Secondary structures of Peptoids in gas phase assessed by Ion Mobily Mass Spectrometry and Molecular Modeling

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Introduction

Peptoids, or poly-N-substituted glycines are peptide reigioisomers.[1] The characteristic feature of these molecules is the side chain appended to the amide nitrogen instead of the α-carbon, as it is found in peptides (**Figure 1**). This structural difference should prevent peptoid backbone to form well-defined structures as α-helix. Though peptoids can form stable secondary structures in solution, mainly helical, as attested by CD and NMR.[2] This secondary structure has been proposed to be responsible for the enantioselectivity exhibited by peptoids in chiral chromatography, though no evidence have been found so far.

However, CD and NMR average the structural information over the entire sample, preventing an analysis of every type of conformations. In this context, Mass Spectrometry (MS) techniques, especially Ion Mobility MS (IMMS), may represent a suitable method to investigate the relationship between primary and secondary structures through the determination of the *Collision Cross Section* (CCS) and by associating molecular modeling.

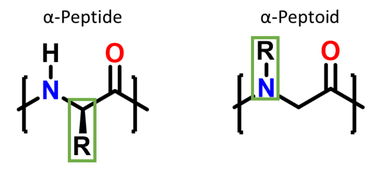

Figure 1: α-Peptide vs. α-Peptoid structure

Experimental section

Peptoids bearing methyl (*Nsar*) or tert-butyl (*NtBu*) side chains are synthesized on solid support using a step by step protocol involving a primary amine and a chloroacetic acid (**Figure 3**).

Ion mobility MS experiments were conducted on a Synapt G2-S*i* (**Figure 2**), in which the mobility cell employs the T-wave technology. A polymer (PEG600, 1000, 2000) calibration was used to determine the experimental CCS (CCS_{exp}) from the arrival time distributions.

Peptoids were diluted in a 1:1 mixture of ACN:MeOH with a concentration around 1 micromolar and directly infused into an Electrospray ionization source (ESI).

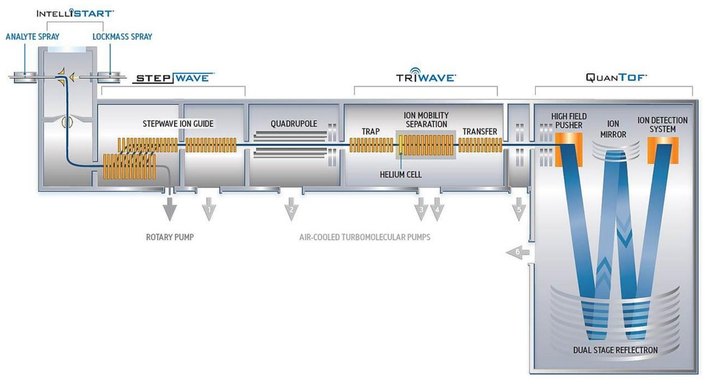

Figure 2: Waters Synapt G2-S*i*

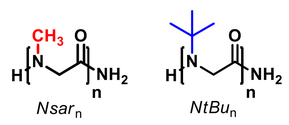

Figure 3: Primary structure of $Nsar_n$ and $NtBu_n$ peptoids

Theoretical section

To obtain candidates structures, we used molecular mechanics and dynamics methods (MM/MD). We employed the PEPDROID force field, a reparametrized version of DREIDING for peptoids based on high-level QM calculations.[3]

Peptoid structures were generated in the Materials Studio 6.0 package and were submitted to multiple quenched MD to fully scan the potential energy surface. Then the most stable structure for each polymerization degree (DP) as well as the helical structure were submitted to a first equilibration MD at 298 K for 10 ns, followed by a second one with the same parameters.

Structures from the second MD were extracted and injected into the Collidoscope program to compute theoretical CCS (CCS_{th}) through the Trajectory Method (TM).[4] This method is currently the most accurate to compute CCS and compare them to the CCS_{exp}.

