Improving Intensity and Sensitivity of MALDI Signals by Using Nanoliter Volume Spots

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Overview

MALDI-TOF mass spectrometry is a concentration-dependent experiment. As such, detection of trace analytes should be enhanced by using small spots of high concentration. In this poster, we introduce and evaluate a method of making nanoliter-volume spots for detecting analytes of limited quantity. We find that better signal intensity can be obtained with nL spots compared to the same quantity of material in µL spots.

Introduction

The quality of MALDI-TOF MS signals normally depends on the concentration of the sample spot that is irradiated by the laser. In many cases, however, the amount of the sample (especially those of biological origin) is limited, and there is considerable difficulty getting high quality MS signals. One way to improve the signal intensity without increasing the sample amount is to make MALDI spots smaller, affording higher concentrations.

Herein, we describe a technique by which nanoliter volume spots (down to 10 nL) can be made for MALDI-TOF detection. The nanoliter syringe and induction-based fluidics (IBF) were employed to insure precise volume deposition, and ionic liquid matrices were used to obtain better homogeneity and reproducibility of signals than would be generated from MALDI spots made with conventional solid matrices. This new approach may have potential for effectively analyzing proteins and other biological samples that are isolated in limited amounts.

Methods

Sample preparation

Bradykinin (BK) solutions of different concentrations (100, 50, 20, 10, 5, 2, 1, 0.5, 0.2 µ M) were prepared in a mixture of acetonitrile/water (1:1, v/v). An ionic liquid matrix (ILM) *n*-butylammonium-α-cyano-4-hydroxycinnamate was dissoloved in a solution of acetonitrile/water (1:1, v/v) to afford a 0.5 M solution. Each time, the BK solution of a specified concentration was mixed with the ILM solution of equal volume. The spotting solution was loaded into a pipet or syringe for the deposition on the MALDI plate by conventional spotting or by the IBF technique, respectively.

Spots deposition

For normal spots (0.5 µL/spot), pipets were used for deposition. For smaller size spots, the IBF nanoliter spotter was used. A syringe containing the sample was placed in the nanoliter wave's inductor. After growth of known volume on the tip, the needle was energized to charge the drop and launch it to the MALDI plate, effecting spherical droplet transmission to the spot where it was aimed (see ThP 030 for details). For deposition of groups of spotting solutions (specified concentration and volume), 12 spots were deposited in adjacent wells of MALDI plate and analyzed.

Mass spectrometry

MALDI-TOF spectra were obtained with an ABI 4700 Proteomics analyzer in the positive-ion, reflector mode. The intensities (or S/N ratio) for the peak corresponding to $[BK + H]^+$ (*m/z* 1060.6) were recorded.

Results

Making nanoliter spots

In cases of normal size spots and nanoliter spots, the matrix formed transparent films on the plate. MALDI from these spots occurred with improved reproducibility (Fig. 1)

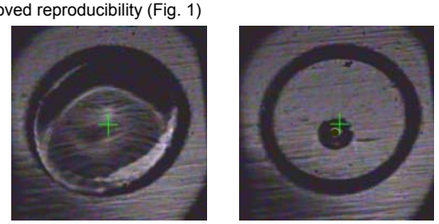

Figure 1. Comparison of images of a normal-size spot (left, 0.5 µL) and nanoliter size spot (right, 10 nL). The images were obtained by the camera on ABI 4700 Proteomics analyzer.

Evaluating MS quality from nanoliter spots with identical analyte concentrations

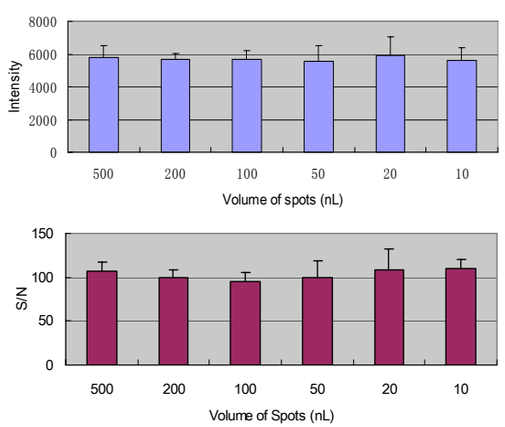

Figure 2. Intensity (top) and S/N (bottom) comparison among spots with 5 µM BK but different spot volumes.

