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Extended Gas-Phase Trapping Followed by Surface-Induced Dissociation of Noncovalent Protein Complexes

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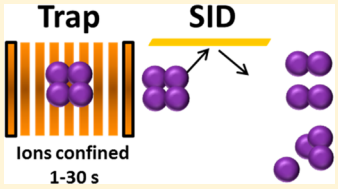
Supporting Information

ABSTRACT: Mass spectrometry has emerged as a useful tool in the study of proteins and protein complexes. It is of fundamental interest to explore how the structures of proteins and protein complexes are affected by the absence of solvent and how this alters with increasing time in the gas phase. Here we demonstrate that a range of protein and protein complexes can be confined within the Trap T-wave region of a modified Waters Synapt G2S instrument, including monomeric (β-lactoglobulin), dimeric (β-lactoglobulin and enolase), tetrameric (streptavidin, concanavalin A, and pyruvate kinase), and pentameric (C-reactive protein) complexes, ranging in size up to 237 kDa. We demonstrate that complexes can be confined within the Trap region for varying lengths of time over the range 1−60 s and with up to 86% trapping efficiency for 1 s trapping. Furthermore, using model systems, we show that these noncovalent complexes can also be fragmented by surface-induced dissociation (SID) following trapping. SID reveals similar dissociation patterns over all trapping times studied for unactivated protein complexes, suggesting that any conformational changes occurring over this time scale are insufficient to cause substantial differences in the SID spectra of these complexes. Intentional alteration of structure by cone activation produces a distinct SID spectrum, with the differences observed being conserved, in comparison to unactivated complex, after trapping. However, subtle differences in the SID spectra of the activated complex are also observed as a function of trapping time.



Mass spectrometry (MS) has emerged as a powerful tool for the structural and conformational analysis of proteins and protein complexes.[1] The development of "soft" ionization methods, such as electrospray ionization (ESI)[2,3] and nano-electrospray ionization (nano-ESI),[4,5] revolutionized the field, facilitating the transfer of proteins and intact protein complexes into the gas phase. These developments have enabled solution-phase topologies and even in vivo active structures to be probed.[6−8]

One of the advantages of mass spectrometry is the fast transmission time from ion production to detection, which can be on the microsecond time scale for quadrupole time-of-flight instruments. It is, however, often advantageous to extend the time ions spend in the gas phase prior to detection, to enable the utilization of activation methods such as electron-transfer dissociation (ETD) or ultraviolet photodissociation (UVPD) or to facilitate gas-phase chemical reactions.[9,10] The ability to extend the time protein ions spend in the gas phase can also increase fundamental understanding of the evolution of protein structure in the absence of solvent. This is particularly advantageous when coupled with other techniques, such as ion mobility (IM), which can clearly distinguish conformational changes occurring as a result of increased time in the gas phase.[11,12] In addition, fragmentation techniques such as electron-capture dissociation (ECD) have also been shown to be a probe for structural changes occurring as a result of increased trapping time.[13,14]

Bellina et al.[15] recently demonstrated that it was possible to trap protonated flavin mononucleotide and cytochrome *c* ions, individually, within the Transfer T-wave region of a modified Synapt G2S (Waters) mass spectrometer for 1 or 2 s, following which the ions could be irradiated with a UV laser for UV photodissociation studies. Furthermore, it has been demonstrated that the peptide substance P can be confined within the Transfer T-wave for up to 4 h, with minimal signal loss.[16]

Here we demonstrate that this approach can be extended to large protein complex ions, enabling ions to be confined within the Trap T-wave region of a Synapt G2S operated in time-of-flight (TOF) mode. Initial proof-of-concept experiments are reported, demonstrating that *m*/*z*-selected protein and protein complex ions, as large as 237 kDa, can be confined within the Trap region for up to 60 s. Furthermore, we demonstrate with an in-house-modified[17] instrument (Figure S1) that ions can be confined within a truncated Trap T-wave region, with high efficiency, following which they can be fragmented by surface-induced dissociation (SID). In this SID experiment, ions collide with a fluorocarbon-coated gold surface.[18] SID is a high-energy, fast deposition process.[19] SID can produce a wider range of product ions and more abundant substructural products reflective of the initial protein complex topology[20,21] in comparison to the commercially available collision-induced

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dissociation, which often proceeds via ejection of a highly charged monomers. Hence, SID is advantageous as a structural probe.

