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Protein Structure in the Gas Phase: The Influence of Side-Chain Microsolvation

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Supporting Information

ABSTRACT: There is ongoing debate about the extent to which protein structure is retained after transfer into the gas phase. Here, using ion-mobility spectrometry, we investigated the impact of side-chain–backbone interactions on the structure of gas-phase protein ions by noncovalent attachment of crown ethers (CEs). Our results indicate that in the absence of solvent, secondary interactions between charged lysine side chains and backbone carbonyls can significantly influence the structure of a protein. Once the charged residues are capped with CEs, certain charge states of the protein are found to undergo significant structural compaction.

Today electrospray ionization (ESI) is routinely applied to characterize proteins via mass spectrometry (MS).[1] There is an ongoing discussion about the extent to which native structural elements can be conserved in the gas phase. In recent years, studies have revealed that not only the protein's primary structure but also higher-order structural elements, up to the quaternary architecture of a protein complex, can be retained in the absence of solvent.[2] A subtle balance between attractive inter- and intramolecular forces, including salt bridges, van der Waals interactions, hydrogen bonds, and the repulsive Coulomb forces between like charges, governs the folding and three-dimensional organization of gas-phase proteins. In this context, one of the crucial determinants has been shown to be the charge state.[3] Low charge states can exhibit compact, globular structures that are governed by a hydrogen-bonding network within the molecule's backbone, whereas high charge states often form extended conformations that are largely dominated by Coulomb repulsion between the charged residues.[4] Intermediate charge states can show a multitude of coexisting conformations.

One method to investigate the overall structure of protein ions is ion-mobility spectrometry (IMS), where ions drift through a buffer gas under the influence of a weak electric field.[2c,5] The time required for ions of a particular mass and charge to traverse the drift cell is related to the orientationally averaged collision cross section (CCS). Therefore, coexisting structures with different CCSs can be spatially separated. Furthermore, the absolute value of the CCS yields direct information on the molecular shape. The conformations of the protein cytochrome *c*, for example, have been studied thoroughly in the gas phase using IM-MS.[3,6] Remaining questions concern the extent to which these gas-phase structures resemble conformations of the solvated protein and the underlying mechanisms leading to structural changes. It has been proposed that one of the crucial steps during transfer from solution to the gas phase is the collapse of charged side chains onto the backbone of the protein ion.[7] Here we investigated the influence of side-chain solvation and side-chain–backbone interactions on the protein's overall structure.

To analyze the impact of side-chain–backbone interactions on the gas-phase structure of proteins, we non-covalently attached different amounts of crown ether (CE) (18-crown-6, 264 Da) to cytochrome *c*. These CEs are known to coordinate strongly to protonated lysine side chains.[8] The proteins or protein–CE complexes were investigated via ion mobility mass spectrometry (IM-MS) using a Waters Synapt G2-S instrument in which ions drift through a buffer gas under the influence of an electric field having the form of a traveling wave.[9] Absolute CCS values for calibration of traveling-wave IMS data were determined using a drift-tube IM-MS apparatus constructed in-house following a design described elsewhere.[10]

A typical mass spectrum of cytochrome *c* with charge 7+ and varying CE content is shown in Figure 1A. Under mild source conditions, complexes with up to five CEs can be observed. In the case of 7+ ions, the mass of the utilized CE yields a shift of m/z 37.7 (=264/7) for each additional adduct. Figure 1B shows IMS arrival time distributions (ATDs) of cytochrome *c* with charges of 5+ to 10+ and varying numbers of CEs attached. To compare the charge states, the drift times were converted to CCSs (given in Å^2).[11] As has been observed previously,[3,12] the molecule's CCS increases with increasing charge, reflecting the unfolding of the compact, globular (G) structure at low charge (5+) into an extended unfolded (U) structure at high charge (10+). It is interesting to observe the effect of CE attachment on the CCS. For states with charges below 6+ (G) or above 9+ (U), the addition of CEs led to a slight increase in CCS. This observation was expected because of the additional mass of the CEs. However, counterintuitive effects were observed for CE complexes in intermediate (I) charge states (charges of 6+ to 9+), as can be seen exemplarily for 7+ and 8+ ions in Figure 1. Here, CE binding leads to dramatic decreases in size, with CCSs up to 30% smaller than those of the bare ions. This indicates significant compaction upon the addition of CEs. A complete overview of the data set for the 5+ to 14+ charge states is given in Figure S1 in the Supporting Information.

