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Elastic properties of poly(ethylene-glycol) studied by molecular dynamics stretching simulations

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Abstract

Unfolding and elastic properties of single poly(ethylene-glycol) (PEG) polymers were studied by molecular dynamics (MD) simulations. The simulations were performed in close resemblance to recent single molecule force spectroscopy experiments, and thus allowed an interpretation of these experiments at the atomic level. In agreement with these experiments, the water solvent was found to drastically affect the elastic properties of PEG. Our simulations explain this effect in terms of local helical structures which are stabilized through water bridges. Stretching forces of more than 100 pN were required to break these water bridges and thereby to destroy these helical structures. © 1999 Elsevier Science B.V. All rights reserved.

1. Introduction

Poly(ethylene-glycol) (PEG) is a synthetic water soluble polymer which has been studied extensively for both its widespread use in various industrial, biotechnical and clinical applications [1,2] and, from a more general viewpoint, as a model system for the study of the complex behaviour of polymers in solution [3].

The properties of macromolecules are governed by their three-dimensional molecular structure. Accordingly, the conformation of PEG in different solvents has been studied extensively. Light scattering experiments unambiguously revealed that PEG does not aggregate in water and methanol [3], and infrared spectroscopy has shown that the *gauche* conformation around the C–C bond dominates the *trans* conformation with increasing water fraction, which suggested formation of a supra-structure of PEG in water solvent [4]. It was suggested that water bridges between PEG oxygen atoms could stabilize such supra-structure.

Recent single molecule force spectroscopy experiments [5] demonstrated that single PEG polymers are more elastic in hexadecane solvent than in water solvent at stretching forces below 100 pN. For larger stretching forces, hexadecane solvated PEG molecules turned out to be stiffer than water solvated PEG. Whereas the elasticity of PEG in hexadecane could be well described by a simple spring, its elastic properties in water turned out to be more complex. The interpretation given in Ref. [5] considers the deformation of helical supra-structures which was modeled through an elastically coupled Markovian two-level system. The model of helical structure

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formation received further support from quantum chemical calculations [6], which suggested a favorable hydration energy for PEG with the C–C bond in *gauche* conformation. Molecular dynamics (MD) stretching simulations have proved to be a powerful tool for the study of the response of polymers to mechanical stress [7,8]. In this Letter, we present extended MD stretching simulations of PEG, which were carried out in order to study the atomic origin of this pronounced solvent effect. The simulations were performed in close resemblance to the AFM force spectroscopy experiments described in Ref. [5] which, therefore, served to check our simulation results against experiment.

2. Methods

A structural model for an extended PEG 18-mer was generated using the molecular editor of Quanta [9]. All simulations were performed with the parallelized MD program EGO [10], which uses the CHARMM force field [11]. EGO allows efficient computation of Coulomb forces using the ‘fast multiple timestep structure adapted multipole method’ [12] so that no artificial truncation of these forces [13] had to be applied. The parameters for dihedral angles, which are critical for the conformational properties of PEG, were taken from the CHARMM library. In particular, torsions of the two relevant dihedral angles around the C–C and C–O bonds were described by a sinusoidal potential with periodicity three, equilibrium values at $-60°$ (*gauche*$^-$), $60°$ (*gauche*$^+$), and $180°$ (*trans*) for both bonds and force constants of 1.6 and 0.2 kcal/mol for C–C and C–O, respectively. These equilibrium values are in good agreement with the angles observed in PEG crystals [14].

Two simulation systems were used to describe PEG solvated in hexadecane and water, respectively. The polar water solvent was described explicitly by surrounding the PEG with 1539 water molecules using SOLVATE [15]. The size and shape of the resulting ‘droplet’ was chosen such that the minimum distance between extended PEG molecule and the system surface was 12 Å. To counterbalance surface tension and evaporation, all molecules in a surface layer of 6Å thickness were subjected to ‘deformable boundary forces’ [11] (approximated by a quartic polynomial as described in Refs. [16,17]). The influence of the apolar hexadecane solvent was considered to be small and, therefore, gas phase simulations were used.

