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Modeling single chain elasticity of single-stranded DNA: A comparison of three models

Shuxun Cui*, You Yu, Zhangbi Lin

State Key Laboratory of Polymer Materials Engineering, Polymer Research Institute, Sichuan University, Kehua North Road 133, Chengdu 610065, PR China

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ABSTRACT

The dominant models of polymers, i.e., the freely jointed chain (FJC) model, the wormlike chain (WLC) model and the freely rotating chain (FRC) model are modified by integrating the inherent single-molecule elasticity obtained from quantum mechanics (QM) ab-initio calculations. The QM modified models have been utilized to generate fitting curves for single-stranded DNA obtained in organic solvent. The analyses on the deviation between the fitting curve and the experimental force curve demonstrate that the QM-FRC and QM-FJC model are suitable for ssDNA, but not the QM-WLC model. We also find that one repeating unit of ssDNA is corresponding to a Kuhn segment in QM-FJC model or two rotating units in QM-FRC. Having close correlation to the inherent elasticity and real molecular structure of the polymer, QM-FJC and QM-FRC are emerging as structure relevant models.

1. Introduction

Mechanical forces exist ubiquitously in every chemical/physical systems, especially in living organisms. However, due to the limitation in characterization, it is hard to directly measure the forces imposed on a specific small molecule. One of the features of polymers is that the extension of a polymer chain can reach to mesoscale or even to macroscale, which can be utilized to bridge the macroscale objects and the small molecules [1]. On the other hand, the mechanical properties of polymer itself are also very important, because the rubbers, plastics, polysaccharides, proteins and nucleic acids are all in the category of polymer. A general understanding on the mechanics of single polymer chains will certainly facilitate the development of materials science and biological science. By linking the important disciplines, the mechanics of individual polymer chains is emerging as one of the key issues in modern sciences.

Having a large number of degrees of freedom, even a single polymer chain has to be treated in terms of statistical physics when force/energy is concerned [2]. In order to analyze the force/energy of a single polymer chain with statistical mechanics, the structure of a polymer chain has to be treated into simplified models. With the pioneering work of Flory and many others, a number of models have been proposed for the theoretical treatment of single polymer chains [2–6]. Three of the models, i.e., the wormlike chain (WLC) model (Eq. (1)), the freely jointed chain (FJC) model (Eq. (2)) and the freely rotating chain (FRC) model (Eq. (3)) have attracted more attention than others [2,5–10].

$$F\frac{l_p}{k_BT} = \frac{R}{L[F]} + \frac{1}{4(1-R/L[F])^2} - \frac{1}{4} \tag{1}$$

$$R = L[F]\cdot\{\coth[(F\cdot l_k)/(k_B\cdot T)] - (k_B\cdot T)/(F\cdot l_k)\} \tag{2}$$

$$R = L[F]\cdot[1 - k_BT/(2F\cdot l_b)] \tag{3}$$

In these equations, R is the end-to-end distance of a polymer chain at a given stretching force F, $L[F]$ is the contour length of the polymer chain being stretched, k_B is the Boltzmann constant, T is the temperature, l_k, l_p and l_b are the Kuhn length, persistence length and the rotating unit length of the polymer chain, respectively. $L[F]$ and l_k, l_p and l_b are free parameters for model fitting.

During the last two decades, the development of single molecule force spectroscopy (SMFS) activated many research fields, especially for the research of single polymer mechanics. A very important progress in this field is that the measured force-extension curve (herein after force curve) of an individual polymer chain has provided the experimental basis to test the validity of the above mentioned physical models. The first verified model is the WLC model, which is successfully applied to describe the single-molecule elasticity of double-stranded DNA (dsDNA) and proteins [11–13]. Later, FJC model was also exploited to fit single chain force curves. The original models presume that the polymer chains are

* Corresponding author.
E-mail address: cuisx@scu.edu.cn (S. Cui).

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inextensible upon stretching and only entropic elasticity is involved. In other words, the $L[F]$ was considered to be equal to the contour length without force stretching, L_0. Actually, the minor variation of bond length and bond angle upon stretching does enlarge the contour length of a polymer. To meet the practical conditions, enthalpic elasticity is integrated into the models, which improved the fitting performance of the models for many polymers [9, 14–23], see Eq. (4).

$$L[F] = L_0 \cdot (1 + F/K_0) \tag{4}$$

In Eq. (4), K_0 is the normalized linear elasticity (or Young's modulus) of the single polymer chain.