All the spots with different volumes in Fig. 2 showed good homogeneity and, when irradiated, afforded reproducible spectra (RSD < 20%), indicating that ILM advantages are retained for nanoliter spots. The data in Fig. 2 indicate that spots of varying volumes but identical concentration produce nearly identical signal intensities.

Comparison of normal and nanoliter spots containing equal analyte amounts

For constant amount of analyte, nanoliter spots showed significant signal enhancement compared to normal size spots (0.5 µ L), owing to increased concentration in the spot (Fig. 3). The effect is quantified by the ratio of the slopes of the straight lines in Fig. 3. The ratio of the slopes is 1 : 4.6 : 8.3 : 18.5. The effect occurs over the increased dynamic range attributable to use of ILM matrices (Fig. 3).

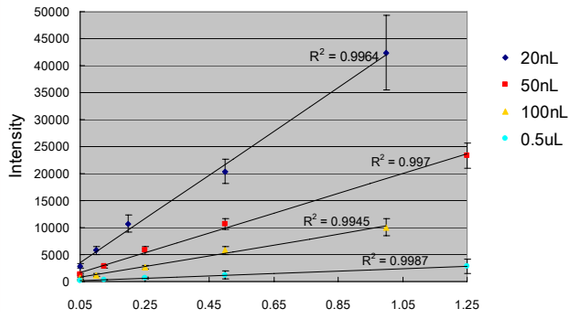

Figure 3. Peak intensity comparison between normal size spots and nanoliter spots containing same concentrations. Volumes are in legend.

With signal intensity enhancement, the spectra obtained from nanoliter spots are clearer (less relative "chemical noise") than those from normal size spots with the same amount of analyte. We expect this advantage will make peak identification easier when the analytes are in a complicated mixture.

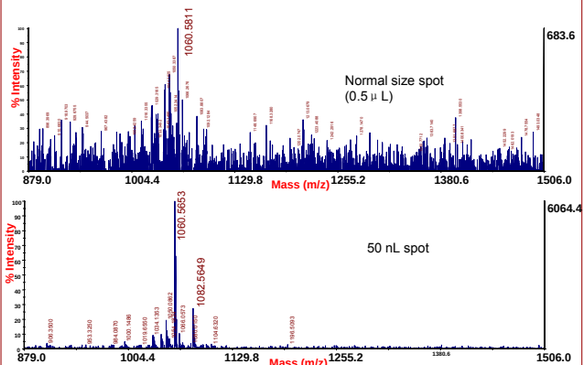

Figure 4. Comparison between spectra obtained from nL and uL spots.

In Fig. 4, we see that for a normal size spot (upper) and a nanoliter spot (bottom), both containing 0.25 pmol bradykinin, the intensity of the molecular ion peak increased by more than 8 times when the MALDI was from the nanoliter spot.

Detection limit

In many cases the sensitivity achievable with ionic liquid matrices (ILMs) was lower than with solid matrices because there are no "hot spots", where there is high concentration of sample owing to the idiosyncrasies of crystallization. One way to raise the sensitivity is to add the same amount of sample to a smaller quantity of matrix to increase concentration and "mimic" the phenomenon of "hot spots." This requires a deposition approach whereby small quantities of liquid can be handled accurately and precisely. Such an approach permits the use of trace amounts of materials, but with more efficiency than when using larger drops as in conventional MALDI. When using *n*-butylammonium CHCA and BK, for example, one can obtain analyte signal from spots with concentrations as low as 50 nM. The resulting detection is of 0.5 fmol BK when a 10 nL spot was used. This is comparable with the detection limit achievable by using CHCA (0.25 fmol), but the approach allows the advantages of ILMs (large dynamic range, high reproducibility) to be maintained.

Conclusions

- The use of nanoliter spots afford significantly improved signal intensity and sensitivity when using a specified amount of analyte. Thus, the sample is used more efficiently.
- The advantage of ionic liquid matrices (the homogeneity, signal reproducibility, wide concentration dynamic range) can be maintained by using nanoliter spots.

Future Work

Future work will focus on exploring the versatility of the method:

- apply to other kinds of analytes
- apply to other matrices (including traditional solid matrices)

Acknowledgements

- This research was supported by the NIH National Center for Research Resources (P41 RR000954).
- Mr. Andrew D. Sauter for cooperation and advice.

References

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55th ASMS Conference – Indianapolis 2007
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