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Review

Native mass spectrometry of photosynthetic pigment–protein complexes

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ABSTRACT

Native mass spectrometry (MS), or as is sometimes called "native electrospray ionization" allows proteins in their native or near-native states in solution to be introduced into the gas phase and interrogated by mass spectrometry. This approach is now a powerful tool to investigate protein complexes. This article reviews the background of native MS of protein complexes and describes its strengths, taking photosynthetic pigment–protein complexes as examples. Native MS can be utilized in combination with other MS-based approaches to obtain complementary information to that provided by tools such as X-ray crystallography and NMR spectroscopy to understand the structure–function relationships of protein complexes. When additional information beyond that provided by native MS is required, other MS-based strategies can be successfully applied to augment the results of native MS.

1. Introduction

Many functional units in biology are high-order complexes formed of proteins and ligands [1]. As an example, the multi-subunit pigment–protein complexes in photosynthetic systems are important and timely examples [2]. Native mass spectrometry (MS), in addition to the traditional tools for protein structural studies, X-ray crystallography and NMR spectroscopy, is emerging as a complementary tool to interrogate protein complexes in their near native states [3,4] and to provide insights that complement those from traditional biology approaches [5].

Efforts to study the relationship between protein structure and function [6] focus on higher order protein complexes [7]. In photosynthesis, these complexes are composed of proteins and cofactors, usually pigments. In photosynthesis, light energy is absorbed by pigment–protein complexes in an antenna complex and is funneled to the reaction center complex. This process relies on the light-absorbing pigment molecules that are arranged at well-defined positions and angles in the pigment–protein complexes to facilitate energy transfer [8]. Motivated by their importance, traditional and novel structural biology methods continue to be developed and applied to these complexes [1]. High-resolution methods (e.g., X-ray crystallography and NMR spectroscopy [9]), and low-resolution methods (e.g., cryo-electron microscopy [10] and small-angle X-ray scattering (SAXS) [11]) are the principal tools. In addition, MS is providing an important complementary approach [12]. Although it does not yet provide high resolution data, it has advantages that protein complexes can be interrogated in their near-native state with consumption of relatively small amounts of sample and with fast turnaround. One strategy in the MS "tool box" is native MS (the definition is given in Section 2.2), which is a fast-growing approach to address questions in the characterization of protein complexes [13]. A summary of native MS based studies, from fundamental MS to critical applications in photosynthetic pigment–protein complexes, is presented here.

2. Mass spectrometry of protein complexes

2.1. Generating ions of protein complexes

The first step in an MS-based study of protein complexes is generating ions of the intact complexes. It was not possible before the 1980s to generate ions in any routine way from non-volatile and labile biomolecules, much less from large bio-molecular complexes. Traditional ionization methods (e.g., electron ionization (EI) and chemical ionization (CI)) are suitable for volatile small molecules, and Fast Atom Bombardment (FAB), the first routine approach to non-volatile biomolecules, is inappropriate for large

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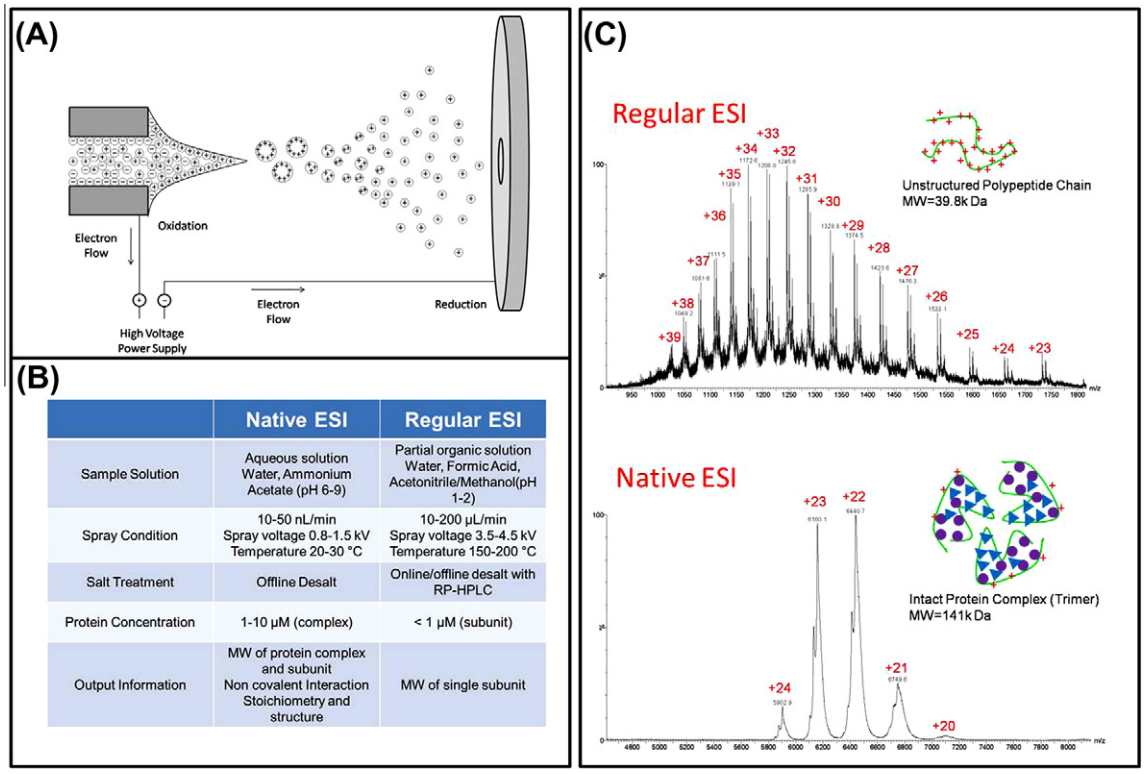