Together with K_0, there are three free fit parameters for each of the above mentioned models. These fit parameters are determined in an asymptotic way by selecting the fitting curve that has minimum deviation to the experimental force curve.

An alternative approach is to use the inherent fit parameters for a certain kind of polymer. Recently, by utilizing the quantum-chemical (QM) ab-initio calculations, Hugel et al. obtained the theoretical elasticity of the repeating unit of single-stranded DNA (ssDNA) [8]. Surprisingly, this elasticity was found to be non-linear, which could be expressed in a polynomial expansion to provide the basis for a numerical fit of the measured force curves, see Eq. (5).

$$F = \sum_{n=1}^{5} \gamma_n (a[F]/a_0 - 1)^n \quad \gamma_1 = 8.44\ \text{nN}, \gamma_2 = 29.5\ \text{nN}, \quad \gamma_5 = 19,637\ \text{nN} \tag{5}$$

a_0 is the length of the repeating unit at zero force for ssDNA, $a[F]$ is the length in the stretched status, γ_1 is the linear elastic modulus, other coefficients are non-linear corrections.

Experimentally, the single chain mechanics of ssDNA can be achieved by atomic force microscope (AFM) based SMFS. Thus, it is very interesting to see which of the theoretical models is suitable in describing the single chain mechanics of ssDNA. In the present work, we attempt to integrate the elasticity of ssDNA from QM results into each model and provide an evaluation on the applicability of each model on ssDNA.

2. Experimental section

2.1. Materials and chemicals

The ssDNA used in this study is a customized sample (IBA GmbH, Germany), which is an oligomer containing 176 bases with random sequence of 1:1 thymine (T) and cytosine (C). All the other chemical reagents are purchased from Sigma or Fluka, and are analytically pure.

2.2. Sample preparation and force measurements

The ssDNA sample (0.1 mmol/L) is diluted 20,000 times in PBS buffer to a concentration of 5 nmol/L. To prepare the sample for measurements, ssDNA is allowed to adsorb onto an amino-functionalized glass slide (Quantifoil Micro Tools GmbH, Germany) for 10 min, followed by rinse with Milli-Q water thoroughly. After that, the sample is mounted in the AFM (MFP-1D, Asylum Research, CA). Prior to the measurements, a drop of liquid is introduced between the V-shaped Si_3N_4 AFM cantilever (Veeco Instruments Inc., NY) and the sample. Then during the AFM manipulation, the data are collected at the same time and transferred to force-extension curves later. The spring constant of the AFM cantilever is measured by thermo excitation method (with an error of less than 20%), ranging from 10 to 30 pN/nm [24]. The stretching velocity applied in this study is 2.0 μm/s if not mentioned otherwise. The details of the AFM instrumentation can be found elsewhere [5,6,25]. The normalization of the force curves is performed as described in the literature [2,5,6,23].

3. Results and discussion

3.1. Integration of QM results into three models

Since the minor changes of bond angle and bond length are already considered in the calculations on one repeating unit, Eq. (5) can be rewritten to describe the whole polymer chain (see Eq. (6)).

$$F = \sum_{n=1}^{5} \gamma_n (L[F]/L_0 - 1)^n \quad \gamma_1 = 8.44\ \text{nN}, \gamma_2 = 29.5\ \text{nN}, \quad \gamma_5 = 19,637\ \text{nN} \tag{6}$$

During stretching, the value of $L[F]/L_0$, starting from 1, increases with the increasing of F. Therefore, $L[F]/L_0$ is a monotonic increasing function of F and vice versa. During the elongation of ssDNA, $L[F]/L_0$ is an ergodic value ranging from 1 to a number corresponding to the rupture of the attachment. Here, we utilize the strength of a typical covalent bond as the upper limit for the stretching force, e.g. 2000 pN [26]. Thus, the upper limit for $L[F]/L_0$ is about 1.12, according to Eq. (6). In the range from 1 to 1.12, any arbitrary value of $L[F]/L_0$ is reasonable and corresponds to a mapping value of F in the fitting curve, which can be calculated with Eq. (6).

