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# INCREASED SEQUENCE COVERAGE BY PEPTIDE MASS FINGERPRINTING USING MALDI IN COMBINATION WITH N-TERMINAL MODIFICATION OF TRYPTIC PEPTIDES

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## INTRODUCTION

Peptide mass fingerprinting (PMF) is a widely used MALDI TOF MS technique for the identification of proteins. PMF relies on the comparison of experimentally obtained peptide masses from enzymatically digested proteins with theoretical peptide masses, obtained through the in silico digestion of proteins contained within protein or translated nucleotide databanks. The most widely used enzyme is trypsin due to its availability in a pure form and compatibility with mass spectrometry. For a typical PMF experiment, data are obtained from an m/z range of 800 Da - 4000 Da. This range is chosen, as few tryptic peptides have masses greater than 4000 Da and in the m/z range from 0 Da - 800 Da many species that are unrelated to the protein are also detected, e.g. matrix ions. An initial theoretical calculation was conducted to ascertain the fraction of tryptic peptides which contain seven or fewer amino acid residues, by randomly selecting 100 proteins from a SWISS-Prot database and theoretically digesting them with trypsin assuming no missed cleavage sites (see Figure 1). From this experiment it can be seen that ca 50% of tryptic peptides have seven or fewer amino acid residues, corresponding to 20.5% of total sequence coverage. Peptides of this size fall within the m/z range of 0 Da - 800 Da. In the work presented here we show a strategy for increasing the mass of small peptides by 572.182 Da through N-terminal derivatization using N-Tris (2,4,6-trimethoxyphenyl) phosphonium-acetic acid N-hydroxysuccinimide ester (TMPP-acOSu). This increase in mass brings smaller peptides into the detection window usually set for MALDI analysis. An added benefit of the use of TMPP-ac is that it directs the fragmentation of singly charged ions in MS/MS experiments.

## EXPERIMENTAL

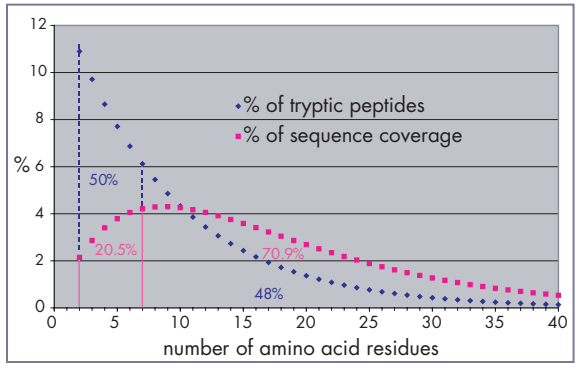


*Figure 1: Theoretical peptide length distribution of tryptic peptides*

### Sample Preparation

Standard tryptic digests, Alcohol dehydrogenase (ADH) (Waters, Milford MA) and bovine serum albumin (BSA) (Waters, Milford MA), were used to evaluate the TMPP-acOSu derivatization. The dry digest standards were reconstituted in 20 mM triethylammonium buffer (1:4 acetonitrile/water) to give a final concentration between 0.5 and 10 pmol/µl. TMPP-acOSu solution was made up in anhydrous acetonitrile, produced using an AccQ fluoro reagent kit (Waters, Milford MA). The TMPP-acOSu concentration was varied to give ca a 20 molar excess. A 20 µL portion of digest solution was combined with 0.2 µL of TMPP-acOSu solution, this mixture was left for 20 min after which it was acidified using 0.2 µL of concentrated trifluoro acetic acid (TFA) (Aldrich, Poole UK).

### Mass Spectrometry

MALDI MS experiments were performed on a Waters® Micromass® MALDI mass spectrometer and a Waters Micromass MALDI Q-Tof™. MS/MS experiments were carried out using a Waters Micromass MALDI Q-Tof. Samples were prepared by mixing 0.5 µL of sample with 0.5 µL of an α-Cyano-4-hydroxycinnamic acid solution (2 mg/mL in 1:1 acetonitrile/TFA (0.1%v/v)) and spotting this mixture onto a stainless steel target plate.

