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Conformational sensitivity of conjugated poly(ethylene oxide)-poly(amidoamine) molecules to cations adducted upon electrospray ionization – A mass spectrometry, ion mobility and molecular modeling study

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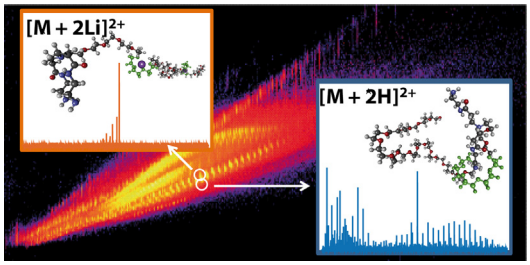
HIGHLIGHTS

- ESI-MS/MS, IMS and molecular modeling were combined to study PEO-PAMAM conformation.
- Protonated and lithiated molecules were studied, with charge states from 2 to 4.
- Protonation mostly occurred on PAMAM, with PEO units enclosing the protonated group.
- Lithium adduction on PEO units lead to more expanded conformations.
- Charge location strongly influenced PEO-PAMAM dissociation behavior.

GRAPHICAL ABSTRACT



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ABSTRACT

Tandem mass spectrometry and ion mobility spectrometry experiments were performed on multiply charged molecules formed upon conjugation of a poly(amidoamine) (PAMAM) dendrimer with a poly(ethylene oxide) (PEO) linear polymer to evidence any conformational modification as a function of their charge state (2+ to 4+) and of the adducted cation (H^+ *vs* Li^+). Experimental findings were rationalized by molecular dynamics simulations. The G0 PAMAM head-group could accommodate up to three protons, with protonated terminal amine group enclosed in a pseudo 18-crown-6 ring formed by the PEO segment. This particular conformation enabled a hydrogen bond network which allowed long-range proton transfer to occur during collisionally activated dissociation. In contrast, lithium adduction was found to mainly occur onto oxygen atoms of the polyether, each Li^+ cation being coordinated by a 12-crown-4 pseudo structure. As a result, for the studied polymeric segment (M_n = 1500 g mol^{-1}), PEO-PAMAM hybrid molecules exhibited a more expanded shape when adducted to lithium as compared to proton.

1. Introduction

Dendrimers have attracted particular attention for their potential as drug delivery vehicles by virtue of their well-defined structure and high drug payload confined within a nanosized

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volume [1]. Poly(amidoamine) (PAMAM) dendrimers, which bear amide functionalities as repeating units, primary amines on the dendrimer terminals and tertiary amines in the interior, are the most intensively investigated dendrimers for drug delivery [2]. We have recently demonstrated that structurally flexible PAMAM dendrimers [3] are effective nanovectors for nucleic acid delivery [4–10]. This promising result encourages us to undertake further investigation on targeted delivery using dendrimers conjugated with specific ligands or antibodies, which can recognize the corresponding receptors or proteins expressed on the cell surface. In this way, nucleic acid molecules can be delivered specifically to the cells of interest, leading to targeted delivery, which can further improve the delivery efficiency and reduce the toxicity by avoiding non-specific interactions and at lower doses. To conjugate targeting moiety to the PAMAM carrier, a poly(ethylene oxide) (PEO) polymer was employed as a linker between these two entities. The choice of PEO was based on the following issues [11]: firstly, PEO is soluble in water thanks to its hydrophilic character; secondly, PEO is neutral and supposed to be inert to avoid any possible interaction with either the targeting moiety or the PAMAM dendrimer; and last but not least, PEO has little toxicity or immunogenicity, allowing further clinical implementation. However, PEO is known for its inherent favorable affinity toward alkali ions, and interactions experienced by PEO-PAMAM molecules with different cations in cell compartments may affect their conformation and hence their delivery properties.

Combining mass spectrometry (MS) with ion mobility spectrometry (IMS) allows individual molecules, produced as naked ions in the gas phase, to be studied in terms of collision cross section (CCS), providing useful information about their conformation [12–14]. Such an IMS/MS coupling was shown to be particularly powerful for enhanced analysis of a wide range of synthetic polymers [15–19] including PEO [20–25], either to benefit from an additional level of separation, and hence improve oligomer resolution, or to evidence conformational changes as a function of the polymer chain length and/or charge state. Using electrospray ionization (ESI) to generate multiply protonated molecules in order to mimic the effect of decreasing pH, and implementing the IMS–MS coupling for ion analysis, we recently revealed that small PAMAM dendrimers experienced shape extension as a function of their charge state [26]. Gas phase ion conformation was controlled by the balance between intramolecular solvation and charge repulsion, the extent of which depended on relative location of cations in multiply charged molecules. Conformational information may also be obtained *via* collision-induced dissociation (CID) experiments since, as previously reported for proteins, conformations of gas phase ions can influence fragment formation [27]. Combining IMS and MS/MS data thus appears as a robust approach in cases where both techniques allow conformational changes to be monitored.

