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Rapid screening of molecular arrays using imaging TOF-SIMS

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Abstract

We explored the application of imaging time-of-flight secondary ion mass spectrometry (TOF-SIMS) to the high-throughput analysis needed for combinatorial chemistry research. Prototypical examples include the characterization of polymer resins, which are chemically modified as part of a combinatorial library synthesis. We studied sample conditioning for various polymer matrices, linker systems, and analytes attached to the linkers and found that the hydrophilicity of the supporting substrates play a very important role in confining the signals to a localized area. We also developed protocols for the high-throughput purpose that use specially designed substrates to hold a large number of resins (as many as 10,000), which avoids cross-contamination among components. Using this approach, we are able to perform chemical assays on polymer resins at a rate of about 1–10 s^{-1}. This analysis has equivalent chemical specificity and sensitivity to but a speed at least an order of magnitude faster than that of ESI–MS or LC–MS.

Keywords: TOF-SIMS; Imaging; Combinatorial chemistry; High-throughput analysis; Polymer resins

1. Introduction

Combinatorial chemistry, which entails the generation of large repertoires of compounds with extensive molecular variations, presents great challenges for analytical techniques. So far, precise molecular structural characterization of synthesized libraries (containing millions of compounds) is not achievable within the same scope of time as the synthesis. We apply time-of-flight secondary ion mass spectrometry (TOF-SIMS) to this task by imaging arrayed samples. Preliminary experiments presented here demonstrate the feasibility of our protocol.

We have been focusing on libraries of solid-phase synthesized small organic molecules (non-peptides). In order to detect those molecules with TOF-SIMS, we found that the covalent linking between the molecules and the polymer matrix has to be clipped [1]. A protocol that involves trifluoroacetic acid (TFA) gas-phase treatment for molecules bound to polystyrene resins via various acid-sensitive linkers was thus developed. In the resulting SIMS images, each resin could be identified by the molecular ions desorbed from it. However, this method only works effectively for certain resins. In addition, as a surface technique, SIMS is extremely sensitive to the sample composition, their physical configurations and matrix effects. All these parameters necessitate a systematic study of SIMS experiments on various combinatorial libraries. Here we investigate these variables, possible mechanisms and choices of substrates.

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2. Experimental

All experiments were performed on a TOF-SIMS instrument equipped with a 25 keV Ga^+ or In^+ LMIG [2]. A low energy electron gun was used for surface charge compensation purposes. In all measurements, ion doses were kept below the static limit.

Resins composed of different linkers were purchased from NOVAbiochem (San Diego, CA) except for the thermal linker, which was synthesized in the laboratory [3]. To simulate members of combinatorial libraries, biotin and stearic acid were covalently attached to the resins. All chemicals used were obtained from Aldrich Chemical (Milwaukee, WI).

Treatments for the resins are divided into three categories based on the linking system. For acid- and base-labile linkers, gas-phase cleavage with suitable volatile organic reagents was used; for thermal- and photo-labile linkers, a delinking process with heating (110 °C for 5 h) or UV radiation (365 nm for 2 h) followed by gas-phase extraction was carried out; and for oxidatively cleavable linkers, the cleavage was conducted in solution. The latter method involves glycerol and a specially built substrate, and is currently under development. The gas-phase reactions were performed in an enclosed 500 ml vessel. The volatile organic reagents were placed at the bottom of the vessel and the resins were held above them. Reagents of 1 and 10 ml were found to work best for cleaving and extracting purposes, respectively, because diffusion of the cleaved compounds was minimized. Treatment time depended on the linker types and varied between several minutes to several hours. Moreover, supporting substrates including silicon, gold (Au), Teflon tape and Para film were all tested to study their effects in SIMS imaging of polymer resins.

3. Results and discussion

Bearing in mind that the most-commonly used matrices: gel-type polystyrene (PS) resins are very rigid systems; either attached or cleaved analytes can hardly diffuse in them. We are also convinced through XPS experiments that most of the analytes are not present on the surface of the resins after synthesis. Therefore, analytes have to be both cleaved and coated to the surface for SIMS detection. Acid-sensitive linkers including Rink, Sasrin, Wang and base-labile linkers like oxime were all treated using the methods mentioned in the experimental section. TFA and hydrazine were employed as the cleaving reagents for acid- and base-labile linkers, respectively. The results for all linkers are similar. An example is shown in Fig. 1a: after a well-controlled gas-phase cleavage, molecular ions of the analytes (stearic acid in this case) are intense and all localize on the original resins.