## RESULTS AND DISCUSSION

MALDI TOF spectra were obtained for an ADH digest and a BSA digest with the samples analyzed before and after TMPP-ac modification. Figure 2 compares the MALDI TOF MS spectra for TMPP-ac modified and un-modified BSA. The mass spectra were deconvoluted and searched against a SWISS-Prot databank using ProteinLynx™ Global SERVER 2.0. In both cases the proteins were correctly identified as the top hit in the database search. In the derivatized sample no unmodified peptides were matched indicating that all peptides in these samples were labelled. A comparison of the search results from the labelled and unlabelled sample shows that some of the peptides matched were homologous, many however, were only found in one of the two spectra. Peptides containing less than seven amino acid residues were only detected in the TMPP modified samples, whereas larger peptides were often seen in both the modified and unmodified samples.

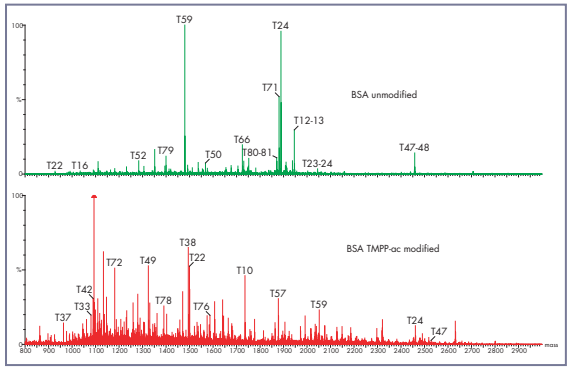


*Figure 2: MALDI TOF spectra of TMPP-ac modified and unmodified tryptic digest of BSA*

The effect of TMPP-ac N-terminal modification on sequence coverage is illustrated with coverage maps for ADH and BSA presented (Figures 3 and 4). Without modification the sequence coverage achieved for ADH was 49.4% and for BSA 34.5%. With the N-terminal modification sequence coverage for ADH was 43.4% and for BSA 62.3%. The peptides matched in the modified sample only were generally small peptides, as these were of low mass and not detected without modification. Peptides matched from the underivatized sample only tended to be larger peptides. Several peptides were matched in both derivatized and underivatized samples.

**ADH**

SIPETQK|GVIFYESHGK|LEHK|DIPVPKPK|ANELLINVK|YSGVCHTDLHAW
HGDWPLPVK|LPLVGGHEGAGVVVGMGENVK|GWK|IGDYAGIK|WLNGSC
MACEYCELGNESNCPHADLSGYTHDGSFQQYATADAVQAAHIPQGTDLA
QVAPILCAGITVYK|ALK|SANLMAGHWVAISGAAGGLGSLAVQYAK|AMGY
R|VLGIDGGEGK|EELFR|SIGGEVFIDFTK|EK|DIVGAVLK|ATDGGAHGVINV
SVSEAAIEASTR|YVR|ANGTTVLVGMPAGAK|CCSDVFNQVVK|SISIVGSYV
GNR|ADTR|EALDFFAR|GLVK|SPIK|VVGLSTLPEIYEK|MEK|GQIVGR|YVV
DTSK|RPPGFSPF

Identified in unmodified ADH only (31.4% coverage, 6 peptides)
Identified in TMPP modified ADH only (25.4% coverage, 5 peptides)
Identified in both TMPP modified and unmodified ADH (18.0% coverage, 5 peptides)
Not present in MS spectrum (25.4% coverage, 5 peptides)
Substitutions identified using MALDI QTof

