

How Big Is Big Enough? Effect of Length and Shape of Side Chains on the Single-Chain Enthalpic Elasticity of a Macromolecule

Zhonglong Luo,[†] Afang Zhang,[‡] Yongming Chen,[§] Zhihao Shen,^{||} and Shuxun Cui^{*,†}

[†]Key Laboratory of Advanced Technologies of Materials, Ministry of Education, Southwest Jiaotong University, Chengdu 610031, China

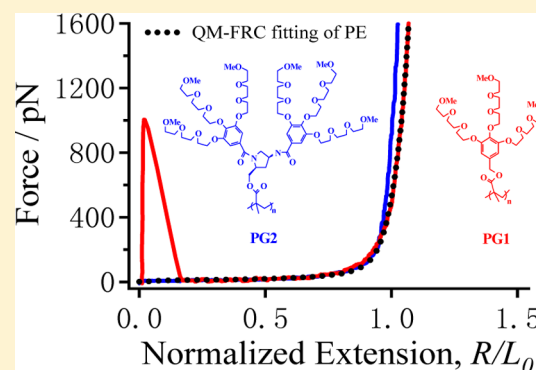
[‡]Department of Polymer Materials, College of Materials Science and Engineering, Shanghai University, Nanchen Road 333, Shanghai 200444, China

[§]School of Materials Science and Engineering, Sun Yat-sen University, Guangzhou 510275, China

^{||}Beijing National Laboratory for Molecular Sciences, Key Laboratory of Polymer Chemistry and Physics of Ministry of Education, Center for Soft Matter Science and Engineering, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

Supporting Information

ABSTRACT: Polymers with a carbon–carbon (C–C) backbone are an important class of polymers, which can be regarded as the derivatives of polyethylene (PE). To investigate the effect of side chains on the single-chain enthalpic elasticity (SCEE) of polymers with a C–C backbone, several polymers with pendants or side chains of different lengths and shapes have been studied by single-molecule AFM. We find that both length and shape of the side chains count: only the side chains that are both long and bulky (i.e., bulky dendrons of second or higher generation as side chains) affect the SCEE. Thus, only rare polymers have special SCEE. For the vast majority of polymers, the SCEE is identical to that of PE, which means that the SCEE is determined by the nature of the C–C backbone. It is expected that this conclusion can also be popularized to all polymers with various backbones. This study is an important update to the understanding of polymers at the single-chain level.



INTRODUCTION

In general, all linear polymers are composed of backbones and side chains. Polymers with a carbon–carbon (C–C) backbone are an important class of polymers, which can be regarded as the derivatives of polyethylene (PE). It has been long recognized that the physical properties of bulk polymeric materials can be adjusted by side chains.¹ For instance, the typical physical properties of polystyrene (PS) are largely different from those of polypropylene (PP) (see Table S1). At the single-chain level, it has been reported that polymers with larger side chains will show a more extended conformation (i.e., more rigid).^{2,3} However, a flexible polymer chain can also be stretched into an extended conformation by a common fluid flow,^{4,5} which actually only exerts a very weak force with a magnitude of ~ 1 pN (see Supporting Information for details).

Recently, studies by the atomic force microscopy (AFM)-based^{6–22} single-molecule force spectroscopy (SMFS)^{23–42} show that at the low force region ($F < \sim 100$ pN) the behavior of a polymer chain is mainly affected by its entropic elasticity, while at the high force region ($F > \sim 300$ pN) it is mainly affected by the enthalpic elasticity (Figure 1). Previous SMFS studies indicated that the single-chain apparent elasticity of polymers can be affected by both surrounding environments

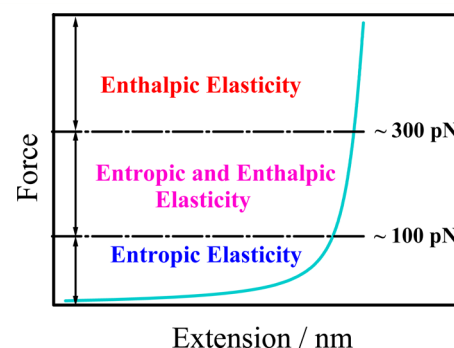


Figure 1. A typical F – E curve showing the elastic behavior of a polymer chain. The low force region ($F < \sim 100$ pN) is mainly affected by entropic elasticity, the high force region ($F > \sim 300$ pN) is mainly affected by enthalpic elasticity, and the middle force region (~ 100 pN $< F < \sim 300$ pN) is affected by both enthalpic and entropic elasticity.

and condensed state.^{29,34,43–46} For example, the single-chain elasticity of poly(N,N -dimethylacrylamide) (PDMA) and

