

MALDI-TOF MS Analysis of Proteins by an Enhanced, Rapid Guanidination Procedure

Steven L. Cockrill, Kelly L. Foster, Justin Wildsmith, Melissa C. Spears, April R. Goodrich, John G. Dapron, Tom C. Hassell, William K. Kappel and Graham B.I. Scott
Sigma-Aldrich Biotechnology, PO Box 14508, Saint Louis, MO 63103 USA

Overview

Purpose

- To demonstrate the utility of the guanidination method for enhanced analysis of proteins by MALDI-TOF MS.

Methods

- Proteins were denatured, reduced, alkylated and digested with trypsin.
- Tryptic peptides were either analyzed directly by MALDI-TOF MS or guanidinated and then analyzed by MALDI.
- Following analysis, unguanidinated tryptic peptides were removed from the MALDI target, guanidinated, and re-analyzed by MALDI.

Results

- As a result of guanidination, sequence coverage was significantly increased for a variety of model proteins.
- Following a sub-optimal MS analysis, samples could be completely removed from the MALDI target, guanidinated and successfully re-analyzed by MS.

Introduction

In proteomics research, trypsin is commonly used for protein digestion to produce peptides with molecular masses in the optimal range for MS analysis. Tryptic peptides containing a C-terminal arginine undergo preferential ionization and are therefore more efficiently detected in MALDI-TOF MS when compared to lysine containing species. To reduce this bias and enhance overall ionization, lysine residues can be guanidinated to convert the ϵ -amine side chain to a homoarginine group as shown in **Figure 1**. Following guanidination, increased MS peak intensity is observed for lysine containing peptides. This results in enhanced ability to identify proteins by providing a larger number of candidates for peptide mass fingerprinting (PMF).

Commonly, the guanidination method is employed on solutions directly after tryptic digestion. In this study, guanidination was also performed on samples after spotting on a MALDI target and analysis. This methodology would facilitate data recovery following generation of an unsuccessful or non-optimal MALDI spectrum.

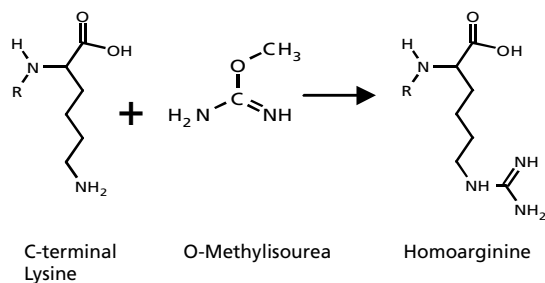
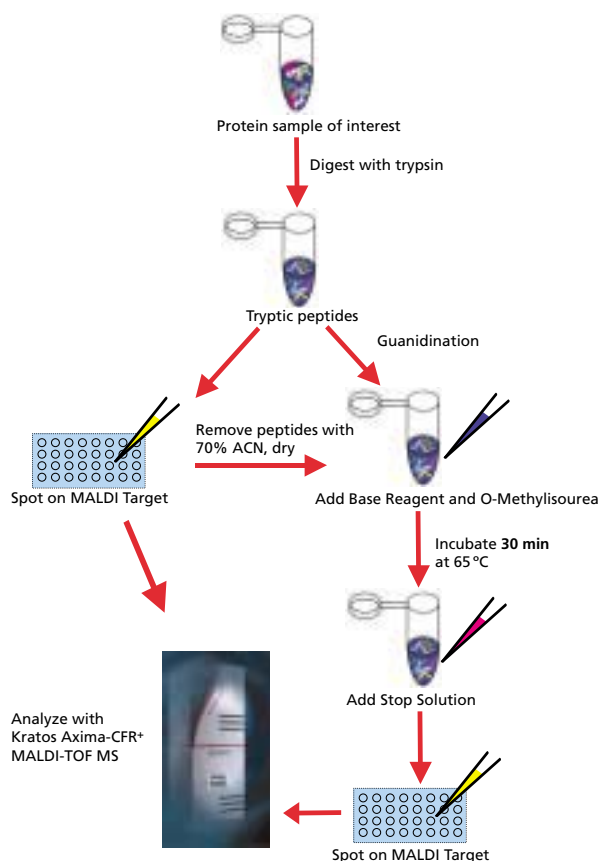


