Salt bridge structure of microhydrated arginine kinetically trapped in the gas phase by evaporative cooling.

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ABSTRACT

Amino acids and peptides generally exhibit zwitterion form with salt bridge (SB) structures in solution but charge solvated (CS) motifs in the gas phase. Here we report a study of non-covalent complexes of protonated amino acid arginine, $ArgH^+(H_2O)_n$ (n = 1-5), produced in the gas phase from aqueous solution with a controlled number of retained water molecules. These complexes were probed by cold ion spectroscopy and treated by quantum chemistry. The spectroscopic changes induced upon gradual dehydration of arginine were assigned by structural calculations to the transition from SB to CS geometries. SB conformers appear to be present for the complexes with as few as 3 retained waters, although energetically CS structures should become prevailing already for $ArgH^+$ with 7-8 water molecules. We attribute the revealed kinetic trapping of arginine in native-like zwitterion forms to evaporative cooling of the hydrated complexes to as low as below 200 K.

INTRODUCTION

Water is one of the few molecules that are vital to our life. The myriads of water functions range from such large-scale ones as stabilizing the globe temperature by oceans to the molecular scale of biology, where water serves as the universal solvent, supports cellular structure, etc. The amazing ability of H_2O to form intermolecular H-bonds makes it an essential part of many types of biomolecules and thus regulates their structure and, at the end, biological functionality. Understanding the structure-function relations remains essential in many fields of life science, but the complexity of the, eventually, endless H-bond network of waters often makes the determination of accurate structures of biomolecules in bulk aqueous solutions challenging even with the time-proven methods like NMR. Although spectroscopy in conjunction with quantum chemistry computations may provide accurate geometries of small to midsize biomolecules isolated in the gas phase,[1–5] these intrinsic structures can substantially differ from the native solution ones, which are truly biologically relevant.

A clear example of how drastically water may conform biomolecules is a comparison of structures of amino acids and peptides in aqueous solution and in the gas phase. In aqueous solutions with neutral or slightly lower pH, these biomolecules adopt zwitterion form with a salt bridge (SB) bond between the protonated N- and deprotonated C-termini. In the gas phase, the C-terminus appears neutral due to the water-mediated N- to C-terminus proton transfer, which happens when the remaining waters cannot anymore stabilize the SB structure. Detection of this critical level of hydration can be performed with charged microhydrated biomolecules in the gas phase, where one can benefit from the finite size of such molecular complexes. Extensive work has been done to investigate the effect of metal cations for stabilization of SB structures of bare amino acids,[6–10] as well as of amino acids solvated by a single water molecule.[11–13] Stabilization of SB structures by microhydration of natively protonated amino acids has been studied on a few occasions theoretically,[14–18] but only little experimentally.[19,20] It was shown that the zwitterion form of the studied amino acids can be stabilized in the gas phase by complexation with metal cations and/or water molecules. Arginine is one of the amino acids that exhibits protonation of its side chain under native conditions in aqueous solution and in the gas phase. This enables mass spectrometry-based

tools for studying SB to charge solvated (CS) transition in microhydrated $ArgH^+$. Theoretical studies suggest that CS structures of protonated arginine solvated by up to 7 water molecules remain energetically more favorable than the SB configurations; further hydration gradually makes SB structures more favorable.[14] Recent cryogenic ion mobility (IM) studies challenge however this suggestion. The experiment detected an essential structural difference for $ArgH^+$ in its complexes with three and with four (and more) water molecules.[21] The difference was tentatively attributed to the transition from CS to SB structures.

Herein we report a joint experiment-theory study that addresses this discrepancy and determines the minimum number of water molecules that retain the hydrated arginine in its native-like zwitterion form. Non-covalent complexes $ArgH^+(H_2O)_n$ with a controlled number of water molecules n = 0–5 were interrogated by cold ion IR spectroscopy and computationally treated with quantum chemistry. The spectroscopic changes induced by the gradual decrease of the level of hydration were attributed to the evolution of arginine from its native-like SB to the intrinsic CS structure. We discuss the role of evaporative cooling in the kinetic trapping of hydrated biomolecules in their native-like structures.

METHODS

Our experimental approach has been described in details elsewhere.[1,22] The hydrated ions are generated directly from solution using a "gentle" mode of a nano-electrospray ion (n-ESI) source[23] and transferred through a metal capillary and three consecutive inline molecular skimmers to a room temperature octupole ion trap (pre-trap) for accumulation and thermalization. Thermalized ionic complexes are mass-selected by a quadrupole mass-filter and guided into a cold octupole ion trap,[24] which is kept at 6 K. The trapped complexes are cooled down to ~10 K by collisions with He buffer gas, which is pulsed into the trap (gas pulse duration is ~1 ms; maximum number density of He in the trap is $\sim 2 \cdot 10^{15}$ cm^{-3}). The cold ions are then irradiated by a pulse of IR light (6±2 mJ/pulse within the 2800-3800 cm^{-1} range and 1.5±0.5 mJ/pulse within the 1500-1800 cm^{-1} range; 1 to 3 cm^{-1} spectral bandwidth), produced by an optical parametric oscillator (OPO, Laser Vision). The absorption of the IR light results in evaporation of a few water molecules. The remaining parent and the reduced fragment complexes are released from the trap and

