Elastic response of poly(ethylene glycol) polymer chains studied using dynamic atomic force microscopy

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Stretching response of polymer chains under external force is crucial in understanding polymer dynamics under equilibrium and non-equilibrium conditions. Here we measure the elastic response of poly(ethylene glycol) using a lock-in based amplitude modulation-AFM with sub-angstrom amplitude in both low and high frequency regime. Appropriate analysis that takes into account the cantilever geometry and hydrodynamic loading effects of oscillating cantilever in liquid, relates X signal of lock-in amplifier linearly to stiffness. Stiffness data extracted from X signal was compared with stiffness from derivative of conventional “static” force extension curves. For stiffness data from X signal, fitting to standard entropic model of WLC gives a physically meaningful value of persistence length and also follows scaling behaviour of WLC. Entropy dominated conformational transition with its characteristic V-shaped signature was observed at around 250 pN. Accurate measurement of stiffness enabled us in understanding the thermodynamics of conformational changes.

INTRODUCTION

Studies that require stretching response of polymers range from non-linear elasticity in biological and polymeric networks and gels[11] to the collapse of single protein[2 3] in protein folding problem. The stretching dynamics of single polymer chains in solutions in the field of microrheology[4 5], to the mechanobiology of protein like titin in muscle contraction[54], stretching mechanics plays a fundamental role. Poly(ethylene glycol) is a water soluble, nontoxic and a biocompatible polymer with diverse applications[7 8]. Biocompatibility and water solubility of PEG hydrogels finds its use in biomedical application such as tissue regeneration[9]. Because of low toxicity and poor immunogenicity covalently attaching PEG with therapeutic proteins makes them useful in drug delivery[10], peptide resistance in aqueous solution for biomaterial preparation[8]. In biology as protein purification agent[8] or an ideal spacer molecule in recognition spectroscopy[11] PEG has numerous useful applications. Most of the mentioned applications stems from PEGs interesting physical properties in aqueous solutions and involve its elasticity. Thus elastic response of this simple but important polymer forms the basis for understanding more complex stretching studies in water and therefore has been the subject of both experiments[12 13] and simulations[14 15] revealing peculiar hydration effects.

In general, elastic response has been extensively studied for synthetic polymers[16 17], polysaccharides[16], polyelectrolytes[18 19] and proteins[20] using single molecule force spectroscopy experiments(SMFS). The commonly used technique of optical tweezers, magnetic tweezers and AFM have been employed to study these experiments[21]. In conventional AFM[22 23] force spectroscopy one end is attached to cantilever tip and other end to sample stage via some chemistry[24], is pulled at a constant velocity generating force versus extension profiles. A non-linear restoring force is generated as a result of decrease in conformational entropy of polymer with increasing extension. Profiles are then modelled with standard entropic models of freely jointed chain(FJC), wormlike chain (WLC) and freely rotating chain (FRC)[25 27] resulting in an estimate of an important parameter called kuhn length or persistence length, a measure of local stiffness of polymer chain. Because of fast changes in conformational entropy of polymers compared to the timescale defined by pulling velocity, these force extension profiles are termed as “quasi-static” profiles[25].

Dynamic force spectroscopy[32] initially developed for high resolution imaging[33 34] was latter used as a direct and independent way of measuring local stiffness and dissipation in single molecule[35 41], tip-surface interaction[41 46 47 53] and confined liquids[48 52 54]. Specifically in case of single molecule experiments, cantilever tip is oscillated either magnetically or mechanically[55] with a very small amplitude at a known frequency while the molecule is slowly being pulled. Changes in oscillation amplitude or frequency shifts due to change in interaction force depending on the mode of operation, are related to the stiffness of molecule. Dynamic methods offer increased sensitivity to local conformational changes[35 37 57 58] and allows for a direct measurement of interaction stiffness. In addition they also decouple elastic response(stiffness) from any dissipative component in the interaction. Appropriate modelling of cantilever-molecule ensemble that would relate changes in experimental parameters like amplitude to the physical quantity such as stiffness is crucial for correct interpretation of results. Studies done so far using dynamic force spectroscopy assumes cantilever beam as point mass with an ideal spring and dashpot in parallel. These approximations may lead to serious misinterpretation of elastic and dissipative response of molecule.
Benidetti et al.\cite{66} considered the equation of motion of whole cantilever beam vibrating in liquid and solved it in presence of molecule tethered to its end under appropriate boundary conditions. With proper choice of off resonance frequency and small amplitudes of oscillation, X-signal of lock-in was shown to be directly proportional to stiffness of molecule. Here we use this X-signal to measure elastic response of PEG and compare it with stiffness derived from quasi-static constant velocity pulling experiment. We show that there is quantitative difference in the measured stiffness from these two experiments. A correct estimate of persistence length results through X-signal.....

