

Negative Ion Mass Spectrometry of Peptides

An Aid to Structure Determination

A thesis submitted for the degree of Doctor of Philosophy (Ph.D.)

by

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Abstract

Amphibians contain a rich chemical arsenal in their skin glands, vital for their defence against predators, both large and small. The peptides secreted by the frogs have a range of biological activities. These include both antibacterial and anticancer activity, others are neuropeptides, while some exhibit antifungal and antimalarial activity.

Peptides are usually sequenced using positive ion mass spectrometry (MS). However, negative ion MS can also provide valuable sequencing information. Under negative ion MS conditions, the presence of a Cys residue is immediately identified by the facile side chain loss of H₂S. The position of the Cys residue is determined by the formation of a side chain induced backbone cleavage ion. When a Cys residue is in the C-terminal position of a peptide, the spectrum is dominated by the loss of both H₂S and CO₂.

Negative ion MS can also be used to identify and sequence intramolecular disulfide bridged peptides. The disulfide bridge is immediately identified by the facile loss of H₂S₂ from the parent anion. Once the disulfide bridge is cleaved, further amide cleavages provide much of the sequence of the peptide, including the residues originally within the disulfide link. When one of the disulfide bridged Cys residues is in the C-terminal position, the major fragmentation is the loss of H₂S₂ and CO₂ from the parent ion.

The negative ion mass spectra of citropin 1.1 and synthetically modified analogues show an unusual loss of an internal Val residue from the (M-H)⁻ parent ion. This rearrangement requires the decomposing anion to have an α -helical structure.

The skin secretions of *Litoria peronii* or Peron's Tree Frog contain five novel peptides which have been named peroneins. Four pro-peroneins are present in the summer secretions only. The biologically active peptides caerulein 1.1, caerin 1.1 and caerin 2.1 are also present in the glandular secretions.

Statement of Originality

This thesis contains no material which has been accepted for the award of any other degree, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

I give my consent to this copy of my thesis, when deposited in the University Library, to be available for loan and photocopying.

Daniel Bilusich

Date

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20 Common Amino Acids

Amino Acid	Abbreviation		Integral Mass	Structure
	3 Letter	1 Letter		
Alanine	Ala	A	71	$\begin{array}{c} \text{CH}_3 \\ \\ \text{---NH---CH---CO---} \end{array}$
Arginine	Arg	R	156	$\begin{array}{c} \text{CH}_2\text{CH}_2\text{CH}_2\text{---NH---C---NH}_2 \\ \\ \text{NH} \\ \\ \text{---NH---CH---CO---} \end{array}$
Asparagine	Asn	N	114	$\begin{array}{c} \text{CH}_2\text{---CONH}_2 \\ \\ \text{---NH---CH---CO---} \end{array}$
Aspartic Acid	Asp	D	115	$\begin{array}{c} \text{CH}_2\text{---CO}_2\text{H} \\ \\ \text{---NH---CH---CO---} \end{array}$
Cysteine	Cys	C	103	$\begin{array}{c} \text{CH}_2\text{---SH} \\ \\ \text{---NH---CH---CO---} \end{array}$
Glutamic Acid	Glu	E	129	$\begin{array}{c} \text{CH}_2\text{CH}_2\text{---CO}_2\text{H} \\ \\ \text{---NH---CH---CO---} \end{array}$
Glutamine	Gln	Q	128	$\begin{array}{c} \text{CH}_2\text{CH}_2\text{---CONH}_2 \\ \\ \text{---NH---CH---CO---} \end{array}$
Glycine	Gly	G	57	$\begin{array}{c} \text{H} \\ \\ \text{---NH---CH---CO---} \end{array}$
Histidine	His	H	137	$\begin{array}{c} \text{HN} \diagup \text{N} \\ \\ \text{CH}_2 \\ \\ \text{---NH---CH---CO---} \end{array}$
Isoleucine	Ile	I	113	$\begin{array}{c} \text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3 \\ \\ \text{---NH---CH---CO---} \end{array}$
Leucine	Leu	L	113	$\begin{array}{c} \text{CH}_2\text{CH}(\text{CH}_3)_2 \\ \\ \text{---NH---CH---CO---} \end{array}$
Lysine	Lys	K	128	$\begin{array}{c} \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{---NH}_2 \\ \\ \text{---NH---CH---CO---} \end{array}$
Methionine	Met	M	131	$\begin{array}{c} \text{CH}_2\text{CH}_2\text{---S---CH}_3 \\ \\ \text{---NH---CH---CO---} \end{array}$
Phenylalanine	Phe	F	147	$\begin{array}{c} \text{CH}_2\text{---} \langle \text{benzene ring} \rangle \\ \\ \text{---NH---CH---CO---} \end{array}$
Proline	Pro	P	97	$\begin{array}{c} \langle \text{5-membered ring} \rangle \\ \\ \text{---N---CH---CO---} \end{array}$
Serine	Ser	S	87	$\begin{array}{c} \text{CH}_2\text{---OH} \\ \\ \text{---NH---CH---CO---} \end{array}$
Threonine	Thr	T	101	$\begin{array}{c} \text{CH}(\text{OH})\text{CH}_3 \\ \\ \text{---NH---CH---CO---} \end{array}$
Tryptophan	Trp	W	186	$\begin{array}{c} \text{CH}_2\text{---} \langle \text{indole ring} \rangle \\ \\ \text{---NH---CH---CO---} \end{array}$
Tyrosine	Tyr	Y	163	$\begin{array}{c} \text{CH}_2\text{---} \langle \text{phenol ring} \rangle \\ \\ \text{---NH---CH---CO---} \end{array}$
Valine	Val	V	99	$\begin{array}{c} \text{CH}(\text{CH}_3)_2 \\ \\ \text{---NH---CH---CO---} \end{array}$

Nominal masses of peptides are used throughout this thesis. Nominal masses are obtained by summation of the integral masses of the amino acid residues.