

Stabilization of Compact Protein Structures by Macrocyclic Hosts Cucurbit[*n*]urils in the Gas Phase

Jong Wha Lee,^{1,2,*} Mi Hyun Park,³ Jeong Tae Ju,³ Yun Seop Choi,³ Soo Min Hwang,³
Dong Jin Jung,³ and Hugh I. Kim^{2,*}

¹Department of Chemistry, Pohang University of Science and Technology (POSTECH), Pohang 37637, Republic of Korea

²Department of Chemistry, Korea University, Seoul 02841, Republic of Korea

³Kyeongbuk Science High School, Pohang 37646, Republic of Korea

Received December 19, 2015; Revised January 23, 2016; Accepted January 26, 2016

First published on the web March 31, 2016; DOI: 10.5478/MSL.2016.7.1.16

Abstract : Characterization of intact protein structures in the gas phase using electrospray ionization combined with ion mobility mass spectrometry has become an important tool of research. However, the biophysical properties that govern the structures of protein ions in the gas phase remain to be understood. Here, we investigated the impact of host-guest complexation of ubiquitin (Ubq) with macrocyclic host molecules, cucurbit[*n*]urils (CB[*n*]s, *n* = 6, 7), on its structure in the gas phase. We found that CB[*n*] complexation induces the formation of compact Ubq ions. Both CB[6] and CB[7] exhibited similar effects despite differences in their binding properties in solution. In addition, CB[*n*] attachment prevented Ubq from unfolding by collisional activation. Based on the experimental results, we suggest that CB[*n*]s prevent unfolding of Ubq during transfer to the gas phase to promote the formation of compact protein ions. Furthermore, interaction with positively charged residues *per se* is suggested to be the most important factor for the host-guest complexation effect.

Keywords : ion mobility mass spectrometry, cucurbit[*n*]uril, ubiquitin, host-guest chemistry

Introduction

The advancement of electrospray ionization (ESI) in combination with ion mobility mass spectrometry (IM-MS) enabled characterization of protein tertiary and quaternary structures using MS.^{1,2} In addition to mass-to-charge ratio (*m/z*), IM-MS provides collision cross-section (Ω_D) of an ion, which is related to the size and shape of the ion. Recent studies utilizing ESI-IM-MS have provided important insights on the structural dynamics of proteins and protein complexes.^{1,2} Limited understanding, however, is available for the biophysical properties that govern the structure of protein ions in the gas phase. Previous reports showed that structural rearrangements of protein ions can occur to some extent in the gas phase, including

rearrangements of the side chains to form salt-bridges and hydrogen bonds.³ Therefore, for assessment of protein structures in solution using MS, it is important to understand biophysical properties of protein ions and prevent their structural rearrangements in the gas phase.

Host-guest chemistry provides a unique means to investigate structural changes of molecules in response to specific interactions. A recent study by Pagel and coworkers showed that noncovalent attachment of a host molecule, 18-crown-6 (18C6), to protein ions caused their structural compaction.⁴ This observation was explained by microsolvation of the charged lysine side chains by 18C6 preventing their structural rearrangements in the gas phase. However, underlying mechanism for the observation has not been characterized in detail. Understanding structural changes of protein ions induced by specific interactions would provide deeper insights into the structural properties of protein ions in the gas phase.

Here, we investigated the influence of host-guest complexation on the structure of ubiquitin (Ubq) ions in the gas phase. Cucurbit[*n*]urils (CB[*n*]s, Figure 1) are macrocyclic host molecules comprised of *n* glycoluril groups.⁵ A CB[*n*] molecule has two positively charged portals for interaction with positively charged groups, and a hydrophobic cavity to accommodate hydrophobic functional groups.⁶ Among the CB[*n*] family, we utilized CB[6] and CB[7], which selectively bind to lysine^{7,8} and

