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Ion mobility-mass spectrometry and orthogonal gas-phase techniques to study amyloid formation and inhibition

Waldemar Hoffmann[1,2], Gert von Helden[2] and Kevin Pagel[1,2]

Amyloidogenic peptide oligomers are responsible for a variety of neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Due to their dynamic, polydisperse, and polymorphic nature, these oligomers are very challenging to characterize using traditional condensed-phase methods. In the last decade, ion mobility-mass spectrometry (IM-MS) and related gas-phase techniques have emerged as a powerful alternative to disentangle the structure and assembly characteristics of amyloid forming systems. This review highlights recent advances in which IM-MS was used to characterize amyloid oligomers and their underlying assembly pathway. In addition, we summarize recent studies in which IM-MS was used to size- and mass-select species for a further spectroscopic investigation and outline the potential of IM-MS as a tool for the screening of amyloid inhibitors.

Addresses
[1] Institute of Chemistry and Biochemistry, Freie Universität Berlin, Takustrasse 3, Berlin 14195, Germany
[2] Fritz-Haber-Institut der Max-Planck-Gesellschaft, Faradayweg 4-6, Berlin 14195, Germany

Corresponding author: Pagel, Kevin (kevin.pagel@fu-berlin.de)

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Introduction

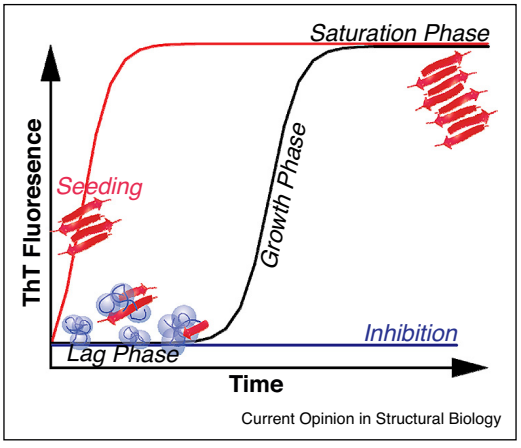
Amyloid formation is a central pathological feature of a variety of neurodegenerative disorders such as Alzheimer's (AD) and Parkinson's disease [1]. In their native state, the involved proteins are usually harmless, soluble and can range from functional, fully folded species to intrinsically disordered structures. Upon environmental changes, such as a change in pH or interactions with lipid membranes, the proteins undergo a conformational transition and self-assemble into insoluble, β-sheet rich amyloid fibrils. These fibrils are the major constituents of amyloid plaques that can for example be found in the brain tissue of Alzheimer's patients and were therefore long considered to be the toxic species that cause these diseases. Over the last few years, however, increasing evidence suggested that not the mature fibrils, but rather early, soluble intermediates, that occur during amyloid formation, are the actual toxic species in various neurodegenerative disorders [2]. Due to their polydisperse, polymorphic, and transient nature these intermediates are challenging to characterize *via* traditional condensed-phase methods like nuclear magnetic resonance (NMR) or circular dichroism (CD) spectroscopy. In most of the cases only ensemble-averaged information is obtained, and as a result, very little is known about the structure of individual oligomers.

Over the last decade, several gas-phase techniques, particularly ion mobility-mass spectrometry (IM-MS), emerged as promising tools to separate and analyze individual oligomeric species without affecting the equilibrium of the ensemble [3]. An IM-MS analysis typically starts with the preparation of the sample under conditions where fibril formation occurs. Soluble oligomers are then carefully transferred into the gas phase using soft ionization techniques such as electrospray ionization (ESI). Packets of these ions are further injected into an ion mobility cell that is filled with an inert buffer gas, through which they migrate due to a weak electric field that is applied. During this drift, compact ions undergo fewer collisions with the buffer gas than extended species and therefore traverse the cell faster, that is with a shorter drift time. As a result, ions are not only separated according to their mass and charge like in conventional MS, but also based on their rotationally averaged collision-cross section (CCS). This analysis based on size and shape enables the analysis of species, which exhibit an identical m/z but differ in their oligomer size or conformation. This review summarizes the recent achievements in which IM-MS and orthogonal techniques were used to obtain detailed structural information of soluble intermediates and to study the mode of inhibition on distinct oligomeric states.