detected by a quadrupole mass spectrometer, which is tuned to transmit alternatively either one of the fragments (e.g., a loss of single water molecule) or the parent complexes. We average 10 measurements at each IR wavelength at the repetition rate of 10 Hz. Each spectrum was recorded, at least, 2 times to ensure its reproducibility; the low number of deuterated complexes (reduced due to the presence of significant fraction of the mixed complexes with H and D atoms) forced us to measure each spectrum 5 to 7 times to ensure an acceptable quality of the data. A wavelength meter WS-5 (High-Finesse) measured the wavelength of the (visible) signal wave of the IR OPO and (only once) of the Nd:YAG pumping laser, thus providing the wavenumber of the generated by the difference frequency mixing IR light with ±0.2 cm^{-1} accuracy.

L-Arginine from Fluka BioChemika (≥99.5%), L-Arginine methyl ester dihydrochloride (≥98%) from Sigma-Aldrich and L-Arginine (α-^{15}N) hydrochloride (≥98%) from Cambridge Isotope Laboratories were used without further purifications. Methanol and ethanol of analytical reagent grade are from Fisher Chemical, water of HPLC Plus grade, deuterated water (≥99.9%) and acetic acid (≥99%) are from Sigma-Aldrich. Arginine was dissolved either in the water/methanol/ethanol (5/3/2) mixture or in the deuterated water (with no methanol) with 0.2 vol% of nondeuterated acetic acid. Arginine concentration in the prepared solutions was 50 μM.

The geometries and vibrational frequencies of the complexes were calculated using the density functional theory (DFT). In particular, we have used the B3LYP exchange-correlation functional, which includes the Lee-Yang-Parr correlation functional[25] in conjunction with a hybrid exchange functional first proposed by Becke[26], along with the basis set denoted 6-311++G**.[27] This basis set includes diffuse functions as well as polarization functions for both hydrogen and heavy atoms. All calculations have been carried out with the Gaussian 16 package.[28]

Semiempirical-based Born-Oppenheimer molecular dynamics simulations have been performed with the CP2K package,[29] where the nuclei are treated classically and the electrons quantum mechanically

within the PM6 semiempirical formalism;[30] the cubic cells of 3 nm side and the time-steps of 0.5 fs were used in the simulations.

RESULTS AND DISCUSSION

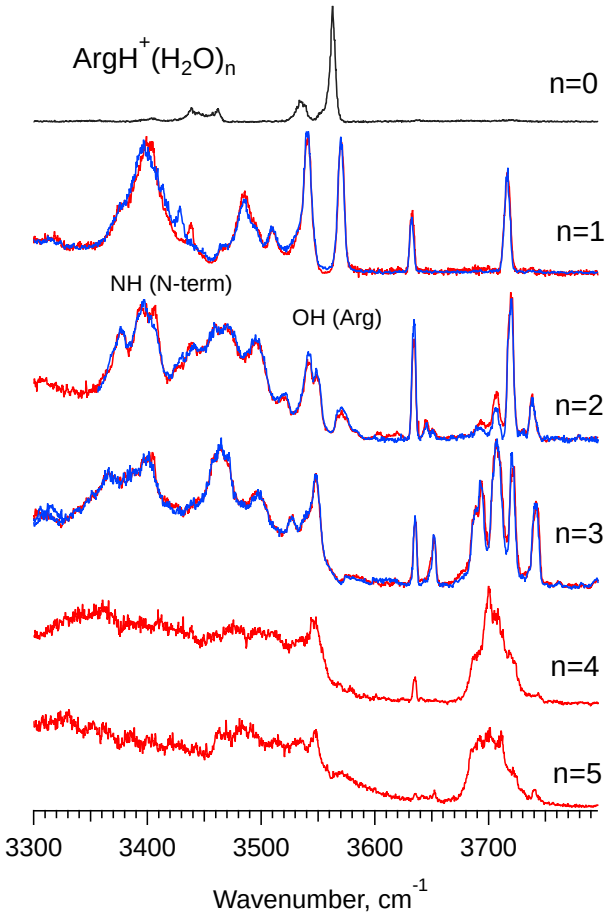

Figure 1. IRMPD spectrum of $ArgH^+$ (black trace) measured by monitoring fragment at *m/z* = 158; IRPD spectra of $ArgH^+(H_2O)_n$ (*n* = 0-5; red traces) and α-^{15}N isotopically labeled $ArgH^+(H_2O)_n$ (*n* = 1-3; blue traces) measured by loss of single water molecule. Vertical green bars emphasize the assigned transitions for $ArgH^+$ in the complexes.

