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APPROACHES FOR IMPROVING PROTEOME CHARACTERISATION WITH MALDI-MS AND MS/MS

Jim Langridge[1], Emmanuelle Claude[1], Richard Tyldesley[1], Dominic Gostick[1], Simon Gaskell[2] Ian Beningfield[2] and Darryl Pappin[3]

[1]Waters Corporation, Manchester, UK., [2]The Michael Barber Centre for MS, UMIST, Manchester, UK., [3]Imperial College, London, UK.

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AIMS

- To improve the detection and identification of peptides in MALDI-TOF-MS
- To improve the fragmentation of singly charged peptides with MALDI MS/MS.
- Automate the derivatisation of tryptic peptides in preparation for analysis by MALDI MS and MS/MS.

INTRODUCTION

- Mass spectrometers have rapidly established themselves as an invaluable tool for identifying and characterising a wide variety of cellular proteins. The approach providing the highest level of automated sample throughput, in terms of samples per hour, is currently MALDI-TOF-MS. This technique provides a peptide mass fingerprint of the protein digests and allows the rapid and accurate identification of the parent protein by comparison to a databank. In the case of low abundance proteins, only a few peptides may be detected and databank searching can lead to an ambiguous result. One of the problems is that arginine containing peptides dominate MALDI Tof spectra[1]. Lysine-terminating peptides in particular are less likely to be detected since the lysine sidechains are low in basic nature and less likely to be ionised (**Figure 1**). The efficiency of the ionisation process can be improved by chemically modifying the lysine residue to homoarginine using a well-studied modification[2] (**Figure 2**). This increases the overall basicity of the peptide, chances of ionisation and therefore detection. Here we demonstrate the process of modifiying peptides in order to increase the overall quality of MALDI-TOF-MS spectra used for peptide mass fingerprinting.
- In peptide mass fingerprinting, if the number of peptides detected is small or if the resulting sequence coverage is poor, it is advantageous to be able to include even short pieces of sequence information to provide added specificity in databank searching. This information can be obtained when the MALDI ionisation technique is coupled with a high performance MS/MS mass spectrometer (MALDI Q-Tof). The amount of sequence information obtained from peptides using MALDI-MS/MS is dependent on the peptide size and the collision applied during the fragmentation process. Additionally, the peptides are singly charged due to the MALDI ionisation process and this often results in incomplete sequence information from the MS/MS data. It would be advantageous in this case if the fragmentation efficiency of these peptides could be increased to recover this information. Here we describe the use of a compound, SMA (Morphiline acetic acid-N-hydroxy succinimide ester), to chemically derivatize N-terminal and Lysine amino acid containing peptides (**Figure 3**) to increase their fragmentation efficiency and therefore the amount of sequence information obtained using MALDI-MS/MS.
- In this paper we will also describe how derivatisation steps can be incorporated into automated sample-preparation protocols with implications for use in high throughput proteomics sample analyses.

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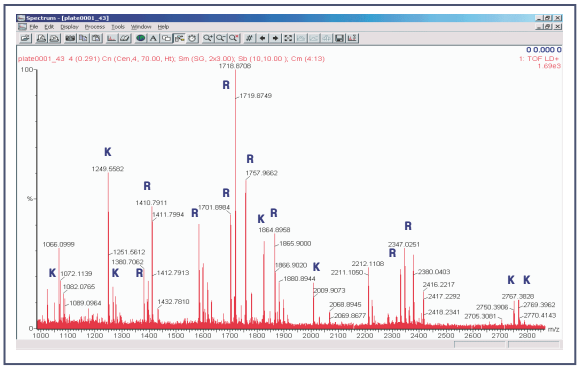

