Biochimica et Biophysica Acta 1834 (2013) 1257–1268

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Review

Advances in ion mobility spectrometry–mass spectrometry reveal key insights into amyloid assembly ☆

L.A. Woods, S.E. Radford *, A.E. Ashcroft **

Astbury Centre for Structural Molecular Biology & School of Molecular and Cellular Biology, University of Leeds, LS2 9JT, UK

ARTICLE INFO

Article history:
Received 30 July 2012
Received in revised form 27 September 2012
Accepted 2 October 2012
Available online 11 October 2012

Keywords:
Amyloid
Ion mobility spectrometry
Mass spectrometry
Oligomer
Ligand binding

ABSTRACT

Interfacing ion mobility spectrometry to mass spectrometry (IMS–MS) has enabled mass spectrometric analyses to extend into an extra dimension, providing unrivalled separation and structural characterization of lowly populated species in heterogeneous mixtures. One biological system that has benefitted significantly from such advances is that of amyloid formation. Using IMS–MS, progress has been made into identifying transiently populated monomeric and oligomeric species for a number of different amyloid systems and has led to an enhanced understanding of the mechanism by which small molecules modulate amyloid formation. This review highlights recent advances in this field, which have been accelerated by the commercial availability of IMS–MS instruments. This article is part of a Special Issue entitled: Mass spectrometry in structural biology.

1. Introduction

Mass spectrometry (MS) has become widely accepted as a tool to analyse biological systems over the past twenty years, subsequent to the pioneering development of electrospray ionization (ESI) [1,2]. The advent of ESI, and other soft ionization techniques such as matrix assisted laser desorption ionization (MALDI) [3], initiated a new field in which a wealth of information about protein structure and macromolecular assemblies can be deduced using MS, including revealing insights about protein/ligand binding and dynamic structure. Whereas the first ESI–MS experiments of biological macromolecules provided accurate molecular mass measurements [2], the technique evolved rapidly such that structural information about proteins and their biomolecular complexes could be obtained, in addition to information about protein stability, dynamics and post-translational modifications [4–12]. The use of ESI–MS in structural biology has been accelerated most recently through the coupling of ion mobility spectrometry (IMS) to MS [13–16]. This approach has enabled the detailed analysis of biological systems, particularly in cases where alternative techniques such as crystallography or nuclear magnetic resonance are unable to be used due to the analyte's poor solubility, large mass, and/or the inability to crystallize, or in cases where the species of interest are present within a heterogeneous mixture [17]. In this review, we describe the principles of mass spectrometry coupled with ion mobility spectrometry (IMS–MS) and discuss how this approach has enhanced our understanding of protein oligomerization during self-assembly into amyloid.

2. Background to ESI–IMS–MS development and application to biological systems

Ion mobility spectrometry (IMS) is able to separate complex mixtures of ions based on their shape and/or charge [18,19], yielding structural information complementary to molecular mass measurements. The technique of IMS relies on separating gaseous ions according to their mobility through a drift-tube filled with a buffer gas [18]. Ions are accelerated through the drift tube by an electric field, wherein ions of differing shapes have different mobilities depending on an ion's characteristic collision cross-section (CCS). Larger, extended ions will experience more collisions with the buffer gas and, as a result, will take longer to traverse the drift tube in comparison with smaller, more compact ions of the same molecular mass which will undergo fewer collisions with the buffer gas and hence will have a greater mobility and a shorter drift time. One caveat to ion mobility analysis is that the CCS data collected from ion mobility analyses give a rotationally averaged value, as the gaseous ions are able to collide with the buffer gas in a number of different orientations. Despite this, when used in conjunction with mass spectrometry, it has been shown that structural features of proteins and protein complexes can be elucidated [20,21].

Abbreviations: CCS, collision cross-section; ESI–MS, electrospray ionization–mass spectrometry; HDX, hydrogen–deuterium exchange; IMS–MS, ion mobility spectrometry–mass spectrometry

☆ This article is part of a Special Issue entitled: Mass spectrometry in structural biology.

