Available online at www.sciencedirect.com

ScienceDirect

International Journal of Mass Spectrometry 272 (2008) 1–11

International Journal of Mass Spectrometry

www.elsevier.com/locate/ijms

Tandem mass spectrometry of doubly charged poly(ethylene oxide) oligomers produced by electrospray ionization

Marion Girod[a], Yannick Carissan[b], Stéphane Humbel[b], Laurence Charles[a,*]

[a] *Université de Provence – CNRS, Laboratoire Chimie Provence – UMR 6264, Spectrométries Appliquées à la Chimie Structurale, 13397 Marseille cedex 20, France, France*

[b] *Université Paul Cézanne – CNRS, Institut des Sciences Moléculaires de Marseille – UMR 6263,13397 Marseille cedex 20, France*

Received 3 July 2007; accepted 10 December 2007
Available online 23 December 2007

Abstract

Electrospray ionization combined with collision-induced dissociation has been applied to characterize fragmentation pathways of doubly charged poly(ethylene oxide) polymers. MS/MS spectra of doubly lithiated precursors were shown to provide the most informative data for this polymer structural analysis. Most fragment ions could be accounted for by applying the mechanisms proposed in the literature for singly charged oligomers. Although MS/MS experiments were conducted in a low collision energy regime, radical cationic fragments were also detected and were shown to further dissociate. This consecutive dissociation was explored performing hydrogen/deuterium exchange experiments and *ab initio* studies. Two different mechanisms had to be envisaged depending on the size of the fragmenting radical cationic species.

Keywords: Doubly charged oligomer; Electrospray; MS/MS; PEG

1. Introduction

Mass spectrometry (MS) has become an increasingly important tool for polymer analysis since the development of soft ionization methods. As oligomers can be detected as intact molecular adducts, data from matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI) mass spectra can be utilized to determine the size of repeat units as well as end group masses, average molecular weights and polymerization degree. Further structural characterization of the polymer repeat unit and end-groups requires oligomer ions to be fragmented, in collision-induced dissociation (CID) or post-source decay (PSD) experiments.

Structural study of low molecular weight poly(ethylene glycol)s (PEG) was first reported by Lattimer, using fast atom bombardment (FAB) to generate precursor ions [1,2]. In this pioneering work, fragmentation pathways of PEG oligomers were proposed and a first nomenclature was established. Four main fragment ion series were described and named A, B, C and D according to the ion structure. In case of asymmetric polymers, the letters A, B, C and D have been restricted to those daughter ions which still contain the intact ω end-group from the precursor whereas A′, B′, C′ and D′ were used to name fragments with the initial α end-group. More recently, CID studies of small PEG polymers using MALDI [3–8], ESI [9,10] or DESI [11] to generate oligomer adducts have been reported. The same fragment ions as those described by Lattimer were observed and nomenclature used to label peaks in the MS/MS spectra has only slightly evolved, according to specific end-group definitions.

In most reported studies, the choice of alkali metal cation to be adducted to PEG molecules was shown to have a great impact in the production of fragment ions under CID conditions [1,2,6–9,12]. MS/MS spectra of lithiated precursors $[M+Li]^+$ were shown to be the most informative for structural analysis of PEG whereas a bare alkali cation was often the only reported daughter ion arising from dissociation of sodium or potassium adducts. It was concluded that structurally significant fragments could only be produced in the case of strong interactions within the ionic adduct, which are known to increase as the cation size decreases.

To our knowledge, PEG fragmentation studies were mainly performed on singly charged molecules. Since multiple charging is readily achieved during ESI of moderate size PEG [13], study

---

[*] Corresponding author. Tel.: +33 4 91 28 86 78; fax: +33 4 91 28 28 97.
E-mail address: laurence.charles@up.univ-mrs.fr (L. Charles).

1387-3806/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2007.12.010

of the MS/MS behaviour of multiply charged oligomers was thus of interest.

2. Experimental

2.1. Chemicals

Methanol and HPLC grade water were purchased from SDS (Peypin, France). All chemicals were from Sigma–Aldrich (St. Louis, MO). Poly(ethylene glycol) hydroxyl terminated, PEG1000, and poly(ethylene oxide) methyl terminated, PEO-Me1100, were first dissolved in water and further diluted using methanolic salt solutions to a final 100 $\mu g\,mL^{-1}$ concentration. LiCl, NaI, KCl, RbI and CsI salts were used at a 10 mM concentration.

2.2. MS

All experiments were performed with a QStar Elite mass spectrometer (Applied Biosystems SCIEX, Concord, ON, Canada) equipped with an ESI source operated in the positive ion mode. The capillary voltage was set at 5500 V and the cone voltage at 80 V. In this hybrid instrument, ions were measured using an orthogonal acceleration time-of-flight (oa-TOF) mass analyzer. A quadrupole was used for selection of precursor ions to be further submitted to CIDs in MS/MS experiments. Nitrogen was used as the nebulizing gas (10 psi), the curtain gas (20 psi) as well as the collision gas. Collision energy was set according to the experiments. Analyst software version 2.1 was used for instrument control, data acquisition and data processing. Direct sample introduction was performed at a 5 μL/min flow rate using a syringe pump.

2.3. Computational details

Geometry optimisations were performed using the density functional theory (DFT) method as implemented into Gaussian 03 Revision C.02 [14]. In this study we used the B3LYP functional for all calculations, using the unrestricted formalism. The basis set used was 6–311g (2d,p). Spin contamination was checked for each calculation: $\langle S2 \rangle$ was always found to be 0.00 for closed shell systems and between 0.75 and 0.76 for doublets. All energy differences for dissociations were corrected of basis set superposition error (BSSE) by use of the counterpoise correction method on the optimal geometries.

3. Results and discussion

3.1. Effect of cationizing agent

When submitted to ESI, PEG1000 oligomers were mostly detected as doubly cationized molecules. Signal from singly charged oligomers was measured to be about 3% of the total ion current (TIC). The propensity of poly(ethylene glycol) oligomers to desorb as doubly charged molecules under electrospray conditions was shown to be independent of the cationizing agent present in solution. The behaviour of doubly charged oligomers was studied in MS/MS experiments as a function of the adducted cation. MS/MS spectra presented in Fig. 1 were obtained after dissociation of $[21\text{-mer} + 2\text{Cat}]^{2+}$, with Cat = Li, Na, K, Rb or Cs, at a 30 eV collision energy (laboratory frame). From Fig. 1b–e, a common fragmentation pattern was observed, which consists of the production of two singly charged daughter ions, the singly charged oligomer $[21\text{-mer} + \text{Cat}]^{+}$, and the bare alkali cation, Cat^{+}. However, the very different abundances measured for these two ions would indicate the bare alkali cation also arises from dissociation of singly charged oligomer which would consecutively eliminate the oligomer molecule as a neutral. Moreover, the production rate of Cat^{+} was observed to increase with the size of the alkali, despite the center-of-mass collision energy decreases. For example, relative intensity of Cat^{+} as compared to the precursor ion was 0.19 when sodium was used as the cationizing agent (that is, in a MS/MS experiment with a center-of-mass collision energy of 0.827 eV) whereas the same ratio calculated from the dissociation of $[21\text{-mer} + 2\text{Cs}]^{2+}$ at 0.680 eV was found to be 273. These data are consistent with those previously reported, indicating the strength of interactions within the ionic adduct increases as the cation size decreases [1,2,6–9,12]. Different results were obtained when lithium was used as the cationizing agent (Fig. 1a). The bare Li^{+} cation could hardly be observed and numerous additional fragments were detected. As for singly charged PEG, MS/MS spectra of doubly lithiated PEG oligomers appear to yield the most informative data. Lithium was thus selected to further study fragmentation pathways of doubly charged PEG oligomers.

