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Revealing the Quaternary Structure of a Heterogeneous Noncovalent Protein Complex through Surface-Induced Dissociation

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Ⓢ *Supporting Information*

ABSTRACT: As scientists begin to appreciate the extent to which quaternary structure facilitates protein function, determination of the subunit arrangement within noncovalent protein complexes is increasingly important. While native mass spectrometry shows promise for the study of noncovalent complexes, few developments have been made toward the determination of subunit architecture, and no mass spectrometry activation method yields complete topology information. Here, we illustrate the surface-induced dissociation of a heterohexamer, toyocamycin nitrile hydratase, directly into its constituent trimers. We propose that the single-step nature of this activation in combination with high energy deposition allows for dissociation prior to significant unfolding or other large-scale rearrangement. This method can potentially allow for dissociation of a protein complex into subcomplexes, facilitating the mapping of subunit contacts and thus determination of quaternary structure of protein complexes.
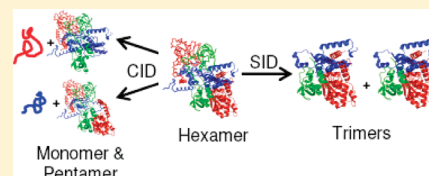
The majority of proteins exist and perform their functions as multimers of varing stoichiometries and architecture.[1] However, very few methods are available that can provide insights into subunit interactions. Native mass spectrometry (MS) is increasingly being used to study noncovalent protein complexes, as many structural features found in solution may be maintained in the gas phase.[2,3] While subunit stoichiometries are readily obtainable by mass measurement alone, the determination of subunit arrangement within protein complexes remains a significant challenge. This is particularly true for heterogeneous complexes with multiple types of subunits. Considerable progress has been made using solution-phase disruption to divide the original protein complex into smaller subcomplexes, which may be readily measured by MS.[4,5] The composition of the stable subcomplexes provides insight on the topology of the protein complex. However, MS activation methods used to date have fallen short of providing subunit topology. Here, we present the first evidence for subunit arrangement obtained directly from gas-phase experiments on a heterogeneous complex via surface-induced dissociation (SID).

We have demonstrated previously the ability of SID to yield unique dissociation pathways for protein complexes, resulting in complementary information to collision-induced dissociation (CID).[6−8] While the SID process is not yet well understood for macromolecules, there is a large body of work concerning SID of small molecules; influential factors such as collision energy, surface composition, and translational-to-vibrational energy conversion have been well-studied.[9−11] The higher effective mass of a surface relative to that of neutral gas atoms used in CID (typically argon) results in significantly higher energy deposited through a single surface collision.[9] As SID is a single-collision activation process, rather than activation via thousands of less energetic collisions as in CID, dissociation pathways other than those of the lowest energies become accessible.

The differences in the collision events in CID and SID result in different but complementary information being obtained from these experiments. CID of multiprotein complexes almost universally produces a highly charged, unfolded monomer and the complementary $(n - 1)$ product ion, regardless of the size, composition, or architecture of the precursor complex.[12] A few notable examples of alternative dissociation have been cited in the literature,[13−16] but there are none that lead to complete determination of assembly structure. The highly charge-asymmetric nature of this dissociation indicates significant structural change of the dissociated monomer.[12] While a single dissociation pathway is generally observed in CID, some structural information can still be gathered. For instance, if a monomer dissociates from the multimer via unfolding, logic suggests surface subunits may be ejected preferentially, allowing determination of the peripheral subunits.[5,17] Although it is rare, there have been instances when core subunits have dissociated as monomers during CID, presumably due to extensive structural change upon activation.[18]

Limited ability of protein subunits to unfold (for example, due to extensive disulfide bonding[15] or buried termini[16]) prior to dissociation appears to be a key factor in obtaining atypical CID product ions. Using ion mobility, Pagel et al. have demonstrated more charge-symmetric product ions to have more compact cross sections, suggesting less extensive rearrangement,[14] unlike highly charged monomer product ions which have very extended structures. However, due to the typically limited dissociation observed by CID, no complete contact map can be established from CID data alone.
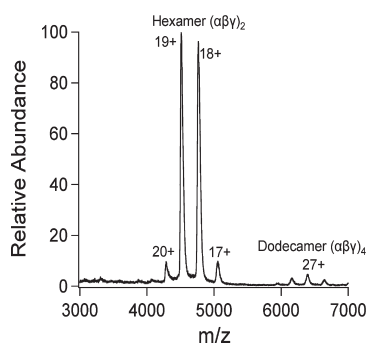
Figure 1. Native mass spectrum of TNH indicating hexameric oligomeric state.

