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Dissecting the Large Noncovalent Protein Complex GroEL with Surface-Induced Dissociation and Ion Mobility–Mass Spectrometry

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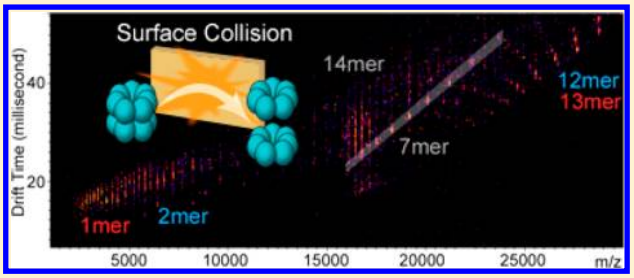
Supporting Information

ABSTRACT: Tandem mass spectrometry is a tool to dissect noncovalent protein complexes into smaller substructures for quaternary structure analysis. The commonly used activation method, collision induced dissociation (CID), often provides limited structural information from the typical dissociation pattern where unfolded monomers are ejected from the protein complex. In contrast, surface-induced dissociation (SID) has been shown to be very effective at dissociating protein complexes with less unfolding than CID. We present here SID of a large noncovalent tetradecamer protein, GroEL (801 kDa). A wide variety of products, including heptamers representative of the native topology, are released from the precursor upon SID, significantly different from the ubiquitous monomer ejection in CID. Enhanced dissociation into heptamers is observed when the charge states of the GroEL precursor are reduced by adding triethylammonium acetate into the spraying buffer. Ion mobility is utilized after SID to separate products overlapping in m/z to simplify the SID spectra. Compact heptamers from the charge-reduced tetradecamer are clearly distinguished from other overlapping species. SID can be very useful for quaternary structure studies of large noncovalent protein complexes, as manifested by the GroEL data where the tetradecamer dissociates into heptamers, reflecting the native topology of the complex.



Mass spectrometry (MS) has become an important tool for structural biology,[1,2] with a growing applicability to quaternary structure analysis of noncovalent protein complexes.[2−5] With well-controlled ionization and instrumental conditions, large and fragile protein complexes can be detected by MS. Molecular weight measurement with MS allows determination of stoichiometry of the complexes with a higher confidence than low-resolution techniques.[6] Ion mobility (IM), a gas-phase separation technique, has been interfaced with mass spectrometry and provides critical information about the conformations of the proteins in the gas phase.[7] Collisional cross sections (CCS) determined from IM experiments suggest that many protein complexes, within the timescale of MS analysis, retain memory of their solution structure in the gas phase[8,9] and can preserve nativelike topology when transferred from a buffer that resembles physiological conditions.[1,10]

Additional information about the protein quaternary structure can be obtained with tandem mass spectrometry (MS/MS) where the noncovalent protein complexes are isolated and dissociated upon activation into smaller substructures in the gas phase.[11] Ideally, subcomplexes that are representative of the native topology will be detected so that the quaternary structure of the precursor complex can be revealed. However, the commonly used MS/MS method, collision-induced dissociation (CID, collision with neutral gas atoms/molecules) often induces unfolding of the protein complexes resulting in ejection of highly charged and unfolded monomers from the complexes,[11−16] with a few exceptions.[17−19] Although this ubiquitous dissociation pattern can be beneficial for further confirmation of stoichiometry, it is uninformative for quaternary structure analysis, especially for large protein assemblies.

In contrast, surface-induced dissociation (SID, collision with a stationary surface) has shown promising results in probing the quaternary structure of protein complexes. Minimal unfolding of a pentameric protein complex upon SID, with much more extensive dissociation into subcomplexes than CID, has been reported.[20] The products released from SID for several pentameric and tetrameric complexes are also shown to be compact and experience less unfolding than in CID.[20−22] While unfolded monomers are usually the dominant products in CID, SID provides meaningful information from additional dissociation products (e.g., for structural elucidation of a heterohexamer protein, shown to be a dimer of $\alpha\beta\gamma$ trimers by SID, and a stacked ring decamer protein).[23,24] The more extensive dissociation with less unfolding observed in SID is attributed to the fast, energetic surface collision, which obviates the restriction of dissociation by the slow, multistep, low barrier processes seen in CID (common instrument platforms for this

