Molecule-specific imaging using focused ion beams is one of the most powerful applications of ToF-SIMS and offers unique surface characterization information. However, many experiments lack the necessary sensitivity to properly take advantage of the sub-100 nm probe size typical of liquid metal ion sources. The yield of biomolecules using Ga\(^{+}\) ions, for example, is very small when compared to that obtained using Cs\(^{+}\) ion or SF\(_{5}\)\(^{+}\) cluster ion sources. Most recently, a C\(_{60}\)\(^{+}\) source with a probe size approaching 1\(\times\)10\(^{-6}\) m has become available and offers promise to expand imaging applications dramatically. Here, we report on imaging experiments on 50\(\times\)10\(^{-6}\) m polystyrene resin particles used in solid phase synthesis of combinatorial libraries and on the assay of the membrane chemistry of single biological cells. Both of these examples allow much higher quality images to be acquired with, in some cases, almost no sample damage. This latter effect opens the possibility of three-dimensional molecule-specific imaging.

Keywords: ToF-SIMS imaging; Cluster sources; Combinatorial chemistry; Biological cells

1. Introduction

Perhaps the most exciting consequence of the enhanced yields associated with cluster ion beams is the possibility of performing greatly improved molecule-specific imaging experiments with ToF-SIMS instrumentation. SIMS images that are more information-rich will allow advances in drug discovery, via high-throughput analysis of combinatorial beads and in cell biology, by examining membrane events such as exocytosis. Preliminary studies using a microbeam carbon cluster source on a number of biomaterials show much-improved contrast over corresponding images acquired using atomic projectiles [1]. Liquid metal ion sources consisting of gold cluster ions offer the possibility of producing higher yields with submicron lateral resolution—perhaps the best choice when optimizing sensitivity and lateral resolution [2]. These sources are just beginning to be tested in an applications environment [3]. Here, we examine the capabilities of a newly developed buckminsterfullerene probe (C\(_{60}\)) probe for imaging combinatorial bead arrays and single cells. This source has been configured to operate with a lateral resolution of 1–5 \(\mu\)m and has already produced high quality images [4]. With measured intensities of up to 10,000 times greater than those obtained using Ga\(^{+}\) liquid metal ion sources, the effective lateral resolution of this source is often equivalent to that obtainable with a Ga source due to the enhanced dynamic range. Two examples are
illustrated in this work. In the first case, we compare the assay of protein molecules bound to 50 \( \mu \)m polymer resin particles used in solid phase combinatorial chemistry experiments. Signal intensities are large enough to directly detect the molecular ion of a protein on a single bead and to acquire sequence information from the fragmentation pattern. In a second example, we examine the possibility of imaging the phospholipid distribution on single biological cells. To test this idea, we report on the assay of freeze-dried Tetrahymena cells using both \( \text{In}^+ \) ion bombardment and \( \text{C}_{60}^+ \) ion bombardment. The results show a better than 50-fold improvement in secondary ion efficiency for the fullerene source. In general, we suggest that this emerging ion source will open many new possibilities for imaging, especially if the ultimate probe size can be reduced to below the current 1 \( \mu \)m limit.

2. Experimental

Sasrin linker-copoly (styrene-1\% divinylbenzene (DVB)) resins were purchased from Bioscience Inc. (Philadelphia, PA). Rink amide methylbenzhydroxylamine (MBHA) resin (75–150 \( \mu \)m, 0.7 mmol/g) and 9-fluorenylmethoxycarbonyl (Fmoc) —protected amino acids were purchased from NOVAbiochem (San Diego, CA). Biotin and other reagents and solvents were obtained from Aldrich (Milwaukee, WI) without further purification.

The ToF-SIMS instrumentation has been described in detail elsewhere [5]. The instrument is equipped with a liquid metal ion gun (LMIG) operated at 15 keV and a \( \text{C}_{60}^+ \) primary ion source operated at 20 keV, both of which are directed at the target at a 40\(^{\circ}\) angle relative to the surface normal. In all measurements, the incident ion dose is kept at or below the static limit of 1 \( \times \) 10\(^{12} \) ions/cm\(^2\). With a repetition rate of 3000 pulses/s, most images are acquired in less than 5 min. The \( \text{C}_{60}^+ \) primary ion beam system is obtained from Ionoptika Ltd. (Southampton, UK) and a detailed characterization has been reported recently [4].

3. Results and discussion

The feasibility of imaging combinatorial resins with \( \text{C}_{60}^+ \) ion beams is examined using biotin-Sasrin linker-copoly (styrene-1\% DVB) supports. The benefits from switching to \( \text{C}_{60}^+ \) from the \( \text{Ga}^+ \) primary ion source are also investigated for comparison purposes. Two images of the biotin resin obtained with \( \text{Ga}^+ \) and \( \text{C}_{60}^+ \) ions are shown in Fig. 1. The particles are placed on a silicon \( \{0 0 1\} \) wafer and are treated with vapor-phase trifluoroacetic acid (TFA) to break the covalent bond between the biotin molecule and the Sasrin linker prior to the mass spectrometry [6]. The total ion intensity and the molecular ion intensity are shown for each projectile. The higher intensities associated with \( \text{C}_{60}^+ \) ion bombardment compensate for the relatively large beam diameter and result in comparable image resolution to that found for \( \text{Ga}^+ \) ion bombardment. Also interesting to note is that in the total ion image obtained with \( \text{C}_{60}^+ \), the signals originating from the polymer are more intense than those measured from the silicon substrate; however, the opposite is observed in the image obtained with \( \text{Ga}^+ \) where only weak signals originate from the polymer. This phenomenon indicates that molecular desorption by \( \text{C}_{60}^+ \) is most effective with soft and porous substrates rather than hard substrates. This result is in contrast to what is observed for atomic projectiles like \( \text{Ga}^+ \) and \( \text{In}^+ \) ions [7]. Presumably, since each carbon atom in the cluster has 333 eV of kinetic energy, the deposited energy stays closer to the surface, even for these low density targets [8].

