

Ionic liquids as matrixes for MALDI-MS

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Introduction

MALDI's success is due in large part to the development of a number of effective matrixes and an increased understanding of their roles and uses. Solid matrixes are more widely used because they are simple and provide good results. Nevertheless, a serious problem remains; namely, analytes cannot be dispersed throughout a solid matrix in a homogenous way [1]. Thus, the operator must search for a "sweet spot," resulting in poor reproducibility and poor quantification, which are important factors particularly in the application of MALDI to proteomics. Room-temperature ionic liquids have high solubilizing power, negligible vapor pressure, and broad liquid range. Like the classical solid matrixes, appropriately designed ionic liquids have strong absorptivity of the laser light and good solubility of samples. We expect that they will produce a more homogenous spot than solid matrices, thus offering an opportunity to achieve better reproducibility and quantification.

Methods and Instrumentation

Synthesis and confirmation of ionic liquids: Ionic liquids of α -cyano-4-hydroxycinnamic acid (CHCA) were prepared by dissolving 0.5 g of acid in 15 mL methanol. After an equimolar amount of base was added, the mixture was sonicated for 5 min then filtered and evaporated by using a rotavaporator to remove the solvent. All products were dried under vacuum for 6 h to remove residual solvents. The molecular weights of all ionic liquids were confirmed with a PerSeptive Biosystems Voyager-RP time-of-flight mass spectrometer, using both positive and negative-ion modes. In the positive-ion mode, 2-(2-Benzotriazolyl)-p-cresol (BTAC) was used as matrix, and in the negative-ion mode, ionic liquids were used as self-matrixes.

Ionic liquids tested as MALDI matrixes: All ionic liquids were dissolved in acetonitrile / water (2:1, V/V, 0.1% TFA) at a concentration of 0.4-0.7 M. Bradykinin, human insulin and poly (ethylene glycol) (PEG, average $M_n \sim 2000$) were chosen as test analytes. Bradykinin and human insulin were dissolved in ionized water at a concentration of 10 μ M while PEG 2000 was dissolved in the mixture of acetonitrile / water (2:1, V/V, 0.1% TFA) at 1 mM. Samples were prepared by first vortexing 4 μ L of matrix solution and 4 μ L of test analyte solution for 1 min and then spotting 1 μ L of mixture solution on a stainless steel plate. MALDI-TOF spectra were obtained with the same instrument in the positive-ion mode.

Quantification: Bradykinin and Tyr-Bradykinin were chosen as analyte and internal standard separately. The analyte solutions were diluted to 100, 200, 300, 400, 500 μ M from a 1000- μ M stock solution; the 100- μ M solution was further diluted to give 20, 40, 60, 80- μ M solutions. The internal standard was kept at a constant concentration of 100 μ M. A 2 μ L volume of the analyte was premixed with 2 μ L of internal standard and 6 μ L of matrix solution to give the spotting solution. For each analyte/internal standard ratio, five sample spots were prepared by loading 1 μ L of the sample/matrix mixture on the MALDI plate. Mass spectra were accumulated for 50 laser shots for each sample spot. This was repeated for the other four sample spots. A noise filter (NF 0.7, where 0.7 is the correlation factor) was applied, and an automatic Y offset was used to set the base line to zero. The peak-height ratios of the analyte relative to those of the internal standard were calculated and averaged from the five measurements at each concentration. Standard deviations and relative standard deviations for average peak-height ratio from different concentrations were also determined.

Results and Discussion

Signal improvement: MALDI-TOF spectra results from positive and negative-ions modes verified the molecular weights of all ionic liquids. Bradykinin, human insulin and PEG 2000 were tested on MALDI-TOF, using the various ionic liquids as matrixes. For most of the ionic liquids, good signals can be obtained with mass accuracy of within 50 ppm, which is the quoted accuracy of our instrument. The signal could be reproduced reliably from different portions of the spot, attesting to the homogeneity of the sample preparation. Moreover, the resolving power obtained for the human insulin (fw 5807.6) was 600

(linear mode), which is two times compared to that obtained when the human insulin was desorbed from solid matrixes. Finally, ionic liquids outperformed the analogous solid matrix in their abilities to promote ion formation at comparable laser intensities. The signal to noise ratio of human insulin in MALDI-TOF spectra has been improved from 3000 for CHCA matrix to 4500 for ionic liquid 6: diethylammonium α -cyano-4-hydroxycinnamate (**Figure 1**).

