

Energetics of cationized PEG oligomers: a mass spectrometric and theoretical size dependence study.

Ph.D. Thesis

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Introduction

Mass Spectrometry plays a very important role in the elucidation of the structure of polymers and biopolymers: it yields highly sensitive, selective, specific and reliable data within a relatively short time. Compared to other commonly used analytical methods (e.g. chromatographic or spectroscopic), it measures not the average properties of a mixture of polymers but provides information on individual molecules instead.

Among mass spectrometric methods, multistage techniques are of particular interest. Its simplest form is called tandem MS and includes three steps: in the first, ions are mass selected, they are then fragmented via excitation with one of several techniques and finally all the ions coming out of this process are separated and detected. This way useful structural information can be obtained, related e.g., in the polymer field, to the end-group distributions or to the sequence of the molecule (e.g. homo- or copolymers). The most widespread form of this technique involves excitation in collisions with an inert gas, the so-called Collision Induced Dissociation (CID) technique. The amount of accessible information with the latter technique, however, depends on our ability to generate a *fast and efficient way to obtain and interpret mass spectra*. Therefore, a detailed understanding of the chemical and physical processes involved in such an experimental setup is required.

The CID technique is expected to be efficient and generate structural information on a wide molecular mass range. It is well known that the degree of fragmentation available with this technique decreases as the molecular ion size is increased. However, the energy imparted into the molecule can be increased, up to a limit, by increasing the collision energy and/or by increasing the number of collisions. It was, indeed, noted that for singly charged ions CID becomes inefficient (does not produce abundant fragments), when the molecular size increases over 2-3000 Da. The need to extend the range of molecules that can be studied by tandem mass spectrometry is so critical that it inspired further research in excitation techniques, and led to the development of various other techniques. However, there is still little knowledge about the efficiency of the fragmentation processes taking place in the collision cell; even if, it is empirically known to be strongly connected to the voltage used to “excite” the molecular ions in the cell.

The ionization step is crucial to obtain data from mass spectrometric experiments. As polymer compounds can be difficult to ionize efficiently by other techniques, an alternative consists in attaching a metal ion to the molecules to allow for their MS analysis. The

coordination propensity of a compound with respect to such an adduct ion relates directly to the binding energy of the newly formed molecular ion, which is important in two aspects. First, the strength of this attachment allows for the molecular ions to survive the ionization process and to be detected. Second, in the case this attachment is sufficiently strong, decomposition of the metal ion will be more difficult than breaking some bonds within the polymer, which would allow a sufficient degree of fragmentation to ensure that informative tandem mass spectra are generated.

To interpret tandem mass spectra usually requires first a detailed description of the fragmentation mechanism. Modelling techniques and especially application of the methods of quantum chemistry, accompanied by appropriate experiments can help describe these mechanisms. Theoretical methods can generally provide information on relatively simple molecules. Details earned from theoretical methods provide us with the necessary background to interpret tandem mass spectra not only of smaller molecules but also those of more complex analytes: understanding the fragmentation processes of oligomers indeed help in interpreting the spectra of polymers, copolymers or even mixtures of polymers.

Aims

The aim of this thesis is to investigate how the size –mass or polymerization degree– affects the structures and various energetic quantities related to cationized oligomers. To this end, a combined experimental (using the tandem mass spectrometric technique) and theoretical approach (using quantum chemistry modelling) was used to study ionization and decomposition of poly(ethylene glycols), $\text{HO}-(\text{C}_2\text{H}_4\text{-O})_n\text{-H}$ (PEG). Beside their great interest in the chemical industry and for pharmaceutical applications, PEGs are commercially easily available and well-characterized compounds. More interesting for our study is that the monomeric unit is made up of 3 “heavy” atoms only, allowing for extensive use of high-level modelling techniques extending to a large polymerization degree.

Synthetic polymers are, nowadays, studied less often than proteins and peptides by mass spectrometric techniques. It is, however, shown in this work that for systematic studies such polymers are excellent model compounds both for practical and theoretical purposes. This observation and the results presented below suggest that following this idea may help disentangle various molecular properties through systematic studies as a function of the polymerization degree.

