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Mass spectrometry of acylated peptides and proteins

Jenny Anne Vazquez

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MASS SPECTROMETRY OF ACYLATED PEPTIDES AND PROTEINS

A thesis submitted in fulfilment of the requirements for the award of the degree

DOCTOR OF PHILOSOPHY

from

UNIVERSITY OF WOLLONGONG

by

JENNY ANNE VAZQUEZ, BA, BSc(Hons), Grad.Dip.Ed.

SCHOOL OF CHEMISTRY

2010

CERTIFICATION

I, Jenny Anne Vazquez, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Jenny Anne Vazquez

5th May 2010

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ABBREVIATIONS

Symbol	Meaning
a	acetyl
ACN	acetonitrile
ACTH	adrenocorticotrophic hormone, clip 18–39
AIDS	acquired immune deficiency syndrome
b	benzoyl
BCA	bovine carbonic anhydrase
BUDA	Boston University Data Analysis (software)
°C	degrees Celsius
C.E.	collision energy
CAD	collision activated dissociation
CE	capillary electrophoresis
CID	collisionally induced dissociation
D	dimensional (as in 2D-gel electrophoresis)
Da	Daltons
DNA	deoxyribose nucleic acid
e	exponent; $\times 10^n$
ECD	electron-capture dissociation
ESI	electrospray ionisation
eV	electron volts
f	trifluoropropionoyl
FTICR	Fourier transform-ion cyclotron resonance
g	glutaroyl
GPI	glycosylphosphatidyl inositol (anchor)
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
i	iodoacetyl
i.d.	internal diameter
IDA	intelligent data acquisition TM
IPA	isopropanol
IUPAC	International Union of Pure and Applied Chemistry
kDa	kilodaltons
kV	kilovolts
kyn	kynurenine
LC	liquid chromatography
LIT	linear ion trap
<i>M</i>	molar (mol/L)
[m+H] ⁺	protonated or positively charged ion
<i>m/z</i>	mass-to-charge ratio
MALDI	matrix-assisted laser desorption/ionisation

mg	milligram
MHC	major histocompatibility complex
min	minute
mL	millilitre
mM	millimolar
MS	mass spectrometry or mass spectrum(a)
MS/MS	tandem mass spectrometry (mass spectrometry/mass spectrometry)
MS ⁿ	multi-stage mass analysis
myr	tetradecano- or myrist- acyl group
nm	nanometres
NMR	nuclear magnetic resonance
NNRTI	nonnucleoside reverse transcriptase inhibitor
o	orthogonal (as in Q-o-TOF)
oco	octano- or capryl- acyl group
ole	n-octadeceno- or ole- acyl group
p	methoxypolyethylene glycol propionoyl
pam	hexadecano- or palmit- acyl group
PEG	used to represent methoxypolyethylene glycol propionate
pH	-log[H ⁺]
pmol	picomole
ppm	parts per million
PSD	post source decay
Q or q	quadrupole
QIT	quadrupole ion trap
R	general symbol used to represent a functional group (e.g. for an amino acid sidegroup.)
RF	radio frequency
RNA	ribose nucleic acid
SDS-PAGE	sodium dodecylsulphate polyacrylamide gel-electrophoresis
SIV	simian immunodeficiency virus
SORI	sustained-off resonance ionisation
ste	octadecano- or stear- acyl group
SWIFT	stored waveform inverse Fourier transform
T	denotes peptide resulting from tryptic digestion
T	Tesla
TFA	trifluoroacetic acid
TIC	total ion current
TOF	time-of-flight
u	unified atomic mass unit
μL	microlitre
μm	micrometre
UV	ultra-violet
V	volts
VIS	visible

PUBLICATIONS

1. Yang J., Gitlin I., Krishnamurthy VM., **Vazquez JA.**, Costello C, Whitesides GM. Synthesis of monodisperse polymers from proteins. *Journal of the American Chemical Society.* **2003** 125:12392-3.

My contribution towards this publication was in providing molecular mass measurements of the monodisperse polymers and identifying a spurious acylation. This work is presented in Chapter 5 of this thesis.

2. Gudiksen KL., Urbach AR., Gitlin I., Yang J., **Vazquez JA.**, Costello CE., Whitesides GM. Influence of the Zn(II) cofactor on the refolding of bovine carbonic anhydrase after denaturation with sodium dodecyl sulfate. *Analytical Chemistry.* **2004** 76:7151-61.

My contribution towards this publication was in providing molecular mass measurements of the native and denatured protein, bovine carbonic anhydrase, and identifying sites of deamidation in the native and denatured protein forms. This work is presented, in part, in Chapter 5 of this thesis.

