Investigating fibril structure and assembly using mass spectrometry

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Introduction

The assembly of normally soluble proteins into pathological aggregates is of great interest due to their role in important illnesses such as Alzheimer's and Parkinson's diseases. The human protein beta-2-microglobulin (β_2m) assembles into amyloid fibrils *in vivo* causing the debilitating condition known as dialysis related amyloidosis (DRA). We have been investigating the mechanism of aggregation of β_2m into amyloid using electrospray ionisation mass spectrometry (ESI-MS), focusing on the conformational properties of aggregation-prone monomers, as well as the structure of the fully assembled fibril.

Investigating fibril structure

Fibrils which have the cross-β structure characteristic of amyloid fibrils found in the joints of DRA sufferers can be formed from wild-type β_2m under acidic conditions *in vitro*. The morphology of the fibrils formed *in vitro* depends on the conditions employed. Incubation at low ionic strength at pH 2.5 yields long (>μm) straight fibrils, while at pH 3.6 short (<500nm) fibrils form. At higher ionic strength (0.2M-0.4M) fibrils which exhibit a curved, nodular morphology are formed. To determine the conformation of β_2m within fibrils with these different morphologies, limited pepsin proteolysis of the fibrils was performed, followed by analysis of the fragments by ESI-MS and tandem mass spectrometry (MS/MS). For comparison, proteolytic degradation of the monomer and seven synthetic peptides representing the entire sequence of β_2m was undertaken. The differing morphologies of the fibrils resulted in distinct digestion patterns as shown in Figure 1.

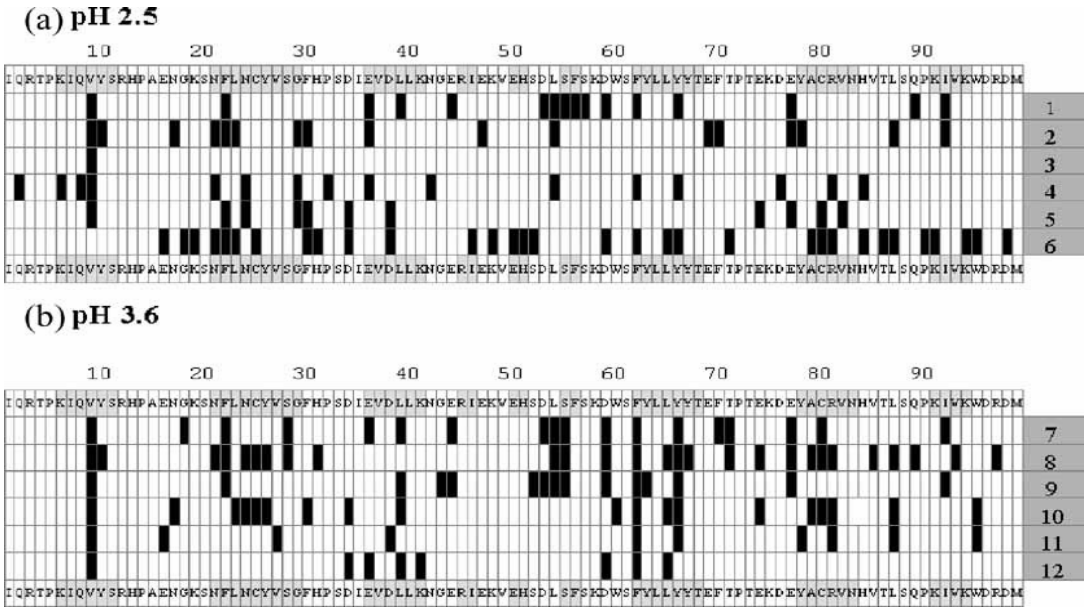

Fig 1. Summary of the cleavage sites observed by ESI-MS(/MS) after pepsin proteolysis of β_2m fibrils of different morphological type. (a) Samples at pH 2.5: (1) and (2) monomeric β_2m, (3) and (4) long, straight fibrils formed at low ionic strength, and (5) and (6) nodular fibrils formed at high ionic strength. (b) Samples at pH 3.6: (7) and (8) monomeric β_2m, (9) and (10) short, straight fibrils formed at low ionic strength, and (11) and (12) nodular fibrils formed at high ionic strength. Each pair of reactions (e.g (1) and (2)) were incubated with pepsin (1:100 w/w) for 15min or for 24hr, respectively, at 25°C. The 99-residue amino acid sequence is also shown. The regions in grey shading indicate the location of β-strands in native β_2m. The vertical bars in (a) and (b) depict the C-terminal residue of an identified peptide fragment and hence indicate the proteolytic cleavage sites.