The signal enhancement capability of \( \text{C}_{60}^+ \) may be quantitatively evaluated by comparing the intensity of the molecular ion \( (\text{M+H})^+ \) of biotin measured with \( \text{C}_{60}^+ \) and \( \text{Ga}^+ \) on two samples. The first sample is prepared as a thin film by depositing 5 \( \mu \)l of a water solution of biotin (1 mg/ml) on a silicon \( \{0 0 1\} \) wafer and the second sample is a biotin-Sasrin linker-copoly (styrene-1\% DVB) resin after linker cleavage with vapor-phase TFA. Because, the quantity and the physical form of the analytes might not be the same, direct comparison between these two samples is not possible. Instead, results from two different ion sources are compared for each case. The enhancement exhibits a strong dependence upon the nature of the substrate: about 35-fold more signal is observed when the ions are desorbed from the silicon substrate while 110-fold more signal is observed when the ions are desorbed from the polymer support. These data complement the data shown in Fig. 1 and lead to the conclusion that unlike \( \text{Ga}^+ \), \( \text{C}_{60}^+ \) is particular effective for ion
desorption from polymer resin surfaces. The calculated values of the disappearance cross-section ($\sigma$), for the molecular ion are $1.5 \times 10^{-14}$ cm$^2$ for Ga$^+$ ion bombardment and $2.3 \times 10^{-15}$ cm$^2$ for C$_{60}^+$ ion bombardment. Note that by combining the 10-fold smaller value of $\sigma$ with the 100-fold increase in signal intensity that the efficiency of secondary ion formation is found to increase by 1000-fold.

The advantage of using C$_{60}^+$ primary ions is particularly striking when attempting to assay libraries of peptides. Because of the relatively high molecular weight of these biomolecules (generally $>500$ amu), very small signals are observed with Ga$^+$ ions. On the other hand, these peptides give rise to substantial molecular ion signals when employing C$_{60}^+$ primary ions. Comparisons were carried out using two types of peptides built on rink amide MBHA resins. For each type, a solution containing peptides cleaved from a single resin was deposited onto a silicon substrate followed by Ga$^+$ and C$_{60}^+$ ion bombardment. As shown in Fig. 2, for the peptide (Phe)$_4$ at $m/z = 606.3$, a clear molecular ion map is obtainable with C$_{60}$ bombardment. In fact, there is an ion signal enhancement of over 20,000 compared to Ga, with even greater enhancements observed for (Phe)$_5$ at $m/z = 752.37$. Since, a single resin is loaded with about one nanomole of peptide, we estimate the limit of detection of C$_{60}^+$ to these peptides is a picomole or better. This improvement in the detection of peptides allows us to explore the possibility of high-throughput imaging of combinatorially synthesized peptide libraries using C$_{60}^+$.

There is also important information in the fragments observed in the protein spectra. A typical example is shown in Fig. 2, where the phenylalanine fragment from (Phe)$_4$ at $m/z = 120$ is imaged and is also localized to the bead. This capability is essential when attempting to characterize large libraries due to the inevitable appearance of isobaric molecular ions. Currently, collision induced dissociation (CID) and MALDI are the two major tools for acquiring sequencing information, however, neither technique can readily obtain such
information directly from a polymer resin surface. Without applying a matrix or other complex treatments, the resins are directly imaged using C$_{60}^+$. The analysis results in the determination of the full sequence of this peptide. We have confirmed that this observation is not specific to any one peptide by verifying the results with a wide range of peptides with varying composition. In addition, we notice that the fragmentation pattern can be adjusted by employing more sophisticated sample treatments. More details will be discussed in a subsequent paper.

In addition to proteins, lipids are biomolecules of great interest because of their role in drug discovery but also because of their suggested role in various diseases. Since, the enhancement factors associated with polymer surfaces are much larger than for metallic substrates, it is reasonable to suggest that the C$_{60}$ probe will be useful for the imaging lipids on single biological cell membranes. To test this idea, we have used a line of Tetrahymena cells [9], which are currently being investigated by our laboratory in other experiments. These cells are interesting because the
membrane structure is a sensitive function of temperature and conjugation (mating) causes morphology changes to the membrane chemistry. We compare the intensity of the phosphocholine head group ion at m/z = 184 acquired using In⁺ ion bombardment and C₆₀⁺ ion bombardment in Fig. 3 for a collection of freeze-dried cells. Note that for C₆₀ the 50-fold increase in signal level results in brighter pixels with more contrast and therefore better effective image resolution. Preliminary experiments using freeze-fractured cells suggest equally improved performance and the ability to distinguish between domains of lipids with differing composition.

4. Conclusion

The increased secondary ion efficiency associated with cluster ion bombardment promises to greatly expand molecular imaging options with ToF-SIMS. The greater signal intensity results in improved image resolution and contrast when compared to atomic ion bombardment. The emergence of the C₆₀⁺ ion source with good focusing properties is particularly exciting since, the yield enhancements are largest with organic matrices, an environment most appropriate for the imaging of combinatorial beads and biological cells. Moreover, there are prospects in the future for the development of cluster ion sources with probe sizes approaching 100 nm, providing comparable resolution to liquid metal ion guns [10].

References