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► **To cite this version:**

Massimiliano Porrini, Clémence Rabin, Josephine Abi-Ghanem, Frédéric Rosu, Leonardo Darré, et al.. Multi-scale Simulations Coupled with Ion Mobility Experiments Reveal the Fate of Nucleic Acids in the Gas Phase. 64th ASMS Conference on Mass Spectrometry and Allied Topics, Jun 2016, San Antonio, United States. hal-01562153

HAL Id: hal-01562153

<https://hal.science/hal-01562153>

Submitted on 13 Jul 2017

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Multi-scale Simulations Coupled with Ion Mobility Experiments Reveal the Fate of Nucleic Acids in the Gas Phase

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Introduction

Native electrospray ionization (ESI) experiments on biomolecules like nucleic acids imply spraying samples from physiological solutions. The desolvation is so gentle to preserve at the best the solution structure [1]. Hyphenating ion mobility (IM) mass spectrometry (MS) to ESI-MS allows one a further separation of the species based on mass, charge and conformation [2]. It has already been suggested experimentally (non-physiological conditions) [3] and theoretically [4] that base *Watson-Crick* h-bonds and stacking are preserved in gas-phase. However, the evaporation of the droplet water molecules as well as the IM milliseconds dynamics (the drift tube for the present study) still pose unanswered questions concerning the final effects on the structure of the nucleic acids.

For instance, how the desolvation and its rate affect the detected conformational families? When and how the final charge state(s) plausibly generated by NH_4^+ cations proton transfer to the backbone phosphate groups are reached in the plume region? Can the nucleic acids ions experience substantial conformational rearrangements during the milliseconds dynamics in the drift tube?

Computer simulations allow one to atomistically visualise biomolecules dynamics and structure [5]; they therefore embody an indisputable complement for examining what happens to the solute molecules while water molecules are evaporating and counterions and co-ions declustering [6-8]. Combining state-of-the-art DFT, semi-empirical (PM7 hamiltonian), all-atom molecular dynamics (MD) and temperature replica exchange MD (T-REMD) simulations with ESI-IMS experimental observations, we are able to provide pivotal insights for answering the above questions. We observed that electrosprayed 12-mer, 24-mer and 36-mer DNA or RNA duplexes, at the low charge states typically obtained from physiological buffers, undergo a severe compaction with respect to the values anticipated for preserved canonical A- or B-helix structures. The challenge is to account for the experimental collision cross sections ($^{DT}CCS_{\text{He}}$) with theoretical calculations [9]. Ultimately, we show that modelling approaches urge simulating desolvation and progressive protonation of the solute, to end up with bare

structures that, by self-solvation, are capable to “click” themselves in a relatively long-time stable conformation(s).

Methods and Material

DFT calculations (6-31G(d,p) basis-sets) implementing MO6-2X functional (with GD3 correction) using Gaussian09 software [10] were run on small-double helices (7-mer, 8-mer and 9-mer) composed of CG base pairs. Semi-empirical calculations, MOPAC-PM7 hamiltonian [11], were run on 12-mer duplexes. Unbiased and “steered” classical simulations were run on the 12-mer, 24-mer and 36-mer. T-REMD were run on the 12-mer, using 18 replica and temperatures spanning a range from 300 K to ca. 600 K, obtaining a rate of successful exchanges of 30%. Amber14 [12] suite of programs and the parmBSC1 force field [13] were used. IMS experiments were carried out on an Agilent 6560 IMS-Q-TOF instrument (pure water, pH=7 and 100 mM $<[\text{NH}_4\text{OAc}] < 150$ mM), and $^{\text{DT}}\text{CCS}_{\text{He}}$ were determined with the drift tube in helium, in soft or harder ion trapping conditions. Theoretical CCSs were calculated with an adequately parameterized EHSS model [9].