Quantification: Tyr-bradykinin was used as an internal standard in the quantification of Bradykinin for experiments in which ionic liquids were used as MALDI matrixes. Good linear relationships were obtained for the tested ionic liquid 1: triethylammonium α -cyano-4-hydroxycinnamate, ionic liquid 2: tripropylammonium α -cyano-4-hydroxycinnamate, and ionic liquid 3: tributylammonium α -cyano-4-hydroxycinnamate (**Figure 2**). This showed ionic liquids as useful matrixes in peptide quantification with MALDI. This result is very surprising. Typically, application of MALDI quantification is not satisfying by using the solid matrix. With special techniques, such as employing a matrix-comatrix to improve the signal reproducibility for quantitative analysis, quantitative measurements by MALDI are possible when appropriate measures are taken. But now, by using the ionic liquids as matrixes, we can get the good quantification results without any additional steps or techniques.

References:

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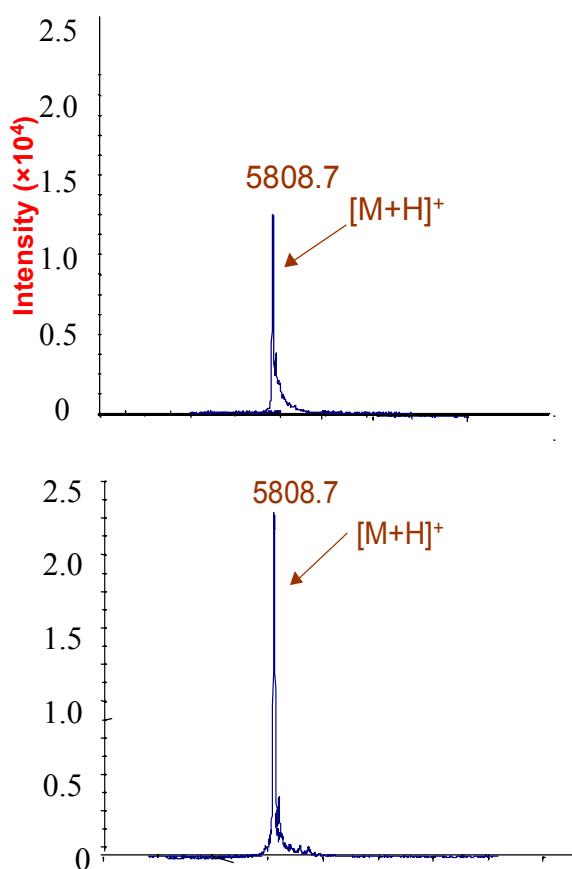


Fig 1. MALDI mass spectra of human insulin in a matrix of (a) CHCA; (b) ionic liquid 6

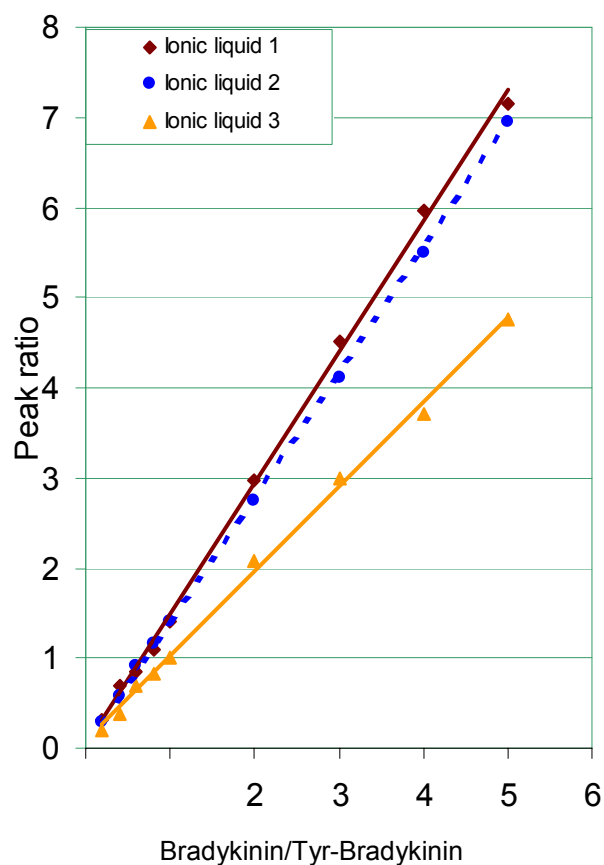


Fig. 2 Quantification of Bradykinin by ionic liquids 1, 2, and 3