Photoionization of Dipeptides with Femtosecond Laser Pulses

Shixin Sun, Vasil Vorsa,[1] and Nicholas Winograd[*]

184 Materials Research Institute Building, Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

Abstract. Imaging time-of-flight secondary ion mass spectrometry is a very important technique for characterizing organic and biological systems. However, the signal is very low due to small amounts of desorbed molecules. During the ion bombardment process, neutral species as well as positive and negative ions are desorbed, with neutral molecules being the main species. Therefore, ionization of the neutral species should improve sensitivity. Here, we report the use of femtosecond laser photoionization of ion beam desorbed dipeptides to enhance the detection sensitivity. Ionization using 200 nm laser pulses is found to break the C-C bond in the C-C=O group or the C-C bond between the functional group and the backbone, which agrees with the ?-cleavage mechanism proposed previously. Photoionization produces 5-10 fold higher dipeptide ion yields than the secondary ion yields achieved with the use of the incident ion beam only.

INTRODUCTON

Imaging time-of-flight secondary ion mass spectrometry (SIMS) is an important technique used to characterize organic, inorganic and biological systems [1,2]. During the ion bombardment process, neutral species as well as positive and negative ions are desorbed, and the yield of neutral species is a few orders of magnitude higher (typically 10^4:1) than that of the ions. Therefore, efficient analysis of the neutral species desorbed would potentially produce more signal compared to desorbed ion detection. Earlier efforts focused on using nanosecond lasers to photoionize neutral species desorbed from surfaces [3,4]. This has been a success with atomic species, however, this results in extensive photo-induced fragmentation in molecular systems.

Ultrashort pulsed lasers are promising new ionization sources for the detection of molecules desorbed from surfaces. The enhanced absorption rates generated by high-power ultrashort pulses make it possible to "outrun" the neutral fragmentation channels, which are prevalent when employing nanosecond excitation of molecular

[1] *Air Products and Chemicals, Inc., 7201 Hamilton Blvd., Allentown, PA 18195*

species. Research [5,6] shows that the degree of fragmentation can be reduced considerably by using ultrashort laser pulses for ionization.

Here, we report the use of femtosecond laser photoionization of ion beam desorbed dipeptides to enhance the detection sensitivity. The ionization mechanism is examined and found to agree with the ? -cleavage mechanism proposed previously [7].

EXPERIMENTAL

The dipeptides were purchased from Sigma and used without further purification. The analytes were pressed into In foil directly and transferred into the vacuum chamber for analysis. The details of the time-of-flight secondary ion mass spectrometer (TOF-SIMS) apparatus have been described previously [3]. Briefly, molecules are desorbed from the surface by a 25 KeV Ga^+ ion beam (Ionoptika) in forms of positive ions, negative ions or neutral species. Ions formed at the sample surface are pulse extracted into a dual-field reflectron TOF mass analyzer by applying a positive or negative potential directly to the sample stage. Ions are separated from each other in the flight tube according to their masses and detected by a micro-channel-plate (MCP) detector. The signal from the MCP detector is sent to a analog-to-digital converter (ADC) whose output is transferred to a PC for storage and later analysis. The sample block is cooled to -125?C with liquid nitrogen to minimize the background signal from sample sublimation.

The Ti:sapphire laser system (Clark-MXR, Inc.) employed in the experiments has been reported elsewhere [8]. Briefly, an Argon ion laser pumps the mode locked Ti:sapphire oscillator which produces 800 nm pulses with ~50 fs pulse width and 3 nJ per pulse energy. The 800 nm pulses are stretched to ~300 ps by multiple passes on a single grating and then amplified by a Ti:sapphire regenerative-amplifier and a Ti:sapphire post-amplifier. Both amplifiers are pumped by the second harmonic of Nd: YAG lasers. The amplified beam has 5.5 mJ per pulse energy. Finally, the beam is compressed to ~100 fs with 3.5 mJ per pulse energy. The output of this laser system is frequency doubled, tripled or quadrupled to produce 400, 267 and 200 nm respectively. The beam is coupled into the analysis chamber by a 25-cm focal length CaF_2 lens. The laser spot size and position over the sample is controlled by moving the lens mounted on an x, y, z manipulator outside the chamber.

RESULTS AND DISCUSSION

The 200 nm photoionization mass spectra of Val-Val and Leu-Trp are shown in Figure 1 and Figure 2 respectively. Neither of these two dipeptides produces significant molecular ions, although molecular ions can be easily seen from SIMS spectra. The base peak in the Val-Val spectrum results from ? -cleavage (Figure 3), which was proposed previously [8].

A previous study shows that for aliphatic amino acids, the lowest ionization energies correspond to the removal of one lone pair electron from the nitrogen atom and form a radical site on the nitrogen atom [7]. The radical site on the nitrogen atom

initiates the reaction by donating an electron to form a double bond between the carbon and nitrogen atoms. The amido group donates another electron, and the C-C bond is broken and a carboxyl group radical is formed. In Val-Val, there are two nitrogen atoms. Due to the electron withdrawing ability of the C=O group, the lone pair ionization energy of the amido group nitrogen is a little bit higher than that of the other nitrogen, so only the C-C bond shown in Figure 1 is broken.