Figure 1: Guanidination Reaction

Materials

- ProteoMass™ Guanidination Kit (Sigma-Aldrich Product Code [MS0100](#))
- Model proteins: Lysozyme, α -Lactoglobulin, α -Lactalbumin, Myoglobin, Apolipoprotein A1, Fetuin, Transferrin, Carbonic anhydrase I, and β -Casein (Sigma-Aldrich Product Codes [L 6876](#), [L 4756](#), [L 6010](#), [M 1882](#), [A 0722](#), [F 3004](#), [T 8158](#), [C 4396](#), and [C 6905](#))
- Trypsin, Proteomics Grade (Sigma-Aldrich Product Code [T 6567](#))
- ProteoPrep™ Reduction and Alkylation Kit (Sigma-Aldrich Product Code [PROT-RA](#))
- Urea Solution, 8M after reconstitution (Sigma-Aldrich Product Code [U 4883](#))
- MALDI matrix, α -cyano-4-hydroxycinnamic acid (Sigma-Aldrich Product Code [C 8982](#))
- ProteoMass Peptide MALDI-MS Calibration Kit (Sigma-Aldrich Product Code [MS-CAL2](#))
- All other reagents used were obtained from or prepared at Sigma-Aldrich

Methods



- Prior to tryptic digestion, samples were reduced and alkylated. Proteins were denatured in 8 M urea, 20 mM Bis-tris propane, pH 8.5 and reduced with tributylphosphine (TBP) at a final concentration of 5 mM. Alkylation was performed with 15 mM iodoacetamide. To prepare samples for enzymatic digestion, 50 mM ammonium bicarbonate was added as a diluent to obtain a final concentration of 1 M urea.
- Aliquots of approx. 100 µg protein were digested with 2 µg trypsin pre-dissolved in 1 mM HCl. Samples were digested for 12–18 hr at 37 °C.
- Guanidination was performed following the instructions provided with the ProteoMass Guanidination Kit. 10 µl of Base Reagent (2.85 M ammonium hydroxide) and 10 µl of O-Methylisourea solution were added to 10 µl of the peptide sample. The mixture was incubated at 65 °C for 30 minutes. The reaction was terminated by adding 30 µl of Stop Solution (10% TFA).
- Samples, either before or after guanidination, were combined with an equal volume of *p*-cyano-4-hydroxycinnamic acid solution (10 mg/ml CHCA in 70% ACN, 0.03% TFA) and spotted onto a MALDI target.
- MALDI-MS data was acquired using a Kratos Axima-CFR⁺ mass spectrometer in positive ion reflectron mode.
- For selected samples, peptides were removed from the MALDI target by pipetting 1 µl of 70% ACN onto the spot, mixing well and removing the solution to a microcentrifuge tube. This was repeated twice more. The washes were combined and dried in a vacuum centrifuge.
- Samples removed from the MALDI target were guanidinated following a similar procedure to the one noted above with the exception that all of the volumes were reduced by 50%.
- The final reaction mixture was dried in a vacuum centrifuge and reconstituted with 2 µl 70% ACN and 1 µl CHCA solution.
- MALDI-MS data was acquired as above .

Results

Increased Sequence Coverage

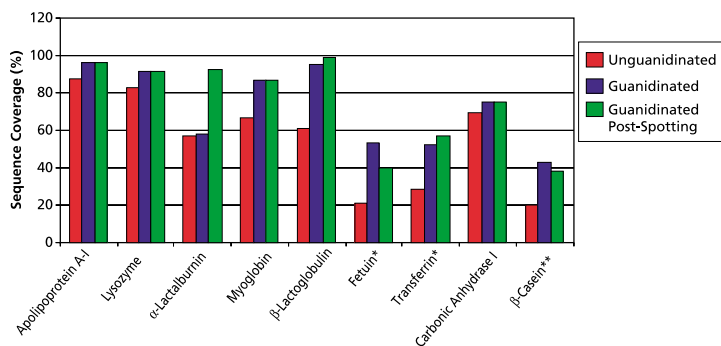


Figure 2: Comparison of sequence coverage for protein tryptic digests before (red) and after guanidination. Samples were guanidinated in solution following the procedure for the ProteoMass Guanidination Kit directly from a tryptic digest (blue) or after removal of peptide spots from a MALDI target (green). In all cases, sequence coverage significantly increased following guanidination.