As described in Eqs. (1)–(3), each original model has two free parameters. One is the contour length of the polymer, $L[F]$. Another is the Kuhn length l_k for FJC, the persistence length l_p for WLC, and the length of the rotating unit l_b for FRC, respectively. By introducing L_0 into the models, we can modify the original models as Eqs. (7)–(9), respectively.

$$F\frac{l_p}{k_B T} = \frac{R/L_0}{L[F]/L_0} + \frac{1}{4(1-(R/L_0)/(L[F]/L_0))^2} - \frac{1}{4} \tag{7}$$

$$R/L_0 = (L[F]/L_0)\cdot\{\coth[(F\cdot l_k)/(k_B\cdot T)] - (k_B\cdot T)/(F\cdot l_k)\} \tag{8}$$

$$R/L_0 = (L[F]/L_0)\cdot[1 - k_B T/(2F\cdot l_b)] \tag{9}$$

R/L_0 is the normalized extension of a polymer chain. Since $L[F]/L_0$ can be an arbitrary value in the proper range, each model now has only one free parameter left. The modified models, which are integrated with the QM results, are called the QM-FJC, QM-WLC and QM-FRC, respectively. In the following section, we will determine the value of l_b in QM-FRC model first.

3.2. QM-FRC fitting results

In the FRC model, a polymer chain consists of many similar freely rotating units [7]. The angle between the two adjacent rotating units is relatively fixed. These presumptions are very close to the real situation of a polymer. Therefore, the two characteristic parameters of FRC model, the length of the rotating unit (l_b) and the angle between the two adjacent units (θ), may be relevant to the real structure of a polymer. For polymers with different backbone structures, the value of l_b varies. The primary structure of ssDNA is more complicated than common synthetic polymers. However, it is reasonable that the rotating unit length of ssDNA is in the range roughly from a typical single covalent bond ($\sim$0.15 nm) to the repeating unit length of ssDNA (0.59 nm) [22].

For a given value of l_b, the QM-FRC fitting curve can be generated in the following procedure. In the range from 1 to 1.12, any arbitrary

value of $L[F]/L_0$ is reasonable and corresponds to a mapping value of F in the fitting curve, which can be calculated with Eq. (6). From this pair of values for $L[F]/L_0$ and F, the corresponding normalized extension of ssDNA, R/L_0, can be calculated with Eq. (9). One value pair of R/L_0 and F corresponds to one point in the fitting curve. In this way, the whole fitting curve can be generated when changing the value of $L[F]/L_0$ from 1 to 1.12. Note that during the fitting process described above, the values of $a[F]$, a_0, $L[F]$ and L_0 are not used directly, which simplifies the calculations involved in the model fitting. An alternate but more complicated procedure for the curve fitting is to provide the inverse function of Eq. (6), and then the fitting curve can be generated by Eq. (3), [27]. Fig. 1 shows the QM-FRC fitting curves of ssDNA for various l_b values.

From Fig. 1 one can find that the lower value of l_b, the higher force is needed in the low force regime. This is reasonable since l_b is the length of the rotating unit of a polymer chain. For a given contour length, lower value of l_b means more rotating units in the chain, which leads to larger number of degrees of freedom in the free status. Upon force stretching, the number of degrees of freedom for a polymer chain will decrease rapidly, approaching to unity at high forces [2,3]. Therefore, the chain that has lower value of l_b will have higher entropic elasticity, which consumes more energy in the low force regime. At high force regime, all the three curves tend to merge together. This is because that the high force regime is mainly governed by the enthalpic elasticity of ssDNA, which is fixed in Eq. (6).

To determine l_b for ssDNA in the QM-FRC model, the experimental single molecular force curve of ssDNA is needed. It is worth noting here that, in the QM calculations, the ssDNA molecule is set in a vacuum condition. However, single molecular force curve of ssDNA was often obtained in aqueous buffer. One should keep in mind that water is a very complicated solvent. With hydrogen-bonding donor and acceptor, this polar solvent strongly influences the properties of solute molecules. It is clear that the physiological function and single chain mechanics of DNA crucially depend on the aqueous environment. Therefore, the inherent elasticity obtained in QM calculations may differ greatly from the experimental one, where the influences of water are imposed to the molecule.

A practicable choice is to carry out the force measurements in organic solvents. The interactions between the common organic solvent molecules and the solute molecules are van der Waals interactions in general, which is the weakest intermolecular interactions. Having a minimum influence from solvent, it is expected that the solute molecules' behavior is close to that under the vacuum condition [28].