Received: February 1, 2016

Revised: March 28, 2016

Published: April 21, 2016

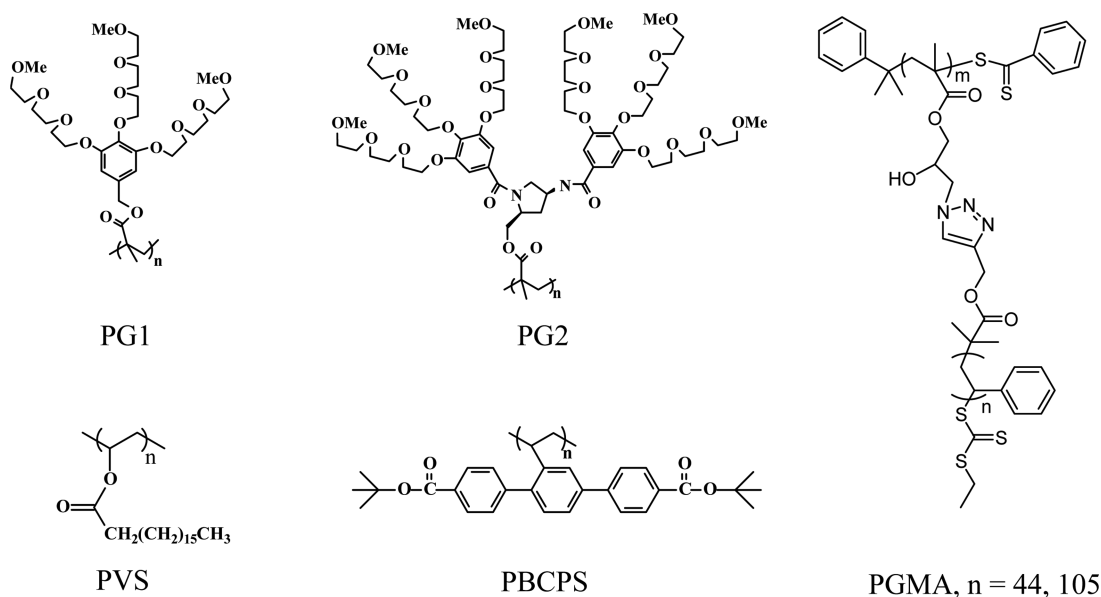


Figure 2. Molecular structures of the polymers with large side chains. PG1 and PG2 are the first- (G1) and second-generation (G2) dendronized polymers. PBCPS is a jacketed polymer with a short bulky side chain. PVS, PGMA-g-PS₄₄, and PGMA-g-PS₁₀₅ are the polymers with thin (short or long) side chains.

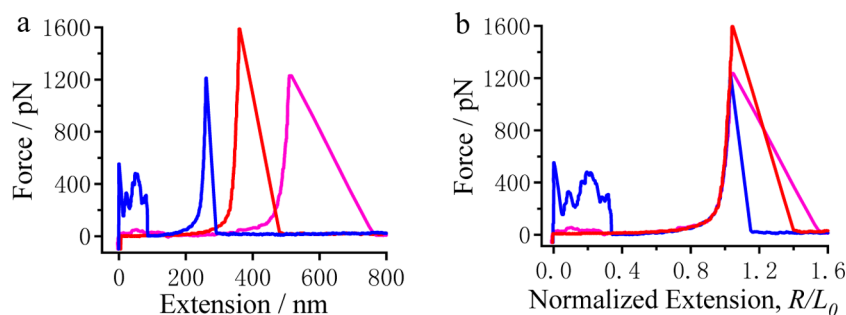


Figure 3. Single-chain elasticity of PG2. (a) Typical $F-E$ curves of PG2 obtained in nonane. (b) Normalized $F-E$ curves of those shown in (a).

poly(*N,N*-diethylacrylamide) (PDEA) can be influenced remarkably by the aqueous solutions (Figure S2).²⁹ Therefore, to avoid the influence from environments, force measurements should be completed under an unperturbed state, e.g., in a vacuum. Taking into account the limits of experimental conditions, however, it is difficult to carry out the experiment under vacuum conditions. In general, the interactions between the nonpolar solvent and the solute molecules are van der Waals forces, which can be ignored in many cases. This presumption has been validated by previous SMFS studies.⁴⁷ Thus, we can choose nonpolar organic solvents to replace the vacuum conditions.^{45,48} In a previous SMFS study, we have found that although the three kinds of polymers (i.e., PS, polyacrylamide (PAAm), and PDEA) have different side chains, they show the same single-chain enthalpic elasticity (SCEE),⁴¹ which is identical to the theoretical SCEE of PE.²⁵ These results imply that the effect of the size and polarity of side chains on the polymer SCEE can be ignored. Considering the limit of only three kinds of polymer samples with relatively small side chains used in the prior work, the conclusions should be also limited. Here in this paper six kinds of polymers with a C–C backbone having pendants or side chains of various lengths and shapes (Figure 2) are studied to find a more rigorous principle for the polymer SCEE ($F > \sim 300$ pN).

MATERIALS AND METHODS

Materials and Chemicals. Six kinds of polymers with the same C–C backbone but side chains of different lengths and shapes are used in this study, including two kinds of dendronized polymers (PG2 and PG1), two kinds of brush-like polymers poly(glycidyl methacrylate) (PGMA) with side chains of different lengths, PGMA-g-PS₄₄ and PGMA-g-PS₁₀₅, one jacketed polymer with a short bulky side chain, poly[2,5-bis(4-carboxylic phenyl)styrene] (PBCPS), and poly(vinyl stearate) (PVS). The polymers PG1, PG2, PBCPS, PGMA-g-PS₄₄, and PGMA-g-PS₁₀₅ are synthesized according to the literature.^{3,49,50} The M_n are measured to be 1.98×10^6 , 2.4×10^6 , 8.63×10^5 , 5.88×10^5 , and 8.74×10^6 g/mol, and the molecular weight polydispersity indexes are 1.88, 2.21, 1.1, 1.1, and 1.1, respectively. PVS ($M_w = 9.0 \times 10^4$ g/mol, analytically pure) is purchased from Aldrich. The molecular structures of these polymers are shown in Figure 2. Deionized (DI) water (>15 M Ω -cm) is used when water is involved. All other chemicals are analytically pure and used without further treatment.