Figure 1 shows IR spectra of $ArgH^+(H_2O)_n$ (*n* = 0-5) measured in the fingerprint spectral region of 3300-3800 cm^{-1}. The peaks above 3600 cm^{-1} are firmly assigned to the OH-stretches of water. Based on known data,[1] the peak at 3570 cm^{-1} for *n* = 1 has been assigned to the free OH stretch of the C-terminus,

which, expectedly, points to CS structure of the singly hydrated arginine. Retaining one more water (n = 2) significantly changes the spectrum. The increased number of the (water) OH-stretch peaks above 3600 cm^{-1} suggests that the complex resides in, at least, two conformational states. The intensity of the free OH stretch transition of the carboxyl at 3570 cm^{-1} transition weakens significantly for n = 2, further decreases for n = 3 and becomes completely unresolved for n = 4 and 5. This decrease implies that in some of the conformers of the $n \geq 2$ complexes either a water binds to the OH of the C-terminus, causing redshift and broadening of this transition, or the C-terminus becomes deprotonated. Although under our experimental conditions the conformers with such structural features appear to be highly abundant, they are not present among the earlier calculated (and recalculated herein) lowest energy conformers of these complexes.[14] The calculations suggest that below n = 7 $ArgH^+$ in the complexes resides in CS states and exhibits the OH group that is free of any non-covalent interactions. In addition, the calculations suggest that for n = 2 and 3 N-terminus remains free of interactions with water molecules but is involved in the intramolecular couplings with an amine of the side chain. This contradicts to the observed redshift of 32 cm^{-1} for the NH-stretch of the N-terminus in some of the complexes with n = 2 and 3, relative to the ones with n = 1. The shift was revealed from spectroscopy of the $ArgH^+$ complexes with isotopically labeled (^{15}N) N-terminus (Fig. 1, blue traces).

These observations prompt us to conclude that for n > 1 the complexes reside not in their global minima states, but rather appear kinetically trapped in solution-like geometries. Conformational search among the structures that reside in numerous local minima of potential energy (PE) surface, especially for such a flexible molecule like Arg, is very challenging. Our attempts to find among the low-energy structures those, whose spectra would reasonably match to the experiment did not succeed, although for n = 3 we found that most stable both CS and SB conformers appear lower in energy than those reported earlier.[14] Regarding this, our subsequent computations were focused not on solving the exact geometries of the experimental complexes, but rather on revealing the characteristic spectroscopic differences between the CS and SB structures of the low PE complexes and on mapping up these differences to the experiment.

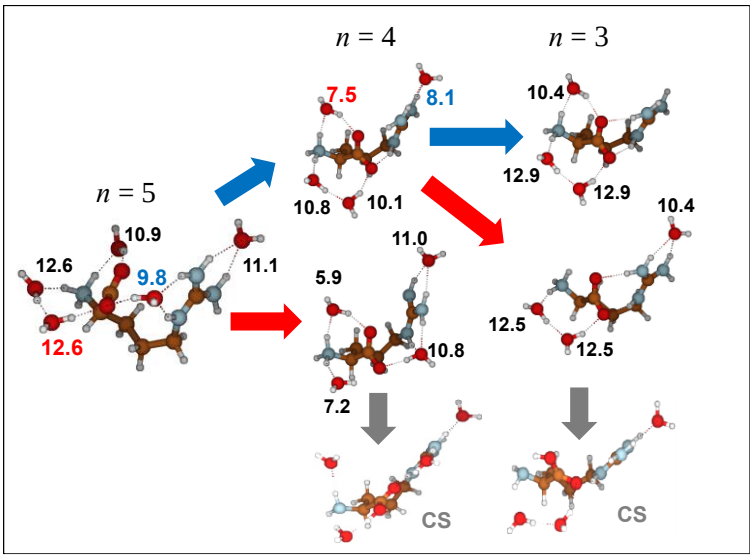

Figure 2. Schematic diagram illustrating generation of SB structures of the $ArgH^+(H_2O)_n$ complexes (shown partially for *n*=5 to 3 only). The numbers near each water molecule indicate the binding energy of this water to the complex (in kcal/mol). The color of a number codes the water molecule that was removed to generate the subsequent smaller complex to which the arrow of the same color points. Vertical grey arrows for *n* = 4 and 3 indicate that the respective SB structures are less stable than the generated from them CS conformers.

To mimic the experimental conditions, the complexes with *n* waters were generated based on the preceding *n* + 1 complexes. It turns out that for *n*=5 the geometry of arginine in the lowest energy SB complex is very similar to the geometry of Arg in the most stable structure of the complex with *n*=8, which is of SB type too.[14] We therefore take the lowest energy SB *n*=5 structure as a starting point for generating the geometries of the subsequent smaller complexes. The geometry of the $ArgH^+(H_2O)_5$ complex taken from ref[14] was, first, reoptimized at the B3LYP/6-311++G** level. Single water molecules were then removed from this structure and the geometries of five such generated *n* = 4 SB complexes were optimized at the same level. Next, a proton was moved from the N-terminus to one of the two oxygens of the C-terminus to generate two templates of CS structures, which were then optimized too. For each pathway, the three (one SB and two CS) structures were optimized and arranged by their PE. The most stable SB structures were then used as templates to generate *n* = 3 structures, and so on, as illustrated in figure 2 (see

Tables S1-S3 for images of the structures). Vibrational spectra were then calculated in a harmonic approximation for all conformers of $ArgH^+(H_2O)_n$ complexes (n = 2-5) generated through this procedure. A comparison of these results with the measured (conformation non-selective) spectra (Fig. S2) does not allow for unambiguous validation of the respective structures. This roots, mainly, from the large size and flexibility of the studied non-covalent complexes.