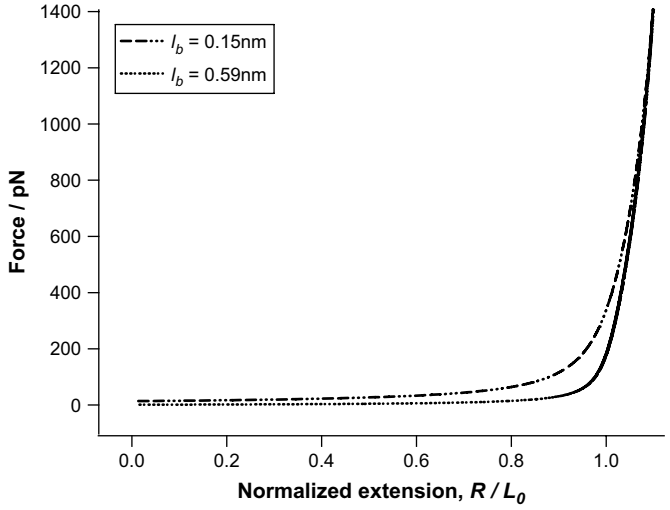

Fig. 1. The QM-FRC fitting curves of ssDNA with various l_b values.

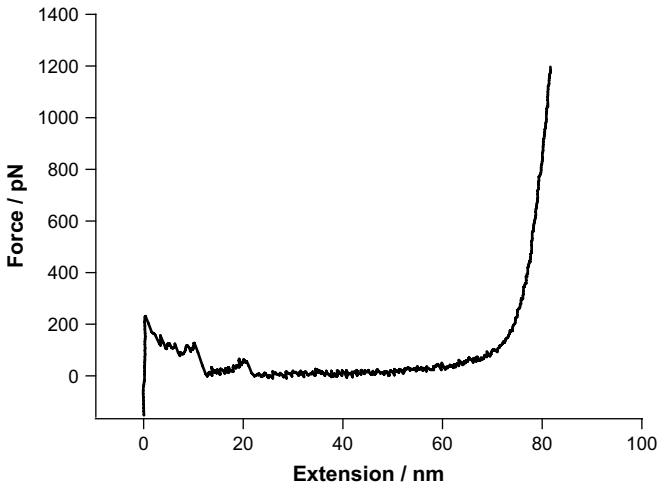

Fig. 2. Typical force curve of a single chain ssDNA obtained in DEBenzene.

We only choose T and C in the oligomer ssDNA to avoid complicated intramolecular structures such as hairpins and loops. We expect that this kind of ssDNA will present the inherent elasticity upon stretching in organic solvent. Fig. 2 shows a typical force curve when a single chain of ssDNA is stretched in a common organic solvent, diethylbenzene (DEBenzene). The force rises monotonically with extension, corresponding to the increasing restoring force during the elastic elongation. As the polymer bridge between the AFM tip and glass substrate ruptures, the force drops rapidly to zero.

The experimental force curve can be normalized to compare with the QM-FRC fitting curve, see Fig. 3. One can find that at high force regime (e.g. larger than 800 pN), there is no remarkable deviation between the experimental curve and QM-FRC fitting curves. This is because that the high force regime is mainly governed by the enthalpic elasticity of ssDNA. This result validates that the non-linear elasticity obtained from the QM calculations can be used to describe the real enthalpic elasticity of ssDNA in organic solvent.

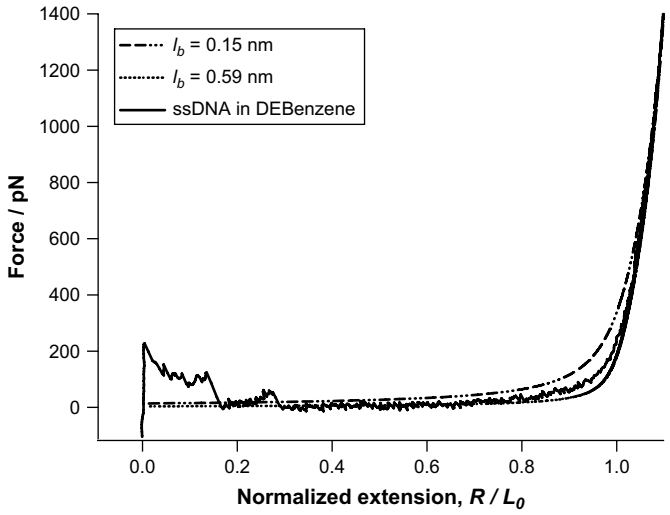

Fig. 3. Single-molecule force curve of ssDNA vs QM-FRC fitting curves with various l_b values.

