



Ga⁺ TOF-SIMS lineshape analysis for resolution enhancement of MALDI MS spectra of a peptide mixture

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Abstract

The use of mass spectrometry to obtain molecular profiles indicative of alteration of concentrations of peptides in body fluids is currently the subject of intense investigation. For surface-based time-of-flight mass spectrometry the reliability and specificity of such profiling methods depend both on the resolution of the measuring instrument and on the preparation of samples. The present work is a part of a program to use Ga⁺ beam TOF-SIMS alone, and as an adjunct to MALDI, in the development of reliable protein and peptide markers for diseases. Here, we describe techniques to prepare samples of relatively high-mass peptides, which serve as calibration standards and proxies for biomarkers. These are: Arg8-vasopressin, human angiotensin II, and somatostatin. Their TOF-SIMS spectra show repeatable characteristic features, with mass resolution exceeding 2000, including parent peaks and chemical adducts. The lineshape analysis for high-resolution parent peaks is shown to be useful for filter construction and deconvolution of inferior resolution SELDI-TOF spectra of calibration peptide mixture.

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1. Introduction

Recent advances in soft ionization using laser desorption and ionization (LDI) for mass spectrometry (MS), including matrix-assisted and surface-enhanced embodiments (MALDI, SELDI), have led to sensitive (fmol/amol) detection of unfragmented singly charged “parent” ions up to hundreds of kDa in mixtures [1]. This has spurred research to characterize the protein

MS profiles associated with the changes in cell or organ states (SELDI [2]). The ultimate goals are to describe disease profiles to allow early, non-invasive medical diagnostics and to permit targeted drug development.

Profiling MS is impeded in part by inadequate control of the MALDI ionization process and in part by the irreproducibility of the complex surface preparations [3]. The result is that ion yields for mixtures may not correlate with surface concentrations. Furthermore, the high-throughput electro-optics used sacrifice resolution (SELDI [2]) to gain sensitivity. Increasing detector gain, or using higher laser powers to increase sensitivity, leads to electronics overload

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and the formation of multiply charged ions or multiple adducts [3]. Therefore, MS profiles are currently limited both in the accuracy of the assigned mass number and in quantitative assignment of the relative concentrations.

In this paper, we use two methods to improve TOF-LDI MS profiling. First, we examine angiotensin, vasopressin and somatostatin peptide samples using higher resolution Ga^+ beam TOF-SIMS hardware [4] to validate mass assignments for profiling. We compiled a set of high-resolution SIMS spectra for these peptides on different substrates and in solution with well-controlled sample preparation protocols. Second, we use these higher resolution lineshapes to construct time-domain shaping and spiking filters to deconvolve their LDI spectrum. The methodology used dates back to the work of Wiener [5], and has been successfully deployed in RADAR and geophysics [6]. The method creates a filter that maps noisy unresolved input with overlapped peaks into resolved, narrower target lineshapes while suppressing the noise [6]. Our preliminary results show that output-energy shaping and spiking filters yield an approximately three-fold improvement to resolution in the LDI spectra of the peptides.

2. Materials and methods

Dry powder, refrigerated, 1 mg peptide samples of angiotensin, vasopressin and somatostatin were obtained from Sigma–Aldrich in sealed glass containers. Substrate foils were comprised of 0.25 mm thick silver (99.998%), gold (99.5%) and copper (99.9985%) (Alfa Aesa). Before incubation with the peptide solution, the foils were cut to 1 cm \times 1 cm, etched for 3–4 min in 20% nitric acid, rinsed with d.i. water, ultrasonicated for 5 min, and then rinsed twice with d.i. water. Etching and washing were done to ensure that the native oxides and surface contaminants were removed and the clean non-oxidized substrate was exposed to bind peptides. Aqueous (d.i. water) 10% (w/v) solutions of the peptides were shaken for 5 min to suspend or dissolve them, incubated with etched substrate foils at 320 K for 40–60 min, purge-dried with nitrogen for approximately 5 min, and stored under nitrogen in desiccators before acquisition of SIMS spectra.

