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Applicability of Imaging Time-of Flight Secondary Ion MS to the Characterization of Solid-Phase Synthesized Combinatorial Libraries

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We employ imaging time-of-flight secondary ion mass spectrometry to perform high-throughput analysis of solid-phase synthesized combinatorial libraries by acquiring mass spectra from arrays of polymer resin particles. To generalize this procedure to various types of resins and their associated chemical linkers, it is necessary to understand the dynamics associated with the analyte molecules during chemical pretreatment steps. Using stearic acid as a model compound, we examine the influence of three classes of linkers—acid or base labile linkers, a thermally labile linker, and a photochemically cleavable linker— all of which are used to anchor one end of the analyte to the polymer resin. With data obtained using secondary ion mass spectrometry, scanning electron microscopy, and X-ray photoelectron spectroscopy, we conclude that an effective treatment of the resin needs to include cleaving the linker and extracting the unbound analyte to the resin surface. We also demonstrate that the hydrophilicity of the polymeric constituents of a resin particle affects the experiments by changing the location of the analyte molecules during resin treatment. With this information, it is possible to utilize imaging TOF-SIMS to assay a range of material supports with assurance that high-quality spectra can be acquired.

Combinatorial chemistry entails the generation of a large repertoire of compounds with extensive molecular variation and has been employed as a strategy for discovering new compounds in pharmaceutical chemistry, biochemistry, and materials science.[1−3] Collections or libraries of more than 1 million members have been created using this methodology. A major challenge is to reduce the time, effort, and cost required to identify promising compounds from the mix. Although magic angle spinning ^{13}C and ^{1}H gel-phase NMR,[4,5,6] single-bead FT-IR,[7,8] and Raman and mass spectral techniques such as liquid chromatography−mass spectrometry and electrospray ionization mass spectrometry have been employed to address this issue,[9,10] analysis speed is still problematic.

We approach the high-throughput assay of combinatorial libraries synthesized on solid-phase polymer resins through the use of imaging time-of-flight secondary ion mass spectrometry (TOF-SIMS). In imaging SIMS, a beam of energetic primary ions is rastered across the surface of a target and secondary ions are desorbed and detected on the fly. The desorption generally occurs from the top few molecular layers of the sample. A mass spectrum is obtained and stored for each pixel in the image (normally 256 × 256 pixels), and this information can be retrieved later to characterize a small fraction of the entire field. In our case, the mass spectrum of molecules adsorbed to the surface of an individual resin particle in an array of thousands of resin particles is obtained from data imbedded in a single image. Preliminary experiments have demonstrated the feasibility of this strategy.[11−13] By using mass spectrometry, it is possible to identify protonated molecules and other structurally significant fragment ions desorbed by the primary ion beam, enabling direct identification of the target molecule. The combination of the TOF detector with a gallium liquid metal ion gun (LMIG) provides femtomole detection limits[14] for most molecules along with submicrometer lateral resolution for imaging. Moreover, this scheme allows assay of a single resin particle in ∼100 ms.

Our early experiments indicated that the quality of the mass spectrum associated with molecules synthesized on the resin was greatly improved by clipping the chemical linker utilized to anchor one end of the molecule. We devised a scheme whereby the array of particles was exposed to a gas-phase mixture of trifluoroacetic acid (TFA). This exposure released the molecule from the resin without allowing diffusion between individual beads. Hence, it was

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possible to acquire molecular ion information and at the same time to utilize the spatial information inherent in imaging TOF-SIMS experiments for parallel assay of bead arrays.

Although this strategy has been modestly successful for assay of peptide libraries, it has not been generally feasible to extend this protocol to arbitrary molecules or to expect reliable data from resin particles of varying composition. In this paper, we examine a number of experimental variables that have an impact on the quality of the resulting mass spectrum. Specifically, we examine the use of three classes of chemical linkers used in library synthesis, including traditional acid or base labile linkers, photochemically cleavable linkers, and thermally cleavable linkers. In addition, using stearic acid as a model compound for assay, these linkers are attached to either a pure polystyrene (PS) resin of hydrophobic character or to a polyethylene grafted-polystyrene (PEG−PS) resin, which is amphiphilic in character. The results show that all of the linkers can easily be cleaved with the appropriate reagent, but that after cleavage, the concentration of stearic acid on the surface of the resin varies over a wide range. For example, we show using X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy that, for PS resins, TFA not only releases bound molecules but extracts them to the bead surface, increasing their effective concentration for TOF-SIMS analysis. For PEG-PS resins, however, this same treatment drives molecules inside the resin, reducing the intensity of molecular ion signals. This observation is explained in terms of the relative hydrophobic and hydrophilic character of reagent and substrate. With this information, we expect that improved protocols may now be developed that allow routine assay of many types of combinatorial libraries with throughputs approaching the projected capabilities[13] of imaging TOF-SIMS methodology.

EXPERIMENTAL SECTION

General Considerations. Resin particles with standard acid- or base-sensitive linkers and photochemically sensitive linkers were purchased directly from NOVAbiochem except for Sasrin, which was purchased from Bioscience. The thermally cleavable linker was synthesized and coupled to polystyrene as noted below. Stearic acid was chosen as a model molecule for testing our procedures since it can be easily attached to a variety of resin particles and linkers. Dichloromethane (DCM) was distilled from calcium hydride. All other reagents and solvents were used as received from Aldrich. Si {001} wafers (Silicon Quest International) and poly(tetrafluoroethylene) (Teflon) tapes (VWR) were cleaned with ethanol (3×), acetone (3×), and hexane (3×) in a sonicator prior to being used as supporting substrates in SIMS experiments.