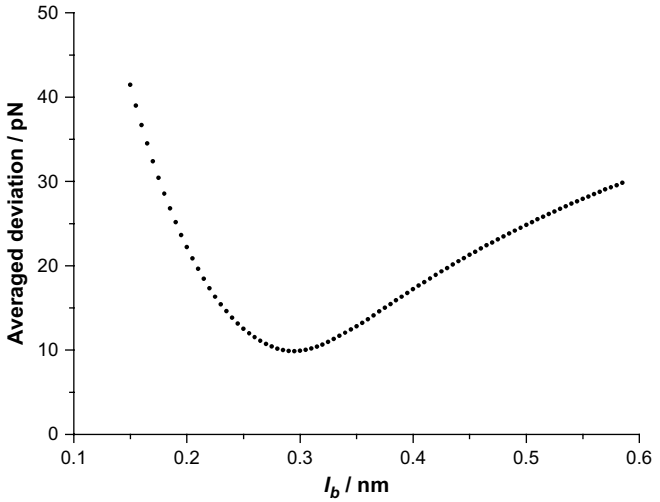

Fig. 4. The averaged deviation of force between the experimental curve and QM-FRC fitting curve as a function of l_b. The minimum deviation is obtained at $l_b = 0.295$ nm.

As shown in Fig. 3, although various l_b values can lead to satisfactory fitting curves for the high force regime, there is a remarkable deviation in the low force regime (e.g. lower than 500 pN). However, Fig. 3 implies that the inherent value of l_b for ssDNA is in the range of 0.15–0.59 nm. To find out the optimum value of l_b for curve fitting, a detailed analysis on the force deviation between the two types of curves is carried out, in which l_b is varied from 0.15 to 0.59 nm with a small increment of 0.005 nm. In the analysis, each point of the experimental force curve in the range from $R/L_0 = 0.6$ to the topmost point of the curve is compared with the QM-FRC fitting curve. The averaged deviation force between the experimental and fitting curves for all points in the compared range, namely $(\sum_{i=1}^{n} |F_{exp} - F_{fit}|)/n$, is plotted against l_b in Fig. 4. There is a saddle point at $l_b = 0.295$ nm, where the value of averaged deviation (9.8 pN) is very close to the standard deviation of the noise in the experimental curve (7.1 pN). In this case, the fitting curve is so close to the experimental curve that the deviation in between is negligible, see Fig. 5. We notice that when the l_b is 0.295 ± 0.005 nm, the resulting fitting curves show almost same performance for the fitting. Similar analyses also show that the optimum value for l_b is 0.295 ± 0.005 nm for different experimental force curves. Therefore, we determine that for ssDNA, $l_b = 0.295$ nm. In this way, there is no free parameter left in Eq. (9). The excellent fitting result in the entire force regime indicates that the QM calculations reflect the inherent single chain elasticity of ssDNA and the QM-FRC model is appropriate for ssDNA.

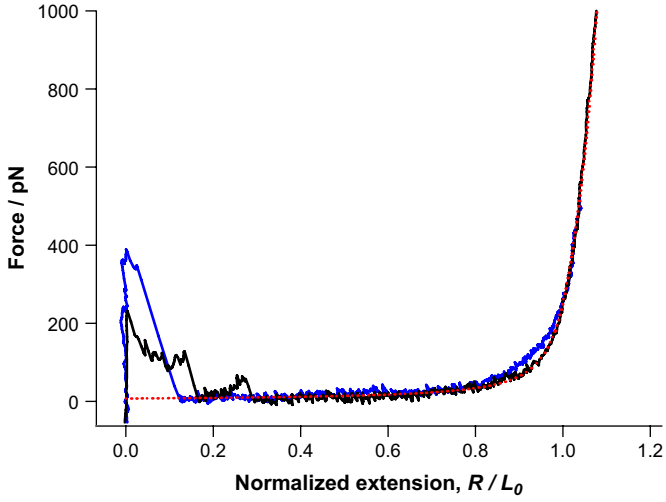

Fig. 5. The QM-FRC fitting curve with $l_b = 0.295$ nm vs the normalized experimental force curve. For comparison, the force curve of ssDNA (blue curve) obtained in aqueous buffer solution is also shown, which presents a remarkable deviation from the fitting curve [28]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. QM-FJC fitting results