Results

The major charge states were -5, -7 and -8 for the 12-mer, the 24-mer and the 36-mer respectively. The $^{\text{DT}}\text{CCS}_{\text{He}}$ values increase with the increasing net charge and decrease with the increasing CG base pairs content. The $^{\text{DT}}\text{CCS}_{\text{He}}$ fall in the following ranges (in \AA^2): $683 < ^{\text{DT}}\text{CCS}_{\text{He}}(12\text{-mer}) < 755$, $1084 < ^{\text{DT}}\text{CCS}_{\text{He}}(24\text{-mer}) < 1157$ and $1426 < ^{\text{DT}}\text{CCS}_{\text{He}}(36\text{-mer}) < 1478$. The theoretical CCS for the corresponding canonical B-helices are far larger (902 \AA^2 , 1694 \AA^2 and 2485 \AA^2), see Figure 1.

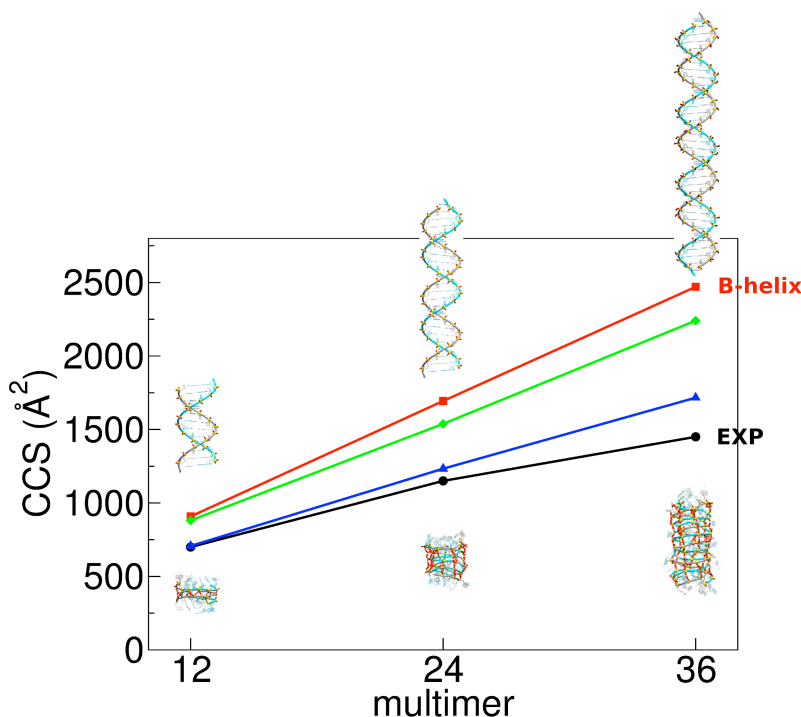


Figure 1. Comparison between experimental (black line) and theoretical CCS values for the 12-mer, 24-mer and 36-mer DNA duplexes. The theoretical values refer to the canonical B-helix duplexes (red line), to the final structures issued from unbiased MD simulations (green line) and the final structures from following restrained minimization (blue line).

We implemented a multi-scale computational approach to clarify the compaction mechanism. DFT is used to properly take into account the *Watson-Crick* base pairs and the stacking interactions. The calculations on B-form small-helices $[d(\text{CGCGGGC})_2]^{2-}$, $[d(\text{CGCGGGCC})_2]^{3-}$ and $[d(\text{CGCGGGCCC})_2]^{3-}$ show a hydrogen bonding network formation between protonated phosphates across the minor groove causing a winding of the helices. The major groove slightly shortens too. With the 12-mer duplexes, this behavior is confirmed using semi-empirical calculations (PM7). The same calculations run with A-form RNA or DNA duplexes show a “zipping” along the major groove. Importantly, despite significant distortions, *Watson-Crick* hydrogen bonds and base stacking are preserved in all the cases.

Implementing the same protocol as the one used by McAllister *et al.* [6] we simulated at atomistic level the desolvation of a 12-mer duplex. The simulations prove that this compaction is likely to happen in the plume region and it is due to the “sticky” NH_4^+ cations that shelter along the grooves, helping the backbone strands to get close each other (Figure 2).

