Pulling Geometry-Induced Errors in Single Molecule Force Spectroscopy Measurements

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ABSTRACT In AFM-based single molecule force spectroscopy, it is tacitly assumed that the pulling direction coincides with the end-to-end vector of the molecule fragment being stretched. By systematically varying the position of the attachment point on the substrate relative to the AFM tip, we investigate empirically and theoretically the effect of the pulling geometry on force-extension characteristics of double-stranded DNA. We find that increasing the pulling angle can significantly lower the force of the characteristic overstretching transition and increase the width of the plateau feature beyond the canonical 70%. These effects, when neglected, can adversely affect the interpretation of measured force-extension relationships. We quantitatively evaluate force and extension errors originating from this "pulling angle effect" and stress the need to correct the pulling geometry when stretching rigid molecules with an AFM.

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AFM-based single molecule force spectroscopy (AFM-SMFS) has become an important tool to study the elasticity and conformations of nucleic acids, proteins, and polysaccharides (1). In AFM-SMFS, two ends of a molecule are anchored to the substrate and the AFM cantilever tip, respectively. The molecule is mechanically stretched and its length and tension are measured with a subnanometer and picoNewton resolution. Regardless of the method used to anchor the molecule of interest, it is typically and tacitly assumed that the pulling direction is always axial to the extension of the molecule, so that the force applied to the molecule is identical to the force deflecting the AFM cantilever, as illustrated in Fig. 1 *a*. However, the actual pulling geometry may be more complicated than the ideal situation in that the attachment point on the substrate may not coincide with the normal projection of the attachment point on the tip, resulting in the molecule being pulled at an angle, as shown in Fig. 1 *b*. In such a case, the force measured through the deflection of the AFM cantilever, F_z, is only a component of the force applied to molecule, F. Similarly, the measured extension, L_z, is only the projection of the distance between the two anchor points. It is noted that the other two components of force F can also cause the deflection of the AFM cantilever. F_x can cause the cantilever to deflect either up or down depending on the direction of the moment it generates, while F_y can twist the cantilever laterally. A question about the significance of the errors in force and extension measurements originating from neglecting this ''angle effect'' was raised by Stuart M. Lindsay some time ago (S. M. Lindsay, private communication, 1998). It appeared that for flexible proteins such as titin, and flexible polysaccharides, such as dextran, which collapse into fairly compact structures, the pulling angle is typically small and such are the errors (3). However, this question has never been addressed for fairly rigid molecules such as double-stranded DNA (dsDNA), collagens, and actin filaments, for which it is likely that the attachment points on the substrate and the AFM tip may be separated by a large distance, resulting in a large pulling angle.

In this letter, we study the effect of pulling geometry on AFM measured force-extension relationships of double-stranded λ-phage DNA, which is a classical subject for force spectroscopy experiments (4–6) and displays a very characteristic overstretching transition that is its mechanical signature (5,7,8). We illustrate how the pulling angle affects the force-extension measurements by repeatedly stretching one DNA duplex at various pulling angles and examining the changes in the overstretching transition, and compare the experimental data with theoretical predictions.

MODELING

A typical force-extension relationship for dsDNA with what is believed to be a minimal pulling angle effect is shown in Fig. 2. We simply approximate its entropic regime by two straight lines. In this model, the molecule is almost fully extended to the length L_0, with zero force, and then the force rises linearly with the extension, which reaches L_1 when the overstretching transition starts. Then the molecule is overstretched by ~70%, at a force $F_{BS} \approx 65$ pN; these data originated from optical tweezers measurements, in which the ''pulling angle effect'' can be controlled to be minimal (5). For modeling purposes we use three linear segments to approximate the force-extension profile from 0 to the end of the B-S transition (L_2), which are described by

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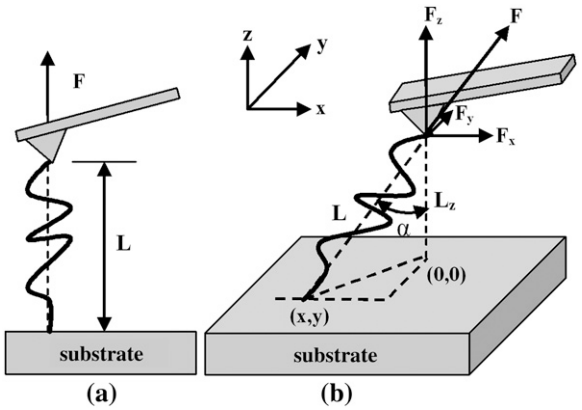

FIGURE 1 Schematic diagram of possible pulling situations in AFM-SMFS. (*a*) Ideal situation; (*b*) general situation.

$$F = \begin{cases} F = 0 & 0 \le L < L_0 \\ F_{BS}\left(\frac{L_1 - L}{L_1 - L_0}\right) & L_0 \le L < L_1 \\ F_{BS} & L_1 \le L \le L_2 \end{cases}. \quad (1)$$

When pulling at an angle, as shown in Fig. 1 *b*, we obtain

$$F_z = F\cos(\alpha); \quad L_z = L\cos(\alpha), \quad (2)$$

where α can be expressed as

$$\alpha = \tan^{-1}\left(\frac{\sqrt{x^2 + y^2}}{L_z}\right). \quad (3)$$

By combining Eqs. 1–3, the relationship between F_z and L_z can be obtained. For simplicity, in the later analysis, we assume that F_z is equal to the force measured by the photodiode, through the deflection of the cantilever.

