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Supercharging protein ions in native mass spectrometry using theta capillary nanoelectrospray ionization mass spectrometry and cyclic alkylcarbonates

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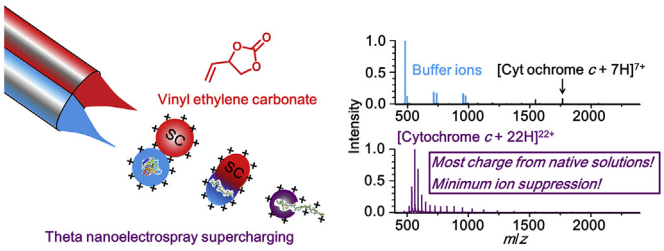
HIGHLIGHTS

- Formation of protein ions from native solutions in highest charge states to date.
- Theta nanoelectrospray ionization supercharging is compatible with biological buffers.
- Formation of highly charged proteins from native solutions is no longer a barrier to obtain high sequence coverage by tandem mass spectrometry.

GRAPHICAL ABSTRACT



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ABSTRACT

Theta nanoelectrospray ionization of protein ions formed from aqueous buffer solutions that are mixed with denaturing solutions containing cyclic alkylcarbonates (*e.g.*, vinyl ethylene carbonate; VEC) results in a significant increase in the extent of ion charging compared to native mass spectrometry. For six proteins, the extent of ion charging can be significantly higher than that obtained using denaturing solutions and alternative native "supercharging" methods. In theta nanoelectrospray supercharging, the extent of charging scales with protein mass in agreement with an analytical scaling relationship for ions with elongated structures. Theta nanoelectrospray supercharging of non-covalent complexes from native solutions results in essentially the complete loss of protein-ligand and protein-protein interactions. Based on circular dichroism spectroscopy, VEC can effectively denature proteins in buffered solutions. These data provide evidence that enrichment of VEC in theta nanoelectrospray ionization generated droplets can denature proteins on the timescale of droplet desolvation and ion formation. This approach can be used to form highly charged protein ions from native solutions containing biological buffers, including some that are considered incompatible with native MS. Forming some protein ions in the highest reported charge states directly from native solutions is no longer a challenge in obtaining primary structural information using tandem mass spectrometry.

1. Introduction

Electrospray ionization (ESI) mass spectrometry (MS) is

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extensively used in the identification of proteins and the analysis of post-translational modifications. Information about the conformations of proteins can be obtained from hydrogen deuterium exchange ESI mass spectra [1–3] and the charge state distributions in ESI mass spectra [4–6]. For ESI, proteins are often partially denatured by using organic solvents and acids in solution, which can result in the formation of protein ions in higher charge states than by use of aqueous solutions at near neutral pH. Protein ions in high charge states are typically formed in extended conformations [7–9] which can fragment more readily by tandem mass spectrometry and be more efficiently detected by some high-performance mass spectrometers (orbitrap and Fourier transform ion cyclotron resonance) to yield more extensive sequence information than those in lower charge states [10–12]. In contrast, ESI of proteins in aqueous solutions at near neutral pH yield protein ions in compact and native-like conformations. Native MS is an important tool in structural biology to obtain information regarding non-covalent complexes, including stoichiometry, dynamics of assembly, and the strength of ligand-protein binding interactions.

Structural information regarding native protein conformations, protein-ligand binding sites, and biomolecular complexes can be obtained by use of various mass spectrometry strategies, such as oxidative labelling [13,14], hydrogen-deuterium exchange (HDX) [1,15–18], limited proteolysis [19,20], and chemical cross linking [21–23]. In such approaches, information about protein structure is 'labelled' in solutions in which the protein and protein complexes are in native structures. Denaturing solution conditions are subsequently used to decode the structural information by use of mass spectrometry and tandem mass spectrometry. For protein ions in sufficiently high charge states, structural information can be directly obtained without chromatography or proteolysis from top-down fragmentation spectra and solution-phase labelling experiments (*e.g.* HDX) [24–27]. Although extensive protein ion sequence coverage by whole protein tandem MS is typically limited to proteins with masses below 30 kDa [10,28–30], near complete protein sequence coverage can be obtained from single isolated charge states of protein ions as large as 67 kDa using very high charge states [10].

