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Energetics of fragmentation for cationized poly(ethylene glycols) oligomers

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Dear Sir,

There are various difficulties studying macromolecular systems. One on these is that the applicable theoretical and experimental methodologies often have significant limitations. A common solution is to use smaller "model" compounds; study them in detail; and extrapolate these results to the larger system. Although very useful, this raises the question, how accurate is the extrapolation? In the present Letter we want to give an example related to activation energies, which have large importance describing fragmentation processes. There are diverse methods determining activation energies in mass spectrometry; many values obtained this way can be found in the literature[1-3]. However, most of these relate to small molecules. Available methods for studying compounds over ca. 200 Da size are less accurate; and there are few reliable values[4-11]. "Common wisdom" suggests that, for the same reaction type, increasing the molecular size should decrease the barrier height (i.e. the critical energy, but not the internal energy needed for fragmentation!): Increasing size typically stabilizes the product ion (and product radical, if any) with respect to the parent compound. With the advance of experimental techniques to study macromolecules, it may become useful to be able to estimate, how molecular size influences the activation energy.

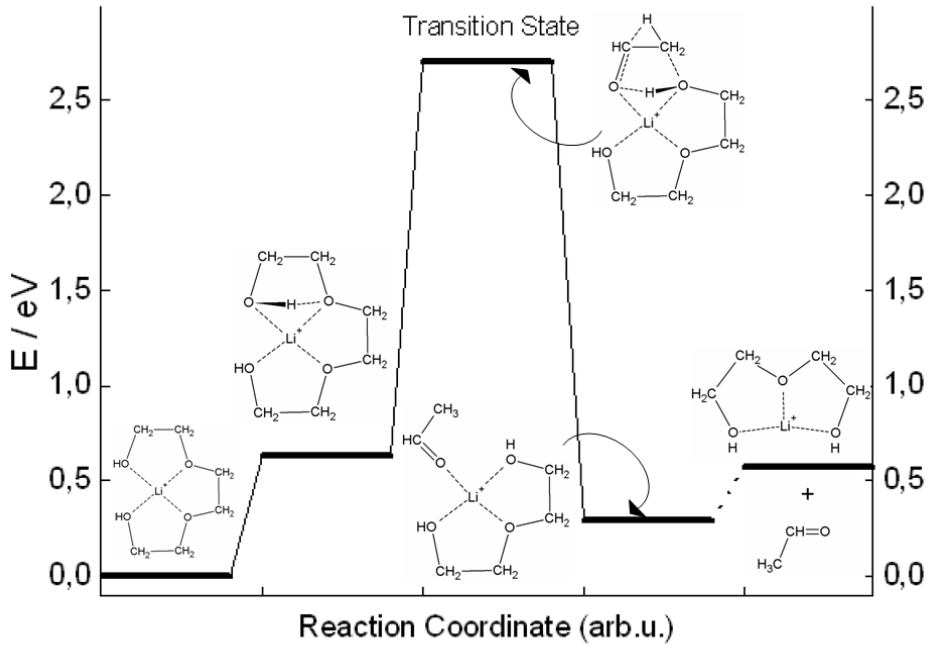
To understand the factors influencing the size dependence of fragmentation energetics, reliable information would be desirable. Theoretical methods achieved the level of accuracy that makes them an accepted source of reliable data. We intend to provide this kind of information using a

well-defined model system. Poly(ethylene glycols) -PEG- oligomers have been selected to study the influence of molecular size on fragmentation energetics. Studying homopolymers (like PEGs) has the advantage that structural features do not change with increasing size. This simplifies studying size effects and makes results easier to interpret[12]. In the case of peptides, which are in the forefront of interest, the presence of various amino acid residues modifies chemical behaviour, which can mask the size effect completely[13].

Quantum chemistry, in particular, density functional theory was used to determine the mechanism of fragmentation. Calculations have been carried out using two different functionals: the popular B3LYP[14-16] and the more recently developed M05-2X[17]. The former has been generally used in the study of reactions of polyatomic molecules. The latter generally gives a more accurate description of loose-type interactions (e.g. H-bonds), and in particular, barrier heights for reaction; in this respect, this functional generally provides barrier heights and fragmentation energies within 0.2 eV with respect to the highest-level ab initio methods[18]. Optimization of the geometry of the structures were performed using the 6-31G(d) basis set, followed by single point energy calculation using the larger 6-311++G(2d,2p). The character of the stationary points was identified using normal mode frequency analysis. Each minimum is genuine (there are no imaginary frequencies), each potential barrier is a first-order saddle point (there is one imaginary-frequency mode).

PEG oligomers are most often studied in mass spectrometry as even electron, positively charged lithium adducts ($[M+Li]^+$).[19] Occasionally protonated, sodiated or other cationized species are also observed, although their fragmentation is not so characteristic. More precisely, in the case of protonated species (much weaker signal than with other adducts), mainly low mass daughter ions are observed limiting the structural information available[20]. On contrary, for sodiated, similarly for potassiated species, loss of the cation is almost the only fragment observed[19]. Concerning lithium cationized PEGs, the main fragmentation process is the loss of one or several monomer units (the latter may be formed either in a one step process or in sequential reactions)[19, 21, 22]. The lowest energy process of lithiated PEG is the loss of a monomer unit (C_2H_4O) from the end of the chain. We have studied the mechanism of this process in detail. There are several alternative reaction channels leading to C_2H_4O loss, the lowest energy channel among these leads to the loss of acetaldehyde, as shown for the trimer in Scheme 1.

