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Molecular depth profiling in ice matrices using C_{60} projectiles

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Abstract

The prospects of molecular sputter depth profiling using C_{60}^+ projectiles were investigated on thick ice layers prepared by freezing aqueous solutions of histamine onto a metal substrate. The samples were analyzed in a ToF-SIMS spectrometer equipped with a liquid metal Ga^+ ion source and a newly developed fullerene ion source. The C_{60}^+ beam was used to erode the surface, while static ToF-SIMS spectra were taken with both ion beams alternatively between sputtering cycles. We find that the signals both related to the ice matrix and to the histamine are about two orders of magnitude higher under 20-keV C_{60} than under 15-keV Ga bombardment. Histamine related molecular signals are found to increase drastically if the freshly introduced surface is pre-sputtered with C_{60} ions, until at a total ion fluence of about 10^{13} cm^{-2} the spectra are completely dominated by the molecular ion and characteristic fragments of histamine. At larger fluence, the signal is found to decrease with a disappearance cross section of approximately 10^{-14} cm^2, until at total fluences of about 10^{14} cm^{-2} a steady state with stable molecular signals is reached. In contrast, no appreciable molecular signal could be observed if Ga^+ ions were used to erode the surface.

Keywords: C_{60}; ToF-SIMS; Ice matrix; Depth profiling; Polyatomic projectile

1. Introduction

Molecular depth profiling using mass spectrometric methods is often hampered by the fact that the eroding ion beam generates damage in the utmost surface layer. As a consequence, the wealth of molecular information generally obtained in static SIMS spectra is generally lost rapidly once the surface is subjected to ion fluences that are large enough to cause significant erosion. In many applications, on the other hand, the analyte molecules are located in or even buried beneath a surface layer that needs to be removed prior to the actual analysis. A particular example of that situation is the detection of biologically relevant molecules in aqueous systems, where the prevailing sample preparation technique involves relatively complex freeze fracture procedures to uncover the desired information. Sputter depth profiling of such a sample would greatly simplify the analysis, but has been proven largely unsuccessful if atomic projectiles are used for sputter erosion. On the other hand, polyatomic projectiles have been shown to greatly enhance the detection efficiency of molecular species in a SIMS experiment [1–4]. Moreover, it has been demonstrated that carbon clusters may be particularly useful in order to allow successful

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sputter depth profiling of organic molecules [5–7]. In the present work, we therefore employ a newly developed fullerene ion source [2] in an attempt to sputter depth profile across a model system consisting of histamine molecules dissolved in a frozen water ice sample. The results indicate that fullerene cluster beams might be promising tools which open new possibilities for molecular localization within biological systems, thus making it possible to obtain high resolution 3-D molecular maps of cellular constituents within layers of single biological cells.

2. Experimental

The experiments were performed in a ToF-SIMS apparatus described in detail elsewhere [8]. The instrument is equipped with a liquid metal Ga^+ and a newly developed C_{60} ion source [2]. Under the employed operating conditions, the fullerene source delivered a pure C_{60}^+ current of about 1 nA into a spot size of about 30 μm, while the Ga^+ current was about 2 nA with a sub-micrometer spot. Depth profiling was performed by alternating between sputter erosion cycles—during which the C_{60}^+ beam was operated in dc mode and rastered across an area of 800 μm × 800 μm—and data acquisition cycles using either the C_{60}^+ or the Ga^+ beam operated in a pulsed mode and rastered across a 500 μm × 500 μm field of view. Great care was taken to align the two ion beams onto the same surface spot using images taken on a TEM grid. Due to the slightly different height of each sample, the alignment needed to be checked on a regular basis by taking Ga^+ images of a spot pre-bombarded with C_{60}^+. Spectra were recorded in single ion counting mode and summed over 50,000 primary ion pulses.

The samples were prepared by depositing about 1 μl of an aqueous solution of histamine (concentration 1.6 mg/ml) onto a silver substrate that had been etched in HNO_3 and thoroughly rinsed in deionized water and methanol. The sample was then frozen by slowly immersing the Cu sample mount block into liquid nitrogen. This method produces relatively thin and homogeneous ice films with visible crystallites of several hundred μm diameter.

Due to the highly insulating nature of the ice sample, charge compensation was employed using a low energy electron beam (20 eV, ∼1 μA) that was switched from pulsed mode during data acquisition to dc mode during the sputtering cycles. While this was absolutely needed to detect a meaningful spectrum using Ga^+ projectiles, it proved to be mostly unnecessary with C_{60}^+ ions. Apparently, the cluster bombardment leads to a much more balanced surface charge, a fact that may be attributed to the high positive secondary ion yields that are obtained with these projectiles.

