

Simple chemical treatments to improve MALDI-TOFMS peptide maps

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Overview

The occurrence of peptides within a peptide map could be altered, using a combination of guanidination and treatment with a detergent.

Introduction

Trypsin digests produce peptides terminating in lysine or arginine. Of these two classes arginine terminating peptides are more readily ionized and hence dominate the MALDI-TOF mass spectrum. Guanidination is commonly used to increase the representation of lysine terminating peptides by converting lysine to homo-arginine. More recently detergent has been shown to alter the distribution of ionized species. We argue here that detergent is probably altering the distribution of peptides within a micelle, so that arginine terminating peptides are removed from the MALDI environment so allowing other less ionizable peptides to ionize. By using a combination of guanidination and detergent treatment all the peptides within a map can be represented.

Methods

Trypsin digests: In solution digests of 1mg/ml protein were prepared in 0.04M ammonium bicarbonate.
SDS Treatment: Equal volumes of digest were mixed with 0.15 % SDS (1.5 mg/ml) in solvent (50% aqueous acetonitrile, 0.1% TFA). Treatments were sonicated for 15 minutes, centrifuged at 13K rpm for 1 minute, then sonicated again for 15 minutes.
Guanidination: Digests were reacted with 100 mg/ml o-methyl isourea hemisulfate in 3.3M ammonium hydroxide. Reaction complete in 1 hour . Reaction mixture was acidified with TFA and zip tip purified.
Samples were co-crystallised on steel targets with 2.5 mg/ml αcyano-4-hydroxy- cinnamic acid in solvent. MALDI-TOF mass spectrometry was performed on an LaserToF RS available from **SAI, Manchester, UK**.

Results

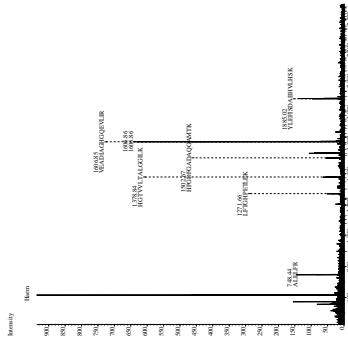


Figure 1: Peptide map, no treatment

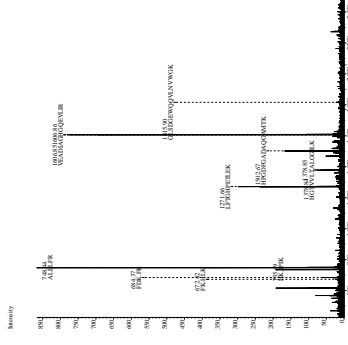


Figure 2: SDS treatment

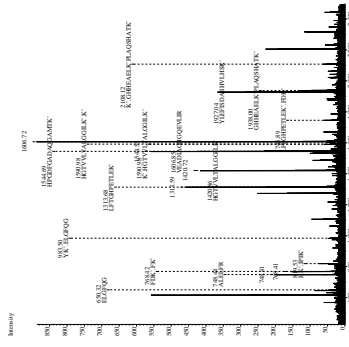


Figure 3: Guanidination treatment

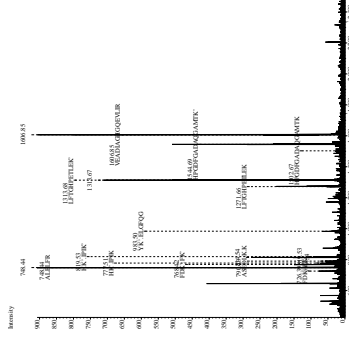


Figure 4: Combined SDS and guanidination treatments

Sequence Hits

GLSDGEWQQVLNVWGK**V****EAD****I****A****G****H****G****Q****E****V****L****R****L****F****T****G****H****P**
E**T****L****K****F****D****K****F****K****H****L****K****T****E****A****E****M****K****A****S****E****D****L****K****K****H****G****T****V****L****T****A****L****G****G****I**
K**K****G****H****E****A****E****L****K****P****L****A****Q****S****H****A****T****K****H****K****I****F****I****K****Y****L****F****I****S****D****A****I****H****V****L****H****S****K****H****P****G****D****F**
G**A****D****A****Q****G****A****M****T****K****A****L****L****F****R****N****D****I****A****A****Y****Y****K****E****L****G****F****Q****G**

Red font: sequence found with no chemical treatment
Underline: sequence found with sds
italic small: sequence found with guanidination
@url: sequence found with combined guanidination and sds treatments

Discussion

From a combination of treatments (guanidination and sds) there are only 2 sequences unaccounted for: TEAMK and NDIAAY, giving a sequence coverage of 92.1%. It is possible that NDIAAY is not seen because there is an absence of a nucleophilic arginine or lysine, and so the peptide competes less favorably for ionisation and subsequent detection. It is also possible that guanidinated TEAMK is not witnessed because it is isobaric with the third isotope of ALELFR.

The three treatments give their own detection advantages:

- 1) SDS is the only treatment to bring forward the sequence GLSDGEWQQVLNVWGK
- 2) Guanidination is the only treatment to contribute KKGHHEAELKPLAQSHATK
- 3) Combined guanidination and sds is the only treatment to reveal ASEDLK

References

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Beardsley RL, Reilly JP, Anal Chem, 74(8):1884
Brancia, F.L., Oliver, S.G., Gaskell, S.J. Rapid Commun. Mass Spectrom. 14, 2070.
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