Open Access

*Reprint requests to Jong Wha Lee and Hugh I. Kim

E-mail: jongwha12@postech.edu and hughkim@korea.ac.kr

All MS Letters content is Open Access, meaning it is accessible online to everyone, without fee and authors' permission. All MS Letters content is published and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0/>). Under this license, authors reserve the copyright for their content; however, they permit anyone to unrestrictedly use, distribute, and reproduce the content in any medium as far as the original authors and source are cited. For any reuse, redistribution, or reproduction of a work, users must clarify the license terms under which the work was produced.

phenylalanine⁹ residues of proteins, respectively. By investigating structural impact of the two hosts with different binding properties, the biophysical properties that influence protein structures in the gas phase can be studied. We generated complex ions of Ubq using ESI, and probed their structures in the gas phase using IM-MS. Ω_D values from the IM measurements provide comprehensive understanding on the influence of host-guest complexation on protein structures in the gas phase.

Experimental

Materials and sample preparation

Ubq from bovine erythrocytes, equine cytochrome c (Cyt c), equine myoglobin (Myb), CB[6], CB[7], and formic acid were purchased from Sigma-Aldrich (Saint Louis, MO, USA). HPLC-grade water and acetonitrile were purchased from Avantor Performance Materials, Inc. (Center Valley, PA, USA) and used as solvents. Protein solutions were prepared as 10 μ M, and CB[6] or CB[7] was added to be 100 μ M concentration.

ESI-IM-MS

ESI-IM-MS experiments were performed with a Synapt G2 HDMS quadrupole travelling wave ion mobility orthogonal time-of-flight mass spectrometer equipped with a Z-spray ionization source (Waters, Milford, MA, USA). The capillary, sampling cone, and extraction cone voltages were set as 2.00 kV, 40 V, and 2 V, respectively. The helium cell and drift cell gas flows were 180 mL min⁻¹ and 30 mL min⁻¹, respectively, with wave voltage and height of 450 m s⁻¹ and 12 V, respectively. Calibration of measured arrival times from IM experiments into Ω_D values was performed by measuring arrival times of Ubq, Cyt c, and Myb and creating a calibration curve using their previously reported Ω_D values.¹⁰ Collisional activation experiments were performed in the trap cell of the Synapt G2 instrument with a gas (argon) flow of 2.0 mL min⁻¹.

Results and Discussion

Structures of Ubq and its complex ions

Figure 2 shows that Ubq ions with various charge states are formed upon ESI of a Ubq solution. With addition of

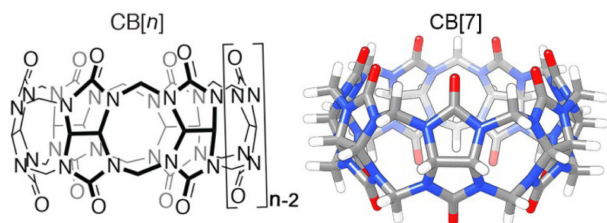


Figure 1. Schematic structure of CB[n] and three-dimensional structure of CB[7].

CB[6] or CB[7] to a Ubq solution, Ubq ions complexed with CB[n] molecules were generated. Up to five CB[6] molecules or four CB[7] molecules were observed to be bound to a Ubq ion, and the charge states of the complex ions spanned from 6+ to 13+. The MS experiments were performed using a denaturing solution (50/50 water/acetonitrile solution with 1% formic acid) for effective complexation of Ubq with CB[n]s. Although denaturation of a protein in solution may influence its structural distributions in the gas phase,¹¹ small differences in the Ω_D distributions of Ubq and its complexes were observed depending on solution conditions (data not shown).

Ω_D values of both Ubq and its complex ions generally increased with increasing charge states (Figure 3). This trend is well known, and can be attributed to strong electrostatic repulsion between positively charged sites resulting in expansion of highly charged ions.¹² On the other hand, comparison between uncomplexed and complexed ions of identical charge state reveals previously unreported trend in their structures. Three trends in Ω_D distributions of the ions were observed depending on their charge states, which are: 1) decrease in Ω_D (low charge states), 2) increase in Ω_D , followed by decrease in Ω_D with further CB[n] attachments (intermediate charge states), and 3) increase in Ω_D (high charge states).

