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A. VERTES✉
G. LUO
L. YE
Y. CHEN
I. MARGINEAN

Laser Pulse Length Dependence of Internal Energy Transfer in UV-MALDI-MS

Department of Chemistry, George Washington University, Washington, DC 20052, USA

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ABSTRACT Recent internal energy (IE) measurements for various analytes in matrix-assisted laser desorption ionization (MALDI) have indicated that the amount of IE transferred to analytes not only depends on the matrix but also on the nature of the analyte. Common matrixes, such as α-cyano-4-hydroxycinnamic acid (CHCA), 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA), and 2,5-dihydroxybenzoic acid (DHB), had been characterized as "cold" or "hot" according to the IEs of analyte ions produced in the corresponding MALDI plume. In this contribution, we present evidence that IE transfer in MALDI depends on the matrix, analyte, as well as on the laser pulse properties. A substituted benzylpyridinium salt as a thermometer molecule (TM) was investigated in CHCA, SA, and DHB matrixes. A nitrogen laser (4 ns pulse length) and a mode locked frequency tripled Nd : YAG laser (22 ps pulse length) were used as excitation sources at various fluences. Survival yields (SYs) of the analyte molecular ions were extracted from the spectra and the corresponding IEs were obtained from Rice–Ramsperger–Kassel–Marcus (RRKM) theory. The SYs indicate that the IEs of analyte ions in MALDI are analyte, matrix, and laser source dependent. The ion generation threshold fluences follow the same order for both lasers: CHCA < SA < DHB, but for the analyte the mode locked $3 \times \omega$ Nd : YAG laser source requires a higher threshold fluence than the nitrogen laser. Despite the higher fluence, the SYs are generally higher (the corresponding IEs are lower) for the $3 \times \omega$ Nd : YAG laser than for the nitrogen laser. The SYs of the TM molecular ions decrease with an increase of fluence for both the ns laser and the ps laser.

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1 Introduction

One of the most significant factors that influence the fragmentation of ions in mass spectrometry is internal energy. The so-called soft ionization methods deposit relatively low amounts of energy into the analyte molecule, thereby reducing the degree of fragmentation. While this effect is beneficial for molecular weight determination, the formation of structurally relevant fragments is necessary for the elucidation of molecular structure. Thus, there is a need to determine the factors that influence the internal energy of ions produced by various soft ionization methods, with the ultimate goal of controlling the degree of fragmentation (e.g., [1–3]). Internal energy distributions for analyte ions generated by electrospray ionization have recently been reported in various publications (e.g., [4, 5]).

Due to the importance of matrix-assisted laser desorption ionization (MALDI) in biomedical analysis, several groups have started to explore the internal energy content of ions [6–9] and neutrals [10] generated by this process. Although most of the recent work is still in the form of conference proceedings [11–14], there are some relevant observations we can already summarize.

There are three major factors responsible for the internal energy content of analyte ions in MALDI, the applied laser fluence, the nature of the matrix and the type of the analyte molecule. Qualitative experiments based on collision-induced dissociation of peptide ions in a TOF/TOF system indicate that α-cyano-4-hydroxycinnamic acid (CHCA) as a "hot" matrix leads to abundant analyte fragmentation whereas 2,5-dihydroxybenzoic acid (DHB) as a "cold" matrix results in fewer or no fragment ions [7]. Similarly, for the deprotonated negative ions of the dinucleotide (AG) analyte there is a strong correlation between fragmentation and the gas-phase basicity of the deprotonated matrix anion [6]. Both of these experiments rely on proton transfer reactions between the primary matrix ions and the analyte molecule, showing that the internal energy of analyte ions depends on the exothermicity of the proton transfer reaction.

In the case of preformed analyte ions, such as benzyl-substituted benzylpyridinium salts, no such reaction is necessary for analyte ion detection and the internal energy measured in different matrixes shows the opposite trend, i.e., CHCA, 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA) and DHB as "cold", "intermediate", and "hot", respectively. Looking at the average internal energy values found for the different matrixes, it is apparent that with ns laser excitation CHCA imparts the least amount of energy (3.69 ± 0.21 eV) on these analytes followed by SA (4.04 ± 0.27 eV) and DHB (4.30 ± 0.29 eV) [8]. Our experiments with ps laser excitation presented here and with other types of analytes further corroborate the results above [12].

✉ Fax: +1-202/994-5873, E-mail: vertes@gwu.edu

The energy transfer between matrix and analyte in non-reactive collisions has also been the subject of theoretical investigations. Both kinetic [15, 16] and molecular dynamics modeling [17, 18] indicate the existence of an energy transfer bottleneck between matrix and analyte. It is, however, the presence of reactive collisions through charge transfer reactions that seems to be responsible for the formation of analyte ions from oligonucleotides [19, 20] and from some peptides [21].

