ABRF 2008 contest
Code: 21094

Summary of strategy and experiments

Firstly, 1/3 of samples A and B were digested with trypsin. And they were analysed by MALDI-TOF (Voyager STR Applied Biosystems) and by nanoLC MS/MS (Qstar Pulsar i Applied Biosystems). At the first glance the results indicated the existence of a single protein "Q15109/RAGE HUMAN".
The digested samples were passed through R2, R3 and graphite columns and MALDI-TOF was performed with the elution from the three columns. These accomplished a good coverage. Also, the same digested samples were injected twice in a nanoLC MS/MS (Qstar) using exclusion list for the second injection. The search engines Mascot and Protein Pilot were used. Protein Pilot allowed the identification of the C-terminal peptide: AVSISIIEPGEEG. The N-terminal peptide was found by performing de novo manual sequencing with the software Analyst. Therefore, the fragment of the protein Q15109/RAGE HUMAN was determined. Later on the C-terminal peptides corresponding to the different truncation proteins in sample B were found in these same files.

The second 1/3 of samples A and B were digested with Glu-C. The analysis of the digested samples by Qstar allowed the identification of a few new peptides and therefore increased a little bit the total coverage of the protein.

The third 1/3 of samples A and B was used to perform an MS of the intact samples both by MALDI-TOF and by ESI MS (Qstar). The spectra from ESI MS were reconstructed using BioAnalyst software. The analysis of both of these experiments showed that sample A contained a single protein, and its molecular weight corresponded to the fragment of the protein previously found and corresponded as well with the N-terminal and C-terminal peptides identified, and that sample B contained a mixture of truncations of the same protein. With this information and going back to the MS/MS files of the first trypsin digestion we were able to identify the C-terminal peptides for the three species in sample B. Also, the three molecular weights found in the ESI MS of sample B corresponded exactly with the sequences determined. At this point, an SDS gel was conformed confirming the previous findings since sample A showed a single band around 36KDa while sample B showed three bands around 12KDa, 24KDa and 36KDa. The bands were digested and analysed by LC/MS/MS, but no new information was found, probably due to the little amount of sample.

Moreover, the spectra of ESI MS (Qstar) for the intact sample B showed not only the species of 12, 24 and 36KDa, but also a 25KDa one, which we could not assign to any truncation or modification of the protein, and since we had no sequence evidence for it, we decided to consider it an artefact.