With a different instrument, conformational transitions as observed here have been shown to depend strongly on the timing parameters, such as the trapping time prior to the

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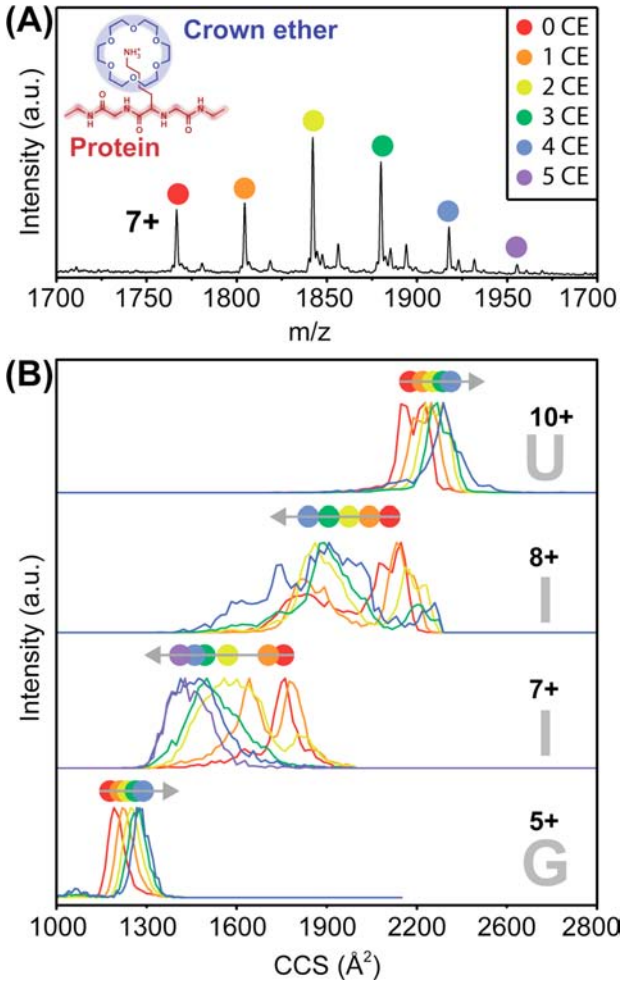

Figure 1. (A) Mass spectrum of crown ether (CE)-complexed cytochrome *c* with a charge of 7+. Complexes with up to five CEs could be observed under mild source conditions. (B) Estimated CCSs from IMS arrival time distributions (ATDs) of CE-complexed cytochrome *c* molecules in charge states corresponding to unfolded (U), intermediate (I), and globular (G) structures. The numbers of CEs non-covalently bound to the molecule are indicated by the color code given in (A). The arrows indicate increasing mass.

injection of the molecules into the drift cell.[6a] In addition, in Synapt instruments, the ion temperature is known to increase significantly during injection into the drift cell.[13] The compaction effect described here for protein–CE complexes was observed with both the traveling-wave and drift-tube IMS instruments, and the ATDs showed no dependence on device timing and IMS injection voltage within the accessible range.

To gain more information on the CE-induced compaction process, tandem IM-MS experiments were carried out on CE-complexed cytochrome *c* ions. A high-pressure collision cell prior to the IMS cell allowed fragmentation of *m*/*z*-selected protein–CE complexes via collision-induced dissociation (CID). Subsequent structural changes of the molecule and its fragments could be observed by IMS analysis. Mass spectra of CE-complexed cytochrome *c* in the 7+ charge state at three different injection energies are shown in Figure 2. Starting with a protein containing four CEs, lower-order complexes could be produced as fragments by increasing the injection voltage (Figure 2, top to bottom). Figure 3 shows ATDs of the resulting complexes for the 5+, 7+, and 10+ charge states. Ions

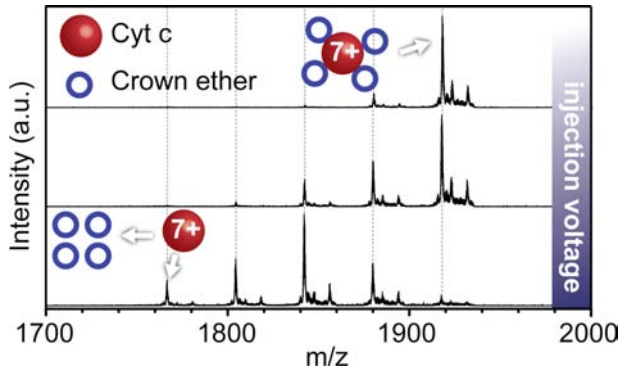

Figure 2. Tandem MS spectra of the 7+ cytochrome *c*–4CE complex at different injection voltages. Increasing the injection voltage (top, 2 V; middle, 8 V; bottom, 16 V) created all of the lower-order complexes.

featuring globular (G, 5+ and lower) or predominantly unfolded conformations (U, 10+ and higher) underwent no significant structural changes when CE was removed from the complex (Figure 3 A,C). However, CE removal from intermediate charge states (6+ to 9+) induced partial unfolding of the molecule, leading to longer drift times and larger CCSs (Figure 3 B). Independent of the charge state, the ATDs obtained after CID were virtually identical to those measured for ions produced directly via ESI (Figure 3, red dashed lines). This indicates that the CID-generated extended conformations observed for the intermediate charge states result from the loss of CE molecules rather than from the energy deposited into the complex during CID.