3.2. MS/MS of doubly charged PEG-Me

Due to molecule symmetry, dissociation of poly(ethylene glycol) adducts can occur through different routes to yield the same fragment ions [1,2]. Since reaction mechanisms can only be evidenced using an asymmetric polymer, a PEG methyl ether polymer (PEG-Me1100) was chosen, with CH_3O– and H– as α and ω end groups, respectively. The MS/MS spectrum of the doubly lithiated 23-mer, detected at *m/z* 529.3, is presented Fig. 2. Most peaks observed in this MS/MS spectrum could have been accounted for, using mechanisms established for the dissociation of singly charged PEG oligomers [1,2], which would only be briefly described here.

The first mechanism, reported as a charge-induced reaction in the case of singly lithiated oligomers, should allow two series of ions to be produced in the case of doubly charged precursors. Indeed, depending on the relative position of the two cations on the oligomer backbone at the time of dissociation, two singly charged products (Scheme 1a) or a doubly charged molecule and a neutral (Scheme 1b) would be formed after ethylene elimination. Peak assignment was achieved based on the expected ion *m/z* values (as indicated in Scheme 1) and validated by isotopic information. Indeed, selection of doubly charged precursor ions was performed in a quadrupole analyzer which resolution was intentionally lowered to allow both the monoisotopic ion and its one ^{13}C counterpart to be simultaneously submitted to dissociation. For peaks measured at *m/z* values lower than that of the precursor ion, doubly charged daughter ions could thus be

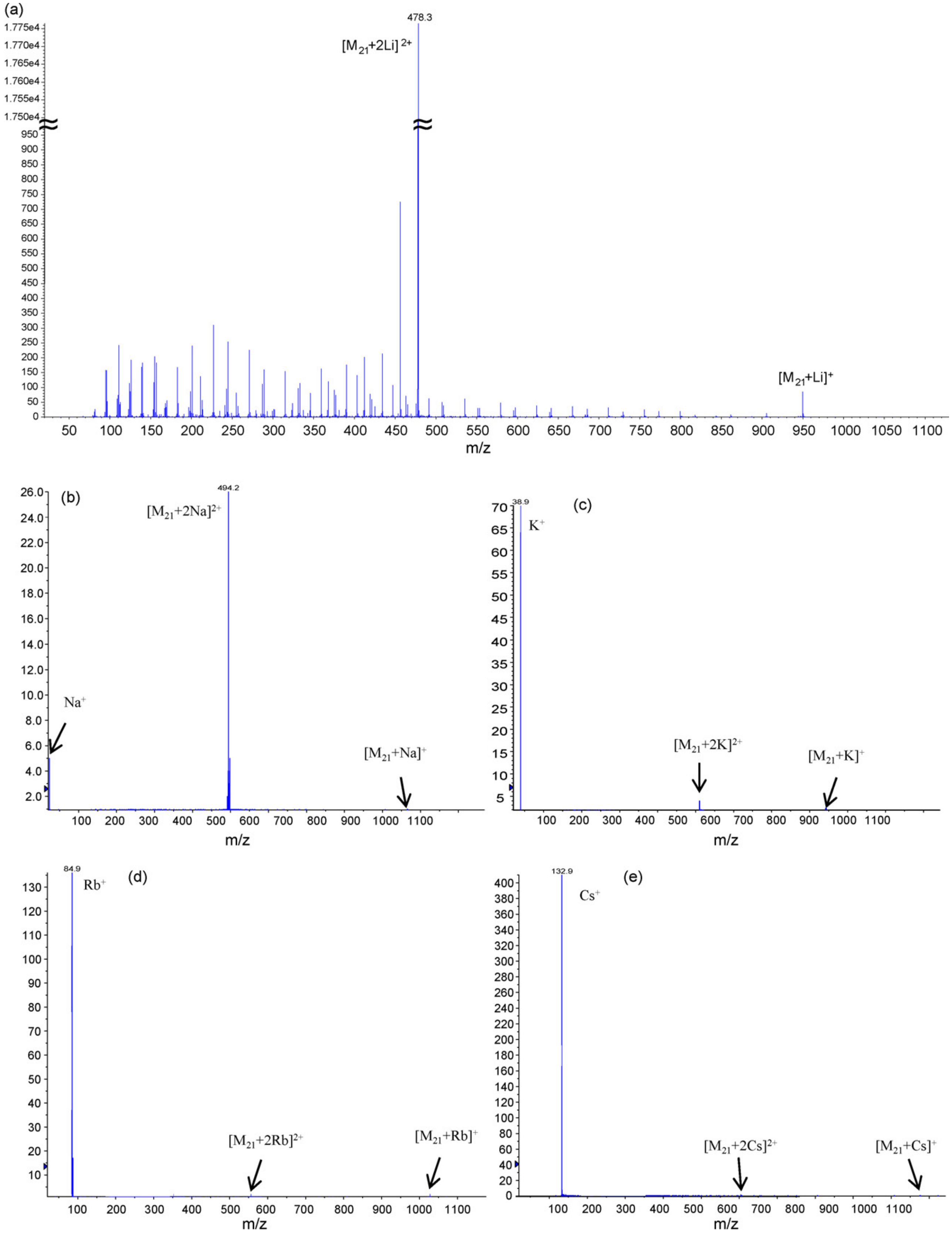


Fig. 1. ESI-MS/MS spectra of doubly charged PEG1000 21-mer with (a) Li^+, (b) Na^+, (c) K^+, (d) Rb^+ and (e) Cs^+ as the cationizing agent.