The charge state distributions of protein ions formed by ESI depend on many factors, including the gas-phase basicity of the components of ESI solutions [31,32] and atmospheric gases [33], instrumental parameters [34,35], and protein ion conformations [36,37]. In native mass spectrometry, the extent of protein ion charging scales approximately with mass to the power of 0.5 [38–40]. This scaling relationship can be derived from the Rayleigh limit equation by assuming that protein ions are ideal spheres with uniform mass density [41]; *i.e.*, the extent of charging can be limited by droplet surface tension and the protein volume. For protonated protein ions formed from denaturing solutions, the extent of protein ion charging scales as $(z\text{-}1) \times \ln(z\text{-}1)$ with mass, where z is the highest observed charge state [7]. This scaling relationship can be derived analytically by modelling the protein ions as line segments with uniformly distributed point charges. For protein ions formed from native-like solutions, the extent of protein charging in ESI can be increased by: (i) doping small molecules (*e.g. m*-nitrobenzyl alcohol [42,43], sulfolane [44,45], or glycerol carbonate [46]) with low vapour pressures at sufficiently low concentrations [*e.g.* 1–5% (v/v)] into solution to ensure that protein conformations prior to ESI are largely unaffected ("native supercharging") [43,47,48]; (ii) increasing the electric field between the ESI emitter and the inlet to the mass spectrometer ("electrothermal supercharging") [49]; and (iii) rapidly mixing a native solution with a denaturing solution by use of dual-channel ESI using a theta capillary [50].

To assess the extent of ion charging and protein denaturation during ESI, myoglobin is a protein standard that has been widely used as a benchmark in native mass spectrometry because the protein contains a non-covalently bound haem ligand that can readily dissociate in solution and during ion transfer to the MS [45,48–50]. Loo and co-workers demonstrated that by addition of 270 mM sulfolane into aqueous ESI solutions, the most abundant charge state of protonated myoglobin can be increased from 8 + to 14+ [45], which was the highest extent of protein ion charging for this common test protein that was reported in the study (Fig. 1). Going et al. discovered that by doping native ESI solutions with 2%(v/v) of glycerol carbonate (GC), which is based on the cyclic alkylcarbonate scaffold (*e.g.*, propylene carbonate [51] and butylene carbonate (BC) [10,11,33]), protein ions can be formed in higher charge states than by use of sulfolane and *m*-nitrobenzyl alcohol (Fig. 1). For example, by addition of 237 mM GC to aqueous solutions containing ammonium acetate, the most abundant charge state of myoglobin increased to 19+, which is ~36% higher than by use of sulfolane (Fig. 1).

In native MS, the addition of semi-volatile organic molecules to aqueous ESI solutions at sufficiently high concentrations can result in the suppression of ion signal, the formation of protein ion adducts, and the denaturation of proteins and protein complexes in solution. Such limitations can be overcome by the introduction of acidic vapours into the ESI source [52,53] and electrothermal supercharging [49]. However, the extent of protein ion charging that has been reported to date for such methods are comparable to or lower than that reported for native supercharging using chemical additives. For example, electrothermal supercharging MS can be used to shift the most abundant charge state of myoglobin from 8 + to 15+, which is slightly higher than by use of native supercharging with sulfolane (Fig. 1).

Protein ion charge state distributions can be shifted to lower or higher m/z values by mixing solutions using several approaches, including extractive electrospray ionization (EESI) [54,55], fused droplet-electrospray ionization (FD-ESI) [56,57] and liquid sampling desorption electrospray ionization (DESI) [58]. Theta nanoelectrospray ionization mass spectrometry [50,59,60] is particularly advantageous because: (i) fast reactions that occur in low to sub microseconds as a result of solution mixing can be monitored by mass spectrometry [61,62], and (ii) instrumental modifications are not required. In theta nanoelectrospray ionization, two nESI chambers are arranged in parallel for mixing two different solutions immediately prior to the detection of ions. Zare and co-workers used microparticle velocimetry measurements to

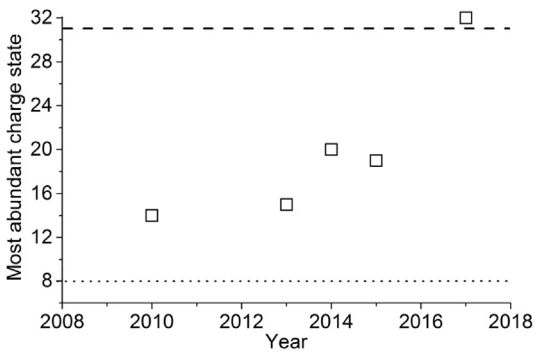

Fig. 1. Plot showing the significant increase in the most abundant charge state of myoglobin that can be formed from native solutions using ESI-MS methods. The data are from Ref. [45] (native supercharging using sulfolane; 2010) [49], (electrothermal supercharging; 2013) [50], (theta nanoelectrospray ionization using acetic acid; 2014) [48], (native supercharging using glycerol carbonate; 2015), and this work (theta nanoelectrospray ionization using vinyl ethylene carbonate). The dotted and dashed lines denote the most abundant charge state of myoglobin formed from a native (8+) and denaturing solution using vinyl ethylene carbonate (31+) [11], respectively.

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