REVIEW

Rapid Surface Heating Promotes Laser Desorption Ionization of Thermally Labile Molecules from Surfaces

Sang Yun Han*

Department of Nanochemistry, Gachon University, Gyeonggi 461-701, Republic of Korea

Abstract : In recent years, matrix-free laser desorption ionization (LDI) for mass spectrometry of thermally labile molecules has been an important research subject in the pursuit of new ionization methods to serve as alternatives to the conventional matrix-assisted laser desorption ionization (MALDI) method. While many recent studies have reported successful LDI of thermally labile molecules from various surfaces, mostly from surfaces with nanostructures, understanding of what drives the LDI process still requires further study. This article briefly reviews the thermal aspects involved in the LDI mechanism, which can be characterized as rapid surface heating. The thermal mechanism was supported by observed LDI and postsource decay (PSD) of peptide ions produced from flat surfaces with special thermal properties including amorphous Si (a-Si) and tungsten silicide (WSi_x). In addition, the concept of rapid surface heating further suggests a practical strategy for the preparation of LDI sample plates, which allows us to choose various surface materials including crystalline Si (c-Si) and Au tailorable to specific applications.

Keywords : Laser desorption ionization (LDI), Rapid surface heating, Thermal mechanism, Matrix-assisted laser desorption ionization (MALDI), Surface-assisted laser desorption ionization (SALDI)

Introduction

Ionization is one of the crucial elements in mass spectrometry techniques. As evidenced by the paradigm shift brought by the advent of ESI (electrospray ionization)[1] and MALDI (matrix-assisted laser desorption/ionization)[2-4] methods, ionization can greatly extend the scope of mass spectrometry. Before the introduction of ionization methods, mass spectrometry was only a tool for mesuring the molecular weights and ion chemistry of small molecules (< 500 Da). However, methods enabling the generation of intact ions with large molecular weights (> 100,000 Da), such as peptides and proteins, have made mass spectrometry an essential and state-of-the-art tool for biomolecular research. Due to the importance, the development of new ionization methods has become an important research area in the field of mass spectrometry,

and this has led to the introduction of ionization techniques that are suitable for many applications, including APPI (atmospheric pressure photoionization),[5] APCI (atmospheric pressure chemical ionization),[6] and DART (direct analysis in real time),[7] DESI (desorption electrospray ionization)[8].

MALDI is a representative laser-induced desorption ionization technique. Its advantages include high sensitivity consuming only sub-femtomole analytes and rapid processing, thus allowing high-throughput characterization of large biomolecules.[2-4] In addition, its capability of localizing biomolecules on specimen surfaces allows mass spectrometric imaging, offering a new opportunity for bioclinical diagnosis.[9] In general, MALDI utilizes excess matrix that is co-crystalized with analytes.[4] This leads to certain limitations to its application. For example, interference arising from matrix peaks in the low mass region of mass spectra hampers its application to small molecules. In addition, the presence of tiny, irregular sample-matrix crystals greatly affects the reproducibility of ionization, limiting the use of MALDI for quantification. Due to such limitations, matrix-free laser desorption ionization (LDI) methods have been sought in recent years.
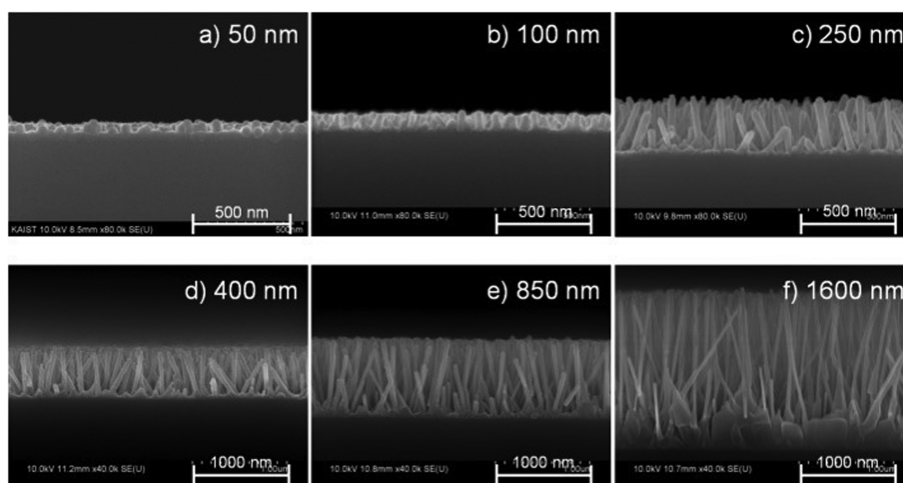
LDI of thermally labile molecules from nano-structured surfaces: ZnO NWs and WO_x NWs