18C6 complexation has previously been shown to cause decreased Ω_D values of protein ions.⁴ Still, Ω_D decrease by CB[n]s is surprising because CB[n]s (c.a. 1 kDa) are much

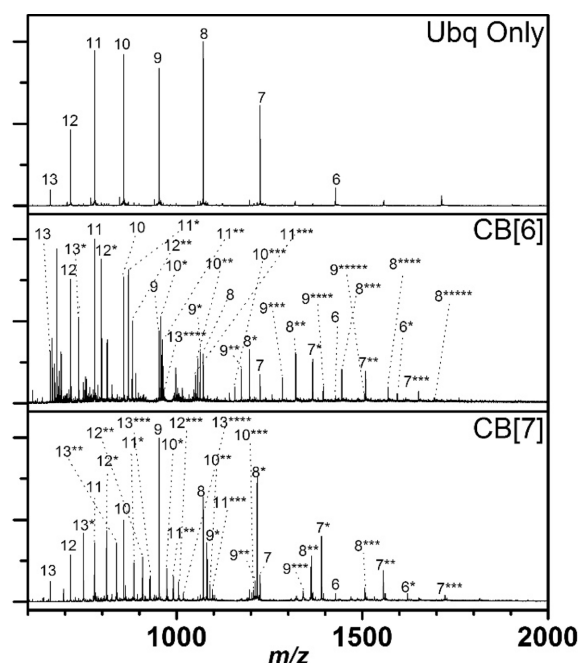


Figure 2. Mass spectra of Ubq (10 μ M), and Ubq with CB[6] (100 μ M) or CB[7] (100 μ M) in 50/50 water/acetonitrile solution with 1% formic acid (positive ion mode). Asterisks (*) denote the number of CB[n]s on the Ubq ions.

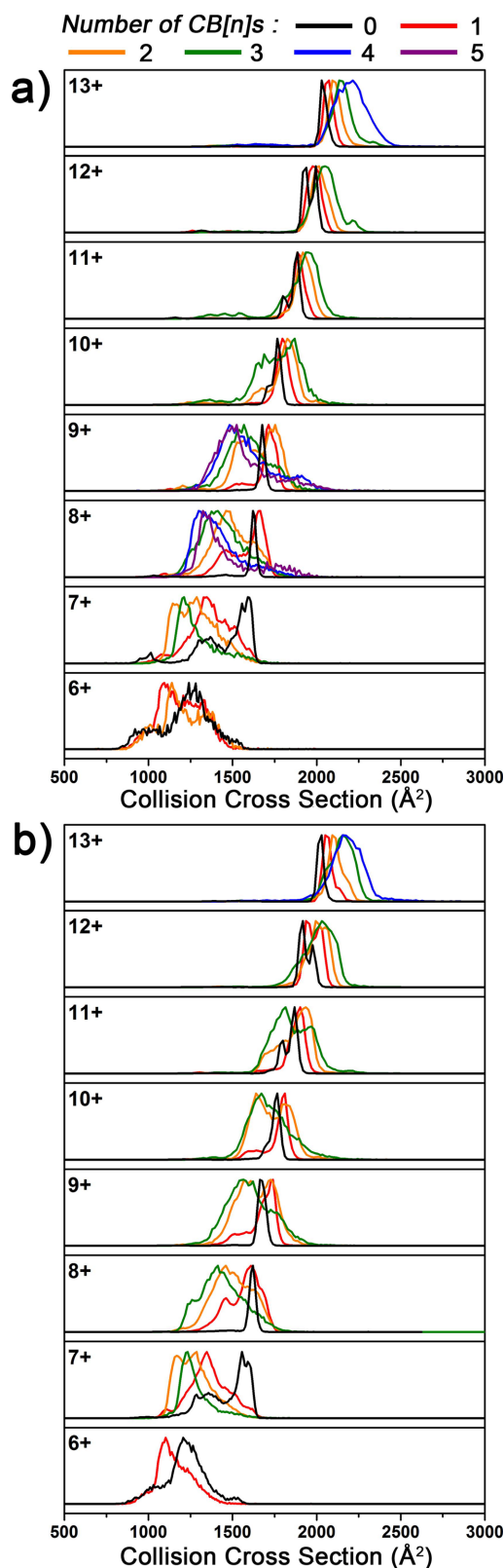


Figure 3. Ion mobility spectra of Ubq and its CB[*n*] complex ions from 50/50 water/acetonitrile solution with 1% formic acid: a) with CB[6], and b) with CB[7].

