Nuclear Instruments and Methods in Physics Research B 315 (2013) 300–303

Contents lists available at SciVerse ScienceDirect

Nuclear Instruments and Methods in Physics Research B

journal homepage: www.elsevier.com/locate/nimb

Secondary ion emission from insulin film bombarded with methane and noble gas cluster ion beams

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ARTICLE INFO

Article history:
Received 1 December 2012
Received in revised form 7 May 2013
Accepted 15 May 2013
Available online 3 July 2013

Keywords:
Secondary ion mass spectrometry
Gas cluster ion beam
Soft-sputtering
Matrix-free ionization

ABSTRACT

Recent advances in large cluster projectiles for secondary ion mass spectrometry (SIMS) allow the intact ions of some protein molecules to be detected without a matrix. However, detailed mechanisms of soft-sputtering and ionization of biomolecules remain unknown. Herein we investigate the secondary ion emission from insulin films under argon, krypton, and methane cluster ion bombardment. The intact insulin ion intensity significantly decreases for $(CH_4)^+_{1500}$ ion bombardment compared with Ar^+_{1500} ion bombardment at the same energy range of 3.3 eV/atom (or molecule), even though collisions with energetic methane clusters should generate numerous protons on the surface, which would enhance the ionization probability through proton attachment. In contrast, the intact ion intensity is almost the same for Ar^+_{2500} and Kr^+_{2500} cluster ion bombardment at the same energy range of 2 eV/atom. These observations suggest that detailed mechanisms for the ionization and sputtering by gas cluster ions should be investigated to enhance the intact ion intensity.

1. Introduction

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) allows imaging and local analysis of material surfaces. TOF-SIMS has a high sensitivity, wide mass range, high mass resolution, and high lateral resolution. In addition to providing information on elements and isotopes, the technique yields direct information about the molecules and can be used to analyze surface species with high molecular masses, which are thermally unstable and cannot be vaporized. Although TOF-SIMS is applicable to almost any type of material, including polymers, the highly energetic projectile ions destroy the chemical integrity of the sample, limiting the detectable intact ions.

Recently, an argon gas cluster ion beam (GCIB) has been applied as a projectile for TOF-SIMS, which dramatically improves these demerits [1,2]. Because the kinetic energy of a cluster is distributed to each constituent atom in the collision process, individual atoms have a smaller kinetic energy than an atomic projectile. The average kinetic energy per constituent in the cluster ion (E_{atom} or $E_{molecule}$) is the value of the energy obtained by accelerating the cluster ion (hereafter called the cluster energy) divided by the number of constituent atoms or molecules of the cluster ion (hereafter called cluster size). For a gas cluster ion, which typically contains several thousands constituent atoms or molecules, the E_{atom} or $E_{molecule}$ should be on the order of several eV, which is the same order as a covalent bonds. Therefore, a gas cluster projectile greatly reduces the beam-induced damage of the sample and improves the efficiency of intact molecule desorption [2]. Currently, the molecular weight of the intact ions detectable from a sample molecule without a matrix is above 20 kDa [3].

To apply this technique to the analysis of macromolecules in practical biosamples, the signal intensity of high mass molecular ions must be further enhanced. The secondary ion intensity, when excluding analyzer-related factors, can be determined from the sputtering yield and ionization probability. The sputtering yield increases with the incident ion energy [4], but is a trade-off with molecular fragmentation. The properties of the bombarding primary ion beams and the chemical nature of the sample surface during a collision with the primary ion influence the ionization probability. Thus, selecting the appropriate primary ion species may flexibly modify the sample surface by primary particles, enhancing the secondary ion intensities.

There are some reports about the effect of the primary ion species on the ion formation efficiency for organic molecules under bombardment by Cs^+, SF_5^+, atomic noble gas ions, etc. [5–9]. Recently, it has been reported that insulin molecules are ionized without fragmentation and emitted into a vacuum upon impact with a neutral SO_2 cluster where $E_{molecule}$ is below 1 eV [10], suggesting that both the chemical effect in a large cluster collision and the physical sputtering processes play important roles in the soft-ionization of proteins.

We have developed a GCIB TOF-SIMS apparatus [11] and reported that intact ions of biomolecules with molecular weights

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0168-583X/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.nimb.2013.05.064

of 20 kDa or higher are detected without using a matrix under Ar GCIB bombardment [2,3]. In our previous study, the physical sputtering process of intact insulin ions by Ar GCIB bombardment was investigated [12]. In this study, krypton and methane as well as argon gas cluster ion beams are used to investigate the secondary ion emission from insulin films. The intact ion intensity in the SIMS spectra is almost the same for Ar and Kr cluster bombardment. Although the collision of an energetic methane cluster should generate many protons on the surface, which may enhance the ionization probability through the proton attachment, the intact ion intensity significantly decreases for methane gas cluster ion bombardment. These observations strongly suggest that detailed mechanisms for the ionization and sputtering of organic molecules by gas cluster ions should be investigated to enhance the intact ion intensity.

2. Experimental

Details of the GCIB TOF-SIMS apparatus are given elsewhere [11–13]. The apparatus consisted of three parts: a cluster-source chamber, an ionization and size-selection chamber, and a sample chamber. All parts were differentially pumped. The neutral cluster beam generated in the cluster source chamber was collimated by a skimmer with a 0.2-mm diameter opening and flowed into the ionization chamber. The clusters were ionized by electron impact from a tungsten filament at an energy of 150 eV, and the cluster ions were extracted at an acceleration voltage of 5 kV. The cluster-size selection by a time-of-flight technique was performed using two pairs of ion deflectors. A gate valve separated the sample chamber from the ionization and size selection chamber. The size-selected cluster ion beam was then used to bombard the sample through a 5-mm diameter aperture and a focusing ion lens.