In all simulations an integration step size of 10^{-15} s was used. To obtain a starting point for the MD stretching simulations, both the gas phase system and the solvated system were equilibrated for 2000 ps, respectively. In all simulations the system was coupled to a heat bath of 300 K by velocity rescaling as described in Ref. [18] with a coupling constant of 10^{13} s^{-1}. The stretching forces, which probe the elastic properties of PEG, and which in the experiments were exerted by the cantilever, were modeled by connecting the oxygen atom of one terminal monomer (‘O1’) to a harmonic potential (‘cantilever’) with a force constant of 0.07 N/m; that value has also been used in the AFM force spectroscopy experiments [5]. The ‘cantilever potential’ was set up such as to act only in pulling direction, i.e., parallel to the polymer axis. Accordingly, motions perpendicular to this axis were not affected, as is also the case in the AFM experiments. The other terminal oxygen atom was kept fixed with a stiff isotropic harmonic potential (force constant 28 N/m).

Subsequently, the PEG 18-mer was stretched by moving the cantilever potential with constant velocity. Force versus extension curves were obtained by recording the pulling force on atom ‘O1’ as a function of polymer extension. That force was computed from the elongation of the spring using Hooke’s law. To study the influence of pulling velocity on the force versus extension curves, we carried out several stretching simulations with pulling velocities ranging from 50 to 5 m/s (corresponding to simulation time spans of 300–3000 ps).

The PEG conformation during stretching was characterized through end to end distance, radius of gyration, and helicity ρ_{helix}. The latter was defined as the number of triples of adjacent angles around C–C bonds that were in the *same gauche* conformation divided by the total number of dihedral angle triples. Here, angles between $-120°$ and $0°$ were defined to be *gauche*$^-$; those between $0°$ and $+120°$ as *gauche*$^+$. Accordingly, ρ_{helix} ranges from 0 for no helicity up to 1 for perfect helical structure. Note that this definition for helicity is rather restrictive, since short helical fragments contribute only weakly

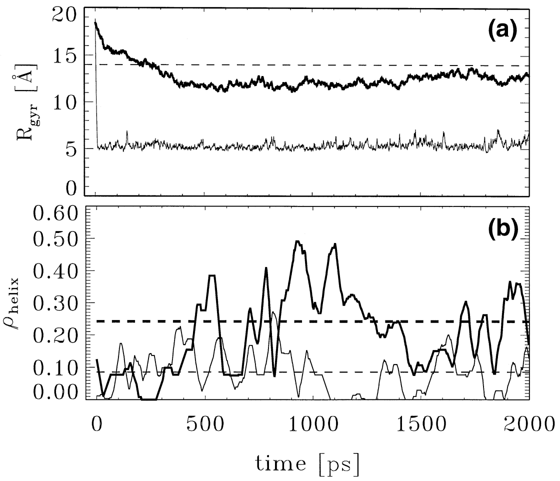

Fig. 1. Radius of gyration (a) and helicity ρ_{helix} (b) for PEG in water (bold lines) and in gas phase (thin lines) during equilibration. The radius of gyration for PEG with perfect helical structure is indicated by the dashed line in (a). The dashed lines in (b) show the averages of the respective helicities.

to ρ_{helix}. Consider, e.g., helical fragments of four monomers that are separated by single monomers. This highly helical structure would be described by $\rho_{\text{helix}} = 0.4$.

For the solvated PEG, formation and rupture of hydrogen bonds between water molecules and PEG oxygen atoms were monitored using a distance criterion (< 4 Å between the heavy atoms) and an energy criterion (sum of van der Waals and electrostatic interaction energy less than -1 kcal/mol) as suggested in Ref. [19]. Water bridges were defined as water molecules that form simultaneous hydrogen bonds to at least two PEG oxygen atoms. Note that we included the more general energy criterion rather than using the common and purely geometric angle criterion (see, e.g., Ref. [20]) in order to quantify the respective local interactions.

Finally, the interaction energy of PEG with all nearby water molecules (distance $\leqslant 4$ Å) was recorded, and the average accessibility α of PEG oxygen atoms to water bridges was assessed by determining for each nearby water molecule the number of PEG oxygen atoms that are close enough to this water molecule to form a hydrogen bond, and by taking the average over all these numbers. Accordingly, values near one indicate that few or no water bridges can be formed, whereas a value of 1.5 or larger indicates that many water bridges are sterically possible.