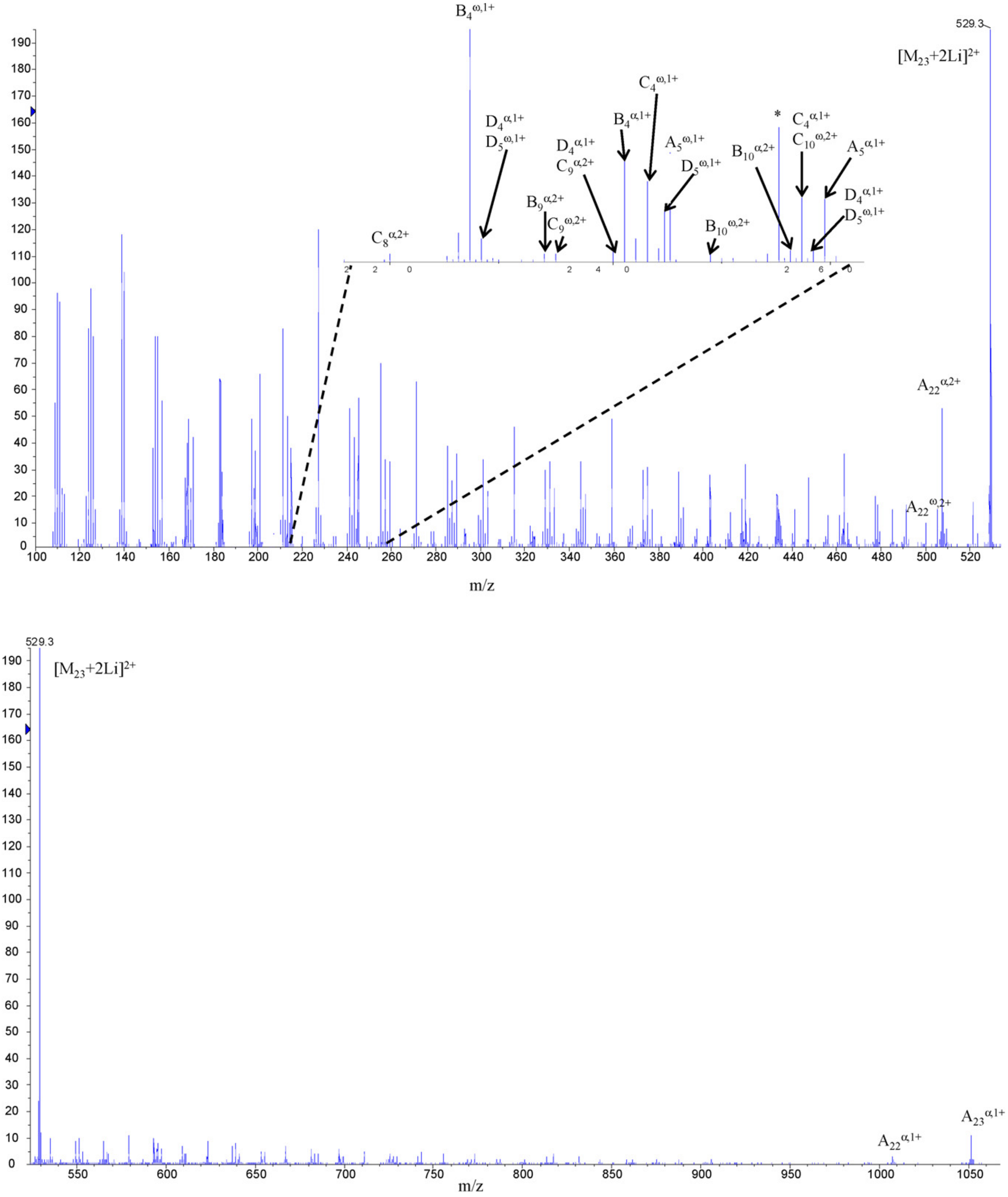
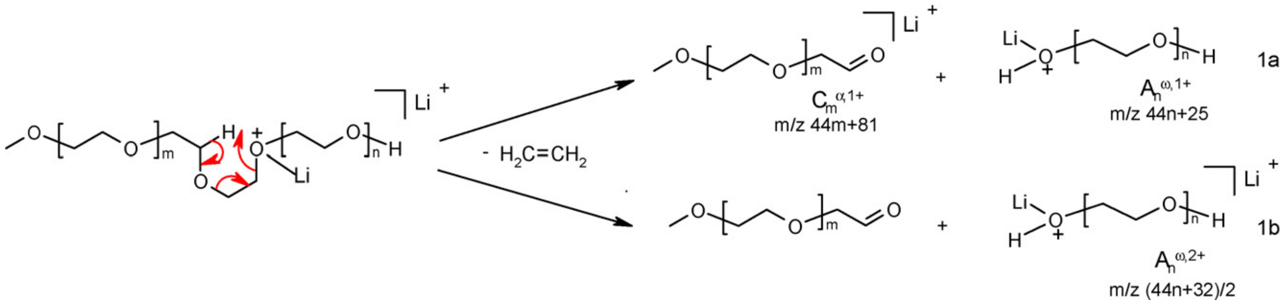


Fig. 2. ESI-MS/MS spectrum of doubly lithiated PEG-Me1100 23-mer (collision energy: 60 eV, laboratory frame).

distinguished from singly charged ones based on their partial isotope pattern. In Scheme 1a and b, the A^{ω} series ions are lithiated dihydroxyl terminated PEG oligomers, i.e., they still contain the ω end group from the precursor ion. The subscript indicates the number of intact monomer units within the A ion structure and the charge state is mentioned in superscript. In Fig. 2, $A_n^{\omega,1+}$ ions were observed from $n = 2–22$ while $A_n^{\omega,2+}$ ions were detected from $n = 13–22$. In Scheme 1a, $A_n^{\omega,1+}$ ions are formed together with $C_m^{\alpha,1+}$ ions, i.e., lithiated formyl-terminated oligomers containing the original α end-group. From the doubly lithiated



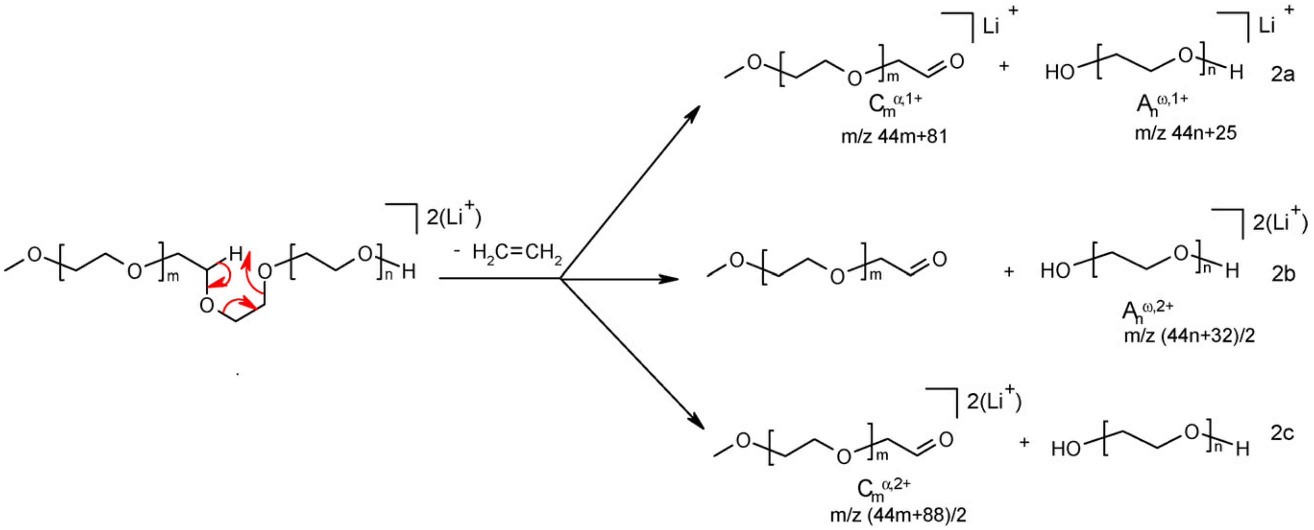
Scheme 1.