EXPERIMENTAL SECTION

β-lactoglobulin A, enolase, concanavalin A, and pyruvate kinase were all purchased from Sigma−Aldrich (St. Louis, MO). C-reactive protein was purchased from CalBioChem (EMD Biosciences, Inc., San Diego, CA) and streptavidin from Thermo Scientific Pierce Biotechnology (Rockford, IL). Protein samples were buffer-exchanged (Microspin 6, Bio-Rad, Hercules, CA) into 100 mM ammonium acetate and diluted to 10 μM complex concentration. For pyruvate kinase, 5% methanol was added to the sample solution to aid in spraying. For charge reduction, 100 mM triethylammonium acetate (Sigma−Aldrich, St. Louis, MO) at 20% by volume in 100 mM ammonium acetate was used; no pH adjustment of buffers was performed and all were approximately pH 6.8.

Nano-ESI MS analysis was conducted by utilizing a modified quadrupole ion mobility time-of-flight (Q-IM-TOF) instrument (Synapt G2S, Waters Corp., Manchester, U.K.) with a customized SID device installed before the IM chamber (SID-IM) as previously described.[17] The instrument was operated in TOF mode for all experiments; a cone voltage of 20−30 V was used, unless otherwise stated, for all studies; and a trap gas flow rate of between 1.5 and 2.7 mL/min (Table S1) was used in all studies (resulting in pressures between 6.65×10^{-3} and 1.09×10^{-2} mbar in the Trap T-wave region). Trap gas flow rate was tuned individually to maximize trapping efficiency for each complex. For MS studies, spectra were averaged over five pulses. For SID studies typical acquisition times were 6−60 min, depending on trapping times utilized and initial signal intensity. Twenty-minute acquisitions proved applicable for most systems.

RESULTS AND DISCUSSION

Initial experiments focused on confining a range of model proteins of increasing oligomeric order and molecular weight within the full-length Trap T-wave region. Individual protein samples were prepared and introduced into the mass spectrometer by nanoelectrospray ionization. The m/z of interest was selected with the quadrupole, and then that sample was confined within the Trap T-wave region by applying a sequence of direct current (dc) potentials to the exit lens. The sequence and automation of the potentials was controlled by an instrument control script in WREnS (Waters Research enabled software), similar to the script reported previously for trapping in the Transfer region.[15,16] All potentials were applied via the embedded power supply of the system. The trapping sequence consists of four steps (Figure S2). The first step is a beam check, during which potentials are set as in standard transmission mode, enabling ion intensity to be monitored. Following the beam check, the trap fill step occurs, during which a stopping voltage of between 10 and 15 V is applied to the exit of the trap cell, enabling ions to be accumulated in this region (Table S1). A fill time of 1−3 s was found to be optimal for all samples studied here, dependent on ion intensity, with longer fill times being used for lower intensity species. When the trap fill time has been reached, the script automatically turns off the source capillary voltage, stopping the ion beam. Following filling, the ions can be confined for a defined length of time; here we apply trapping times between 1 and 60 s. Throughout the trapping time the capillary source voltage is held at 0 V, stopping the spray of ions, and the exit is held at the raised potential, confining the ions. The final step is extraction, in which the potential applied to the Trap T-wave exit lens is lowered, enabling the trapped ions to be axially ejected and travel throughout the rest of the instrument before being detected in the TOF analyzer. This process enables extended trapping times in comparison to operating in ETD mode, which enables ions to be confined for only up to 1 s.[22]

Monomeric β-lactoglobulin (8+), dimeric β-lactoglobulin (13+), enolase (19+), streptavidin (15+), concanavalin A (21+), and pyruvate kinase (32+) were all individually successfully confined within the Trap T-wave region. A maximum trapping efficiency of 86% was determined for the 8+ charge state of monomeric β-lactoglobulin (Table S2). In general, trapping efficiency was observed to decrease with increasing molecular size (Table S2), and it was determined that an increase in the stopping potential (15 V for pyruvate kinase in comparison to 10 V for β-lactoglobulin) and Trap gas argon flow (2.5 mL/min for pyruvate kinase in comparison to 1.5 mL/min for β-lactoglobulin) was required for efficient trapping of higher charged and higher molecular weight species. A decrease in trapping efficiency is generally observed upon increasing trapping time for all systems studied here, which is also accompanied by a reduction in signal-to-noise upon increased trapping (Figure 1).

