

Structural characterization of PAMAM dendrimers: Molecular modeling and ion mobility mass spectrometry investigation

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Introduction

Dendrimers are monodisperse hyperbranched polymers with a well-defined structure (Figure 1). Their step-by-step synthesis allows a control of the molecular structure, going from a generation (G) to the following increasing the number of surface groups. One of the most studied dendrimer is poly(amidoamine) (PAMAM), especially in the context of drug delivery. PAMAM dendrimers are biocompatible, water soluble and have a nanometric size, which are interesting properties for nanocarriers of drug molecules. Dendrimers can be used to transport drug molecules by covalently linking them to the surface groups of the dendrimer or by the formation of non-covalent complexes. Two possibilities exist for the later: encapsulation inside the dendrimer or electrostatic interaction at the surface [1]. The type of non-covalent complex will have an impact on the nanodrug properties and it is important to distinguish them. Up to now, the techniques usually used (solubility, equilibrium dialysis, NMR) cannot clearly identify the structure [2]. A way to fully describe the structure of the complexes would be to use Ion Mobility Mass Spectrometry (IMMS) and molecular modeling. But first, it is necessary to characterize the structure of the dendrimers alone to validate the method.

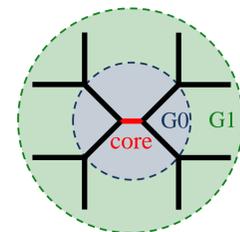


Figure 1: general structure of dendrimers

Experimental section

PAMAM with cystamine core (CYS) (Figure 2) G0 to G3 and with ethylene diamine core (EDA) (Figure 3) G0 and G1 were synthesized by a two step divergent method: alkylation of the amine with methyl acrylate, then amidation with ethylene diamine [3]. EDA PAMAM G2 and G3 are commercial products.

Ion mobility MS experiments were performed on a Waters Synapt G2-Si (Figure 4), equipped with Travelling Wave Ion Mobility (TWIM). Experimental collision cross sections (CCS_{exp}) were obtained via a polymer calibration (PEG 600, 1000, 2000 and 3350, and PLA 3300 and 5500) [4]. Dendrimer solutions were prepared at a concentration of 50 micromolar in a 1:1:1 mixture of H₂O:MeOH:ACN and infused directly in the ESI source.

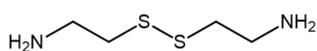


Figure 2: cystamine

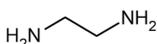


Figure 3: ethylenediamine

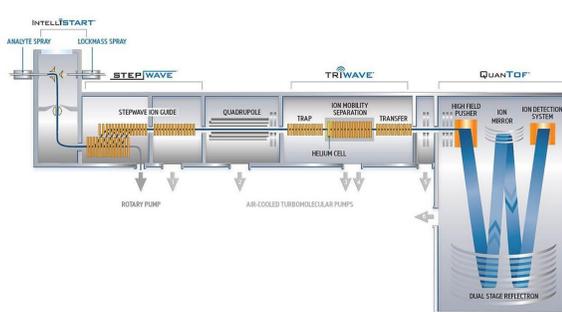


Figure 4: Waters Synapt G2-Si mass spectrometer

Theoretical section

PAMAM ions observed in IMMS were also studied by molecular mechanics and dynamics (MM/MD) with partially reparametrized DREIDING force field and COMPASS charges. The different charge states of PAMAM were protonated at terminal amine, maximizing the distance between the protons. The structures were optimized by two consecutive annealing from 5 to 800K for 50 cycles. The most stable structure for each charge state and generation was then submitted to an equilibration MD at 298K for 20 ns followed by a second identical dynamic. 200 frames from the second dynamic were injected into Collidoscope program to calculate the theoretical CCS (CCS_{th}) by the Trajectory Method (TM)

