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► **To cite this version:**

Nicolas Sommerer, Christophe Hirtz, Jean-Pascal Gimeno, François Chevalier, Delphine Centeno, et al.. Unexpected proteomics and biochemical complexity of human salivary α -amylases in 2D gels. 17th International Mass Spectrometry Conference, Aug 2006, Prague, Czech Republic. . hal-04239153

HAL Id: hal-04239153

<https://hal.science/hal-04239153>

Submitted on 18 Oct 2023

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



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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 :
 100 μ g of proteins, pI 4-7, MW 15-150kDa, Colloidal Coomassie Blue staining

In-gel protein digestion :
 0,1 μ g of trypsin 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:

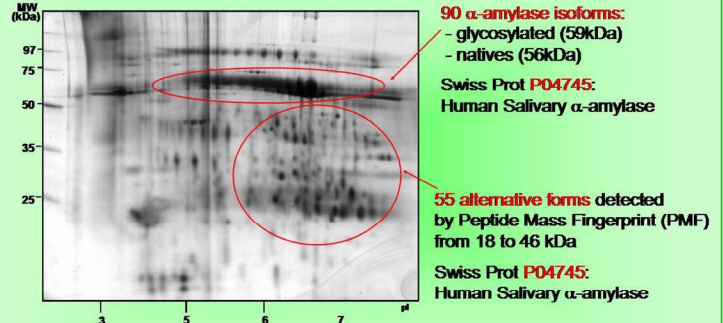


Fig. 1: 2D gel of salivary protein extract

Results

P04745 sequence (amino acid number)

Sequence coverage (detected peptides)

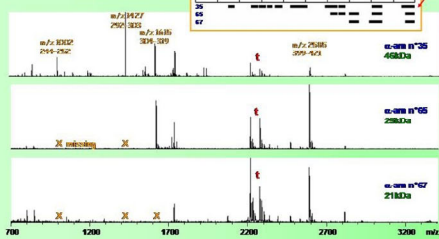


Fig. 2: tryptic PMF of N-term deleted α -amylases

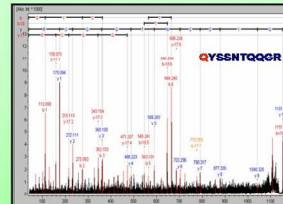


Fig. 5: α -amylase N-term Q is pyroglutamylated

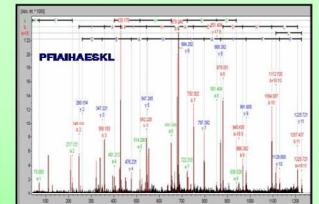


Fig. 6: α -amylase C-term identified after 2% Formic Acid digestion

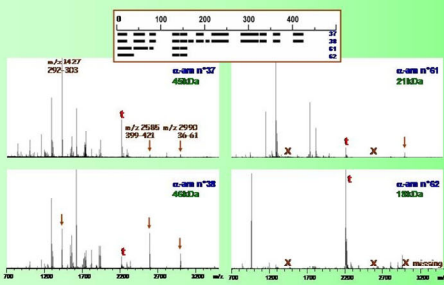


Fig. 3: tryptic PMF of C-term deleted α -amylases

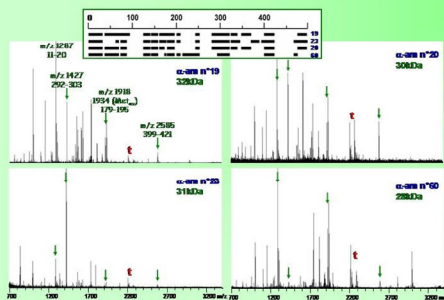


Fig. 4: tryptic PMF of internally truncated α -amylases

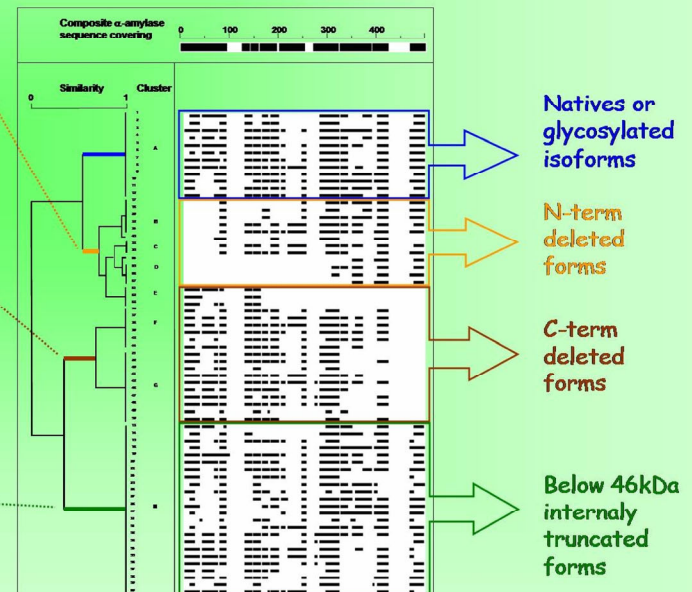


Fig. 7: sequence coverage hierarchical clustering

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

- [1] J. J. Zakowski, et al., *Crit. Rev. Clin. Lab. Sci.* 21, 283 (1985)
- [2] C. Hirtz, et al., *J. Physiol. Biochem.* 61, 469 (2005)
- [3] C. Hirtz, et al., *Proteomics* 5, 4597 (2005)