Experimental and Computational details

Collision Induced Dissociation tandem mass spectrometry experiments have been carried out using different types of instruments: triple quadrupole, quadrupole time-of-flight and quadrupole ion trap with electrospray ionization technique. Using the concept of Survival Yield (ratio of the number of parent ions with the overall number of ions, parent and daughters ions, observed on the spectrum), energy-dependent studies as a function of the polymerization degree have been carried out.

Density functional theoretical (DFT) calculations were performed to elucidate the fragmentation mechanisms of lithium cationized poly(ethylene glycols) and how the energetics is modified as a function of polymerization degree. This modelling technique was also used to study the structure of cationized PEGs (with lithium, sodium, potassium and cesium). The levels of theory used were changed to assess the validity of the results. The B3LYP and M05-2X functionals were utilized with the following basis sets: 6-31G(d), Lanl2dz, TZVP as well as 6-311++G(2d,2p).

Results and Conclusions

Influence of the size –or mass– on the structure and energetics of synthetic polymers affects their tandem MS behaviour significantly. It is of importance from both practical and fundamental aspects:

- **The collision energy required to obtain similar fragmentation degree was observed to increase linearly with the mass of the polymer.** The collision energy needed to fragment the molecular ions has been related to their mass (i.e. their degree of polymerization) and has led to an excellent linear correlation. To this end we measured the fragmentation energetics of lithium cationized PEGs containing from 5 monomers up to 100 monomers (250 Da up to ~4.5 kDa). This study was performed at a variety of experimental conditions by using trapping and quadrupole-type instruments. A consequence of the use of different instruments is that the experimental conditions varied to a large extent: e.g. the nature and pressure of the collision gas, the experimental timescale and the range of voltages applied for excitation were all changed. Other compounds (ca. poly(tetrahydrofuran) as well as a set of peptides) were also measured to generalize our findings. This finding has both theoretical and practical importance. From a theoretical point of view it suggests fast internal energy randomization up to at least 4.5 kDa; so that statistical rate theories are applicable in this range. These results also suggest an easy method for instrument tuning for high-throughput structural characterization through tandem MS: after a standard compound is measured, the optimum excitation voltage is in a simple proportion with the mass of any structurally similar analyte at constant experimental conditions.
- **The fragmentation energetics of lithium cationized PEGs, namely the structure of the transition states associated with various fragmentation processes were determined by means of computational chemistry techniques.** This mechanistic study provides us with quantitative estimates of the activation energies for the most important processes observed. Moreover, starting with PEG 2 and going up to 6 monomers, barrier heights for these processes were computed using two DFT methods. Both methods suggest that the barrier height for the main fragmentation path monotonously decreases with the increase of polymerization degree, leading to values in

the range of 2.3 up to 3 eV (far higher than what is known for e.g. biopolymers). This observation may be related to the concept of electrostatic catalysis: the cation has an effect on the activation energy for fragmentation. This effect depends on the polymerization degree till the solvation shell completion, which strongly suggests that the molecular fragmentation parameters remain constant after the saturation of the shell. Note that these estimates are particularly significant as no such information for polymer compounds is available in the literature.

- **The structure of cationized PEGs has been determined by means of quantum chemistry methods.** Density functional theoretical methods including several basis sets and two functionals were used to collect information on the structure and energetic parameters of PEG coordinated by alkali metal ions. The oligomer chain is found to form a spiral around the alkali cation, which grows to roughly two helical turns when the oligomer size increases to about the dekamer for each alkali ion. Above this size the additional monomer units do not build further the spiral for Li^+ and Na^+ ; instead, they form less organized segments outside or next to the initial spiral. The distance of the first layer of coordinating O atoms from the alkali cation is 1.9 – 2.15 Å with 50 atoms for Li^+ , 2.3 – 2.5 Å with 60 for Na^+ , 2.75 – 3.2 Å with 70 for K^+ and 3.5 – 3.8 Å with 110 for Cs^+ complexes. The collision cross sections with He increase linearly with oligomer size with no sign of leaning toward the $2/3^{\text{rd}}$ power dependence characterizing spherical particles. The binding energy of the cation to the oligomer increases up to about 10 monomers, where it levels off for each adduct ion, indicating that this is approximately the limit of the oligomer size that can be influenced by the alkali cation. The binding energy – degree of polymerization curves are remarkably parallel for the four cations. The limiting binding energy at large polymerization degree is about 5.64, 4.77, 3.69 and 3.25 eV for Li, Na, K and Cs, respectively. When available, geometrical features are compared with X-ray and neutron diffraction data on crystalline and amorphous phases of conducting polymers formed by alkali-metal salts and PEG showing very good agreement.