Results and discussion

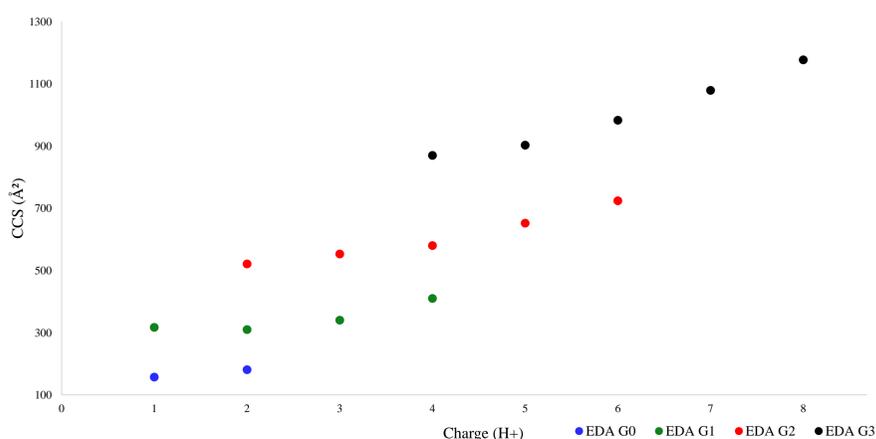


Figure 5: experimental CCS evolution of different generations of EDA PAMAM with the charge state. Colors are the same as in figure 6

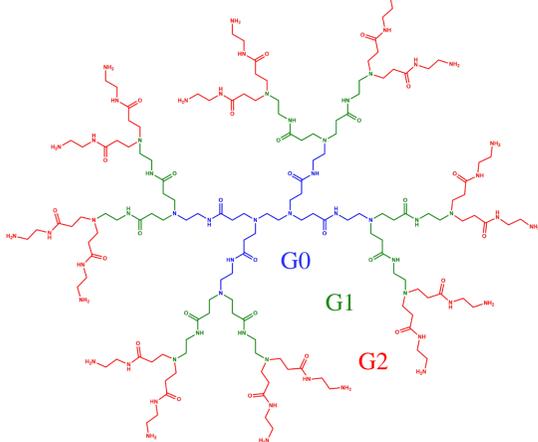


Figure 6: EDA PAMAM G2 with the different generations highlighted

Figure 5 shows that the CCS_{exp} increases with the number of charges at the surface of the dendrimer, for each of the 4 generations studied. This increase in CCS, and so the expansion of the 3D structure with the charges can be explained by the tendency of the ions to minimize the coulombic repulsion between the charged branches, going from globular structure to fully extended. Structure of these ions were calculated by MM/MD, along with the corresponding CCS_{th} in order to characterize them.

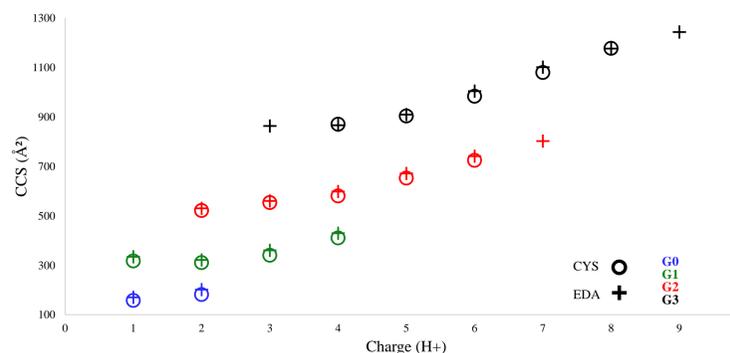


Figure 7: Experimental CCS of CYS and EDA PAMAM from G0 to G3

Comparison between CYS and EDA PAMAM shows that the core have a weak influence on the structure and its modification with charges (Figure 7). The comparison between experiment and theory also reveals a good agreement for CYS PAMAM.

