International Journal of Mass Spectrometry 390 (2015) 132–136

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Probing asymmetric charge partitioning of protein oligomers during tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 3 May 2015
Received in revised form 18 August 2015
Accepted 20 August 2015
Available online 3 September 2015

Keywords:
Protein complexes
Proteomics
Electron transfer dissociation
Asymmetric charge partitioning

ABSTRACT

Dissociation of gaseous protein complexes produced by native electrospray often induces an asymmetric partitioning of charge between ejected subunits. We present a simple asymmetric charge partitioning factor (ACPF) to quantify the magnitude of asymmetry in this effect. When applied to monomer ejection from the cytochrome *c* dimer and β-amylase tetramer, we found that the ~60–70% of precursor charge ending up in the ejected monomers corresponds to ACPFs of 1.38 and 2.51, respectively. Further, we used site-specific fragmentation from electron transfer dissociation (ETD) to identify differences in fragmentation and characterize domains of secondary-structure present in the dimer, ejected monomers, and monomers obtained directly from electrospray ionization (ESI). We found evidence of structural changes between the dimer and ejected monomer, but also that the ejected monomer had a nearly identical set of fragment ions produced by ETD as the ESI monomer with the same charge state. Surprisingly, ACPF values for ETD fragment ions generated directly from the dimer revealed that the fragments undergo asymmetric charge partitioning at over twice the magnitude of that observed for ejection of the monomer.

1. Introduction

Native electrospray ionization (ESI) coupled with mass spectrometry has proven extremely useful for the analysis of large assemblies of biological macromolecules [1,2]. Because many non-covalent interactions are maintained during ionization, it is possible to study protein-protein [3,4], and protein-ligand [5] complexes by mass spectrometry (MS) that would be destroyed under denaturing conditions. The stoichiometry of these macromolecular assemblies plays a critical role in many cellular processes [6], including disease states [7], making the direct observation of an intact complex of high interest in the study of biological and gas-phase phenomena alike.

Beyond high-accuracy intact mass measurement, gas-phase fragmentation can provide further identification and characterization of the intact complex [8,9] as well as individual subunits [10,11]. In addition, fragmentation using electron capture dissociation (ECD) can cleave covalent protein backbone bonds while maintaining nearby non-covalent interactions [12]. Recent studies have utilized this unique property to provide information about the higher-order structure of gaseous proteins [13–15] and protein complexes [8,16]. Similarly, native electron capture dissociation (NECD), a new sub-type of radical dissociation that is induced by heating the transfer capillary instead of the addition of exogenous electrons, was used to probe the gas-phase unfolding of the cytochrome *c* dimer [17,18].

On the other hand, threshold dissociation of protein complexes by collisional or photon-based activation often liberates one or more monomers with a disproportionately high charge, in a process first described by Light-Wahl et al. in 1994 [19]. The effect has been studied by many labs since, and termed "Asymmetric Charge Partitioning" (ACP) [20–22]. It can be attributed to, among other reasons, the unfolding of one monomer prior to or during ejection, causing it to acquire far more charge than would be expected based on its portion of the mass of the intact complex [23]. Studies by the Robinson group have further showed a close correlation between the ratio of surface areas of the ejected monomer and the intact complex and the overall degree of charge transfer [24]. Experimental results from several labs on a variety of systems [25] have also reproduced the effect and found ACP to be dissociation method [26] and sometimes charge state [23] dependent. Theoretical studies have also been performed, using molecular dynamics [27] and other modeling methods [28].

Here, we introduce a simple metric for quantifying ACP, and use electron transfer dissociation (ETD) [29], a radical fragmentation technique similar to ECD, to probe the secondary structures in the gas-phase cytochrome *c* dimer, its ejected monomer, and the

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http://dx.doi.org/10.1016/j.ijms.2015.08.021
1387-3806/

analogous monomer produced *via* electrospray. We not only find evidence of the structural changes between the dimer and ejected monomer, but also of an ACP upon dissociation of the fragment ions formed by ETD. This observation provides the first characterization of ACP as a general phenomenon for any sub-structural element liberated from a protein complex.

2. Material and methods

Horse heart cytochrome *c* was purchased from Sigma Aldrich (St. Louis, MO, USA) and used without further purification. Cytochrome *c* samples were sprayed at 5 μL/min from a 0.26 mg/mL solution in 100 mM ammonium acetate on an Orbitrap Elite mass spectrometer (Thermo Scientific, Bremen, Germany). β-Amylase samples were purchased from Sigma Aldrich (St. Louis, MO, USA) and were buffer exchanged into 100 mM ammonium acetate using 30 kDa molecular weight cutoff filters (Millipore, Billerica, MA, USA). Samples were sprayed and analyzed with a modified Q-Exactive HF mass spectrometer (Thermo Scientific, Bremen, Germany) as described previously [10].

ETD was performed with fluoranthene as the reagent using an N_2 carrier gas. When ETD was performed for precursor charge reduction without fragmentation (ETnoD) [30], the reaction time was increased (generally >5 ms) to maximize the signal of the targeted reduced-charge species. When ETD was performed for backbone cleavage, ETD reaction times were set to maximize fragment ion production while minimizing reduced precursor ion signal, generally 2–10 ms depending on the original precursor charge state. CAD indicates RF-induced collisional activation and was performed in an ion trap. CAD was used to produce the ejected monomer as well as to break non-covalent bonds after ETnoD (*i.e.* supplemental activation) in selected experiments. All data were analyzed manually with the freely available software, mMass [31]. Ion yields were normalized by charge to account for the linear detection bias in the Orbitrap analyzer [32].