Sample Preparation. PG2 and PG1 are dissolved in DI water to a concentration of 10 and 50 mg/L, respectively. PBCPS, PVS, PGMA-g-PS₄₄, and PGMA-g-PS₁₀₅ are dissolved in tetrahydrofuran to a concentration of 1.0, 1.0, 0.5, and 0.5 mg/mL, respectively. The quartz slides, which are treated by a hot piranha solution (98% H₂SO₄ and 35% H₂O₂, 7:3, v/v) for 30 min, are used as the substrates. (Warning: piranha solution is extremely oxidizing and should be handled with extreme care!) To prepare the sample for SMFS, a few drops of the sample solution are allowed to adsorb onto the clean substrate for ~ 1 h. Then, the sample is thoroughly rinsed with the abundant pure

solvent to remove the loosely adsorbed molecules, followed by drying with air flow. After that, the sample is instantly used in the force measurements. An organic solvent, nonane, is used as the liquid environment in this study.

Force Measurements. A commercial AFM (NanoWizard II, JPK Instruments, Germany) is used in the force measurements. All of the force measurements are carried out at room temperature (RT, ~ 23 °C). Prior to the force measurements, the spring constant of each AFM cantilever (MCLT model, Bruker Corp., CA) is tested by employing the thermal excitation method (from 35 to 55 pN/nm). The stretching velocity is $2.0 \mu\text{m/s}$ if not mentioned otherwise. The details of the AFM instrumentation can be found in refs 13, 43, and 51.

RESULTS AND DISCUSSION

PG2, which is the second-generation dendronized polymer, is studied first due to the most bulky pendants among the samples (Figure 2). The typical F – E curves of PG2 obtained in a nonpolar solvent, nonane, are shown in Figure 3a. In each F – E curve, the force rises monotonically with elongation of the chain until the breakage of the molecular bridge between the tip and substrate, where the force descends to the noise level of the apparatus rapidly. On account of the polydisperse characteristic of the polymers and the stochastic position where the AFM tip picks, the apparent contour lengths of the elongated chains vary (Figure 3a). To compare the F – E curves of different contour lengths, the F – E curves are normalized by their extension at a given force, e.g., 500 pN (see Figure 3b). The normalized F – E curves of PG2 can be superposed well in the entire force region, indicating that the F – E curves present the single-chain elasticity of PG2.⁴⁷

The F – E curves of PG2 with different pulling speeds (0.2 – $10 \mu\text{m/s}$) are also obtained. After normalization, there are no evident differences among them (see Figure S3). This result indicates that the time scale of the movement of the segments in the chain is much shorter than that in the force measurements, suggesting that the force measurements are carried out under the quasi-equilibrium condition.^{24,45}

It is well-known that the behaviors at the single-chain level of many macromolecules can be roughly described by several physical models, e.g., the freely rotating chain (FRC) model, the worm-like chain (WLC) model, and the freely jointed chain (FJC) model.^{25,42,52} In particular, the modified forms of these models can describe the single-chain elastic behavior of many polymers semiquantitatively even if they are only uncomplicated approximations.^{24–26,52} Recent reports showed that the experimental single-chain F – E curves of polymers with a C–C backbone can be described well by the FRC model.^{24,41,52} Considering that the bond angles and bond lengths are increased upon elongation, quantum-chemical (QM) calculations are used to obtain the theoretical SCEE of the polymers.²⁵ By integrating the QM results into the FRC model, the QM-FRC model is obtained (see Supporting Information for details).⁴¹ The QM-FRC model of PE can fit the F – E curves of the polymers with a C–C backbone (e.g., PS; see Figure S4) well while l_b (the length of the rotating unit) is 0.154 nm, which is identical to the length of a C–C bond.⁴¹ This result indicates that the influences on the polymer SCEE from the side chain of these polymers (PS, PAAm, and PDEAm) are similar to the H atoms of PE, which can be ignored. We try to use the same QM-FRC model to describe the single-chain elasticity of PG2 because the polymer PG2 has the same backbone to PE. A QM-FRC fitting curve is generated and plotted together with the F – E curve of PG2 (see Figure 4).

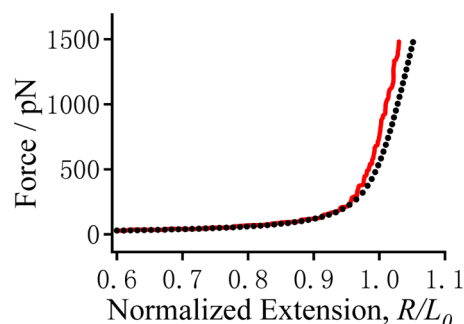


Figure 4. Typical F – E curves of PG2 obtained in nonane (solid curve) and the QM-FRC fitting curve of PE with $l_b = 0.154$ nm (dotted line).