Figure 1. MALDI-TOF-MS spectrum with Lys and Arg containing peptides indicated

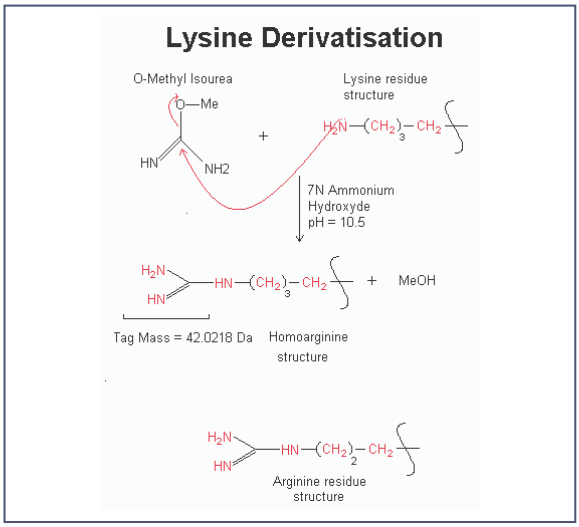

Figure 2. Lysine derivatisation reaction

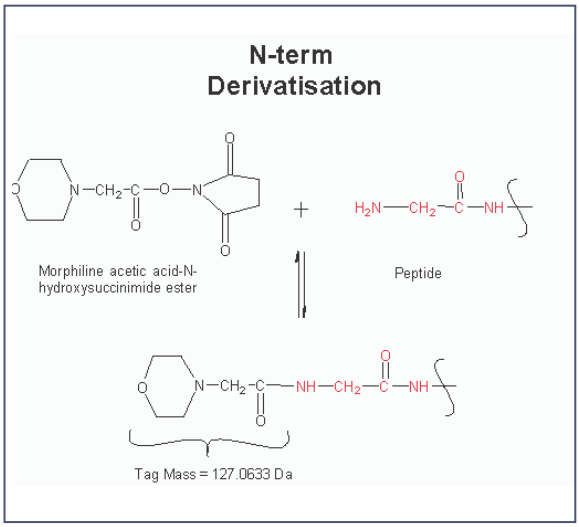

Figure 3. N-term derivatisation with SMA

MATERIALS AND METHODS

Digestion and Derivatisation

- The Guanidation method. A solution of Alcohol Dehydrogenase was prepared in the pmole/µL concentration range and digested with trypsin (Sigma, Poole, UK) at 37°C. The peptides were then derivatised by the method outlined in **Figure 4**. 7M Ammonium hydroxide and O-Methyl Isourea was added to 1-2µL of the tryptic digest and incubated at 65 °C. The peptides were dried down under vaccum and resuspended in 0.1% TFA. Incorporation of the method into this preliminary automated protocol still required several manual intervention stages.
- The SMA method. Solutions of Alcohol Dehydrogenase and Phosphorylase B (Sigma) were prepared at the pmole/µL concentration range and digested with trypsin at 37°C. The peptides were then derivatised by the method outlined in **Figure 5**. The derivatisation steps were repeated and the reaction was stopped with 0.1% TFA.

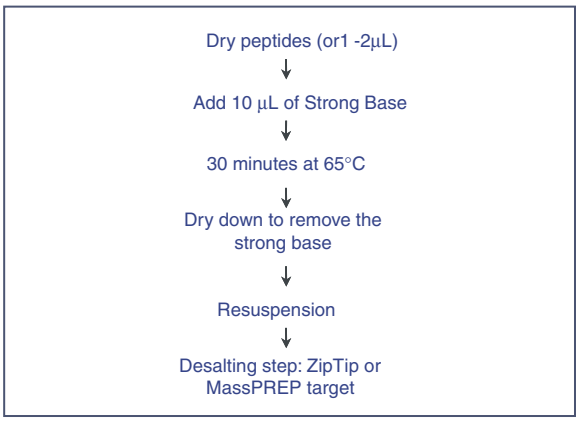

Figure 4. Guanidation method

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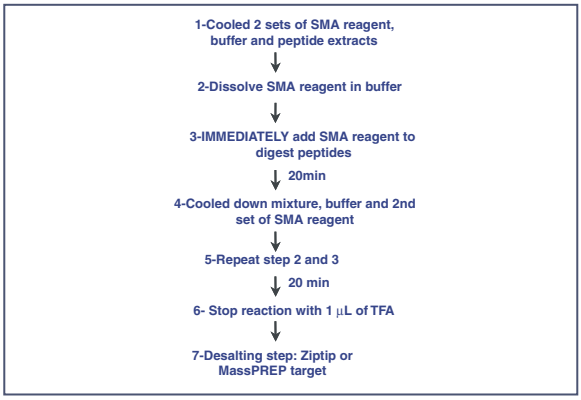