Procedure for Coupling Stearic Acid to Sasrin, Rink Amide, Wang, Oxime, and Hydroxyethyl Photolinker Resins. Although these resins have different sizes (200−400 to 100−200 mesh), loadings (0.25−1.07 mmol/g), and polymer matrixes (copoly(styrene−1% DVB) and polyethylene-grafted-polystyrene), their coupling procedures are identical. The resins (0.05 mmol) were swelled in DCM (1 mL) for 30 min. To the resin solution were then added stearic acid (28.4 mg, 0.1 mmol, 2 equiv), DCC (100 μL, 0.1 mmol, 2 equiv), and catalytic DMAP (1.5 mg, 0.25 equiv) in 1 mL of 1:4 (v/v) mixture of DMF/DCM. After being stirred at room temperature for several hours, the particles were filtered, washed intensively with DCM (3×), *i*-PrOH/DCM (3×), and DCM (6×), and then dried in vacuo. The coupling yield was 50−70% for various resins as determined by weight.

Preparation of Stearic Acid-Coupled Acetal-Thermally Labile Linker-Copoly(styrene−1% DVB) Resin. A linker that can be broken with application of modest heat was designed and synthesized onto aminomethyl resins (Sigma-Aldrich). The hydroxyl functionality in the molecule allows further modification with analytes containing carboxylic acid groups. Upon heating at 110 °C for 3 h, the linker breaks and releases the attached molecules. Coupling with stearic acid is achieved through ester bond formation in a fashion similar to that described for the Sasrin resin.

General Procedure for Gas-Phase Release of Analyte Molecules. Volatile reagents are placed at the bottom of a 500-mL glass chamber while the resins are held 5 cm above the solvent surface. Since the glass chamber is sealed with Parafilm, the vapor from the reagents saturates the chamber and reacts with the resins. Typical treating time varies from 20 min to several hours depending upon the type of linkers.[15,16]

XPS Measurements. The XPS data were obtained using a Kratos XSAM 800 PCI with a nonmonochromatic Mg Kα source at 20-mA current, 14-kV electron accelerating voltage, and 40-eV pass energy. The sample is prepared by covering a 5 mm × 5 mm carbon tape with the resins of interest. The XPS experiments are performed on two kinds of samples before and after a 20-min gas-phase cleavage with TFA. Bromine and chlorine peaks are measured and compared for the bromoacetic acid 2-chlorotrityl resins. For stearic acid-Sasrin resins, the spectra in the range of 271−296 eV are compared.

SEM Measurements. The SEM data were obtained using a JEOL JSM-6300F field emission scanning electron microscope operated at 500-V accelerating potential. The working distance is 15 mm, and the objective aperture is 40 μm. The SEM experiments are performed on stearic acid-Sasrin resins before and after a 20-min gas-phase cleavage with TFA.

Imaging TOF-SIMS Measurements. The TOF-SIMS instrumentation has been described in detail elsewhere[17] and is briefly discussed below. A 25-keV liquid metal (Ga^+) ion gun (LMIG) with a dc beam current of 1 nA, a spot size of 100 nm, and a pulse length of 30 ns is used to desorb ions from the uppermost layers of a sample within an ultrahigh vacuum environment. In all measurements, the incident ion dose is kept at or below the static limit of 1×10^{12} ions/cm^2. With a repetition rate of 3000 pulses/s, most images are acquired in less than 5 min. The focus and shape of the beam are adjusted to achieve the best images of the resins. To maximize signal intensities from the polymer resins, charge compensation is performed by irradiating the sample with a pulsed beam of 30-eV electrons having a dc current of 50 nA. The electron beam is allowed to strike the sample for 50 μs after each LMIG pulse, during which period the sample stage voltage is held at 0 V.