The development of LDI methods was prompted by the discovery of DIOS (desorption ionization on porous

Figure 1. SEM images of ZnO NWs of 50 nm diameter with different NW lengths (25 ~ 1600 nm) utilized for the LDI study, Ref 18.†

silicon) phenomena.[10] It was found that laser irradiation could cause soft desorption ionization of intact biomolecules from the nanoporous surfaces of crystalline Si (c-Si) formed by photo-electrochemically etching. In contrast, LDI is hardly observed from the flat surfaces of c-Si that do not have such surface porosity of sub-micrometer size. Since then, LDI from nanostructured surfaces, including porous Si surfaces formed by various methods,[11] clathrate-structured Si surfaces,[12] Si nanowires (NWs),[13,14] Si nanoposts,[15,16] and so on, has been extensively studied. Likewise, LDI of thermally labile molecules was successful from the surfaces of nanostructures,[17] and thus the existence of nanostructures has been considered to be indispensable. However, the role of surface nanostructures in the LDI mechanism is not evident and still requires further study.

Although not plenty, there have been efforts to understand the mechanism involved in LDI of thermally labile molecules from nanostructured surfaces.[11,18-20] A study investigated the NW length dependence of LDI efficiency using ZnO NWs with a diameter of 50 nm with well-defined nanowire lengths of 25 to 1600 nm, where ZnO NWs possessed the same material properties as those of bulk ZnO (Figure 1). Careful study was performed by investigating the laser power dependence of LDI efficiency and LDI threshold behaviour for drug molecules as a function of NW length.[18]

In the aforementioned study, LDI efficiency exhibited a strong length dependence, where LDI of drug molecules was found to be most enhanced on 250 nm-long NWs, corresponding to an aspect ratio of 5. But LDI did not occur from the flat surfaces of ZnO, *i.e.* without any surface nanostructure. In accordance with previous observations, this indicated that the presence of surface nanostructures is essential to cause LDI from ZnO surfaces. It was speculated that the nanostructures serve as
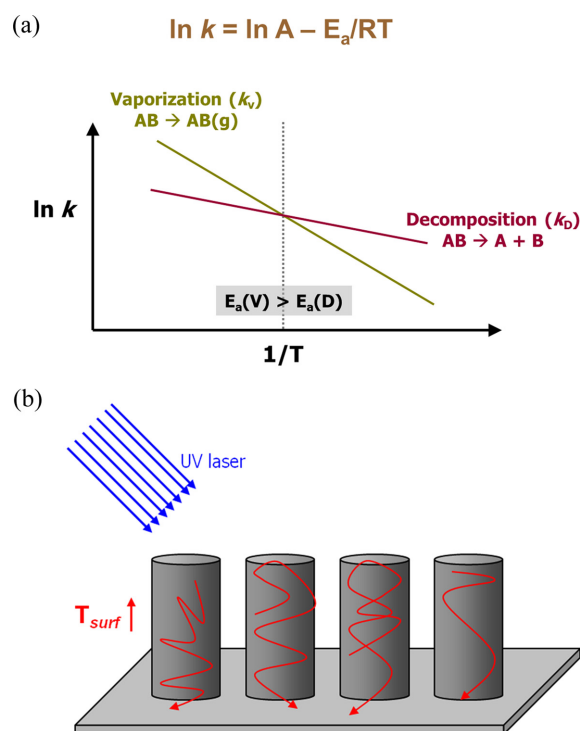
Figure 2. a) Kinetics consideration of the competition between desorption (V) and decomposition (D) on surfaces of thermally labile molecules. b) Rapid surface heating upon laser irradiation due to transient heat trapping in nanostructured surfaces.