Ga^+ TOF-SIMS spectra were acquired for 5 min on a TRIFT II spectrometer (PHI) at pressures of $(2\text{--}4) \times 10^{-8}$ Pa, using a 15 kV primary ion beam of 3.47×10^{11} ions/cm², rastered over an area of 4×10^{-4} cm². Several areas were scanned on the same foil to assess reproducibility. Mass axis calibration was done with at least three masses (Ga, Ag, and C_5H_{10}) to an accuracy better than 3 mDa using Win-cadence 3.3 software (PHI). Spectra of a calibration mixture of seven peptides (vasopressin, somatostatin, dynorphin, ACTH(1-24), bovine insulin-B, human insulin, and hirudin) deposited on a hydrophobic chip surface were acquired on a SELDI spectrometer (CIPHERGEN) at EVMS under usual peptide profiling settings (laser-power 210, 10 Hz rep-rate, sensitivity 7, and a detector voltage 1650 V).

3. Results and discussion

TOF-SIMS often fragments large molecules, so special substrates and preparation methods are used to reduce this effect [7,8]. Fig. 1 shows representative TOF-SIMS results for individual peptides on the various substrates. Silver substrates gave the best spectral reproducibility, resolution, and S/N for parent peptide ions. On gold, there appears to be less efficient uptake of the peptides than on silver, under the same etching conditions, resulting in generally lower S/N for the parent peaks. For copper, only vasopressin appears to bind (with limited efficiency) (Fig. 1(c)); we did not observe a parent peak signal for the other peptides on copper. At a 15 kV Ga^+ beam energy, our resolution is approximately 2500, but the effective resolution is limited by the natural width of the isotopic distribution. This also limits the S/N since the peak counts are spread over these distributions. Signal is further distributed among adducts. As seen in Fig. 1(a) and (b), we observed silver and gold adducts with reproducible isotopic ratios for angiotensin. In general, adducts having one Na and up to three Ag atoms have been observed for all peptides deposited on Ag foils.

Since SIMS detects only the topmost analyte monolayer (~ 10 Å), competition for sites limits uptake of heavier components in concentrated sample mixtures, due to their slower transport, occupation of larger binding regions, and larger conformational barriers to surface binding. High concentrations may also

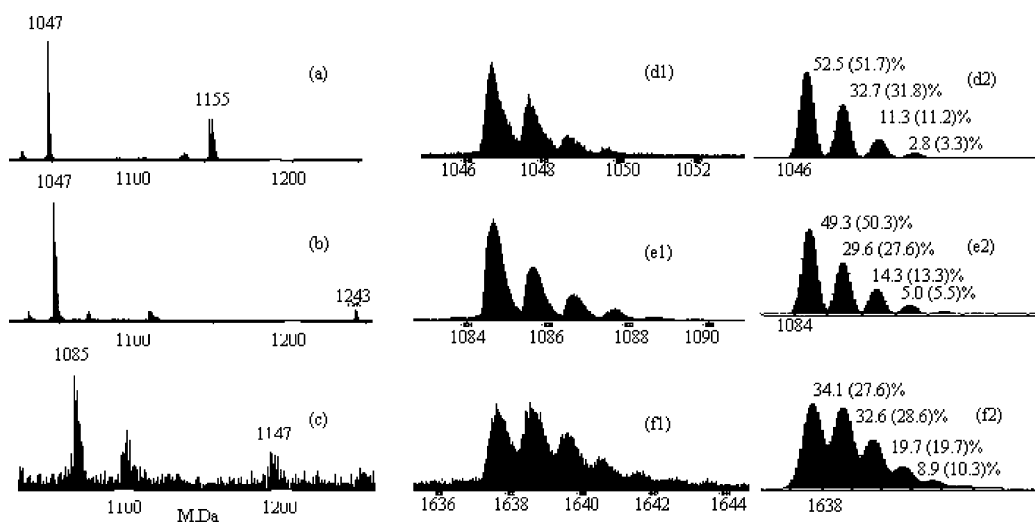


Fig. 1. Parent peak and first substrate adduct for human angiotensin II peptide deposited on etched (a) Ag, (b) Au and (c) vasopressin on Cu. Parent peaks for angiotensin (d1, resolution 2600, C50H71N13O12), vasopressin (e1, resolution 2400, C46H65N15O12S2) and somatostatin (f1, resolution 2300, C76H104N18O19S2) and corresponding calculated [9] isotopic distributions (d2, e2, f2). Theoretical [9] relative peak intensities are shown for the first four most abundant peaks in the distributions (d2, e2, f2) with experimental ratios in parenthesis. Note: sodium (23 Da) adducts are observed in all spectra, in addition to Ag (107, 109 Da), Au (197 Da) and Cu (63, 65 Da).