3. Van Rhijn, I.; Young, D. C.; De Jong, A.; **Vazquez, J.**; Cheng, T.-Y.; Talekar, R.; Barral, D. C.; Leon, L.; Brenner, M. B.; Katz, J. T.; Riese, R.; Ruprecht, R. M.; O'Connor, P. B.; Costello, C. E.; Porcelli, S. A.; Briken, V.; Moody, D. B. CD1c bypasses lysosomes to present a lipopeptide antigen with 12 amino acids. *Journal of Experimental Medicine.* **2009**, 206, 1409-1422.

My contribution to this paper was the characterisation of the lipopeptide antigen. The sequencing of this peptide was essential to the progression of the research which led to the publication of this paper. This work is presented in Chapter 4 of this thesis.

4. Moody, B.D., Rhijn, I.V., Young, D.C., Costello, C.E. (2008) Patent: Methods and Compositions for Immunomodulation. USA: 20080226587

My contribution to this patent was the sequencing of the 12-mer lipopeptide (described in publication 3) which is one of two compositions contained this patent. This work is presented in Chapter 4 of this thesis.

5. **Vazquez, JA.**, Berg, EA., Panepinto MJ., Catherine E. Costello, CE. Mass Spectrometry of Acylated Peptides. (In preparation.)

The content of this paper is presented in Chapter 3 of this thesis.

ABSTRACT

The majority of naturally occurring proteins are modified in some manner, with many biological systems requiring these modifications in order to function properly. Acylation is one such type of modification. For example, the neuropeptide ghrelin, which plays a critical role in appetite stimulation, is octanoylated on its ser-3 residue. Many proteins are palmitoylated at one or more cysteine residues, with the lipid moiety essential for membrane binding. It is likely that many biological systems rely on protein acylations, and it would be beneficial to develop techniques that allow a facile detection of naturally occurring acylations.

Synthetically acylated peptides and proteins (a class of monodisperse polymer) have many potential uses. Synthetically acylated peptides and proteins are being developed for use as therapeutic agents and also as chemical standards. Synthetically acylated peptides, for example, have the potential for use as vaccines for diseases such as hepatitis and HIV. The ability to successfully characterise these types of semi-synthetic molecules is imperative in their development process. In this work, mass spectrometry is explored as a means of analysing acylated peptides and proteins.

A number of synthetically acylated peptides were examined using a range of mass spectrometry techniques in order to identify characteristic fragmentations. Acylated peptides were fragmented using electrospray ionisation or matrix-assisted laser desorption/ionisation combined with collisionally induced dissociation tandem mass spectrometry, and also using matrix-assisted laser desorption/ionisation post source decay mass spectrometry. Acylated peptides were observed to fragment in a similar manner to their unacylated counterparts, with the degree of fragmentation observed dependant on the length of the acyl chain (i.e. higher collision energies were required to

illicit the same degree of fragmentation with increasing chain length). The presence of an acylation on an N-terminal amino acid allowed the formation of a b_1 product ion, not normally observed in the spectrum of unmodified peptides. A number of useful marker ions for acylation at serine, tyrosine and cysteine residues were observed, including, acyl carbenium ions and acylated immonium ions. The neutral loss of the acyl moiety was also commonly observed. The tandem mass spectrometry conditions required to produce marker ions were explored.

Two lipopeptides, synthesised as by-products in the production of N-terminally acylated HIV protein Nef₁₋₆ (acyl-GGKWSK), were characterised. These lipopeptides were found to be twelve residues long, with an unusual ether-linked kynurenine at position seven, and contained either a stearyl or oleoyl moiety at their N-terminal glycine residues (ste/ole-GGKWSK-O-kyn-SKWSK). The successful characterisation of these molecules has allowed continued investigations into their use as immunological agents.

A range of novel monodisperse polymers, acylated at their lysine residues, were analysed intact or subsequent to enzymatic digestion, using high performance liquid chromatography and tandem mass spectrometry. Analogous tandem mass spectra were observed irrespective of the acylating agent, however, chromatographic elution times were found to be dependant on the identity of the acyl moiety. Acylated lysine carbenium ions were observed in the tandem mass spectra, 17 u lower in mass than the calculated value for an acylated lysine immonium ion.

This work demonstrates the ability of mass spectrometry to enable the characterisation of a wide range of acylated peptides and proteins. The use of a variety of mass spectrometry and commonly employed analysis techniques (such as enzymatic digestion) further aid in the characterisation of acylated peptides and proteins.

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