MATERIALS AND METHODS

Measurements of the elasticity of dsDNA were carried out on our custom-made AFM instrument (9,10). This AFM is built around a high precision piezoelectric *XYZ* stage (Physik Instrumente, Karlsruhe, Germany), which is equipped with three capacitive sensors that provide an open-loop resolution of 0.1 nm in the *Z* axis and 1 nm in the *X* and *Y* axes. Double-stranded λ-phage DNA digest in a solution of 10 mM Tris+HCl, 1 mM EDTA, pH 8 was purchased from Sigma (St. Louis, MO). Eighty microliters of DNA solution (60 ng/μl) supplemented with 150 mM NaCl was deposited onto a freshly-evaporated gold substrate. After the sample was incubated for 2–3 h, it was gently rinsed 3–5 times with the buffer solution, and attached to the *XYZ* stage. Untreated silicon nitride AFM tips (Microlever from Veeco, Woodbury, NY) were employed for the pulling measurements. These cantilevers have a nominal spring constant of 10 mN/m and an actual spring constant of ~20 mN/m as measured in solution using the energy equipartition approach (11).

RESULTS AND DISCUSSION

During the pulling experiment, a single dsDNA molecule was identified by recording its characteristic overstretching transition, after which the AFM tip was lifted from the sample surface to avoid extra molecule attachment. The molecule was then stretched repeatedly at different horizontal locations by controlling the motion of the *XYZ* stage under the AFM tip. A force-extension profile was recorded at each location. If the attachments of the DNA molecule at both the substrate and the AFM cantilever tip are strong, such a measurement can be repeated up to 100 times. We note that although similar effects were observed in several different experiments performed on different DNA molecules, the data shown in this letter was literally obtained on one single DNA duplex that was repeatedly stretched at 51 matrix locations, with a pulling time interval of ~30 s. Three of the experimentally measured force-extension curves are shown in Fig. 3. By determining the plateau forces, defined as the force in the center of the width of the plateau, the location with the highest plateau force (~90 pN) is identified as the case with a zero or a minimal angle and is designated as position (0,0). This curve is shown in blue in Fig. 3. We note

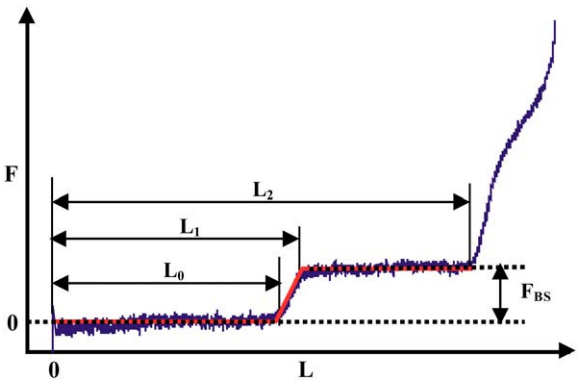

FIGURE 2 Illustration of the typical force-extension curve for dsDNA and the approximation of the curve with three linear segments.

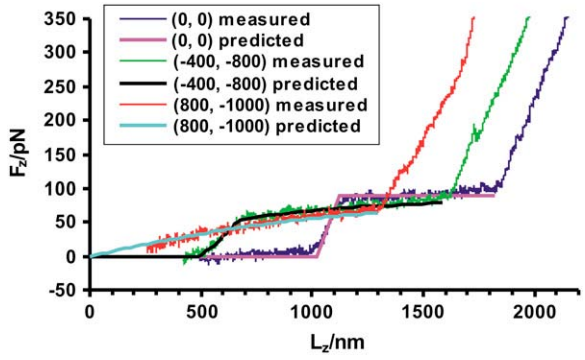

FIGURE 3 Comparison between experimental measurements and theoretical predictions at three different pulling angles. The solid smooth lines represent the theoretical predictions.

