

# Removal of monovalent cation adducts using a matrix additive during MALDI-TOF-MS analysis of peptides

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## Overview

MALDI-TOF-MS analysis of peptides often can be impaired by the presence of monovalent cations, such as sodium and potassium. Cations induce the formation of matrix adducts that often suppress the overall ionization of analyte and overlap with low abundance sample components thus affecting analysis in the mass range below 1400 Da. Also, cations adduct to peptides thus shifting the mass and interfering with identification of parent proteins, from database searching, and post-translational modifications. Common solid phase extraction techniques often result in significant sample loss. Here we present a study that describes a method for cation adduct removal by application of a zwitterionic surfactant blend.

## Introduction

Proteins in a purified or complex sample are commonly identified by enzymatic proteolysis followed by mass spectrometry (MS) analysis and sequence database matching. Naturally, reduction of sample complexity is essential for obtaining highly sensitive and accurate results. One source of sample complexity is adduction of monovalent cations to peptides. Monovalent cations such as sodium and potassium ions are contaminants that originate from commonly used buffers or from incompletely deionized water. During matrix-associated laser desorption ionization time-of-flight (MALDI-TOF) MS analysis<sup>1, 2</sup>, these cations can associate with peptides and cause the formation of adduct clusters in the spectrum. The adduct cluster peaks repeat at intervals of (M-1) Da, where M is the molecular mass of the cation.

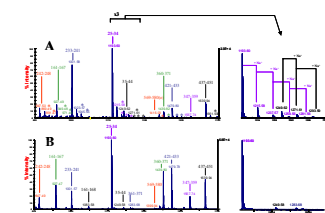
The presence of cation adduct clusters in MALDI-MS spectra can easily complicate a peptide mass fingerprint (PMF) analysis. Adducts reduce the sensitivity of the analysis by partitioning the signal intensity arising from a single peptide into various adduct cluster peaks. Adduct clusters can also suppress the signal of an overlapping or neighboring peak of low abundance. Monovalent cation adduction can also preclude the characterization of PTMs.

This study describes experiments that demonstrate the capacity of a proprietary zwitterionic surfactant blend to sequester monovalent cation adducts. Application of the surfactant blend as a matrix additive for MALDI-TOF-MS analysis markedly reduces the complexity of sodium-rich peptide samples. Application of this surfactant blend also eliminates the problem of sample loss associated with many solid-phase extraction protocols

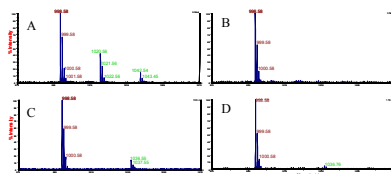
## Methods

All experiments were performed on an Applied Biosystems Voyager DE-STR MALDI-TOF-MS instrument. Analysis of tryptic digests of bovine serum albumin (BSA) (1.5  $\mu$ M) were performed to assess the efficiency of cation adduct removal, the recovery of proteolytic fragments and the statistical scores of sequence database search (Mascot, Matrix Science) by the zwitterionic surfactant blend versus solid phase extraction. A quantitative analysis that demonstrates a concentration dependent relation between removal of cation adduct and surfactant concentration was performed using Bradykinin as the test analyte. All experiments were performed using alpha-cyano-4-hydroxycinnamic acid as the matrix. Solid phase extraction using C18 ZipTips (Millipore) were used according to manufacturer's directions.

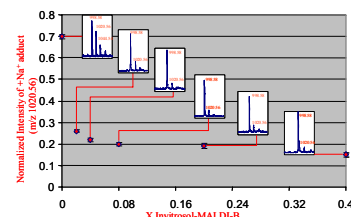
## Results and Discussion



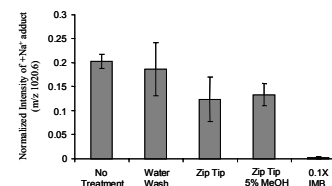
**Figure 1.** MALDI-TOF-MS analysis of the PMF for BSA. Spectra of trypsinized BSA in the absence (Spectrum A) and presence (Spectrum B) of 1X IMB between m/z 825 and 1700 are shown. The numbers above each mass-ion identify the corresponding region of amino-acid sequence for BSA. The font color is coordinated with identified Na<sup>+</sup> adduct(s). The spectra on the right are expanded views of the spectra between m/z 1190 and 1310.