Results & discussion

We compare polyalanine peptide ions and polysarcosine peptoid ions (**Figure 4**). These two types of ions are regioisomers, except that the C terminal extremity is a carboxylic acid and a amide function for peptides and peptoids, respectively. In solution, polyalanine (poly-Ala) peptides adopt α-helical conformations, stabilized by intramolecular H-bonds. However, when transferred to the gas phase for (IM)MS analysis, poly-Ala ions fold into globular species in order to stabilize the proton borne by the terminal amine nitrogen atom (Nter), which is the most basic site.[5] The evolution of the collision cross section (CCS) vs the degree of polymerization (DP) reflects this globular shape (**Figure 4**). Indeed, the projected surface (which can be seen as the CCS in first approximation) of a perfect cylinder (helical shape) evolves as a power of DP^1 (**Figure 5 A**) while the projected surface of a perfect sphere evolves as a power of $DP^{2/3}$ (**Figure 5 B**).[6] The exponent obtained when performing a power fit on the CCS of poly-Ala is really close to the expected one (0.651 vs. 0.667), confirming the globular shape of poly-Ala ions in gas phase. For poly-sarcosine (poly-*Nsar*) ions, also protonated at the terminal amine (Nter), a similar behavior is observed (**Figure 4**). However, the exponent is slightly lower than that of poly-Ala, indicating a relatively much folded structure. We thus performed MD simulations with our PEPDROID force field and obtained the low lying energy structures corresponding to loops, fully stabilizing the proton (**Figure 6**). Thus, the charge totally governs the secondary structure of gaseous poly-*Nsar* and poly-Ala ions.

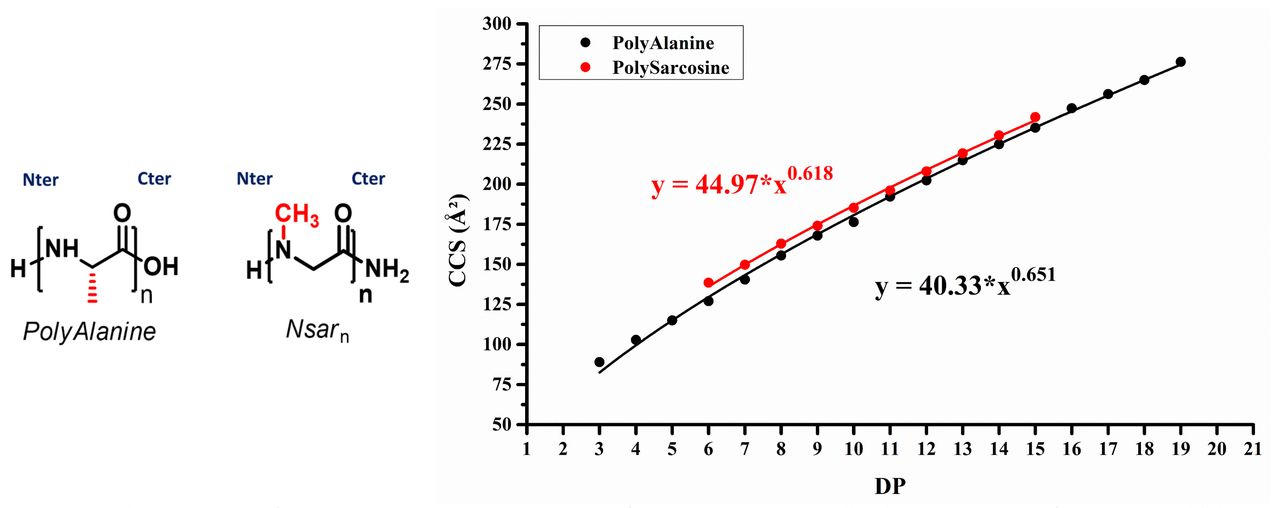

Figure 4: Primary structure of poly-Ala and poly-*Nsar* and the evolution of the collision cross section (CCS) against the degree of polymerization (DP). Poly-Ala CCS are obtained from Clemmer's database.