RESULTS AND DISCUSSION

Typical force versus extension curves of PEG in PBS buffer recorded with single molecule force spectroscopy experiments are shown in Figure 1(a). Statistical models like wormlike chain (WLC), freely jointed chain (FJC) and freely rotating chain (FRC) have been used in the past to describe such force extension curves. In FJC and FRC models length $L$ of the polymer is considered to be made up of discrete and uncorrelated rigid segments of length $b$ called kuhn length/bond length. A more physical and widely used model of WLC treats the polymer as a continuous chain with a characteristic length scale over which directional correlation persist in the chain called persistence length $l_p = b/2$. These models account for entropic dominated contributions to elasticity. Energetic contributions due to C-C backbone stretching becomes dominant in high force regime (> 500 pN) and would required extending above models \cite{29, 31} with additional parameters. Unambiguous estimate of such a parameter is possible only in very high force regime (> 1 nN) \cite{31} and since force more than 500 pN are hardly explored such extensions are ignored in our analysis. Equation 1 describes the marko sigga interpolation formula\cite{27} of WLC relating force $F$ to extension $z$ of polymer chain

$$ F = \frac{k_b T}{l_p} \left( \frac{1}{4(1 - \frac{z}{L})^2} - \frac{1}{4} + \frac{z}{L} \right) $$\hspace{1cm} (1)

with boltzmann constant $k_b$ and temperature $T$ in kelvin.

As shown earlier\cite{12, 15}, our experimental data is well described by WLC model (eq 1) and least square fitting to individual force curves yielded free parameters i.e the persistence length $l_p$ and contour length $L$. PEG is stretched to different contour lengths $L$ as seen in figure 1(a) varying from 80 to 150 nm depending on the point of attachment with cantilever tip and polydispersity of polymer sample. Despite large variation in contour lengths persistence length changed marginally over different force curves. Figure1(b) shows force versus normalized extension curves normalized by their respective contour lengths. Because elasticity scales as $1/L$ individual force curves superimpose over one another and such a procedure indicates that elasticity of single molecule with fixed persistence length is measured. A typical value of $l_p = 0.061 \pm 0.003$ nm was found from fitting WLC to normalized force extension data as shown in figure 1(c).

WLC fits reasonably well but the persistence length obtained is significantly lower than the C-C bond length (0.16nm) and this unphysical value of $l_p$ then suggests that entropic model of WLC may incorrectly describe our data even though it fits well because persistence length was chosen as an adjustable parameter. As shown previously\cite{59, 60, 63} persistence length acquires a force dependence and tend to decrease with increasing force as long wavelength bending modes on length scales $\varepsilon (= k_b T/b < b)$ less then kuhn length are progressively suppressed. Although such a decrease is always possible\cite{12} but a value lower than C-C bond length could also possibly come from changing hydration of PEG chains at higher force as shown in recent simulation\cite{15}.

To address these questions stiffness profiles of PEG chain using dynamic force spectroscopy were directly measured along with quasi-static force extension curves. For this the cantilever was simultaneously oscillated with a very small amplitude while the cantilever is pulled slowly at a constant velocity. In conventional amplitude modulation technique changes in oscillation amplitude and phase shift with respect to drive are monitored with a lock-in amplifier at a frequency close to or way below the resonance frequency. Within the framework of point mass model of cantilever, amplitude changes and phase shifts are mapped to stiffness and dissipation of molecule. Experiments done so far measuring viscoelasticity of single molecule are mainly done on resonance revealing large dissipation signals that qualitatively follows the behaviour of elastic signal\cite{40–42}. Such a crosstalk could arise from large phase shifts coming solely from changes in elastic response or what is called "altered boundary condition". Typically to avoid such a crosstalk and make a direct linear measurement of elastic response off resonance small amplitude operation are suitable because low frequency make amplitude response more sensitive to elastic response ($A(\omega) \sim \frac{1}{\omega^2}$) \cite{35, 56}. These amplitude changes are very sensitive to conformational changes of the polymer and hence improves the resolution. However the measurement is still based on assumption of point mass model and may lead to errors if not operated at true off resonance condition\cite{35, 43}. To this end Benidetti et al.\cite{66} have solved for vibrating cantilever beam in liquid with molecule coupled to its end and shown that under appropriate choice of off resonance frequency and very small oscillation amplitude in phase X-signal from lock-in amplifier is directly related to stiffness/elastic response and out of phase Y-signal to dissipative response. Such a treatment neatly decouples elastic and dissipative part of interaction without any inbuilt assumptions.

