Revealing the Structure of Tryptophan in Microhydrated Complexes by Cold Ion Spectroscopy.

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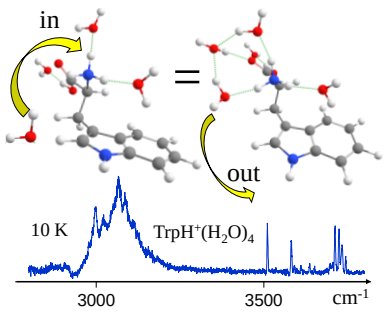
ABSTRACT

Geometry of biomolecules isolated in the gas phase usually differs substantially from their native structures in aqueous solution, which are the only truly relevant to life science. To compromise the high resolution of cold ion spectroscopy that can be achieved in the gas phase and the key role of intermolecular hydrogen bonds that shape biomolecules in water, we study protonated tryptophan microhydrated by one to six water molecules. IR/UV spectra measured with the same instrument under similar conditions appear to be identical for the complexes of the same size produced by soft dehydration and by cryogenic condensation methods. This observation points to the lack of a kinetic trapping in the dehydration/rehydration processes. Quantum chemistry computations allow for unambiguous assignment of the measured IR spectra to the most stable conformers of the complexes. The calculations reveal that retaining as few as four water molecules still conserves most of the $TrpH^+$ native structural features.

TOC Graphic



Knowledge of 3D structures of biomolecules is of fundamental importance in many fields of life science. Solving the structures that these large species adopt under native conditions remains one of the biggest challenges that drives development of new techniques for structural determinations. Cold ion spectroscopy (CIS)[1–5] in combination with quantum chemistry computations is a recent approach that can solve geometries of small to midsize (e.g., amino acids to decapeptide) biomolecules.[4,6–11] Although the method demonstrates high accuracy and conformational resolution, it can only determine structures of biomolecules isolated in the gas phase, but not in solution. The relation between these intrinsic and the biologically relevant native structures often remains vague. Intermolecular non-covalent interactions of a biomolecule with water molecules may compete with the intramolecular non-covalent bonds, leading to substantial alterations of its 3D structure. As a compromise between the high accuracy of structural determinations achievable in the gas phase and the need to determine 3D structures in bulk solution, one can study in the gas phase the biomolecules that are hydrated by a few water molecules.[12–21] This approach relies on the assumption that already a few remaining solvent molecules allow for retaining the main characteristic features of such structures in the gas phase.

Charged microhydrated biomolecules can be conveniently generated directly from solution using, for instance, the "gentle" electrospray ionization sources.[22] Vibrationally resolved spectra of such complexes can be used for validating computed structures. A molecule embedded into its water envelope may exhibit some sufficiently high internal energy barriers for a complex (or the bare ion) to remain kinetically trapped in its native-like geometry at some step of the desolvation.[16,23,24] This geometry will not be the lowest energy one, which makes conformational search among many computed gas-phase structures extremely time-consuming. Failure of the search may point to either an insufficient pool of the tested structures or a certain deficiency of the computations.

Experiment may assist in resolving this ambiguity. Microsolvated complexes can be generated not only by an incomplete desolvation, but also by condensing solvent molecules onto bare ions cooled in a

cryogenic trap.[25,26] Full desolvation of ions requires their substantial internal heating; the temperature of bare ions in ESI sources can reach 500 K.[27] This makes it more likely for an ion to overcome the native-to-intrinsic structural barriers, than in the case of a gradual desolvation at low pressure, when the ion temperature is maintained low by evaporative cooling. The subsequent condensation of solvent molecule onto cold bare ions will bring their structure back to that in the complexes with retained waters only, if the energy barriers for these re-arrangements are smaller than the ion internal energy. The similarity of the ionic conformers in the complexes generated by the two methods can be assessed through comparison of the respective IR or UV spectra, yet prior to any computations. Identical spectra for the same complexes generated by the two different techniques will point to the adiabatic pathway in both directions: desolvation and condensation. The computed lowest energy structures then should properly reflect the intrinsic structure of a bare ion but approach to its native-like geometry upon increasing the level of hydration. Here we explore this approach with protonated amino acid tryptophan ($TrpH^+$). It is a benchmark aromatic biomolecule known for its high yield of UV fluorescence in aqueous solutions.[28,29] Structure and photophysical properties of the gas-phase protonated bare and singly hydrated Trp have been a subject of numerous studies.[1,12,30–36] Briefly, the most stable conformers of isolated $TrpH^+$ exhibit a proton-π interaction between the protonated N-terminus and the nearby indole ring. The coupling enables ultrafast barrierless transfer of the proton to the ring in the electronic excited state, which results in broadening of UV transitions.[12,33] In one of the two conformers of singly hydrated $TrpH^+$ the water molecule sticks between the two groups, blocking the proton transfer. This lengthens the lifetime of the excited state and sharpens UV transitions in the respective conformer.[12,36] Retaining a second water on $TrpH^+$ increases the intensity of the sharp transitions,[12] although no full conformational analysis of this complex has been done so far.