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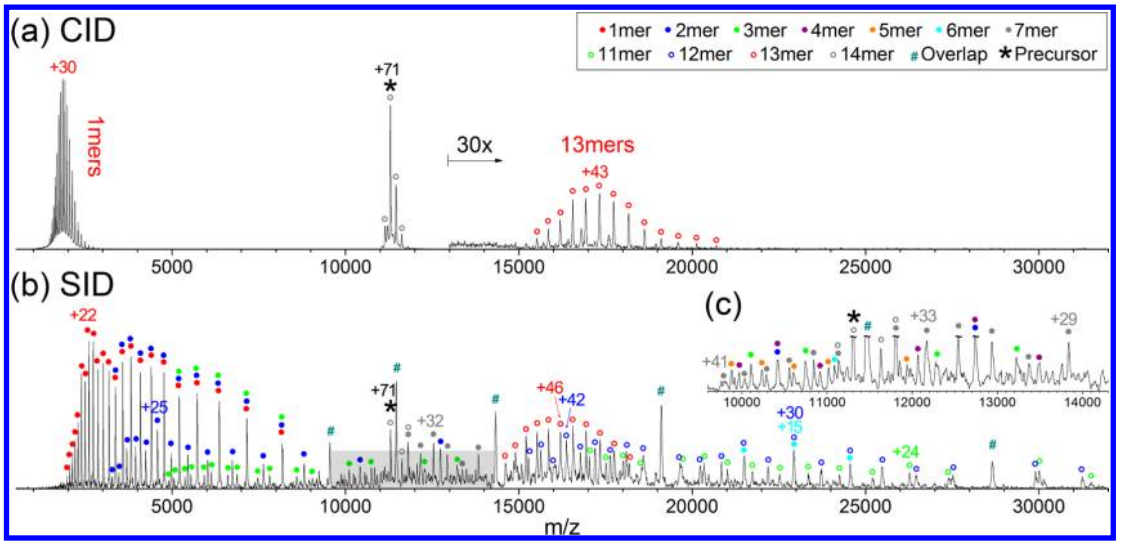

Figure 1. (a) CID of GroEL tetradecamer +71 at an acceleration voltage of 160 V. (b) SID of GroEL tetradecamer +71 at an acceleration voltage of 180 V. Major peaks are labeled following the legend at the top. The inset spectrum is a zoom-in view of the region shaded in the middle of the full SID spectrum, with more assignments for the peaks at low abundances. Charge states of several peaks discussed in the text are selectively labeled with the corresponding colors of the dots in the legend. Ejection of highly charged monomers is the predominant dissociation pathway in CID. In contrast, SID shows extensive dissociation into a wide variety of products.

type of analysis allow acceleration voltages up to a few hundred volts).[13,25]

GroEL is a large tetradecameric protein complex (801 kDa) that consists of two stacked heptamer rings, as shown by crystallography.[26] It has served as a model system to study the fundamental behaviors of very large protein complex ions in the gas phase.[27−30] CID and IM experiments in MS platforms have also been used to study the ligand binding properties of GroEL.[31−33] However, CID only causes monomer ejection from the tetradecamer[17,27,34] and does not provide effective dissociation for the large assembly. In the work presented here, GroEL is dissociated into a wide variety of products by SID, including heptamers that are reflective of the native topology of the tetradecamer complex. The charge state of GroEL was reduced by adding triethylammonium acetate into the spraying buffer, which has been shown to suppress the unfolding of protein complexes in the gas phase,[21,35] so that the dissociation could be more representative of the native quaternary structure. IM was utilized after the dissociation to separate the products in drift time, in order to simplify interpretation of the SID spectra where many species overlap in m/z.