For tryptophan and tyrosine, the lowest ionization energy corresponds to the removal of an electron from the functional group. The ionization potential for tryptophan is 7.2 eV [9], corresponding to the energy required to remove one electron from the functional group. The ionization potential for leucine is 8.5 eV [9], corresponding to the energy required to remove one electron from the N atom. In Leu-Trp, due to the electron withdrawing capability, the energy required to remove one electron from the functional group is increased. Therefore, the energies required to remove one electron from the functional group or from the N atom are similar, so the fragmentation from both ends can be seen in the spectrum, with the functional group signal intensity (130 amu) a little bit higher.

Comparing the spectra of SIMS (data not shown) and photoionization, it is very obvious that molecular ion signal can be easily detected in SIMS experiments, which means that some of the molecules are desorbed and ionized intact. However, there are no molecular ions detected in photoionization experiments. The reason for excessive fragmentation has to be further investigated. In addition to comparing mass spectra, it is useful to compare total ion yields formed by SIMS and photoionization. Comparing the spectra of SIMS (data not shown) and photoionization, it is very obvious that the total ion yield resulting from photoionization is much higher than that from SIMS. For Leu-Trp, it is 25 fold higher.

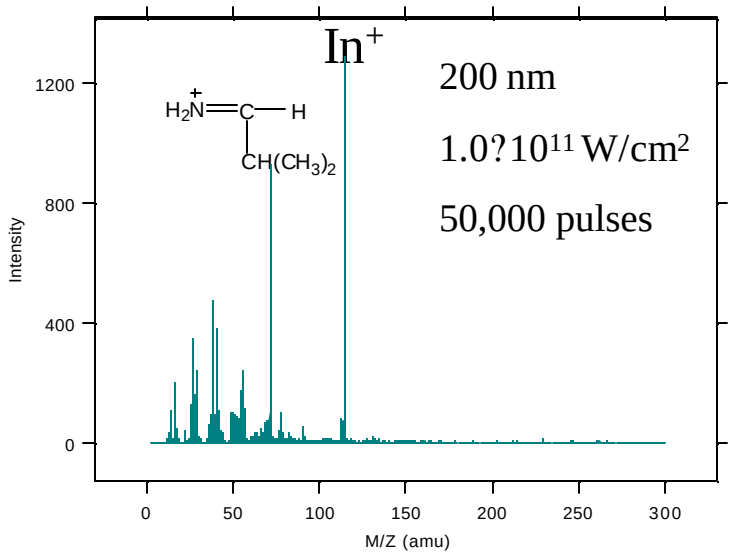

Figure 1. Laser photoionization spectrum of Val-Val

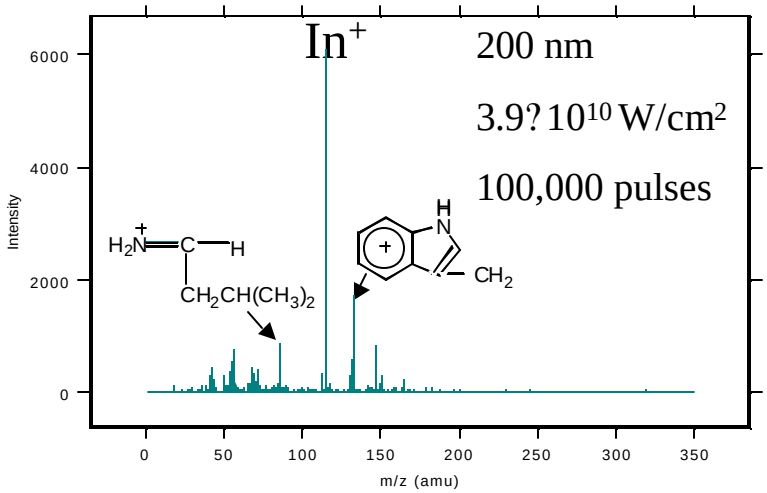

Figure 2. Laser photoionization spectrum of Leu-Trp

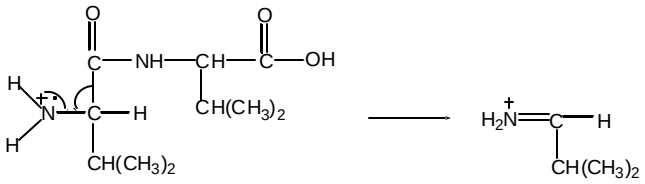

Figure 3. ? -cleavage mechanism

CONCLUSIONS

For photoionization of dipeptides with 200 nm femtosecond laser pulses, there is no molecular ion signal detected and the ? -cleavage is the main fragmentation mechanism. When comparing the total signal intensity produced with photoionization and SIMS, photoionization produces more signal.

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