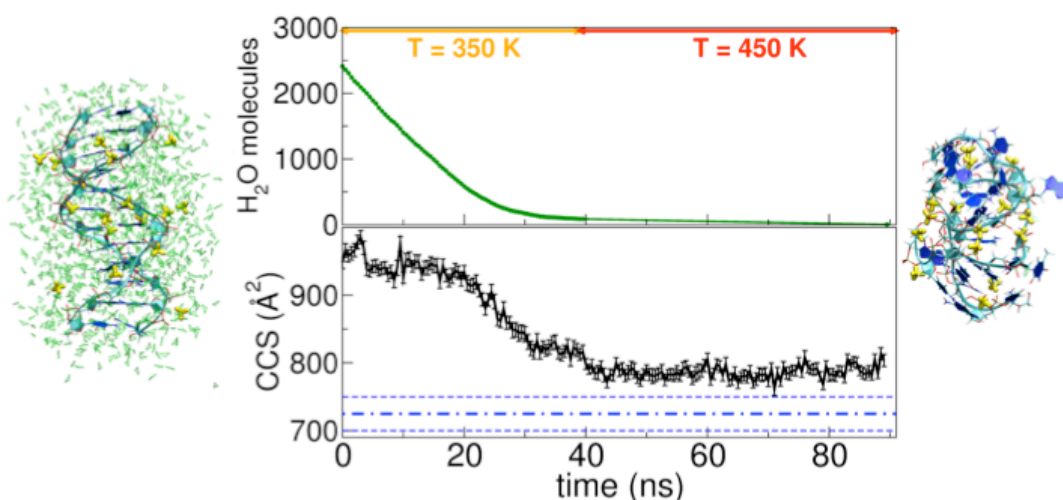


Figure 2. Results of the desolvation process of a Dickerson-Drew 12-mer duplex immersed in a 2500 water molecules droplet together with 17 NH_4^+ cations (net charge = -5). Top graph: number of water molecules composing the droplet upon evaporation and temperatures used for the MD simulations. Bottom graph: trend of the CCS values along the desolvation. The initial and final desolvated structures, together with the ammonium cations, are reported on the left and right side, respectively.

From the CCS trend (Fig.2 lower graph), it is evident that the major shrinking begins at around 20 ns, that is when the evaporation rate slows down from being linear (Fig. 2

upper graph). These results show that, unlike proteins, running unbiased MD all at once *in vacuo* is not enough realistic in the case of nucleic acids, leading to structures too elongated whose CCS values (green line in Figure 1) are compatible only with higher charge states, like the -6 and -7 obtained by Bowers' team [14, 15].

For sequences longer than the 9-mer, we then mimicked the grooves “zipping” (proven by QM and desolvation calculations) by steering the duplexes MD trajectories to explore other regions of the configuration space. Restraints between mating phosphate groups across both grooves were imposed during the initial minimization, then removed prior to 1- μ s gas phase dynamics at 300 K. The configurations now sampled in the gas phase have the CCS in agreement with the $^{DT}CCS_{He}$ values (blue line in Figure 1). As it is shown in Figure 2, despite the longer is the sequence the larger is the discrepancy between ^{THEO}CCS and $^{DT}CCS_{He}$, we conclude that the compaction is due to additional favorable hydrogen bonds forming between phosphate groups, neutralised by NH_4^+ counterions proton transfer.

Conclusions

Nucleic acids behave differently from proteins upon electrospray [16]: the phosphate groups tend to favor “outside-in” structures at low charge states. Modelling techniques, other than overcoming the important issue of poor sampling to simulate biomolecules in the IMS chamber, urge the development of protocols and methods that can better describe what happens during the ESI process. The major structural changes occur during the desolvation and charge state generation, especially in the case of nucleic acids, for which the non-dramatic collapse of the side chains onto the backbone [17] can not happen, but instead the compaction is rather due to self-solvation operated by protonated phosphate groups.

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