larger than 18C6 (c.a. 0.3 kDa), making it possible that compaction caused by CB[*n*]s is not sufficient to counteract the size increase due to their attachment. Despite this possibility, Ω_D decreased for low charge state ions (Figure 3), which suggests that size increase due to CB[*n*] attachment is overwhelmed by innate properties of the hosts to promote the formation of compact protein ions. On the other hand, CB[*n*] complexation increased the Ω_D values of intermediate or high charge state ions, indicating that CB[*n*]s are unable to promote compaction of protein ions with high charge density. Interestingly, multiple CB[*n*] complexation can cooperate to promote the formation of compact Ubq ions with intermediate charge states. For example, despite the 60% increase in mass caused by the complexation, 8+ and 9+ charged Ubq ions with five CB[6] molecules have Ω_D values that are smaller than that of uncomplexed Ubq ions. These results suggest that CB[*n*]s are highly efficient for promoting the formation of compact protein ions.

Then, the structures of CB[6] and CB[7] complex ions were compared (Figure 3). It was observed that the structures of CB[6] and CB[7] complex ions are similar. Especially, the structures of 6+ to 9+ charged complexes are highly similar. This result implies that CB[6] and CB[7] share the most important factor driving the generation of compact complex ions. The capability to interact strongly with positively charged groups is the most probable candidate, because CB[6], CB[7], and 18C6 all share this property. Although CB[7] preferentially interacts with hydrophobic guests in solution, the binding property of CB[7] was reported to be altered during transfer to the gas phase to interact strongly with positively charged guests.¹³ In addition, even if CB[7] is bound to a hydrophobic guest through one of its two portals, the other portal can accommodate another positively charged group simultaneously.^{13,14}

Some differences were observed between CB[6] and CB[7] complex ions at higher charge states (Figure 3). The Ω_D values of 10+ to 12+ charged ions with three CB[7] attachments are smaller than those with three CB[6] attachments, suggesting that CB[7] is more effective in stabilizing compact structures of protein ions than CB[6], despite its larger size. A possible cause is the simultaneous interactions of CB[7] with multiple residues, as discussed above, which would allow CB[7] to further promote the generation of more compact Ubq ions.¹⁴

Collisional activation of Ubq ions

Noncovalent interactions between CB[*n*]s and their guests can be sufficiently strong to endure dissociation upon collisional activation.^{7,13,15} Thus, it is possible to monitor structural transitions of host-guest complexes in the gas phase caused by collisional activation. Figure 4 shows that Ubq complex ions unfold when collisionally activated. It was observed that ions with higher numbers of