a structural barrier that momentarily traps absorbed laser energy such that it does not easily dissipate into the bulk substrates. This can lead to a rapid increase of the surface temperature. In view of simple kinetics consideration using Arrhenius equation ($\ln k = \ln A - E_a/RT$), there is a competing relation between the dissociation (k_D) of molecules on surfaces and intact desorption, *i.e.*

vaporization (k_v) from the surfaces upon surface heating. As the general activation energy for desorption ($E_a(V)$) is higher than that of dissociation ($E_a(D)$), $E_a(V) > E_a(D)$, rapid surface heating favours intact desorption of molecules from surfaces.[21] The results suggested that the thermal mechanism, *i.e.* rapid surface heating,[21-23] may play a key role in intact LDI of thermally labile molecules[11,18-20] (Figure 2). A similar study using surfaces with length controlled WO_x NWs showed pronounced formation of LDI ions of peptides, leading to the same conclusion.[24]

LDI from flat surfaces of materials with very low thermal diffusivity: a-Si and WSi$_x$

Rapid surface heating as a possible driving force for LDI from surfaces raises an interesting question. If it is indeed due to rapid surface heating caused by the presence of surface nanostructures, what would happen if a surface possesses certain material properties that prevent heat, *i.e.* absorbed laser energy, from being easily dissipated into the bulk substrate so that rapid surface heating can occur without the assistance of surface nanostructures? Examples of such materials would be those with very small thermal conductivity (K) such as amorphous Si (a-Si) or very large heat capacity (C_p) such as WSi$_x$, which thus have a very low thermal diffusivity ($\kappa = K/\rho C_p$, where ρ is density). Very low thermal diffusivity itself may facilitate transient trapping of absorbed laser energy near the surface.

In this regard, LDI from flat surfaces, *i.e.* without any surface nanostructure, of a-Si was explored.[25] a-Si has very similar material properties to those of c-Si. In particular, they have similar UV absorption of 10^6 cm^{-1} and heat capacity of about 1.65 Jcm^{-3}K^{-1}. However, a-Si and c-Si possess distinctly different thermal conductivities of 5.5 and 150 Wm^{-1}K^{-1}, respectively. The difference in K can result in a significant difference in the depth of conductive heating ($D = [Kt/C_p\pi]^{1/2}$) for the two materials. As for a-Si, the depth of conductive heating is estimated to be 90 nm for 7 nsec, a characteristic time duration of a pulsed laser. But c-Si may have a much longer depth of about 450 nm. It should be noted that LDI from flat c-Si surfaces has rarely been reported. A simulation with a heat diffusion equation[25,26] predicted that, upon irradiation of laser pulses with a pulse width of 7 nsec and laser power of 6 MW/cm^2, the surface temperature of a-Si can increase to peak temperature as high as 1400 K at around 9 nsec, while the surface temperature of c-Si can only reach 300 K. The theoretical estimates show that the very low thermal conductivity of a-Si can result in a rapid increase of the surface temperature upon pulsed laser shining, and thus may promote LDI of thermally labile molecules from the surfaces.

In an experiment, pronounced LDI of various peptides with molecular weights up to 1500 Da took place from the

(a)

