MALDI-PSD-TOFMS determination of milk-protein quality by *phosphopeptide mapping* and *charge directed fragmentation*

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Overview

A tryptic digest of "dirty" casein gave a poor score for its identity
Hypothesis: "poor score was due to contaminant peaks"

a) What are the contaminant peaks?
b) Can they be removed to improve the ID score?

APPROACHES

- Whole protein checked (linear TOF)
- Guanidination: to increase K peptide representation
- IMAC extraction of phosphopeptides: to reduce the data set
- CDF-PSD on a harmonic field reflectron: to sequence the unassigned peaks

CONCLUSIONS

- Whole protein qualitatively correct
- Guanidination increased score, but not significantly
- IMAC can remove contaminant peaks
  - Improved ID score to *CAS*1
  - Isolated a new peptide from *CAS*B
  - Isolated other none casein peptides (?acidic peptides)
- CDF-PSD can sequence major contaminant peptides

Introduction

SAI were requested to analyse a casein tryptic digest. A MALDI-TOFMS spectrum of the digest had a number of unassigned peaks, Figure 1a (top), one of which dominates the spectrum (Peak 1 @ 1959 m/z). An investigation was made to 1) improve the score by removing the contaminants, and 2) identify the nature of the contaminants.

Methods

All materials were from Sigma, Poole, UK.

Digest: 0.5mg/ml technical casein, digested with 5% w/v DPCC treated trypsin, in 0.05M ammonium bicarbonate (AMBC). *Guanidination*: sample diluted 1:1 with O-methylisourea sulfate 100mg/ml in 0.5M Na_2CO_3. Incubation at 37^{o}C for 2 hours.

Sulphonication: sample diluted 1:1 with 50mg/ml 4-sulfophenyl isothiocyanate in 0.04M AMBC. Incubation for 2 hours at RT.

*S*amples were cleaned up on C-18 z-tips (Millipore) prior to analysis.

I*MAC*: samples were prepared as per instructions in the kit from Pierce.

All mass spectrometry was performed on a ***LaserToF RS***, SAI, UK, with a high performance, harmonic field reflectron MALDI-TOF-MS (Ref: S Thompson et al, Proc 47th ASMS 1999, "Ion optical properties of quadratic mirrors", p3066).

Results

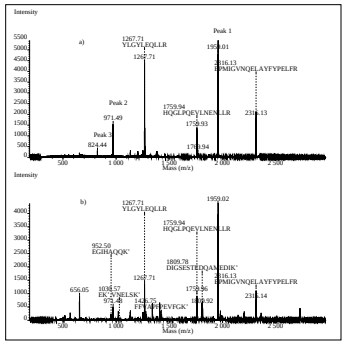

Figure 1: Trypsin digest of casein a) untreated, b) guanidinated.

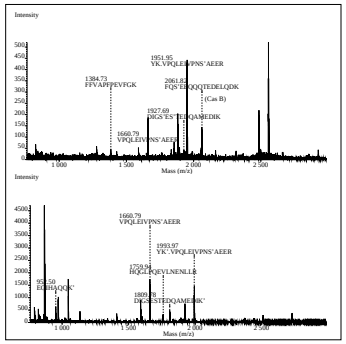

Figure 3: MALDI-TOFMS of phosphopeptide extraction of the casein tryptic digest. (a) Untreated, (b) guanidinated.

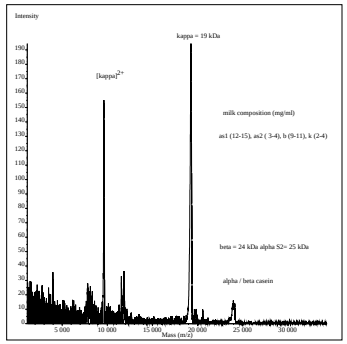

Figure 2: MALDI-TOFMS of whole casein protein

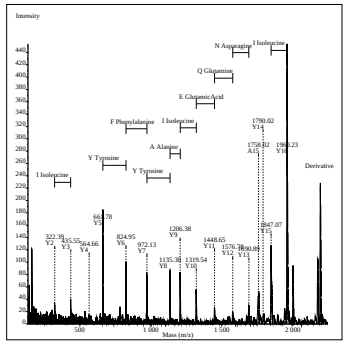

Figure 4: PSD of Sulphonicated mystery peak, m/z 1959.

Treatment	***Search Restriction***	***Score***
a) None	Open	0.37
b) Guanidinated	Open	0.58
c) IMAC of (a)	Phosphoserine	0.88
d) IMAC of (a)	Phosphoseine & taxa	1.31

Table 1: CAS1 identification scores for casein digest, guanidinated digest, and phosphopeptide extracts. Score >1 is highly significant.

Discussion

MALDI-TOFMS was used to ascertain the quality of a milk protein. Figure 1a (top) shows the original MALDI-TOFMS spectrum of a casein digest. There are unassigned peaks, one of which dominates (Peak 1 @ 1959 m/z). Figure 1b (bottom) shows the guanidinated spectra. A protein database search of the guanidinated data was made. CAS1 (alpha casein) was the top third hit and scored low (Table 1). Guanidination has correctly identified four additional lysine peaks (Fig 1 b) and improved the score (Table 1).

A linear MALDI-TOFMS of the whole protein (Figure 2) gave good qualitative agreement to the proteins expected composition. Bovine milk casein is composed of alpha (25 kDa, 75%, CAS1 and CAS2), beta (24kDa, 22%, CASB) and kappa (19kDa, 3%, CASK) casein. Kappa casein is the dominant protein in the linear data (Figure 2) though it was beta and alpha casein in an SDS-PAGE data (not shown). Kappa casein is a glycoprotein, which may effect both its mobility in PAGE and ionisation in MALDI.

IMAC isolated phosphopeptides from the peptide map. Peptide class selection improves score chances by reducing the size of the database searched. Figure 3 shows the MALDI-TOFMS of a phosphopeptide extract of the casein digest (a, top) and the guanidinated casein digest (b, bottom). IMAC has detected a CASB peptide, m/z 2061, not visible in the original map. No peptides of CAS2 nor CASK were present. Table 1 shows how guanidination, and phosphopeptide restriction with and without taxa restriction, steadily improves the score, even in the presence of contaminant peaks. A search of non casein peaks from IMAC were resubmitted individually into the search engine (not shown). All were plausible milk proteins and one, FOL1 is a known milk protein. Their identification however is highly speculative, since the original proteins are not known to phosphorylate in the postulated peptides.

A sulphonication of the digest was made using 4-sulfophenylisothiocyanate. Figure 4 shows the PSD spectra of the sulphonicated derivative of the mystery peak at m/z 1959. The LaserToF PSD sequencing tool was used to ascertain the most likely sequence (Figure 4). The resulting masses were searched using MASCOT and a top hit for a casein peptide, IGVNQELAYFYPELFR, was found. The peptide could only be the result of a faulty trypsin cut. In fact, within the full protein this peptides N terminal is preceded by M. Trypsin should not cut here. The MALDI-sulphonication-PSD confirms that the mystery peptide is a casein peptide and is a result of aberrant protease activity, possibly due to endogenous milk proteases (the trypsin has behaved normally before and since this work). Peaks 2 and three were proven to be further degradation products of peak 1: FYPELFR (M+H = 971.49) and YPELFR (M+H = 824.43).