3. Results and discussion

3.1. Monomer ejection from the dimer of cytochrome c

Fig. 1 depicts spectra of cytochrome *c* monomers ejected from dimeric precursors of selected charge states. In the top panel, the 13+ charge state of horse heart cytochrome *c* dimer was isolated

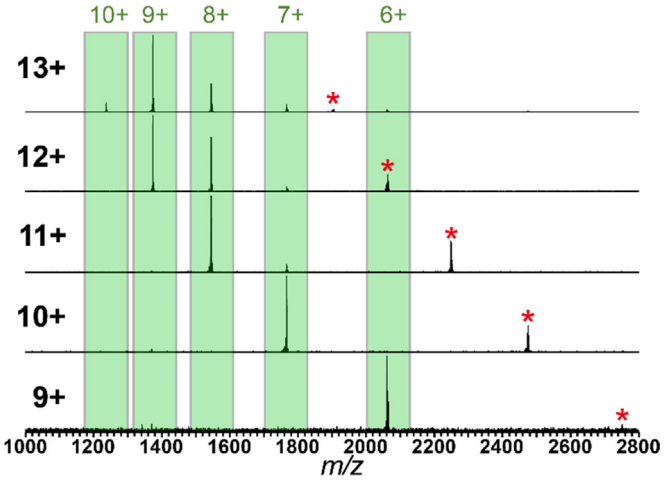

Fig. 1. Ejected monomer species observed from isolation of the 13+ through 9+ species of the cytochrome *c* dimer (precursor charge state listed at far left). The 12+ to 9+ parent ions (indicated by an asterisk) were formed by charge reduction (ETnoD) of the 13+ during the ETD process.

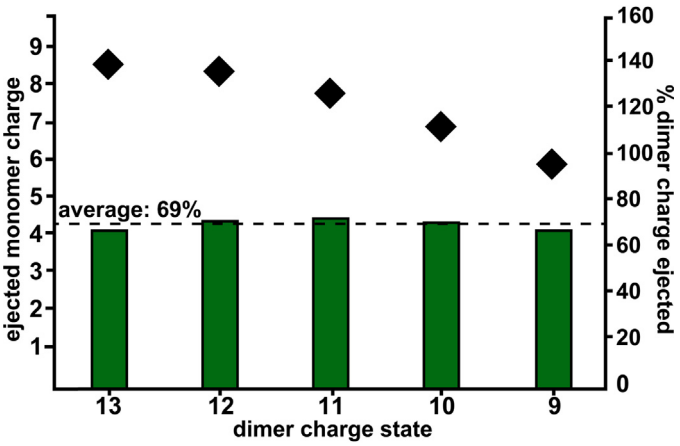

Fig. 2. Black diamonds indicate the intensity-weighted average charge of the ejected monomers (left axis) observed after isolation and fragmentation of each dimer charge state (*x*-axis). The green bars represent the percent of charge from the dimeric precursor that is observed in each monomer for each monomer ejection experiment (right axis). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and activated with CAD to eject monomer ions of charge states 10+ to 6+. To prevent overlap of dimer and monomer signals, the isolated 13+ dimer was subjected to ETD at reaction times optimized for formation of the reduced-charge precursors that have not fragmented (ETnoD) [30]. Each of the 12+ to 9+ reduced-charge dimer species was then isolated individually, and activated with CAD to eject monomers. No significant yields of *c*- or *z*-fragment ions were observed relative to signals from monomer ejection. Each dimer charge state is therefore a product of the parent 13+ ion; however, isolation and activation of the ESI-formed 11+ ion produced similarly charged ejected monomers (Supplementary Fig. 1).

Fig. 2 shows the intensity-weighted average charge of ejected monomers resulting from each dimer charge state (black diamonds). The green bars underneath indicate the percent of the precursor charge represented by the weighted-average monomer charge. Despite the general downward trend of ejected monomer charge (Fig. 2), the overall percent remains remarkably constant, varying from 66 to 72%. Thus, the magnitude of ACP does not change even with a 31% decrease in precursor charge density, indicating that it is more affected by the relative unfolding of the two subunits than it is by the Coulombic strain of charges on the precursor for the 13–9+.

Monomer charge states from all of the ejected dimers show significant evidence of ACP. A previous study by Williams and coworkers [23] found significant differences between the monomer ejection from cytochrome *c* dimers formed directly by ESI when compared to those formed by gas-phase deprotonation of isolated higher charge states. Specifically, they found that when the 15+ cytochrome *c* dimer is deprotonated to form the 13+, it produces symmetrically ejected monomers. The current results do not show any evidence of this effect: the doubly reduced 11+ dimers produced nearly identical ejected monomers when compared to those produced from the ESI-formed 11+. The apparent discrepancy in results can be attributed to several factors, including the higher pressure of the ion trap (~4 mTorr) when compared to that found in an ICR instrument and the difference in proton transfer/electron transfer reagent (diethylamine instead of the fluoranthene used here). The magnitude of ACP can also vary greatly for complexes electrosprayed out of different solution conditions and at different concentrations; a previous study by Smith et al. [33] showed no ACP with dissociation of cytochrome *c* dimers under denaturing conditions. Indeed, the full mass spectrum of cytochrome *c* prior to isolation and dissociation (Supplementary Fig. 2) shows