The internal energy content of the ions and neutrals is also crucial in the pulsed laser deposition (PLD) [22] of organic molecules and in the matrix-assisted pulsed laser evaporation (MAPLE) of synthetic and biopolymers [23]. As expected, the presence of the matrix results in lower or no degradation of the deposited polymer material indicating lower internal energy content. Interestingly, resonant infrared radiation with no matrix present produces chemically intact deposits whereas ultraviolet radiation yields layers of modified composition. These observations also point to the important role of internal energy in PLD and MAPLE experiments.

Survival yield measurements on various peptides and other thermometer molecules (TM) indicate a strong correlation between ionization mechanisms and internal energy. Additional data is needed to uncover the relative contribution of matrix properties (optical and thermal) and the thermodynamics of the charge transfer reaction between matrix and analyte.

Recently we reported IE values measured for a set of TMs in MALDI [8]. One of the main conclusions of the study was that the ionization process is not only matrix, but also analyte dependent. To further explore the energy transfer processes in MALDI, we describe the effect of dramatically changing the pumping rate by using a significantly shorter laser pulse.

2 Experimental

The methods, instrumentation, and materials used in this study have been discussed previously [8]. Here we mainly focus on the differences in the experiments described in this paper.

The 3-methoxy-benzylpyridinium salt (3MO-BP) was used as a MALDI analyte in three different matrixes: CHCA, SA, and DHB. A nitrogen laser (337 nm, 4 ns pulse length) and a mode locked $3 \times \omega$ Nd : YAG laser (355 nm, 22 ps pulse length) were used at fluences slightly above the MALDI ion generation threshold. The analyte ion current peaks corresponding to the molecular and fragment ions were integrated in time and the survival yields (SYs) were calculated as $SY = \Sigma I_M/(\Sigma I_M + \Sigma I_F)$, where I_M and I_F are the abundances of the molecular ion and the fragment ions, respectively. The SY values were converted to experimental rate coefficients for the unimolecular decomposition of the TMs, $k_{exp}(E)$.

Theoretical rate coefficients for the unimolecular decomposition reaction were calculated using RRKM theory [24] for different internal energies. The critical energies needed in these calculations were obtained from literature [4, 25], while the vibrational frequencies were calculated at AM1 level, using PC Spartan, version 02 (Wavefunction, Irvine, CA). The internal energies were obtained by projecting the experimental rate coefficients on the RRKM curve.

3 Results and discussion

The SYs in the three studied matrixes for nitrogen and mode locked $3 \times \omega$ Nd : YAG laser excitation were measured. Representative mass spectra obtained by the two different lasers from DHB matrix are shown in Fig. 1. The enhanced molecular ion (M^+) yield and the lower yield of fragment ions (F^+) with the ps laser are clearly visible. In the case of the ns laser, the matrix suppression effect is also observed.

Compared to the ns case, with the ps pulses significantly higher laser fluence is necessary for the desorption/ionization of the analyte (Fig. 2). The relative excess of analyte ion threshold fluences with the two lasers, $100 \times [F_{thr}(ps)/F_{thr}(ns) - 1]$, are 68%, 61%, and 145% for CHCA, SA, and DHB, respectively. This observation prompts a very interesting question. Why do we need 61% to 145% more photons (or energy) to generate analyte ions when the optical pumping rate is increased by a factor of ~ 200? A possible answer can

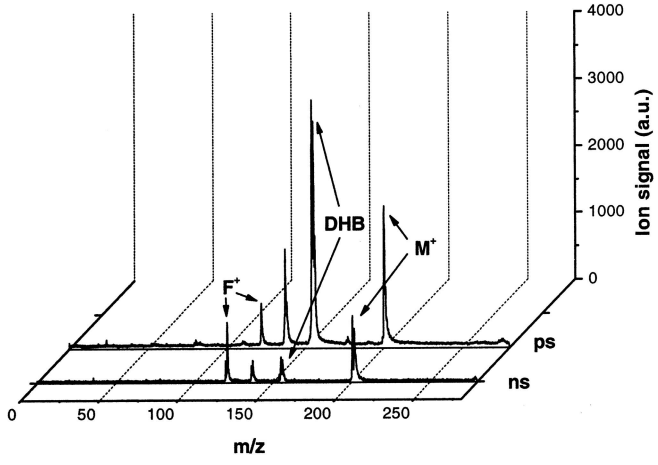

FIGURE 1 Comparison of MALDI mass spectra of 3MO-BP from DHB matrix produced by ns and ps laser