PMF Comparison: Myoglobin

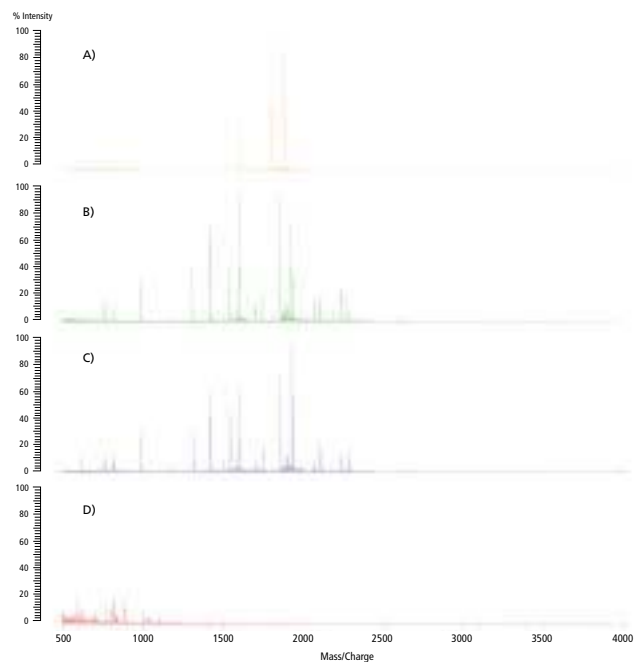


Figure 3: MALDI-TOF MS analysis of a tryptic digest of myoglobin. The peptides were analyzed A) without guanidination, B) with guanidination following the procedure in the ProteoMass Guanidination Kit, and C) with guanidination following removal of the sample from the target. A greater number of peptides were identified in the guanidinated samples resulting in a significant increase in sequence coverage. Spectrum D demonstrates that the peptides were largely removed from the target. The peaks observed in this final spectrum are consistent with matrix clusters.

PMF Comparisons: Transferrin and β-Lactoglobulin

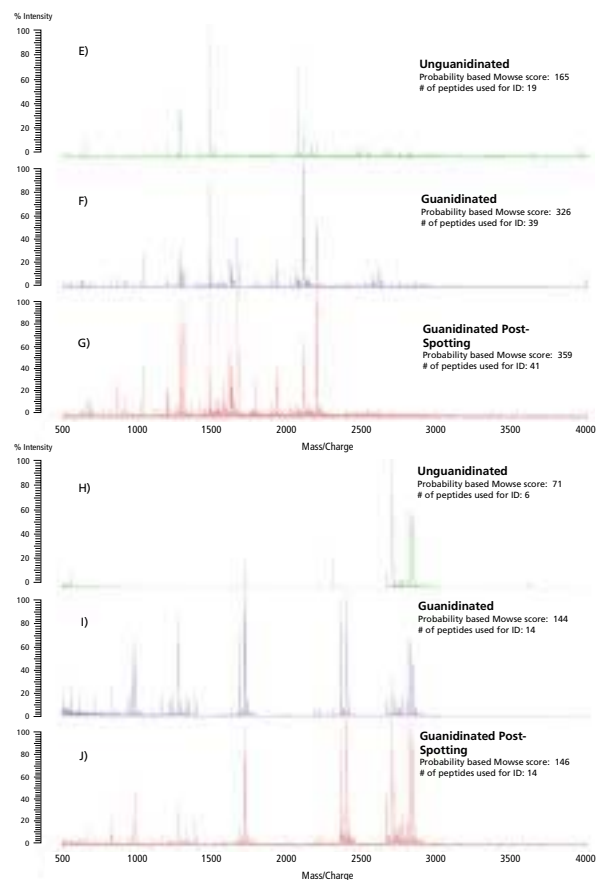


Figure 4: MALDI-TOF MS analysis of tryptic digests of Transferrin (E - G) and β-Lactoglobulin (H - J). The peptides were analyzed without guanidination (E, H), with guanidination directly following digestion (F, I), and with guanidination following removal of the sample from the target (G, J). Guanidination provides enhanced MALDI spectra of protein digests which results in increased confidence in protein identification. Probability based Mowse scores were obtained using the Mascot search engine at Matrixscience.com.

Conclusions

- Guanidination of C-terminal lysine residues results in increased sequence coverage for a variety of protein types including glycosylated and phosphorylated proteins.
- Following an original MALDI analysis, peptides could be removed from the MALDI target, guanidinated in the presence of the MALDI matrix, and re-analyzed to obtain a larger number of identifiable peptides.
- Sample information can be recovered, and even enhanced following this modified guanidination procedure.
- Guanidination is a fast and reliable tool for the protein mass spectrometrists to facilitate protein identification with a higher level of confidence.

