Identification of primary and secondary structures of Peptoids: Association of Molecular Modeling and Ion Mobility Mass Spectrometry

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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 Collision Cross Section (CCS) and by associating molecular modeling.

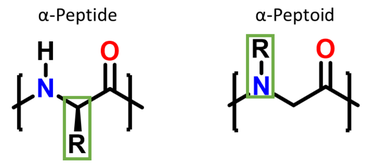

Figure 1: α-Peptide vs. α-Peptoid structure

Experimental section

Peptoids bearing (S)-1-phenylethyl (*Nspe*) side chains are synthesized on solid support using a step by step protocol involving a primary amine and a chloroacetic acid.

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 micromolar and directly infused into an Electrospray ionization source (ESI).

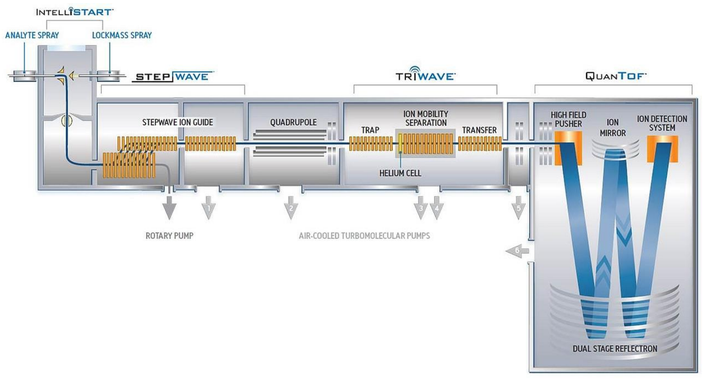

Figure 2: Waters Synapt G2-S*i*

Theoretical section

To obtain candidates structures, we used molecular mechanics and dynamics methods (MM/MD). The forcefield we employed is a reparametrized version of DREIDING for peptoids based on high-level QM calculations.

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 conditions.

Structures from the second MD were extracted and injected into the Collidoscope program to compute theoretical CCS (CCS_{th}) through the Trajectory Method (TM) [3]. This method is currently the most accurate to compute CCS and compare them to the CCS_{exp}.

Results and discussion

Non-acetylated peptoids *Nspe*$_n$ H$^+$ **Acetylated peptoids Ac*Nspe*$_n$ H$^+$**