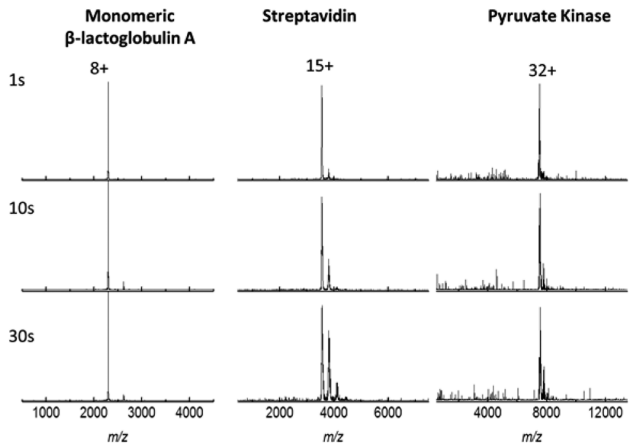

Figure 1. Spectra obtained following trapping for 1, 10, and 30 s. Spectra shown are combined over five extraction pulses.

Furthermore, for all proteins and protein complexes studied, charge stripping is observed following trapping, and increases with increasing trapping time (Figure 1 and Figure S3). Charge stripping was observed regardless of whether argon, helium, or xenon was used within the Trap T-wave region for trapping studies (data not shown), and we speculate that this may be a result of ion-neutral reactions occurring upon trapping.

In order to perform SID experiments, the instrument configuration was modified such that the full-length Trap T-wave was replaced with a truncated Trap with an SID device placed immediately after (Figure S1), as previously described.[17] In this configuration the trapped ions can be fragmented by SID and the dissociation spectra can be acquired. SID is advantageous for the study of protein complexes, as the dissociation spectra are reflective of the topology of the precursor. Furthermore, previous studies have shown that distinct fragmentation spectra can be obtained before and after

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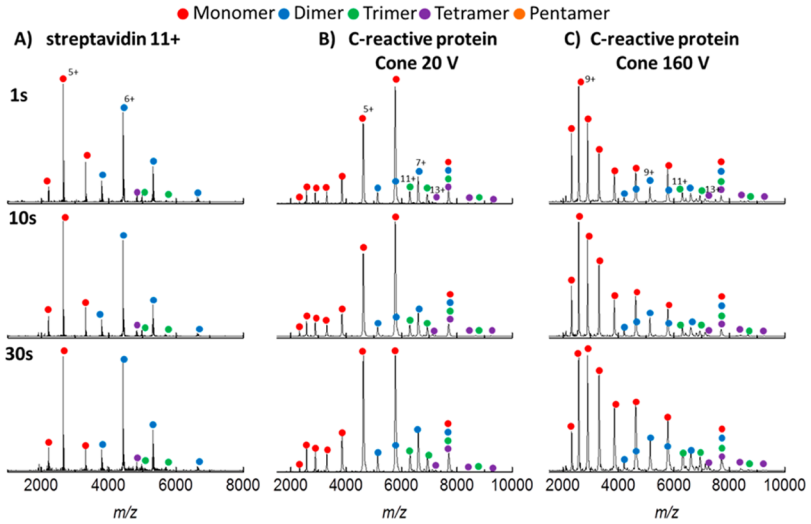

Figure 2. SID spectra obtained following trapping for 1, 10 or 30 seconds for A) 11+ Streptavidin at a collision energy of 550 eV (ΔV = 50, times 11 charges) and a cone voltage of 20 V, B) 18+ C-reactive protein (CRP) pentamer at a SID collision energy of 1440 eV with no cone activation (cone voltage 20 V) and C) 18+ C-reactive protein (CRP) pentamer at a SID collision energy of 1440 eV, using a high cone voltage (160 V) to activate the complex. In all cases a fill time of 1 second was used.

in-source activation of protein complexes, highlighting that SID can distinguish conformational changes of protein complexes.[23]

The first system under trapping SID study was the tetrameric protein complex streptavidin. In order to retain initial conformations more reflective of solution-phase topologies, samples were prepared with the charge reducing reagent triethylammonium acetate (TEAA), as reduced charge states of protein complexes are thought to adopt more nativelike conformations.[24] Under charge-reducing conditions, the 11+ charge state of streptavidin was chosen for further analysis. In the full-length trap, at this charge state, a trapping efficiency of 88% was obtained for 1 s trapping. With the truncated trap, it was found the ions could be trapped with similar efficiency (84% for 1 s trapping). Furthermore, it was observed that ions could be trapped for up to 30 s with less than 4% charge stripping (Figure S4).