(b)
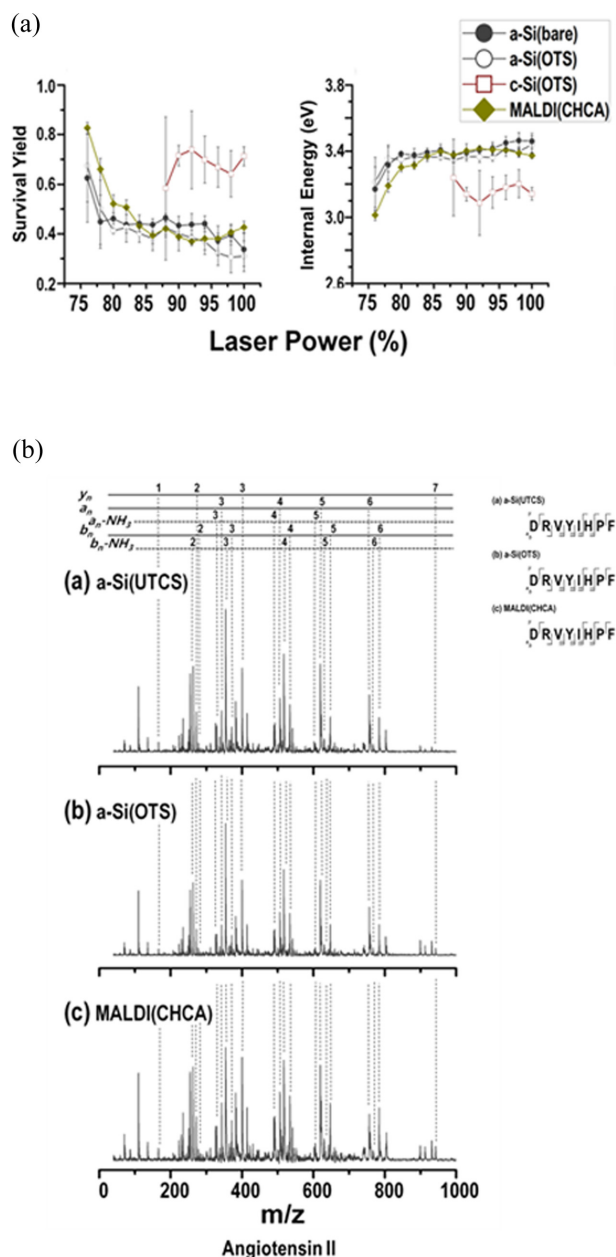
Figure 3. a) Survival yield and internal energy of BP ions produced by LDI from the a-Si surfaces, b) TOF-TOF mass spectrometry of Anigiotensin II., Ref. 25 †; OTS (octadecyltrichlorosilane) and UTCS (undecyltrichlorosilane) are surface coating materials. CHCA(α-cyano-4-hydroxycinnamic acid) is the matrix used for MALDI.

flat surfaces of a-Si, while LDI from the flat surfaces of c-Si was not reported, indicating that rapid surface heating is indeed involved in the LDI mechanism.[25]

On the other hand, to look at the thermal mechanism in more detail, the internal energy of benzylpyridium (BP) ions, known as thermometry ions, produced by LDI from the a-Si surfaces was studied by measuring the survival

yield after laser-induced desorption in comparison with theoretical RRKM calculations (Figure 3(a)).[27,28] In the results, the internal energy of LDI ions clearly reflected the surface temperature, which supported that LDI is most likely driven by rapid surface heating. A supplementary study of surface passivation with inert molecular layers, which might lower the desorption activation energy ($E_a(V)$), enhanced the LDI efficiency, where it was found that an increase in internal energy was not accompanied. All the results are in stong agreement with the prediction from kinetics consideration based on Arrhenius relation.[21] In the study, it was also demonstrated that laser-induced thermal desorption facilitates postsource decay of peptide ions, allowing peptide sequencing suitable to TOF/TOF mass spectrometry (Figure 3(b)). The choice of the thermal properties of materials to induce rapid surface heating and thus to enhance LDI was successful, which in turn supports that the thermal mechanism may indeed govern LDI of thermally labile molecules.

As for the case of very large heat capacity (C_p), LDI from the flat surfaces of WSi$_x$ was examined. WSi$_x$ has a moderate thermal conductivity. However, it has a very large heat capacity (309 Jcm^{-3}K^{-1}), and hence may have a conduction depth (D) of only about 11 nm. Indeed, pronounced generation of intact LDI ions of peptides was observed.[31]

LDI from surfaces supported by insulator layers: Si/SiO$_2$ and Au/glass

In fact, the choice of materials with extreme thermal properties such as a very low K and a very high C_p is rather limited; in many cases they are difficult to produce and have limited applications. For broader applications, it would be more beneficial to use surface materials that are amenable to surface chemistry, for example, Si and Au, for which well-known thiol and silane chemistry allows chemical modifications for various applications.