EXPERIMENTAL SECTION

GroEL lyophilized power (Sigma, Milwaukee, WI) was dissolved at 20 μM (monomer concentration) in buffer A (20 mM tris-HCl, 50 mM potassium acetate, 0.5 mM ethylenediaminetetraacetic acid, 5 mM magnesium chloride, pH 7) with 2 mM adenosine-5′-triphosphate (ATP). An acetone precipitation step was used to precipitate the protein, separating it from the impurities in the sample that do not precipitate.[36] The sample was shaken slowly on a vortex for 1 h. Then, methanol was added to 20% of the final volume and the sample was shaken for another hour. After that, the protein was precipitated with 50% acetone by volume. The precipitate was resolublized in buffer A with 2 mM ATP, at a final protein concentration of 20 μM for the monomer, as recommended for refolding.[37] The solution was shaken slowly on a vortex for 1 h at room temperature and then concentrated using a micro-centrifugation device (Amicon Ultra 0.5 mL, 30 kDa cutoff, Millipore, Billerica, MA). The concentrate was diluted to 20 μM of GroEL monomer with buffer A containing 2 mM ATP and shaken again for 1 h followed by the filtration step. The shaking and filtration were repeated four times to maximize refolding efficiency of the tetradecamer complex. The solution was eventually concentrated to give 6 μM of GroEL tetradecamer, which was then buffer exchanged into 200 mM ammonium acetate using a size exclusion spin column (Micro Bio-Spin 6, BioRad, Hercules, CA). For charge reduction, 100 mM triethylammonium acetate (Sigma, Milwaukee, WI) was added to the protein solution (in 200 mM ammonium acetate) to 20% of the volume.

The instrument used in the work is a modified Synapt G2 mass spectrometer (Waters Corporation, Manchester, United Kingdom). The descriptions of the original instrument[38] and the custom modification[20,21] can be found elsewhere. Details of the SID device are described in a previous report.[24] Briefly, a custom SID device was inserted immediately before the ion mobility cell of the instrument, after a shortened trap stacked-ring ion guide. The DC voltages on the SID lenses can be tuned either to allow a fly-through of the ions for MS and CID experiments or to direct the ions onto the surface for collision with subsequent steering of the products to downstream optics for collection. The current instrument setup is able to perform CID or SID experiments after quadrupole m/z selection and separate the CID/SID products in the IM cell. The acceleration voltage in CID is defined by the "Trap CE" setting in the instrument tune page, which is the potential difference between the DC offsets of the quadrupole and the trap traveling wave ion guide before the IM cell. The acceleration voltage in SID is defined by the potential difference between the DC offset of the trap traveling wave ion guide and the surface, which can be adjusted using the "Trap bias" setting in the instrument tune page. The acceleration voltages are listed with the spectra presented here to serve as a qualitative comparison of CID and SID for large protein complexes within voltage limits in the commercial instrument.

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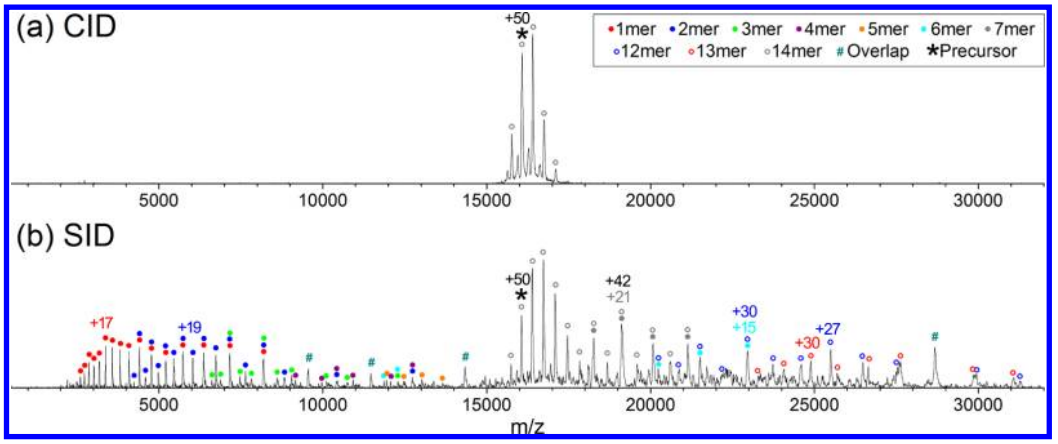

Figure 2. (a) CID of GroEL tetradecamer +50 at an acceleration voltage of 200 V. (b) SID of GroEL tetradecamer +50 at an acceleration voltage of 180 V. Major peaks are labeled following the legend at the top. Charge states of the products are labeled on representative peaks with colors that correspond to the dots in the legend. Charge states of the tetradecamer are shown in black in order to distinguish them from the charge state of heptamers, which are shown in gray. The charge-reduced precursor hardly dissociates in CID, even at the maximum acceleration voltage available in the instrument. However, distinct heptamers, which are representative of the subunit architecture of the native conformation, can be observed in SID and are of comparable or higher intensity than several of the other products.