23-mer, $C_m^{\alpha,1+}$ ions with $m = 1–16$ were produced. Fragmentation reactions shown in Scheme 2 would produce the same $A_n^{\omega,1+}$ and $C_m^{\alpha,1+}$ ion series as those defined in Scheme 1. As the result of a charge-remote mechanism, the sites of both lithium attachments to the molecule are uncertain in contrast to the situation depicted in Scheme 1. Consequently, a third route could be envisaged (Scheme 2c) yielding $C_m^{\alpha,2+}$ ions detected from $m = 8–20$ in Fig. 2. The A^{ω} ion series can also be produced as singly or doubly charged molecules according to the reaction presented in Scheme 3. Vinyl-terminated $B_m^{\alpha,1+}$ oligomers ions would be formed complementary to $A_n^{\omega,1+}$ ions (Scheme 3a) and were detected from $m = 1–19$ while their doubly charged homologues, alternatively produced according to Scheme 3b, were observed from $m = 9–21$.

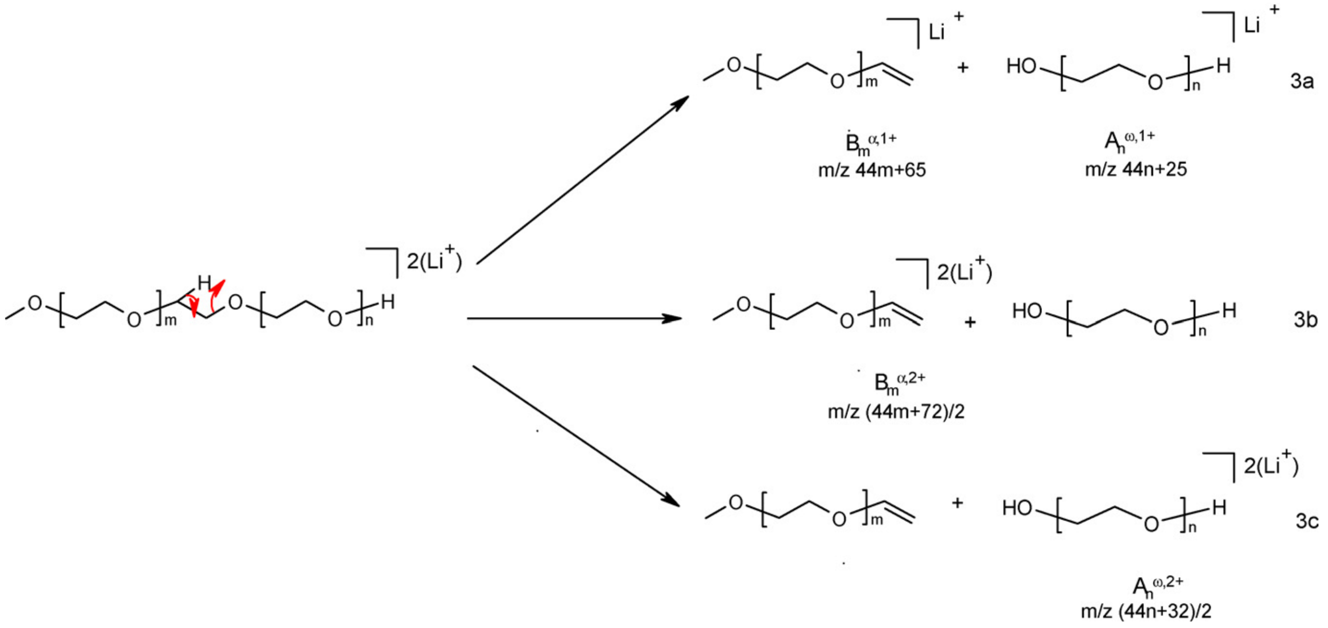
Another route to produce singly and doubly charged C^{α} is presented in Scheme 4. After elimination of a hydrogen molecule, three sets of products can be formed depending on the relative position of the two cations at the time of the dissociation. The same reaction could lead to $C_m^{\alpha,1+}$ ions together with $B_n^{\omega,1+}$ (Scheme 4a) or to the production of doubly charged molecules such as $C_m^{\alpha,2+}$ (Scheme 4b) or $B_n^{\omega,2+}$ (Scheme 4c). B_n^{ω} were detected from $n = 2–19$ with charge state +1 and from $n = 10–21$ with charge state +2.

From the charge-induced dissociation described in Scheme 5, ethylene elimination would result in the formation of new product ion series: two singly charged products, $A_m^{\alpha,1+}$ and $C_n^{\omega,1+}$ ions (Scheme 5a) or one doubly charged molecule, $A_m^{\alpha,2+}$, and one neutral (Scheme 5b). Data from Fig. 2 indicate $[M_{23} + 2Li]^{2+}$ dissociate according to Scheme 5 to yield $A_{2–23}^{\alpha,1+}$ ions, $C_{1–16}^{\omega,1+}$ ions and $A_{13–22}^{\alpha,2+}$ ions. Note that the peak observed at *m/z* 1051 was labelled $A_{23}^{\alpha,1+}$ based on its structure but this ion would actually be produced as the precursor ion eliminate a Li^+ cation.