The results observed here indicate that the local environment of charged side chains in the gas phase can severely influence the structure. What can give rise to the observed CE-induced compaction? The addition of CE does not alter the overall charge, and a slight change in the positions of the CE-complexed side chains or the associated changes in local dielectric environment should not significantly affect the overall role of Coulomb repulsion. A more important factor that could have an influence on the structure is the alteration of the interactions between the protonated basic side chains and the protein backbone. In a condensed-phase protein, the charged side chains are involved in salt bridges, coordinate to carbonyl groups of the backbone, or interact with solvent molecules. In the absence of a solvent environment, the side chains will take part in intramolecular interactions and can coordinate to the backbone carbonyl groups, which would then no longer be available to be involved in potentially structure-stabilizing hydrogen bonds. The added CE can take over the role of the solvent, with the result that the side-chain–backbone interaction is reduced. This effect is schematically indicated in Figure 4, where the charged protein is depicted as a cartoon. Secondary structure elements such as β-sheets and α-helices are depicted in red, unordered structure is depicted as gray strands, and CEs are represented by blue rings. The solvation shell of the molecule is represented as a light-blue sphere. The upper part of the figure shows the stepwise evolution of the protein on its way to the gas phase after ionization via ESI as suggested previously.[7] After evaporation of the solvent, the charged side-chain residues collapse onto the molecule's peptidic backbone, forming hydrogen bonds with backbone carbonyls or creating salt bridges with anionic residues. As a result, hydrogen bonds within the protein's backbone can be disrupted. The

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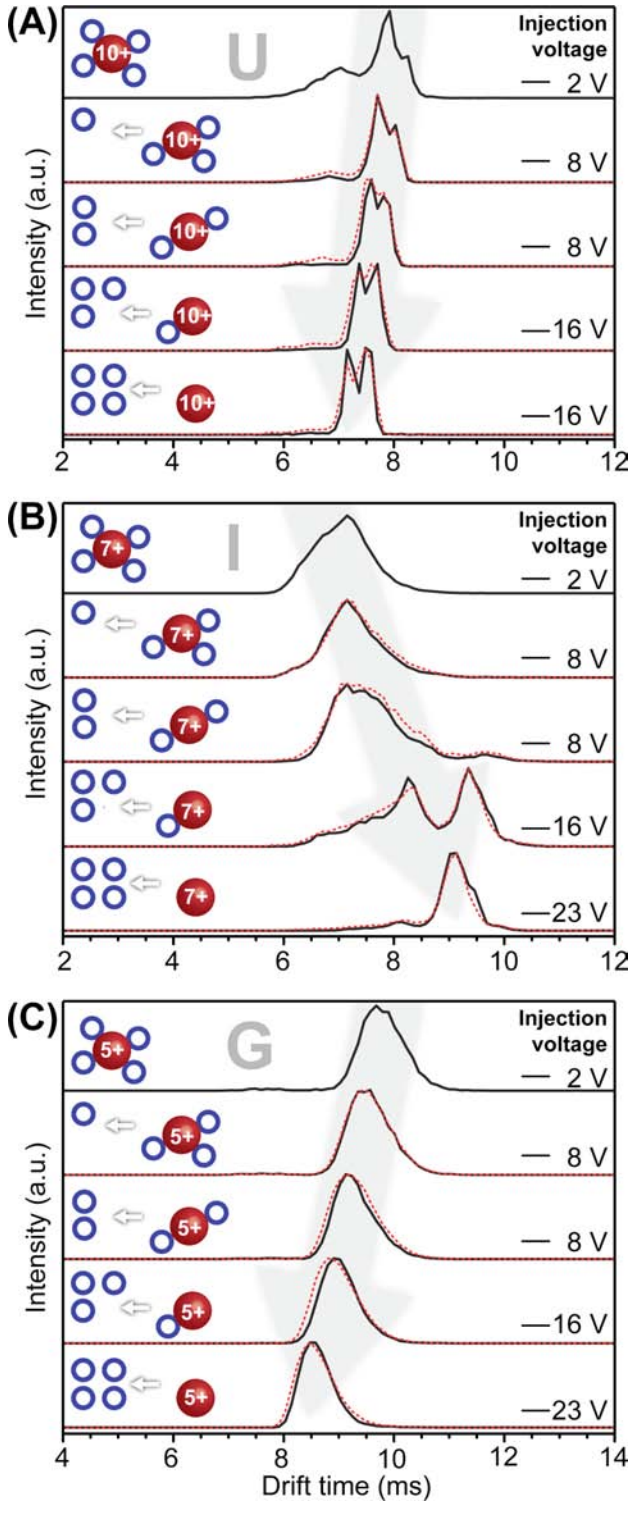

