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ESI-IMS–MS: A method for rapid analysis of protein aggregation and its inhibition by small molecules

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ABSTRACT

Electrospray ionisation-ion mobility spectrometry–mass spectrometry (ESI-IMS–MS) is a powerful method for the study of conformational changes in protein complexes, including oligomeric species populated during protein self-aggregation into amyloid fibrils. Information on the mass, stability, cross-sectional area and ligand binding capability of each transiently populated intermediate, present in the heterogeneous mixture of assembling species, can be determined individually in a single experiment in real-time. Determining the structural characterisation of oligomeric species and alterations in self-assembly pathways observed in the presence of small molecule inhibitors is of great importance, given the urgent demand for effective therapeutics. Recent studies have demonstrated the capability of ESI-IMS–MS to identify small molecule modulators of amyloid assembly and to determine the mechanism by which they interact (positive, negative, non-specific binding, or colloidal) in a high-throughput format. Here, we demonstrate these advances using self-assembly of Aβ40 as an example, and reveal two new inhibitors of Aβ40 fibrillation.

1. Introduction

Amyloidosis contributes to more than 50 human disorders including Alzheimer's disease (AD) [1], the most common form of dementia worldwide [2]. The accumulation of the amyloid-β peptide (Aβ) in extracellular plaques in the form of highly ordered amyloid fibrils is a hallmark of AD, but it is the pre-fibrillar oligomers that are thought to be the major neurotoxic species [3]. Due to the complex mechanisms involved in AD and other amyloid diseases, there are currently few therapies available. Indeed, as the toxic species in many of these disorders remain elusive, current therapies focus on ameliorating symptoms, rather than preventing disease progression [4]. The identification and characterisation of the potentially toxic oligomers populated *en route* to amyloid fibrils is a significant challenge due to the heterogeneous, transient and lowly-populated nature of these species. ESI-IMS–MS has the unrivalled ability to study such systems given its unique potential to detect and identify multiple ions present at low concentrations within the same sample, based on their mass-to-charge ratio (m/z) [5–9]. When coupled to IMS, further separation of ions of the same m/z ratio but different collision-cross sectional areas (CCS) is enabled, allowing different conformational states of isobaric protein oligomers to be characterised simultaneously [5,9–14]. Changes in protein conformation, and appearance and subsequent disappearance of oligomeric states, can be monitored over time [5,13,15–17]. Furthermore, as native ESI-IMS–MS allows the preservation of protein-ligand complexes, the binding interactions of small molecules to amyloid peptides/proteins can be observed,

Abbreviations: Aβ, amyloid-β peptide; Aβ40, amyloid-β peptide residues 1–40; AD, Alzheimer's disease; ADH, alcohol dehydrogenase; AFM, atomic force microscopy; CCS, collision-cross sectional area; Cl-NQTrp, chloronaphthoquinine-tryptophan; CsI, caesium iodide; DMSO, dimethyl sulfoxide; EGCG, (−)-epigallocatechin gallate; ESI-IMS–MS, electrospray ionisation-ion mobility spectrometry–mass spectrometry; HDMS, high-definition mass spectrometry; hIAPP, human islet amyloid polypeptide; HTS, high-throughput screen; m/z, mass to charge ratio; ROCS, Rapid Overlay of Chemical Structures; (T)EM, (transmission) electron microscopy.

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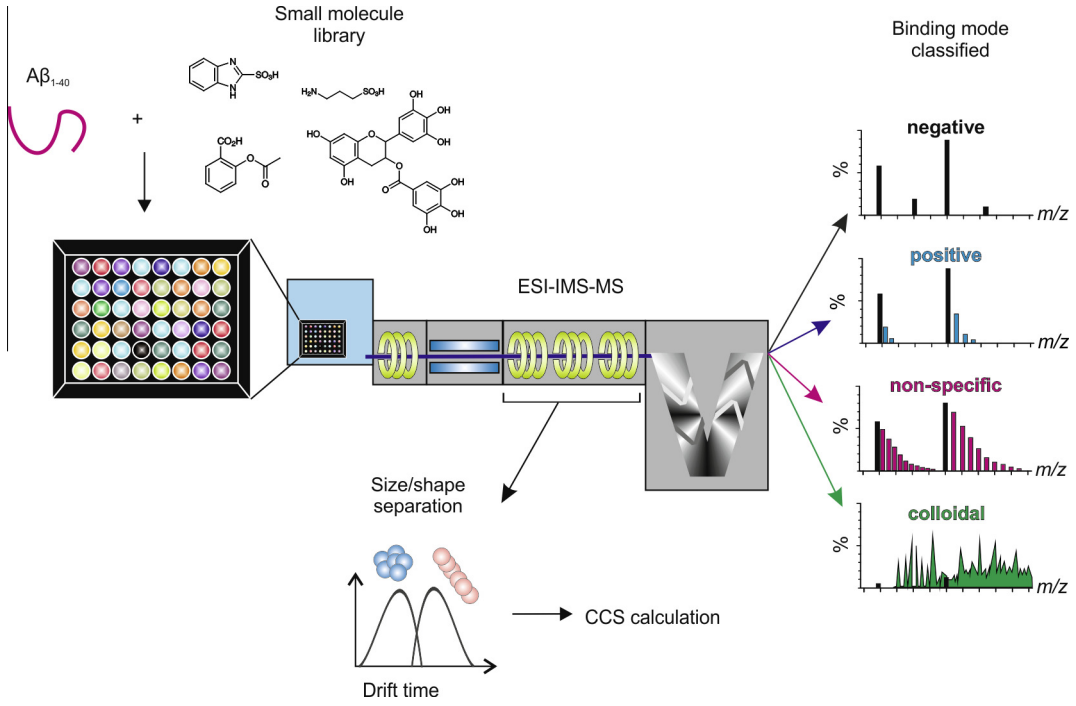