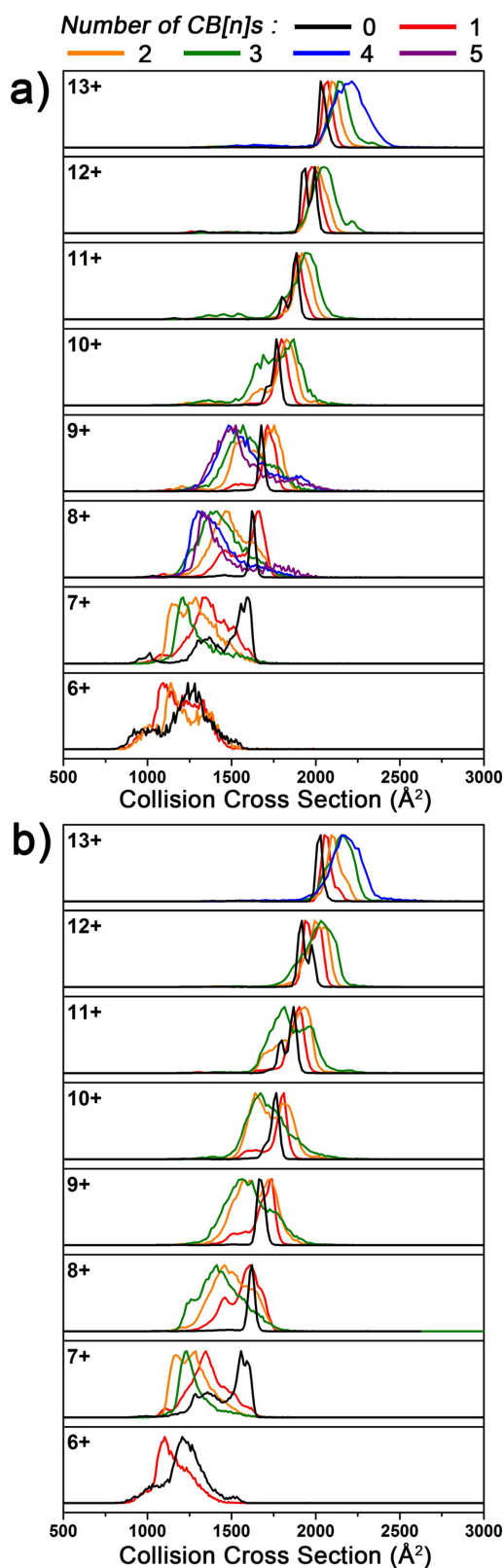


Figure 4. Ion mobility spectra of Ubq and its CB[*n*] complex ions after applying 20 or 40 V of collision energy (CE): a) CB[6], b) CB[7].

CB[*n*]s were less prone to unfolding by collisional activation. For example, 8+ Ubq ions with three and four CB[*n*] attachments unfold incompletely at 20 V of collision energy (lab frame), whereas Ubq ions with two or less CB[*n*] attachments unfold almost completely at the collision energy. These experimental results indicate that CB[*n*]s can stabilize Ubq ions against unfolding by collisional activation. On the other hand, when 8+ ions were unfolded using sufficient energy (40 V), the Ω_D values of the complexed ions with more CB[*n*] attachments generally exhibited greater Ω_D values than complex ions with fewer CB[*n*] attachments. This suggests that CB[*n*]s cannot promote gas-phase compaction of significantly unfolded ions, even if the ions are of low or intermediate charge states. Similar results were observed for ions with different charge states (data not shown).

Mechanism of compact Ubq ion formation by CB[*n*]s

It is important to consider the stage during ionization and MS analysis at which CB[*n*]s most critically affect Ubq conformations. While CB[*n*] complexation can affect protein structures in solution,^{7,15} we observed that the Ω_D distributions of Ubq complex ions were largely independent on solution conformation of Ubq. Additionally, it was observed that CB[*n*]s cannot refold ions that have undergone significant unfolding in the gas phase (Figure 4). These results, combined with our collisional activation studies, imply that CB[*n*]s prevent unfolding of Ubq ions during transfer to the gas phase, rather than directly inducing compaction in solution or in the gas phase.

A possible reason that CB[*n*]s prevent unfolding of Ubq is due to the mass increase by their attachments. One of the most prominent factors that drive protein unfolding during transfer to the gas phase is the increase in effective ion temperature by acceleration under an electric field. Mass increase also increases the vibrational degrees of motion, resulting in reduced effective ion temperature. However, mass increase appears to be a minor factor because 18C6 also generates compact ions despite its small mass.⁴ Likewise, Williams and coworkers showed that small anions such as perchlorate (ClO_4^-), iodide (I^-), and sulfate (SO_4^{2-}) can cause the formation of compact protein ions.¹⁶ Thus, stabilization of compact protein structures can occur without significant contribution from the increase in mass.