At high forces, the value of $\coth[(F \cdot l_k)/(k_B \cdot T)]$ approaches to unity (see Eq. (8)). In this case, the QM-FJC model can be rewritten in the following form.

$$R/L_0 = (L[F]/L_0) \cdot [1 - k_B T/(F \cdot l_k)] \quad (10)$$

Note that this equation is very similar to the QM-FRC model in Eq. (9). Therefore, it is reasonable to presume that $l_k = 2l_b$. Here in this section, $l_k = 0.59$ nm is adopted for QM-FJC model. The limitation of Eq. (10) is that the force should be high enough so that the $\coth[(F \cdot l_k)/(k_B \cdot T)]$ approaches to unity. For most accuracy, we will utilize Eq. (8) for the curve fitting. The fitting result is shown in Fig. 6. The superposition of the QM-FJC fitting curve and the experimental curve indicates that QM-FJC is also suitable for describing the single chain mechanics of ssDNA and the presumption of $l_k = 0.59$ nm is valid.

Since both QM-FJC model ($l_k = 0.59$ nm) and QM-FRC model ($l_b = 0.295$ nm) can generate very good fitting curves for ssDNA, it is interesting to see whether there is a difference between the two fitting curves. As shown in Fig. 7, there is a small deviation between the fitting curves of the QM-FJC model and QM-FRC model. At forces higher than 25 pN, the two fitting curves begin to merge together. Below 25 pN, the deviation between the two curves is perceptible. However, the maximum deviation in between is about 5 pN, which is smaller than the noise of the experimental curve. Therefore, the deviation between the two models is negligible in the whole force range. In other words, these two models are equivalent in fitting force curves obtained in AFM, especially in the range higher than 25 pN [7].

In the above QM-FJC fitting, the value of the Kuhn length, l_k, is determined by the presumption that $l_k = 2l_b$. To further confirm the value of this important parameter, a similar analysis is carried out

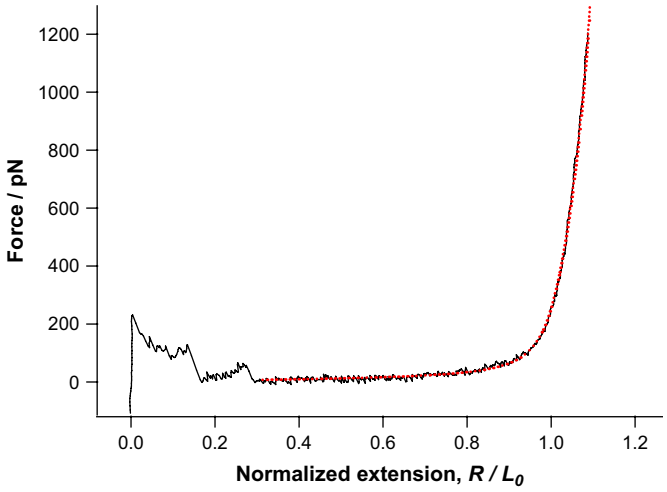

Fig. 6. The QM-FJC fitting curve with $l_k = 0.59$ nm vs the normalized experimental force curve.

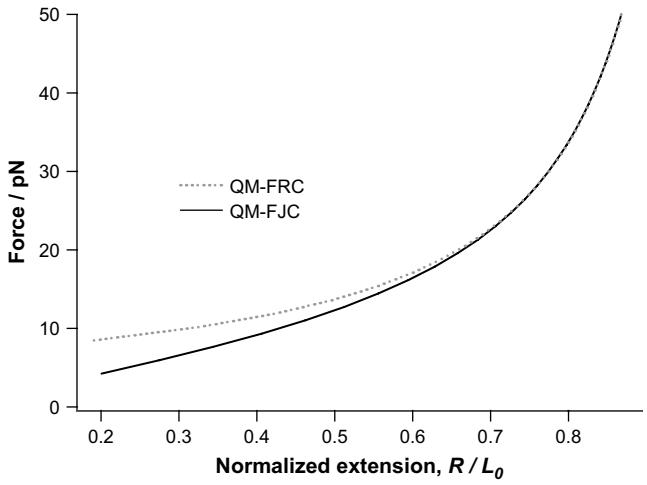

Fig. 7. The comparison between the fitting curves generated by QM-FJC model ($l_k = 0.59$ nm) and QM-FRC model ($l_b = 0.295$ nm). The data are zoomed in to show the deviation.