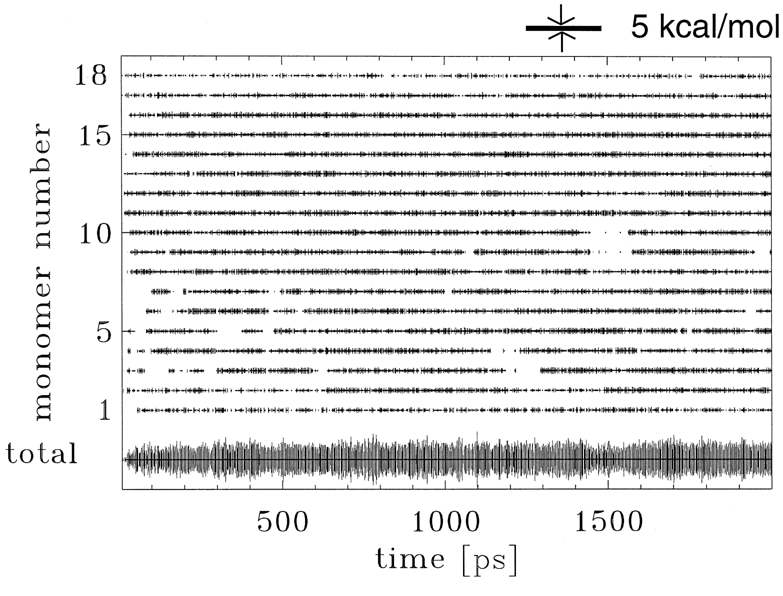

Fig. 2. Strength of water bridges between PEG oxygen atoms for each of the monomers during equilibration, measured in kcal/mol and indicated by the thickness of the 18 traces. The sum over all monomers is shown at the bottom of the figure. A scale is shown at the top.

3. Results and discussion

3.1. Equilibration

During equilibration the end to end distance of the (initially extended) gas phase PEG chain dropped rapidly to nearly zero within less than 10 ps. Fig. 1a shows a corresponding decrease of the radius of gyration to about 5 Å (dotted line). In contrast, the average monomer extension of solvated PEG stabilized after a relaxation time of 400 ps at a value of 2.0 Å, and the radius of gyration at $R_{\mathrm{gyr}} \approx 12$ Å (bold line, Fig. 1a). That value is only slightly smaller than the value of a PEG with fully helical structure (dashed line), and it did not change significantly for the remaining 1600 ps of the simulation.

Fig. 1b shows the helicity, ρ_{helix}, of the polymer during equilibration. For gas phase PEG (thin line) ρ_{helix} fluctuated between 0 and 0.2 with an average of 0.08 (dashed, thin line), whereas the helicity of the solvated PEG (bold line) varied considerably between 0.1 and 0.5 after 400 ps with an average value of 0.25 (dashed, bold line). These data indicate almost no helicity for gas phase PEG, but suggest that helical structure elements occur frequently in solvated PEG. The upper snapshot in Fig. 5 shows a representative example of such helical structure; also shown are selected water molecules which form water bridges between PEG oxygen atoms, and which are considered to be a possible driving force for helix formation. As can be seen, water molecules can bridge simultaneously to up to four oxygen atoms.

Fig. 2 quantifies the strength of the water bridges for each of the 18 monomers during equilibration. Here, the strength is measured in terms of interaction energies, denoted by the thickness of the respective traces. As can be seen, the strength of the water bridges stabilized after a relaxation phase of around 300 ps. Also visible are transient ruptures and re-formations of water bridges. The total water bridge energy (bottom line in the figure) was 2.5 kcal/mol per monomer. The equilibrated structures were used for the subsequent stretching simulations.