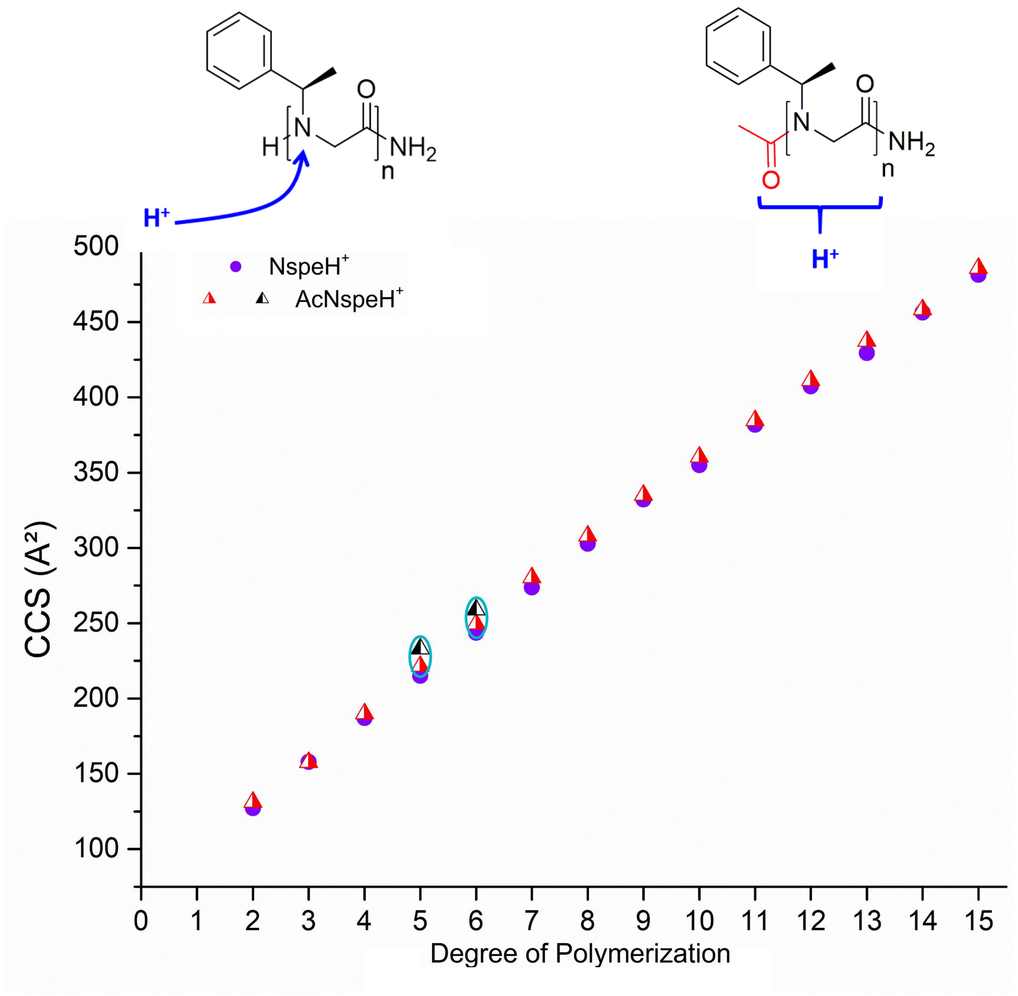

Figure 3: Evolution of the CCS for acetylated (▲◭) and non-acetylated (●) peptoids for DP ranging from 2 to 15. Two drift times are obtained for Ac*Nspe*$_5$ and Ac*Nspe*$_6$.

We observe a linear trend of the CCS_{exp}, either for acetylated or non-acetylated *Nspe*$_n$ peptoids. The most stable peptoid structures generated with our forcefield are loops over the proton until DP9 and then start forming a helix outside the loop (**Figure 4**). The CCS_{th} for these structures is within 2% of the CCS_{exp}. Therefore, we can conclude that peptoid gas phase structures are governed by the charge beard by the terminal amine.

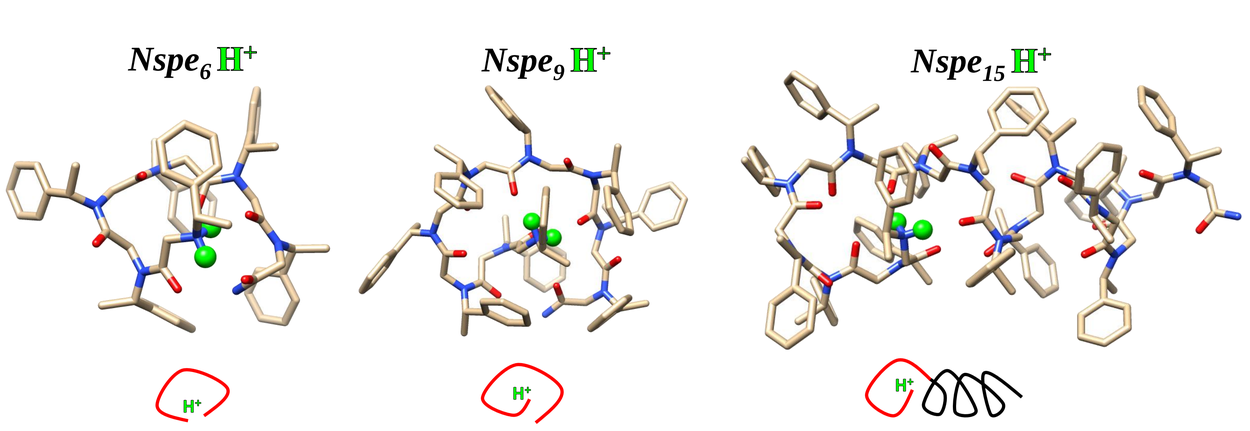

Figure 4: Structural evolution of the most stable structures (*Nspe*$_n$) generated with our forcefield. For DP lower than 9, a loop structure allows a full stabilization of the charge. When the DP is higher, additional units start forming a helix outside the loop.

However, for acetylated ones, we obtain asymmetric arrival time distributions for only two DP (5 and 6) which we deconvoluted (**Figure 3 and 5**). CCS_{th} of the most stable structure and also for a helical structure are in good agreement with CCS_{exp} suggesting that these peptoid could conserve the solution structure in gas phase (**Figure 5**). From these results, it is difficult to conclude about the conservation of the helical structure in gas phase since we only observe potentially a helical structure for two DP.