As shown in Figure 4, however, it is surprising that there is a marked deviation between the QM-FRC fitting curve of PE (which can fit the F – E curve of PS perfectly; see Figure S4) and the F – E curve of PG2, especially in the high force region ($F > 300$ pN), where the F – E curve is apparently steeper than the fitting curve. This result indicates that the SCEE of PG2 is strongly influenced by its bulky dendron pendants. That is to say, the effect of the G2 side chain on the polymer SCEE cannot be ignored in this case. Previous studies by AFM imaging showed that the large side chains (i.e., the bulky dendrons of second or higher generation) would make the main chain rather stiff.^{2,50} The possible reason may be that the polymer chain will be extended by the steric force from the dendron side chain even if there is no external force. Recently, Schlüter et al. have found that the main chain will be cut into short fragments during the process of synthesis of higher-generation dendronized polymers (PG5), which may be induced by Coulombic forces, shear forces, and steric forces.⁵³ However, for the neutral polymer PG2 (Figure 2) in nonpolar solvents, the Coulombic forces can be ignored.^{53,54} The shear forces mainly originate from the stirring of the solution, which is not applied in the current study. Therefore, the SCEE of PG2 is mainly influenced by the steric forces from the pendants.⁵⁴

Note that in the QM calculations for PE, H atoms (the smallest one among all groups) are set as the side chains. However, the size of the G2 side chain is much bigger than that of the H atom. Thus, we think that the discrepancy between the fitting curve and F – E curve in Figure 4 should originate from the QM calculations. For the polymer PG2, the elastic moduli may be influenced by the bulky side chain. Thus, the elastic moduli (γ_1 , γ_2 , and γ_3 ; see Supporting Information for details) obtained from the QM calculations of PE may not be suitable for PG2. Thus, it is reasonable that the F – E curve of PG2 is not described well by QM-FRC model of PE in the high force region. To obtain the correct fitting result, the elastic moduli should be calculated by the QM calculations with the real structure of PG2. However, because of the limit of the computing capacity at present (which is usually used to calculate the molecules with tens of atoms), it is difficult because there are more than 200 atoms in each repeating unit of PG2.⁵⁰ Meanwhile, the steric force from the large side chains exists only in the molecules under very crowded conditions, i.e., in a polymer chain. Thus, the steric force from the large side chain will not exist in the expected QM result with only one repeating unit. As a result, the QM calculations of PG2, which must consider the steric force from the huge side chain, would be impractical at present. However, it is expected that we can obtain a new set of elastic moduli for PG2 through the way of

modifying the values of γ_1 , γ_2 , and γ_3 on the basis of $l_b = 0.154$ nm for a C–C backbone polymer. To achieve a minimum difference between the F – E curve of PG2 and the fitting curve, we adjust the γ values over and over again. One of the optimal solutions is obtained, through which a good fitting curve for PG2 can be generated (see Supporting Information for details). This artificial fitting method is practical since accurate QM calculations are difficult for PG2 at present. The large difference between the γ values for PG2 and PE further illustrates that the SCEE of PG2 is influenced greatly by its huge side chain.

Since the huge G2 side chain is an extreme case and there is a tremendous gap between the sizes of G2 and H atom, a series of polymers with side chains of different lengths and shapes are needed to observe the side-chain effects. Four other kinds of polymers (i.e., PVS, PBCPS, PGMA-*g*-PS₄₄, and PGMA-*g*-PS₁₀₅; see Figure 2) have been studied further in this study. PVS is a polymer that has an alkane side chain (–C₁₇H₃₅). PBCPS is a jacketed polymer with a short bulky side chain.⁴⁹ PGMA-*g*-PS₄₄ and PGMA-*g*-PS₁₀₅ with long thin side chains are brush-like polymers.⁵⁰ It is helpful to note that there is a characteristics, i.e., the large side chains, shared by all these polymers, especially PGMA-*g*-PS₄₄ and PGMA-*g*-PS₁₀₅.

The typical F – E curves and those after normalization of PVS obtained in nonane are shown in Figures S5a and S5b, respectively. To obtain the optimum value of l_b for the F – E curve, a detailed analysis of the force deviation between the F – E curve and the QM-FRC fitting curve is carried out (Figures S5c and S5d). Statistical analysis shows that the values of l_b of all the F – E curves of PVS follow a narrow distribution (Figure S5e). We find that the F – E curve of PVS can be fitted well by the QM-FRC model of PE with the optimum value of l_b (0.1525 nm, see Figure S5b). This result indicates that the side chains of PVS cannot influence the polymer SCEE. The possible reason may be that the side chain of PVS is not long enough. Thus, we investigate the polymers PGMA-*g*-PS₄₄ and PGMA-*g*-PS₁₀₅, which have much longer side chains. However, the F – E curves of them can also be described well by the QM-FRC model of PE (Figures S6 and S7), which means that the polymer SCEE cannot be influenced by the side chains of these polymers. Considering that the length of the side chains of these two brush-like polymers (PGMA-*g*-PS₄₄ and PGMA-*g*-PS₁₀₅) are already very long, we can conclude that the polymer SCEE will not be influenced by the thin side chains, no matter if they are short or long.

According to the above results, we speculate that the polymer SCEE may be influenced by a bulky side chain. Thus, PBCPS, which has a bulky side chain, is investigated here. However, we find that the polymer SCEE cannot be influenced by the side chain of PBCPS (Figure S8). The possible reason may be that the side chain of PBCPS is bulky but not long enough to influence the polymer SCEE.

To compare the SCEE of these four kinds of polymers, the normalized F – E curves are plotted together (see Figure 5). The superposition of the normalized F – E curves indicates that these four kinds of polymers share the same SCEE. This result is further confirmed by the very close value of l_b for each of the polymer samples (see Figure S9 for details). These results imply that the steric forces of the side chain of these polymers are not big enough to influence their SCEE. It is possible that there are some conformations which will avoid the steric effect of the side chains.