3. Results and discussion

A series of typical mass spectra that are encountered during the acquisition of a C_{60}^+ depth profile is shown in Fig. 1. Prior to any dc bombardment of the surface (Fig. 1a), a static SIMS spectrum is obtained which mainly shows a series of water clusters and, hence, closely resembles that measured on a pure ice surface. In particular, practically no signal is visible that can be attributed to the histamine molecules dissolved in the ice sample. We attribute this finding to the fact that the short time period needed to transfer the samples through air from the LN_2 bath into the vacuum load lock leads to the deposition of a thin ice layer covering the actual sample. In a static SIMS experiment, the analyte molecule, therefore, would not have been detectable at all. The situation when the surface has been bombarded by ions up to a total C_{60}^+ ion dose of about $3 \times 10^{13}\,cm^{-2}$ is shown in Fig. 1a. It is immediately evident that the nature of the spectrum has completely changed. In fact, the spectrum is now dominated by the molecular ion $(M + H)^+$ (mass 112) of histamine and characteristic fragment ions at masses 81, 82, 83, and 95. The same holds true if the surface is analyzed by Ga^+ projectiles, with the exception that the signal intensities and, hence, the secondary ion yields are lower by about two orders of magnitude. If the surface is further bombarded by C_{60}^+ ions, the histamine related molecular signals start to decrease again and seem to reach a steady state level in the limit of high dose. The spectrum obtained under these conditions, i.e. at a total C_{60}^+ ion dose of about $3 \times 10^{14}\,cm^{-2}$, is shown in Fig. 1c. It is seen that the ice matrix related signals have recovered to their initial values, but the histamine related signals are still clearly visible.

In order to obtain depth profile information, the dose dependence of the $(M + H)^+$ peak and the