* Corresponding author. Tel.: +44 113 343 3170; fax: +44 113 343 7486.
** Corresponding author. Tel./fax: +44 113 343 7273.
E-mail addresses: S.E.Radford@leeds.ac.uk (S.E. Radford), A.E.Ashcroft@leeds.ac.uk (A.E. Ashcroft).

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http://dx.doi.org/10.1016/j.bbapap.2012.10.002

The use of conventional ion mobility drift tubes coupled to MS has produced excellent results but has been limited to home-built instrumentation [16,22–24]. Over the past decade, much effort has been directed at producing commercially viable instrumentation. This has led to the development of ion mobility separation based on either a differential IMS setup [25,26] or a travelling-wave IMS system (Fig. 1) [15]. In particular, travelling-wave IMS–MS has been found to be promising for protein analysis, showing good sensitivity and separative power, and it can be used to measure the mass and to estimate CCS value for each individual component within a sample. Although it is possible to acquire CCS values directly from drift-times using conventional IMS [27], calibration of the travelling-wave IMS device with ions of known CCS is required [28–30]. Once the travelling-wave IMS device has been calibrated, CCSs can be estimated for previously uncharacterised species. In the case of a protein, a CCS can be derived for each of its charge state ions. A folded, native-like protein conformer may only give rise to two or three charge state ions, all with similar CCSs; however, an unfolded protein conformer could generate a wide charge state distribution, with the higher charge state ions having much larger CCSs than the lower charge state ions, a phenomenon due in part to Coulombic repulsions between the charges of the highly charged ions. Therefore, for each protein conformer, the CCS of the lowest charge state ions is usually the smallest and therefore the one used to define that particular conformer. Using this approach, IMS–MS has revealed valuable information on a range of biological systems [31–34]. One area that has benefited greatly from such analyses is that in which transient intermediate species are lowly populated and short lived, such as in amyloid fibril formation, as discussed below.

3. Amyloidosis: a challenging biomolecular assembly system ideal for analysis by ESI–IMS–MS

More than 25 different proteins or peptides, whose aggregation into fibrils is associated with amyloidosis in vivo, have been identified to date [35]. In different amyloid disorders, fibrils accumulate in specific regions of the body causing the variety of symptoms associated with individual diseases [36,37]. Due to the prevalence of these diseases, gaining insights into how amyloid deposits form and how their production can be halted is crucial. To achieve this, in vitro techniques have been employed to provide information. The advantage of such experiments is that amyloid formation can be induced to occur on relatively rapid timescales, rather than the decades or more generally required for amyloid to become symptomatic in vivo. Accordingly, decreasing the pH, incubating with protein denaturants such as sodium dodecyl sulphate, exposing the protein or peptide to metal ions, introducing destabilising mutations into the protein sequence, and/or agitating the solution vigorously have all been used to initiate rapid fibril formation in vitro [37–39]. The formation of amyloid-like fibrils under these conditions can be confirmed using imaging methods such as electron microscopy (Fig. 2a) or atomic force microscopy [35], complemented by X-ray fibre diffraction to confirm the presence of the well-characterized cross-β structure of amyloid (Fig. 2b) [37]. Additionally, small organic molecules known to bind to amyloid fibrils are commonly used to detect their presence: Congo red is particularly useful in this role as upon excitation with plane-polarised light the dye exhibits apple-green birefringence when bound to the regular array of β-strands within the cross-β structure of amyloid [40] (Fig. 2c, d). Thus, Congo red birefringence is an excellent diagnostic