result in multi-layer formation or polymerization, leading to greater sample fragmentation and lower *S/N* for parent peaks of higher molecular weight analytes [4]. For the 4:1:1 solutions of angiotensin, vasopressin and somatostatin incubated on gold and silver, the observed ratios of total parent intensities in SIMS spectra were 4.0:0.6:0.5 (Au) and 4.0:1.2:0.4 (Ag), which almost reproduces their known relative concentrations in solution (but the heavier peptide peak was smaller by a large margin). Thus, for complex mixtures fragmentation patterns of higher weight species have to be interpreted from TOF-SIMS spectra [4].

Isotopic distributions for peptides with known primary sequences can be predicted theoretically [9] and yield excellent agreement with experiment, as seen in Fig. 1(d2–f2), for both peptide peaks and their adducts with substrate isotopes. Isotopic signatures help to distinguish contaminants from adducts. Fig. 2(a) shows the SELDI spectrum of a seven-peptide mixture having two “regular” patterns near each parent peak that trail towards higher mass. These patterns originate from the formation of adducts with the matrix (sinapinic acid, 224 Da) and with sodium (23 Da). The behavior in LDI is very similar to the SIMS observations, suggesting that the lineshape of LDI peaks can

be reasonably approximated as an unresolved isotopic distribution.

Although parent peaks for this calibration peptide mixture are well separated in the SELDI spectrum, this will seldom be the case for biological samples. However, if a peak in the LDI spectrum can be assigned to a single peptide for which we have a SIMS profile, or whose isotope pattern can be computed with confidence, we can use such a lineshape as a “target” to create a time-domain filter. This filter can then be convolved with the observed spectrum to create a resolved record. Our work has shown that isotopic peaks of lower intensity (starting from the fourth or fifth maximum in the pattern) deviate from the prediction [9] causing the filter to underestimate small peaks in mixtures. Therefore, for deconvolution of low intensity peaks in mixtures, we have found it beneficial to construct target lineshapes from experimentally measured SIMS patterns.

Using experimental SIMS spectra, we resolved eight isotopes for vasopressin and 10 for somatostatin, and constructed deconvolution filters for SELDI data. The SELDI data was spline-interpolated and the SIMS data was locally integrated so the time sampling intervals match. For finite-length time-domain output-energy filters [6] quasi-random artifacts appear

