

Symmetrical Gas-Phase Dissociation of Noncovalent Protein Complexes via Surface Collisions

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Nanospray mass spectrometry is rapidly becoming a viable method for the analysis of multimeric protein complexes. The soft ionization conditions afforded by nanospray provide for gentle transfer of proteins from physiologically relevant solutions into the gas phase, making it possible to maintain many of the weak, noncovalent interactions that exist between protein subunits. This has allowed macromolecules of great size and complexity to be examined by mass spectrometry, including intact ribosomes,^{1,2} virus particles,³ and enzyme assemblies.⁴

The chemical architecture of a given assembly can be further investigated through tandem mass spectrometry (MS/MS). Unfortunately, the interpretation of tandem mass spectra for large biological complexes is hindered by a relatively crude understanding of their dissociation. Upon activation of protein complexes, the primary observations have been an asymmetric partitioning of both mass and charge. For example, dissociation of homotetrameric complexes of concanavalin A and avidin and heterotetrameric hemoglobin by Smith and co-workers^{5,6} yielded monomers and trimers as the primary products. Despite accounting for only one-quarter of the complex in terms of mass, the monomer species retained approximately half of the overall charge. Asymmetric partitioning is not limited to tetramers as it has been observed for several protein complexes, regardless of size or number of subunits.^{7–9}

Smith and co-workers proposed that this dissociation pattern was similar to the fission of electrospray droplets.⁶ In this model, the monomer fragment ions have a disproportionately high surface area and therefore retain an uneven amount of charge. Heck and co-workers¹⁰ and later Williams and co-workers^{11,12} demonstrated that charge separation also occurs asymmetrically in homodimers, complexes that should theoretically partition evenly. Williams and co-workers proposed that one of the monomeric subunits is unfolding during the dissociation process, and because of the increased surface area of the elongated subunit, there is a Coulombically favorable charge transfer from the folded subunit to the unfolded one. Klassen and co-workers have also observed evidence of proton transfer in a pentameric protein complex, proposing asymmetric dissociation to be an entropically favorable pathway facilitated by charge enrichment of a single subunit.¹³ Recently, Robinson and co-workers have correlated the degree of charge partitioning to the relative surface areas of the unfolded monomer and the remaining complex.¹⁴ Despite these advancements, questions remain about the preferential unfolding of a single subunit and the driving force behind this process.

The mode of ion activation is likely to play a key role in the unfolding of the ejected monomer. In the present work, we have studied the asymmetric dissociation of protein complexes by surface-induced dissociation (SID) as well as by traditional collision-induced dissociation (CID). Unlike CID, SID is a fast, single step activation process in which internal energy is deposited into

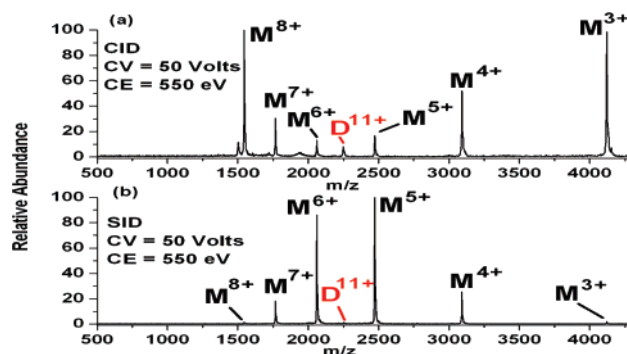


Figure 1. Comparison of tandem mass spectra for (cyt C)₂¹¹⁺ by CID (a) and SID (b). The precursor ion is shown in red for clarity. The voltage difference between the source hexapole and the collision cell (CID) or the surface (SID) is listed as ΔV . The collision energy (CE), the product of ΔV and the precursor ion charge state, is also given for each spectrum.

the projectile ion in a matter of picoseconds.^{15,16} Consequently, the dissociation products might differ significantly from those produced by multiple collision slow-heating methods.

We have modified a quadrupole time-of-flight mass spectrometer (Waters Q-TOF II) in order to activate ions by CID and SID within the same instrument.¹⁷ This modification allows us to eliminate any discrepancy that might arise from different source conditions and observation time frames, and make a direct comparison between the two activation methods. In this work we examine cytochrome C, a protein whose gas-phase structural properties have been extensively studied, including analysis of cytochrome C homodimers by Williams and co-workers.^{11,12} We electrosprayed 200 μM cytochrome C (Sigma-Aldrich) using a home-built nanospray source from a buffer solution of 10 mM $\text{NH}_4\text{Ac}/\text{NH}_4\text{HCO}_3$, pH 7. Cytochrome C forms nonspecific homodimer aggregates at high concentrations. The dimer peaks are broad (see Supporting Information), a commonly observed feature of electrosprayed protein complexes that likely results from the presence of multiple protein-solvent adducts.