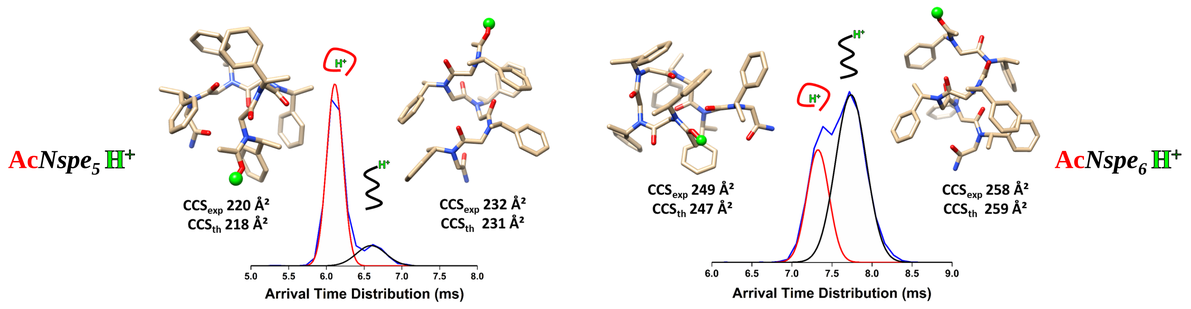

Figure 5: Asymmetric arrival time distributions for AcNspe$_5$ and AcNspe$_6$ with the associated CCS_{exp}, CCS_{th} and structures.

We hence investigated parameters that could help us decipher whether we have a conservation of the helical structure in gas phase. We based our approach on the computation of effective asphericity (Ω_{asp}) which is defined in **equation 1**.

$$\Omega_{asp} = \frac{\Omega_{exp} - \Omega_{sphere}}{\Omega_{linear} - \Omega_{sphere}} \qquad \textbf{eq. 1}$$

The effective asphericity helps to determine, on a scale from 0 to 1, whether the structure is extended or globular. A value of 0 means a globular structure while a value of 1 means a full extended structure. This method was previously established by Counterman *et al.* [4] for poly-alanine peptides (**Figure 6**). To determine Ω_{sphere} and Ω_{linear}, we computed CCS_{th} for peptoids of DP2 to 20 in fully globular shape and fully extended. We then computed the Ω_{asp} for the acetylated and non-acetylated peptoids.

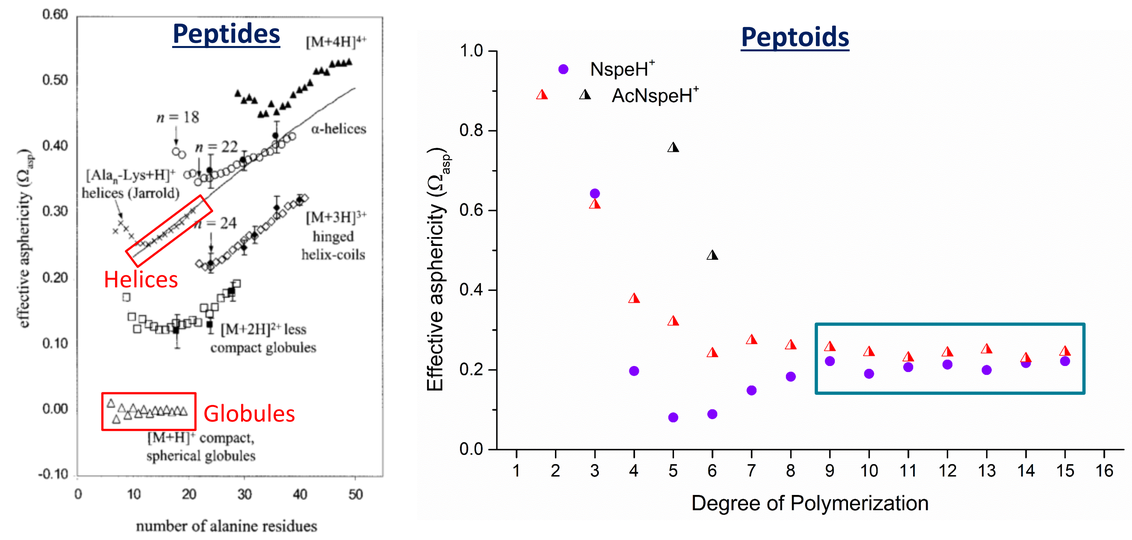

Figure 6: Effective asphericites for polyalanines peptides (from ref. [4]) and for peptoids.

The Ω_{asp} starts at high-value for low DP, then decreases and reaches a plateau from DP 9 to DP 15. It seems that the amine acetylation mainly influences low DP value (3 to 8) by slightly increasing the Ω_{asp}. However, these low values would mean that *Nspe*$_n$ peptoids form a globular structure which evolves in a less compact one, but not helical either. These results are consistent with results obtained from the most stable structures (**Figure 4**).

Conclusion

The evaluation of peptoid secondary structures in gas phase is a challenging task which required 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. For acetylated peptoids of DP6 and 6, this seems to be more complicated since we obtain two drift times, associated to a loop and a helical structure. By applying peptide parameters such as effective asphericities, peptoids would not adopt helical structures in gas phase but rather less compact globules.

Aknowlegdments

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