Comparative evaluation of different surfaces for peptidome extraction by magnetic bead separation and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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Introduction

Peptidome profile of human fluids (urine, sera, plasma, tears, etc.) is nowadays an extended interesting tool that permits the identification of novel disease-associated biomarkers (Pisitkun 2006, Villanueva, 2004). However a wide range of pre-analytical considerations in variability and efficacy terms must be born in mind (Fiedler 2007). Our aim is to standardize different magnetic bead separation protocols followed by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry, for every studied fluid that will allow us to get the most reproducible information contained in such fluids, considering different variables which have been described as variability and reproducibility factors.

Material and methods

Magnetic Beads with different surface functionalities (hydrophobic interaction, cation exchange and metal affinity) have been evaluated for peptide extraction from the fluids used (urine, plasma and sera). Performance and variability has been calculated for all the characteristic mass signals obtained from the samples that have been processed after filters and different sampling proceedings. A MALDI-TOF mass spectrometer (Ultraflex; Bruker Daltonics) was used for peptidome profiling with minimal modifications of the standard parameters.

Results

Evaluation of sampling performance. We considered the previously described factors (3)(Fiedler 2007) which are freeze-thaw cycles and PH neutralization effect as an important source of variability in the accuracy and reproducibility of the relative peak intensities as well as the number of them in the evaluation of the results of three different urine samples processed after and between three freeze-thaw cycles and with and without PH correction before freezing. We did not observe any considerable adverse influence in peaks signal number at a global analysis peaks level. We have evaluated additionally the process in variability coefficient terms considering both automated and manual mode. For this purpose we have processed four samples of urine from different healthy volunteers by triplicate and during three consecutive days in order to evaluate reproducibility in the way proposed, obtaining a reduction of one half of variability coefficient using the automatic extraction. In addition we carried out an evaluation process of different surface functionalities for peptide extraction as well as an adapted protocol in volume and incubation times for each surface, obtaining the best protocol in our hands for every fluid and surface considered.

Conclusions

Although we do not disregard to consider in the future to study different sources of variability (Fiedler 2007) when more deeply analysis are performed for individual peaks, we can conclude basing ourselves on the contradictory literature and on our own experience that these variables are lab-depending. Nevertheless for pattern studies we will process all samples including in our experiments in the exact same conditions in order to get the best reproducibility. Moreover comparing processing methods, we obtained a result of a clear reduction of variability when samples were processed in an automatic way (52% for manual and 25% for automatic mode of variability coefficient).

References

Pisitkun, T., Johnstone R., Knepper MA., Moll. Cell. Proteomics 2006; 5:1760-71.

Villanueva J., Philip J., Entenmberg D., Chaparro CA., Tanwar MK., Holland EC., et al. Anal Chem. 2004; 76:1560-70.

Fiedler GM., Baunmann S., Leichtle A., Oltmann A., Kase J., Thiery J., et al. Clinical Chemistry. 2007; 53:421-8.