Figure 5. Derivatisation protocol - SMA

Automated sample preparation

The protein samples were processed on a MassPREP robotic liquid handling system (Waters Corp, Manchester, UK) (**Figure 6**) that performs all the chemistry steps to produce consistent and reproducible in-solution tryptic digests. This robotic procedure includes the tryptic digestion, derivatisation and spotting of the peptides (2.0µL) onto 96-position MALDI target plates. Aliquots of the chemically modified digests were loaded onto MassPREP target plates, dried and desalted by repeated washing with water. Matrix (α-cyano 10mg/mL) was then deposited onto the dried peptide spots.

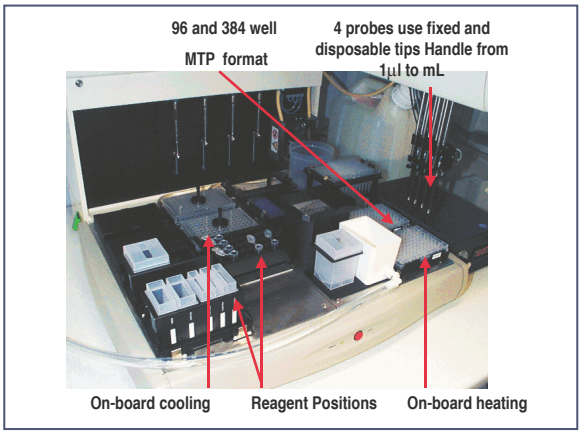

Figure 6. Deck layout of the MassPREP digestion robot

MATERIALS AND METHODS

Mass Spectrometry - MALDI-TOF-MS

Data were acquired on a benchtop Micromass M@LDI (Waters Corp., Manchester, UK) mass spectrometer. This instrument is optimised for high throughput protein identification by peptide mass fingerprinting. The system is reflectron based with a 2.3M effective flight path. The source design incorporates "time lag focusing" and the dual MCP detector is complemented with a 2GS/sec ADC. The quality of data obtained is significantly enhanced by the use of a real time data selection algorithm.

Mass Spectrometry - MALDI MS/MS

All MALDI MS/MS data were acquired using a Micromass Q-Tof Ultima MALDI (Water Corp., Manchester, UK) hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer. The key features of this MALDI system are: the mass spectrometer is fitted with MALDI 96 well sample stage; the laser used (LSI 337ND) runs at variable repetition rate and with adjustable power density; a high pressure source to provide collisional cooling of the ions. The instrument can be run in both MS and MS/MS mode with precursor ions for the MS/MS experiments being fragmented in the collision cell using Argon collision gas. The collision process is controlled by setting a cell voltage proportional to mass and fine tuned so that a even distribution of fragment ions are produced.

RESULTS AND DISCUSSION

Guanidation

An experiment was set up to investigate the effect on peptide detection with MALDI-TOF-MS after proteins were modified with the guanidation derivatisation method previously outlined (**Figure 3**). **Figure 6** shows MALDI-TOF-MS spectra acquired from both unmodified and modified preparations of Alcohol dehydrogenase.

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The spectrum representing the unmodified digest shows a number of peaks representing peptides have been detected, some with high intensities, mainly peptides containing a C-terminal arginine, and many with much lower relative intensities mainly containing a C-terminal lysine. In contrast, the spectrum for the modified protein digest illustrates that a number of the low-abundance peptides have increased in their relative intensities as a result of the derivatisation procedure (**Figure 7**– blue arrows). This can be confirmed by the shift in mass (42Da) for the peaks corresponding to the conversion of lysine residues to homo-arginine residues (**Figure 7**. Blue arrows). The peptides containing C-terminal arginine residues and no internal lysine residues (black arrows) remain unmodified.