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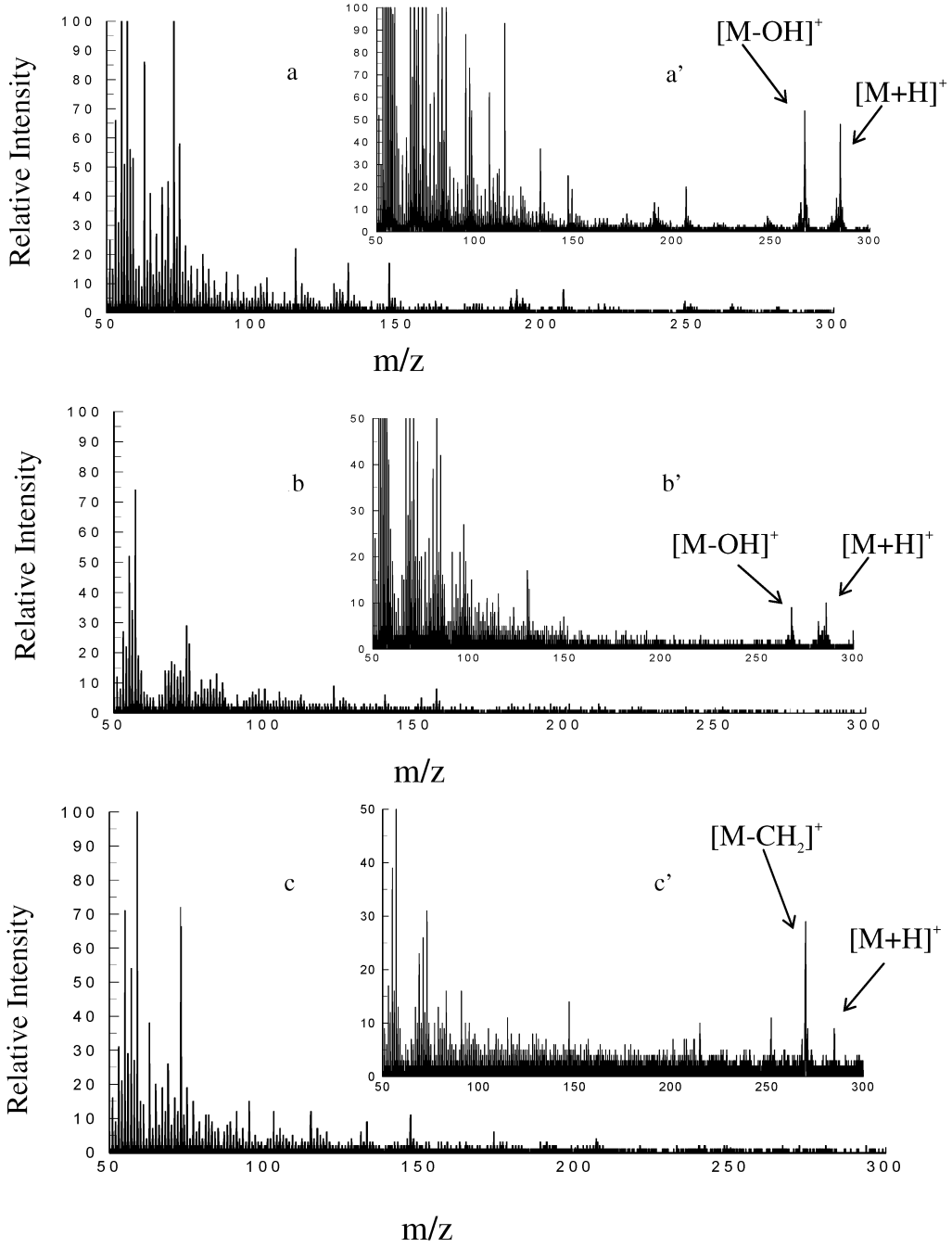

Figure 1. SIMS spectra of PS resins functionalized with stearic acid through various linkers—Wang, thermally labile, and photosensitive linkers before (a, b, c) and after (a′, b′, c′) linker cleavage using the methods described in the Experimental Section. In all cases, no protonated molecule $[M + H]^+$ of stearic acid (*m*/*z* 285.28) is observed before linker cleavage, but after the molecules are clipped from the linker, ions of $[M + H]^+$ (*m*/*z* 285.28), $[M - OH]^+$ (*m*/*z* 267.26), or $[M - CH_2]^+$ (*m*/*z* 270.26) are detected in the SIMS experiments. (a, a′) Stearic acid anchored on the PS beads through acid-sensitive Wang linker. (b, b′) Stearic acid on thermally labile linker. (c, c′) Stearic acid on hydroxyethyl-photosensitive linker.

RESULTS AND DISCUSSION

The essential goal when imaging TOF-SIMS is utilized for the assay of dense arrays of polymer resins is to acquire a mass spectrum that rapidly leads to the molecular weight determination of the analyte molecules. Since this technique is based upon the desorption and ionization of surface molecules after impact of the primary ion, there are several obvious factors that need to be established. The molecules need to be desorbed intact with adequate sensitivity for rapid detection, and they need to retain their spatial integrity to permit the parallel assay of massive arrays.

Desorption of molecules induced by ion bombardment is believed to occur by a correlated liftoff mechanism associated with a cascade of moving atoms within the solid.[18] Effective desorption occurs when the target molecule is located very near the surface of the sample and when it is not strongly bound to substrate atoms.[19] The restriction of desorption to surface molecules has important implications for sensitivity. For example, a medium-

