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Mass spectrometric methods to analyze the structural organization of macromolecular complexes

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ABSTRACT

With the development of soft ionization techniques such as electrospray ionization (ESI), mass spectrometry (MS) has found widespread application in structural biology. The ability to transfer large biomolecular complexes intact into the gas-phase, combined with the low sample consumption and high sensitivity of MS, has made ESI-MS a method of choice for the characterization of macromolecules. This paper describes the application of MS to study large non-covalent complexes. We categorize the available techniques in two groups. First, solution-based techniques in which the biomolecules are labeled in solution and subsequently characterized by MS. Three MS-based techniques are discussed, namely hydroxyl radical footprinting, cross-linking and hydrogen/deuterium exchange (HDX) MS. In the second group, MS-based techniques to probe intact biomolecules in the gas-phase, e.g. side-chain microsolvation, HDX and ion mobility spectrometry are discussed. Together, the approaches place MS as a powerful methodology for an ever growing plethora of structural applications.

1. Introduction

Probing protein interaction networks at atomic detail is vital to a mechanistic understanding of biological function. Classical structural analysis techniques able to provide atomic resolution information, i.e. X-ray crystallography and NMR spectroscopy, can encounter difficulties for large macromolecular systems, systems with flexible or unstructured regions, low protein abundance, membrane associated systems, and transient interactions. Cryo-electron microscopy (EM) has seen a revolution in its capability in recent years [1]. The advent of direct electron detectors and high power microscopes now enables structures to be obtained with resolutions of $\geqslant$3.5 Å (at least for highly symmetric and stable complexes) but results in a relatively low-resolution, i.e. 3.5 Å only for highly symmetric and stable complexes [2] for samples available only in low amounts and without the need for crystal formation. Biological mass spectrometry (MS) [3] has gained widespread application in the analysis of proteins and protein complexes following the development of electrospray ionization (ESI) more than 20 years ago [4]. In addition to proteomics approaches [5], MS has played a critical role in structural biology [6,7]. Remarkable advances in MS instrumentation and its application have allowed the structure, function and properties of complex biological systems to be probed both in the gas-phase and in solution.

Biomolecules can be covalently labeled in solution by several approaches such as hydroxyl radical footprinting [8], cross-linking [9], and hydrogen/deuterium exchange (HDX) [10]. The modified biomolecules can then be characterized via bottom-up or top-down MS [11,12]. In the bottom-up approach, peptides generated by enzymatic cleavage of proteins are introduced into the mass spectrometer, whereas in top-down MS, intact protein ions are introduced into the mass spectrometer and subsequently fragmented in the gas-phase. Alternatively, label-free methods such as limited proteolysis [13,14] combined with MS have been used to report on structure–function relationships of biomolecules and their topology. Under native-like conditions, limited proteolysis of a globular protein can reveal flexible regions with relatively large conformational dynamics (e.g. loops) since unstructured and extended conformations are highly susceptible to protease cleavage whereas folded regions are protected from digestion [15]. In a recent impressive example, limited proteolysis was coupled with a targeted proteomics workflow to enable the analyses of conformational changes in more than 1000 yeast proteins directly in a biological context [16].

The key distinguishing feature of ESI-MS, the ability to ionize macromolecules while maintaining weak non-covalent interactions, has enabled the study of large biological protein complexes in the gas-phase [17]. Biomolecular ions produced by ESI have

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low internal energy and are thereby less likely to be fragmented upon transfer from solution to the gas-phase. This immediately opens the question of the extent to which the solution-phase structure is preserved in the gas-phase. As the final desolvation step of biomolecular ions upon ESI is too fast to be measured experimentally (in the nano/picosecond range), the stepwise structural evolution of a globular protein, cytochrome *c*, (Fig. 1) surrounded by a monolayer of water molecules was simulated by molecular dynamics (MD) [18,19]. These simulations showed that the final water molecules are removed from the protein ions in the nanosecond time scale with no changes in the protein structure (B and C in Fig. 1). The exterior of the native protein changes within ~10 picoseconds as charged side-chains form a network of electrostatic interactions on the protein surface. The charges on the protein side-chain residues collapse onto the backbone, forming a transient exterior-collapsed "near-native" conformation (D in Fig. 1). This structure is stable for up to milliseconds. The hydrophobic interactions are lost in the millisecond time scale (E in Fig. 1), followed by loss of the electrostatic interactions (F in Fig. 1) as a result of "drying" the native conformer. The ions then rearrange to find their most stable structure on a longer time scale, i.e. seconds to minutes. Experimental evidence is required to validate the structural changes proposed to occur for cytochrome *c* in the gas-phase by MD. A number of notable advances in MS instrumentation and technique development has allowed the examination of biomolecular ions with minimal structural changes during ion transfer to the gas-phase [20]. Ion mobility spectrometry-mass spectrometry (IMS-MS) experiments enable ions to be probed on the millisecond time scale [21], (E–G in Fig. 1 and inset). Ion-molecule/ion reactions such as HDX techniques have been used for studying protein ions in the microsecond [22] to millisecond [23] time scale (i.e. D and E–G, respectively, in Fig. 1 and inset). Side-chain microsolvation of cytochrome *c*, via non-covalent attachment of crown ethers to its protonated sites, mimics attachment of the last solvent molecules before complete evaporation of solvent (B in Fig. 1 and inset), thereby leaving the protein in a more native-like state [24].