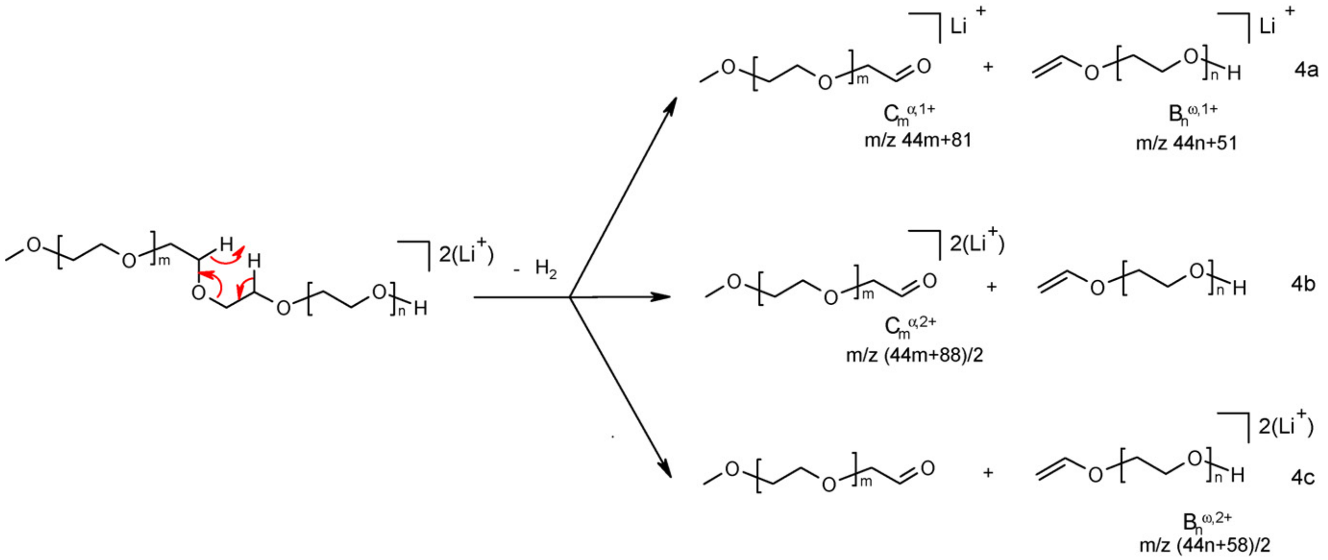
The formation of A, B and C series ions can be accounted for by multiple dissociation reactions, the main of which being described above. Therefore, relative ion abundances in the MS/MS spectra could not be used to figure out which set of products is preferentially formed in each of the presented reactions. Moreover, all singly charged fragments could also arise from consecutive dissociations of $A_{23}^{\alpha,1+}$, which has the same structure as a singly charged PEG-Me oligomer and is formed at low collision energy. However, such contribution to the daughter ion abundance is assumed to be quite low. Indeed, MS/MS experiments performed on the singly charged 23-mer precursor ion, obtained from in-source dissociations of $[M_{23} + 2Li]^{2+}$, showed that a pretty high collision energy (>100 eV, laboratory frame) is required to produce few fragments of low intensity. This indicates a higher fragmentation efficiency of doubly charged polymer adducts, as compared to their singly charged homologues, as previously reported [13,15].



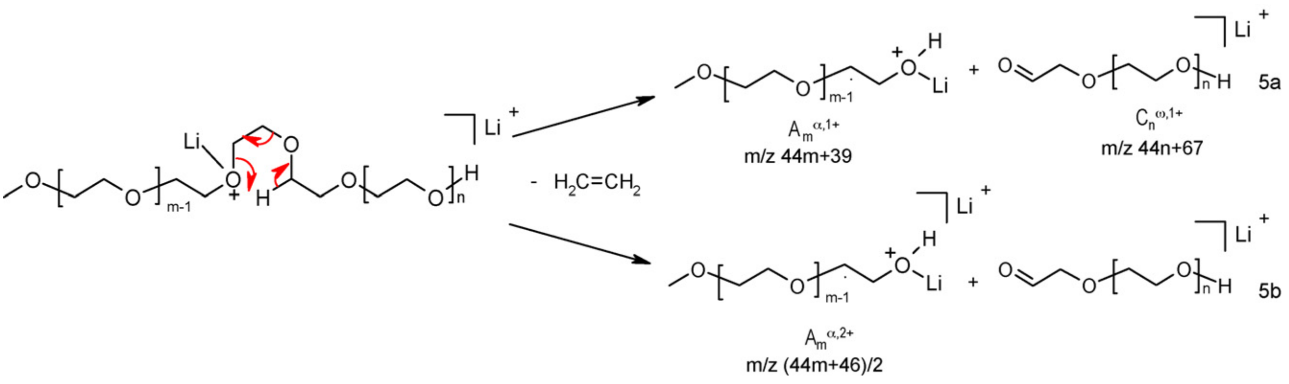
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.

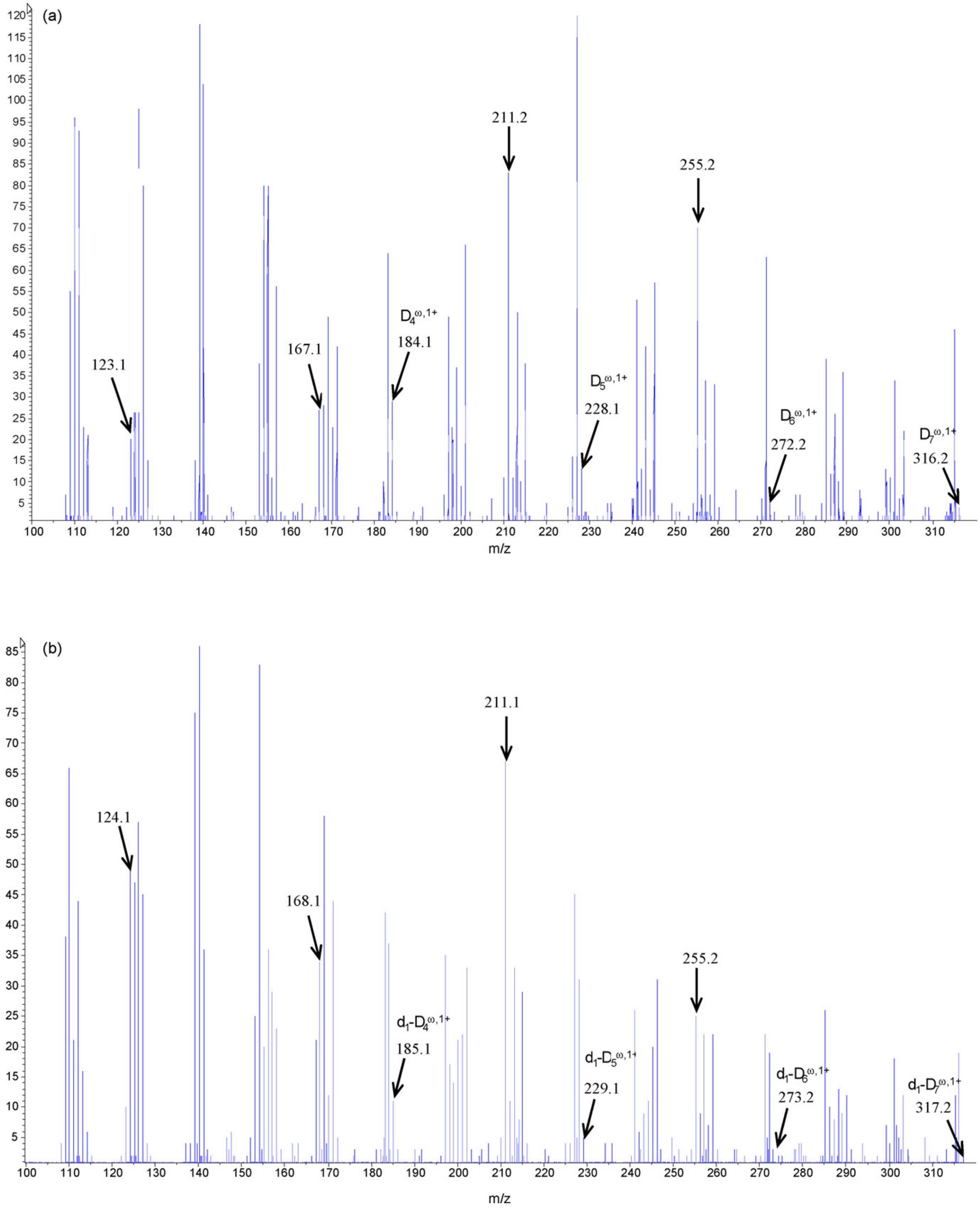
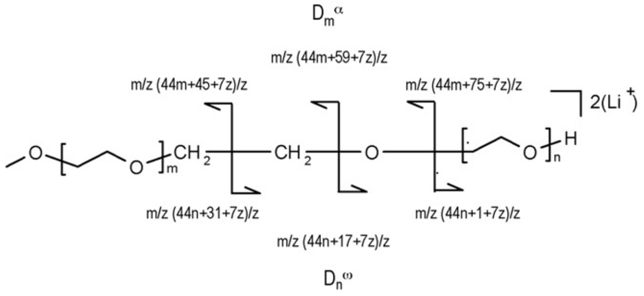