3.2. Stretching simulations

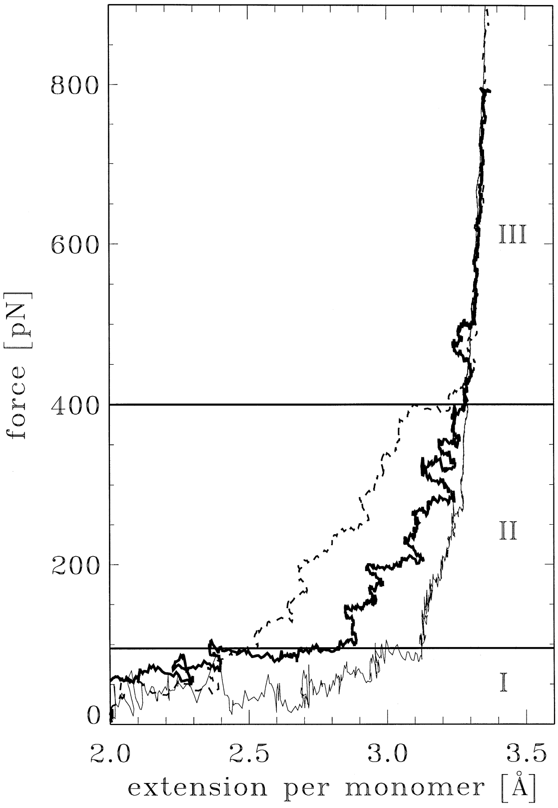

Fig. 3. Force versus extension curves for PEG in water for pulling velocities 50 m/s (dashed) and 7.5 m/s (bold line), respectively, as well as for PEG in gas phase (thin line). Three force regimes (I, II, III) are distinguished and discussed in the text.

Fig. 3 shows force versus extension curves obtained for solvated PEG at a pulling velocity of 7.5 m/s (bold line) and for gas phase PEG (thin line). Three force regimes can be distinguished, a 'low force' regime (I) below 100 pN, an intermediate regime (II) between 100 and 400 pN, and a 'high force' regime (III) above 400 pN. Whereas in the low and high force regimes the two systems show similar elastic behaviour, significant solvent effects show up in the intermediate regime. In particular, stretching of solvated PEG seems to require considerably larger forces than stretching in gas phase.

Fig. 3 also compares the force versus extension curve of solvated PEG at the pulling velocity 7.5 m/s (bold line) with a curve obtained at the much larger pulling velocity of 50 m/s (dashed line). Obviously, at such large velocities, increased forces are required, particularly in the intermediate regime. We attribute this effect to Stokes' frictional forces.

Indeed, at pulling velocities between 10 and 5 m/s no velocity dependence of stretching forces can be detected (data not shown), and, hence, frictional forces can be assumed to be negligible here. This finding is in agreement with simulations of solvated ligand-receptor complexes or polysaccharides [8,7,21,22], which also showed vanishing frictional forces for velocities below 10 m/s.

For unbinding processes an energy barrier has to be overcome and typically gives rise to an additional logarithmic dependency of unbinding forces on the pulling velocity (as discussed, e.g., in Refs. [22–24]), which can complicate direct comparisons of nanosecond simulations with millisecond experiments. For PEG stretching, however, we do not expect significantly changed force versus extension curves at the experimental timescales of milliseconds, since here no large single energy barriers have to be overcome. Indeed, comparison with Fig. 4 in Ref. [5] shows very good agreement of our computed force versus extension curves with experimental data. We therefore base the subsequent structural analysis on a simulation carried out at 7.5 m/s.

We now ask how the observed stretching forces arise from molecular interactions. To explain the observed solvent effect, we focus at the interactions