Here we perform this structural analysis and extend it to protonated tryptophan microhydrated either by retaining or condensing of up to six water molecules. For each method of the generation and size of the complexes their IR and/or UV cold ion spectra were measured with the same instrument under

identical experimental conditions. The partially assigned by the experiment IR spectra were used for validating the low-energy structures calculated by quantum chemistry methods. We, finally, analyze the validated structures of the complexes and outline their relation to the native structure of tryptophan.

Our experimental approach, except the cryogenically cooled ion pre-trap, has been described in details elsewhere[21] (see also Supporting Information (SI)). Briefly, the hydrated ions are generated directly from solution using a "gentle" mode of an electrospray ion source.[22] Alternatively, the same complexes can be generated by condensing water vapor onto the ions stored in a cryogenic ion pre-trap. After selecting the ions of interest with a quadrupole mass-filter, they are transferred to a 10 K ion trap for performing IR photodissociation (IRPD) action spectroscopy.

Structures of the complexes were generated via molecular dynamics annealing and then clustered to the groups of similar structures. Geometries and potential energies of the lowest-energy conformers in each group were refined and then the harmonic spectra and free energies were calculated using density functional theory (see SI for details).

Figure 1 shows IRPD spectra of $TrpH^+(H_2O)_n$ (n=1-6) complexes produced by a gentle dehydration of electrosprayed droplets (blue traces). These spectra are almost perfectly identical to the respective spectra measured in the 3 μm spectral region for the complexes generated by the low-temperature condensation of n=1 to 4 waters onto the electrosprayed bare ions (red traces; see also figure S1). It is essential that the compared spectra have been measured on the same instrument with the same method and under the identical conditions. For instance, the recent studies of singly and doubly hydrated protonated glycine, which were produced by the two methods but in two different laboratories, revealed certain ambiguity in the interpretation of the data.[19,37,38] The ambiguity, potentially, could arise from the method of preparation, but also from the difference in the used spectroscopic techniques/experimental conditions. Our modified instrument enables direct assignment of spectral differences, if any, exclusively to the method of microhydration.

The spectral congestion, which increases with the size of the clusters, may hide the fine differences between the IR spectra measured for the same but differently produced complexes. On the other hand, UV spectra of aromatic molecules often exhibit extremely high sensitivity to structural differences of their non-covalent complexes.[39,40] Regarding this, for the complexes with *n*=4-6 produced by the two methods we compared their respective UV spectra (Figure S2).