The protein was ionized with a nanoelectrospray setup through glass capillaries[24] with an inserted platinum wire at an applied voltage of 1.0−1.5 kV. The sampling cone was set to 100−150 V at room temperature. Backing pressure was 5.6 mbar. Argon gas flow in the trap cell was 4 mL/min in SID and 10 mL/min in CID for optimum focusing of the high m/z region. Helium cell and IM cell (nitrogen) gas flows were 120 mL/min and 60 mL/min, respectively. The injection voltage either from the trap cell (for CID) or from the surface (for SID) to the helium cell was 60 V. The helium cell DC voltage was 25 V. The IM cell traveling wave was set to a 200 m/s wave velocity and 18 V wave height. The pressure in the time-of-flight analyzer was 6.7×10^{-7} mbar. Mass spectra were acquired in the TDC mode.

RESULTS AND DISCUSSION

Extensive Dissociation Can Be Observed in SID of GroEL Tetradecamer. The +71 charge state produced by spraying from the ammonium acetate buffer for GroEL tetradecamer was selected by the quadrupole and activated with CID and SID, respectively (Figure 1). Major products from CID and SID are identified based on their measured mass (Table S1 of the Supporting Information). CID of the GroEL tetradecamer at an acceleration voltage of 160 V (Figure 1a) shows a strong signal for monomer ejection with monomer charge states centered around +30. The complementary tridecamers are also observed at high m/z with charge states centered around +43. The dissociation of GroEL into monomers and tridecamers in CID is consistent with previous findings in the literature.[17,27] At higher acceleration voltages in CID (up to 200 V within the limit of the instrument), dissociation into dodecamers can also be observed in the high m/z region, which can be attributed to the loss of monomers from the secondary dissociation of tridecamers.[17] The high-charge states of the monomers indicate that the monomers are unfolded.

In contrast, SID of the precursor at an acceleration of 180 V features extensive dissociation into a variety of products up to m/z 32000 (Figure 1b). The prevalent pathway is still the dissociation into monomers (red dots) and tridecamers (red rings), but with the monomers carrying less charge and the tridecamers carrying more charge than the ones in the CID spectrum. Additional SID products can be detected, such as dimers, trimers, undecamers, and dodecamers. At a lower acceleration voltage of 160 V in SID, monomers and tridecamers are still the dominant products in the spectrum but at lower abundances (Figure S1b of the Supporting Information).

It is noted that there are a few peaks with enhanced abundance across the SID spectrum, which are labeled with the number signs. They do not follow the regular continuous charge state distributions and show up at abnormally high intensities because they are the m/z that could potentially contain overlapping species with all possible oligomeric states (i.e., monomer, dimer with twice the charge, heptamer with 7 times the charge, etc.). The overlapping species can ideally be distinguished by the separation in drift time. However, many of the products do not appear as well-resolved spots in the IM−MS plot (Figure S2a of the Supporting Information). The broad drift time distribution, which is likely due to partial unfolding of the products, results in coalescence of the various oligomeric species in the drift time axis and makes it difficult to resolve possible overlapping oligomers with the same m/z.

Despite the low abundances and complexity from peak overlapping, the middle region of the SID spectrum (expanded view in Figure 1c) contains numerous products that are well-resolved in m/z. Tetramers, pentamers, hexamers, and heptamers are explicitly identified in this region with good accuracy (experimental mass listed in Table S1 of the Supporting Information). Some of the peaks are overlapping in this region and difficult to unambiguously assign, especially the hexamers which can overlap with all dimers and trimers. However, most of the heptamers have unique m/z that can be discriminated from other species. The heptamer peaks within the m/z range of 9800−14000 correspond to charge states from +41 to +29, which are centered at around half the charge of the tetradecamer precursor (+71). This indicates that the tetradecamer dissociates into heptamers in a symmetric manner, consistent with the native conformation of the complex which consists of two heptamer rings. The heptamer product ions are also present in higher abundance relative to the tetramer, pentamer, and hexamer ions observed in this

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region. This implies that aside from the ejection of small subunits (monomers, dimers), there is a tendency for GroEL to dissociate in a manner representative of the native conformation over these other pathways. It is noted that there are also some hexamers with charge states around +15 (m/z 21000−25000, cyan dots), products that presumably originate from secondary dissociation of heptamer in a highly asymmetric manner where the leaving monomer took half of the charge of the heptamer. Thus, the products reflecting the native conformation can further dissociate with excess internal energy under the experimental conditions. Despite the complexity of the SID spectrum and the unfolding of many of the product ions for the +71 GroEL precursor, it is apparent that the fast and energetic activation process in SID allows many more dissociation pathways to be accessed than the monomer ejection pathway prevalent in CID.

