Metalo-peptides, a cautionary tale!

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Introduction

Isobaric peptides

- Peptide mass fingerprinting (PMF) makes use of search engine mass-comparisons of trypsin digests to theoretical maps.
- Not all peptides in a PMF are recognised by simple matching. For example, modified peptides.
- One such peptide is present in cytochrome c. This protein is a haem-protein which following trypsin digestion generates a haem-bound tryptic-peptide (Russel and Edmondson, 1997).
- It is easily mistaken for another peptide in the PMF, an isobaric impostor. (Beardlsey et al 2002, Keough et al 2000).
- The work presented here alerts workers to the possible occurrence of metalo-peptides in the PMF's of metalo-proteins.
- We show strategies for the characterisation of suspected isobaric impostors.

Methods

- A standard trypsin digest of horse heart cytochrome c in 0.1M ammonium bicarbonate solution was performed.
- Guanidination followed the methods of Keough (2000) and Beardsley (2002) with a few modifications.
- Peptide maps were mass analysed using the SAI LaserTof LT3 in reflectron, positive ion mode.
- MS/MS of the proposed isobaric species was conducted using seamless PSD.

Results and Discussion

Evidence that IFVQK.CAHTVEK is an isobaric impostor

- The digest was correctly identified as cytochrome c from 13 peptides giving 78% coverage.

Observed Mass (Da)	Theory Mass (Da)	Residue from	to	Error Da	Error ppm	Amino Acid Sequence
779.46	779.45	79	86	0.02	20.5	MIFAGIK
1168.61	1168.62	27	38	0.00	−4.3	TGPNLHGLFGR
1350.71	1350.72	88	99	−0.01	−8.9	TER.EDLIAYLK
1433.72	1433.77	25	38	−0.05	−32.8	HK.TGPNLHGLFGR
1470.65	1470.68	39	53	−0.03	−21.1	TGQAPGFTYTDANK
1478.77	1478.82	88	100	−0.05	−33.1	TER.EDLIAYLK.K
1478.77	1478.82	87	99	−0.05	−33.1	K.TER.EDLIAYLK
1495.64	1495.69	60	72	−0.05	−35.4	EETLMEYLENPK
1598.70	1598.77	38	53	−0.07	−45.7	K.TGQAPGFTYTDANK
1633.58	1633.81	8	22	−0.23	−142.6	IFVQK.CAQCHTVEK
1761.73	1761.91	7	22	−0.18	−100.5	K.IFVQK.CAQCHTVEK
2081.02	2081.02	55	72	0.00	0.5	GITWK.EETLMEYLENPK
2209.17	2209.11	55	73	0.05	24.4	GITWK.EETLMEYLENPK.K

Table 1: Peptide map of cytochrome c: red font indicates those identified peptides with uncharacteristically large mass error.

Mass error evidence

- IFVQK.CAHTVEK and its mis cut K.IFVQKCAHTVEK, were observed to have greater mass error (Table 1).

Lysine evidence

- Lysine count is low. There is only one lysine as determined by guanidination, Figure 1, whereas IFVQK.CAHTVEK has two.

Isotopic clusters

- Closer inspection of the 1633 peptide shows there are two smaller preceding peaks, which are indicative of an iron isotopic cluster (Figure 2).

Swiss-Prot interrogation

- The Swiss-Prot entry for cytochrome c describes the covalent attachment of haem to the protein, via Cys 14 and Cys 17. A tryptic digest of cytochrome c would result in these cysteines being contained in the peptide CAQCHTVEK. (Figure 6).

Calculation of correct modification

- The covalent attachment of haem (C34H32N4O4.Fe) to this peptide gives a monositopic mass of 1631.6 Da . This mass corresponds to a peak found at 1631.58, the first of two preceding smaller peaks of the 1633.58 specie.

Confirmatory PSD

- The PSD of 1631.6 gave a spectra that contained a fragment with the same mass as haem, Figure 3.

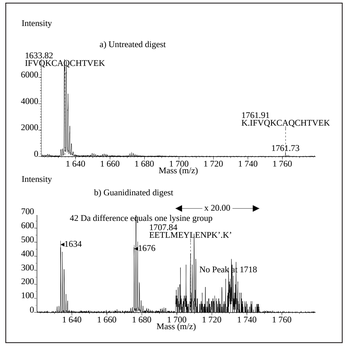

Figure 1: Counting lysines by guanidination.
a) untreated digest, b) guanidinated digest. One lysine group corresponds to a mass shift of 42 Da.