The pathway of amyloid formation

Even though the proteins that are involved in amyloid formation differ significantly in their size and sequence, they share similar assembly characteristics [3]. The pathway usually follows a nucleation-dependent mechanism, which can be divided into three parts: a lag-, growth- and saturation-phase (Figure 1). Dye molecules such as

Figure 1



Features of amyloid formation. The typical amyloid formation pathway can be divided into three stages, a lag-, growth- and saturation phase (black trace). During the lag phase, transient and highly interconverting intermediates are found, but no fibrils are present. Once a so-called nucleus is formed, fibril formation is initiated (growth phase), and an autocatalytic growth phase leads to mature fibrils that can be observed at the saturation phase. The addition of nuclei in the form of seeds eliminates the lag phase and directly initiates the growth phase (red trace). In the presence of small inhibitors, no fibril formation occurs (blue trace).

thioflavin T (ThT) are commonly used to monitor the kinetics of fibril formation in real-time. ThT binds to amyloid fibrils via intercalation, which results in a change in the structure of the dye and an increased fluorescence [4]. It is believed that protein aggregation is initiated by an unfolding/misfolding event of the monomer, forming species that are capable of undergoing oligomerization (lag phase) [3]. These oligomers can further exchange subunits or undergo conformational transitions, but once a so-called nucleus is formed, fibril formation is facilitated by an autocatalytic growth (growth phase) up to the presence of mature fibrils (saturation phase). The autocatalytic growth can be further accelerated by fibril fragmentation, providing additional fibril ends for monomer association, whereas end-joining of two fibrils might reduce the rate at which mature fibrils are formed [5]. A detailed understanding of the underlying structures in each phase is crucial for identifying potential targets and the successful development of drugs.

The duration of the lag phase is highly-dependent on environmental conditions [6] or the presence of small molecules that can inhibit fibril formation (Figure 1, blue trace) [7••]. Also the dynamics of the oligomers can lead to different behavior as was for example demonstrated for beta-2 microglobulin (WT) and its H51A alloform [8•]. Both proteins form oligomers that are indistinguishable via IM-MS (same CCSs), but the lag phase of the H51A alloform is significantly longer than that of the WT. IM-MS and real-time MS using ^{15}N-enriched proteins were performed to study subunit exchange at the end of the individual lag phases. H51A oligomers were shown to undergo no, or a barely measurable exchange, which indicates that these oligomers are kinetically trapped species. WT oligomers, on the other hand, exhibited a rapid subunit exchange, and this behavior accounts for the short lag phase of WT beta-2 microglobulin [8•].

In the presence of pre-formed nuclei that can act as fibrillar seeds, the entire lag phase can be eliminated (Figure 1, red trace) [3]. The fibrillar seeds presumably act as a template for monomers to refold and assemble into amyloid fibrils. For example, fibrillar amyloid-β40 (Aβ40) can cross-seed the human islet amyloid polypeptide (hIAPP). The reverse, however, does not occur, that is hIAPP seeds are not capable of cross-seeding Aβ40 monomers. The cross-seeding phenomenon is therefore in many cases sequence-dependent. If both peptides are co-incubated another effect termed co-polymerization occurs. Here, mixed Aβ40/hIAPP-oligomers and mixed fibrils are observed. For that reason the cross-seeding phenomenon has to be distinguished from co-polymerization [9].

Assembly and architecture of fibrils

In principle, all peptides and proteins are able to form amyloids under certain conditions [6]. However, certain amino acids (AAs), exhibit a higher propensity towards fibril formation than others [10]. Recently, it was proposed that the tendency to form amyloid fibrils, microcrystals or unordered aggregates is linked to the propensity of isolated AAs to form large meta clusters [11•]. Hydrophobic residues, like isoleucine or phenylalanine, form extended oligomers which are larger in size than expected for an idealized spherical growth of the oligomers [12]. Hydrophilic AAs (serine, asparagine), on the other hand, assemble into more compact oligomers. Peptides, containing multiple hydrophobic AAs (extended AA meta-clusters) are prone to fibril formation, whereas sequences rich in hydrophilic residues (compact AA meta-clusters) are more likely to form microcrystals. This correlation in the assembly characteristics was used to establish a novel approach to score and predict the aggregation propensity of peptides.

Due to extensive research in the last 25 years, the structure of mature fibrils is well understood today. X-Ray diffraction and NMR structures are available for a couple of smaller peptides [13,14] up to fibrils of the full-length Aβ peptide [15,16]. All known structures share a common fibrillar core-structure, often referred to as a 'steric-zipper', which is formed by two β-sheets, each β-strand stacked in-register [14]. The protruding side-chains of each strand interdigitate like a zipper, forming a dry, tightly self-complementing interface. This highly ordered arrangement explains the stability of amyloid