Exploring the Folding Pattern of a Polymer Chain in a Single Crystal by Combining Single-Molecule Force Spectroscopy and Steered Molecular Dynamics Simulations

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Supporting Information

ABSTRACT: Understanding the folding pattern of a single polymer chain within its single crystal will shed light on the mechanism of crystallization. Here, we use the combined techniques of atomic force microscopy (AFM)-based single-molecule force spectroscopy (SMFS) and steered molecular dynamics (SMD) simulations to study the folding pattern of a polyethylene oxide (PEO) chain in its single crystal. Our results show that the folding pattern of a PEO chain in the crystal formed in dilute solution follows the adjacent re-entry folding model. While in the crystal obtained from the melt, the nonadjacent folding with large and irregular loops contributes to big force fluctuations in the force-extension curves. The method established here can offer a novel strategy to directly unravel the chain-folding pattern of polymer single crystals at single-molecule level.

INTRODUCTION

Analysis and characterization of the folding pattern of polymer chains in their single crystals is an exceedingly challenging task, mainly due to the long-chain nature of the polymer molecules leading to highly complex and various growth forms. Despite techniques such as neutron scattering1 and IR spectroscopy2 having been used to indirectly probe the chain folding pattern of polymer single crystals, single-molecule-level experimental verification of the chain folding pattern has only been gained so far in silico3 due to the complexity of the sample system and the limitation of detecting methods. With the appearance of those elegant single-molecule manipulation techniques, such as atomic force microscopy (AFM),4,5 optical tweezers,6 magnetic beads,7 and so on, the study of molecular interactions at the individual molecule level becomes possible. Among those single-molecule manipulation techniques, AFM can work in both aqueous solution and organic solvent, making it a powerful technique in the investigation of molecular interactions in biological and synthetic polymer systems.8–10 Fruitful information can be derived from the force spectrum of polymers. Due to the fact that some force spectra can be correlated well with the force-induced structure/conformation change of a polymer chain, the force spectrum can also be termed a force fingerprint of the specific polymer. For example, the sawtooth pattern in the force spectrum may represent the unfolding of multiple polyproteins,9,11 the sequence-dependent unbinding of RNA–protein complexes,12 or detachment of multiple polymer loops from the substrate;8,13 long plateau-containing force curves may reflect the unwinding of the helical structures13,14 or detachment of train-like polymer fragments from the substrate.8,15 This information has deepened our understanding of the relationship between molecular structure and mechanical properties of individual polymer chains. Apart from the great progress in single-molecule detection techniques, great advancement has also been made in the field of steered molecular dynamic (SMD) simulations, in which the force-induced conformation transition can be revealed at the atomic level, representing a powerful tool complementing and guiding single-molecule experiments in vitro.16–18

Here, we used AFM-based single-molecule force spectroscopy and steered molecular dynamics (SMD) simulations to explore the folding pattern of a polyethylene oxide (PEO) chain in its single crystal prepared by solution or melt crystallization. SMD simulations have been performed on adjacent re-entry19 and nonadjacent folds20,21 of PEO crystal
systems. SMFS results on PEO single crystals grown from dilute solution and the melt phase were compared with those SMD simulations, and different folding patterns were identified for those two types of polymer crystals.

■ EXPERIMENTAL SECTION

Materials and Sample Preparation. Thiol-terminated poly-ethylene oxide (HS-PEO-OC8H17, Mn = 48.8 kDa/g/mol, PDI = 1.05) was purchased from Polymer Source Inc. Amyl acetate and dimethylsulf-oxide were purchased from Aldrich. N-((3-Maleimidopropanamido) succinimide ester (AMAS) was purchased from Thermo Scientific (Pierce). 3-Aminopropyl(dimethyl)methoxysilane was purchased from Fluorochem, UK. All materials were used as received. PEO single crystals were prepared from solution and melt by the self-seeding method, which was adapted from the previous work by Li et al.22 and Wang et al.23 The process of immobilization of the PEO single crystals has been described in detail in the Supporting Information and our previous work.24

SMD Simulations. The steered molecular dynamics simulations were performed in the NPT ensemble (1 atm press and 298 K) using the programs VMD1.8.7,25 NAMD2.7,26 and the CHARMM32 ether force field,27,28 with an integration time step of 1 fs. Van der Waals (vdW) energies were calculated using a smooth (12 Å) cutoff and the programs VMD1.8.7,25 NAMD2.7,26 and the CHARMM32 ether force field,27,28 with an integration time step of 1 fs. Van der Waals (vdW) energies were calculated using a smooth (12 Å) cutoff. We set up the crystal structure model by MS Modeling (the direction of crystal lattice c corresponding to z axis); 5 times length of one crystal lattice along z axis was used so the length was nearly equal to the thickness of the crystal as estimated in the AFM experiment (∼10 nm), and constrained atoms in the outside chains (along z axis) to mimic the expanded crystal. After energy minimization and a 2 ns equilibration of the simulation system, we randomly fixed four terminal carbon atoms (as the fixed atom) at one side of the crystal, and pulled one chain of the opposite surface by attaching a harmonic spring (with a force constant of 7 kcal/mol/Å2) in between the terminal carbon (as the SMD atom) and the stretching point at a constant speed of 20 m/s along z axis.