Fig. 3. Details of the low mass range (*m*/*z* 100–320) of the ESI-MS/MS spectrum of doubly lithiated PEG-Me1100 23-mer (a) before and (b) after H/D exchange.



Scheme 6.

Dissociation pathways described in the literature to lead to cationic fragments from singly charged PEG oligomers [1,2] were thus observed to occur from doubly charged precursors, except for the so-called E fragment arising from an internal elimination of two ethylene oxide groups. In addition, numerous doubly charged daughter ions were observed. However, singly charged fragments still dominated the MS/MS spectrum, as already reported in CID of highly charged polymers [15]. Interestingly, although reported to proceed with maximum efficiency at high (keV) collision energy [12], elimination of radical species was also observed in the present experiments. These decompositions produce radical cations, systematically reported as D ions regardless of the nature of the bond actually cleaved [1–3,6]. The same notation has been adopted for its simplicity (Scheme 6). As for previous series, depending on the position of the two Li^+ on the precursor ion relatively to the cleaved bond, D_m^{α} and D_n^{ω} could be expected as singly or doubly cationized radical species. However, since D series mainly contain low mass ions, only singly charged species could be detected as $\mathrm{D}_{1\text{–}7}^{\alpha,1+}$ and $\mathrm{D}_{3\text{–}7}^{\omega,1+}$.

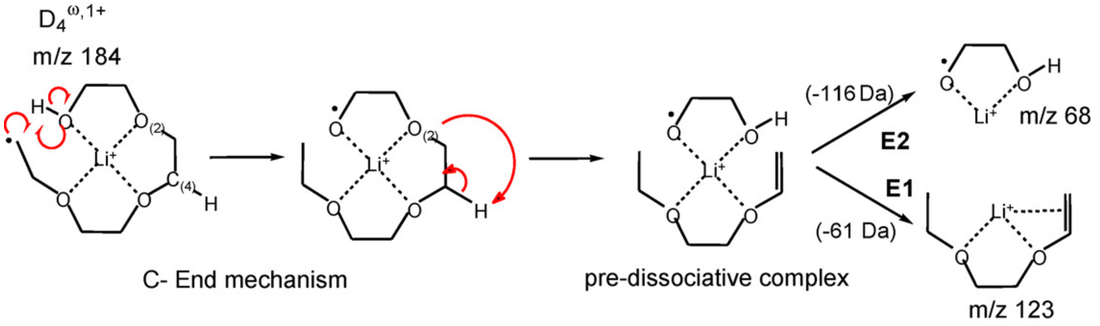
In addition, a new series of singly charged ions was observed in the low mass range of the MS/MS spectrum at *m*/*z* 123, 167, 211 and 255 (Fig. 3a). Accurate mass measurements suggest that those $\mathrm{D}_{4\text{–}7}^{\omega,1+}$ ions arising from the homolytic cleavage of the O–C bond (Scheme 6) would consecutively eliminate a $\mathrm{C_2H_5O_2}$ radical species. To tentatively describe this reaction mechanism, conformation of these dissociating $\mathrm{D}_{4\text{–}7}^{\omega,1+}$ radical cations was explored in the following *ab initio* study.

3.3. *Ab initio study of $\mathrm{D}_{4\text{–}7}^{\omega,1+}$ radical cations*

The $\mathrm{D}_{4\text{–}7}^{\omega,1+}$ radical ion species generated after homolytic cleavage of a O–C bond contain different numbers of oxygen atoms, each of which is a potential binding site for the lithium cation [16,17]. Conformation of these ions corresponding to energy minima in each case are displayed in Fig. 4. All structures present the same basic features. The lithium cation is buried deep in the oligomer chain and is coordinated to as many oxygen atoms as possible. The oligomer chain appears to be quite flexible and warps itself around the lithium cation [18]. The coordination layer takes the shape of a square or a square based pyramid for *n* equals 4 or 5, respectively. For $n \geq 6$, all oxygen atoms cannot coordinate the lithium for steric issues: only five oxygens coordinate the lithium forming a square based pyramid. The atoms of the coordination layer strongly interact with the lithium. The Li–O distance varies between 1.95 and 2.18 Å. No significant variation of this distance was found when the number of oxygen atoms increases. Li^+ always remains tightly bound to the oligomer chain. Based on atomic spin densities calculated for the each $\mathrm{D}_n^{\omega,1+}$ radical cations, the unpaired electron is located at the C atom of the terminal $\mathrm{CH_2}$ group.

From these calculated conformations, a fragmentation mechanism was proposed as described in Scheme 7 for $\mathrm{D}_4^{\omega,1+}$. It consists of a hydrogen radical transfer from the hydroxyl end-group to the terminal C atom ($\mathrm{C}_{(8)}$ in the case of the $\mathrm{D}_4^{\omega,1+}$ ion) followed by a proton transfer from $\mathrm{C}_{(4)}$ to $\mathrm{O}_{(2)}$. This mechanism was named the “C-End mechanism”. The resulting lithium ion-bound pre-dissociative complex could then lead either to the cation detected at *m*/*z* 123 (E1) or to the distonic radical cation $\mathrm{Li}^+\cdots{}^\bullet\mathrm{O{-}CH_2{-}CH_2{-}OH}$ expected at *m*/*z* 68 (E2). The first reaction was calculated to be less endothermic ($\mathrm{E1} = +162.2\,\mathrm{kJ\,mol^{-1}}$) than the competing dissociation ($\mathrm{E2} = +180.3\,\mathrm{kJ\,mol^{-1}}$). The additional complexation of Li^+ by the double bond in the *m*/*z* 123 product is likely to account for its highest stability. The interaction energy between a double bond and a Li^+ ion was calculated on the simplest possible model (Li^+ on ethylene). The interaction energy was found to be $93.2\,\mathrm{kJ\,mol^{-1}}$ at this level. However, due to geometrical constraints in the actual system, the stabilization is surely much lower than this ideal value, but still substantial.