However, the stiffening effects from the side chains have been observed by the traditional measurements for many

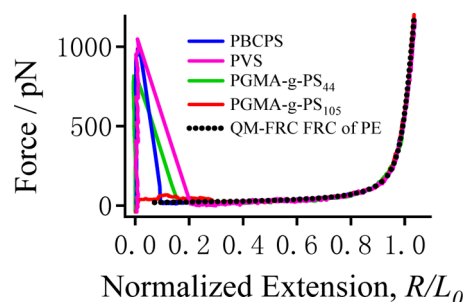


Figure 5. Normalized F – E curves of four kinds of different polymers, including PVS (pink), PGMA-*g*-PS₄₄ (green), PGMA-*g*-PS₁₀₅ (red), and PBCPS (blue). All the experimental F – E curves can be fitted well by the QM-FRC model of PE ($l_b = 0.154$, dotted line).

polymers,^{55–58} i.e., small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS),^{59–61} and AFM imaging.^{54,62} The physical properties of the bulk polymeric materials, such as melting temperature (T_m) and tensile strength, will be influenced remarkably by the sizes of the side chains.¹ For example, the typical physical properties of PP and PS are largely different (see Table S1). The marked difference between them indicates that the physical properties of the polymer change remarkably when the methyl groups of the polymer are substituted by the phenyl groups. At the single-chain level, the stiffening effects from the side chain have been found by SAXS.⁵⁴ In the traditional measurements, the conformation of the polymer chain will be influenced by a minute change of the side chain. A flexible polymer chain could be extended by a weak force from a common fluid flow (~ 1 pN; see Figure S1). However, in AFM force measurements, the noise signal is ~ 10 pN, which is much larger than the forces in the traditional measurements. Therefore, it is reasonable that the influence of the side chains cannot be observed by SMFS if the steric forces from the side chains are smaller than the noise signal.

From Figure 2, one can observe that there is a marked gap between the side chains of PBCPS and PG2, though both of them are bulky. Therefore, another polymer, PG1 (Figure 2), which also has a dendron side chain (the size of G1 side chain is about one-half of that of G2), is investigated in this study. Interestingly, the typical F – E curve of PG1 obtained in nonane can be fitted well by the QM-FRC model of PE ($l_b = 0.154$; Figure 6 and Figure S10). This result indicates that the effect of the side chains of PG1 on SCEE can be ignored, too. However, one should keep in mind that the G1 side chain is more bulky than that of vast majority of polymers. Thus, investigation of

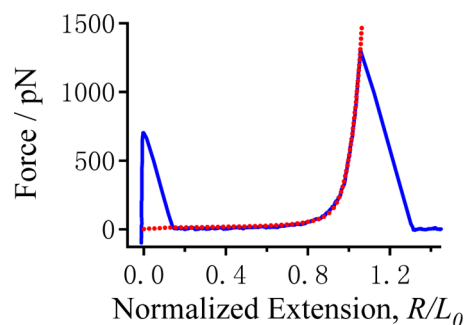


Figure 6. Typical F – E curves of PG1 (solid curve) obtained in nonane and the QM-FRC fitting curve of PE ($l_b = 0.154$, dotted line).

additional kinds of polymers with a C–C backbone one by one will be unnecessary.

To compare the polymer SCEE of these two kinds of dendronized polymers, the typical F – E curves of PG1 and PG2 are plotted together and normalized to the same extension, showing a marked difference between them in the high force region (Figure 7, $F > 300$ pN). A comparison of the structures

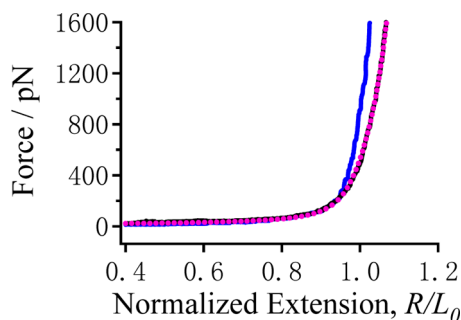


Figure 7. Typical F – E curves of PG2 (blue curve) and PG1 (black curve) obtained in nonane and the QM-FRC fitting curve at $l_b = 0.154$ nm (dotted line).

of PG1 and PG2 (Figure 2) shows that the differences between the side chains of them are already very small (only one generation). The G1 side chain is also bulky, but not long enough. Therefore, it is possible that the G1 side chain is the upper limit of the side chain which cannot influence the SCEE of the polymers with a C–C backbone. In other words, if the size of the side chains of the polymer is smaller than (or similar to) G1, the influence of the side chains at the single-chain level will not be observed by SMFS. Considering the size of G1, we believe that for vast majority of polymers with a C–C backbone the SCEE is determined by the nature of the backbone.

CONCLUSIONS

In this paper, we have studied the effect of side chains on the single-chain enthalpic elasticity (SCEE) of a macromolecule. Six kinds of polymers with side chains of different lengths and shapes are investigated by SMFS in the nonpolar solvent, where the van der Waals interactions between solvent molecules and polymer chain can be ignored. We find that both length and shape of the side chains count: The polymer SCEE will be influenced remarkably only when the side chains are both long and bulky. Thus, only rare polymers have special SCEE, for instance, the second- or higher-generation dendronized polymers. For vast majority of polymers, the effect of the side chains cannot be observed by SMFS, and the SCEE is determined by the nature of the backbone. As a result, the SCEE of most polymers with a C–C backbone is identical to that of PE. The backbone determined SCEE is called the inherent elasticity. It is expected that this conclusion can also be popularized to all polymers with various backbones. This study is an important update to the understanding of polymers at the single-chain level.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.6b00247.