Owing to the particular structure of the PEO-PAMAM hybrid molecules, useful information regarding location of adducted charges should be obtained in MS/MS of lithiated or protonated species. On the one hand, as described from the pioneering work of Lattimer [28,29], PEO oligomers are best structurally characterized by MS/MS when cationized with lithium, in contrast to any other alkali adducts for which release of the naked alkali was the sole reported dissociation pathway. Due to strong interactions between multiple oxygen atoms of the PEO chain wrapped around the lithium cation [30,31], typical charge-assisted cleavages of in-chain bonds yield product ions carrying one or the other end-group [32], regardless of the precursor ion charge state [33]. As a result, so-obtained CID spectra typically exhibit various distributions of fragments spaced by 44/*z*. On the other hand, due to the presence of numerous amine groups, PAMAM dendrimers have mostly been analyzed by mass spectrometry as protonated species, and their activation was reported to induce two typical neutral loss sequences [34]: (i) loss of 1-(2-aminoethylamino)ethanol (102 Da) followed by elimination of *N*-methylene-1,2-ethanediamine and ketene (that is a total mass of 114 Da), or (ii) release of 5-aminopiperidin-2-one (114 Da) prior to the 102 Da neutral loss. Both dissociation routes could be envisaged either as charge-assisted or charge-remote processes.

In this study, lithiated and protonated PEO-PAMAM molecules produced by ESI were subjected to MS(/MS) and IMS experiments to evidence any conformational changes as a function of the nature and the number of adducted cations. Molecular dynamics simulations were performed to establish reliable molecular conformations for the different lithiated/protonated species. Because lithium is not usually found in biological cells and the role of the solvent is no longer considered in these techniques operating on gas-phase species, data obtained here are not expected to be directly transposable to the drug delivery context of this study. Nevertheless, they should shed light on the intrinsic behavior of the investigated PEO-PAMAM molecules and allow the robustness of this analytical approach to be validated.

2. Experimental

2.1. Chemicals

Methanol was from SDS (Peypin, France), formic acid and lithium chloride were from Sigma Aldrich (St. Louis, MO), and deuterated methanol (CD_3OD, 99.8%) was purchased from Eurisotop (Saint-Aubin, France). All chemicals were used as received without further purification. Polyalanine used for CCS calibration was from Sigma–Aldrich.

2.2. Synthesis

PEO-PAMAM (**1**) was synthesized according to the five-step synthetic sequence depicted in Scheme 1, starting from the functionalized PEG1500 (**2**) [35]. The two last steps followed the synthetic procedure for PAMAM synthesis described previously [6]. Details of each synthesis step, as well as all chemicals used, can be found in Supplementary information.

2.3. Mass spectrometry and ion mobility spectrometry

High resolution MS, MS/MS and traveling wave ion-mobility mass spectrometry (TWIM MS) experiments were performed with a Waters Synapt G2 HDMS quadrupole/time-of-flight (Q/ToF) tandem mass spectrometer (Manchester, UK), using the following parameters: ESI capillary voltage: +2.8 kV; extraction cone voltage: optimized for each sample in the +50 to +200 V range; desolvation gas (N_2) flow: 500 L h^{-1} (N_2); transfer CE: swept between 20 and 80 eV; trap gas flow: 2 mL min^{-1} (Ar); helium cell gas flow: 180 mL min^{-1}; ion mobility cell gas flow: 90 mL min^{-1} (3.45 mbar N_2); source temperature: 35 °C; desolvation temperature: 35 °C; IM traveling-wave height: 40 V; and IM traveling wave velocity: 650–1000 m s^{-1}. Data analyses were conducted using the MassLynx 4.1 and DriftScope 2.1 programs provided by Waters. The drift timescale of the TWIM MS experiments was converted to a collision cross-section scale, following the calibration procedure described by Smith et al. [36]. Briefly, the corrected collision cross sections of protonated polyalanine oligomers, obtained from published work [37], were plotted against the drift times (arrival times) of the corresponding ions measured in TWIM MS experiments at the same traveling-wave velocity, traveling wave height, and ion-mobility gas flow setting used for the PEO-PAMAM analytes. PEO-PAMAM samples were solubilized in methanol, subsequently diluted in methanol acidified with 1.0% (v/v) formic acid or methanol supplemented with lithium chloride (1 mM) to an