AMIDE HYDROGEN DEUTERIUM EXCHANGE OF TRANSLATION INITIATION FACTORS FOLLOWED BY ULTRA-HIGH RESOLUTION FT-ICR MASS SPECTROMETRY

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Amide hydrogen deuterium exchange (HDX) of proteins and protein complexes followed by mass spectrometry (MS) has reached a mature state. Nevertheless, the standard protocol relies on protein digestion using pepsin, the specificity of which is not well established. Therefore, systematic MS/MS of individual peptide is a prerequisite for the complete peptic peptide identification, which limits the further analysis as only MS/MS-identifiable peptides can be included in the exchange analysis step. Ultra-high resolution mass spectrometry enables highly accurate mass measurements that could, in principle, simplify the identification procedure. Nevertheless, our first attempts showed that mass accuracy alone was not sufficient for unambiguous identification, due to the common case of more than one peptide with the same or very close mass. This can arise even for small proteins of around 10 kDa. A statistical analysis of peptide cleavage sites by pepsin[1] was published and used in our approach as a basis for the development of a statistical tool predicting the probability of a given peptide to be present in a pepsin proteolytic digest. Using this calculated probability as a filter, a peptide reference list can be established without any MS/MS identification. The method has been verified by analysis of a series of peptide ions from five proteins of different sizes (11 kDa to 91 kDa) with standard q-TOF MS/MS fragmentation method. Out of more than 50 peptide ions examined by MS/MS, all have proven to be correctly identified with our new method.

Once validated, this method has been applied to data on the yeast eIF3 subunits. The yeast eIF3 complex involved in translation initiation in eukaryotes is composed of five subunits, three of which have been shown to interact with each other, whereas the precise binding regions remain to be elucidated. We aim on resolving the interactions of these 3 subunits: eIF3i (37 kDa), a C-terminal domain of eIF3b (11.3 kDa) and a domain of eIF3g (16 kDa). At present, two heterodimers formed by eIF3i with either eIF3g domain or eIF3b domain have been already characterized by liquid chromatography, and are investigated using HDX-MS. The current results regarding the interaction and dynamics of these two protein complexes will be presented.

[1] *Hamuro Y, Coales SJ, Molnar KS, Tuske SJ, Morrow JA. Specificity of immobilized porcine pepsin in H/D exchange compatible conditions. Rapid. Commun. Mass spectrum. 2008, 22, 1041.*