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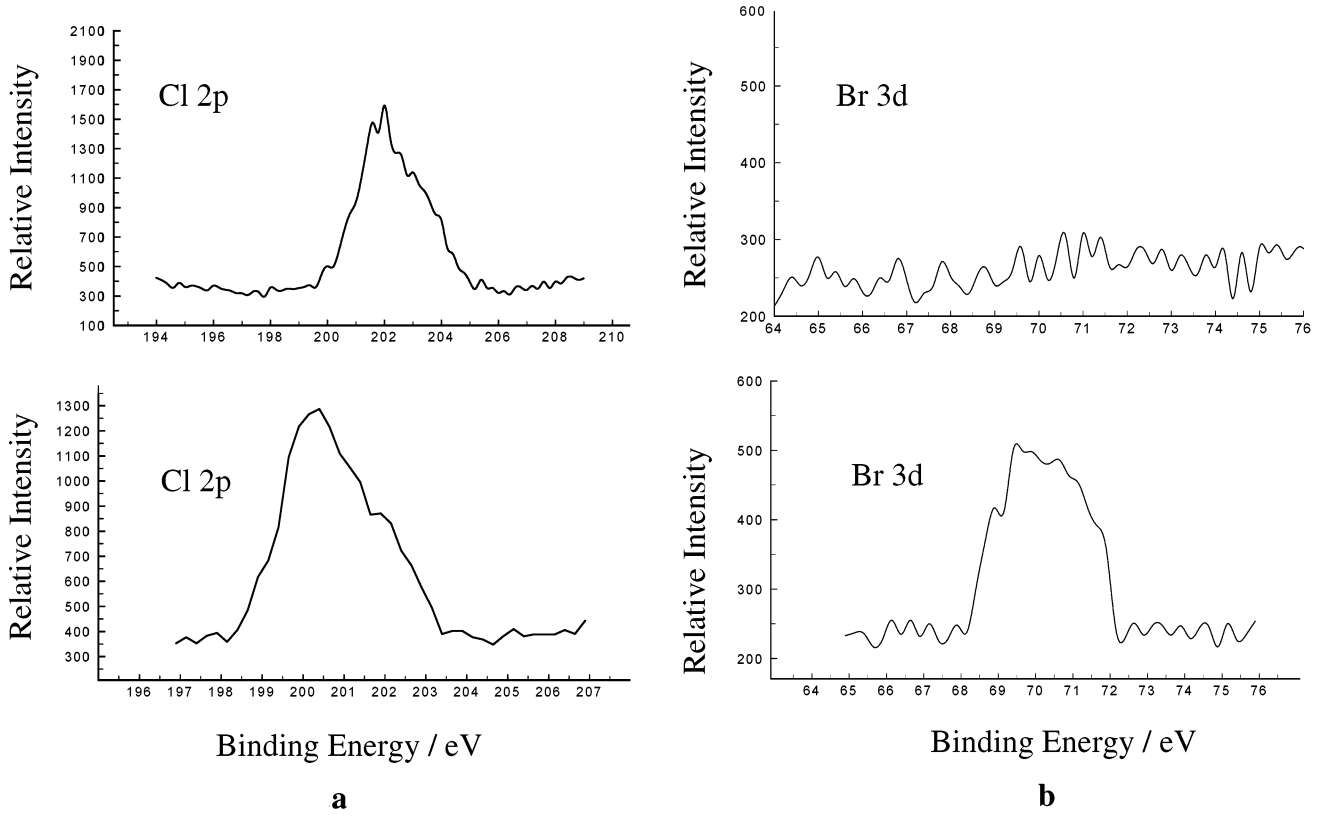

Figure 2. XPS spectra measured on bromoacetic acid 2-chlorotrityl resins at the topmost 25 Å of the samples.(a) Comparisons of chlorine signals before (top graph) and after (bottom graph) gas-phase TFA treatment. Their signal intensities are of the same order. (b) Comparisons of bromine signals before (top graph) and after (bottom graph) gas-phase TFA treatment. Bromine is detected only after such treatment, indicating that its spatial distribution as well as the analyte molecule containing Br element has changed.

loaded 100-μm resin particle containing 800 μmol/g analyte holds 5×10^{-10} mol of material. Assuming a random distribution of molecules, ~1% resides near the surface and is accessible for SIMS detection. Moreover, to avoid damage by the ion beam, the total dose is restricted to less than 1% of the number of surface sites. Hence, there will be less than 5×10^{-14} mol of material available for detection, even under the best of circumstances.

Molecular desorption from surfaces is only efficient if the chemical bond between the adsorbate molecule and the substrate is sufficiently weak to allow the cooperative uplifting mechanism to operate efficiently. Traditional linker moieties employed in solid-phase combinatorial library syntheses have already been shown to inhibit molecular desorption.[13,20] Therefore, clipping the linker is necessary for successful detection of the analytes. This effect is illustrated for stearic acid attached to copoly(styrene−1% DVB) resins through Wang, thermally labile, and photosensitive linkers, respectively. As shown in Figure 1a, b, and c, when the linkage bond is still intact, only fragment peaks of stearic acid below m/z 150 are detected. After bond cleavage with appropriate methods as described in the Experimental Section, intense protonated molecules ($[M + H]^+$ at m/z 285.28) are observed for all three linkers as shown in Figure 1a′, b′, and c′. Although there are still unidentified peaks below m/z 150, the protonated molecular ions are easily identified in the higher mass region.

Only the surface molecules are detected by SIMS, of course, so it is essential to be sure that any sample treatment does not preferentially remove these species. Analyte molecules are present throughout the bulk of the porous polymer support as well as on the surface. To address this issue, we have utilized XPS to determine the surface concentration of specific atomic constituents before and after chemical treatment. Since XPS responds quantitatively to the surface concentration of these elements, it is possible to detect changes in the location of analyte molecules. An example of this type of measurement is shown in Figure 2 using bromoacetic acid 2-chlorotrityl resins (copoly(styrene−1% DVB)) as a model. Here, one Br atom is located on the molecule and one Cl atom is located on the linker. The signal intensities of these two elements are then compared before and after gas-phase cleavage. The Cl signals are of the same order of intensity (1700 vs 1300 cts) (Figure 2a), indicating that the amount of linker does not change significantly during the reaction, whereas Br signals are detected only after gas-phase treatment (Figure 2b). This result implies that, concomitant with cleavage, analyte molecules are extracted to the surface from the bulk.