As the size of the precursor ion increases from $n = 4$ to $n = 5$, the elimination of the 2-hydroxyethoxy radical (61 Da) was further shown to be the preferential route over the formation of a distonic radical cation by an amount of energy of $104\,\mathrm{kJ\,mol^{-1}}$. This might account for the higher intensity measured for *m*/*z* 167 as compared to *m*/*z* 123 despite the lower abundance of $\mathrm{D}_5^{\omega,1+}$ as compared to $\mathrm{D}_4^{\omega,1+}$ precursor ions. This result could



Scheme 7.

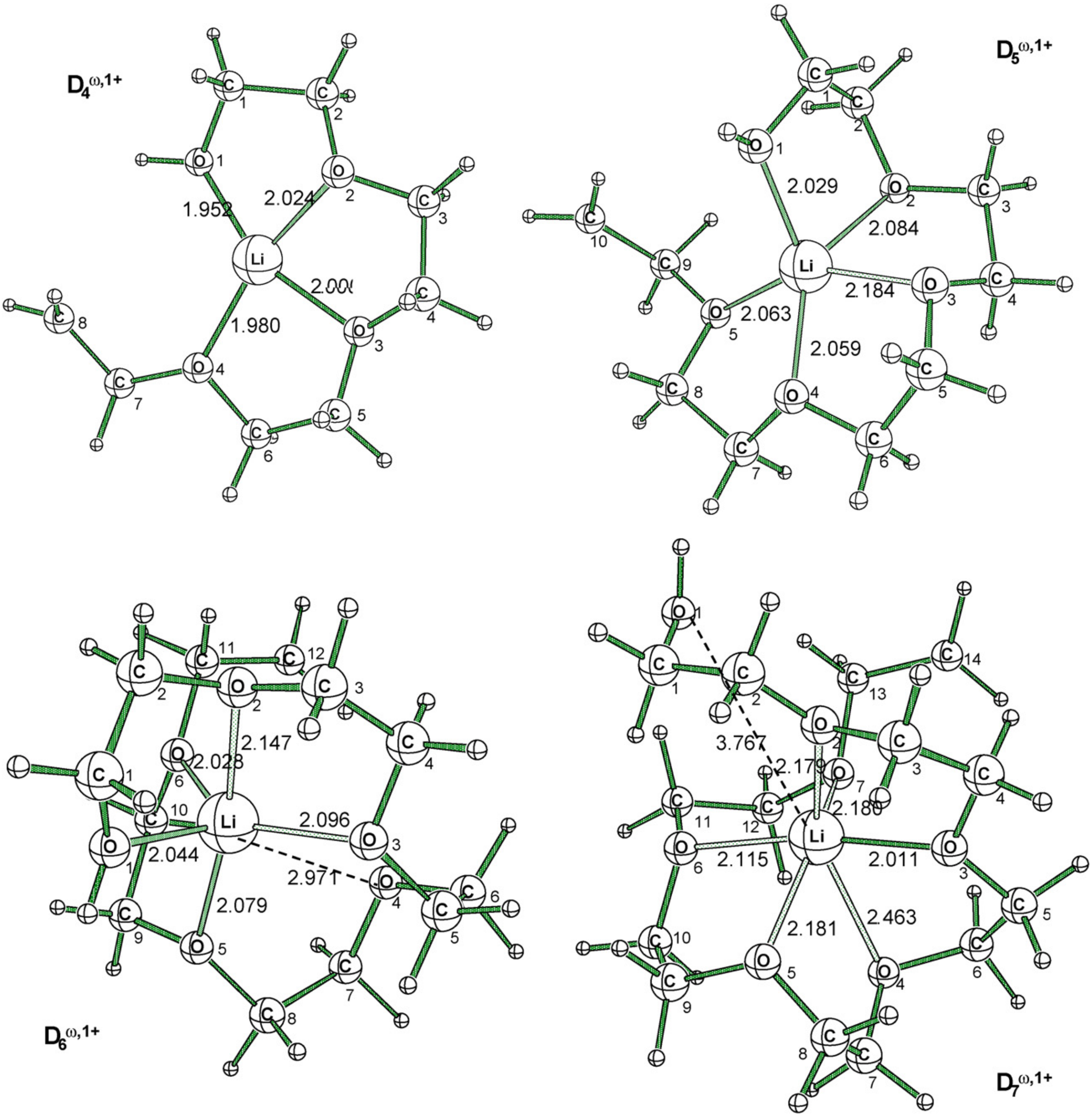
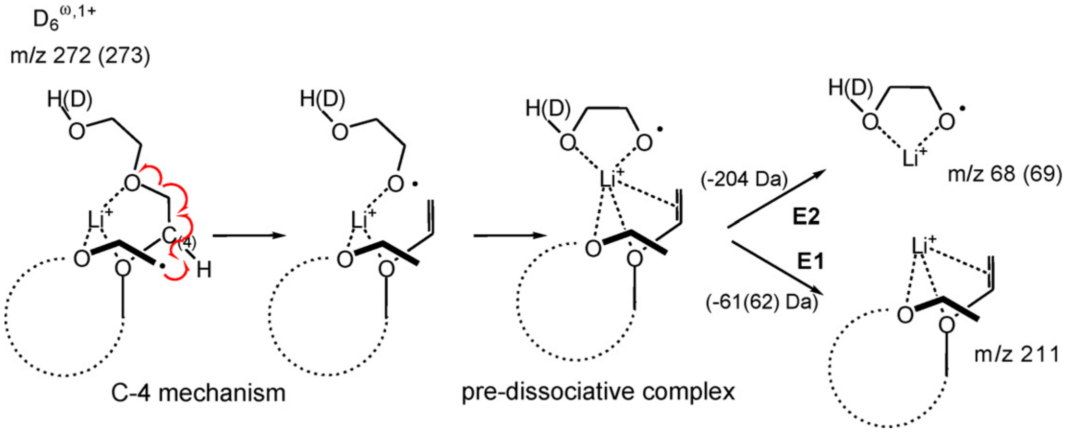


Fig. 4. B3LYP/6-311g(2d,p) geometries of the lowest energetic structures of fragment ions $D^{\omega,1+}$ (bond lengths are given in Angstroms).

be explained by an increase in the number of stabilizing Li–O bonds in the so-formed cation. The same trend is observed for the loss of $^\bullet$O–CH$_2$–CH$_2$–OH radical as the number of ethylene oxide units increases in $D_n^{\omega,1+}$ (Fig. 5).