Fig. 1. Schematic of the ESI-IMS–MS experimental procedure. The protein of interest is mixed individually with small molecules from a compound library in 96-well plate format. Via a Triversa NanoMate automated nano-ESI interface, the samples are infused into the mass spectrometer, wherein separation occurs based on the mass to charge ratio (m/z) and collisional cross-sectional area (CCS). A non-interacting small molecule will produce a spectrum the same as that generated by the peptide alone (black). A small molecule that specifically interacts with the peptide will produce a binomial distribution of bound peaks (blue) [45]. A non-specific ligand will bind but result in a Poisson distribution of bound peaks (pink) [45]. A colloidal inhibitor will produce a range of overlapping peaks due to self-association of the small molecule (green).

concomitant with changes in the relative abundances and distributions of oligomeric species present. These changes can then be correlated to alterations in fibril formation rate or yield [5,7,9,17–20] allowing identification of novel inhibitory compounds. The specific conformational states to which inhibitors bind can also be determined [5,13,17], and the mode of inhibition can be elucidated by simple analysis of the resulting spectra [18].

Here we demonstrate the power of ESI-IMS–MS as a method able to provide rapid and accurate analysis of protein aggregation and its inhibition, using self-assembly of Aβ40 into amyloid fibrils as an example system. The basis of the experimental set up is shown in Fig. 1. A further example, using amylin involved in type II diabetes mellitus, can be found in Young et al. [18].

2. Methods

2.1. Sample consideration

The most important parameter to consider in sample preparation for analysis by ESI–MS is the buffer in which the aggregation process is to be studied. Most *in vitro* biochemical techniques used to study amyloid assembly utilise involatile buffers that are incompatible with ESI–MS. This leads to issues with efficient ionisation of the sample and extensive adduct formation [13], reducing the quality of the resulting spectra. It is necessary, therefore, to conduct MS experiments in aqueous, volatile buffers such as ammonium acetate, ammonium formate or ammonium bicarbonate. *Note:* Simply replacing a non-volatile buffer with an MS-compatible buffer at the same pH and ionic strength may not yield the same rate of, and/or products of, aggregation. Ion composition, as well as ionic strength and pH, can influence aggregation parameters. We suggest, therefore, that the aggregation process under these conditions should be characterised prior to analysis by ESI–MS, using solution assays (e.g. dye binding assays, light scattering, or imaging of aggregates via electron microscopy (EM)/atomic force microscopy (AFM) (reviewed in [21])), to confirm that the assembly mechanism is similar in the non-volatile and ESI–MS-compatible buffers of equivalent ionic strength and pH.

Proteins stored or purified in non-volatile buffers, such as Tris·HCl, should be stringently buffer-exchanged, and concentrated if necessary, prior to analysis by ESI–MS. Working protein concentrations of low micromolar range are typical.

2.2. Sample and small molecule preparation

For the current study, an ESI-IMS–MS screen of the interactions of small molecules with Aβ40 at pH 6.8 was undertaken.

1. Aβ40 was expressed recombinantly and purified as described previously [18,22]. *Note:* Synthetic peptide could be used in place of recombinant peptide [6,9], which yields similar results (data not shown). However many preparations contain impurities that may complicate MS-based analyses and affect aggregation [23]. Therefore, care should be taken in ensuring sufficient sample clean-up.
2. Importantly, in the context of this screen, the final stages of purification involved size exclusion chromatography (Superdex™ 75 GL 10/300 column, GE Healthcare, UK) with a volatile mobile phase (50 mM ammonium bicarbonate, pH 7.8) and peptide-containing fractions were lyophilised. This step yields pure peptide, free from buffer salts, which can be diluted directly into MS compatible buffers and therefore requires no further buffer exchange. Pure recombinant Aβ40 peptide (containing an additional N-terminal methionine not present in wild-type Aβ40 produced by the cleavage of amyloid precursor protein) was then resolubilised in DMSO at 3.2 mM and diluted into 200 mM ammonium acetate, pH 6.8, 1% (v/v)