In contrast to CID, SID typically produces more charge-symmetric product ions, implying less structural change prior to dissociation.[6−8] The high-energy, single-step activation of SID and the apparent absence of large-scale structural rearrangement before dissociation permits subcomplexes within the protein to be retained intact. Identification of these subcomplexes may enable mapping of the subunit contacts for the entire protein complex.

In the present work, we have conducted dissociation studies of toyocamycin nitrile hydratase (TNH), a heterogeneous, Co-binding, noncovalent protein complex with three unique subunits (α, 21.2 kDa; β, 10.1 kDa; γ, 11.5 kDa).[19] In this case, MS shows the complex to be hexameric (Figure 1) and SID produces trimeric product ions representative of the substructure of the original protein complex (Figure 2b). This direct observation of substructure via gas-phase dissociation has not been reported previously and illustrates the potential for SID in structural biology.

■ EXPERIMENTAL SECTION

Experiments were performed on a quadrupole time-of-flight mass spectrometer (Waters QTOF2) previously modified to incorporate an SID device between the mass selecting quadrupole and the hexapole CID collision cell;[20] thus, both CID and SID experiments may be performed within the same instrument. TNH was expressed and purified in-house[19] and buffer exchanged into 100 mM ammonium acetate using Micro Biospin columns from Bio-Rad (Hercules, CA). Solutions of ~20 μM TNH were nanoelectrosprayed via a home-built source with capillaries pulled in-house on a P-97 micropipet puller (Sutter Instruments, Hercules, CA).

■ RESULTS AND DISCUSSION

A mass spectrum of TNH indicating hexameric stoichiometry is shown in Figure 1. Representative CID and SID MS/MS spectra for the 19+ hexameric precursor are shown in Figure 2a, b, respectively. As expected, CID produces only highly charged monomers and the corresponding pentameric products. SID produces a drastically different dissociation pattern than CID; most notably, the dominant dissociation pathway of the 19+ hexamer is dissociation into charge-symmetric 9+ and 10+ $\alpha\beta\gamma$ trimers. The variety of product ions in the SID spectra ultimately leads to more structural information than is produced by CID. Energy-resolved SID data facilitate delineation of the observed dissociation pathways and lead to improved understanding of the complex quaternary structure. With increasing collision energy (Figure 3b), the trimer abundance decreases while the abundance of the $\alpha\beta$ dimer increases. Similarly, at still higher collision energies, the $\alpha\beta$ dimer abundance begins to decrease while the abundance of the individual α and β monomers increase. These results imply sequential dissociation of the complex after the initial dissociation breaks the hexamer into two trimers. A schematic of these dissociation pathways can be seen in Figure 2c, and a table of all product ions observed by SID and CID may be found in Supporting Information.

Only α or β monomers are ejected from the hexameric complex by CID. At lower collision energies, the β subunit is
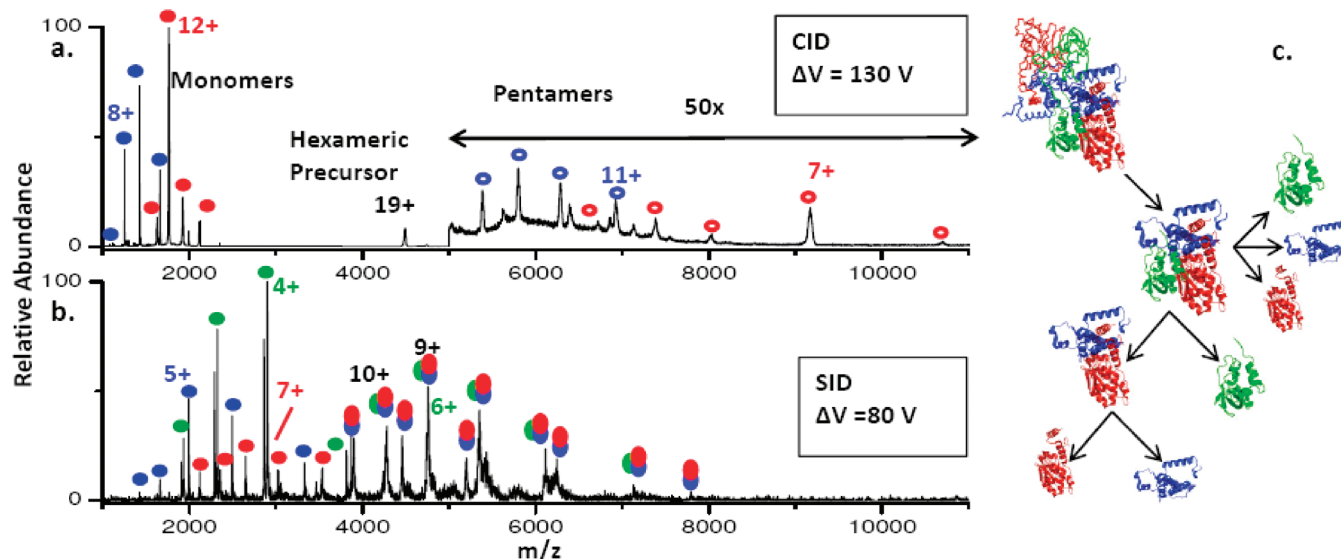

