USING MECHANICAL FORCES TO STUDY NANO-BIO MATERIALS PROPERTIES

Ching-Hwa Kiang and Wuen-shiu Chen

Department of Physics and Astronomy, Rice University, Houston, TX 77005, USA

INTRODUCTION

Single-molecule manipulation and force measurement is a newly developed technique that allows us to investigate biomolecules and materials on a nanometer scale [1–5]. This technique is also capable of investigating biomolecular interactions directly unlike the bulk studies.

We studied the mechanical properties of a single-stranded DNA, polydeoxyadenylate, poly(dA) by pulling single poly(dA) molecules while monitoring the force exerted on it. We used atomic force microscope (AFM) to stretch poly(dA), which has been shown to have distinct plateaus in their force-extension curves [6, 7]. The overstretched region of the poly(dA) force curves were fit with with either worm-like-chain (WLC) model or extensible freely-jointed-chain (eFJC) model to reveal their elastic properties. We showed that, under high force, poly(dA) has a unique elastic behavior that is different from that of double-stranded DNA (dsDNA). The elastic property of poly(dA) is also different from that of single-stranded DNA (ssDNA) with other sequences. This highly stretched state may play an important role in many biological processes.

EXPERIMENTAL

Materials

Sixty μl solution containing 10 μg/ml single-stranded poly(dA) (Sigma) prepared in Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) with 150 mM NaCl was allowed to absorb onto a freshly exposed gold substrate with an incubation time of 4 hours. The sample was then washed by buffer solution to remove unbounded poly(dA)

Apparatus and Procedures

Figure1 illustrates the experimental setup of single-molecule manipulation. A single poly(dA) molecule was picked up by a microcantilever and stretched using AFM (Veeco). The extension of poly(dA) is controlled by the position of piezo stage and the force is detected via the deflection of the laser beam. The molecule was pulled at 250 nm/s, stayed at a fixed position for 60 seconds, and relaxed to its original state at the same speed. The time series data were converted to force-extension curve of poly(dA) and analyzed with the WLC and eFJC models using programs written in MATLAB.

RESULT AND DISCUSSION

Figure 2 shows five relaxation curves of poly(dA). The unique force-extension curve of poly(dA) shows two plateaus, which is consistent with that observed in Ref.[6]. To compare the elastic property of poly(dA) to dsDNA, we used the inextensible WLC model since dsDNA is best described by an WLC model,

$$F = \frac{1}{P_{ds}\beta}\left[\frac{1}{4(1-\frac{x}{b_{ds}})^2} - \frac{1}{4} + \frac{x}{b_{ds}}\right], \qquad (1)$$

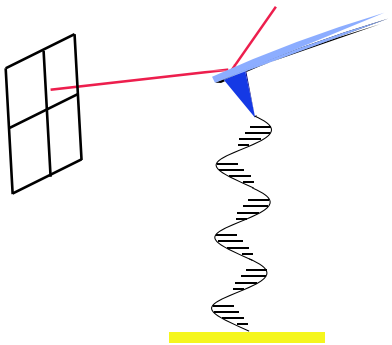

FIG. 1: Illustration of single-molecule manipulation experiments using AFM

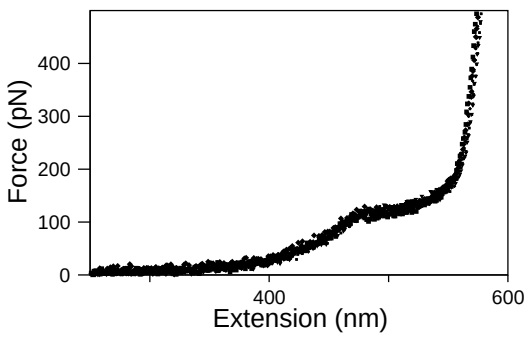

FIG. 2: Five overlapping force-extension relaxation curves of poly(dA).