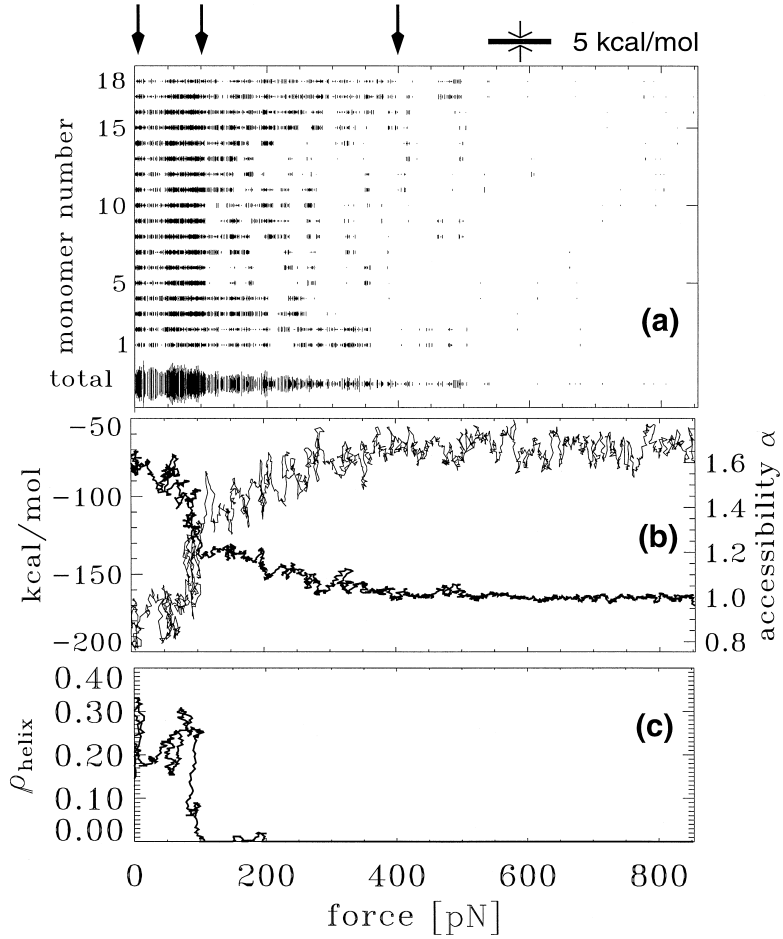

Fig. 4. (a) Interaction energies of water bridges to individual monomers as a function of pulling force during a stretching simulation with pulling velocity 7.5 m/s; (b) total interaction energy between PEG and all surrounding water molecules closer than 4 Å (thin line), average accessibility α of PEG oxygen atoms to water bridges (bold line); (c) helicity ρ_{helix}.

between PEG and water solvent and at the helicity of the polymer. Fig. 4 summarizes the main results, which were extracted from a stretching simulation at pulling velocity 7.5 m/s. Note that in the figure all observables are plotted as a function of the pulling force acting on PEG. Three representative snapshots of the PEG structure, shown in Fig. 5, have been taken at the force values indicated by the arrows at the top of the figure. Strikingly, all the observables shown in Fig. 4 exhibit a sudden change at the beginning of the intermediate regime (cf. Fig. 3) at pulling forces of 100 pN. The interaction energies between water molecules which form water bridges to PEG monomers (cf. Fig. 4) drop abruptly; many water bridges even ruptured, particularly to monomers 5–10. The fraction of affected hydrogen bonds can be estimated by inspection of the corresponding – and quite significant – decrease of the total water bridge energy [(a), bottom line]. The second snapshot in Fig. 5 shows the PEG structure immediately after that event. Comparison with the initial structure (top) suggests that particularly those water bridges ruptured which involved three or more PEG oxygen atoms.

The total energy that is required to induce such drastic conformational change can be estimated from the jump by approx. 40 kcal/mol of the total interaction energy of PEG with all surrounding water molecules within 4 Å distance (thin line in Fig. 4b). This value presumably overestimates the energy actually required, since the energy from newly formed hydrogen bonds between water molecules is not taken into account. A rough estimate from Fig. 3 of the mechanical work transferred to each monomer through stretching, namely 0.9 Å · 100 pN = 1.3 kcal/mol, yields a total of 23 kcal/mol. This value indicates that, indeed, rupture of hydrogen bonds between solvent and PEG is the main effect. That these are to a large fraction involved in water bridges between PEG oxygen atoms is indicated by the drop from 1.5 to 1.3 of the average accessibility α of PEG oxygen atoms to water bridges (bold line in Fig. 4b).

At the same time the helicity ρ_{helix} (Fig. 4c) vanished, and the polymer extended considerably by 0.5 Å per monomer, as can be seen from Fig. 3 as well from the snapshots in Fig. 5. These data suggest that it is the drastic change of the PEG structure which determines its elastic properties and is induced by the rupture of a series of water bridges, in particular those, which involve three or more PEG oxygen atoms and thereby stabilize helical structures.