Spectacular advances in mass spectrometric instrumentation have led to the field of "native MS" which focuses on the structural and functional analysis of the dynamics and interactions in proteins and within protein complexes [25–27]. This review highlights MS-based techniques that are able to reveal solution and gas-phase information on the structure, function, and properties of macromolecular assemblies. A brief description of each technique is provided along with its capabilities and current limitations. As native mass spectra of macromolecular assemblies lie in high mass-to-charge ratio (m/z) ranges, the tuning and calibration of the mass spectrometer to higher mass ranges over m/z 20,000 is required and methods to achieve this are reported. As an example of a native mass spectrum of a macromolecular assembly, the methods used to acquire a mass spectrum of GroEL, are described in experimental detail and more recent achievements of native MS in the analysis of large complexes are highlighted.

2. Solution-phase MS-based techniques

In addition to the well-known field of proteomics, combining other techniques with mass spectrometry has found direct

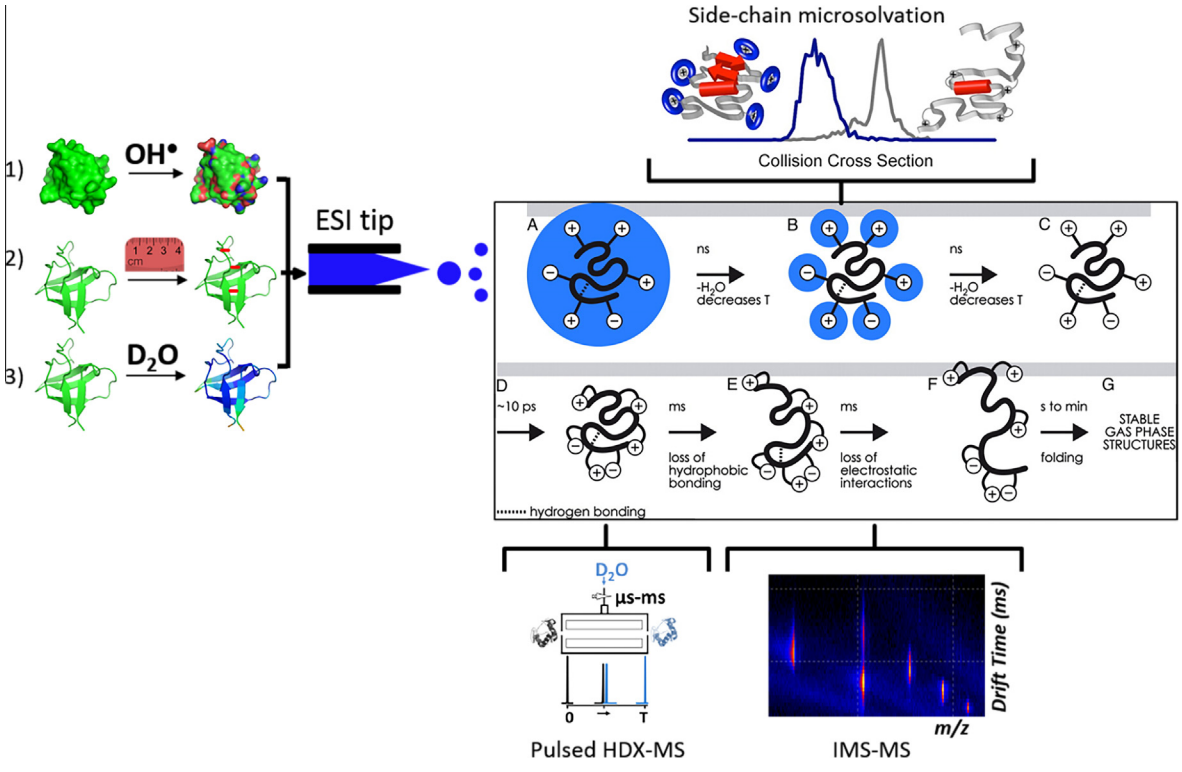

Fig. 1. MS-approaches that can be used to study biomolecules. Left: biomolecules can be covalently labeled in solution by OH· footprinting (1), cross-linking (2), and HDX (3). Right: the stepwise structural evolution of a globular protein, cytochrome c, after ESI from MD simulations (reproduced from [14]. Copyright (2008) National Academy of Sciences, U.S.A.). (A) is the protein ion surrounded by a monolayer of water molecules; (B) the final water molecules around the charged side-chains; (C) bare ions with their native structure; (D) the near-native structure with protein ions' charges collapsed onto the backbone; (E) protein ion with loss of its hydrophobic core; (F) the protein ion with loss of electrostatic interactions; (G) further changes in protein conformations may occur to form a stable structure in the gas-phase. Insets: techniques to capture biomolecule ions after ESI at steps B (side-chain microsolvation, taken from Ref. [19] with permission), D (pulsed HDX, taken from Ref. [17] with permission), and E–G (IMS-MS) are also shown. Side-chain microsolvation preserves the native structure. Pulsed HDX-MS captures near-native protein ions. Protein ions are confined in a linear ion trap (shown as a box) and a short pulse of D_2O is introduced into the chamber at different time intervals (shown for times zero and T ms) to capture snapshots for gradual evolution of deuterated peaks. IMS-MS probes ions which may lose some of hydrophobic core/electrostatic interactions depending on the experiment time scale.

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