The results shown in Figure 1 indicate that the long, straight fibrils formed at pH 2.5 (low ionic strength) show enhanced protection from limited proteolysis (only one cut site at valine 9) relative to the fibrils formed at pH 3.6. The protected fibril core is reduced from ~90 residues in the long, straight fibrils to only ~30 residues in the nodular forms. Only the A strand (first 11 residues) of β_2m is unprotected in all fibril types.

Investigating fibril assembly

As the assembly mechanism of β_2m fibrils remains unclear, the identification and characterisation of early oligomeric species was undertaken. Using ESI-MS the formation of β_2m oligomers was monitored over time at pH 2.5 and 3.6 and validated using analytical ultracentrifugation (AUC) and Thio-T fluorescence (Thio-T). The oligomeric species detected at each time point for both pH values is shown in Figure 2.

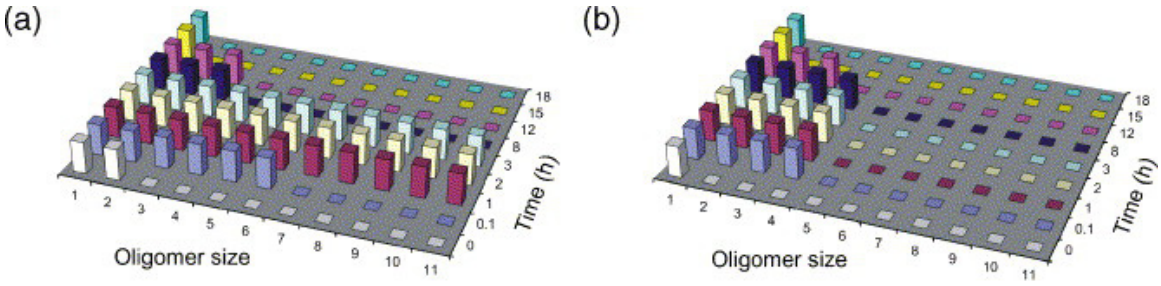

Fig 2. Oligomer distributions observed during β_2m assembly measured by nanoESI-MS over a range of *m/z* 3200–5500. (a,b) Summary of species detected during fibril assembly at 37°C at (a) pH 3.6 and (b) pH 2.5 using nanoESI-MS. Note that as the concentration of each oligomer cannot be quantified precisely by nanoESI-MS, the population of individual species is denoted as either present or absent in this chart.

The MS data, confirmed by AUC, shows that in the lag phase of pH 2.5 fibril assembly (~7 h as monitored by Thio-T fluorescence) monomeric to tetrameric species are formed (Fig. 2b). By contrast, at pH 3.6 wherein nodular fibrils are formed without a lag phase, an extensive series of oligomeric forms are detected (Fig. 2a), consistent with a mechanism involving monomer addition. This work highlights the power of ESI-MS to identify protein aggregation intermediates in complex heterogeneous systems in real time.

Investigating aggregation-competent conformations

In the next step of this work we are using hydrogen-deuterium exchange (HX) monitored by MS to investigate rarely populated, partially unfolded conformations of monomeric β_2m within the native ensemble. Comparison of the data, with parallel experiments using NMR, is beginning to unravel the complex dynamics of β_2m and to define the role of these conformational changes in fibril assembly.

Publications

Myers, S.L., Thomson, N.H., Radford, S.E. & Ashcroft, A.E. (2006) Investigating the structural properties of amyloid-like fibrils formed *in vitro* from β_2-microglobulin using limited proteolysis and electrospray ionisation mass spectrometry. *Rapid Commun. Mass Spectrom*. **20**, 1628-1636.

Smith, A.M., Jahn, T.R., Ashcroft, A.E. & Radford, S.E. (2006) Direct observation of oligomeric species formed in the early stages of amyloid fibril formation using electrospray ionisation mass spectrometry. *J. Mol. Biol.* **364**, 9-19.

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