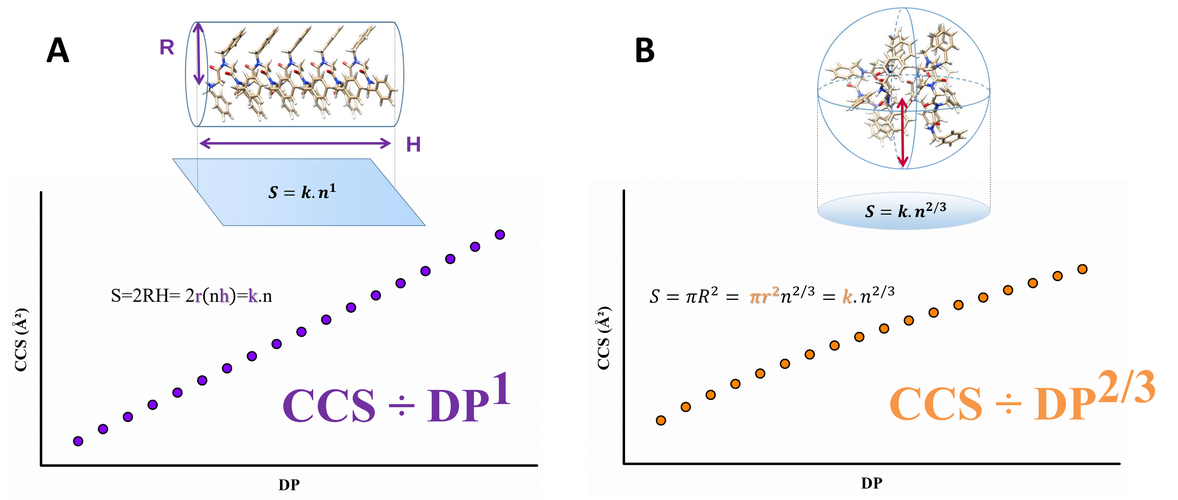

Figure 5: Theoretical models for the CCS evolution of (**A**) a perfect cylinder (helical) and (**B**) a perfect sphere (globular).

Next we focused on peptoid ions bearing bulky side chains, such as tert-butyl (*NtBu*, **Figure 3**). Due to the steric hindrance, this side chain should constrain the backbone into a well-defined structure. The evolution of the CCS against DP for poly-*NtBu* starts as a power around 0.667 till DP 10. The exponent value of the power fit is 0.702, indicating a much ordered structure. Indeed, for DP lower than 10, MD calculations indicate that a loop structure is formed around the proton, as seen with poly-*Nsar* while for DP11, a hybrid loop-helix is obtained. This is due to the balance between electrostatic stabilization and steric hindrance.