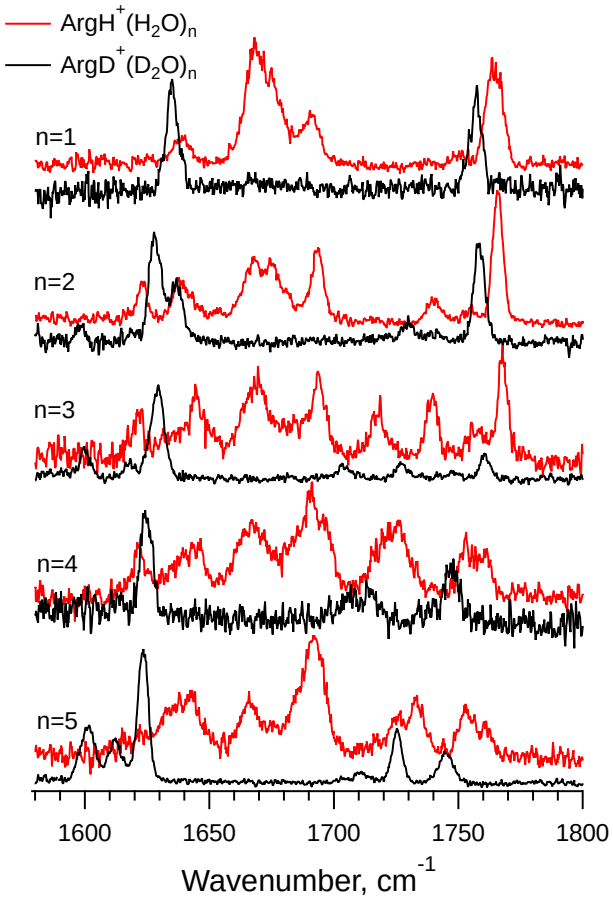

Figure 3. IRPD spectra of $ArgH^+(H_2O)_n$ (red traces) and of its deuterated (OH and NH groups) analogue $ArgD^+(D_2O)_n$ for n = 1-5.

In order to reduce the spectral complexity, we measured spectra of $ArgD^+(D_2O)_n$, (n = 1-5) complexes with all deuterated NH and OH groups. Deuteration significantly redshifts all the transitions associated with these groups, such that the few remaining peaks in the region of 1580-1650 cm^{-1} can be

safely assigned to the redshifted C–N antisymmetric stretches of the guanidinium group.[31] Based on the available data,[1] the remaining and only slightly redshifted (8 cm^{-1}) peaks in the 1700-1770 cm^{-1} region can now be assigned to a CO stretch of the H-bonded C-terminus. Detection of only one strong peak (1765 cm^{-1}) in this spectral region for the complex with n = 1 implies the presence of only one conformer (conformational family), which was assigned above to a CS structure. We therefore assign this peak to C=O stretch of the H-bonded C-terminus. One more redshifted ($\Delta\nu$ = 27 cm^{-1}) weak peak and two more redshifted peaks ($\Delta\nu$ = 28 cm^{-1} and $\Delta\nu$ = 49 cm^{-1}) of comparable intensity were detected for n = 2 and 3, respectively, pointing to the increase of the number of conformers upon increasing the level of hydration. The position of the highest-frequency peak remains nearly the same for n = 1-3, which allows its assignment to C=O stretch for n = 2 and 3 too. This assignment is further confirmed by spectroscopy of the microhydrated modified arginine that was methylated on the C-terminus (Fig. S3). The methylation forbids SB structures, while a gradual (n = 1-5) hydration of the CS structures almost does not influence the frequency and relative intensity of the C=O stretch. In contrast, the relative intensity of this peak in the spectra of $ArgH^+(H_2O)_{1-5}$ complexes (Fig. 3) gradually drops to zero upon hydration for n = 4 and 5, while the relative intensities of the two redshifted ($\Delta\nu$ =12 cm^{-1} and $\Delta\nu$ =42 cm^{-1}) peaks are increasing, respectively.

