Using ion mobility mass spectrometry to probe amyloid systems

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Introduction

More than twenty five proteins or peptides are associated with amyloid disease. The precise mechanisms by which these unrelated soluble protein or peptide monomers assemble into highly-ordered fibrillar deposits is unknown. Amylin (also known as islet amyloid polypeptide) is the amyloid peptide associated with type II diabetes. It is found *in vivo* as amyloid deposits in the pancreatic islets of sufferers and its self-aggregation is thought to be a pathogenic factor in the disease. Ataxin-3 is the protein associated with the neurodegenerative polyglutamine (polyQ) disease spinocerebellar ataxia type 3 (also known as Machado-Joseph disease). PolyQ diseases are a group of inherited neurodegenerative disorders caused by aggregation of specific proteins with expanded polyQ regions.

The work described in this report highlights two recent examples of how electrospray ionisation-ion mobility spectrometry-mass spectrometry (ESI-IMS-MS) can be used to probe amyloid systems that are otherwise difficult to study. ESI-IMS-MS represents a powerful technique for elucidating the structures of species within complex, heterogeneous samples. It enables separation and subsequent identification of individual components within complex mixtures, within a single experiment. In the study of amylin presented here, ESI-IMS-MS was used in parallel with molecular modelling to characterise the soluble oligomeric species of fibril-forming peptides. In the study of ataxin-3, ESI-IMS-MS was used with limited proteolysis, to provide insights into its structure and dynamics.

Results

For three of four peptides derived from the amyloidogenic core of amylin (Figure 1), oligomers >20-mer were detected using ESI-IMS-MS. This is the first time that oligomers of amylin fragments have been reported. For the non-fibril-forming peptide (GAIL), used as a control, no species larger than dimer were observed. An IMS calibration allowed collision

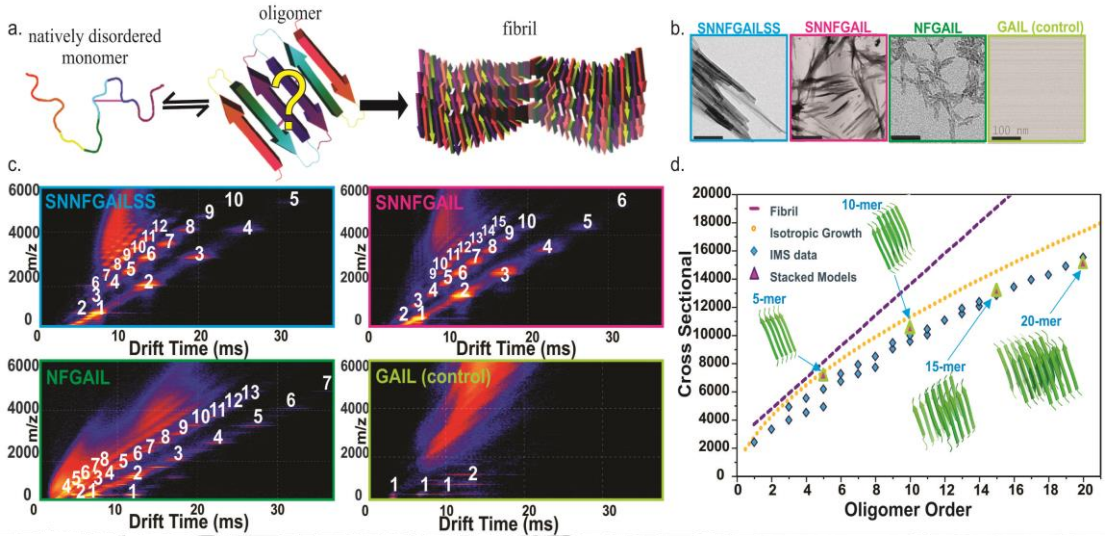

Figure 1: (a) hypothesis of amyloid progression, **(b)** EM images of peptide fibrils, scale bar is 100 nm. **(c)** ESI-IMS-MS data showing oligomers formed by peptides. **(d)** comparison with modeled structures.

cross-sections (CCS) of the oligomers to be estimated. These CCS were compared with molecular models to reveal a possible mechanism of amyloid fornation by the self-

assembling peptides. CCSs observed were comparable with single β-sheet models for the low-order oligomers, with higher-order oligomers fitting better with multiple β-sheet models. For ataxin-3 (Figure 2), ESI-IMS-MS revealed that the full-length protein populates a wider range of conformational states than the isolated Josephin domain (JD), most likely due to the flexibility of the C-terminal domain. ESI-MS spectra showed that the major products of JD and ataxin-3, after limited proteolysis, are mostly the same. These observations suggest that the C-terminal domain and the JD do not exhibit significant or long-lived interactions.

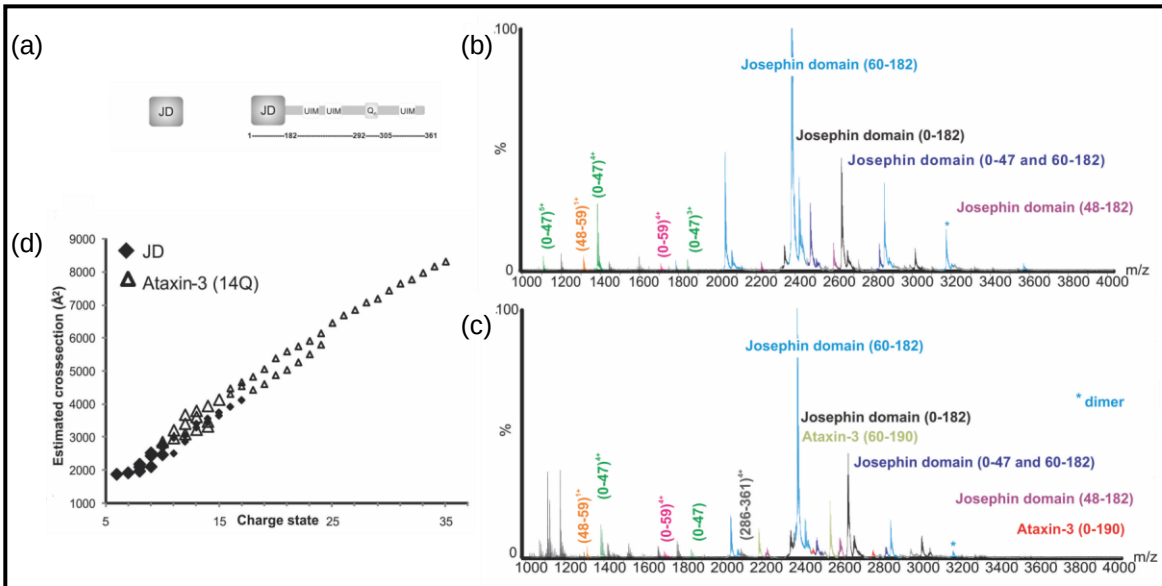

Figure 2: schematics of **(a)** the Josephin domain (JD, left) and ataxin-3 (right). ESI-MS spectra following limited proteolysis of **(b)** the JD, and **(c)** ataxin-3. **(d)** ESI-IMS-MS estimated CCS for JD and ataxin-3.

Publications

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