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Protein structure evolution in liquid DESI as revealed by selective noncovalent adduct protein probing

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ABSTRACT

Previous experiments based on charge state distributions have suggested that liquid desorption electrospray ionization (DESI) is capable of preserving solution phase protein structure during transfer to the gas phase (*Journal of the American Society for Mass Spectrometry* 21 (2010) 1730–1736). In order to examine this possibility more carefully, we have utilized selective non-covalent adduct protein probing (SNAPP) to evaluate protein structural evolution in both liquid DESI and standard ESI under a variety of conditions. Experiments with cytochrome c (Cytc) demonstrated that methanol induced conformational shifts previously observed with ESI are also easily observed with liquid DESI. However, undesirable acid-induced unfolding becomes apparent at very high concentrations of methanol in liquid DESI due to acetic acid in the spray solvent, suggesting that there are conditions under which liquid DESI will not preserve solution phase structure. The effects of ammonium acetate buffer on liquid DESI SNAPP experiments were examined by monitoring structural changes in myoglobin. Heme retention and SNAPP distributions were both preserved better in liquid DESI than traditional ESI, suggesting superior performance for liquid DESI in buffered conditions. Finally, liquid DESI SNAPP was used to study the natively disordered proteins α, β, and γ synuclein with SNAPP. α-Synuclein, the main component of fibrils found in patients with Parkinson's disease, yielded a significantly different SNAPP distribution compared to β and γ synuclein. This difference is indicative of highly accessible protonated basic side chains, a property known to promote fibril formation in proteins.

1. Introduction

Properly folded, biologically active proteins have secondary and tertiary structures essential to their role in the cell. A change in protein conformation is often associated with a change or loss in protein function. Determination of protein structure has been an area of interest especially given the therapeutic aspect of using small molecules or other conditions to activate, deactivate, or alter the function of proteins with the ultimate goal of treating disease. Traditionally, protein structure has been examined by solid and liquid phase techniques such as crystallography [1], NMR [2], FRET [3], fluorescence [4], and circular dichroism [5]. In recent years, mass spectrometry has been increasingly used to both directly and indirectly probe protein structure. Charge state distributions [6,7], selective non-covalent adduct protein probing (SNAPP) [8], covalent labeling [9], H/D exchange [10], ion mobility [11], electron capture dissociation (ECD) [12], and radical directed dissociation (RDD) [13] have all been successfully used to examine protein structure and monitor how conformational changes occur in response to external stimuli. These mass spectrometry based techniques all rely on electrospray ionization (ESI), which is particularly important for those methods that examine protein structure in the gas phase. For experiments where protein structure is probed in vacuo, the ideal outcome is successful transfer of the solution phase structure into the gas phase with high fidelity. Unfortunately, conditions which favor this outcome typically reduce sensitivity significantly relative to standard ESI.

Recently, Chen and Miao reported that liquid DESI could be used to ionize proteins with apparent retention of solution-phase structure and enhanced sensitivity relative to ESI [14]. Liquid DESI, a technique related to solid surface DESI developed by Cooks and coworkers [15,16], consists of a simple experimental setup (see Fig. 1) where the sample and electrospray solutions are decoupled. The sample of interest is dissolved in one solution and pushed through a flat, open ended tube at a steady flow rate. A separate spray solvent, typically consisting of 50:50:1 water:methanol:acetic acid, is then electrosprayed and directed by high speed gas flow toward the sample solution tube. Charged droplets emerge from the intersection of the source and sample solutions, eventually generating protonated ions for detection in the mass spectrometer. Importantly, Chen and coworkers demonstrated that although denaturants such as methanol and acetic acid

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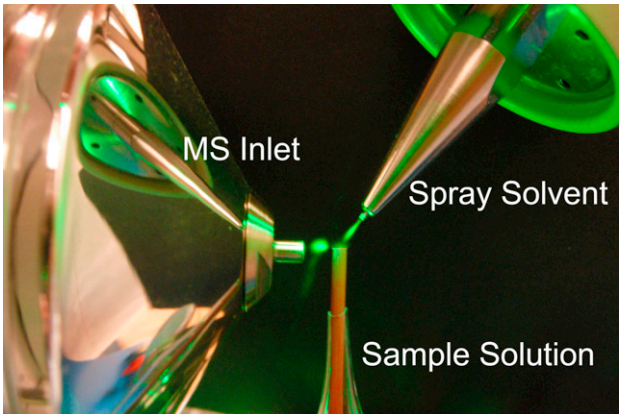

Fig. 1. Composite image of the liquid DESI source setup. A green laser pointer is used to illuminate the droplet clouds between the ionization source, sample tube, and mass spectrometer inlet. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

are used in the spray solvent, the protein charge states observed from liquid DESI ionization were similar to those obtained from much more "native" solutions [17]. In comparison, charge state distributions for proteins electrosprayed directly from 50:50:1 water:methanol:acetic acid exhibit significant shifts indicating denaturation of the protein. The apparent lack of denaturation in liquid DESI is of interest because decoupling the spray and sample solvents potentially enables maximization of ion count simultaneously with preservation of protein structure. However, subtle structural changes are difficult to detect by shifts in charge state distribution, suggesting that a more sensitive method may be required to reveal whether smaller changes in structure take place in liquid DESI.