Figure 3. Structural changes upon CE removal. (A, C) No significant structural changes were observed when CE was removed from low-charge G or high-charge U cytochrome *c* ions. (B) Cleavage from intermediate charge states induced partial unfolding of the molecule, resulting in an increase in arrival time. Regardless of charge, no difference between the CID-generated CE complexes and those generated directly via ESI (red dashed lines; 2 V injection voltage) was observed.

disappearance of these structure-defining bonds can lead to destabilization of secondary and tertiary structure elements, resulting in structural changes in the protein. This situation is

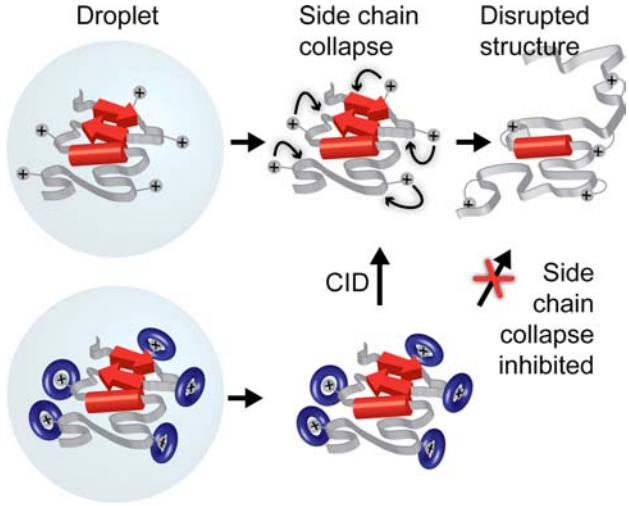

Figure 4. Effect of CE side-chain microsolvation on the molecule's structure. Secondary structure elements are shown in red, unordered structure is shown in gray, solvent is shown in light blue, and CEs are shown as blue rings. Collapse of the side chain after evaporation of the solvent is inhibited when the side chain is capped with a CE. See the discussion in the text.

illustrated in the upper-right corner of Figure 4. However, when the charged side chains are capped with CE, the collapse after evaporation of the solvent is inhibited, leaving secondary structure elements intact (Figure 4, lower-right). As a consequence, a compact and presumably more native-like structure is observed for CE-complexed proteins in intermediate charge states.

Further support for the proposed mechanism comes from a previous study on the much smaller peptide gramicidin S.[14] With a combination of gas-phase IR spectroscopy and theory, it was found that the charged NH_3^+ groups of the two Orn residues[15] strongly interact with the peptide backbone, leading to a disruption of intramolecular β-sheet-like hydrogen bonds. In contrast, when the side-chain–backbone interaction is inhibited through CE complexation, the hydrogen-bonding pattern that governs the secondary structure remains essentially unaffected. In addition, IM-MS experiments on other CE-complexed proteins such as the β-sheet-rich protein ubiquitin (8.5 kDa) and the non-covalent protein–ligand complex myoglobin (17 kDa) revealed compaction of intermediate charge states similar to that found for cytochrome *c*. This finding suggests that the observed effect is general and not limited to cytochrome *c*.

In this study, we have demonstrated that the gas-phase structure of cytochrome *c* (and other proteins) can be drastically altered by the non-covalent attachment of CE molecules. On the basis of our results, we postulate that interactions between protonated lysine side chains and backbone carbonyls are in direct competition with the intramolecular hydrogen bonding that determines the protein's secondary and tertiary organization. Non-covalent attachment of CEs to the charged side chains of the protein can compensate for these effects by solvating the positively charged ionic groups in a similar way to solvent molecules in the condensed phase. In a more general context, this implies that side-chain microsolvation by CEs can be used as a tool to manipulate and tune protein structures in the gas phase.

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■ ASSOCIATED CONTENT

Supporting Information

Experimental details and IMS data for CE-complexed cytochrome *c* in the 5+ to 14+ charge states. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(15) Ornithine (ORN) is a nonproteogenic amino acid whose side chain is similar to that of lysine but shortened by one methylene group.