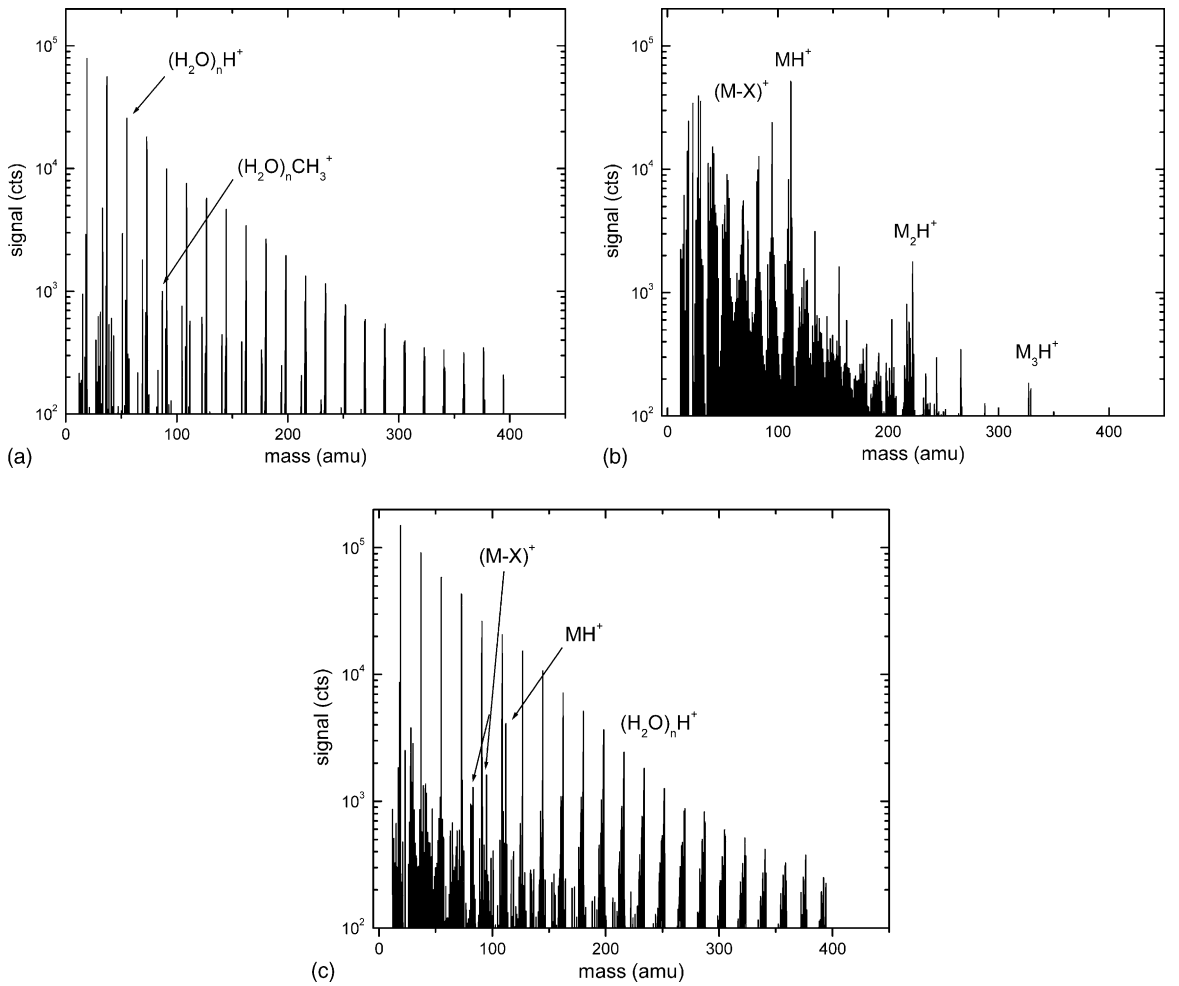

Fig. 1. ToF spectra of frozen histamine/ice sample taken with 20-keV C_{60}^+ primary ions. (a) No pre-bombardment; (b) pre-bombardment to a total fluence of 3×10^{13} cm^{-2}; (c) pre-bombardment to a total fluence of 3×10^{14} cm^{-2}.

$(H_2O)H^+$ peak intensities representing the histamine molecule and the ice matrix, respectively, is plotted in Fig. 2. It is seen that the initial increase of the molecular signal is accompanied by a strong decrease of the matrix related signal. In fact, both signals seem to exhibit complementary trends in the dose range up to 10^{14} cm^{-2}. This finding suggests that the observed signal variations are caused by a real depth profile which is characterized by a histamine enriched surface-layer. In view of the applied sample preparation technique, this layer may be produced by water evaporation during the relatively slow sample cooling process. Alternatively, the histamine surface enrichment may have already been present in the liquid phase, an effect that has been demonstrated in microtome experiments earlier and is attributed to enhanced adsorption at the liquid–gas interface [9].

The exponential decay of the molecular signal that is observed in a fluence range $1-3 \times 10^{14}$ cm^{-2} seems to indicate the accumulation of bombardment induced surface damage. The straight line fit indicated in Fig. 2 yields a disappearance cross section of about 1.4×10^{14} cm^2, a value that relates reasonably to the range typically observed for other molecules [4,10–13]. The fact that a steady state molecular signal is observed at fluences above 3×10^{14} cm^{-2} indicates a competition between damage production and sputter removal rate that tends to balance at a certain damage

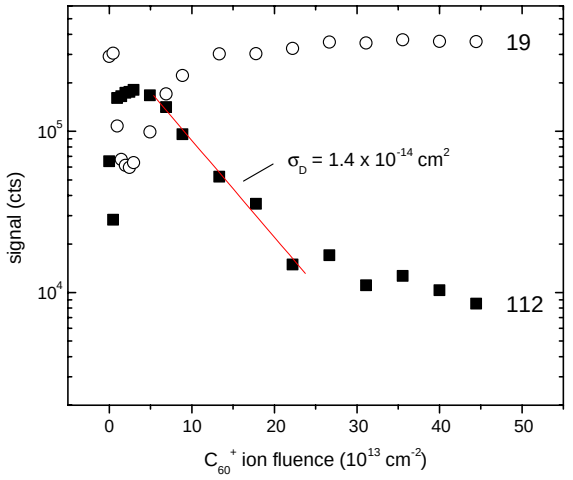

Fig. 2. Dependence of histamine molecular ion $(M + H)^+$ and ice matrix $(H_2O)H^+$ signals on C_{60}^+ pre-bombardment fluence.

level. It should be noted that this observation is not made if Ga^+ ions are used to erode the surface. In fact, no appreciable molecular ion signal could be detected at all if only Ga^+ pre-bombardment was applied. If the molecular signal is uncovered by C_{60}^+ pre-bombardment, switching to Ga^+ bombardment results in an exponential signal decay with a disappearance cross section about three times larger than that observed in Fig. 2. We attribute this difference to the high sputtering yield generated by the cluster ion beam. In fact, both molecular dynamics computer simulations [14] as well as preliminary experimental data obtained using a quartz microbalance [15] show that the sputtering yield under 20-keV C_{60}^+ bombardment is by at least two orders of magnitude higher than that observed with 15-keV Ga^+ projectiles. As a consequence of the fast erosion rate, the damage generated by the C_{60} projectiles must, therefore, be removed at comparable rate as how it is produced. In contrast, due to the much lower sputtering yield—at comparable damage cross section—the damage production largely dominates for the atomic Ga projectiles.

4. Conclusions

Using the new C_{60}^+ ion probe, depth profiling of biochemically relevant molecules in frozen aqueous matrices appears to be feasible. Moreover, our experiments indicate that it is possible to combine C_{60} sputter erosion and Ga probe data acquisition, thus, permitting to utilize the high lateral resolution obtained with liquid metal ion sources for 3-D molecular localization in layered samples. These findings bear great implications for the analysis of many biologically relevant systems. As an example, it appears to be possible to obtain high resolution 3-D maps of molecular constituents within single biological cells, a task which to-date requires extremely complex freeze-fracture techniques to expose the cell interior to static SIMS analysis.

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