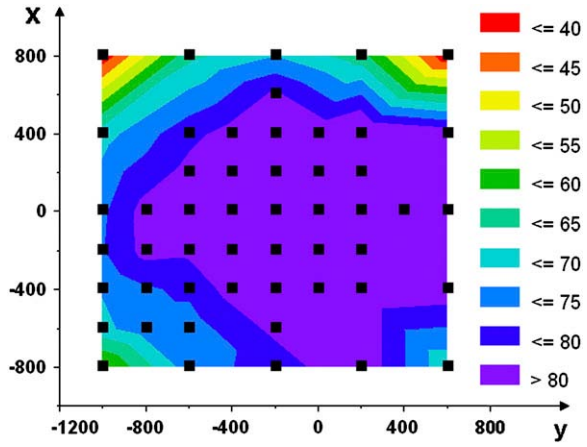

FIGURE 4 The contour plot of the measured plateau forces (in pN) at various locations (in nm). The black dots represent the locations where measurements were taken.

that our maximum plateau force of 90 pN is higher than the 65 pN measured in the experiments using optical tweezers (5). While 65 pN is representative of DNA duplexes carrying single-strand breaks, higher plateau forces have been measured for intact duplexes (12,13). Thus, it is our contention that the pulling experiments conducted in this study were performed on an intact duplex. By fitting the (0,0) curve with the three linear segments described in the previous section, L_0 was determined to be 1020 nm; L_1, 1120 nm; and L_2, 1820 nm. It should be noted that the width of the B-S transition plateau for curve (0,0) is measured to be 700 nm, which represents a 68.6% elongation of the molecule length and is in good agreement with the reported data obtained by optical tweezers (5). At location (−400, −800) the width of the B-S transition plateau becomes longer (910 nm) and the plateau force becomes smaller (71 pN). As can be seen in Fig. 3, the measured experimental curve for this pulling location (*green*) is in good agreement with the predicted curve (*black*). At location (800, −1000), the experimental (*red*) and predicted (*cyan*) curves only show a portion of the B-S transition plateau, as the molecule was already under significant tension before pulling occurred. At this location, the width of the B-S transition plateau is 1290 nm and the plateau force is 39 pN, as measured from the theoretical curve.

Fig. 4 is a contour plot of the measured plateau forces (F_{BS}) at various pulling locations. It can be clearly seen that, although errors in measured plateau forces related to the measurement locations are minimal for measurements taken at positions close to position (0,0), these errors increase dramatically as the distance from the cantilever to the substrate attachment site becomes larger. As a result, the interpretation of measured force-extension relationships at these locations can be adversely affected.

In this letter, we studied the effect of the pulling geometry on force-extension measurements of dsDNA by AFM-SMFS. The results presented herein indicate that the pulling geometry can significantly alter the measured characteristic curves of single rigid molecules studied with an AFM. As a result, we stress the importance of minimizing the pulling angle in future experiments. A method to minimize pulling angles in AFM-SMFS is currently being investigated and will be reported in a separate publication.

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REFERENCES and FOOTNOTES

1. Fisher, T. E., P. E. Marszalek, and J. M. Fernandez. 2000. Stretching single molecules into novel conformations using the atomic force microscope. *Nat. Struct. Biol.* 7:719–724.
2. Reference deleted in proof.
3. Carrion-Vazquez, M., P. E. Marszalek, A. F. Oberhauser, and J. M. Fernandez. 1999. Atomic force microscopy captures length phenotypes in single proteins. *Proc. Natl. Acad. Sci. USA.* 96:11288–11292.
4. Bustamante, C., Z. Bryant, and S. B. Smith. 2003. Ten years of tension: single-molecule DNA mechanics. *Nature.* 421:423–427.
5. Smith, S. B., Y. J. Cui, and C. Bustamante. 1996. Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules. *Science.* 271:795–799.
6. Rief, M., H. Clausen-Schaumann, and H. E. Gaub. 1999. Sequence-dependent mechanics of single DNA molecules. *Nat. Struct. Biol.* 6:346–349.
7. Marko, J. F. 1998. DNA under high tension: overstretching, undertwisting, and relaxation dynamics. *Phys. Rev. E.* 57:2134–2149.
8. Williams, M. C., J. R. Wenner, L. Rouzina, and V. A. Bloomfield. 2001. Effect of pH on the overstretching transition of double-stranded DNA: Evidence of force-induced DNA melting. *Biophys. J.* 80:874–881.
9. Lee, G., K. Abdi, Y. Jiang, P. Michaely, V. Bennett, and P. E. Marszalek. 2006. Nanospring behavior of ankyrin repeats. *Nature.* 440:246–249.
10. Marszalek, P. E., A. F. Oberhauser, Y. P. Pang, and J. M. Fernandez. 1998. Polysaccharide elasticity governed by chair-boat transitions of the glucopyranose ring. *Nature.* 396:661–664.
11. Florin, E. L., M. Rief, H. Lehmann, M. Ludwig, C. Dornmair, V. T. Moy, and H. E. Gaub. 1995. Sensing specific molecular-interactions with the atomic-force microscope. *Biosens. Bioelec.* 10:895–901.
12. Clausen-Schaumann, H., M. Rief, C. Tolksdorf, and H. E. Gaub. 2000. Mechanical stability of single DNA molecules. *Biophys. J.* 78:1997–2007.
13. Leger, J. F., G. Romano, A. Sarkar, J. Robert, L. Bourdieu, D. Chatenay, and J. F. Marko. 1999. Structural transitions of a twisted and stretched DNA molecule. *Phys. Rev. Lett.* 83:1066–1069.