Such extraction phenomena are further proved using XPS measurements on stearic acid-Sasrin resins (copoly(styrene−1% DVB)). Before gas-phase TFA cleavage, the XPS spectrum (Figure 3a) of the resin shows a shakeup peak at 292 eV, which is indicative of the unsaturated bonds in the aromatic ring of polystyrene matrix. After gas-phase cleavage, this 292-eV peak disappears; instead, a new peak at 290 eV from the C═O stretch of stearic acid appears (Figure 3b). Two control spectra are also taken on pure Sasrin resins (Figure 3c) and neat stearic acid (Figure 3d). It is obvious that the XPS spectra from untreated

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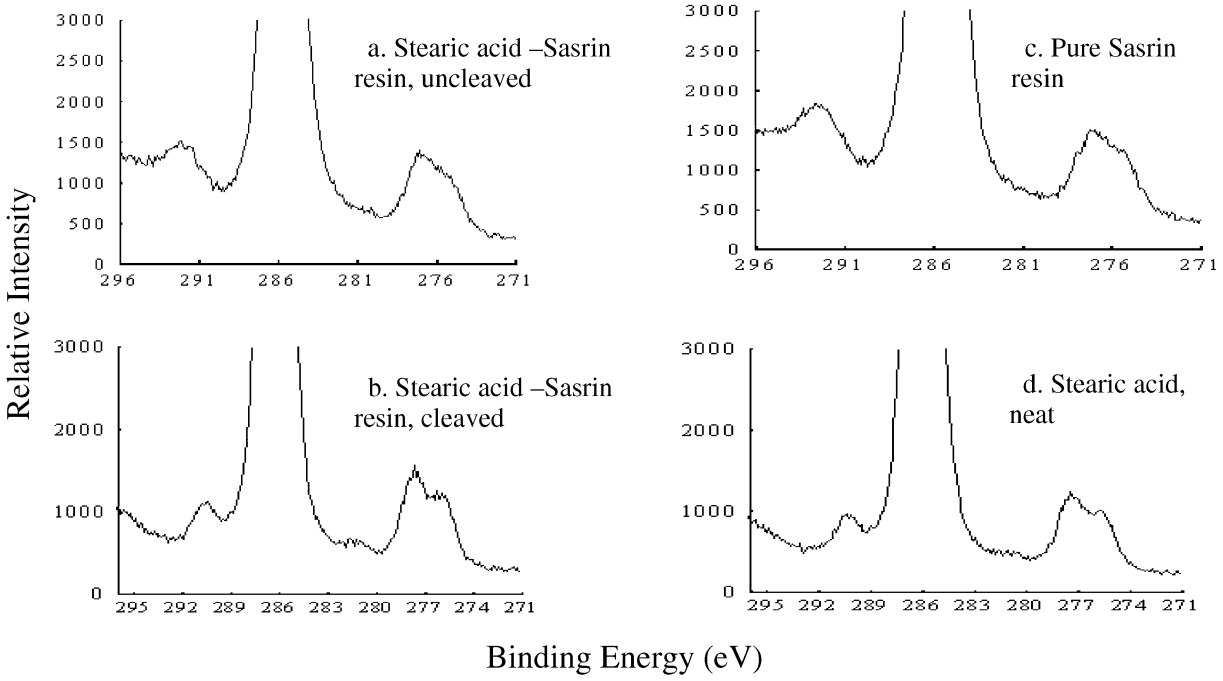

Figure 3. XPS spectra measured on stearic acid-Sasrin resins at the topmost 25 Å of the samples. (a) Stearic acid-Sasrin resin without gas-phase cleavage. (b) Stearic acid-Sasrin resin after gas-phase TFA cleavage. (c) A control sample of Sasrin resin without stearic acid coupled to it. (d) A control sample of neat stearic acid.

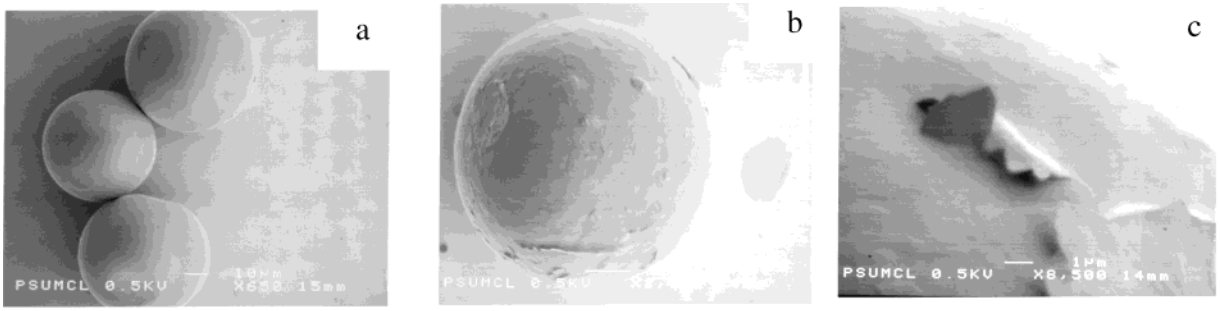

Figure 4. SEM measurements on stearic acid-Sasrin resin. (a) Before gas-phase TFA cleavage. (b, c) After gas-phase cleavage.

stearic acid-Sasrin resins and pure Sasrin resins are almost identical; in contrast, the spectrum from cleaved stearic acid-Sasrin resins resembles the one from neat stearic acid. Again, this result implies that untreated stearic acid-Sasrin resin does not present much stearic acid on the surface; however, the same resin is pretty much covered by stearic acid after gas-phase TFA treatment.