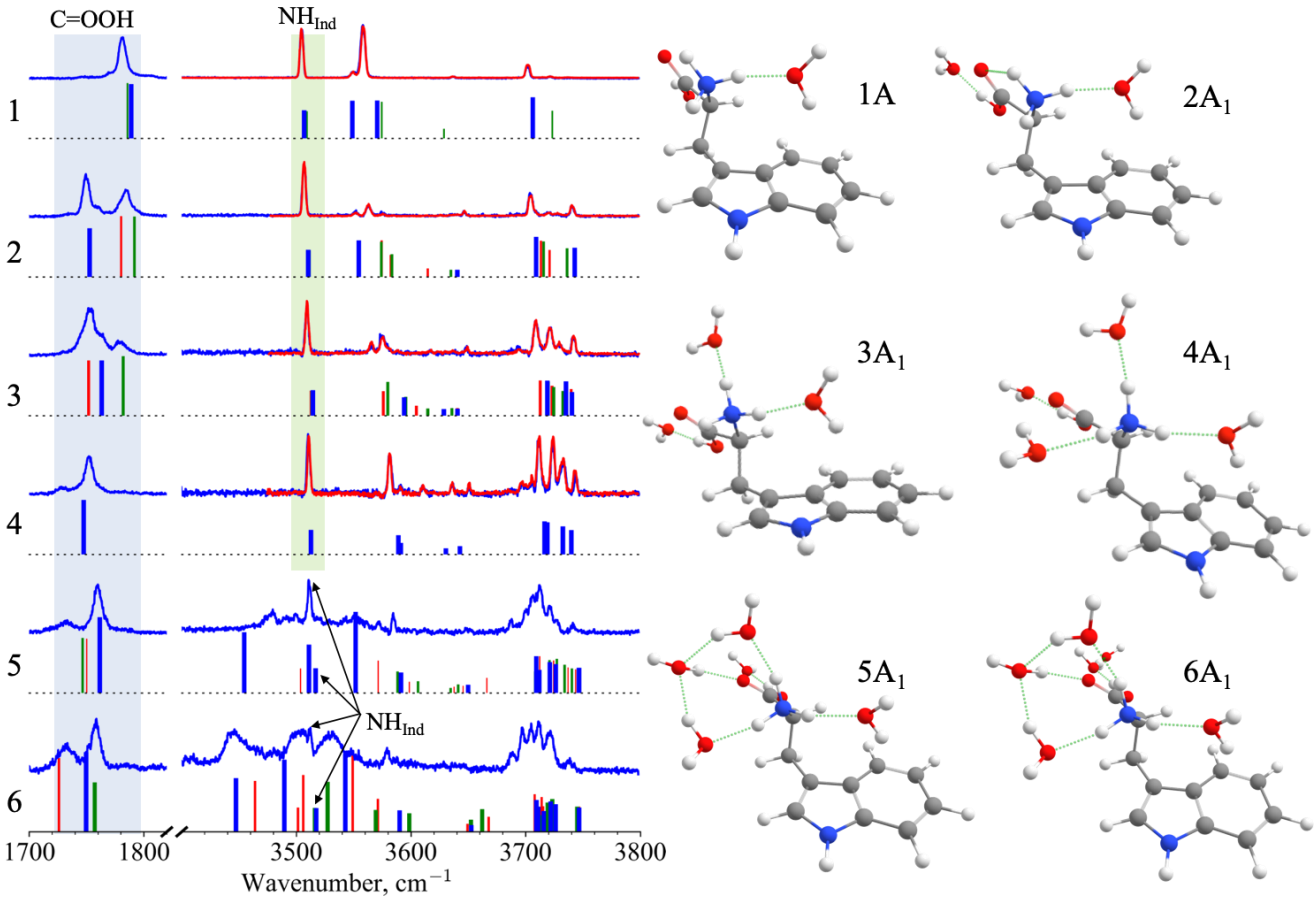


Figure 1. IRPD spectra of $TrpH^+(H_2O)_n$ complexes generated by retaining (blue traces) and by condensing (red traces) of water molecules. The sticks show the spectra calculated for the low-energy conformers: blue – the most stable structures, green and red – the low-energy structures selected for the best match to the experimental traces (see Table S4). The vertical green bar (for n=1-4) highlights and the arrows (for n=5 and 6) point to the transitions assigned by both experiment and calculations to NH-stretch of the indole ring in the most stable structures; the blue bar highlights the calculated C=OOH stretch transitions. The thickness of the sticks roughly reflects the thermal population of the respective conformers at T=300 K. The computed frequencies are scaled by the factors 0.968, and 0.944 for the 6 μm and 3 μm spectral regions, respectively. The most stable structures with their labels are shown on the right.

Again, within the accuracy of the measurements, for each *n* the pairs of the spectra look identical. This implies that, at least, for each *n*=1-6 $TrpH^+$ adopts the same structures regardless of the method hydration. We rule out any kinetic trapping of gas-phase $TrpH^+$, since the geometries of the protonated bare and singly hydrated tryptophan have been solved as the lowest energy gas-phase conformers. This allows us to suggest that the most stable generated complexes with n=1-6 waters under the conditions of our experiment reside in the global potential energy minima.