Schematic of a random-coiled polymer is stretched by a weak force via a fluid flow, the details of the QM-FRC model fitting, the F – E curves of the polymers, and the histograms of the fitting parameter, l_b (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail cuishuxun@swjtu.edu.cn; Phone/Fax +86-28-87600998 (S.C.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation of China (21222401 and 21574106).

REFERENCES

- (1) Bower, D. I. *An Introduction to Polymer Physics*; Cambridge University Press: London, 2002; p 168.
- (2) Popa, I.; Zhang, B. Z.; Maroni, P.; Schluter, A. D.; Borkovec, M. Large Mechanical Response of Single Dendronized Polymers Induced by Ionic Strength. *Angew. Chem., Int. Ed.* **2010**, *49*, 4250–4253.
- (3) Yan, Y. C.; Shi, Y.; Zhu, W.; Chen, Y. M. Highly Efficient Synthesis of Cylindrical Polymer Brushes with Various Side Chains Via Click Grafting-onto Approach. *Polymer* **2013**, *54*, 5634–5642.
- (4) Bensimon, A.; Simon, A.; Chiffaudel, A.; Croquette, V.; Heslot, F.; Bensimon, D. Alignment and Sensitive Detection of DNA by a Moving Interface. *Science* **1994**, *265*, 2096–2098.
- (5) Wan, Z. Z.; Li, L.; Cui, S. X. Capturing the Portrait of Isolated Individual Natural Cellulose Molecules. *Biopolymers* **2008**, *89*, 1170–1173.
- (6) Fisher, T. E.; Marszalek, P. E.; Fernandez, J. M. Stretching Single Molecules Into Novel Conformations Using the Atomic Force Microscope. *Nat. Struct. Biol.* **2000**, *7*, 719–724.
- (7) Muller, D. J.; Dufrene, Y. F. Atomic Force Microscopy as a Multifunctional Molecular Toolbox in Nanobiotechnology. *Nat. Nanotechnol.* **2008**, *3*, 261–269.
- (8) Oberhauser, A. F.; Marszalek, P. E.; Carrion-Vazquez, M.; Fernandez, J. M. Single Protein Misfolding Events Captured by Atomic Force Microscopy. *Nat. Struct. Biol.* **1999**, *6*, 1025–1028.
- (9) Rief, M.; Gautel, M.; Oesterhelt, F.; Fernandez, J. M.; Gaub, H. E. Reversible Unfolding of Individual Titin Immunoglobulin Domains by AFM. *Science* **1997**, *276*, 1109–1112.
- (10) Bull, M. S.; Sullan, R. M. A.; Li, H. B.; Perkins, T. T. Improved Single Molecule Force Spectroscopy Using Micromachined Cantilevers. *ACS Nano* **2014**, *8*, 4984–4995.
- (11) Balzer, B. N.; Gallei, M.; Hauf, M. V.; Stallhofer, M.; Wiegler, L.; Holleitner, A.; Rehahn, M.; Hugel, T. Nanoscale Friction Mechanisms at Solid-Liquid Interfaces. *Angew. Chem., Int. Ed.* **2013**, *52*, 6541–6544.
- (12) Junker, J. P.; Rief, M. Evidence for a Broad Transition-State Ensemble in Calmodulin Folding from Single-Molecule Force Spectroscopy. *Angew. Chem., Int. Ed.* **2010**, *49*, 3306–3309.
- (13) Nash, M. A.; Gaub, H. E. Single-Molecule Adhesion of a Stimuli-Responsive Oligo(ethylene glycol) Copolymer to Gold. *ACS Nano* **2012**, *6*, 10735–10742.
- (14) Hugel, T.; Seitz, M. The Study of Molecular Interactions by AFM Force Spectroscopy. *Macromol. Rapid Commun.* **2001**, *22*, 989–1016.
- (15) Livadaru, L.; Netz, R. R.; Kreuzer, H. J. Stretching Response of Discrete Semiflexible Polymers. *Macromolecules* **2003**, *36*, 3732–3744.
- (16) Rief, M.; Oesterhelt, F.; Heymann, B.; Gaub, H. E. Single Molecule Force Spectroscopy on Polysaccharides by Atomic Force Microscopy. *Science* **1997**, *275*, 1295–1297.
- (17) Zhang, W.; Zhang, X. Single Molecule Mechanochemistry of Macromolecules. *Prog. Polym. Sci.* **2003**, *28*, 1271–1295.

