protein molecule during the ablation event, mainly by solid state vibrational energy transfers and by gas phase collisions after sublimation.

A Model for Laser Induced Polymer Sublimation

Before beginning to discuss the laser induced polymer sublimation process, a few words should be said about nomenclature. The original name for this process was laser desorption. Desorption is the opposite of adsorption, ie. the removal of a molecule adsorbed to a surface. Because of the volume excitation produced by laser irradiation of a relatively volatile matrix it is inappropriate for the phenomenon discussed above. A word that more clearly indicates the physical phenomenon occurring is sublimation, defined as the process of changing from a solid to a vapor without an intervening liquid state. Ablation, ie. the removal of material from a surface without reference to the mechanism of that removal or the final physical state of the removed material, is a useful term in describing the results of the laser's interaction with the matrix, but is sufficiently general to fit any model proposed. Spallation, ie. the removal of material from a surface by the ejection of solid fragments, may be occurring, but it is difficult to imagine this process producing the effects observed. Therefore, the authors have chosen to use "sublimation" rather than "desorption" to describe the process.

One of the major events that must occur for the duration of the laser pulse is the removal of material from the surface, ie. ablation. This ablation can take two forms: 1) the removal of large pieces of solid (spallation); and 2) the rapid sublimation of a small volume of matrix. In order for protein molecules to be observed as gas phase ions, they cannot have left the surface as part of a large piece of cold, solid material. Therefore the mechanism that is producing gas phase ions is a rapid phase change of matrix from a solid to a gas. If this process happens in a sufficiently short time, the gas formed will initially be a very dense, thick gas that will expand supersonically into the vacuum. This expanding gas will have sufficient density to carry the protein molecules embedded in it away from the surface, taking them into the gas phase. Such a process will result in protein

molecules being expelled from the surface with some characteristic velocity that is determined by the gas expansion and not by the molecular mass of the protein.

A similar model to this has been proposed by Nelson *et al* [11] to explain their observations of intact biopolymers ablated from thin (10 μm) frozen water layers, using a visible laser at irradiances one hundred times higher than those used for absorptive matrices such as sinapinic acid. In this case, the laser radiation is absorbed by the substrate, resulting in rapid local heating and spallation. Nelson, *et al* use the term volatilization to describe the formation of gas phase biopolymers, rather than sublimation. This usage may be more accurate in the case they examine, because of the possibility of the formation of a liquid phase intermediate in the solid to gas phase change of a water matrix.

The mechanism for proton transfer during the ablation event cannot be unequivocal assigned on the basis of experimental data to date, although some type of chemical ionization mechanism is very likely. The observation of strong singly (or doubly) protonated (positive) or deprotonated (negative) ion signals suggests that the charging mechanism is not be very efficient. The fact that different matrices produce different intensity ratios of singly to doubly charge protonated ions suggests that the detailed interaction of the matrix with the protein may be important. The observation that the charging pattern of the protein is independent on its primary sequence, ie. the number of basic or acidic amino acid residues in a protein do not systematically affect the singly-to-doubly protonated ratio, indicates that the extent of protonation (or deprotonation) is not dependent on the same acid/base chemistry as is observed in aqueous solutions of the proteins.

The final step in the laser induced sublimation and ion formation process is ion extraction. If the ions are initially formed in a thick gas of matrix, then the ions will not be extracted by the electric field until the gas has expanded sufficiently to allow relatively collision-free acceleration of the ions. Because there is very little direct experimental evidence concerning the exact nature of the plume of material ejected, e.g. the ratio of material spallated to sublimed matrix, it is not meaningful to make detailed calculations of the gas density at any given time after the laser pulse. Also, the exact meaning of the parameter "mean free path", which is frequently used in discussing ion extraction from a gas, is not clear. This difficulty arises because of the extended geometry of a polypeptide chain. Different polypeptide conformations, such as a tangled ball versus and long straight chain, have very different inter-molecular collision cross-sections. On the basis of a very simple calculation (assuming complete sublimation and a 10^6 fold expansion of a 10 nm thick layer of matrix) , a minimum distance that a gas plume evolved from the solid matrix would have to expand to allow some extraction is approximately 100 microns. This expansion distance indicates that there should be a kinetic energy defect in the ions produced caused by the lack of acceleration during this distance. Such a model would suggest that the best energy resolution (or mass resolution) should be obtained near threshold irradiance when the smallest volume possible is being sublimed. At higher irradiances, the large volume of gas produced should change the distance from the surface at which ions are extracted, resulting in peak broadening and time-of-flight shifts to longer times.

Acknowledgements

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