In support of our suggestion, Figure 1 compares the measured IR spectra with those computed for the low-energy conformers of $TrpH^+(H_2O)_n$. Isotopic labelling of the indole ring ($N^{14}\rightarrow N^{15}$) allows for direct assignment (Fig. S3) of the indole NH-stretch transition. Upon dehydration its frequency redshifts only little from 3512 cm^{-1} for *n*=6 to 3504 cm^{-1} for the bare ion, which allows us to use this transition as a reference for scaling the calculated frequencies. For *n*=1 they match well to the transitions in the IRPD spectra; the measured spectra, the calculated frequencies and the two most stable structures appear to be nearly identical to the data earlier reported by Molina et al. For $TrpH^+(H_2O)_2$, in addition to the IRPD spectrum in figure 1, we explicitly measured conformer-selective IR-UV depletion spectra of this complex (figure 2).

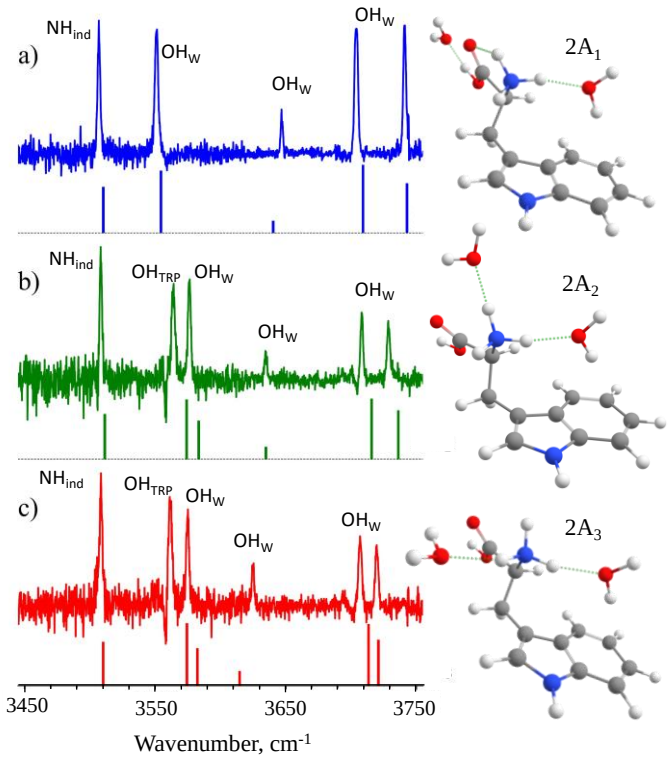


Figure 2. Conformer-selective IR-UV depletion spectra of $TrpH^+(H_2O)_2$ complex, measured with UV wavenumber fixed at (a) 35176, (b) 35243, and (c) 35281 cm^{-1}. The computed spectra of the three most stable conformers $2A_1$, $2A_2$ and $2A_3$ with relative free energy of 0, 0.67 and 0.82 kcal/mol, respectively, are shown by sticks; the respective structures are shown on the right. The complexes were generated from solution by incomplete dehydration.

One highly and two low-abundant conformers of $TrpH^+(H_2O)_2$ complex were found while detecting the fragment at m/z=188 Th (loss of two waters and ammonia). The scaled vibrational frequencies calculated for the most stable computed complex, named $2A_1$, match very well to the spectrum of the most abundant conformer, thus validating the calculated geometry (figure 2a). The experiment – theory match is equally good between the two conformers of lower abundance (figures 2b and 2c) and the next two low-energy computed structures (named $2A_2$ and $2A_3$). Similar to the most stable conformer of $TrpH^+(H_2O)_1$, the main common structural feature of the conformers $2A_{1-3}$ is the insertion of one water molecule between the N-terminus and the indole ring (Fig. 2). As it was discussed above, the insertion interrupts the proton–π coupling that allows fast proton transfer to the indole ring.[12,36] The validated herein structures of the three $TrpH^+(H_2O)_2$ conformers provide a rational explanation of this phenomenon. In these structures the

positive charge of NH_3^+ group is solvated by two H-bonds, leaving one hydrogen atom of the N-terminus free of non-covalent interactions. The unfavorable position of this atom clearly forbids, however, the proton–π coupling.