Increasingly large forces up to 400 pN are required to further extend the polymer by 0.4 Å per

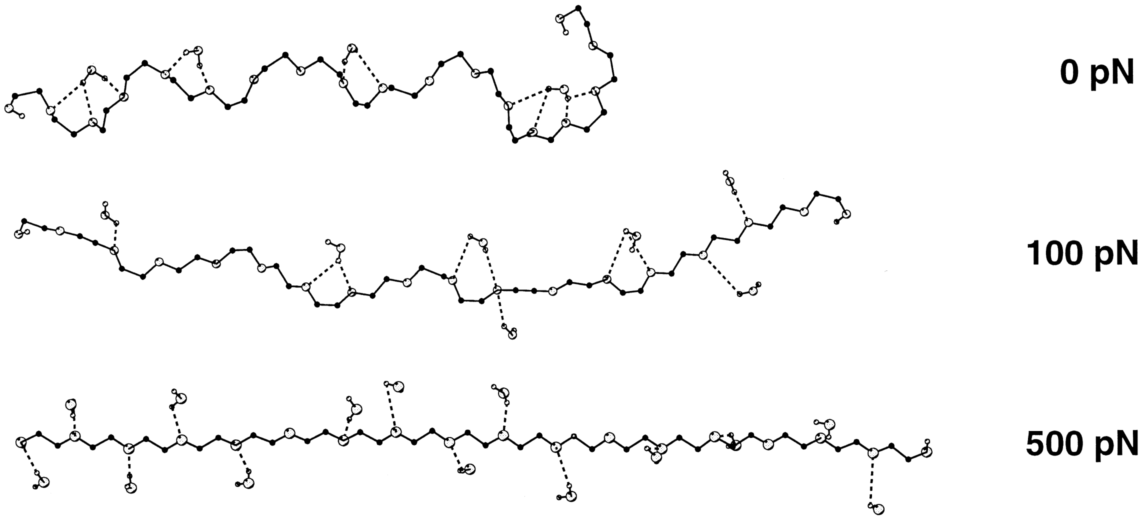

Fig. 5. Three representative snapshots of solvated PEG at zero (top), small (mid), and large (bottom) pulling force. Hydrogen bonds between water molecules and PEG oxygen atoms are marked through dashed lines. Only carbon atoms (black), oxygen atoms (light), and polar hydrogen atoms (white, small) are shown; non-polar hydrogen atoms are not shown.

monomer to almost its maximum length. In this intermediate force regime the water bridges visible in the second snapshot of Fig. 5, which survived the main unfolding step, ruptured, as can also be seen from the slow increase of the interaction energy of PEG with nearby water molecules (Fig. 4b). Due to the absence of water bridges in gas phase, here the polymer is much stiffer in that regime.

In the high force regime, at forces above 400 pN, the force extension curves (Fig. 3) for gas phase PEG (dotted line) and solvated PEG (solid line) are almost identical. Obviously in this regime water bridges do not significantly contribute to the molecular elasticity, which instead is dominated by intramolecular processes such as deformations of bond angles and torsion angles. In addition, as can be seen from Fig. 4b, in this force regime the total interaction energy of PEG with surrounding water molecules (thin line) does not significantly change any more, as is also the case for the average accessibility of PEG oxygen atoms for water bridges (bold line), which stabilized at nearly unity. The latter value indicates that at this stage formation of water bridges was almost completely suppressed, since the distance of adjacent PEG oxygen atoms exceeded the reach of single water molecules.

4. Conclusions

Our extended MD stretching simulations provided insight into the effect of water solvent on the elastic properties of PEG at the atomic level. In particular, the observed stiffening of PEG in water is explained to arise from the formation of local helical structure elements that are stabilized by water bridges between adjacent PEG monomers. Accordingly, larger stretching forces are required for solvated PEG in comparison to gas phase PEG to rupture these water bridges and thereby to unfold the helical structure elements. In gas phase PEG, no supra-structure was observed. At a pulling force of around 100 pN the helicity of solvated PEG completely disappeared. At forces above 400 pN no differences in the elasticities were observed for gas phase PEG and solvated PEG indicating that in this force regime only intra-molecular interactions like deformations of bond angles and torsion angles contribute to the elasticity. Comparison of the computed force versus extension curves with those from recent single molecule force spectroscopy experiments [5] shows good agreement and confirms that the elastic properties of PEG in water solvent are well described by our simulations. Furthermore, the comparison with experiment shows that the gas phase simulations provide a good model for PEG in apolar solvents like hexadecane.

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