

Constructing a comprehensive catalogue of human salivary gland-derived intact proteome using top-down MS

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Overview

- Develop a top-down based intact accurate mass and time (AMT) tag approach
- Quantitatively analyze the proteins secreted from human parotid (PS) and submandibular/sublingual gland (SMSL)
- Elucidate the localization of salivary proteins as well as their post translational modifications

Introduction

Human saliva proteins, originating from three major pairs of salivary glands (submandibular, sublingual, and parotid), are synthesized follow a well-defined secretory pathway: firstly synthesized in the acinar cells of the glands, and then transited in the Golgi apparatus and stored in secretory granules before releasing from the cell into the duct system and secreting into the mouth.

Previous investigations have revealed the major human salivary proteins comprising acidic proline-rich proteins (aPRPs), basic proline-rich proteins (bPRPs), glycosylated proline-rich proteins, statherins, histatins, cystatins, amylases, and mucins. Most of these proteins have been found bearing posttranslational modifications, such as N-terminal signaling peptide removal, proteolysis, phosphorylation, and glycosylation.

We here presented a top-down based intact accurate mass and time (AMT) tag approach: an accurate intact mass tag database of about 70 human saliva proteins was generated utilizing different fragmentation methods (i.e., CID, HCD, and ETD) on Velos LTQ-Orbitrap. Saliva samples from six healthy individuals were then analyzed using an ultra sensitive 12T FTICR-MS instrument, and quantitative study was done using this intact. For proof-of-principle experiments, we quantitatively analyzed the proteins secreted from human parotid (PS) and submandibular/sublingual gland (SMSL) to elucidate the localization of salivary proteins, as well as their post translational modifications.

Significant different patterns from SMSL gland and PS gland were resolved with high reproducibility using only 2 µg (~ 2 µl) of saliva sample from each healthy individual. This method can potentially be applied for rapid and correct identification of biomarkers in a few micro-liters of human saliva.

Methods

Adult saliva donors of various ethnic and racial backgrounds, ranging in age from 22 to 30 y, were recruited from the general population, and samples were collected at the UCLA Medical Center with full donor consent, using procedures in accord with the Medical Institution Review Board and the Office of Protection for Research Subjects, as previously described.

Intact AMT tag approach

- Step 1: Intact protein database**
- High resolution LC-MS/MS using LTQ-Orbitrap Velos
 - Combined UStags and ProSightPC for protein identification
 - Generating an intact saliva protein database
- Step 2: Intact protein quantitation**
- High resolution RPLC-MS using 12T FTICR-MS
 - Optimized intact protein AMT tag-based quantitation method
 - Intact protein comparison between saliva PS and SMSL glands (biology insights)

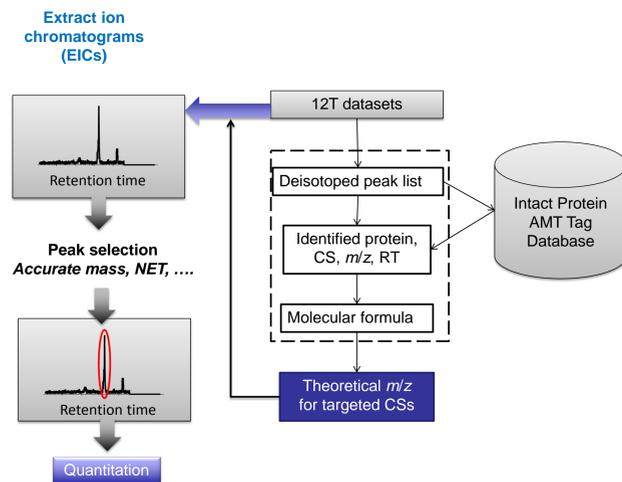


Figure 1. Schematic representative of quantitative protein identification using the intact AMT tag approach