A close inspection of the UV spectrum (Fig. S4) for *n*=2 reveals a broadband low-intense absorption beneath the sharp peaks that must arise from the conformers with a short-living electronic excited state. Such structures were earlier identified for singly hydrated $TrpH^+$.[36] Similar conformers were also found among the calculated herein for *n*=1 and 2 structures with low free energy (named $1B_1$ and $2B_1$; Fig. S5), although the very low level of the broadband UV absorption did not allow us to measure their IR spectra. These structures originate from the first excited conformer of bare $TrpH^+$, which differ from the most stable one, mainly, by the position of carboxyl/amine moieties, relative to the reminder of the molecule.[34] Microhydration does not remove this structural difference, enabling two distinct families (*n*A and *n*B) of the complexes (see the supplementary file "Structures"). IR depletion of the narrow UV peaks assigned to conformers 2A makes our experiment low-sensitive to the conformers 2B, such that they may contribute only little to the spectra in figure 2.

The very good match between the computed and the measured IR spectra of the complexes with *n*=0-2 makes us confident in the used level of theory. Regarding this, for the *n*=3-6 complexes we limited the conformational search to low-energy structures and our measurements to conformer-nonselective IRPD spectroscopy only.

The observed similarity of the spectra measured for the dehydrated and rehydrated complexes of the same size implies that, upon removing some single water molecules from a complex, it may undergo some barrierless (low-barrier) structural rearrangements to the smaller low energy conformers. Our calculations enable mapping the network of such barrierless transitions from the complexes with *n*=6 to bare $TrpH^+$. Figure 3a shows that, upon gradual dehydration, the most stable conformer of the largest studied (*n*=6) complex may relax to the most stable conformer of $TrpH^+$ exclusively through the most stable conformers of the intermediate sizes. In support of this suggestion, we treated the $2A_1 \rightarrow 1A \rightarrow 0A$ transition, which

does require some large structural changes, directly by performing DFT optimization of the structures after removing one appropriate water molecule each time. The optimization, indeed, yields the $1A_1$ and $0A_1$ conformers as the most stable structures of each size.

The calculated geometry of $TrpH^+$ in the nA_1 complexes evolves almost gradually upon dehydration, with the most prominent changes in the orientation of the indole and COOH groups relative to the NH_3^+ one. Figure 3b plots the dihedral angles between the C-NH_3 and C-indole (θ_1) and between the C-NH_3 and COOH bonds (θ_2) for n=0-6. Both angles exhibit sharp changes after removing the fourth and the subsequent water molecules. The more than 100° change of θ_2 between the bare $TrpH^+$ and the n=4 complex is unlikely in bulk water for this internal rotation of the two large parts of the ion. Reasonably assuming that the geometry of $TrpH^+$ is closer to its native structure in the largest studied herein complex of n=6, this change implies that $TrpH^+(H_2O)_4$ is the smallest complex, in which tryptophan should still resemble its native structure. The particular role of the $4A_1$ has a rational explanation. In this complex four water molecules occupy all four highly hydrophilic hydration sites (NH_3^+ and OH of the C-terminus), such that the subsequent 5th and 6th waters energetically prefer coupling to these four waters, but not directly to $TrpH^+$ (e.g., to NH of imidazole). This limits the impact of the subsequent hydration on the structure of the ion. In addition, the occupation of all hydration sites leads to the lower number of the low-energy conformers for n=4 than for the complexes of the adjacent sizes (3, 5 and 6), which makes the $4A_1$ conformer an exclusive bottleneck in the n=0↔6 hydration/dehydration pathways.