Conversely, analytes cleaved from thermal- and photo-labile linkers on PS resins by heating or UV radiation were not detectable by TOF-SIMS. To circumvent this problem, a gas-phase extraction with dichloromethane was employed. The cleaved analytes were then drawn out of the resins and residues were formed around each resin on the supporting substrate. Fig. 1b is of such a case, where stearic acid was on thermal linker resins. Molecular ions were only detected from the residues on the supporting substrate, not from the surface of the resins.

Spatial distribution is important for identifying many samples in imaging SIMS. Therefore, we investigated the influence of the substrates on signal migration. Since the organic reagents are hydrophilic, we observed that hydrophobic substrates, such as Teflon tape and Para film confined the cleaved analytes to the

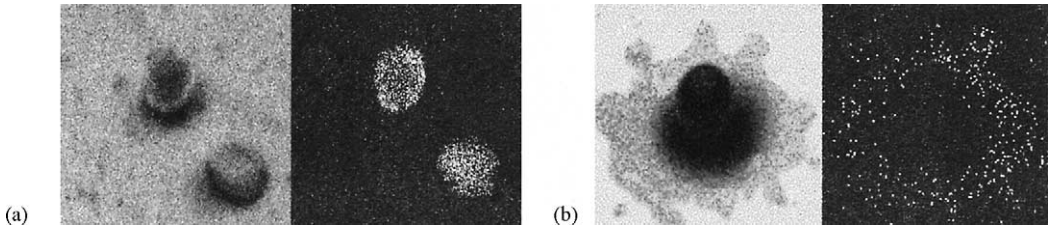

Fig. 1. (a) 300 μm^2 field of view. Stearic acid–oxime–PS resins treated with gas-phase hydrazine: (left) total ion image; (right) molecular ion image. (b) 250 μm^2 field of view. Stearic acid thermal linker–PS resins after gas-phase extraction: (left) total ion image; (right) molecular ion image.

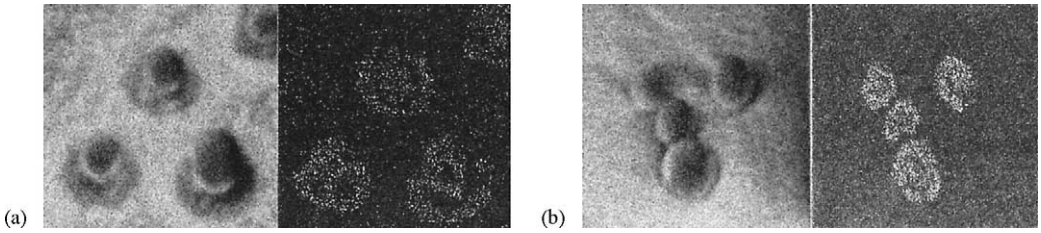

Fig. 2. 300 μm^2 field of view. Stearic acid–Wang–PS resins on Si (a) and on Teflon tape (b), respectively. Both are over-treated with vapor-phase TFA. The ones on the left in each set were total ion images, and the ones on the right in each set were molecular ion images.

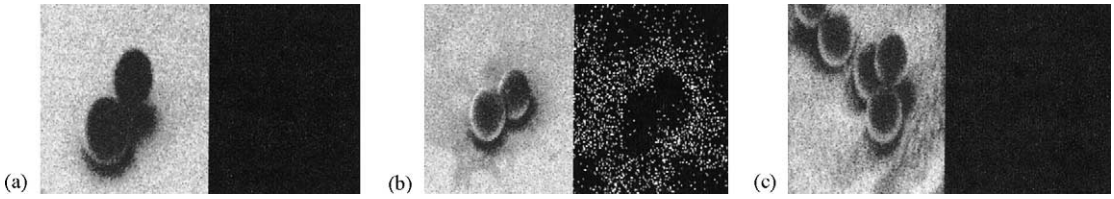

Fig. 3. 400 μm^2 field of view. Stearic acid–Wang–PEG-PS resins on Si (a) and (b) and Teflon tape (c). (b) and (c) were vapor-phase treated with TFA for an elongated period of time, but the treatment time for (a) was just right. Again, the left pictures of each set were total ion images and the right ones were molecular ion images. For (a) and (c), no molecular ion signals were detected.

resins (in the case of acid- and base-labile resins) even when the gas-phase treatment was over-done. However, hydrophilic substrates like silicon (Si) and gold (Au) do not repel the organic reagents and cleaved analytes migrate freely on such substrates. Fig. 2a and b are images of over-treated stearic acid–Wang resins placed on Si and Teflon substrates. The signal-confinement capability of Teflon is clearly demonstrated.

