Unexpected proteomics and biochemical complexity of human salivary α-amylases in 2D gels

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Unexpected Proteomics and Biochemical Complexity of Human Salivary α-Amylases in 2D gels

Nicolas Sommerer[1], Christophe Hirtz[1,2], Jean-Pascal Gimeno[1], François Chevalier[1], Delphine Centeno[1], Valérie Rofidal[1], Marc Tauzin[1], Thierry Bécue[1], Dominique Deville de Perière[2] & Michel Rossignol[1]

1/ INRA – UR Protéomique, Montpellier, France *2/ UM1 – UFR Odontologie*, Montpellier France

Although human salivary α-amylase is described as a very conserved and stable protein [1], a large scale mapping of human salivary proteome by MALDI-TOF Peptide Mass Fingerprinting allowed to identify more than a hundred of α-amylase spots [2]. A careful review of identification results and MALDI mass spectra revealed unexpected forms of α-amylase [3].
2D-gels spots were digested with trypsin or other specific proteases and analyzed by MALDI-TOF, MALDI-TOF/TOF and nanoLC-MS/MS. various α-amylase forms, some being little or largely deleted at the C-terminus or N-terminus, while others showing internal deletions.

Material and methods

2D Electrophoresis :
100µg of proteins, pI 4-7, MW 15-150kDa, Colloidal Coomassie Blue staining

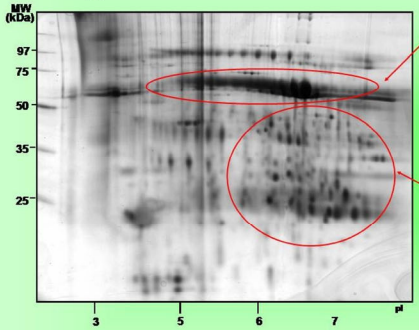
In-gel protein digestion :
0,1µg of trypsine or chymotrypsin or 2% Formic Acid

Mass Spectrometry :

Bruker's UltraFlex II MALDI-TOF/TOF

LC-Packings' Ultimate nanoLC ABI's Q-Trap 4000

Among 350 detected salivary proteins:



90 α-amylase isoforms:
- glycosylated (59kDa)
- natives (56kDa)

Swiss Prot P04745:
Human Salivary α-amylase

55 alternative forms detected by Peptide Mass Fingerprint (PMF) from 18 to 46 kDa

Swiss Prot P04745:
Human Salivary α-amylase

Fig. 1: 2D gel of salivary protein extract

Results

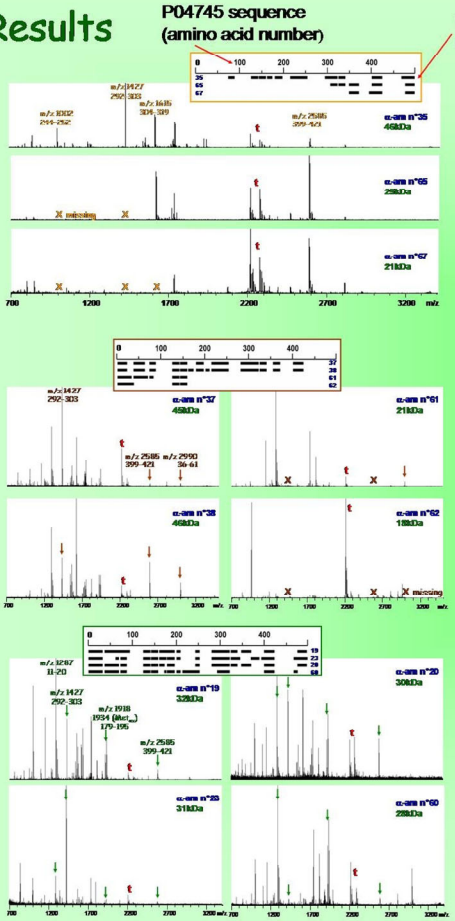


Fig. 2: tryptic PMF of N-term deleted α-amylases

Fig. 3: tryptic PMF of C-term deleted α-amylases

Fig. 4: tryptic PMF of internaly truncated α-amylases

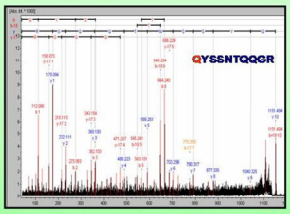


Fig. 5: α-amylase N-term Q is pyroglutamylated

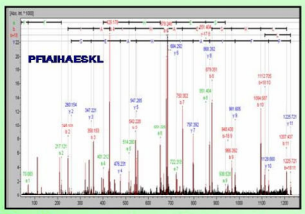


Fig. 6: α-amylase C-term identified after 2% Formic Acid digestion

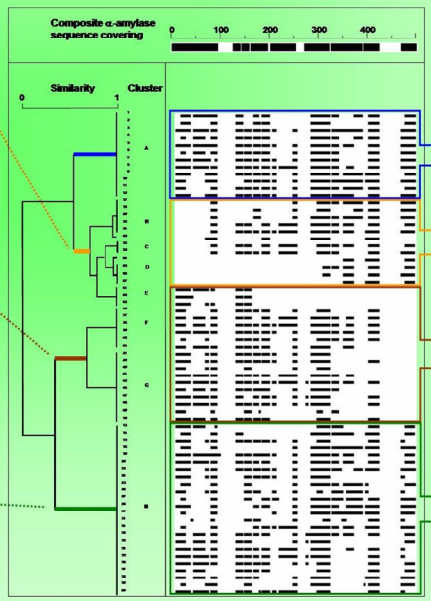


Fig. 7: sequence coverage hierarchical clustering

Conclusion :

- Human Salivary α-Amylase shows multiple forms
- N-term or C-term deletions have been characterized
- Some α-Amylase forms may present internal truncations
- Exhaustive MS/MS fragmentation of tryptic and chymotryptic peptides is on the way to further characterize this complex pattern

References
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