Biochimica et Biophysica Acta 1834 (2013) 1239–1256

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Review

Native ion mobility-mass spectrometry and related methods in structural biology ☆

A. Konijnenberg [a], A. Butterer [a], F. Sobott [a,b,*]

[a] Biomolecular Mass Spectrometry group, Department of Chemistry, University of Antwerp, Belgium
[b] Center for Proteomics (CFP-CeProMa), University of Antwerp, Belgium

ARTICLE INFO

Article history:
Received 16 September 2012
Received in revised form 19 November 2012
Accepted 29 November 2012
Available online 14 December 2012

Keywords:
Native mass spectrometry
Nanoelectrospray ionization
Ion mobility
Covalent labeling
H/D exchange
Cross-linking

ABSTRACT

Mass spectrometry-based methods have become increasingly important in structural biology — in particular for large and dynamic, even heterogeneous assemblies of biomolecules. Native electrospray ionization coupled to ion mobility-mass spectrometry provides access to stoichiometry, size and architecture of noncovalent assemblies; while non-native approaches such as covalent labeling and H/D exchange can highlight dynamic details of protein structures and capture intermediate states. In this overview article we will describe these methods and highlight some recent applications for proteins and protein complexes, with particular emphasis on native MS analysis. This article is part of a Special Issue entitled: Mass spectrometry in structural biology.

1. Introduction

Mass spectrometry (MS) is a relative newcomer to the family of structural biology methods, which have in common that they attempt to characterize in detail key molecules or complexes involved in biological processes in vitro (or rather in this case in vacuo). This development started with the advent of electrospray ionization (ESI) more than two decades ago, and led first and foremost to a sheer explosion of techniques and applications in the field of proteomics. While the Omics techniques are predominantly used to determine the presence and the quantity of (all) components of a complex sample, often in a high-throughput fashion, structural biology traditionally focuses on targeted, in-depth studies of individual components and tries to infer function from structure.

So how does MS fit into this picture? While playing a crucial role in proteomics, one can question how a gas-phase technique would be able to provide reliable data on protein structures. A steadily growing body of evidence over the last years [1–7] – particularly from the laboratory of Carol V. Robinson [8,9] – has shown that mass spectrometry is indeed capable of analyzing intact, noncovalent complexes, provided that non-denaturing conditions are carefully maintained in the sample solution, during the electrospray process and while in the vacuum of the instrument. This remarkable ability of ESI-MS and other adjunct gas-phase techniques has found numerous diverse applications in supramolecular chemistry and, importantly, also in biology. "Native" mass spectrometry has become a valuable addition to the toolbox of biophysical methods, which provide low-resolution, complementary structural data, but taken together are capable of giving crucial insights into complex and dynamic biological structures.

Besides native MS approaches, covalent labeling of biomolecules (including H/D exchange) together with non-native analysis, used to obtain footprints of native structures, is increasingly catching the attention of scientists in this aspect. In this overview article we investigate these methods and highlight some recent developments and applications from the field of protein conformations and protein complexes, with particular emphasis on native MS analysis.

2. Native MS methods

Before discussing current applications of native MS, it is useful to gain an understanding of the parameters which make MS non-denaturing, and what type of information native mass spectra contain.

2.1. Non-denaturing ionization

2.1.1. The mechanism of electrospray ionization

In electrospray ionization (ESI) ions are generated from droplets, by means of a fine dispersion of liquid. A potential difference between the spray capillary which contains the sample, and the entrance aperture to the vacuum of the mass spectrometer, drives both the formation of ions as well as their liberation from bulk solvents. For ESI in positive ion mode, which is typically used for peptide and protein MS, this means that positively charged (multiply protonated) analytes are enriched in the capillary tip. Here electrostatic forces help to overcome the surface tension so that a spray of charged droplets is generated,

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

* Corresponding author at: Department of Chemistry, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerpen, Belgium. Tel.: +32 32 65 33 88.
E-mail address: frank.sobott@ua.ac.be (F. Sobott).

1570-9639/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbapap.2012.11.013

from which the analyte ions are eventually set free. John B. Fenn (1917–2010) received the 2002 Nobel prize in Chemistry for the application of this ionization method to peptides and proteins.

The introduction of MS for native structural studies was greatly facilitated by the development of a miniaturized version of Fenn's electrospray by Wilm and Mann [10,11]. Nanoflow electrospray ionization (nanoESI, nESI) proves to be superior for structural studies due to the fact that the much smaller spray orifices used (1–10 μm diameter) enable a much better dispersion of the liquid into nanodroplets with a favorable surface-to-volume ratio (for a schematic view of the source layout and ionization process see Fig. 1), abolishing the need for harsh desolvation conditions. Furthermore nESI is more tolerant to non-volatile salts and buffers, in certain cases up to the mM concentration range [12]. Importantly nESI also requires smaller sample volumes and concentrations (as little as 1 μL, 0.5–10 μM for "static" flow). This also reduces the need for volatile solvents, making it easier to use purely aqueous solutions, which represent a more native environment for the biological analytes. Moreover, the smaller orifice also enables lower flow rates than in ESI (1.2–30 μL/h vs. up to 30 mL/h). Alternatives for the classic glass capillary setup have become commercially available, allowing high throughput analysis of samples [13], for example automated protein–ligand screening [14]. The Nanomate (Advion, Ithaca/NY, USA) consists of a robotic inlet system that allows fully automated direct infusion of individual samples, but can also be used online for coupling to nanoLC.