*Figure 3: Sequence coverage map for alcohol dehydrogenase*

**BSA**

DTHK|SEIAHR|FK|DLGEEHFK|GLVLIAFSQYLQQCPFDEHVK|LVNELTEF
AK|TCVADESHAGCEK|SLHTLFGDELCK|VASLR|ETYGDMADCCEK|QEP
ER|NECFLSHK|DDSPDLPK|LKPDPNTLCDEFK|ADEK|K|FWGK|YLYEIAR|
R|HPYFYAPELLYYANK|YNGVFQECCQAEDK|GACLLPK|IETMR|EK|VLAS
SAR|QR|LR|CASIQK|FGER|ALK|AWSVAR|LSQK|FPK|AEFVEVTK|LVTDL
TK|VHK|ECCHGDLLECADDR|ADLAK|YICDNQDTISSK|LK|ECCDKPLLEK|
SHCIAEVEK|DAIPENLPPLTADFAEDK|DVCK|NYQEAK|DAFLGSFLYEYS
R|R|HPEYAVSVLLR|LAK|EYEATLEECCAK|DDPHACYSTVFDK|LKHLVD
EPQNLIK|QNCDQFEK|LGEYGFQNALIVR|YTR|K|VPQVSTPTLVEVSR|SLG
K|VGTR|CCTKPESER|MPCTEDYLSLILNR|LCVLHEK|TPVSEK|VTK|CCTE
SLVNR|RPCFSALTPDETYVPK|AFDEK|LFTFHADICTLPDTEK|QIK|K|QTA
LVELLK|HKPK|ATEEQLK|TVMENFVAFVDK|CCAADDK|EACFAVEGPK|L
VVSTQTALA

Identified in unmodified BSA only (8.6 % coverage, 4 peptides)
Identified in TMPP modified BSA only (36.4 % coverage, 30 peptides)
Identified in both TMPP modified and unmodified BSA (25.9 % coverage, 13 peptides)
Not present in MS spectrum (29.2 % coverage, 30 peptides)

*Figure 4: Sequence coverage map for bovine serum albumin*

The total coverage achieved when the information from the TMPP-ac modified and the unmodified experiments were combined was 74.6% for ADH and 70.8% for BSA. Therefore, in this case combining data from the derivatized and underivatized analyses, the overall sequence coverage is much greater than that achieved in the separate experiments.

As well as a means of increasing sequence coverage, TMPP-ac modification is also a useful tool for directing fragmentation in MALDI MS/MS experiments. Figures 5 and 6 show an example of a MS/MS data obtained from a tryptic peptide of ADH (GVIFYESHGK) both with and without modification. The fragmentation observed from singly charged peptides is notoriously sequence dependent often leading to spectra that are very difficult to interpret. The MS/MS spectra generated with a TMPP-ac tag are more predictable, as the charge is localized on the modifier. The associated MS/MS spectra typically show a strong N-terminal fragment ion series such as the a-type ions.

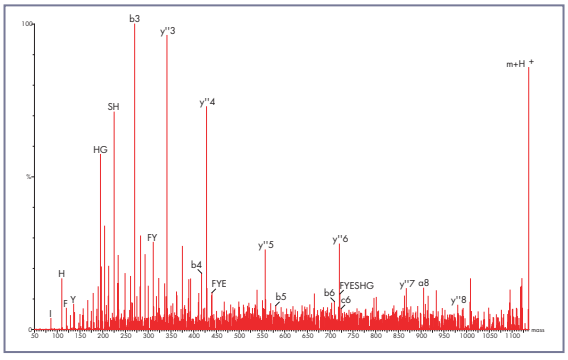


*Figure 5: MS/MS Spectrum of unmodified GVIFYESHGK (tryptic peptide from ADH)*

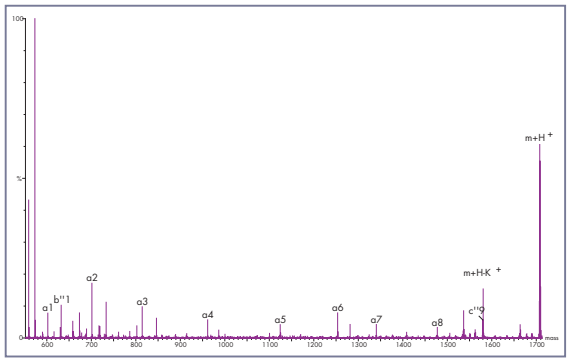


*Figure 6: MS/MS Spectrum of TMPP-ac modified GVIFYESHGK*

## CONCLUSIONS

- An increase of sequence coverage from 49.4% to 74.6% for ADH and 34.5% to 70.8% for BSA was achieved by TMPP-ac modification of tryptic peptides.
- This method improves MALDI MS/MS fragmentation. This will be used to show a greater success rate for protein identification on complex protein mixtures.
- Further work will concentrate on the modification of gel-isolated proteins that are low in molecular mass giving rise to few tryptic peptides.

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