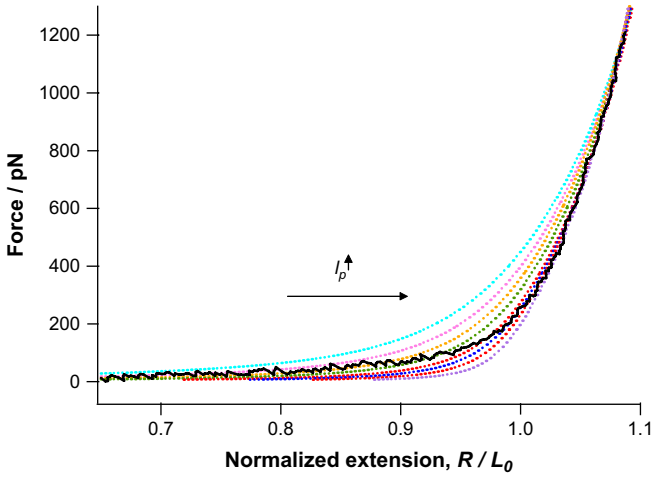

Fig. 9. The QM-WLC fitting curve (dotted line) with various values of l_p vs the normalized experimental curve (solid line). The data are zoomed in to show the deviation. The values of l_p are 0.3, 0.5, 0.8, 1.2, 2, 3, 5 and 10 nm, from left to right, respectively.

to find out the optimum value of l_k. The averaged deviation between the experimental force curve and the QM-FJC fitting curve as a function of l_k is shown in Fig. 8. The saddle point is exactly at 0.59 nm, which verifies the value of l_k.

3.4. QM-WLC fitting results

For a certain value of l_p, QM-WLC fitting curves can be generated by Eq. (7). To find out the proper l_p, the value changes from 0.3 nm to 10 nm. As shown in Fig. 9, the high force regime can be fitted well only if l_p is larger than 2 nm. In the case that l_p is larger than 2 nm, however, a large deviation can be observed in the low force regime. That is to say that there is no such a value of l_p leads to good performance in the whole force range. This result may imply that the WLC model is not suitable for ssDNA, even if the QM results are integrated. The WLC model assumes a continuum without fine structure, which differs from the reality of a polymer chain [2]. This may be the main reason for the failure of the QM-WLC fitting for ssDNA.

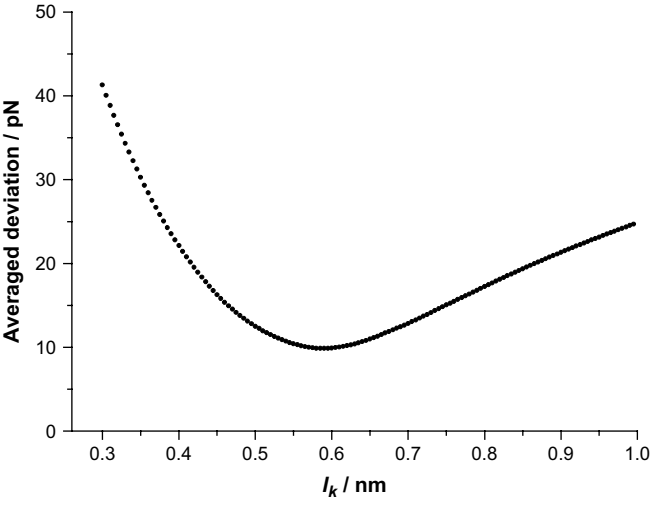

Fig. 8. The average deviation between experimental force curve and the QM-FJC fitting curve as a function of the Kuhn length, l_k. Calculating method is same to that of Fig. 4.