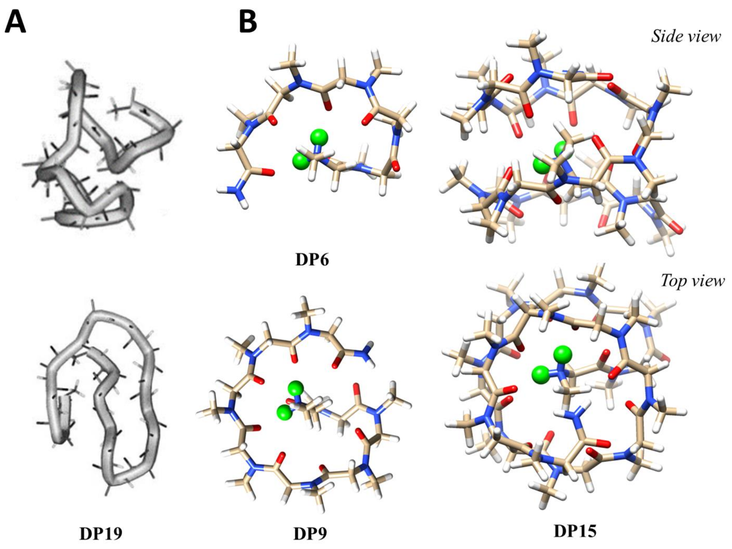

Figure 6: (**A**) Structures of poly-Ala_{19} from Ref. 5 generated by MD. (**B**) Structures of poly-sarcosine at different DP generated by MD. Hydrogens at Nter have been highlighted in green. All poly-sarcosine structures from loops over the protonated terminal amine.

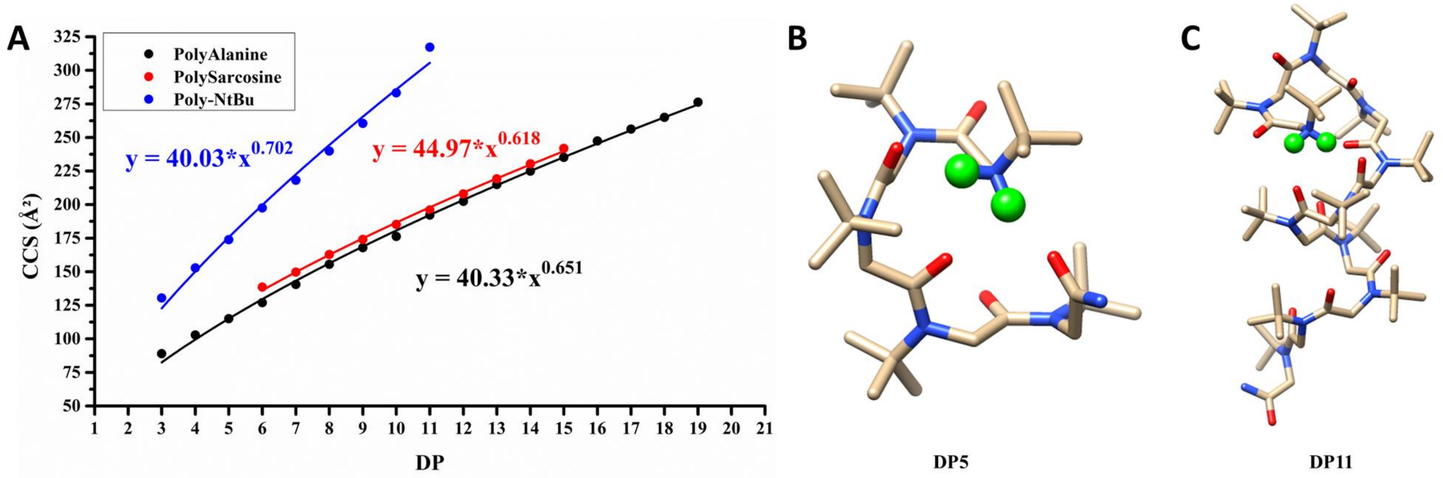

Figure 7: (**A**) Comparison of the CCS evolution against DP for poly-alanine, poly-sarcosine and poly-tert-butyl. Lowest energy structures for (**B**) $NtBu_5$ protonated at terminal amine and (C) $NtBu_{11}$ forming a hybrid loop-helix structure. Hydrogens on terminal amine have been highlighted in green while other hydrogens have been omitted for clarity.

Conclusion

The evaluation of peptoid secondary structures in the gas phase is a challenging task which requires robust theoretical and experimental methods. Indeed, the gas phase structure is governed by the charge, leading to loop structures to fully solvate this charge. However, this may not be a rule of thumb. Peptoids bearing bulky side chains are able to overcome the proton stabilization at relatively high DP (> 10). Since the proton is located at the most basic site, removing this specificity, for example by acetylating the terminal amine, could prevent the formation of loops.

Aknowlegdments

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