SID of Charge Reduced GroEL Tetradecamer Better Reflects Native Topology of the Protein Complex. Suppressed unfolding of protein complexes upon activation of reduced charge states has been observed in the literature.[21,35,39] Therefore, the GroEL tetradecamer was charge-reduced by the addition of trimethylammonium acetate (TEAA) into the spraying buffer, with the intention of better revealing native topology of the complex from dissociation data when unfolding is suppressed. The center of the charge state distribution of GroEL tetradecamers shifted from +71 to +50 after adding TEAA (m/z shifted from 11500 to 16000). CID of the +50 GroEL tetradecamer did not show any remarkable dissociation, and only charge stripping is observed (Figure 2a). Significant charge stripping occurs even without significant activation after quadrupole selection. The remarkable charge stripping behavior for the charge reduced GroEL (even more extensive in SID at lower acceleration voltages as shown in Figure S3 of the Supporting Information) may be attributed to the properties of buffer and salt adducts on the protein and remains to be examined. The unlabeled peaks at low abundances between the tetradecamer peaks in the CID spectrum are likely from the impurities in the sample. They are speculated to be truncated forms of the protein as they are observed even under low activation conditions (no dissociation) and have an experimental mass lower than the theoretical mass of GroEL. However, this species does not contribute significantly to the products in the other spectra, and it is not the emphasis of the current work.

Even though CID of the complex is suppressed at a reduced charge state, effective dissociation is still observed in SID of the +50 GroEL tetradecamer (Figure 2b). The labeled peaks are identified based on their experimental mass (Table S1 of the Supporting Information). Compared with the SID of the +71 tetradecamer, the monomer ejection with formation of the complementary tridecamer for the +50 precursor constitutes a much smaller portion of the products in the spectrum. The complementary dimer and dodecamer pair is detected at comparable abundance. More interestingly, the heptamers in the m/z range of 18000 to 21000 (+22 to +19, gray dots) that represent the native topology of the tetradecamer complex are also observed at similar intensities to other products, different from the SID spectrum of the +71 precursor in which the heptamers are only a minor species (Figure 1b).

Although some of the heptamers are overlapping with some residual tetradecamers in the SID spectrum (Figure 2b), they are identified in the highlighted region as distinct compact spots in the IM−MS plot (Figure 3a). The compactness of the

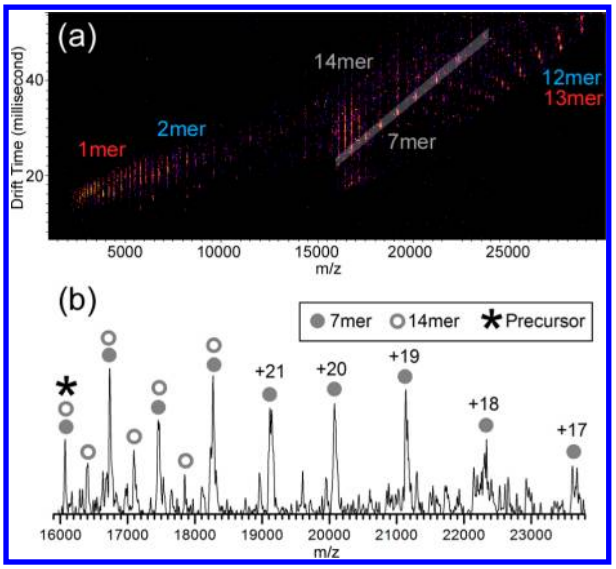

Figure 3. (a) IM−MS plot of the SID spectra for GroEL tetradecamer +50. The separation in drift time (vertical axis) assists in discrimination of species that are overlapping in m/z (horizontal axis). Distinct spots in the IM−MS plot indicate narrow distribution of the drift times, thus suggesting compact conformations of the ions. "Stretched lines" in the vertical axis indicate broader distributions of drift times, which suggest that ions at that m/z may have unfolded into extended conformations. The major dissociation products are labeled at their approximate location in the IM−MS plot. The middle region highlighted in the spectrum clearly shows a series of well-resolved spots corresponding to heptamers. (b) The extracted spectrum from the highlighted region showing the identified heptamer products from the +50 precursor, with significant removal of the interference from overlapping tetradecamer species.