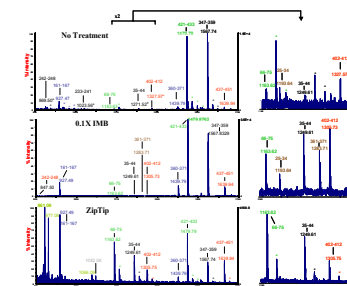
**Figure 2.** MALDI-TOF-MS of Bradykinin. Bradykinin (m/z 998.58) was analyzed in the presence of 50 mM NaCl (A) or 50 mM KCl (C) the sodium adducts (m/z 1020.56, 1042.54) and potassium adduct (m/z 1036.55) are identified by green font. When Bradykinin in 50 mM NaCl (B) or 50 mM KCl (D) are co-spotted with 0.4X IMB 1:1 (v/v) the adducts are markedly reduced or eliminated.



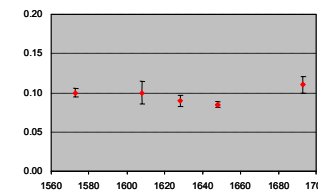
**Figure 3.** Plot of normalized intensity of +Na<sup>+</sup> adduct versus concentration of IMB. Bradykinin was analyzed in the presence of 250 mM NaCl and varying concentrations of IMB. The normalized intensity of the first sodium adduct peak (m/z 1020.56) was measured by the formula (intensity of m/z 1020.56/ intensity of m/z 998.58 + intensity of m/z 1020.56), and plotted against the concentration of IMB. The values on the X axis represent fractions of 1X concentration of IMB. A representative spectrum (linear mode MALDI-MS) for each data point is shown in the plot. The ion intensity of m/z 998.58 in each spectrum is  $\sim 1 \times 10^4$ . Error bars represent standard deviations (n=3).



**Figure 4.** Comparative analysis of common adduct removal techniques versus IMB. The normalized intensity of the first sodium adduct peak (m/z 1020.56) was measured by the formula (intensity of m/z 1020.56/ intensity of m/z 998.58 + intensity of m/z 1020.56). The relative values of the sodium adduct peak are plotted for comparison, where each technique is labeled on the X axis. Error bars represent standard deviations (n=3).



**Figure 5.** Comparative analysis of BSA digest by MALDI-MS. 200 fmol of BSA tryptic digest were analyzed directly (top spectrum), after mixing with IMB (middle spectrum), or after ZipTip (bottom spectrum). The numbers above each mass-ion identify the corresponding region of amino-acid sequence for BSA. The font color is coordinated with identified Na<sup>+</sup> adduct(s). The spectra on the right are expanded views of the spectra between m/z 1150 and 1350. Brown colored mass-ions indicate unique peaks to each spectrum, grey colored mass-ions indicate matrix cluster peaks.



**Figure 4.** Plot of normalized intensity of +Na<sup>+</sup> adduct versus laser intensity. The normalized intensity of the first sodium adduct peak (m/z 1020.56 Da) was measured by formula (intensity of m/z 1020.56/intensity of m/z 998.58 Da + intensity of m/z 1020.56 Da), and plotted against the laser intensity of the acquisition. The ion intensity of m/z 998.58 Da in each spectrum is  $\sim 1 \times 10^4$ .

## Conclusions

The results of the present study demonstrate that our new method for cation adduct removal is effective even in salt concentrations as high as 250 mM. Relative ion intensities between the Bradykinin parent ion and the adduct ion were compared under various surfactant concentrations in 250 mM NaCl. The results demonstrate an inverse linear relation between the normalized intensity of the Bradykinin sodium adduct peak and the surfactant concentration. We hypothesize that the zwitterionic surfactant is capable of sequestering cations and prevents cation association with matrix molecules during matrix crystallization. We tested the surfactant as a MALDI matrix additive for peptide mass fingerprint analysis of a BSA tryptic digest in the presence of 50 mM NaCl. As predicted, the surfactant removed sodium adducts and significantly improved Mascot scores and sequence coverage (114 and 64% versus 96 and 46%). Further, a comparative analysis demonstrates that the surfactant is capable of removing sodium cation adducts more effectively and with markedly better recovery yields than reverse-phase solid phase extraction.

## References

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- (2) Hillenkamp, F.; Karas, M. *Methods Enzymol* **1990**, *193*, 280-295.
- (3) Leite, J.F.; Hajivandi, M.; Diller, T.; Pope, R.M. *Rapid Commun. Mass Spectrom* **2004**, *18*, 2953-2959