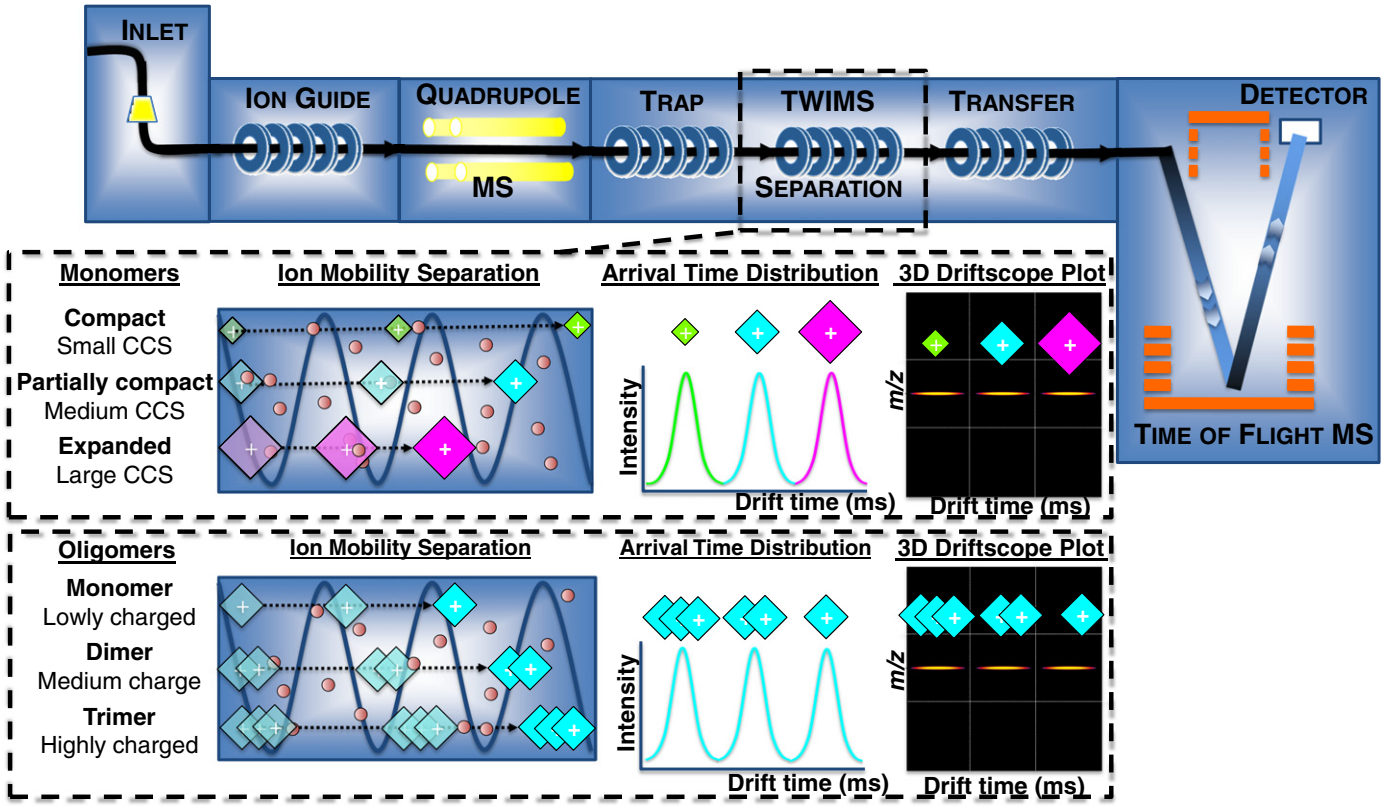

Fig. 1. Schematic showing commercially available travelling-wave ion mobility spectrometry (TWIMS) integrated with an orthogonal acceleration quadrupole-time-of-flight mass spectrometer. Ions are separated in the TWIMS device based on their mobility through the drift cell; ions with a large collision cross-section (CCS) experience more collisions with the buffer gas molecules present in the drift cell and have a longer drift time than more compact ions of the same mass and same charge. Ions of the same *m/z* but different mass and different charge, for example a mixture of protein oligomers including a monomer with one charge, a dimer with two charges etc., are separated based on both their CCS and their number of charges. Typically, for a given *m/z*, the more highly charged ions have a shorter drift time, as they are propelled through the drift cell faster.
Figure adapted from [15].