We therefore recorded the X and Y signal simultaneous
with force extension curves as shown in figure 2(a). Force extension curve and X-signal show good correlation with peaks occurring at same position. Y-signal show no variation with extension except a jump corresponding to detachment of molecule. Featureless Y-signal means no internal dissipation due to backbone deformation as expected for a flexible polymer like PEG. This also confirms the fact that choice of off resonance frequency is appropriate. Stiffness profile generated from X-signal via eq 3 is plotted as a function of force on a log-log scale. For force larger than 70 pN i.e high force regime 15 such traces were overlaid as shown in figure 2(b) and a linear fit with slope 1.4 indicates that stiffness scales with force with an exponent 1.4. Stiffness of a WLC chain scale with force as 3/2 in high force regime irrespective of contour length length or persistence length. Therefore scaling analysis clearly shows that stiffness extracted from X-signal in general follows a wlc behavior.

Further figure 3 shows dynamic stiffness profiles plotted as a function of extension along with stiffness from the derivative (dF/dx) of wlc fit to force extension curves. These two different data sets show significant deviation from each other in their curvatures and peak value. Curvature is a measure of persistence length $p$, and therefore dynamic stiffness measurement would result in a different persistence length. Dynamic stiffness asymptotically approaches its contour length with a typical peak value that is 2-3 times less than force derivative implying increased resolution/sensitivity. Data collected in both low (500-700 Hz) and high frequency regime (1700-1900 Hz) show similar behaviour indicating that "static" entropic stiffness is measured. Dynamic stiffness is then fitted with derivative of equation 1 as shown in figure 4 with a fixed persistence length of 0.4 nm. Persistence length is consistent with both experiment[12] and molecular dynamics simulation[15] done in low force regime and is not less than the size of monomer ($\sim 0.35$ nm). Reason for increased resolution is partly due to increased sensitivity of amplitude changes in dynamic measurement to local conformation changes of the polymer[37, 58]. However amplitude response of cantilever in liquid is also marred with the hydrodynamic load of liquid on the cantilever i.e viscous drag on the cantilever and added mass that drags along the cantilever. This leads to broadening of resonance and shift in resonance frequency to a low frequency respectively[61]. It is imperative to account for such hydrodynamic forces in order for amplitude changes to achieve their optimum resolution. Hydrodynamic contribution is a constant addition to actual stiffness in X-signal and is easily separated giving us good resolution. Studies done so far that accounts for hydrodynamic effects or measure stiffness through frequency shifts with active Q control have shown similar quantitative behaviour as well as increased resolution[39, 62, 65]. Nevertheless stiffness is still interpreted using ad hoc point mass model and can give us different persistence length.

This is because for a point mass model stiffness is inversely proportional to amplitude in off resonance condition but in the present analysis X-signal is directly proportional to stiffness. Recently[15] molecular dynamics simulation were performed to thermodynamically explain static force extension profiles. They showed that elasticity of PEG is dominated by non entropic hydration effects with water entropic contribution cancelling with entropic FJC chain. Simulation however neglects conformational entropy of PEG backbone due to gauge to all trans transition. We show in figure 4 a typical V-shaped minima in stiffness versus force plot signifying the entropy dominated conformational transition[63, 64]. This entropic contribution would need to be included to correctly map the thermodynamics of stretched PEG molecules. Note that minima also incorrectly appears in dissipation signal due to on resonance amplitude and phase measurement[65].