Following trapping for between 1 and 30 s, the streptavidin ions were fragmented with SID at a collision energy of 550 eV (Figure 2A). Similar SID spectra were obtained for beam-type experiments (Figure S5A) and those involving trapping for 1−30 s, with strikingly similar distributions of intensities across all products (Table S3). In SID studies no "beam check" was performed in the trapping experiments; beam-type SID was acquired as a separate acquisition. Direct comparisons, however, cannot be made between beam-type and trapping spectra due to differences in the optics manipulation between these two acquisition modes. Discussion instead will focus on spectra obtained following trapping for different lengths of time. The similar SID fragmentation observed for 11+ streptavidin, even after trapping for 30 s, suggests that any global conformational changes occurring as a result of increased time spent in the gas phase are insufficient to cause a change in SID signature for streptavidin. In order to further probe this in the future, it would be of interest to perform ion mobility following trapping, to provide a direct measurement of conformational change as a result of time spent in the gas phase.

Next the pentameric protein complex C-reactive protein (CRP) was studied. CRP was chosen for further investigation as previous studies have demonstrated this protein can be activated by in-source activation. Furthermore, these conformational changes can result in strikingly different SID spectra.[23] CRP is, therefore, an ideal complex for study here. The 18+ charge state of CRP was confined within the truncated Trap T-wave region for between 1 and 30 s, and for all trapping times, charge stripping was limited to less than 10% (Figure S6). Following trapping, the ions were fragmented by SID, with a collision energy of 1440 eV (Figure 2B). SID energies for both CRP and streptavidin were chosen to minimize remaining precursor. Subtle differences are observed in the SID spectra for 18+ CRP following trapping for different lengths of time, with higher intensity monomer being observed following trapping for longer times and in comparison to beam-type studies (Table S4 and Figure S5B). Furthermore, upon trapping an increase in higher charged monomers (m/z 2000−3900) is observed in comparison to SID spectra obtained without trapping. In addition, the 5+ monomer is seen to increase in intensity relative to the 6+ monomer following trapping for increased times. It is interesting to note that the changes in SID spectra are much less dramatic than those observed upon cone activation, in which one activates the complex by using a higher cone voltage (Figure S5B,C). In-source activation has been shown to shift the collision cross section of the complex and cause a resulting change in the SID spectra.[23] Interestingly these differences in SID dissociation are preserved upon trapping (Figure 2C). If the complex is preactivated by cone activation and then trapped, the SID spectra following trapping are strikingly different from those of the unactivated precursor. Furthermore, the spectra for the activated complex change as a function of trapping time, with the monomer shifting toward lower charge states and a corresponding decrease in the relative intensity of the high charge state monomers. This shift to lower charge states could be indicative of a collapse or cooling of the ions upon trapping. The results, however, suggest that the conformational changes CRP undergoes upon increased time in the gas phase are less severe than those resulting from in-source activation, consistent with the significantly lower ΔV and lower pressure experienced in trapping as opposed to in-source activation.[23] SID was also performed post-trapping for 1, 10, and 30 s for dimeric β-lactoglobulin and tetrameric

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concanavalin A; in both cases the SID spectra following trapping for different times are similar, regardless of trapping time (Figures S7 and S8, and Tables S5 and S6). Coupling of trapping with ion mobility within this instrument would directly inform on changes in conformation through the measurement of collision cross sections, and future studies will explore this.

In summary, protein and protein complex ions as large as 237 kDa have been successfully confined within the Trap T-wave region of a modified commercial instrument. Ions can be fragmented by SID following trapping. Interestingly, the SID spectra appear similar following trapping over all time points studied here, with only subtle differences in the charge state distributions of monomeric products being observed for CRP and more obvious differences occurring following increased trapping for the activated CRP. The similar SID spectra suggest no gross structural changes are occurring over this time scale, and the conformational changes that do occur are insufficient to cause substantial differences in the SID of these complexes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b03479.

Eight figures and four tables with schematic of instrument and trapping procedure, additional spectra, calculated trapping efficiencies for trapping in the full-length trap, and SID product relative intensities (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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