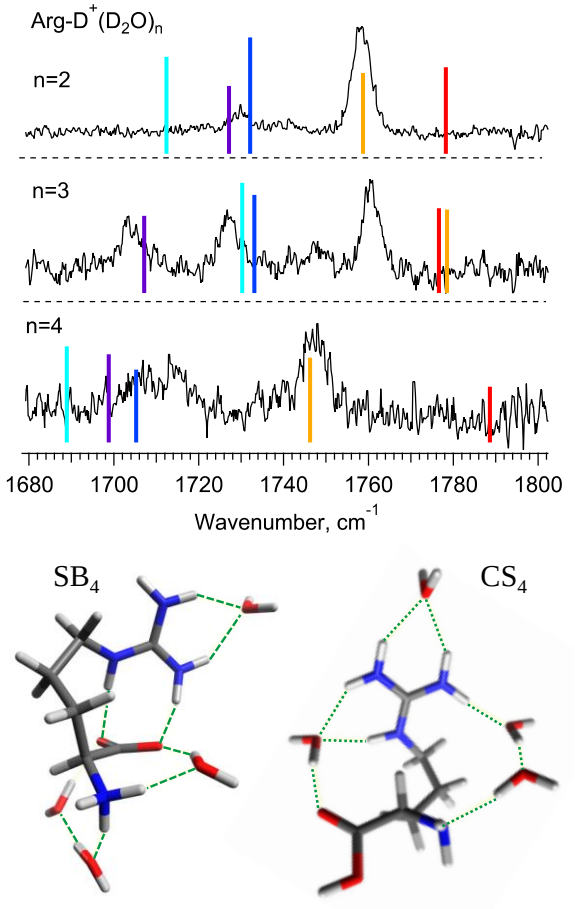

Figure 4. IR transitions of the $(O\text{-}C\text{-}O)^-$ and C=O stretch vibrations, calculated for the locally most stable SB (blue, light blue and violet sticks) and CS (red and orange sticks) conformers of $ArgD^+(D_2O)_n$ complexes (n = 2-4), respectively. The transitions are overlapped with the respective experimentally measured spectra. The frequencies of the transitions are scaled by the factor 1.015. The globally lowest-energy structures of the n = 4 SB and CS complexes are shown at the bottom for comparison.

Figure 4 compares these experimental spectra with the C=O stretch and of the $(O\text{-}C\text{-}O)^-$ antisymmetric stretch vibrations of the C-terminus calculated for the locally most stable SB and CS conformers generated by water removal/proton transfer, as described above. These vibrations are unique and characteristic for each type of conformer. The complexes may remain trapped in the local minima and be observed, if the energy barriers are unfavorable for relaxation to the CS structures with lower PE. Similar to a few earlier studied cases,[32,33] for all the computed complexes the frequencies of the $(O\text{-}C\text{-}O)^-$ vibrations exhibit a clear trend to be redshifted from the C=O ones. In particular, there are no candidate CS conformers to match to

the most redshifted peaks (~1710 cm^{-1}) in the spectra of complexes with n = 3 and 4. In opposite, there are no SB conformers to match to the highest frequency peaks assigned to the C=O stretch in CS structures. Based on this, we may assign the most redshifted peaks in the spectra of the complexes with n = 3 and 4 to SB structures. This also implies the presence of kinetically trapped zwitterionic SB conformers for all larger complexes, although we have no direct evidence of this.

The fact that these zwitterionic SB conformers survive ESI and the room-temperature pretrapping is not trivial to explain. To illustrate the dynamics of the desolvation and of the zwitterionic SB → CS transition, we performed semi-empirical (PM6) MD simulations for the zwitterionic SB complexes with n = 3-6 at a few different temperatures (Fig. S4). The simulations indicate that at T = 300-450 K the lifetime of the complexes is on the timescale of 10-100 ps. This is by far too fast for the complexes to avoid full dehydration and zwitterionic SB to CS relaxation during the 50 ms storage in the room temperature ion pre-trap. Calculations with the ion temperature of 200 K result however in a complete freezing of the dynamics over the limited 500 ps timescale of the simulations. In order to estimate the lifetime of the ion complexes under our experimental conditions, we measured the abundances of all the complexes that could be detected (n = 0-45) in the ion distributions for the cases of the trapped and non-trapped complexes in the ion pre-trap (Fig. 5).

