Molecular Characterization of Protein "Misfolding"-Aggregation Pathways by Mass Spectrometry

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A large variety of cellular processes are based on the formation and dynamics of multi- and supramolecular protein assemblies, and several diseases, previously thought to be unrelated, such as cancer and neurodegenerative diseases, are characterised by "misfolded" protein aggregates. Chemical structures and reaction pathways of pathophysiological aggregates are only poorly characterised at present. "Soft-ionisation" mass spectrometry (MS), such as HPLC-electrospray-MS, is often unsuitable to direct analysis of reaction pathways and intermediates in aggregation. Recently, ion mobility- MS (IM-MS) has been emerging as a new tool for analysis of protein aggregation due to its *concentration- independent* gas phase separation capability. First applications of IM-MS to the *in vitro* oligomerization of α-synuclein (αSyn) and ß-amyloid (Aß), key proteins for Parkinson's disease and Alzheimer's disease, enabled the identification of hitherto unknown degradation and aggregation products. Time- dependent studies of the *in vitro* oligomerization- aggregation of αSyn provided the first identification of a specific autoproteolytic fragmentation, previously observed by gel electrophoresis but not identified, particularly a highly aggregation-prone fragment by cleavage at Val71/Thr72 in the ß-breaking triplett Val-Val-Thr in the central aggregation domain [1]. The corresponding recombinant αSyn(72-140) fragment showed substantially faster aggregation and high neurotoxicity compared to the intact protein. Recently, the development of combined (online) affinity- MS methods [2] enabled first direct ("top-down") structural studies *in vivo,* such as from brain homogenate. Applications of affinity-MS will be discussed using epitope-specific αSyn- and ß-amyloid (Aß)- antibodies [3] for the characterization of oligomers and interactions of Aß, αSyn and ß-glucocerebrosidase, the target enzyme for Gaucher's Disease, a neurological lysosomal storage disorder [4]. These results indicate ion mobility- MS and affinity- MS as powerful tools for the molecular elucidation of structures and intermediates of polypeptide aggregation. Corresponding structures thus obtained provide a basis for (i), the detailed study of oligomerization- aggregation pathways; (ii), the design of peptides capable of inhibiting or modifying aggregation; and (iii), the development of specific methods for quantitative protein determinations in biological fluids.

[1] Vlad, C. et al., (2011) *ChemBiochem.* **12**, 2740-2744.
[2] Dragusanu, M., et al. (2010) *J. Am. Soc. Mass Spectrom.* **21**, 1643-1648.
[3] McLaurin, J., et al. (2002) *Nature Med.* **8**: 1263-1269
[4] Przybylski, M. et al./Univ. Konstanz & Centogene (2012) Eur. & US Patent Applications.