However, the mechanism described in Scheme 7 is hard to envisage for systems larger than $n = 5$: in the conformations obtained for $D_{6-7}^{\omega,1+}$ (Fig. 4), the distance between the hydroxyl group and the terminal C atom is so large that the required H radical transfer cannot be possibly considered. An alternative pathway (Scheme 8) would consist of a concerted mechanism where the hydrogen radical transfer occurs from the $C_{(4)}$ to the terminal C atom, allowing a radical-induced cleavage of the $O_{(2)}$–$C_{(3)}$ bond and the formation of a double bond between $C_{(3)}$ and $C_{(4)}$. This alternative mechanism was called the "C-4 mechanism". The so-obtained lithium-bond pre-dissociative complex would then eliminate the HO–CH$_2$–CH$_2$–O$^\bullet$ radical part, giving rise to *m/z* 211 and *m/z* 255 from $D_6^{\omega,1+}$ and $D_7^{\omega,1+}$, respectively. The 2-hydroxyethoxy radical part of the lithium-bond pre-dissociative complex formed from $D_6^{\omega,1+}$ or $D_7^{\omega,1+}$ would still contain the hydroxyl ω end-group of the PEG precursor ion. Since this hydroxyl end-group contains the only exchangeable hydrogen atom, the "C-4 mechanism" assumption could be tested performing H/D exchange experiments.

MS/MS data of the doubly lithiated 23-mer, detected at *m/z* 529.8 after ESI of the CH$_3$OD solution of PEG-Me1100, are presented in Fig. 3b. As expected, $D_{4-7}^{\omega,1+}$ obtained from the dis-



Scheme 8.

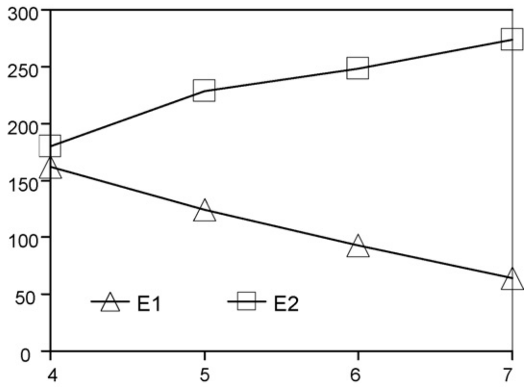


Fig. 5. Dissociation energies for Li-bond pre-dissociative complexes of $\mathrm{D}_n^{\omega,1+}$ systems as a function of n. Relative energies ($\mathrm{kJ\,mol^{-1}}$) are obtained at the B3LYP/6-311g(2d,p) level.

sociation of $\mathrm{d_1}$-PEG-Me1100 precursor ion were detected with a 1 u mass shift. Comparing Fig. 3a and b, the increase of *m/z* 124 and *m/z* 168 ion intensity indicates that $\mathrm{d_1\text{-}D_4^{\omega,1+}}$ at *m/z* 185 and $\mathrm{d_1\text{-}D_5^{\omega,1+}}$ at *m/z* 229 would still eliminate a 61 Da radical (i.e., $^{\bullet}\mathrm{O{-}CH_2{-}CH_2{-}OH}$), as did their respective non-deuterated homologues. In contrast, such a 1 u mass shift was not observed from *m/z* 211 and *m/z* 255, suggesting $\mathrm{d_1\text{-}D_6^{\omega,1+}}$ and $\mathrm{d_1\text{-}D_7^{\omega,1+}}$ both dissociated by losing a $\mathrm{DO{-}CH_2{-}CH_2{-}O^{\bullet}}$ radical (62 Da) according to the proposed “C-4 mechanism”.

4. Conclusion

Mechanisms proposed in the literature for dissociation of singly charged poly(ethylene oxide) oligomers were shown to apply to doubly charged PEO molecules, giving rise to fragments with different charge states. The choice of the alkali metal cations adducted to PEO oligomers was also very important in the case of doubly charged species and lithiated precursors were shown to yield the most informative MS/MS data. Radical cationic fragments were detected although low energy collisions were performed. A new ion series was proposed to arise from a consecutive fragmentation of these D ions. Calculated conformation of these dissociating species suggested two different mechanisms had to be envisaged depending on the size of the D radical cation, which were further validated by results from H/D exchange experiments.

Acknowledgements

This work was supported by the French Research Agency (ANR-06-JCJC-0112). L. Charles acknowledges support from Spectropole, the Analytical Facility of Aix-Marseille University, by allowing a special access to the instruments purchased with European Funding (FEDER OBJ2142-3341). S. Humbel acknowledges support from the Centre Régional de Compétence en Modélisation Moléculaire de Marseille, the Computing Facility of Aix-Marseille University.

References

[1] R.P. Lattimer, J. Am. Soc. Mass Spectrom. 3 (1992) 225.
[2] R.P. Lattimer, J. Am. Soc. Mass Spectrom. 5 (1994) 1072.
[3] A.T. Jackson, H.T. Yates, J.H. Scrivens, G. Critchley, J. Brown, M.R. Green, R.H. Bateman, Rapid Commun. Mass Spectrom. 10 (1996) 1668.
[4] A.R. Bottrill, A.E. Giannakopulos, C. Waterson, D.M. Haddleton, K.S. Lee, P.J. Derrick, Anal. Chem. 71 (1999) 3637.
[5] A.R. Botrill, A.E. Giannakopulos, A. Millichope, K.S. Lee, P.J. Derrick, Eur. J. Mass Spectrom. 6 (2000) 225.
[6] A.J. Hoteling, K. Kawaoka, M.C. Goodberlet, W.M. Yu, K.G. Owens, Rapid Commun. Mass Spectrom. 17 (2003) 1671.
[7] S. Okuno, M. Kiuchi, R. Arakawa, Eur. J. Mass Spectrom. 12 (2006) 181.
[8] A.T. Jackson, M.R. Green, R.H. Bateman, Rapid Commun. Mass Spectrom. 20 (2006) 3542.
[9] R. Chen, L. Li, J. Am. Soc. Mass Spectrom. 12 (2001) 832.
[10] R. Chen, X.L. Yu, L. Li, J. Am. Soc. Mass Spectrom. 13 (2002) 888.
[11] A.T. Jackson, J.P. Williams, J.H. Scrivens, Rapid Commun. Mass Spectrom. 20 (2006) 2717.
[12] T.L. Selby, C. Wesdemiotis, R.P. Lattimer, J. Am. Soc. Mass Spectrom. 5 (1994) 1081.
[13] A.T. Iavarone, E.R. Williams, Int. J. Mass Spectrom. 219 (2002) 63.
[14] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr, T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels,

M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Ciolowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian 03, Revision C.02, Gaussian Inc., Wallingford, 2004.

[15] A.T. Jackson, S.E. Slade, J.H. Scrivens, Int. J. Mass Spectrom. 238 (2004) 265.

[16] G. Vonhelden, T. Wyttenbach, M.T. Bowers, Science 267 (1995) 1483.

[17] J.L. Wu, M.J. Polce, C. Wesdemiotis, J. Am. Chem. Soc. 122 (2000) 12786.

[18] G. Vonhelden, T. Wyttenbach, M.T. Bowers, Int. J. Mass Spectrom. Ion Proc. 146 (1995) 349.