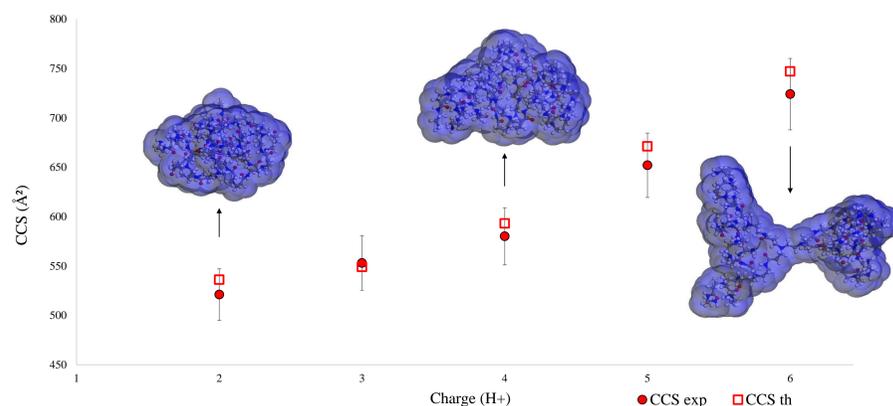


Figure 8: comparison between experimental and theoretical CCS for EDA PAMAM G2. Errors bars represent 5% of the experimental value, which is the usually admitted error. The structures come from the 2nd dynamic with the solvent accessible surface area (SASA) with He as solvent in blue.

The good agreement between CCS_{exp} and CCS_{th} (Figure 8) allows to determine the 3D structure of the ions in the gas phase. As supposed based on the experimental values, the ions with a low number of charges can stabilize them and stay globular, but the structure expands when the coulombic repulsion became too high and separates the branches of the dendrimer. As the CCS_{th} are calculated in He, the *solvent accessible surface Area (SASA)* with He as solvent was calculated. This surface can be seen as the surface on which the gas can collide and would be linked to the CCS. Indeed, figure 7 shows that the SASA increases with the expansion of the structure, and so with the increase of the number of charges.

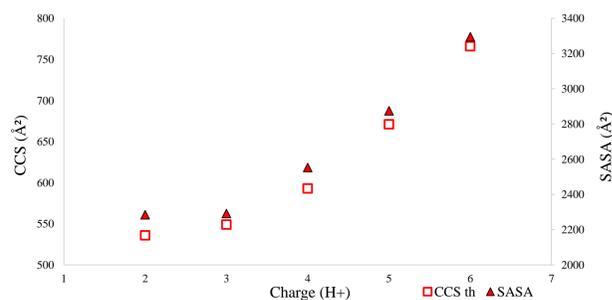


Figure 9: comparison between theoretical CCS and calculated SASA for EDA PAMAM G2

There is a correlation between the two theoretical parameters (Figure 9), so the SASA could be use for a quick qualitative comparison between theoretical structures and experimental CCS. Note that the CCS_{th} were averaged on 200 frames of the 2nd dynamic and the SASA was calculated only on the first frame of the same dynamic.

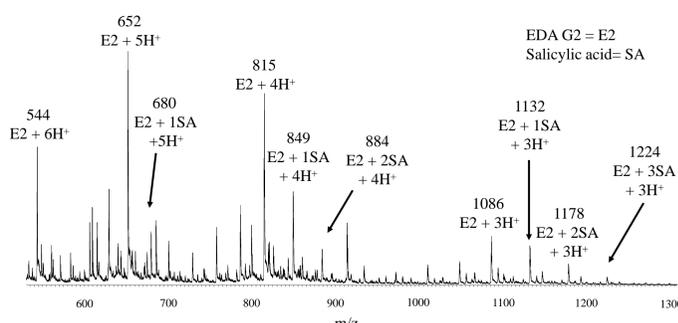


Figure 10: ESI-MS spectra of a solution 1:10 EDA G2 : salicylic acid in H₂O

Figure 10 shows non covalent complexes between EDA PAMAM G2 and salicylic acid. The stoichiometry changes with the charge state and complexes are fragile. They are non-specific and created by the interaction between protonated amine and carboxylic acid at the surface. No inclusion complexes were observed irrespective of the experimental conditions, the generation of PAMAM and the guest.

Conclusion

IMMS and molecular modeling allow to investigate the 3D structure of PAMAM ions in the gas phase; especially with a special attention paid to the charge state influence. The combination of theoretical and experimental data appears useful to characterize dendrimers and could also be used for investigating their non covalent complexes, if those are stable enough to survive the IMMS.

Acknowledgments

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