Single-Molecule Force Spectroscopy. All AFM experiments were carried out on a NanoWizardII BioAFM (JPX instrument AG, Berlin, Germany) using gold-coated Si3N4 AFM tips (Biolever, Olympus Japan) and Si3N4 tips (MSCT, Bruker Corp.). Cantilevers were calibrated in solution using the thermal noise method29 to obtain the spring constant (typically around 0.06 N/m for MSCT and 0.03 N/m for Biolever). All experiments were performed in amyl acetate at room temperature with a pulling velocity of 2 μm/s. The experimental details have been described in our previous work.24 For a random picking experiment, the same AFM tip can be used up to three times (i.e., extracting three individual molecules out of the single crystal) without significantly affecting the experimental result, while for the gold nanoparticle positioning experiment, the AFM tip can normally be used only once. All data were analyzed using custom software written in Igor Pro (WaveMetrics).

■ RESULTS AND DISCUSSION

SMD Simulations on the Pulling of Adjacent Re-Entry and Nonadjacent Re-Entry Folding Chains Produce Different Force Patterns. To describe the folding patterns of polymer chains in their crystals, different models have been suggested, such as the adjacent re-entry folding,19 the nonadjacent folding (including the switchboard),20,21 and so on. There are many arguments on the correctness of those models. To test those suggested models, SMD simulations were performed by assuming ideal adjacent re-entry and nonadjacent re-entry folding for the PEO chains within the single crystal, respectively. It needs to be pointed out that although the SMD simulation was performed at high pulling speed (due to the limitation of computing power) this will not affect the accuracy of the pattern of force changes along the lateral pulling direction.16–18 For the adjacent re-entry folding, the adjacent re-entry folding mode the nonadjacent PEO fragments are connected by the amorphous loops containing various number of repeat units 1, 3, and 5, respectively (shown in Figure 1B). Figure 1C shows the pulling of six stems of PEO fragments (three folds) out of its single crystal. The arrows (a, b, c) show the unfolding of top loops that contain 5, 3, and 1 repeat units, respectively.
Information Movie). The valley regions, which are still well above zero, correspond to the unraveling of the loop at the amorphous region that locates on the top crystal surface.

The patterns on the unfolding curves are quite regular for the adjacent re-entry folding mode, though more complicated nonadjacent re-entry folding mode is observed (see Figure 1D). On the valley regions, the unbinding force decreases \((c\rightarrow a)\) with the increase of loop sizes \((1\text{ to } S)\) on the top surface of the crystal, while on the peak regions, the force is clearly enhanced by the bigger bottom loops. When these facts are put together, we can understand the bigger force fluctuations observed in nonadjacent re-entry folds, that is, the length of the loops located on both crystal surfaces significantly affects the regularity of patterns on the stretching curves.