This result is further demonstrated with SEM experiments. Figure 4a is an image of uncleaved stearic acid-Sasrin resins, and the surface of the resins appears smooth. However, in Figure 4b and c (closeup image) of gas-phase cleaved resins, we see deposits on the surface, which could be stearic acid that is cleaved and extracted from the bulk of the resin. Although not every combination of polymer matrix and linker responds to the linker-cleaving reactions in the same fashion, resin sample treatment needs to cleave the analyte from the linker, extract the analyte from the interior of the resins, and retain spatial information.

Since the quality of the assay is dependent upon the linker and the dynamics associated with the analyte molecule during cleavage, it is essential to evaluate several linking strategies. Accordingly, we next examine the behavior of acid/base-labile, thermally labile, and photosensitive linkers as they affect the stearic acid-copoly(styrene−1% DVB) model system.

Acid- and base-labile linkers, as implied by the name, are cleaved by acid or base reagents such as TFA, HF, or hydrazine. Conventionally, the reaction is carried out in the liquid phase. Gas-phase treatment, however, is found to cleave the linker without causing cross-contamination among samples, and thus is our method of choice. Images of the desorbed stearic acid protonated molecules linked via the base-labile linker oxime, is shown in Figure 5a. For this case, the stearic acid is cleaved and extracted to the resin surfaces by gas-phase hydrazine. Such gas-phase sample treatment works equally well for all polystyrene-based acid/base-labile linkers that we have tested. However, an extended treatment is found to not only extract the analyte onto the resin surface but also to wash the molecules onto the supporting substrates.

The thermally labile linker is clipped by heating at 110 °C for 3 h. The SIMS analysis of the cleaved resin does not directly detect any analyte signal from the surface. After exposure to gas-phase dichloromethane, however, a strong protonated molecule peak is

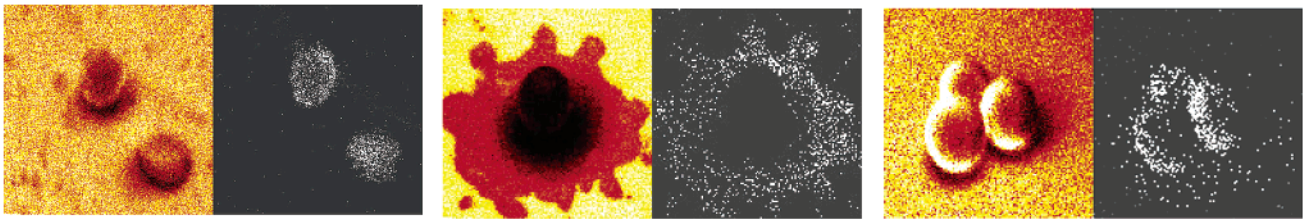

Figure 5. SIMS images measured on three types of polystyrene resins after linker cleavage. Stearic acid is coupled to all three resins through different linkers including a base-labile linker oxime (a), a thermally labile linker (b), and a photosensitive linker (c). All images are 300 μm $\times$ 300 μm. Color images are of total ions in the spectra; black and white images are of only the stearic acid protonated molecules at *m*/*z* 285.28. (a) Stearic acid-oxime-PS resins after gas-phase hydrazine treatment. Stearic acid is desorbed from the surface of the resins. (b) Stearic acid-thermally labile linker-PS resin after heat cleavage and gas-phase extraction with dichloromethane. Stearic acid is desorbed from the residue around the resin but not the resin itself. (c) Stearic acid-photosensitive linker-PS resins after UV cleavage. Stearic acid is desorbed from both the residues and the surfaces of the resins.

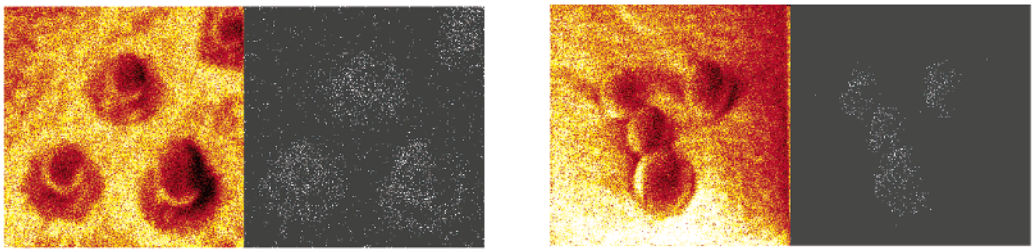

Figure 6. SIMS images that depict dependence of the migration of cleaved molecules on the hydrophilicity of the substrate. The left-hand image shows total ions while the right-hand image shows only the stearic acid protonated molecules at *m*/*z* 285.28. (a) The resins are placed on silicon substrate. Stearic acid is desorbed not only from the resins themselves but also from the residues formed on a hydrophilic Si substrate by extended gas-phase TFA treatment, which washes some cleaved molecules off the beads. (b) The resins are placed on a hydrophobic Teflon film; stearic acid is only desorbed from the surface of the resin particles even after extended gas-phase TFA treatment due to the repulsion between TFA and the substrate. All images are 300 μm $\times$ 300 μm.

observed. For this situation, roughly 10 times as much reagent is required to extract enough analyte for detection, when compared to the oxime-PS-linked resins. As shown in Figure 5b, the analyte is found only in the residue that has been washed from the bead by the extraction process.