Results

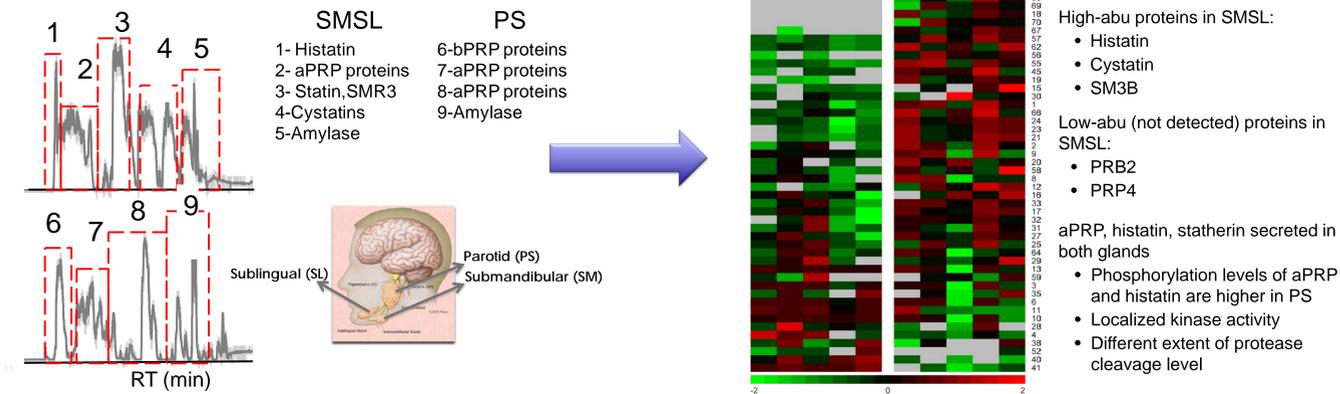


Figure 2. Overall comparison between SMSL and PS intact proteins. Highly abundant proteins in SMSL include histatin, cystatin, SM3B, SM3A, and their isoforms. Low abundant (or not detected) proteins in SMSL are mainly basic proline-rich proteins, such as PRB2 and PRB4. These results indicated secretion of salivary proteins in different glands follows a well defined pathway.

Salivary acidic proline-rich proteins (aPRPs)

- Secreted by both PS and SMSL glands
- Phosphorylation level is higher in PS than in SMSL (kinase involved in PS is more active)
- O-glycosylation and phosphorylation may be co-regulated
- Protease cleavage after PTM occurs
- Protease cleavage is more extensive in SMSL

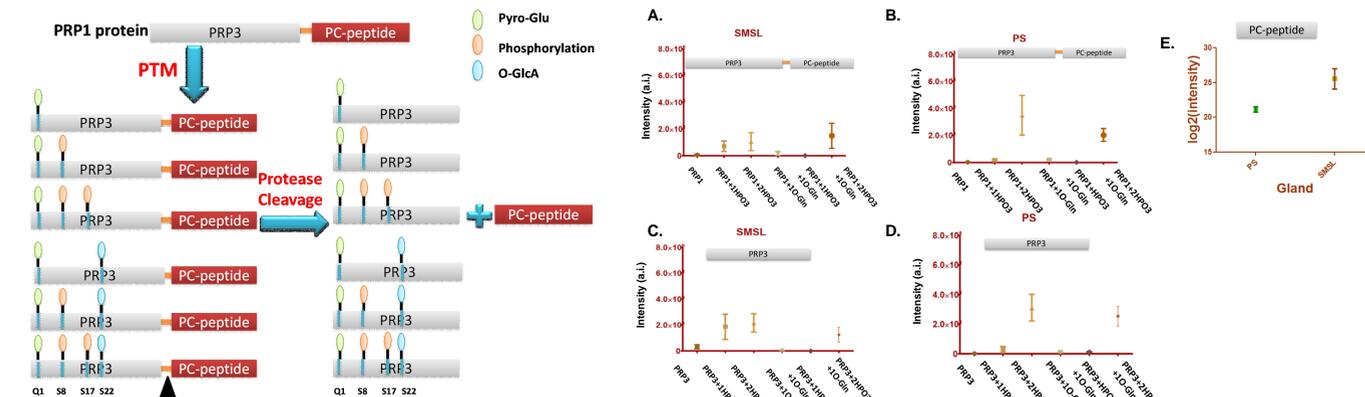


Figure 3. Two major groups of aPRP protein isoforms were observed (illustrated in left panel): one group with mass around 15 kDa, and another group with mass around 11 kDa. Proteins with mass around 15 kDa were identified as intact aPRP protein (PRP1) and its isoforms, while proteins with mass around 11 kDa were found as partially cleaved N-term isoforms of PRP1 proteins at residue 106 (also known as PRP3 proteins). The C-terminal fragment was also observed. In addition, our intact AMT tag approach provides relative quantitation information for different aPRP protein isoforms in different glands (in right panel, A-E).

Conclusions

We demonstrated an intact protein accurate mass and time (AMT) tag approach for confidently identifying and quantifying salivary proteins in different glands.

- High sensitivity and high resolution 12T FTICR-MS allowed us to use a10 ppm filter to more confidently identify and quantify proteins.
- Our in-house developed intact AMT tag pipeline demonstrated high reproducibility.
- For the first time, we observed the new O-ser glycosylations on aPRP proteins at the intact protein level, and correlated this PTM with phosphorylation.
- Our results provide by far the most comprehensive view of intact aPRP proteins and their convertase cleavage products.
- In summary, we provide an accurate method to monitor up to 70 intact saliva proteins for biomarker discovery in the context of human disease, using less than 2 µl of an individual's saliva sample.

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