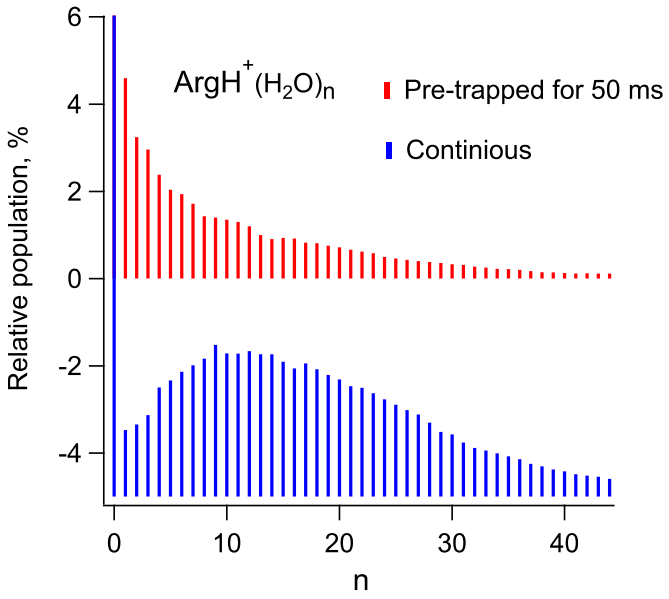

Figure 5. Relative population of $ArgH^+(H_2O)_n$ complexes for $n \leq 45$: after 50 ms storage in the room temperature ion pre-trap (red), and continuously detected as they are generated by ESI source. (blue). The two distributions have been derived from mass spectrometric measurements and were normalized to the total number of ions (100%) in each distribution. The intensities of the fully dehydrated ions (60% for the pre-trapped ions and 12% for continuously measured ions) were truncated for graphical clarity.

This allows for calculating the fraction of water molecules that were evaporated from all the complexes during the trapping. Based on this, we, roughly, estimated (see SI for details) the rate of single water evaporation in the trap, k_1, to 0.02 ms^{-1}, which gives ≃12 ms for the lifetime of, for instance, n=4 complexes. This result prompts us to conclude that, the evaporative cooling keeps the temperature of the ions in the pre-trap much lower than T = 300 K. Our MD simulations of evaporation in a microcanonical NVE ensemble (number of atoms-volume-energy being constant; see SI for details) suggest that the lifetime of 10 ms for the n = 4 complexes requires their internal temperature of ∼150 ± 35 K. This rough estimate is consistent with calorimetric studies of protonated water clusters which indicate that balancing collisional heating by evaporative cooling stabilizes internal ion temperature at as low as ≃170 K.[34] An increase of heating accelerates the evaporation to keep the ion temperature close to this asymptotic value. We therefore conservatively propose that the evaporative cooling of the $ArgH^+(H_2O)_n$ complexes may keep their internal

temperature below ~200 K. It must be this significant lowering of temperature that enables not only to retain water molecules for long time, but also arginine in these complexes to be kinetically trapped in zwitterionic forms, which are the most stable conformers in aqueous solutions.

A substantial evaporative cooling should be general for microhydrated ions. Regardless of the initial temperatures of the ions, boiling off waters keeps the complexes cold, provided the complexes originally own a sufficient number of water molecules to evaporate. Along with the specific intermolecular non-covalent interactions, evaporative cooling might be the key factor that protects the native-like structures of microhydrated biomolecules before a prompt cryogenic cooling. For instance, a recent spectroscopic study proposed evaporative cooling as the reason for preventing unfolding of multiply protonated microhydrated protein ubiquitin in the gas phase.[22]

CONCLUSIONS

To sum up, we suggest that a high final level of hydration of biomolecules may not be a must to reveal the main features of their native-like structures. Small water complexes always originate from the large electrosprayed ones, in which the native structural motives of the embedded biomolecules can be retained by the remaining network of H-bonds. Next, evaporative cooling to $\lesssim 200$ K hinders the transition from the native solution-like to the intrinsic gas-phase structures. Our study examples this: zwitterionic forms of Arg which are common in solution, but do not exist in the gas phase, were identified in the complexes of this protonated amino acid with as few as 3-4 water molecules. Although more extensive calculations are required to identify these structures, the relatively small level of hydration that suffice for kinetic trapping should encourage the use of microhydrated complexes in hunting for native structures of biomolecules.

ASSOCIATED CONTENT

Supporting Information.

Calculated structures of $ArgH^+(H_2O)_n$ (n = 0-8) complexes, energetics of computed structures, results and discussions of molecular dynamics simulations, approach for estimating temperature of hydrated $ArgH^+$ complexes.

AUTHOR INFORMATION

The authors declare no competing financial interests.

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REFERENCES

(1) Boyarkin, O. V. Cold Ion Spectroscopy for Structural Identifications of Biomolecules. *Int. Rev. Phys. Chem.* **2018**, *37* (3–4), 559–606. DOI:10.1080/0144235X.2018.1547453.

(2) Mucha, E.; Marianski, M.; Xu, F.-F.; Thomas, D. A.; Meijer, G.; von Helden, G.; Seeberger, P. H.; Pagel, K. Unravelling the Structure of Glycosyl Cations via Cold-Ion Infrared Spectroscopy. *Nat. Commun.* **2018**, *9* (1), 4174. DOI:10.1038/s41467-018-06764-3.

(3) Menges, F. S.; Perez, E. H.; Edington, S. C.; Duong, C. H.; Yang, N.; Johnson, M. A. Integration of High-Resolution Mass Spectrometry with Cryogenic Ion Vibrational Spectroscopy. *J. Am. Soc. Mass Spectrom.* **2019**, *30* (9), 1551–1557. DOI:10.1007/s13361-019-02238-y.