Materials and methods

Sample preparation

The experiments were performed using a commercial AFM (Model JPK Nanowizard II). Micro-fabricated commercially available gold coated cantilevers, with dimensions length $= 300 \pm 5 \mu m$, width $= 35 \pm 3 \mu m$ and thickness $= 2 \pm 0.5 \mu m$, are used. The stiffness is calibrated using thermal noise method[67]. The stiffness of the cantilever used in the measurement is $0.57 \ \text{N/m}$. The range of resonance frequency is around $8.9 \ \text{kHz}$ in...
FIG. 2: (a) A typical force versus extension curve (shown at the top) with their corresponding X and Y signal (at the bottom) is shown. (b) Scaling of stiffness versus force on a log-log scale in force range from 70 to 430 pN with a scaling coefficient 1.4.

FIG. 3: Comparison of stiffness from derivative of the fitted wlc curve from static force extension data (blue) and directly through X-signal (red) for both (a) low frequency (500–700 Hz) and (b) high frequency (1700–1900 Hz) regimes.

FIG. 4: (a) Dynamic stiffness from X-signal fitted to a derivative of wlc model (equation-1) with fixed persistence length of 0.4 nm (b) Comparison of stiffness from derivative of force extension curve and directly from X-signal.

water. The cantilever is oscillated with free amplitude in the range of 0.5 – 2 Å, at a frequency which is less than one third of resonance. The flatness of the frequency response in this region is ensured for off-resonance operation. The cantilever is held in place by a spring and its base is attached to a dither piezo. A sinusoidal drive voltage is supplied to dither using the lock-in SR830 (Stanford Research Systems). After completing the sample preparation protocol, using the cantilever deflection as signal for feedback control, the tip is auto-approached towards the surface. The point of approach was defined to be the tip applying below 100 pN of force on the sample during approach. Experiments were performed on a 10 × 10 μm² grid in a single run with total of 64 or 128 measurements. The attachment of polymer to the tip was achieved by non-specific interaction between the tip and sample. The tip was retracted from the surface at a constant speed of 50 nm/s. The stiffness and dissipation of the polymer is measured while it is being pulled.

FIG. 5: Schematic

Data analysis

In contrast to point-mass model approximation for cantilever dynamics, many theoretical treatments have considered geometric details of cantilever beam. This formalism is used for calibration of cantilever stiffness in liquid environments. The resulting equation of motion of the cantilever is fourth order partial differential equation in space and time. To solve this equation, the boundary conditions at the free and fixed ends of the cantilever are used. Benedetti et al. have used this formalism in the context of quantifying the dissipation in single molecules measured by dithering the cantilever tip in off-resonance conditions. In this work, the data analysis used for calculating dissipation (γi) and stiffness (ki) from measured quantities is based on this formalism. The equation of motion is

\[-\tilde{\rho}S \frac{\partial^2 z}{\partial t^2} - \gamma_c \frac{\partial z}{\partial t} = EI \frac{\partial^4 z}{\partial x^4}\]  \hspace{1cm} (2)

Where z(x,t) is cantilever displacement at position x from the base (x = 0) and at time t. The z(x,t) performs a simple harmonic motion and can be represented by a rotating vector having R having components X and Y given by X = Rcos(θ) and Y = Rsin(θ). The amplitude and phase of the oscillations is given by \(R^2 = X^2 + Y^2\) and \(\theta = \tan^{-1}(Y/X)\). After applying suitable boundary conditions, the protein stiffness and dissipation can be calculated by recording X and of tip oscillations.

\[X = \frac{A}{2k_iL}(-3k_i + \tilde{\rho} S \omega^2)\]  \hspace{1cm} (3)

and

\[Y = \frac{A}{2k_iL}(3\gamma_i + \gamma_c L)\]  \hspace{1cm} (4)

We record X and Y using lock-in amplifier and use equations 2 and 3 to estimate the stiffness (ki) and dissipation.
CONCLUSION

In conclusion, we have shown that elasticity of poly(ethylene) glycol is entropic dominated well described by coarse grained entropic model of wlc. A more meaningful persistence length of 0.4 nm results from such a model analysis. Well predicted conformational transition exists around 250 pN with its characteristic V-shape and is entropy determined. Dynamic force spectroscopy offers more sensitive measurement of conformational landscape and therefore correctly predicts the underlying thermodynamics.

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