The secondary ions were extracted into a reflectron with a flight tube length of 1.8 m (KNTOF-1800-SL203, Toyama, Kanagawa, Japan) by an acceleration voltage of −2 kV. The reflectron was used in both the linear (flight length: 0.9 m) and reflection modes. The secondary ions were detected by a doubly stacked MCP (Hamamatsu Photonix, F4655) detector in the reflection mode to precisely measure ion flight time, except for intact insulin ions, which were detected by a post-acceleration detector in the linear mode.

In our experiment, the pulse width of the incident beam after size selection spread to more than 10 μs at the sample position, which was insufficient for use as a trigger in the TOF measurements. Thus, we employed a device to gate the secondary ion beam into short pulses [13]. The pulse widths of the secondary ions were shortened to 200 ns–1 μs when a short pulse voltage was applied to the wires of the comb gate device. The mass of a secondary ion was calculated from the flight time between the comb gate device and the detector. To measure intact insulin ions, the linear TOF mode and a pulse width of 1 μs at a comb gate device were adopted to obtain a sufficient ion count. Under these conditions, the mass resolution ($m/\Delta m$) was approximately 15 at $m/z \sim 5800$.

Neutral gas clusters were generated by adiabatic expansion from a nozzle with a 0.1-mm diameter aperture and a 60-mm expansion and cooling zone. The methane (10% concentration) was seeded in Ar (90% concentration) for cooling. The elemental component in the cluster ion beams was assumed from the change in the component of the background gas in the sample chamber when the beam was on and off, which was measured by the quadrupole mass spectrometer (QMS) equipped with a sample chamber.

The sample was a thin film of human insulin (MW: 5808, purity: 99.5%, Peptide Institute Co., Japan). An aqueous solution of the chemical (1 g/L) was dropped on a silicon substrate and dried in vacuum. To promote the hydrophilicity of the surface and to yield a uniform film thickness (~50 nm), the silicon surface was exposed to an air plasma before sampling.

The cluster ion fluence while measuring a SIMS spectrum was below 2.5×10^{12} ions/cm^2. The secondary ion intensities were normalized by the primary ion current. All the measurements were performed at room temperature in a high vacuum. The base and the working pressure in the sample chamber were below 1×10^{-6} Pa and 1×10^{-5} Pa, respectively.

3. Results and discussion

Fig. 1 shows the typical TOF spectra of the GCIB generated from Ar gas, Kr gas, and methane-Ar mixed gas. The ion beams are accelerated at a voltage of 5 kV and then pulsed by the first ion deflector to a width of 15 μs with a repetition frequency of 1500 Hz. The cluster mass is determined from the flight time between the first ion deflector and the sample position. These broad TOF peaks indicate a wide size distribution of the generated cluster ions. The cluster sizes of Ar and Kr are estimated from the values of the cluster mass divided by the atomic mass of the Ar and Kr, respectively. The peak cluster sizes of Ar and Kr before size selection are ca. 1750 and ca. 3350 atoms/cluster, respectively. The size distributions of the spectra are very broad from ca. 600 to ca. 6000 atoms/cluster (Ar) and from ca. 2000 to ca. 8000 atoms/cluster (Kr).

Fig. 2 shows the background intensity of the mass peaks at m/z of 16 and 40 in the sample chamber when the GCIB continuously enters the sample chamber and are shut off by the gate valve. The background pressure is 1.1×10^{-5} Pa when Ar GCIB enters, 7.7×10^{-6} Pa when the GCIB is produced from methane and Ar mixed gas enters, and 1×10^{-6} Pa when the GCIBs are shut off. The ion beam currents for the Ar GCIB and the GCIB generated from methane and Ar mixed gas are 16 and 10 nA, respectively. When the GCIB produced from methane and Ar mixed gas enters the sample chamber, CH_4^+ increases in the background residue gas. The increase of Ar^+ is only 3% of that of CH_4^+, indicating that the main component in this GCIB is methane, although 90% Ar is included in the source gas.

Fig. 3a and b shows the secondary ion spectra under bombardment on a Ag surface by Ar GCIB and the GCIB generated from the methane and Ar mixed gas cluster ion bombardment at an acceleration voltage of 5 kV, respectively. The selected cluster mass is 36,000 Da. These spectra include secondary ion species due to

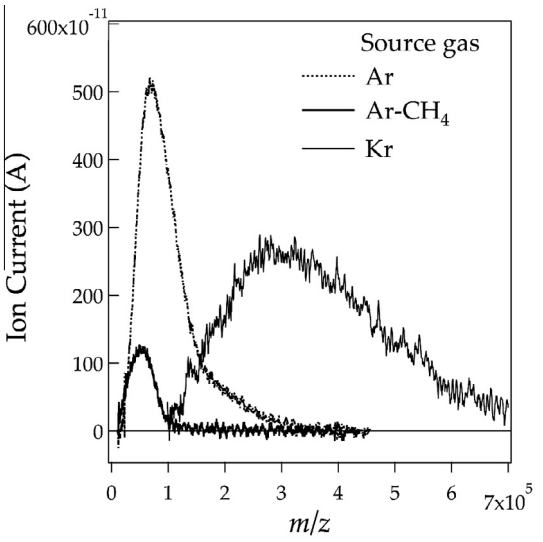

Fig. 1. TOF spectra of the GCIBs generated by the adiabatic expansion of Ar gas, Kr gas, and methane-Ar mixed gas before size selection.