(4) Martens, J.; van Outersterp, R. E.; Vreeken, R. J.; Cuyckens, F.; Coene, K. L. M.; Engelke, U. F.; Kluijtmans, L. A. J.; Wevers, R. A.; Buydens, L. M. C.; Redlich, B.; Berden, G.; Oomens, J. Infrared Ion Spectroscopy: New Opportunities for Small-Molecule Identification in Mass Spectrometry - A Tutorial Perspective. *Anal. Chim. Acta* **2020**, *1093*, 1–15. DOI:10.1016/j.aca.2019.10.043.

(5) Greis, K.; Kirschbaum, C.; von Helden, G.; Pagel, K. Gas-Phase Infrared Spectroscopy of Glycans and Glycoconjugates. *Curr. Opin. Struct. Biol.* **2022**, *72*, 194–202. DOI:10.1016/j.sbi.2021.11.006.

(6) Bush, M. F.; Forbes, M. W.; Jockusch, R. A.; Oomens, J.; Polfer, N. C.; Saykally, R. J.; Williams, E. R. Infrared Spectroscopy of Cationized Lysine and ε-N-Methyllysine in the Gas Phase: Effects of Alkali-Metal Ion Size and Proton Affinity on Zwitterion Stability. *J. Phys. Chem. A* **2007**, *111* (32), 7753–7760. DOI:10.1021/jp071902q.

(7) Bush, M. F.; Oomens, J.; Saykally, R. J.; Williams, E. R. Effects of Alkaline Earth Metal Ion Complexation on Amino Acid Zwitterion Stability: Results from Infrared Action Spectroscopy. *J. Am. Chem. Soc.* **2008**, *130* (20), 6463–6471. DOI:10.1021/ja711343q.

(8) Bush, M. F.; Omens, J.; Williams, E. R. Proton Affinity and Zwitterion Stability: New Results from Infrared Spectroscopy and Theory of Cationized Lysine and Analogues in the Gas Phase. *J. Phys. Chem. A* **2009**, *113* (2), 431–438. DOI:10.1021/jp807470p.

(9) Forbes, M. W.; Bush, M. F.; Polfer, N. C.; Oomens, J.; Dunbar, R. C.; Williams, E. R.; Jockusch, R. A. Infrared Spectroscopy of Arginine Cation Complexes: Direct Observation of Gas-Phase Zwitterions. *J. Phys. Chem. A* **2007**, *111* (46), 11759–11770. DOI:10.1021/jp074859f.

(10) Smith, Z. M.; Steinmetz, V.; Martens, J.; Oomens, J.; Poutsma, J. C. Infrared Multiple Photon Dissociation Spectroscopy of Cationized Canavanine: Side-Chain Substitution Influences Gas-Phase Zwitterion Formation. *Int. J. Mass Spectrom.* **2018**, *429*, 158–173. DOI:10.1016/j.ijms.2017.08.009.

(11) Bush, M. F.; Prell, J. S.; Saykally, R. J.; Williams, E. R. One Water Molecule Stabilizes the Cationized Arginine Zwitterion. *J. Am. Chem. Soc.* **2007**, *129* (44), 13544–13553. DOI:10.1021/ja073796b.

(12) Burt, M. B.; Decker, S. G. A.; Atkins, C. G.; Rowsell, M.; Peremans, A.; Fridgen, T. D. Structures of Bare and Hydrated [Pb(Aminoacid-H)]+ Complexes Using Infrared Multiple Photon Dissociation Spectroscopy. *J. Phys. Chem. B* **2011**, *115* (39), 11506–11518. DOI:10.1021/jp2068655.

(13) Lemoff, A. S.; Bush, M. F.; O'Brien, J. T.; Williams, E. R. Structures of Lithiated Lysine and Structural Analogues in the Gas Phase: Effects of Water and Proton Affinity on Zwitterionic Stability. *J. Phys. Chem. A* **2006**, *110* (27), 8433–8442. DOI:10.1021/jp057436r.

(14) Gao, B.; Wyttenbach, T.; Bowers, M. T. Protonated Arginine and Protonated Lysine: Hydration and

Its Effect on the Stability of Salt-Bridge Structures. *J. Phys. Chem. B* **2009**, *113* (29), 9995–10000. DOI:10.1021/jp903307h.

(15) Gochhayat, J. K.; Dey, A.; Pathak, A. K. An Ab Iniio Study on the Micro-Solvation of Amino Acids: On the Number of Water Molecules Necessary to Stabilize the Zwitter Ion. *Chem. Phys. Lett.* **2019**, *716*, 93–101. DOI:10.1016/j.cplett.2018.12.021.

(16) Hwang, T. K.; Eom, G. Y.; Choi, M. S.; Jang, S. W.; Kim, J. Y.; Lee, S.; Lee, Y.; Kim, B. Microsolvation of Lysine by Water: Computational Study of Stabilized Zwitterion. *J. Phys. Chem. B* **2011**, *115* (33), 10147–10153. DOI:10.1021/jp202850s.