Initial results

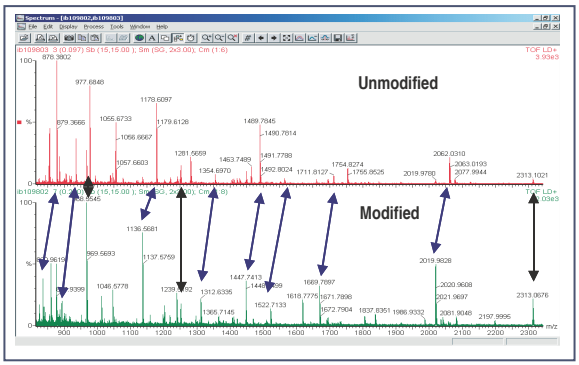

Figure 7. MALDI-TOF-MS spectra from unmodified peptide digest (top) and modified digest (bottom). Blue arrow: Lysine residues
Black arrow: Arginine residues

SMA

An initial investigation was conducted to investigate the automation of the SMA chemistry on the MassPREP digestion robot.. A preparation of Alcohol dehydrogenase in the pmole/µL range was derivatised and digested in an automated fashion (MassPREP; Waters Corp., Manchester) and analysed by MALDI-TOF-MS (Micromass M@LDI; Waters Corp, Manchester).

The resulting spectra is shown in **Figure 8**. Initial inspection of the spectrum indicates that a number of the peptides in the digest mixture have been derivatised by one or more molecules of the SMA compound. The attachment of SMA molecule is shown by a 127Da or 254Da shift up the m/z scale for the attachment of 1 and 2 molecules respectively. The relative intensities of the derivatised peptides has also changed indicating a difference in the ionisation efficiency of these derivatised peptides versus their underivatised predecessors.

A second manual experiment was carried out in which the fragmentation behaviour of the derivatised peptides was examined. Digest samples from normal and SMA modified solutions of Alcohol Dehydrogenase and Phosphorylase B were analysed. A peptide from each digest (non derivatised and derivatised) was selected for MALDI MS/MS analysis on a Micromass Q-Tof Ultima MALDI and the fragmentation patterns were compared. A tabulated summary for the results obtained for alcohol dehydrogenase and Phosphorylase β are presented in ***Figure 9***. ***Figure 10*** represents a comparison of the MS/MS spectra from the underivatised Phosphorylase B peptide at 1550.77 m/z and the corresponding derivatised peptide at 1678.16m/z. The non-modified peptide fragmented to produce 12 y'' ions that provided clear sequence information in addition to an extra 2 b ions. Inspection of the spectrum derived from the derivatised peptide however, showed there were 12 y'' ions but also that there was a significant increase in the number of b ions from 2 to 6. **Figure 11** represents a comparison of the MS/MS data from a non-derivatised peptide at 968.49m/z and the derivatised peptide at 1095.53m/z from the Alcohol dehydrogenase digest.

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In this case we also observed an increase in the number of b ions observed (from 2 to 5) in the MS/MS for the non-derivatised peptide versus the derivatised peptide however the number of y'' ions observed decreased.

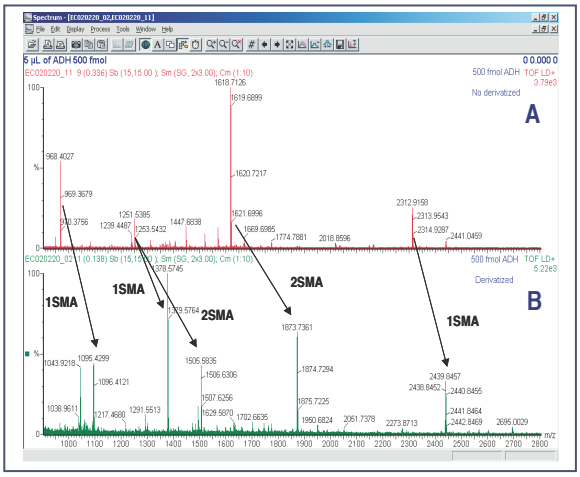

Figure 8. MALDI MS analysis of an alcohol dehydrogenase tryptic digest, prior (A) and post (B), automated SMA derivatisation