Previous works suggested that if there is a proper mechanism on surfaces that can provide rapid surface heating, promotion of LDI of thermally labile molecules can be expected. To corroborate this, LDI on SOI (silicon-on-insulator) wafers was examined.[32] Commercial SOI wafers are widely used in the semiconductor industry, where an insulator layer, silicon dioxide (SiO$_2$), is embedded in a Si wafer, tens to a few hundred nanometers down from the wafer surface. In other words, a thin layer of c-Si is formed on top of the embedded insulator layer in the SOI wafers. It can thus be expected that, upon laser irradiation, the laser energy absorbed by c-Si, the top layer of the SOI wafer, is likely to be trapped momentarily in the layer due to the adjacent insulator layer, which can create rapid surface heating required for LDI processes. Again, LDI of peptide molecules was found to be pronounced from SOI wafers with 50 and 100 nm thick top c-Si layers.
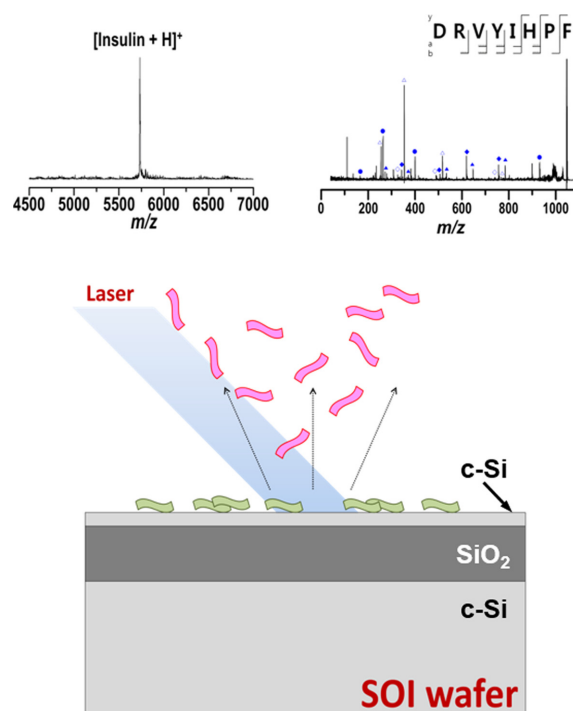
Figure 4. LDI from SOI (Silicon-on-insulator) wafer: LDI mass spectrum of insulin, LDI-TOF/TOF spectrum of angiotensin II., Ref. 32. †

With appropriate additives, LDI of insulin (5729.6 Da) was also successful. In addition, postsource decay of peptide molecules was also found to occur at higher laser power (Figure 4). These results support that rapid surface heating is indeed the main driving force for observed LDI phenomena.

The success of LDI on commercial SOI wafers opens a new gateway for the preparation of LDI surfaces with various surface materials. The c-Si surface, the top layer of the SOI wafer, offers a suitable surface for device patterning and modification of the surface property by silane chemistry.

In many nanobio applications, Au surfaces are very useful as a starting surface to realize various interfacial properties by immobilization of biomolecules using thiol chemistry. In a similar manner, LDI from Au on glass was also examined. For the study, 50 and 100 nm Au coated slide glasses were employed and LDI of peptides from the surfaces was successfully observed. Notably, Au and Si surfaces are suitable for desorption ionization in TOF-SIMS (time-of-flight secondary ion mass spectrometry). The two surfaces offer multi-modal sample plates that are applicable both for LDI MS and TOF-SIMS.[33]

Perspectives

This article briefly reviews the thermal mechanism

involved in the process of LDI of thermally labile molecules. A series of previous reports clearly showed that rapid surface heating is a crucial element responsible for the LDI process. Understanding of the mechanism would be helpful for the development of LDI plates that would be applicable to real mass spectrometry applications. By avoiding the utilization of excess matrix, LDI mass spectrometry can potentially overcome the shortcomings in MALDI MS arising from the matrix. However, LDI methods accompany issues requiring further technical developments, including binding of analytes to surfaces, limited salt tolerance, and aggregation of anaytes on surfaces, which are caused by the lack of a matrix. However, continuing efforts in this field include optimizing the surface properties to lower the desorption activation energy, using additives to avoid aggregation of analytes, creating surface nanostructures to enhance the non-thermal contribution, and uniform loading of analytes over LDI surfaces. Such work will deliver breakthroughs and will eventually lead to matrix-free LDI methods that are suitable for real mass spectrometry applications.

Acknowledgments

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