SNAPP is a simple mass spectrometry based method for investigating protein structure that excels at identifying conformational changes. SNAPP is a comparative method that utilizes non-covalent attachment of probe molecules during ESI to monitor protein structure. SNAPP has been successfully used to reveal the effects of metal ions on the structure of α-synuclein [18], to monitor structural changes induced by single amino acid mutations [19], and to distinguish small structural differences in highly homologous proteins from different species [20]. 18-Crown-6 (18C6) is typically the probe molecule used in SNAPP experiments due to its ability to non-covalently bind to the protonated side chains of lysine and arginine, or to the N-terminus. Side chains which are not buried or sequestered by intramolecular interactions are generally available to bind 18C6. Side chain availability is dictated by protein structure, and changes in protein conformation lead to different numbers of 18C6 adducts. In a typical experiment, 18C6 is simply added to a protein solution and then electrosprayed directly.

Herein, SNAPP will be utilized to examine the effects of the ionization process on protein structure for both liquid DESI and standard ESI. This work is the first use of SNAPP on proteins generated from a liquid DESI source. A methanol induced conformational shift in the protein cytochrome c (Cytc) will be used as a model system to compare the two ionization processes. This system has been previously studied by SNAPP in ESI [8] and is examined here by SNAPP in liquid DESI. The protein structure of myoglobin ionized by liquid DESI from ammonium acetate buffer is examined by SNAPP to evaluate the performance of liquid DESI with additional buffer. The results with both Cytc and myoglobin suggest that liquid DESI is a viable method for examining protein structure. Finally, liquid DESI is used to study dynamic structural differences in the natively disordered synuclein proteins. α-Synuclein is the principal component of amyloid fibrils associated with neurodegenerative Parkinson's disease and undergoes a high rate of fibril formation relative to the highly homologous proteins, β and γ synuclein [21]. Examination of these three proteins by liquid DESI SNAPP is performed in order to gain insight into the link between fibril formation rate and dynamic protein structure.

2. Experimental

Horse heart cytochrome c and myoglobin were purchased from Sigma–Aldrich (St. Louis, MO). The proteins α, β, and γ synuclein were purchased from ProSpecBio (East Brunswick, NJ). Distilled water was purified by a Millipore Direct-Q filtration system before use. Methanol, acetic acid, and ammonium acetate were purchased from Thermo-Fisher Scientific (Waltham, MA). 18-Crown-6 was purchased from Alfa Aesar (Pelham, NH).

2.1. ESI and DESI setup

All ESI spectra were acquired on a Thermo LTQ mass spectrometer with the standard IonMax™ ESI source supplied with the instrument. Liquid DESI spectra for myoglobin were acquired on a Thermo LTQ mass spectrometer and liquid DESI spectra for Cytc were acquired on a Thermo LCQ mass spectrometer. The liquid DESI source was constructed in-house using a partially modified nozzle assembly from an existing Thermo ESI source. The assembly was modified by removing its enclosure and replacing the existing silica capillary (200 μm O.D., 100 μm I.D.) with a larger capillary (240 μm O.D., 100 μm I.D.) in order to increase sheath gas velocity at the spray tip. The spray assembly was properly grounded and attached to a firmly secured aluminum lab jack. The assembly was oriented in such a way that the emitted solvent plume impacted the flat cut end of a securely mounted portion of PEEK tubing (0.0625 in. O.D., 0.005 in. I.D.). A syringe pump was used to deliver sample solution through this tubing at 3 μL/min. The samples consisted of 5 μM protein and 100 μM 18C6 (1:20 ratio) in water unless otherwise noted. A second syringe pump was used to pump the spray solvent through the ESI needle at 3–6 μL/min. The spray solvent consisted of 50:50:1 water:methanol:acetic acid unless otherwise noted. The nitrogen gas pressure going into the spray assembly was set to 80 psi. A picture of this setup is shown in Fig. 1. Sheath gas pressure, sample and spray flow rates, relative angles, and distances between the elements of the setup were optimized for ion count. For SNAPP experiments with both ion sources, ionization conditions and ion optic voltages were initially optimized to improve signal and 18C6 attachment to the protein. These conditions were then kept constant throughout the experiments. The optimal ion optic voltages were not substantially different between the two sources.

3. Results and discussion

Cytc is known to undergo a conformational shift when exposed to increasing amounts of methanol [22], and is therefore an excellent test subject to examine the effects of ionization in liquid DESI. Fig. 2 shows fluorescence and circular dichroism spectra for solutions of Cytc and 18C6 as a function of increasing methanol content at neutral pH. Cytc contains a single tryptophan residue which fluoresces at 350 nm when excited at 285 nm. In the native protein structure this amino acid is buried within the hydrophobic core and does not fluoresce due to FRET quenching by the covalently bound heme group [22,23]. In Fig. 2a the measured fluorescence of Cytc at 350 nm increases dramatically when the protein is exposed to >60% methanol. The increase in fluorescence results from a structural rearrangement increasing the distance between the tryptophan residue and heme group. The native fluorescence of the amino acid is shown as a control (red line) and is also noted to