Quantitative proteomics using MALDI-TOF MS analysis

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Introduction

Most proteomics technologies deal with the identification of proteins. Only one using mass spectrometry, ICAT (1), allows quantification. We have developed a method for quantification of different proteins using MALDI-TOF MS detection. Relative quantification was obtained by mixing two or more differentially tagged proteoms. Several protein tagging strategies, which modify the ε-amine of lysine were established. These modifications are carried out under non-denaturing conditions. The first method established was tagging with acetyl and propionyl groups. Two other methods using NHS-ester chemistry were developed with Cy3/Cy5 dyes and four different positively charged quaternary ammonium groups (2) (charge tag, CT). This chemistry can either be used on purified proteins or directly on serum. The detection of modified serum was combined with the affinity separation system ClinProt™ (Bruker Daltonics).

Methods

Principle of quantification:

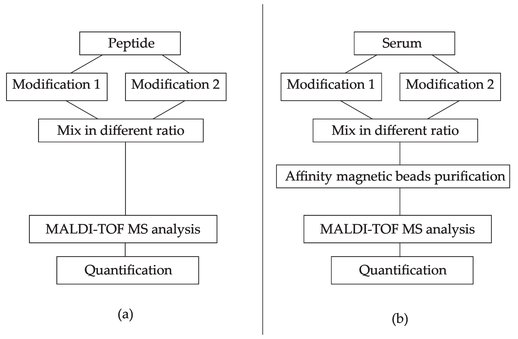

Figure 1: Principle of the quantification on purified protein (a) and on serum (b)

The first step is a chemical modification of peptides. Three different methods were used (figure 2).

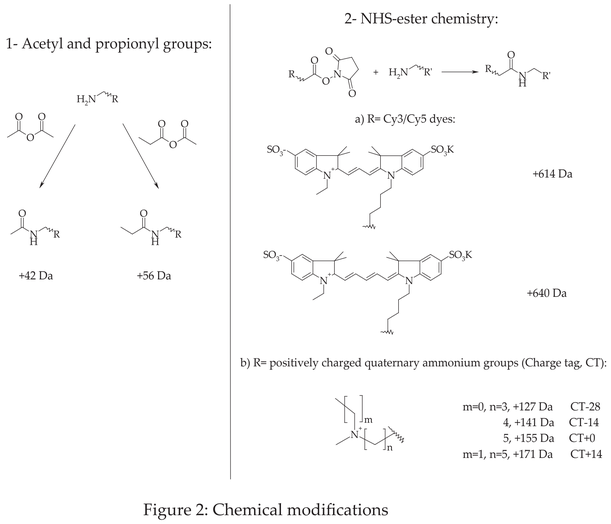

Figure 2: Chemical modifications

After modification differentially tagged proteoms were mixed. All liquid handling was done robotically. Before analysis by MALDI-TOF MS, serum sample were fractionated by affinity magnetic beads separation (ClinProt™, Bruker Daltonics).

Results

Relative quantification on peptide

Two modified samples were mixed together. Similar tags have similar properties and were used in comparative experiments. For each quantification we obtained linear correlation with R^2 around 0.99 (figure 3).

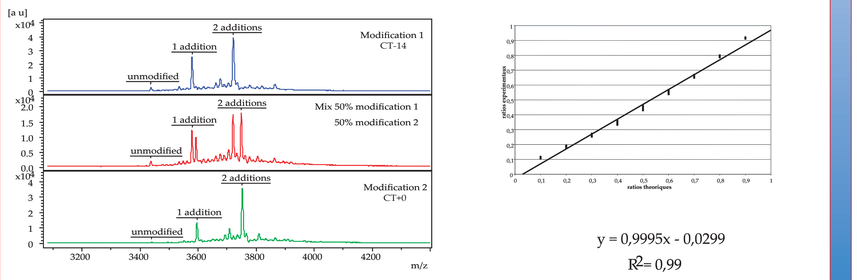

Figure 3: Spectra and relative quantification of salmon calcitonin (MW=3431.9 Da) modified with positively charged quaternary ammonium groups. The spectrum at the top is the first modification (CT-14), the spectrum at the bottom is the second modification (CT+0) and the spectrum in the middle is the mix of the two modifications with a ratio of 1:1.

The quantification of peptides by MALDI-TOF MS is effective. This modification chemistry is effective on primary amines. The peak intensity ratio of derivatives obtained from the same MALDI target spot were directly related to the ratio of analyte concentration.

Relative quantification on human serum

The modification was carried out directly on human serum. In this case, in one sample several unknown peptides could be studied. We tested the quantification of three peptides chosen arbitrarily. For these quantifications we obtained linear correlations with R^2 around 0.9 (figure 4).

