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# Unexpected Proteomics and Biochemical Complexity of Human Salivary $\alpha$ -Amylases in 2D gels



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Although human salivary  $\alpha$ -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  $\alpha$ -amylase spots [2]. A careful review of identification results and MALDI mass spectra revealed unexpected forms of  $\alpha$ -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  $\alpha$ -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 $\mu$ g of proteins, pI 4-7, MW 15-150kDa, Colloidal Coomassie Blue staining

In-gel protein digestion :  
 0,1 $\mu$ 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:

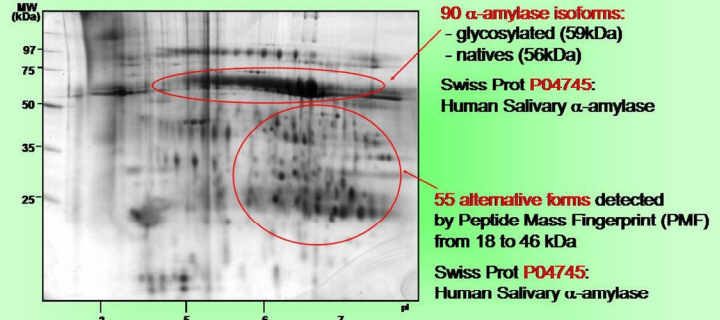


Fig. 1: 2D gel of salivary protein extract

## Results

P04745 sequence (amino acid number)

Sequence coverage (detected peptides)

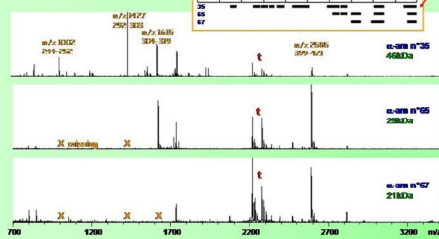


Fig. 2: tryptic PMF of N-term deleted  $\alpha$ -amylases

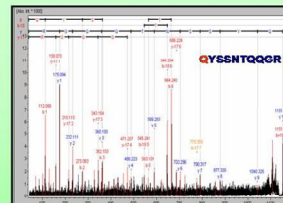


Fig. 5:  $\alpha$ -amylase N-term Q is pyroglutamylated

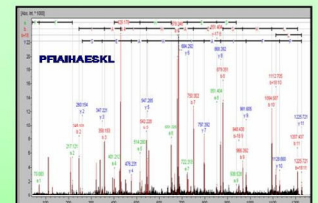


Fig. 6:  $\alpha$ -amylase C-term identified after 2% Formic Acid digestion

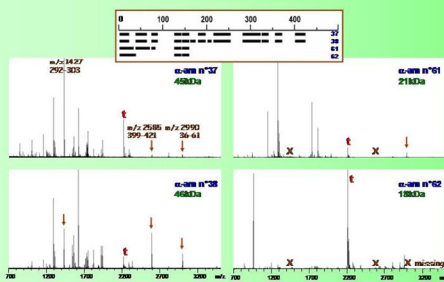


Fig. 3: tryptic PMF of C-term deleted  $\alpha$ -amylases

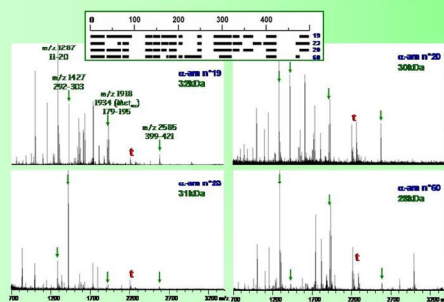


Fig. 4: tryptic PMF of internally truncated  $\alpha$ -amylases

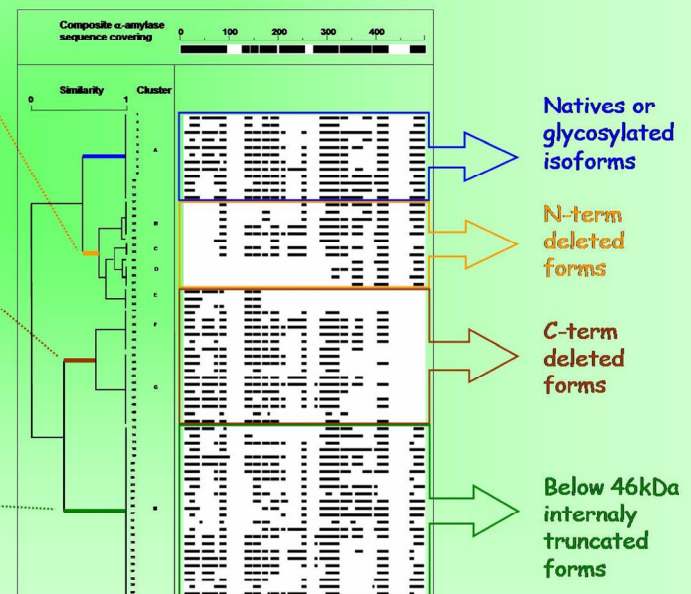


Fig. 7: sequence coverage hierarchical clustering

## Conclusion :

- Human Salivary  $\alpha$ -Amylase shows multiple forms
- N-term or C-term deletions have been characterized
- Some  $\alpha$ -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)