Photosensitive linkers such as hydroxyethyl-photolinker AM require a third type of cleaving protocol. Here, the procedure involves the combination of UV radiation and the use of ethanolamine as a catalyst. Resins are first soaked in ethanolamine and then transferred to the silicon substrate, followed by UV radiation at 356 nm for 3 h. Several resins functionalized with stearic acid are imaged with SIMS as shown in Figure 5c. There is a stearic acid signal detected in the residue around every resin. However, unlike the thermally labile linker, analytes are desorbed mainly from the resin itself.

The spatial distribution of the cleaved analytes is critical for our experiments, and hence, the dynamical behavior of the analyte molecules associated with each protocol is essential to understand. We attribute the observed phenomena to the interaction between polymer matrixes and the gas-phase solvent material. The polystyrene-based resin, for example, is hydrophobic in character and swells in solvents of the same nature. Upon exposure to hydrophilic reagents such as TFA, hydrazine, and ethanolamine, it shrinks and closes up. Polystyrene resins normally repel hydrophilic reagents; however, the reaction energetics drives the reagent into the bead, resulting in cleavage of the linker. Because, for such hydrophilic reagents, the free energy at the surface is lower than inside the polystyrene resin, the cleaving reagents are pushed along with the cleaved analytes back to the surface. This complex process helps to explain the distribution of molecules associated with acid/base-labile and photosensitive linkers. In contrast, for the thermally labile linker, only hydrophobic reagents including dichloromethane and chloroform are able to penetrate the resin for extraction purposes. Eventually, the entire resin is saturated with the extracting reagents and further treatment washes cleaved analytes out, forming a residue around the resin. The two mechanisms also explain the different experimental parameters employed.

Migration of the analyte on the supporting substrate can happen during sample treatment and may cause cross-contamination. To constrain such movement, we investigated the supporting substrates, which are divided into two categories according to their hydrophilicities.[20−22] As established in various experiments, hydrophobic substrates such as Parafilm and Teflon strongly repel hydrophilic reagents such as TFA, hydrazine, and ethanolamine and therefore prevent them from transferring to the substrates. Hydrophilic substrates including silicon and gold do not possess such confining capability with any solvent that we have tested. Images of polystyrene-based stearic acid-Wang resins placed on Si and Teflon substrate, are shown in Figure 6a and b, respectively. On Si, cleaved stearic acid is brought to the substrate by excessive TFA, but on Teflon, stearic acid stays on the resin because of the repulsion between TFA and Teflon.

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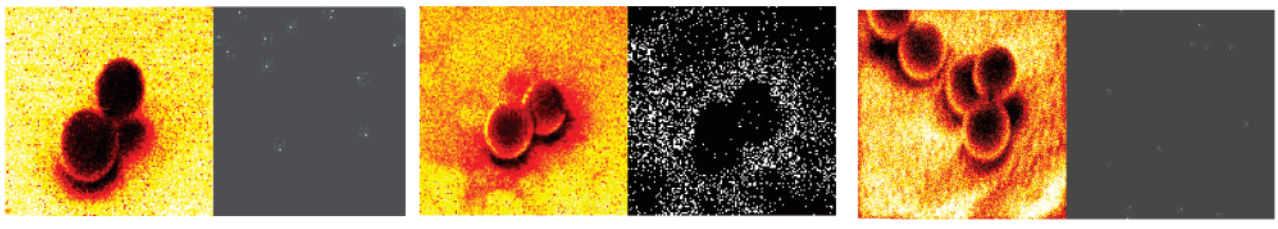

Figure 7. SIMS images measured on gas-phase cleaved stearic acid-Wang linker-PEG-PS resins placed on a Si and Teflon substrates, respectively. The period of TFA gas-phase treatment varies in these cases. Color images are of total ions in the spectra; black and white images are of only the stearic acid protonated molecules at *m*/*z* 285.28. (a) A 300 μm $\times$ 300 μm image of the resins that are placed on silicon substrate. After a well-controlled gas-phase TFA treatment, no stearic acid is detected. (b) A 450 μm $\times$ 450 μm image of the resins that are placed on silicon substrate. After being treated with gas-phase TFA for an elongated period of time, some stearic acid is brought to the substrate and signals come from the residues, but not from the surface of the resins. (c) The image is 450 μm $\times$ 450 μm and on Teflon film. After being treated with gas-phase TFA for an elongated period of time, no residue formed on the substrate and no stearic acid is detected.