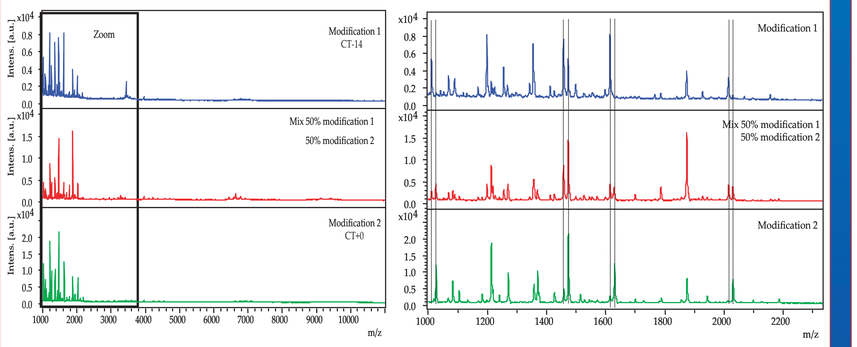

Figure 4: Spectra of human serum modified with positively charged quaternary ammonium groups. The spectrum at the top is the first modification (CT-14), the spectrum at the bottom is the second modification (CT+0) and the spectrum in the middle is the mix of the two modifications with a ratio of 1:1

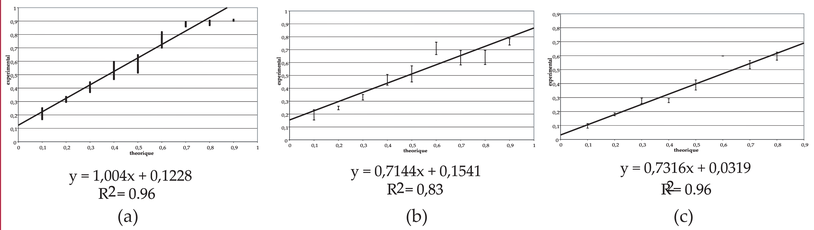

Figure 5: Relative quantification of peptides in human serum after chemical modification with positively charged quaternary ammonium groups. (a) peptide with a mass of 1873 Da, (b) peptide with a mass of 1473 Da and (c) peptide with a mass of 1213 Da.

The relative quantification with human serum is less precise than with purified peptides. The main difference between the two experiments is the purification step. This step was done manually, which introduces variability.

Relative quantification on digested peptide

For human serum quantification only small peptides (up to m/z 4000) can be used. At higher mass the resolution in not sufficient to differentiate 14 Da. In this case, samples had to be digested with endopeptidases like GluC or trypsin. Peptide are digested after chemical modification. The quantification obtained correlates linearly with R^2 around 0.99 (figure 6).

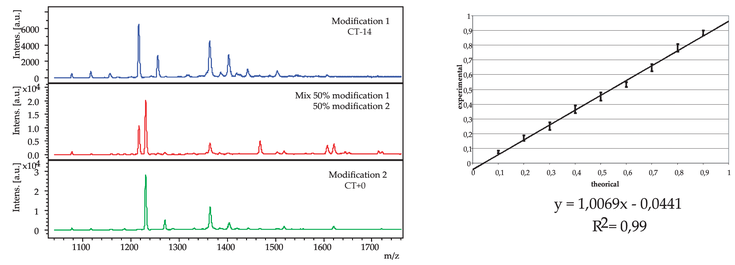

Figure 6: Spectra and relative quantification of bovine insulin (MW=5733,5 Da) modified with positively charged quaternary ammonium groups and digested with endopeptidase GluC. The spectrum at the top is the first modification (CT-14), the spectrum at the bottom is the second modification (CT+0) and the spectrum in the middle is the mix of the two modifications with a ratio of 1:1.

The chemical modification does not inhibit the digestion with endopeptidase GluC. In this case, quantification is also successful. The peak intensity ratio of modifided peptides correlated well with the ratio of analyte concentration.

Absolute quantification

Three modifications were mixed together. Two modifications were at different ratios while the third has always the same quantity (figure 7) as an intenal standard for the quantification.

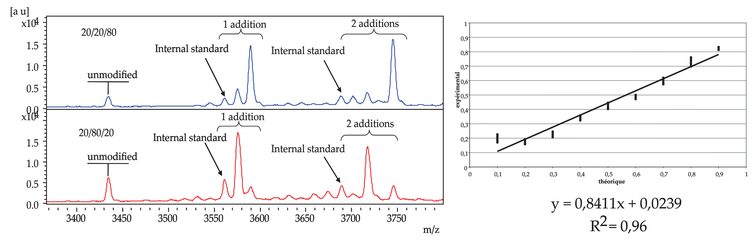

Figure 7: Spectra of absolute quantification of salmon calcitonin (MW=3431.9 Da) modified with three positively charged quaternary ammonium groups. Different ratios of modification 1 (CT-28) / modification 2 (CT-14) / modification 3 (CT+0) are shown. Modification 1 is the internal standard.

The absolute quantification using MALDI-TOF MS is possible. The peak intensity ratios correlate well with the ratio of analyte concentration. We obtained linear correlation with R^2 around 0.96.

Conclusion

Quantification of proteins and peptides by MALDI-TOF MS using chemical modifications was shown. This method is accurate and sensitive for peptides or bigger poteins after endopeptidase digestion.

References

1- Gygi, et al. (1999). 'Quantitative analysis of complex protein mixtures using isotope-coded affinity tags.' Nat Biotechnol **17** (10): 994-9
2-Bartlet-Jones, et al. (1994). 'Peptide ladder sequencing by mass spectrometry using a novel, volatile degradation reagent.' Rapid Commun. Mass Spectrom. **8** :737-742

Acknowledgement
EU FP6-funded Integrated Project MolTools.