spots in the drift time axis suggests that the heptamer product ions have compact conformations after their formation by SID of the tetradecamer, in contrast to the wide drift time distributions of the remaining undissociated tetradecamers (stretched vertical lines in the middle of the spectrum in Figure 3a), which are indicative of extended conformation due to unfolding. With the use of software provided by the instrument manufacturer, a tandem mass spectrum (Figure 3b) is reconstructed that corresponds to the highlighted region in Figure 3a. This allows better viewing of the heptamer products at a reduced level of overlapping species in m/z with reduced background noise. The presence of heptamers at low abundances in the high m/z region also becomes evident owing to the removal of interferences by IM separation after extraction. There are some remaining tetradecamers, especially at lower m/z (close to the precursor m/z), because of the broad drift time distribution of the tetradecamer that are "leaking" into the extracted spectrum. The fact that the heptamers are lying in a single slope in the IM−MS plot is reminiscent of our previous experience with SID products of smaller protein complexes.[20,21] The heptamers over a range of charge states presumably adopt the same compact conformation, thus their drift times only scale with charge. The extent of native conformation retained in the heptamer product, remains to be determined; it is possible that compact heptamers, void of unfolding, have further collapsed from their native conformations. On the basis of our previous observations[21] on SID of several other smaller complexes, subcomplexes released in SID sometimes collapse into more compact conformations than the prediction from crystal structures, presumably to a lower overall

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free energy. For SID in contrast to CID, it is clear that more than one product distribution exists (i.e., monomer/tridecamer, dimer/dodecamer, trimer/undecamer, and heptamer/heptamer); although unfolding is not entirely eliminated through SID, there is a clear increase in the relative abundance of compact heptamer products, especially from the charge-reduced precursor. This increase can be attributed to the suppressed unfolding and suppressed secondary dissociation at lower charge states, thus better revealing the products that are informative of the structure of the native protein complex.

Alternatively, the difference in dissociation pattern may arise from the difference in precursor conformation. There has been some evidence that the GroEL tetradecamer collapses in the gas phase at high charge states.[29,30,40] Our previous work has shown that SID spectra are sensitive to precursor conformation of the protein complexes, where the distorted and partially unfolded precursors result in SID spectra featuring more unfolded monomer products that are analogous to typical CID spectra.[13,24,41] Unfortunately, the current empirically derived methodology for determination of experimental CCS is prone to error if the sample is significantly beyond the mobility range of the calibrants.[42] Thus, it is difficult to compare the CCS of the GroEL precursor across a range of charge states to reveal if there is any subtle collapse in the conformation at higher charge states. Nonetheless, SID spectra of the charge-reduced GroEL tetradecamer, with the additional confirmation from IM separation, clearly show enhanced dissociation into heptamers that are the physiological relevant substructure of the large complex.

CONCLUSION

The data show that SID provides rich information for structural analysis of a large protein complex, GroEL tetradecamer, where CID does not cause sufficient dissociation into structural subunits. A variety of products that are not observed in CID can be detected in the SID spectra. In particular, the cleavage of GroEL tetradecamer into heptamers of significant abundance is more apparent when the precursor ion is charge reduced prior to SID. This enhancement of dissociation to heptamers is most likely due to the suppressed unfolding and suppressed secondary dissociation of heptamers at lower charge. The heptamer is a direct reflection of the native quaternary structure of GroEL tetradecamer, which consists of two stacked heptamer rings. The ability to dissect large protein assemblies to subcomplexes representative of the native topology suggests that SID is very useful and can provide complementary information for quaternary structural analysis of large protein complexes.

ASSOCIATED CONTENT

Supporting Information

SID spectra at different acceleration voltages, IM−MS spectra for CID/SID, and the experimental mass list as mentioned in the manuscript are included in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval of the final version of the manuscript. The initial research was performed when all coauthors held positions at the University of Arizona. The final data presented here were acquired at Ohio State University.

Notes

The authors declare no competing financial interest.

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