(17) Kim, J. Y.; Ahn, D. S.; Park, S. W.; Lee, S. Gas Phase Hydration of Amino Acids and Dipeptides: Effects on the Relative Stability of Zwitterion vs. Canonical Conformers. *RSC Advances*. Royal Society of Chemistry January 1, 2014, pp 16352–16361. DOI:10.1039/c4ra01217h.

(18) Li, H.; Hu, A.; Jiang, J.; Luo, Y. Systematic Study on Hydrated Arginine: Clear Theoretical Evidence for the Canonical-to-Zwitterionic Structure Transition. *J. Phys. Chem. A* **2017**, *121* (18), 3598–3605. DOI:10.1021/acs.jpca.7b02008.

(19) Blom, M. N.; Compagnon, I.; Polfer, N. C.; Von Helden, G.; Meijer, G.; Suhai, S.; Paizs, B.; Oomens, J. Stepwise Solvation of an Amino Acid: The Appearance of Zwitterionic Structures. *J. Phys. Chem. A* **2007**, *111* (31), 7309–7316. DOI:10.1021/jp070211r.

(20) Xu, S.; Nilles, J. M.; Bowen, K. H. Zwitterion Formation in Hydrated Amino Acid, Dipole Bound Anions: How Many Water Molecules Are Required? *J. Chem. Phys.* **2003**, *119* (20), 10696–10701. DOI:10.1063/1.1620501.

(21) Hebert, M. J.; Russell, D. H. Hydration of Guanidinium Ions: An Experimental Search for Like-Charged Ion Pairs. *J. Phys. Chem. Lett.* **2019**, *10* (6), 1349–1354. DOI:10.1021/acs.jpclett.9b00268.

(22) Zviagin, A.; Kopysov, V.; Nagornova, N. S.; Boyarkin, O. V. Tracking Local and Global Structural Changes in a Protein by Cold Ion Spectroscopy. *Phys. Chem. Chem. Phys.* **2022**, *24* (14), 8158–8165. DOI:10.1039/D2CP00217E.

(23) Zviagin, A.; Kopysov, V.; Boyarkin, O. V. Gentle Nano-Electrospray Ion Source for Reliable and Efficient Generation of Microsolvated Ions. *Rev. Sci. Instrum.* **2022**, *93* (11), 114104. DOI:10.1063/5.0119580.

(24) Boyarkin, O. V.; Kopysov, V. Cryogenically Cooled Octupole Ion Trap for Spectroscopy of Biomolecular Ions. *Rev. Sci. Instrum.* **2014**, *85* (3), 033105. DOI:10.1063/1.4868178.

(25) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev. B* **1988**, *37* (2), 785–789. DOI:10.1103/PhysRevB.37.785.

(26) Becke, A. D. Correlation Energy of an Inhomogeneous Electron Gas: A Coordinate-space Model. *J. Chem. Phys.* **1998**, *88* (2), 1053. DOI:10.1063/1.454274.

(27) Krishnan, R.; Binkley, J. S.; Seeger, R.; Pople, J. A. Self-consistent Molecular Orbital Methods. XX. A Basis Set for Correlated Wave Functions. *J. Chem. Phys.* **2008**, *72* (1), 650. DOI:10.1063/1.438955.

(28) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; et al. Gaussian 16, Revision C.01. Gaussian, Inc.: Wallingford, CT 2016.

(29) Vandevondele, J.; Krack, M.; Mohamed, F.; Parrinello, M.; Chassaing, T.; Hutter, J. Quickstep: Fast and Accurate Density Functional Calculations Using a Mixed Gaussian and Plane Waves Approach. *Comput. Phys. Commun.* **2005**, *167* (2), 103–128. DOI:10.1016/J.CPC.2004.12.014.

(30) Stewart, J. J. P. Optimization of Parameters for Semiempirical Methods V: Modification of NDDO Approximations and Application to 70 Elements. *J. Mol. Model.* **2007**, *13* (12), 1173–1213. DOI:10.1007/S00894-007-0233-4/FIGURES/10.

(31) Hong, Z.; Wert, J.; A. Asher, S. UV Resonance Raman and DFT Studies of Arginine Side Chains in Peptides: Insights into Arginine Hydration. *J. Phys. Chem. B* **2013**, *117* (24), 7145–7156. DOI:10.1021/jp404030u.

(32) Oomens, J.; Steill, J. D. Free Carboxylate Stretching Modes. *J. Phys. Chem. A* **2008**, *112* (15), 3281–3283. DOI:10.1021/jp801806e.

(33) Steill, J. D.; Oomens, J. Gas-Phase Deprotonation of p -Hydroxybenzoic Acid Investigated by IR Spectroscopy: Solution-Phase Structure Is Retained upon ESI. *J. Am. Chem. Soc.* **2009**, *131* (38), 13570–13571. DOI:10.1021/ja903877v.

(34) Schmidt, M.; Von Issendorff, B. Gas-Phase Calorimetry of Protonated Water Clusters. *J. Chem. Phys.* **2012**, *136* (16), 164307. DOI:10.1063/1.4705266.