	Peptide Mass	Sequence	Number of SMA	Derivatise	% Coverage Number of Amino	% Coverage B+Y ions	% Coverage B ions	% Coverage Y ions
Phospho B	1550.7	IGEEYISDL DQLR	1	No	54	39	23	54
				yes	100	65	62	69
	1053.5	VIFLENYR	1	No	88	63	50	75
				yes	100	50	50	50
	1426.8	HLQIIYEINQ R	1	No	91	59	64	55
				yes	91	59	73	45
	1678.9	IGEEYISDL DQLRK	2	No	50	32	29	36
				yes	79	47	43	50
ADH	968.8	EALDFFAR	1	No	88	63	50	75
				yes	75	50	63	38
	1618.8	VLGIDGGE GKEELFR	2	No	93	87	80	93
				yes	80	50	50	53
	1569.8	SIGGEVFID FTKEK	3	No	79	61	57	64
				yes	71	38	50	21

Figure 9. Summary of the SMA derivatised peptides analysed by MALDI MS/MS showing the coverage of sequence related ions (b &y'')

Peptide IGEEYISDLDQLR from Phosphorylase B

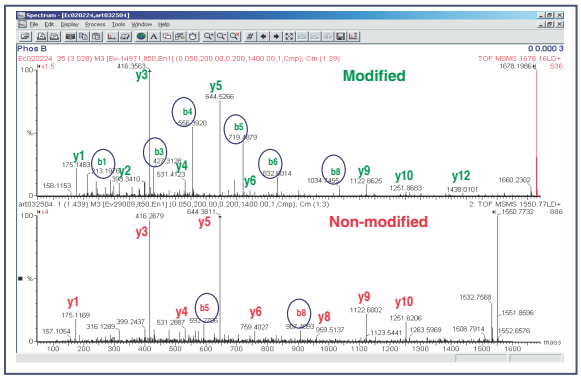

Figure 10. MS/MS spectra from SMA derivatised and non-derivatised peptides, showing the improvement in the MS/MS fragmentation pattern

Peptide EALDFFAR from ADH

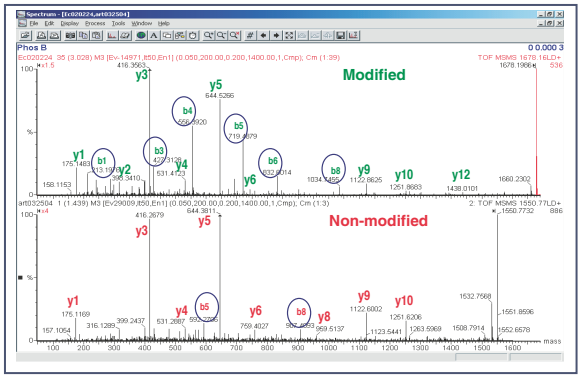

Figure 11. Comparison of derivatised and non-derivatised peptides from ADH.

CONCLUSIONS

- Successful conversion of C-terminal lysine-containing peptides to homoarginine residues for standard samples has been shown using an automated MassPREP procedure.
- Investigation of the MALDI-TOF mass spectra showed an improvement in the intensity of C-terminal lysine containing peptides, when derivatised
- Successful derivatisation of protein digests with SMA has been demonstrated using an automated method
- The SMA derivatisation process has been demonstrated to alter the fragmentation behaviour of tryptic peptides. Mixed results have been obtained with regard to improving the sequence information contained within MALDI-MS/MS data.

REFERENCES

1 Krause E, Wenschuh Hn Jungblutt PR, *Anal. Chem.* 1999; **71**: 4160

2 Hughes WL Jr, Saroff HA, Carney AL. *Am Chem Soc*; 1949; **7**: 2476

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Author to whom all correspondence should be addressed:
Jim Langridge
Waters Corporation
(Micromass UK Limited)
Floats Road, Wythenshawe
Manchester, M23 9LZ
Tel: + 44 (0) 161 946 2400
Fax: + 44 (0) 161 946 2480
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