The reaction proceeds through a quite complex, concerted mechanism. The sequence of intramolecular events was followed by calculating the Intrinsic Reaction Coordinate (IRC) and viewing the geometry changes along the path. First, a strong hydrogen bond is formed between the terminal hydroxyl group and an ether oxygen. This is an intermediate structure (i.e. local minimum). Subsequently this hydrogen shifts to bind more strongly to the mid-chain ether oxygen, forming a new terminal OH group; while at the same time the terminal oxygen starts to form a carbonyl bond. This initiates (in a concerted fashion) a 1-2 hydrogen shift between two carbon atoms; and this leads to the highest-energy point along the path (transition state, TS, Scheme 1). The height of the barrier for the trimer is 2.54 eV at the B3LYP/6-311++G(2d,2p) level of theory. (Here and elsewhere in the text we quote values determined at this level; the Table shows results using M05-2X as well.) On the downhill side of the potential barrier, the newly formed bonds strengthen which leads to the formation of a loosely bound ion-molecule complex between a lithiated PEG dimer and acetaldehyde. This complex breaks with only a small energy requirement (and no energy barrier) into the products.



Scheme 1: Mechanism of fragmentation of the PEG trimer, as calculated at the B3LYP/6-311++G(2d,2p)//B3LYP/6-31G(d) level of theory.

Regarding the mechanism described, we would like to make a few further comments. (1) The critical energy of fragmentation of PEG trimer is 2.54 eV. Compared to most organics it is very high; about two times higher, than that observed for most peptides[6, 8, 9, 12]. This does explain, why do PEG oligomers require far larger collision energy for fragmentation than most other protonated compounds[12] of similar mass. (2) It is somewhat surprising that the TS is quite loose, the activation entropy is estimated to be only 7.9 J/mol/K based on frequency analysis and the rigid-rotor harmonic oscillator approximation. This corresponds to an Arrhenius type pre-exponential factor of 2.3 10^{13} s^{-1}. (3) The reaction is facilitated by the presence of the Li^+ cation. In the absence of Li^+ the barrier is 0.3 eV higher. This suggests a charge-induced process. (4) The type of cation does not have a significant effect on the reaction mechanism: In the case of a sodium adduct the barrier height is similar (higher by ~0.2 eV). (5) The early suggestion[19], that the reaction proceeds through the transfer of the alcoholic H only was also studied. This reaction channel leads to ethylene oxide and requires 0.6 eV higher critical energy (this difference is well beyond the "error bar" of the method), so it can not compete efficiently with the low energy process leading to acetaldehyde.

Probably the most significant finding in the present communication is the size dependence of the critical energy. First of all, it has been established, that the reaction proceeds through the same low energy channel for other oligomers as well (from the dimer to the hexamer). Increasing the size of the PEG does not change the main characteristics of the process. Independently of molecular size, monomer loss remains the lowest energy channel (both experimentally[23] and according to the calculations: other channels were found to require more energy). The activation energies for various PEG oligomers are shown in Table 1. This shows that the critical energies decrease

somewhat with molecular size, but the change is relatively minor (7-16% depending on the level of calculations, see Table 1 for details).

No. of monomers	B3LYP (in eV)		M05-2X (in eV)	
	Barrier height	Li^+ binding energy	Barrier height	Li^+ binding energy
2	2.70	3.35	2.98	3.71
3	2.54	3.90	2.90	4.12
4	2.42	4.15	2.80	4.28
5	2.25	4.53	2.78	4.68
6	2.28	4.69	2.77	4.89

Table 1. Critical energies of fragmentation and Li^+ binding energy in eV as a function of the number of monomers and at two different levels of theory: B3LYP/6-311++G(2d,2p)//B3LYP/6-31G(d) (abbreviated as B3LYP) and M05-2X/6-311++G(2d,2p)//M05-2X/6-31G(d) (abbreviated as M05-2X) where A//B means an energy calculation at level A at the geometry optimized at level B.

To increase confidence in the results, we have repeated the calculations using a different dft functional, M05-2X as well, which has been found to describe loose bonds (like hydrogen bonds) better and yield more reliable barrier heights than the more conventional B3LYP method. The results are given in Table 1. These show qualitatively the same trend; although the activation energies are a little higher (by ca. 0.35 eV); and the change with molecular size is smaller. Results using both functionals show that the limiting value for the critical energy is reached around the tetramer or pentamer.

Activation energies can be compared to the binding energy of the lithium cation (lithium affinities) (Table 1; while values for the 10-, 14- and 18-mer are 5.68, 5.65 and 5.70 eV, respectively using the M05-2X functional). It can be seen that the lithium affinity increases very strongly with oligomer size, and the limiting value is reached only around the 10- or 12-mer. This is a far larger change than that of the activation energy. Note that lithium affinity is larger (or much larger) than the critical energy of fragmentation (monomer loss). This explains why, in contrast to Na^+ or Cs^+, the Li^+ cation is not "lost" in a fragmentation; resulting in a structurally useful MS/MS fragmentation. As Na^+ or Cs^+ affinities are significantly smaller; in these cases loss of Na^+ or Cs^+ instead of a monomer EG unit may become dominant, resulting in MS/MS spectra that, for structure elucidation, are nearly useless.

Results presented in this communication indicate that PEGs (and likely other polyethers) require high (ca. 2.5-3 eV) critical energy for fragmentation. This explains the experimental observation that their MS/MS fragmentation requires far larger collision energies than needed for peptides and most other organics of similar size. In general, larger oligomers require somewhat lower activation energies for fragmentation. However, this is only a minor effect; critical energies among various oligomers decrease by only about 10%. We believe this is the first systematic study to determine

how much the activation energy for decomposition depends on molecular size in even-electron ions. As we lack data for other systems it is difficult to generalize; but seems most likely that the size dependence of critical energies has a minor influence on mass spectrometric fragmentation behaviour.

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