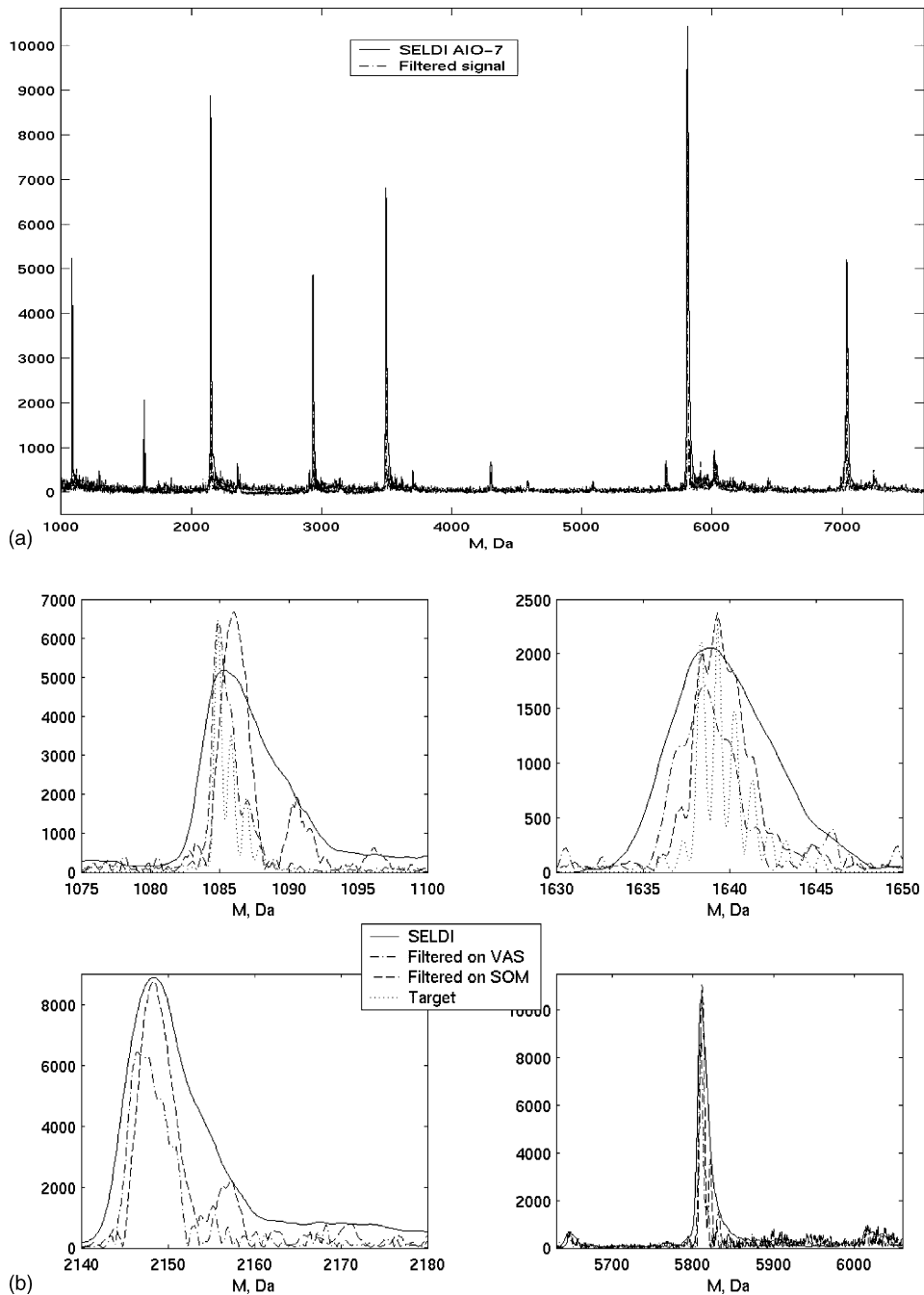


Fig. 2. (a) SELDI calibration spectrum for all-in-one (AIO-7) calibration mixture of seven peptides (solid), and results of output-energy filter deconvolution (dashed) on the basis of target lineshapes (shown dotted in part b) for vasopressin and somatostatin. (b) Comparison of SELDI peak (solid) and deconvolution results (dashed: filter built on somatostatin; dot-dashed: filter built on vasopressin) for vasopressin (1084 Da), somatostatin (1637 Da), dynorphin (2147 Da) and human insulin (5807 Da). Note the detection of small adduct peaks near the parent insulin peak.

around the peaks. We corrected them by choosing filters built on the same source and target lineshapes, but having different lengths (energy-delays). The artifacts are randomly distributed functions of the time-delays. Cross-correlation of the filter outputs conserves the desired target while suppressing the artifacts.

For the work in this paper, four shifted filters were constructed and convolved with SELDI spectra taken with lower resolution; the result is shown in Fig. 2. The output of the filter (Fig. 2(a), dashed) shows an almost three-fold increase in resolution for each broad SELDI peak (Fig. 2(a), solid), with relative intensities matching its target lineshape. We both detect and resolve small adduct peaks (Fig. 2(a) and (e)), and reasonably extrapolate the filter to higher masses (up to 7 kDa), as evidenced by the good match to the expected theoretical isotopic patterns [9] (e.g. for dynorphin and human insulin Fig. 2(d) and (e)). Increasing the number of points per isotopic peak and decreasing the resolution of the target can reduce the amplitude of the artifacts. The trade-off between computation time and resolution will be dealt with elsewhere.

4. Summary

We established a reliable experimental protocol for sample preparation and repeatable detection of parent peaks for several peptides using Ga^+ TOF-SIMS. The best results were obtained on an etched silver substrate. High molecular weight (broad isotopic distribution), low surface concentration, and the presence of impurities are the major factors limiting S/N for parent peak detection. Using TOF-SIMS spectra to construct correlative time-domain output-energy filters, along

with a method to suppress filtering artifacts, yields successful de-convolution of low-resolution LDI spectra. Optimum filter extrapolation strategies will be addressed in future work.

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