It is important to discuss briefly how electrospray works and how it can be used to maintain "native", non-covalent complexes from solutions intact throughout the ionization process. Once the initial solvent droplet is emitted from the nESI capillary tip, the solvent evaporates, reducing the droplet size of up to a point where the charge on the surface of the droplet approaches the Rayleigh limit, the maximum amount of charges a droplet of a certain size and composition can contain while still being stable [15]. When the repulsive forces of the charges on the droplet overcome the attractive forces of the solvent (around 75–95% of the Rayleigh limit) the droplet undergoes fission. The fission event generates new smaller droplets that undergo the same process until the droplet has been reduced in size to a point where on average only one analyte molecule, if any, and some buffer or other remaining, non-volatile solution components are present in the nm-size droplet [16]. The low sample concentrations used for nESI (<ca. 50 μM) avoid that multiple proteins or complexes end up in the same final droplet, which can lead to the detection of oligomer artifacts.

There has been some debate about the mechanism of the final step in ESI which sets the analyte ion free. For larger ions such as proteins and peptides, the so-called charge residue model (CRM) has been proposed [17]. In this model it is believed that in the final stages of solvent evaporation the droplet "dries up" and the charge is transferred onto the surface of the analyte, resulting in multiply charged species. The charges are normally linked to excess protons present in the droplet due to the effect of the (positive, for cations) spray voltage, but instead adducts can also be formed with other nonvolatile ions such as omnipresent cations (e.g., sodium), causing an increase in the analyte mass and peak heterogeneity. Volatile buffers such as ammonium acetate (AmAc) or bicarbonate (AmBic) are often used when spraying under "native" conditions. These buffers allow the pH to be adjusted and increase the ionic strength of the solution, and unlike traditional biochemical buffers which are non-volatile and tend to cluster to the analyte, they do not cause peak broadening.

The fact that nESI is one of the softest desorption/ionization techniques available and that the ions only leave their "native" solvent environment late in the process, together with the speed of phase transition, apparently ensure that large and fragile noncovalent complexes can be preserved and detected intact with a careful tuning of the instrument. For a more detailed discussion of the nESI nanoflow electrospray ionization the reader is directed to an excellent review paper by Kebarle [15].

2.1.2. Charge state distributions and their information content

It is characteristic for electrospray spectra that they display a range of charge states (i.e., multiply protonated species) in a Gaussian-like distribution. The origin of the charge state distribution has been ascribed to the availability of ionizable sites [18], the surface tension of the solvents used (and thus the Rayleigh limit [19]), intramolecular interactions in the folded protein [20], the solvent accessible surface area (SASA [21]) and Coulombic repulsion [22]. The observed charge state series however are more likely due to a combination of the aforementioned factors [23].

Charge state distributions also provide structural information. Unfolded proteins will have a much larger surface available for protonation

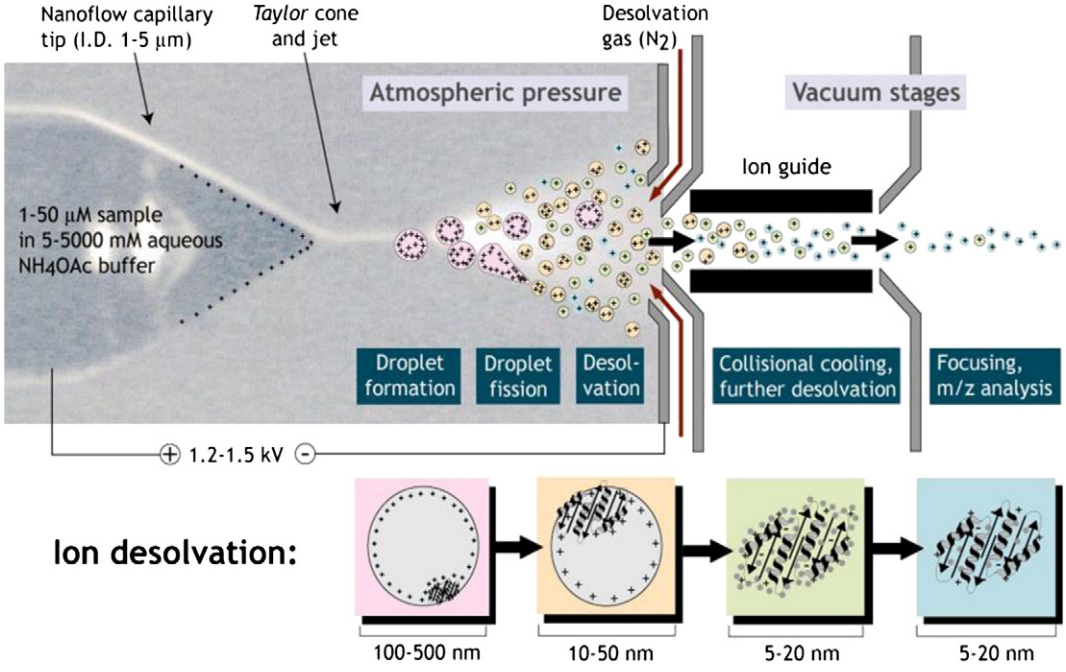

Fig. 1. Schematic overview of the nanoflow electrospray ionization process. Top: depiction of the key elements of a typical atmospheric pressure nanoflow source including the desolvation stages. Bottom: schematic representation of the desolvation process for a noncovalent protein dimer during transition from the solution nanodroplet to the gas-phase desolvated ion.