- (18) Shan, Y. P.; Wang, H. D. The Structure and Function of Cell Membranes Examined by Atomic Force Microscopy and Single-Molecule Force Spectroscopy. *Chem. Soc. Rev.* **2015**, *44*, 3617–3638.
- (19) Puchner, E. M.; Gaub, H. E. Single-Molecule Mechanoenzymatics. *Annu. Rev. Biophys.* **2012**, *41*, 497–518.
- (20) Shi, W. Q.; Zhang, Y. H.; Liu, C. J.; Wang, Z. Q.; Zhang, X.; Zhang, Y. H.; Chen, Y. M. Toward Understanding the Effect of Substitutes and Solvents on Entropic and Enthalpic Elasticity of Single Dendronized Copolymers. *Polymer* **2006**, *47*, 2499–2504.
- (21) Scholl, Z. N.; Yang, W. T.; Marszalek, P. E. Direct Observation of Multimer Stabilization in the Mechanical Unfolding Pathway of a Protein Undergoing Oligomerization. *ACS Nano* **2015**, *9*, 1189–1197.
- (22) Otten, M.; Ott, W.; Jobst, M. A.; Milles, L. F.; Verdorfer, T.; Pippig, D. A.; Nash, M. A.; Gaub, H. E. From Genes to Protein Mechanics on a Chip. *Nat. Methods* **2014**, *11*, 1127–1130.
- (23) Cui, S. X.; Liu, C. J.; Wang, Z. Q.; Zhang, X. Single Molecule Force Spectroscopy on Polyelectrolytes: Effect of Spacer on Adhesion Force and Linear Charge Density on Rigidity. *Macromolecules* **2004**, *37*, 946–953.
- (24) Cui, S. X.; Yu, J.; Kuhner, F.; Schulten, K.; Gaub, H. E. Double-Stranded DNA Dissociates into Single Strands When Dragged into a Poor Solvent. *J. Am. Chem. Soc.* **2007**, *129*, 14710–14716.
- (25) Hugel, T.; Rief, M.; Seitz, M.; Gaub, H. E.; Netz, R. R. Highly Stretched Single Polymers: Atomic-Force-Microscope Experiments Versus ab-initio Theory. *Phys. Rev. Lett.* **2005**, *94*, 048301.
- (26) Janshoff, A.; Neitzert, M.; Oberdorfer, Y.; Fuchs, H. Force Spectroscopy of Molecular Systems Single Molecule Spectroscopy of Polymers and Biomolecules. *Angew. Chem., Int. Ed.* **2000**, *39*, 3212–3237.
- (27) Li, H. B.; Cao, Y. Protein Mechanics: from Single Molecules to Functional Biomaterials. *Acc. Chem. Res.* **2010**, *43*, 1331–1341.
- (28) Li, H. B.; Linke, W. A.; Oberhauser, A. F.; Carrion-Vazquez, M.; Kerkvliet, J. G.; Lu, H.; Marszalek, P. E.; Fernandez, J. M. Reverse Engineering of the Giant Muscle Protein Titin. *Nature* **2002**, *418*, 998–1002.
- (29) Wang, C.; Shi, W. Q.; Zhang, W. K.; Zhang, X.; Katsumoto, Y.; Ozaki, Y. Force Spectroscopy Study on Poly(acrylamide) Derivatives: Effects of Substitutes and Buffers on Single-Chain Elasticity. *Nano Lett.* **2002**, *2*, 1169–1172.
- (30) Rivas-Pardo, J. A.; Alegre-Cebollada, J.; Ramirez-Sarmiento, C. A.; Fernandez, J. M.; Guixé, V. Identifying Sequential Substrate Binding at the Single-Molecule Level by Enzyme Mechanical Stabilization. *ACS Nano* **2015**, *9*, 3996–4005.
- (31) Baumann, F.; Bauer, M. S.; Milles, L. F.; Alexandrovich, A.; Gaub, H. E.; Pippig, D. A. Protein-DNA Chimeras for Nano Assembly. *Nat. Nanotechnol.* **2015**, *11*, 89–94.
- (32) Zou, S.; Schonherr, H.; Vancso, G. J. Stretching and Rupturing Individual Supramolecular Polymer Chains by AFM. *Angew. Chem., Int. Ed.* **2005**, *44*, 956–959.
- (33) Kong, N.; Peng, Q.; Li, H. B. Rationally Designed Dynamic Protein Hydrogels with Reversibly Tunable Mechanical Properties. *Adv. Funct. Mater.* **2014**, *24*, 7310–7317.
- (34) Li, I. T. S.; Walker, G. C. Interfacial Free Energy Governs Single Polystyrene Chain Collapse in Water and Aqueous Solutions. *J. Am. Chem. Soc.* **2010**, *132*, 6530–6540.
- (35) Shang, X.; Shan, Y. P.; Pan, Y. G.; Cai, M. J.; Jiang, J. G.; Wang, H. D. The Force of Transporting a Single Amino Acid into the Living Cell Measured Using Atomic Force Microscopy. *Chem. Commun.* **2013**, *49*, 8163–8165.
- (36) Zhao, W. D.; Liu, S. H.; Cai, M. J.; Xu, H. J.; Jiang, J. G.; Wang, H. D. Detection of Carbohydrates on the Surface of Cancer and Normal Cells by Topography and Recognition Imaging. *Chem. Commun.* **2013**, *49*, 2980–2982.
- (37) Cheng, B.; Cui, S. X. Supramolecular Chemistry and Mechanochemistry of Macromolecules: Recent Advances by Single-Molecule Force Spectroscopy. *Top. Curr. Chem.* **2015**, *369*, 97–134.
- (38) Li, Q.; Scholl, Z. N.; Marszalek, P. E. Capturing the Mechanical Unfolding Pathway of a Large Protein with Coiled-Coil Probes. *Angew. Chem., Int. Ed.* **2014**, *53*, 13429–13433.
- (39) Xue, Y. R.; Li, X.; Li, H. B.; Zhang, W. K. Quantifying Thiol–Gold Interactions Towards the Efficient Strength Control. *Nat. Commun.* **2014**, *5*, 4348.