So far, we have only investigated PS resins; however, polyethylene-grafted-polystyrene (PEG-PS) resins are also of current interest. Grafted-polyethylene chains alter the physical and chemical aspects of

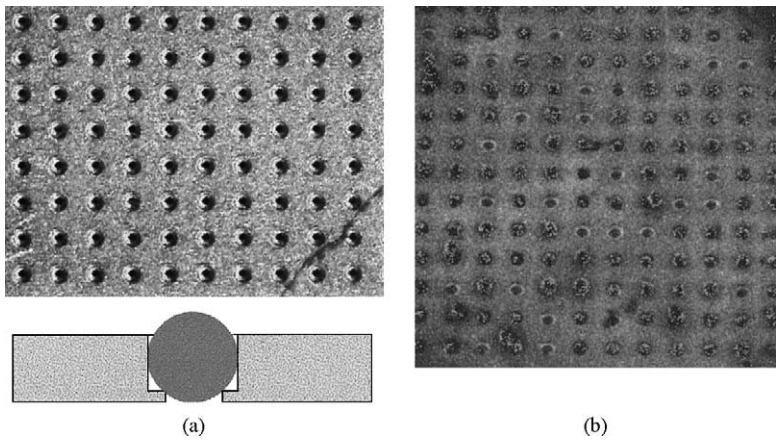

Fig. 4. (a) 800 μm × 1000 μm field of view. The optical micrograph and the cross-section of the specially designed resin arraying substrate. (b) 1400 μm^2 field of view. SIMS image of an array of stearic acid–Wang–PS resins.

the polystyrene resins, making it amphiphilic instead of hydrophobic [4]. As a consequence, PEG-PS resins swell in solvents with a wide range of hydrophilicities: from water to dichloromethane; in contrast, PS resins only swell in hydrophobic solvents like dichloromethane and chloroform.

The effects of polymer matrices on imaging SIMS experiments are compared in Fig. 3. Using substrates to control the spatial distribution of the cleaved molecules, we observed that unlike PS resins, molecules cannot be desorbed from the surface of PEG-PS resins (Fig. 3a and c); instead, they need to be washed from the resins for detection (Fig. 3b). Provided that the cleaving reagent for the acid-labile linker Wang is hydrophilic TFA, for PEG-PS resins, it is found to penetrate into the matrices with the cleaved analytes and leave nothing on the surface; for PS resins, the process is exactly the opposite and the cleaved analytes are brought to the surface.

With confidence in handling different types of resins and understanding reaction mechanisms, we further attempted chemical assay of polymer resins using specially designed and fabricated substrates: circular chips constructed for 50 μm resins and containing up to 10,000 beads/cm^2. Considering that the largest field of view of our mass spectrometer is about 2 mm × 2 mm, as many as 400 beads can be assayed in one TOF-SIMS image. Fig. 4a shows the cross-section of an individual hole in the chip, where the openings on both ends are for loading purposes. A typical example is shown in Fig. 4b using the stearic acid–Wang–PS resin model system. In this picture, the red color denotes the presence of stearic acid and the blue color denotes the presence of ions associated with the arraying disk. 10 Ga^+ ion pulses of 10 ns duration were applied to each pixel at the rate of 3000 pulses s^{-1} and the whole image was acquired in about 1 min.

4. Summary and future directions

Imaging TOF-SIMS is shown to be a feasible technique for the high-throughput analysis of resins synthesized by combinatorial chemistry. The diversity of the resins used in such synthesis results in a very complex situation for sample preparation. Several forces, including the attraction or repulsion between the reagents and the linker, the reagents and the polymer matrices are involved in the cleaving and extracting process of molecular ion signals. As noted earlier, under certain conditions, it is impossible to desorb the molecular ions from the surface of the resins. Our future work will focus on solving this non-ideal problem by restricting the cleaved molecules to an area as small as possible around each resin using substrates composed of both hydrophobic and hydrophilic parts. Enhancing the molecular ion signals is also a goal and we are working on it by improving our instrument with focused ion sources using cluster ion beams. A 10-fold signal increase has already been observed for some molecules.

Acknowledgements

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