	Native ESI	Regular ESI
Sample Solution	Aqueous solution Water, Ammonium Acetate (pH 6-9)	Partial organic solution Water, Formic Acid, Acetonitrile/Methanol(pH 1-2)
Spray Condition	10-50 nL/min Spray voltage 0.8-1.5 kV Temperature 20-30 °C	10-200 μL/min Spray voltage 3.5-4.5 kV Temperature 150-200 °C
Salt Treatment	Offline Desalt	Online/offline desalt with RP-HPLC
Protein Concentration	1-10 μM (complex)	< 1 μM (subunit)
Output Information	MW of protein complex and subunit Non covalent Interaction Stoichiometry and structure	MW of single subunit

Fig. 1. Native MS of protein complexes. (A) Electron spray (ESI) process; (B) the comparison between native ESI and regular ESI; (C) regular ESI and native ESI spectra of Fenna–Matthews–Olson (FMO) antenna protein samples.

biomolecules. The introduction of electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) positioned MS to evolve into a more important method for macromolecular studies owing to both its sensitivity and tolerance of sample heterogeneity. Proteins can be analyzed in solution by ESI [14] or as solids formed by co-crystallization with UV-absorbing organic acids by using MALDI [15]. Protein complexes can also be approached using both ionization methods [16,17], although most efforts directed at protein complexes come from using ESI-MS [18]. For MALDI, preserving non-covalent interactions of protein complexes in the solid phase is problematic, and the singly-charged large protein ions have m/z values usually beyond the optimum range of most commercial instruments. One prospect to overcome this is to combine cross-linking with MALDI [19] to determine the stoichiometry, composition of protein complexes and cross-linking yield.

The focus of this review is ESI. A standard setup involves flowing an analyte solution to the end of a capillary held at high electrical potential (Fig. 1A) [20]. A parallel gas flow surrounds the capillary to aid nebulization of the emerging analyte solution. Because the electrical potential is high, positive ions (in the positive-ion mode of ESI) accumulate at the tip of the solution, causing the solution to form a "Taylor cone" [21]. The stream of solution is drawn out as small, charged droplets that move away each other owing to electrostatic repulsion of the excess positive (or negative) ions within each droplet. According to the charge-residue mechanism [22] of ESI, solvent evaporation continually reduces the size of charged droplet until the Coulombic repulsions between crowded positive ions overcome the droplet surface tension (called the Rayleigh limit). Droplets undergo fission and form even smaller droplets [23]. Evaporation and fission are repeated in several cycles until non-solvated gas-phase ions are produced.

2.2. Comparing regular ESI and native ESI (native MS)

Several factors, including pH of the solution, concentration of non-volatile salts, and fraction of organic solvent affect the protein in ESI. Using a typical ESI solvent (50% acetonitrile, 50% water, 0.1% Formic Acid, pH = 1–2), causes the majority of proteins to be in a denatured state, where the proteins are largely unfolded and have their basic side chains exposed to solvent [24] (Fig. 1B). The ESI spectra of denatured proteins show a broad charge-state distribution (charge envelope) centered at low m/z (Fig. 1C). Because non-covalent interactions between proteins and pigments/ligands are usually destroyed in their denatured state, preservation of the native state of a protein and the even more demanding maintenance of a non-covalent protein complex are problematic when using typical ESI solvents.

To preserve the native state and the non-covalent interactions in a protein complex, it must be in aqueous solution at physiological pH and appropriate ionic strength. An ESI approach called native MS (or native ESI) meets these requirements [25,26]. Proteins are placed in an aqueous solution containing a volatile salt (e.g., ammonium acetate) and directly sprayed by using ESI. In the gas phase, the native-like proteins carry fewer charges than denatured or unfolded states owing to the fewer exposed basic residues in the folded form. As a consequence, the protein ions exhibit a narrow spread of a charge envelope or distribution; the m/z of the protein in this case falls at a higher value (Fig. 1C). Non-covalent interactions are thus preserved.

Another advance in ESI that benefits studies of protein complexes is nano ESI (nESI) [27]. nESI generates smaller droplets than does regular ESI by spraying the sample through a capillary with a smaller diameter (e.g., ~1 μm inner diameter) than normally used for ESI. Ionization conditions (spray voltage, capillary temperature,