The 11+ dimer (D^{11+}) of cytochrome C was selected and fragmented by CID to yield the spectrum displayed in Figure 1a. Three dissociation pathways are evident for the 11+ dimer precursor ion: formation of the 3+/8+, 4+/7+, and 5+/6+ complementary monomers. While the higher order symmetry of the precursor ion in the gas phase is not known, it is composed of two polypeptide chains of identical amino acid sequence. Despite this, it is clear from the CID spectrum in Figure 1a that the most asymmetric dissociation pathway (the $\text{M}^{3+}/\text{M}^{8+}$ monomer pair) is dominant. As stated above, this is presumably due to a breaking of symmetry in the complex by an unfolding of one of the subunits. It should be noted that CID of cytochrome C dimers in a Q-TOF performed by Heck and co-workers yielded high m/z ions of much lower abundance than their complementary low m/z ions, a phenomenon

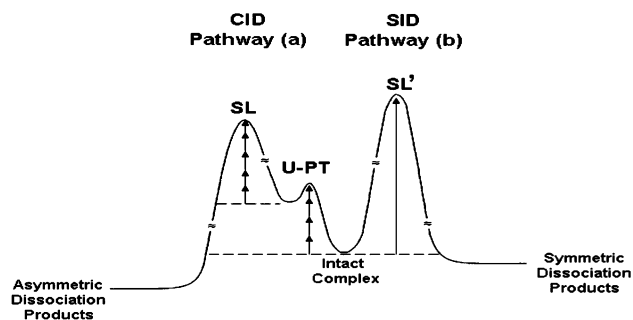


Figure 2. Simplified reaction diagram for collision-induced (a) vs surface-induced dissociation (b) of noncovalent protein complexes. SL = subunit loss, U-PT = unfolding/proton transfer. See text for details.

also observed in early experiments in our lab. This observation has been noted in the literature and attributed to the decreased ability to focus ions of high m/z and the lower velocity of these ions as they impinge upon the detector.^{10,11} This high m/z discrimination was observed with the manufacturer's suggested tuning (see Supporting Information), and overcome through the tuning of source conditions and reducing the DC potential on the tube lens prior to the TOF mass analyzer. CID in this case results in 3+ and 8+ ions of approximately equal abundance. Regardless of tuning parameters, the 8+ fragment is more abundant at this CID energy than the 7+ or 6+ fragment ions.

In order to explore the effects of an "energy sudden" surface collision on protein complexes, the 11+ dimer was collided with a fluorinated self-assembled monolayer on gold.¹⁸ The SID spectrum of the 11+ dimer, shown in Figure 1b, reveals predominantly symmetric charge partitioning. In this case, the predominant pathway is the formation of monomer products with five and six protons, while the 3+ and 8+ monomer ions that dominated the CID spectrum account for a small percentage of the total ion current. This symmetric dissociation pattern is observed over a broad range of collision energies (see Supporting Information).

We propose that the observation of more symmetric charge partitioning is a direct result of the energy-sudden activation provided by SID. CID slowly adds internal energy through multiple collisions, over which time subunit unfolding can gradually occur. Given that the activation time for ion-surface collisions is on the picosecond time scale, it is possible that subunit unfolding does not occur, or occurs to a far lesser extent, before dissociation yields a more symmetric product distribution.

Figure 2 suggests a simplified reaction pathway for two competing dissociation processes. Pathway a represents slow, low-energy dissociation of a protein complex as previously shown by Klassen and co-workers, in which monomer unfolding/proton transfer (U-PT) precedes subunit loss (SL).¹³ Pathway b represents a single step activation process in which little or no unfolding occurs before subunit loss (SL'). Dissociation via pathway a occurs via a multistep activation in which the conformation changes with collisions, and the structure of the complex after the n th collision is different than that of the original precursor ion. In contrast, in pathway b sufficient energy is deposited in a single step to overcome the energy threshold for subunit loss. The transition state structure for subunit loss in these two pathways is unlikely to be the same, thus the charges and subunit structures of the product ions should also be different.

A conclusion that may be drawn from the present and published CID data is that it appears one monomer unfolds to a greater extent than other subunits within the same complex. While it has been debated as to what drives this asymmetric partitioning (i.e., does unfolding facilitate proton transfer or does proton-transfer promote

unfolding)^{11,13,14} it is likely that an increasing number of noncovalent bonds are broken as the complex undergoes multistep collisional heating. Ultimately, the unfolded monomer will dissociate from the remaining complex, taking with it a substantial fraction of the overall charge.

In the case of ion-surface collisions, the collision partner is larger, and ions make more direct contact upon collision. A large percentage of the protein's overall surface area will make physical contact with the surface, and the collision is more energetic in the center-of-mass reference frame. This may provide the possibility that multiple noncovalent interactions in the interfacial subunit regions are disrupted nearly simultaneously leading to ejection of the colliding monomer before substantial unfolding.

While the mechanisms of this process are still being investigated, the current work provides insight into the time scale of the subunit unfolding process, the prospect of subunit dissociation without unfolding, and the effect of activation time frame on the dissociation patterns of protein complexes. This work is ongoing and currently being applied to oligomeric systems of greater size and complexity to determine if symmetric dissociation is observed and if valuable information about the nature of subunit interactions can be extracted from such studies. For biological tetramers, such as transthyretin, SID leads to more symmetric charge and mass partitioning than does CID.¹⁸

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Supporting Information Available: Mass spectrum for cytochrome C and an inset of the selected precursor ion; energy-resolved CID and SID spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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