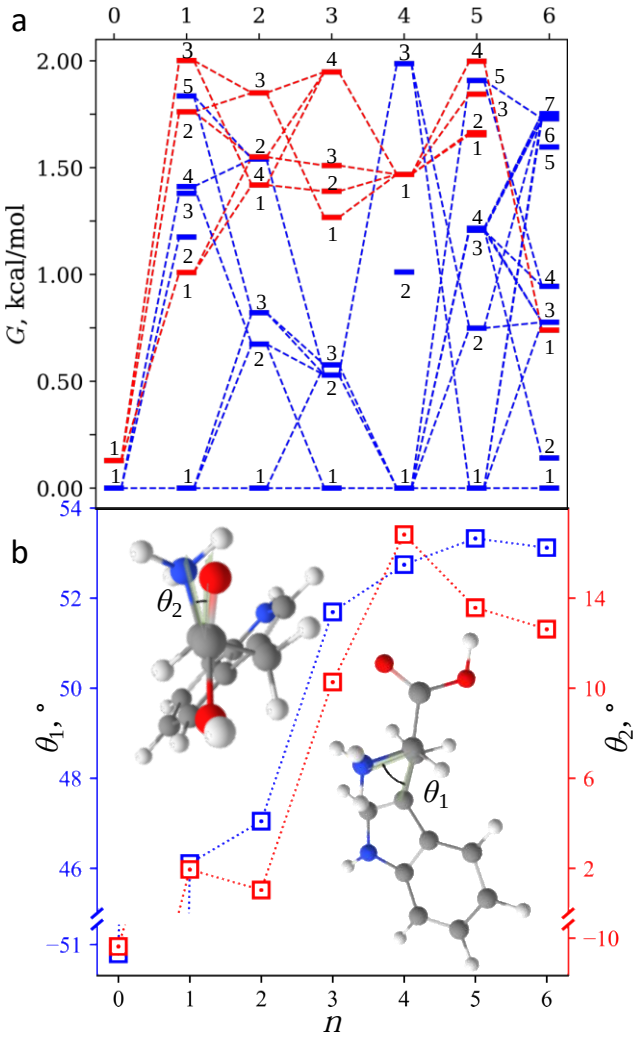


Figure 3. (a) Calculated free energies (G < 2 kcal/mol) of A (blue bars) and B (red bars) types of $TrpH^+(H_2O)_n$ conformers, shown for each *n* relative to the free energies of formation (ΔG_f) of the lowest nA_1 conformer from isolated $TrpH^+$ and *n* water molecules; ΔG_f= 0, -7.32, -11.92, -15.43, -18.05, -18.9, -19.9 kcal/mol for *n*=0-6, respectively. The numbers near the bars indicate subscripts of the conformer labels. Dashed lines connect the conformers, for which the $n \leftrightarrow n+1$ transitions (except $1A_1 \leftrightarrow 0A_1$) do not require any significant rearrangements in the $TrpH^+$ backbone or a migration of water molecules. (b) Evolution of characteristic dihedral angles of $TrpH^+$ structure upon change of *n*.

In conclusion, cold ion IR/UV spectra of $TrpH^+(H_2O)_n$ (n=1-6) appear to be identical for the complexes of the same size generated by retention and by condensation of water molecules. This observation points to the lack of kinetic trapping of $TrpH^+$ in the complexes under our experimental conditions. Consistently, the calculated three lowest free-energy conformers of $TrpH^+(H_2O)_2$ have been

assigned and validated by IR-UV conformer-selective spectroscopy; their geometry explains the known blocking effect of hydration on the fast proton transfer in isolated tryptophane. The IRPD spectra of the larger complexes are also consistent with the spectra calculated for the respective most stable conformers. Structural changes induced by a gradual dehydration may follow the barrierless pathways from the largest studied complex (n=6) to the fully dehydrated $TrpH^+$. The changes remain smooth and gradual for down to n=4 bottleneck structure, which implies that tryptophan hydrated by four waters yet should retain most of the features of its geometry in aqueous solutions. Overall, our study demonstrates that the gas-phase spectroscopy of a gradually hydrated/dehydrated small to midsize biomolecules, when combined with quantum chemistry computations, can provide important hints for solving their native-like structures.

ASSOCIATED CONTENT

Supporting Information.

Materials and methods, IR/UV spectra of $TrpH^+(H_2O)_n$, calculated lowest energy structures of the complexes for n=1-3, relative energies of the structures, all optimized structures with their computed vibrational spectra (file "Structures").

AUTHOR INFORMATION

The authors declare no competing financial interests.

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