4. Conclusions

There are three free parameters in each of the three dominant statistical mechanics models of polymer. One free parameter can be fixed by integrating the single molecular elasticities of ssDNA, which is obtained from quantum mechanics calculations, into the models. By utilizing a normalized extension (R/L_0) in the model, the contour length of a polymer chain at a given stretching force, $L[F]$, is no longer a free parameter. The optimum value for the last free parameter can be found out by selecting the minimum averaged deviation between the fitting curve and the experimental force curve. We find that both QM-FRC and QM-FJC models can generate excellent fitting curves for ssDNA, where QM-WLC model fails. For forces higher than 25 pN, the QM-FRC and QM-FJC models are consistent with each other. For forces lower than 25 pN, the deviation between the two models is within the range of noise in experimental force curve. Therefore, these two models are actually equivalent for ssDNA. Moreover, we find that for ssDNA, the optimum value of the Kuhn length in QM-FJC is 0.59 nm, which is exactly the length of a repeating unit of ssDNA. The rotating unit length of ssDNA in QM-FRC model is 0.295 nm, which is exactly the half of a repeating unit. This may imply that there are two rotating units, likely the sugar ring and the phosphate group, in one repeating unit of ssDNA.

The consistence between the real physical parameter and the modeling parameter may suggest that both QM-FJC and QM-FRC are structure relevant models. Further insight awaits studies on other polymer systems.

Acknowledgements

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References

[1] Zou S, Schoenherr H, Vancso GJ. Angew Chem Int Ed 2005;44:956–9.
[2] Janshoff A, Neitzert M, Oberdorfer Y, Fuchs H. Angew Chem Int Ed 2000; 39:3212–37.
[3] Flory P. Statistical mechanics of chain molecules. Munich: Hanser; 1989.

[4] Kratky O, Porod G. Recl Trav Chim Pays-Bas 1949;68:1106.
[5] Hugel T, Seitz M. Macromol Rapid Commun 2001;22:989–1016.
[6] Zhang WK, Zhang X. Prog Polym Sci 2003;28:1271–95.
[7] Livadaru L, Netz RR, Kreuzer HJ. Macromolecules 2003;36:3732–44.
[8] Hugel T, Rief M, Seitz M, Gaub HE, Netz RR. Phys Rev Lett 2005;94:048301.
[9] Shi W, Zhang Y, Liu C, Wang Z, Zhang X, Zhang Y, et al. Polymer 2006;47:2499–504.
[10] Nakajima K, Watabe H, Nishi T. Polymer 2006;47:2505–10.
[11] Bustamante C, Marko JF, Siggia ED, Smith S. Science 1994;265:1599–600.
[12] Rief M, Gautel M, Oesterhelt F, Fernandez JM, Gaub HE. Science 1997; 276:1109–12.
[13] Cao Y, Li H. Nat Mater 2007;6:109–14.
[14] Clausen-Schaumann H, Rief M, Tolksdorf C, Gaub HE. Biophys J 2000;78:1997–2007.
[15] Cui S, Liu C, Wang Z, Zhang X, Strandman S, Tenhu H. Macromolecules 2004; 37:946–53.
[16] Gunari N, Schmidt M, Janshoff A. Macromolecules 2006;39:2219–24.
[17] Li H, Liu B, Zhang X, Gao C, Shen J, Zou G. Langmuir 1999;15:2120–4.
[18] Liu C, Cui S, Wang Z, Zhang X. J Phys Chem B 2005;109:14807–12.
[19] Oesterhelt F, Rief M, Gaub HE. New J Phys 1999;1:6.
[20] Ortiz C, Hadziioannou G. Macromolecules 1999;32:780–7.
[21] Rief M, Clausen-Schaumann H, Gaub H. Nat Struct Biol 1999;6:346–9.
[22] Smith S, Cui Y, Bustamante C. Science (Washington, DC) 1996;271:795–9.
[23] Zhang W, Zou S, Wang C, Zhang X. J Phys Chem B 2000;104:10258–64.
[24] Florin E, Rief M, Lehmann H, Ludwig M, Dornmair C, Moy V, et al. Biosens Bioelectron 1995;10:895–901.
[25] Zhang D, Ortiz C. Macromolecules 2005;38:2535–9.
[26] Grandbois M, Beyer M, Rief M, Clausen-Schaumann H, Gaub HE. Science 1999; 283:1727–30.
[27] Neuert G, Hugel T, Netz RR, Gaub HE. Macromolecules 2006;39:789–97.
[28] Cui S, Albrecht C, Kühner F, Gaub HE. J Am Chem Soc 2006;128:6636–9.