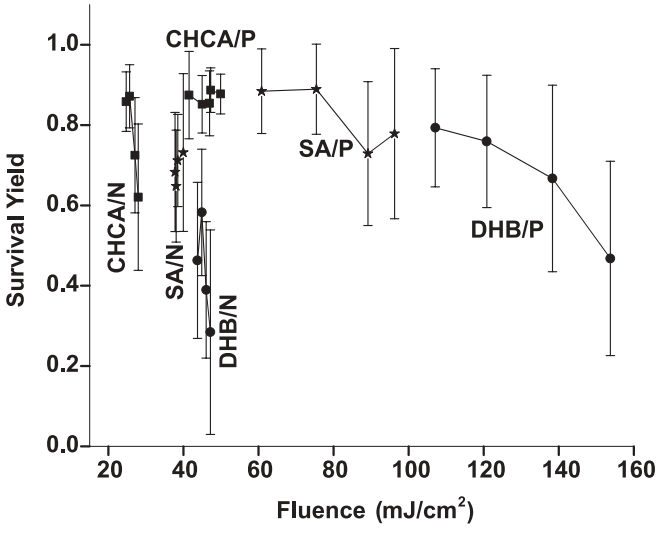

FIGURE 2 Survival yield of 3MO-BP molecular ions desorbed from CHCA (■), SA (⋆), and DHB (•) matrixes with ns and ps lasers. The labels indicate the matrix and the laser used, e.g., CHCA/N stands for CHCA matrix with ns laser

be formulated based on the well-established phenomenon of changing from ladder switching to ladder climbing in case of the shorter pulses [26].

Even at these higher fluences, the SY values are generally higher for the ps laser. For both lasers, the SYs of the molecular ions decrease with increasing laser fluence indicating that at higher fluences larger amounts of energy is transferred and deposited as internal energy in the TM ions. The SYs measured with ps laser excitation, however, decline more slowly than SYs observed with the ns laser. In the three different matrixes the 3MO-BP molecular ions exhibit descending SYs in the order CHCA>SA>DHB, implying that internal energies gained by this analyte follow the reverse order.

The internal energy values for 3MO-BP ions were obtained from the SYs using the RRKM theory (Fig. 3). Although the differences between the values are compressed due to the logarithmic nature of the internal energy vs. SY relationship, the values for the ps laser are slightly lower than the corresponding internal energies for the ns laser. Increasing laser fluence results in an increase of the analyte internal energy, although less so for the ps laser than for ns excitation. For both laser sources, the internal energy of analyte ions in the three different matrixes followed the CHCA < SA < DHB.

In the literature, qualitative estimates are available for the internal energy of peptide ions desorbed with ns laser from the same three matrixes [7]. Those results indicate the opposite trend. In an earlier report, we have suggested a resolution to this apparent contradiction by pointing to the different nature of ion formation for peptides and for TMs [8]. Most peptide ions in MALDI are the result of proton transfer reaction from the matrix, whereas the TMs used in our studies (organic salts) are assumed to be present in the solid phase as preformed ions. If we accept this premise, the TMs only have to be released from the solid phase by the phase transition of the matrix. Indeed, the laser fluence needed for the onset of phase transition seems to follow the same order as the fluence needed for the observation of TM ions.

To explain the difference in internal energy transfer between the ns and ps excitation, one needs to consider the vast difference in pumping rate. While the ns excitation results in ladder switching and matrix fragmentation, the ps laser primarily promotes ladder climbing and higher population of the electronically excited states for the matrix. The late onset of phase transition in these highly excited systems may be an indication of reduced exciton-phonon coupling in these systems.

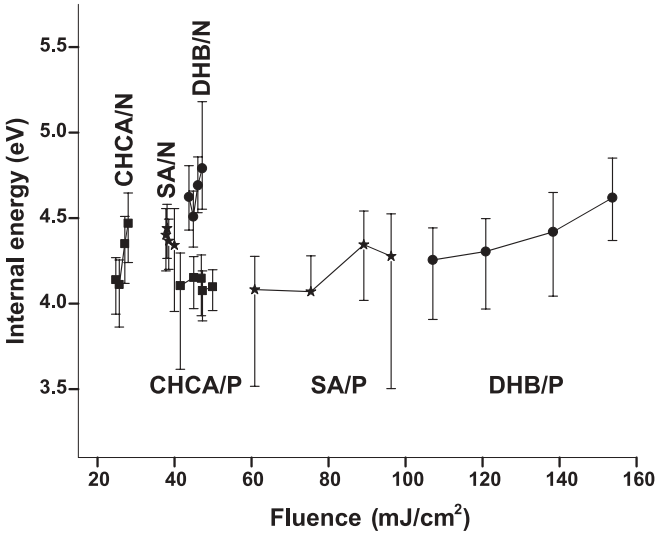

FIGURE 3 Internal energy of 3MO-BP as a function of the laser fluence desorbed with ns and ps lasers from three different matrixes. Same labels as per Fig. 2

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