These results are important for theoretical reasons as they all exhibit a clear tendency of the changes of properties with the size of the polymer and provide us information concerning the nature of the changes. For practical purposes, the Degrees of Freedom effect is important as after a standard compound is measured, the optimum excitation voltage is proportional with the

mass of any structurally similar analytes at constant experimental conditions, facilitating high-throughput applications. The calculated details of the structure and energetics of cationized PEGs are useful for the experimentalist: they help in the optimization of e.g. ionization conditions.

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List of communications.

Publications related to the PhD thesis.

1. **A. Memboeuf**, A. Nasioudis, S. Indelicato, F. Pollreis, A. Kuki, S. Kéki, O.F. van den Brink, K. Vékey, L. Drahos; “*Size effect on fragmentation in tandem mass spectrometry*”. Analytical Chemistry, 2010, 82, 2294-2302.
2. **A. Memboeuf**, L. Drahos, G. Lendvay, K. Vékey; “*Energetics of fragmentation for cationized poly(ethylene glycols) oligomers*”. Rapid Communications in Mass Spectrometry, 2010, 24, 2471-2473.
3. A. Kuki, L. Nagy, **A. Memboeuf**, L. Drahos, K. Vékey, M. Zsuga, S. Kéki; “*Energy-dependent collision-induced dissociation of lithiated poly(tetrahydrofuran): effect of the size on the fragmentation properties*”. Journal of the American Society for Mass Spectrometry, in press, available on-line.
4. **A. Memboeuf**, K. Vékey, G. Lendvay; “*Structure and energetics of poly(ethylene glycols) cationized by Li^+ , Na^+ , K^+ , Cs^+ : a first principles study*”. European Journal of Mass Spectrometry, in revision.

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5. **A. Memboeuf** and S. Aubry; “*Targeted Energy Transfer between a rotor and a Morse oscillator: a model for selective chemical dissociation*”. *Physica D-Nonlinear phenomena*, 2005, 207, 1-23.
6. J. Sztaray, **A. Memboeuf**, L. Drahos, K. Vékey; “*Leucine Enkephalin – a mass spectrometry standard*”. *Mass Spectrometry Reviews*, in press, available on-line.
7. N. PraveenGanesh, C. Candia, **A. Memboeuf**, G. Lendvay, Y. Gimbert, P.Y. Chavant; “*Improved dimethylzinc-promoted vinylation of nitrones with vinylboronic esters*”. *Journal of Organometallic Chemistry*, in press, available on-line.
8. J. Song, **A. Memboeuf**, R.M.A. Heeren, K. Vékey, O.F. van den Brink; “*Discrimination between charge-catalyzed and charge-independent fragmentation processes of cationized poly(*n*-butyl acrylate)*”. *Rapid Communications in Mass Spectrometry*, accepted 2010/08/28.
9. A. Nasioudis, **A. Memboeuf**, R.M.A. Heeren, D.F. Smith, K. Vekey, L. Drahos, O.F. van den Brink; “*Discrimination of polymers by using their characteristic collision energy in tandem mass spectrometry*”. *Analytical Chemistry*, submitted on 2010/07/24.

Lectures related to this thesis:

1. 57th American Society for Mass Spectrometry: 31st May-4th June 2009, USA: *poster*
«MS of poly(ethylene glycols): evidence of structural and energetic interdependence.»
2. 27th Informal Meeting in Mass Spectrometry: 3rd-7th May 2009, AUSTRIA: *oral*
«Fragmentation behaviour of PEG oligomers on different types of instruments.»
3. MTA napok, 3rd-5th December 2008, HUNGARY: *oral*
«MS of poly(ethylene glycols): evidence of structural and energetic interdependence.»
4. 25^{èmes} Journées Françaises de Spectrométrie de Masse: 8th-11th September 2008, FRANCE: *oral*
«Quantum chemistry of poly(ethylene glycols) fragmentation: a size dependence study.»
5. 26th Informal Meeting in Mass Spectrometry: 4th-8th May 2008, ITALY: *oral*
«Size dependence study of poly(ethylene glycols) fragmentation.»