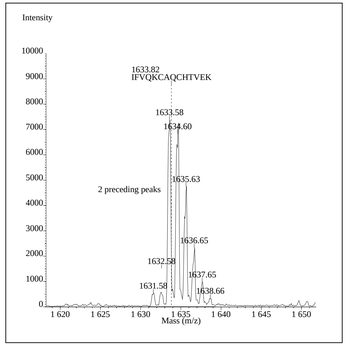

Figure 2: Isotopic cluster of the suspected haem peptide. Spectrum is labelled with the isobaric impostor

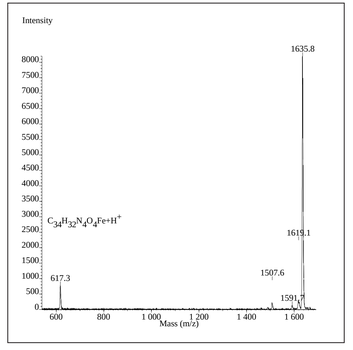

Figure 3 PSD of suspected haem peptide, showing loss of haem group

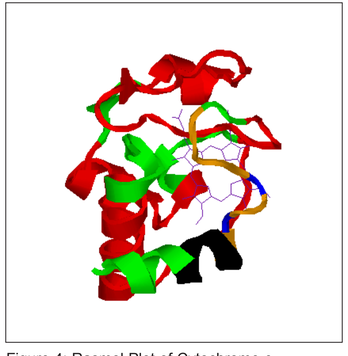

Figure 4: Rasmol Plot of Cytochrome c.
Matched and unmatched peptides: matched peptides (red), unmatched (green), misidentified (black), re- identified orange), haem binding sites (blue), haem (purple)

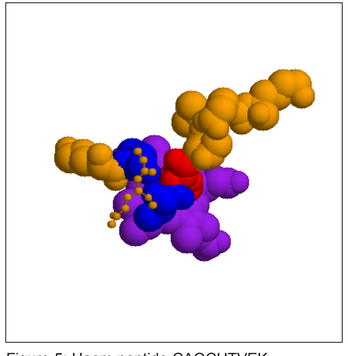

Figure 5: Haem peptide CAQCHTVEK.
The two cys residues (blue) form MALDI stable thioether bonds with haem(purple), whereas His (red) forms an iron ligand. (A and Q are represented as stick and ball for clarity)

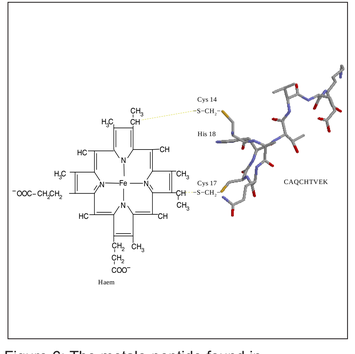

Figure 6: The metalo-peptide found in cytochrome c.

Summary

- Figure 4 is a Rasmol representation of cytochrome c tryptic peptides showing those matched, unmatched and misidentified (the good, the bad and the ugly). **The peptide commonly assigned in PMF experiments as IFVQKCAQCHTVEK is in fact haemo-CAQCHTVEK.** Figures 5 and 6 give different representations of this metalo peptide.

Conclusions

- Database searching of peptide mass fingerprints can sometimes result in the mis-assignment of isobaric impostors.
- Alerted by its uncharacterisitic high mass error, we identified one such peptide.
- Further characterisation by spectral interpretation for iron clusters, seamless PSD and chemical derivitization revealed this peptide to be the metalo peptide haemo-CAQCHTVEK.

References

Russell D H, Edmondson R D (1997) J Mass Spectrom. 32, 236-276.
Beardsley RL and Reilly JP (2002), Anal. Chem. 74, 1884-1890
Keough T, Lacey MP Yougquist RS (2000), Rapid Comm. in Mass Spectrom. 14, 2348-2356
Rasmol: www.umass.edu/microbio/rasmol/
PDB: Berman J, Bourne PE et al. Nucleic Acids Research, 28, 235-242 (2000)

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