**Chain Folding in Solution-Growth PEO Single Crystal Is Different from That in Melt-Crystallized One.** Apart from SMD simulations, we have also used single-molecule force spectroscopy to experimentally investigate the folding patterns of PEO chain in single crystals prepared from the dilute solution and melt, respectively. To pull a single PEO chain out of its crystal, we have developed a method for the immobilization of the single crystal and for the attachment of the polymer chain end to the AFM tip as described in our previous study.\(^{24}\) In brief, thiol-labeled PEO single crystals, which were prepared from its dilute solution, were immobilized by one side on silicon substrate via a short cross-linker \((N-(\alpha\text{-maleimidoacetoxy})\text{succinimide ester, AMAS})\), leaving the other side of the crystal surface exposed for picking up by the AFM tip. The SMFS pulling experiments were performed on the crystal surface with a gold-coated AFM tip under the guidance of AFM imaging (Figure S1 show the AFM images of PEO single crystal prepared from dilute solution and the melt). In other words, by AFM imaging, a specific single crystal can be found, then the AFM tip is brought into contact with the specific area on the crystal surface then separated from it. During contact, the thiol end of the polymer chain can get attached to the AFM tip via gold–thiol chemistry. The PEO molecule can then be pulled out of the crystal during separation. Force–extension curves with a sawtooth-like plateau were obtained during the pulling experiments, as shown in Figure 2A. Such curves take about 90% in total. Since it is widely accepted that single crystals formed in dilute solution should fold more regularly, we intend to compare our SMFS result with those SMD simulations on adjacent re-entry folding systems. Due to the huge difference in stretching velocity between the SMD and AFM-SMFS experiments, it is impossible to quantitatively compare the forces (i.e., the absolute forces are not comparable). However, the patterns on the stretching curves are quite similar and the force plateaus are relatively flat (shown in Figure 2B), which indicate that the sizes of the loops on the crystal surfaces should be relatively small and regular, supporting the adjacent re-entry folding model. It needs to be pointed out, however, that the periodicity of the sawtooth pattern on stretching curves obtained in SMFS experiments is not as regular/obvious as that in SMD simulations. This may due to the following facts: (1) for the current SMD simulation on the pulling of adjacent re-entry folds (Figure 1), the folding mode is idealized, and the size of loops in the amorphous region is very small and uniform; (2) in real solution-crystallized single crystals, the polymer chains can adopt the adjacent re-entry folding mode, but the sizes of loops that connect the adjacent stems may show some variation;\(^{19,20}\) (3) the force fluctuation in the sawtooth pattern of the stretching curve obtained by SMFS is of similar magnitude to that of thermal noise; as a result, some patterns are not recognizable. SMD simulation results of the pulling of adjacent re-entry folding but with bigger loop sizes (containing six repeat units of PEO) show that the loop-size change (from one to six) will affect the gap between adjacent peaks (i.e., the periodicity of the force pattern); please see Figure S2 in Supporting Information for more detail. This is because the originally bigger loop will get shortened to the minimum length before it enters the crystal phase during stretching as shown in the Supporting Information Movie. In addition, there are some force–extension curves (14% in 1662 curves) which show deviation from the force plateau at short and long extensions (shown in Figure 2A and C). This may be ascribed to irregular folding at the ends of the polymer chain.

![Figure 2](image)

For the single crystal obtained from melt, PEO chains were individually pulled out of its single crystal, which was crystallized on the silicon substrate. Typical force curves show a plateau with very large fluctuations (see blue curves in Figure 3A,B) similar to the SMD simulations on irregularly folded systems (shown in Figure 1). Therefore, it is not difficult to imagine that part of the loops on such crystal surfaces should...
To find out the magnitude of fluctuation on the force plateau more accurately, more than 200 stretching curves were used to construct histograms of force distribution on the retraction baseline (the right region of the stretching curve after the force plateau, as shown in Figure 3A) and the force plateaus, respectively, as shown in Figure 3C. From the figure, we can see that the noise level on solution- and melt-crystallized samples is almost the same since the force distribution of the retraction baseline is indistinguishable (see left histograms marked with dotted line in Figure 3C), while the force distribution of the force plateau from the melt-crystallized sample is broader than that of the solution-crystallized one (see right solid histograms in Figure 3C) indicative of bigger fluctuations. By superimposing the center of the histogram of the baseline with the histogram of the force plateau, we can see more clearly the difference of the fluctuation magnitude. The force fluctuations on solution-crystallized sample (colored in red) are close to that of the baseline (i.e., the noise level), as shown in the Figure 3D top traces, while the fluctuation from the melt-crystallized sample is much bigger since the force distribution is much broader (see bottom traces in blue in Figure 3D).

To put all these results together, we can estimate that the adjacent re-entry folding mode plays a predominant role in the crystal grown from solution and nonadjacent re-entry with large and irregular loops occurs in the crystal prepared from the melt.

■ CONCLUSIONS

In summary, the folding pattern of a PEO chain in its single crystal has been explored for the first time at single molecule level by a good combination of single-molecule force spectroscopy and SMD simulations. The results demonstrate that adjacent re-entry folding is the predominant mode in the crystal prepared from dilute solution, whereas nonadjacent re-entry with large and irregular loops on the crystal surfaces occurs in the crystal from the melt. We believe that this study has provided a new physical insight into the polymer crystallization and the combined techniques represent a novel strategy to directly investigate the folding pattern/mechanism in other polymer single crystals as well.

■ ASSOCIATED CONTENT

* Supporting Information
Description of sample preparation, AFM images on single crystals prepared form solution and melt, together with SMD simulations on adjacent re-entry folds with different loops sizes.
This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was funded by the NSFC (20974039, 21221063, 91127031) and NSFC-DFG joint project (TRR61), and the National Basic Research Program (2013CB834503). The work was also supported by SRF for ROCS (SEM), the Program for
New Century Excellent Talents in University (NCET), the State Key Laboratory, and the High Performance Computing Center of Jilin University. We thank Prof. Zhigang Wang (JLU) for helpful discussions and suggestions on SMD simulations.

REFERENCES