Figure 2. Comparison of (a) CID and (b) SID spectra for TNH. In the SID pathway schematic (c), each subunit in the structure (the homologous thiocyanate hydrolase) is indicated by a different color for clarity: α, red; β, blue; and γ, green. In (a) and (b), monomeric product ions are indicated by a solid circle of the corresponding color, while the complementary pentamers are indicated by an open circle of the same color as the monomer. In (b), the data from the homologue shown in (c) has been applied to the labeling scheme, as such, subcomplexes are indicated by clusters of the appropriate color circles.
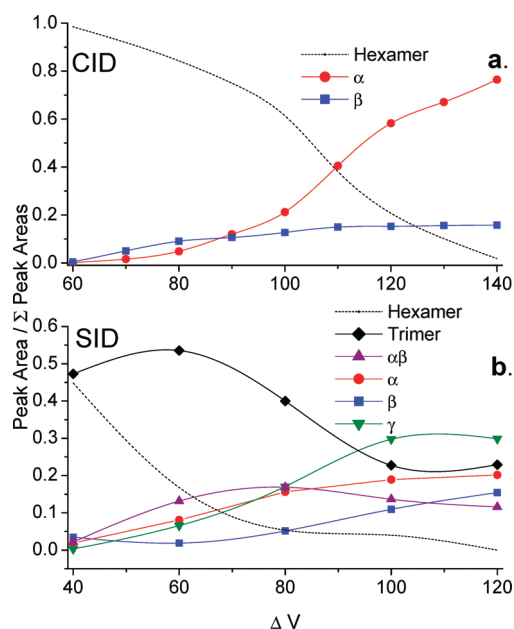
Figure 3. Energy-resolved (a) CID and (b) SID results.

most readily ejected; however, at increasing collision energies, the α subunit is preferentially ejected (Figure 3a). The specific combination of factors that controls which subunit unfolds and/ or dissociates first via CID is unknown; we expect mass to be a contributing factor,[15] and the β subunit is the smallest subunit of this complex. It is curious that ejection of the γ subunit is not observed by CID, as the α subunit is nearly twice the mass of γ and yet dissociation of α dominates. This may be due to stronger interactions of the γ subunit with the rest of the hexamer or it may be that the γ subunit is buried within the hexamer, restricting its ability to unfold and dissociate.

Evidence of Co binding to the α subunit is observed in both CID and SID spectra, with both the holo- and apo-species present (see Supporting Information). The α subunit is known to bind one Co ion in the active site.[19] The relative abundance of the apo-species increases with collision energy. Apo- and holo-α are more readily distinguished by SID than by CID as the lower charge states facilitate better resolution of the two species. Assuming that the secondary and tertiary structure around the active site must remain somewhat intact to keep the Co bound, this supports the hypothesis that dissociation by SID occurs prior to extensive rearrangement. Preservation of subunit—metal interactions upon SID of a complex may be of great use for other ligand—protein interaction studies.

While a high-resolution structure of TNH is not available and the precise nature of the subunit interfaces is undetermined, the protein is homologous to other nitrile hydratases and thiocyanate hydrolases. Therefore, we utilized the X-ray crystal structure of thiocyanate hydrolase (PDB ID 2DD5) as a model to illustrate the dissociation pathway (Figure 2c). In thiocyanate hydrolase, the α—β interface is substantial, while the interaction of the γ subunit with the rest of the trimeric subcomplex is less extensive. If the subunit interfaces within TNH are similar, this would be a plausible explanation of why γ dissociates readily from trimeric product ions. The observation of the γ monomer only as a second generation product of SID suggests that interactions within the hexamer help stabilize the

γ subunit or that the γ subunit lacks surface access within the hexamer.

CONCLUSIONS

This is the only study to date demonstrating an ion activation method capable of yielding extensive dissociation, as well as the release of intact subcomplexes, thus providing relevant substructure information on a noncovalent, hetero-oligomeric protein complex. The capacity to produce intact, charge-symmetric subcomplexes suggests that dissociation occurs faster than subunit unfolding and that a significant degree of secondary and tertiary structure is maintained up to the point of dissociation and for some period of time afterward. Identification of trimeric substructure in TNH provides insight into a protein with little previous structural characterization and indicates a promising advancement of MS as a tool for structural biology.

ASSOCIATED CONTENT

S **Supporting Information.** Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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