- (40) Aioanei, D.; Lv, S.; Tessari, I.; Rampioni, A.; Bubacco, L.; Li, H. B.; Samori, B.; Bruciale, M. Single-Molecule-Level Evidence for the Osmophobic Effect. *Angew. Chem., Int. Ed.* **2011**, *50*, 4394–4397.
- (41) Wang, K. F.; Pang, X. C.; Cui, S. X. Inherent Stretching Elasticity of a Single Polymer Chain with a Carbon-Carbon Backbone. *Langmuir* **2013**, *29*, 4315–4319.
- (42) Smith, S. B.; Cui, Y. J.; Bustamante, C. Overstretching B-DNA: The Elastic Response of Individual Double-Stranded and Single-Stranded DNA Molecules. *Science* **1996**, *271*, 795–799.
- (43) Pang, X. C.; Cui, S. X. Single-chain Mechanics of Poly(N,N-diethylacrylamide) and Poly(N-isopropylacrylamide): Comparative Study Reveals the Effect of Hydrogen Bond Donors. *Langmuir* **2013**, *29*, 12176–12182.
- (44) Shi, W. Q.; Wang, Z. Q.; Cui, S. X.; Zhang, X.; Bo, Z. S. Force Spectroscopy on Dendronized Poly(p-phenylene)s: Revealing the Chain Elasticity and The Interfacial Interaction. *Macromolecules* **2005**, *38*, 861–866.
- (45) Luo, Z. L.; Cheng, B.; Cui, S. X. Effects of Water on The Single-Chain Elasticity of Poly(U) RNA. *Langmuir* **2015**, *31*, 6107–6113.
- (46) Liu, C. J.; Cui, S. X.; Wang, Z. Q.; Zhang, X. Single-Chain Mechanical Property of Poly(N-vinyl-2-pyrrolidone) and Interaction with Small Molecules. *J. Phys. Chem. B* **2005**, *109*, 14807–14812.
- (47) Bao, Y.; Qian, H. J.; Lu, Z. Y.; Cui, S. X. The Unexpected Flexibility of Natural Cellulose at a Single-Chain Level and its Implications to The Design of Nano Materials. *Nanoscale* **2014**, *6*, 13421–13424.
- (48) Bao, Y.; Qian, H. J.; Lu, Z. Y.; Cui, S. X. Revealing the Hydrophobicity of Natural Cellulose by Single-Molecule Experiments. *Macromolecules* **2015**, *48*, 3685–3690.
- (49) Qu, W.; Zhu, X. Q.; Chen, J. H.; Niu, L.; Liang, D. H.; Fan, X. H.; Shen, Z. H.; Zhou, Q. F. Synthesis and Characterization of a Mesogen-Jacketed Polyelectrolyte. *Macromolecules* **2014**, *47*, 2727–2735.
- (50) Li, W.; Zhang, X. C.; Zhao, X.; Zhang, X. Q.; Zhang, A. F. Doubly Dendronized Chiral Polymers Showing Thermoresponsive Properties. *J. Polym. Sci., Part A: Polym. Chem.* **2013**, *51*, 5143–5152.
- (51) Oesterhelt, F.; Rief, M.; Gaub, H. E. Single Molecule Force Spectroscopy by AFM Indicates Helical Structure of Poly(ethylene-glycol) in Water. *New J. Phys.* **1999**, *1*, 6.1–6.11.
- (52) Cui, S. X.; Yu, Y.; Lin, Z. B. Modeling Single Chain Elasticity of Single-Stranded DNA: A Comparison of Three Models. *Polymer* **2009**, *50*, 930–935.
- (53) Yu, H.; Schluter, A. D.; Zhang, B. Z. Main-Chain Scission of a Charged Fifth-Generation Dendronized Polymer. *Helv. Chim. Acta* **2012**, *95*, 2399–2410.
- (54) Zhang, B. Z.; et al. The Largest Synthetic Structure with Molecular Precision: Towards a Molecular Object. *Angew. Chem., Int. Ed.* **2011**, *50*, 737–740.
- (55) Svergun, D. I.; Koch, M. H. J. Small-Angle Scattering Studies of Biological Macromolecules in Solution. *Rep. Prog. Phys.* **2003**, *66*, 1735–1782.
- (56) Pollack, L.; et al. Time Resolved Collapse of a Folding Protein Observed with Small Angle X-ray Scattering. *Phys. Rev. Lett.* **2001**, *86*, 4962–4965.
- (57) Zheng, Z. L.; Kuang, F. Y.; Zhao, J. Direct Observation of Rotational Motion of Fluorophores Chemically Attached to Polystyrene in Its Thin Films. *Macromolecules* **2010**, *43*, 3165–3168.
- (58) Luo, Z. L.; Zhang, G. Z. Sedimentation of Polyelectrolyte Chains in Aqueous Solutions. *Macromolecules* **2010**, *43*, 10038–10044.
- (59) Dubois, E.; Boue, F. Conformation of Poly(styrenesulfonate) Polyions in the Presence of Multivalent Ions: Small-Angle Neutron Scattering Experiments. *Macromolecules* **2001**, *34*, 3684–3697.
- (60) Milas, M.; Rinaudo, M.; Duplessix, R.; Borsali, R.; Lindner, P. Small-Angle Neutron-Scattering From Polyelectrolyte Solutions-from Disordered to Ordered Xanthan Chain Conformation. *Macromolecules* **1995**, *28*, 3119–3124.

(61) Maier, E. E.; Krause, R.; Deggelmann, M.; Hagenbuchle, M.; Weber, R.; Fraden, S. Liquid-Like Order of Charged Rodlike Particle Solutions. *Macromolecules* **1992**, *25*, 1125–1133.

(62) Li, W.; Zhang, A. F.; Feldman, K.; Walde, P.; Schluter, A. D. Thermoresponsive Dendronized Polymers. *Macromolecules* **2008**, *41*, 3659–3667.