Most reasonable explanation is that the interaction with positively charged sites is critical for the structural compaction, because this is the only common factor between CB[*n*]s, 18C6, and small anions. Similar effects provided by these three different types of molecules further suggest that specific binding sites are unimportant for the effects, and presence of the interactions *per se* is most important. Weak dependence of our experimental results on solution conditions support that CB[*n*]s exert a general impact on protein structure, rather than inducing local changes by

binding to specific sites. Therefore, our experimental results demonstrate that host-guest complexation of CB[*n*]s generates compact Ubq ions by their binding to positively charged sites, which stabilizes the positive charges and reduce electrostatic repulsion to prevent elongation of the protein.

Conclusions

We have studied the gas-phase structures of host-guest complex ions comprising a protein and macrocyclic hosts. It was found that CB[*n*]s can promote the formation of compact Ubq ions by interacting with positively charged residues, demonstrating the dominant role of positively charged residues on protein structures in the gas phase. Additionally, our study implies that solvation of positively charged sites using host molecules can be an effective method to study structural properties of protein ions in the gas phase, and to aid the transfer of proteins to the gas phase with their compact structures retained.

Acknowledgments

This work was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare of Korea (grant no. HT13C-0011-040013), a Basic Research Program (No. 2013R1A1A2008974) through the National Research Foundation (NRF) of Korea funded by the Ministry of Science, ICT, and Future Planning (MSIP), and a Basic Science Research Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Education (No. 20100020209).

References

1. Uetrecht, C.; Rose, R. J.; van Duijn, E.; Lorenzen, K.; Heck, A. J. R. *Chem. Soc. Rev.* **2010**, 39, 1633.
2. Lanucara, F.; Holman, S. W.; Gray, C. J.; Eyers, C. E. *Nat Chem* **2014**, 6, 281.
3. Breuker, K.; McLafferty, F. W. *Proc. Nat. Acad. Sci. USA* **2008**, 105, 18145.
4. Warnke, S.; von Helden, G.; Pagel, K. J. *Am. Chem. Soc.* **2013**, 135, 1177.
5. Assaf, K. I.; Nau, W. M. *Chem. Soc. Rev.* **2015**, 44, 394.
6. Dearden, D. V.; Ferrell, T. A.; Asplund, M. C.; Zilch, L. W.; Julian, R. R.; Jarrold, M. F. *J. Phys. Chem. A* **2009**, 113, 989.
7. Heo, S. W.; Choi, T. S.; Park, K. M.; Ko, Y. H.; Kim, S. B.; Kim, K.; Kim, H. I. *Anal. Chem.* **2011**, 83, 7916.
8. Zhang, H.; Grabenauer, M.; Bowers, M. T.; Dearden, D. V. *J. Phys. Chem. A* **2009**, 113, 1508.
9. Logsdon, L. A.; Urbach, A. R. *J. Am. Chem. Soc.* **2013**, 135, 11414.
10. Ruotolo, B. T.; Benesch, J. L. P.; Sandercock, A. M.; Hyung, S.-J.; Robinson, C. V. *Nat. Protoc.* **2008**, 3, 1139.
11. Shi, H.; Pierson, N. A.; Valentine, S. J.; Clemmer, D. E. *J. Phys. Chem. B* **2012**, 116, 3344.
12. Shelimov, K. B.; Jarrold, M. F. *J. Am. Chem. Soc.* **1997**, 119, 2987.
13. Lee, J. W.; Lee, H. H. L.; Ko, Y. H.; Kim, K.; Kim, H. I. *J. Phys. Chem. B* **2015**, 119, 4628.
14. Lee, J. W.; Shin, M. H.; Mobley, W.; Urbach, A. R.; Kim, H. I. *J. Am. Chem. Soc.* **2015**, 137, 15322.
15. Lee, J. W.; Heo, S. W.; Lee, S. J. C.; Ko, J. Y.; Kim, H.; Kim, H. I. *J. Am. Soc. Mass Spectrom.* **2013**, 24, 21.
16. Merenbloom, S.; Flick, T.; Daly, M.; Williams, E. J. *Am. Soc. Mass Spectrom.* **2011**, 22, 1978.