The motion of analyte molecules is also strongly affected by the nature of the polymer matrix. For example, grafted polyethylene chains alter the physical and chemical aspects of the polystyrene resins, making it amphiphilic instead of hydrophobic.[23] As a consequence, PEG−PS resins swell in solvents with a range of hydrophilicities that extends from water to dichloromethane. Such variations in polymeric properties lead to fundamental differences in how they react to the same kind of sample treatment. This variation is illustrated by the bead image of stearic acid-Wang linker-functionalized PEG−PS resins on Si and Teflon substrates shown in Figure 7a and b. Unlike PS resins, analytes cannot be desorbed from the surface of PEG−PS resins (Figure 7a and c); they need to be washed out of the resins for detection (Figure 7b). The reason behind this behavior is that hydrophilic TFA penetrates into the polymer matrix, extracting the cleaved analytes with it and leaving nothing on the surface of the resin.

Investigating the behavior of different resins associated with sample pretreatment for imaging TOF-SIMS allows us to generalize our protocols for high-throughput analysis of solid-phase synthesized combinatorial libraries. Figure 8 demonstrates this strategy using the model system stearic acid-Sasrin linker PS resins. More than 100 resins are characterized in this image based on molecular ions desorbed from the resin surface. The resins are randomly distributed on a specially fabricated chip, which is a gold-plated nickel disk 1 cm in diameter. The chip contains holes that match the size of the target resins (50 μm in this case) and could be chemically assayed with imaging TOF-SIMS on one side and screened against a variety of receptors on the other side. The analysis speed of this approach is determined by the instrumental parameters and the density of the arrays. Considering the density of the chip (10 000 resins/cm^2) and the field of view of the mass spectrometer (2 mm $\times$ 2 mm), as many as 400 resins can be assayed in one TOF-SIMS image. Since a typical image is acquired in less than 3 min, assay of the entire array of 10 000 resins could be completed in ~1 h by moving the sample into an appropriate section in 25 2 mm $\times$ 2 mm tiled images.

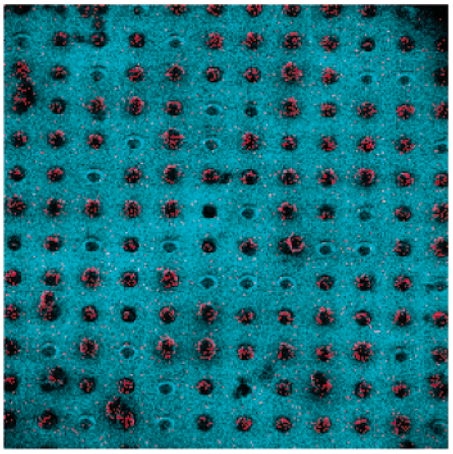

Figure 8. Stearic acid-Sasrin-PS resins randomly distributed on the specially fabricated arraying chip described in text. Image size is 1400 μm $\times$ 1400 μm and taken after gas-phase TFA treatment. Signals of stearic acid protonated molecules, and substrates are displayed in red and in blue, respectively. Signals from all resins are spatially resolvable.

CONCLUSIONS AND PROPOSALS

Imaging TOF-SIMS certainly has the potential to rapidly assay large arrays of resin particles used in the synthesis of combinatorial libraries. As we have shown, several steps are required to attain mass spectra with enough sensitivity to make the approach practical within the combinatorial chemistry arena. Of special note is that it is essential to cleave the chemical bond that holds one end of the analyte molecule to the resin. During this cleavage process it is also necessary to make sure that an adequate number of analyte molecules remain on the surface of the resin and are thus available for analysis. For the pretreatments considered here using a variety of linkers, the analyte molecules have been shown to be surprisingly mobile. The hydrophilicity of the resin particle also plays an important part in determining where the molecules go. In general, we believe that the results presented here will add to our ability to design successful protocols that ensure reliable imaging of the targeted arrays.

In this work, stearic acid has been employed as a model compound. The concepts that have emerged using stearic acid are applicable to many other classes of libraries as well. We have employed the same ideas of hydrophobicity to acquire high-quality protonated molecular ion spectra from proteins with $m/z < 1500$,[24] biotin,[19] and other small molecules. It has also been important to catalog the behavior of several types of linker compounds since the chemical or physical properties of a specific library may

(23) Park, B.-D.; Lee, Y. S. *React. Funct. Polym.* **2000**, *44*, 41−46.

require a specific cleaving temperature, time, or acid/base strength.

This information should also prove valuable as the scope of these assays broadens. For example, we have recently been able to examine resin particles that are bar-coded with different concentrations of Br and Cl. From the intensity of the mass spectral signals, each resin can be identified, leading to a self-deconvolution strategy.[25] Obviously, the dynamical processes discussed here will have an impact on this sort of strategy. Finally, we are in the process of performing similar imaging experiments using a tightly focused C_{60} cluster ion source.[24] With this projectile, we are finding protein signals that are more than 1000-fold larger than when using a Ga ion projectile. All of these developments suggest that the imaging strategy discussed here can be a valuable addition to the repertoire of tools available for combinatorial chemistry research.

(24) Xu, J. Y.; Szakal, C. W.; Martin, S. E.; Peterson, B. R.; Wucher, A.; Winograd, N. Molecular-specific Imaging with Mass Spectrometry and a Buckminsterfullerene Probe: Application to Characterizing Solid-phase Synthesized Combinatorial Libraries, submitted.

(25) Xu, J. Y.; Cheng, J.; Chun, S.; Fenniri, H.; Winograd, N. Barcoded Resins for High-throughput Mass Spectrometric Decoding of Combinatorial Libraries, manuscript in preparation.

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