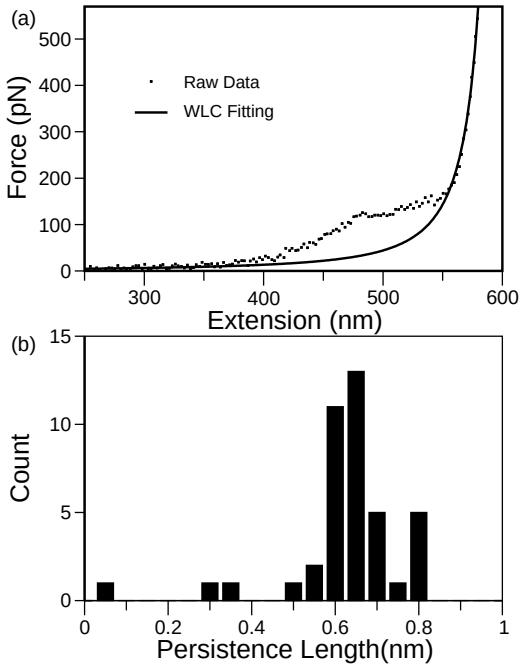

FIG. 3: WLC fitting of poly(dA). (a) A force-extension curve fitted with WLC. (b) Histogram of the persistence length from WLC fit.

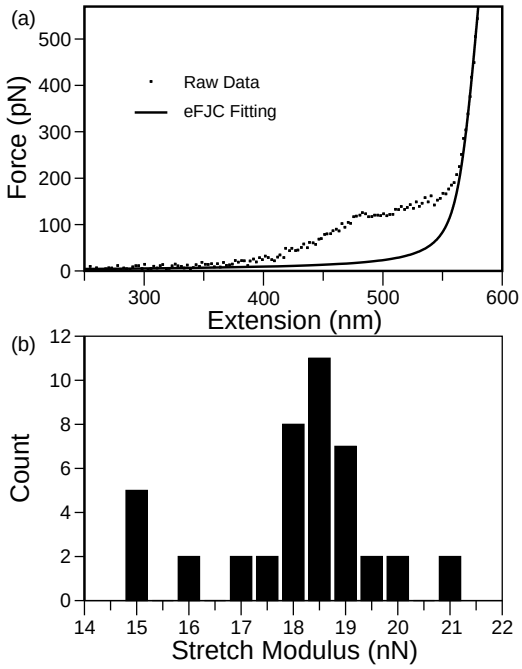

FIG. 4: eFJC fitting of poly(dA) (a) A force-extension curve fitted with eFJC. (b) Histogram of stretch modular from eFJC fit.

where P_{ds}, and b_{ds} are the persistence length and contour length of dsDNA, respectively. F is the force and x is the extension. $P_{ds} = 50$ nm for dsDNA, and this result is similar to the previous estimate [3]. The fitting region of poly(dA) is between 300 pN and maximum force value to ensure the transition part did not affect the accuracy of our fitting Figure 3 shows one example of WLC fitting. Averaging 40 relaxation curves of poly(dA), we obtained $P_{ds} = 0.7$ nm, which is different from dsDNA and more like ssDNA.

To compare the elastic property of poly(dA) to that of ssDNA, whose elastic behavior can be approximated using the extensible freely jointed chain model (eFJC) ,

$$x = b_{ss}\left[\coth(2\beta P_{ss}F) - \frac{1}{2\beta P_{ss}F}\right]\left[1 + \frac{F}{K_{ss}}\right], \quad (2)$$

where P_{ss}, b_{ss}, and K_{ss} are the persistence length, contour length, and stretch modulus. For ssDNA, we have $P_{ss} = 0.75$ nm and $K_{ss} = 2200$ pN.

We fit the relaxation of poly(dA) in the same region with eFJC model with $P_{ss} = 0.75$ nm. Fig. 4 shows one example of the fitting. We found that the stretch modulus of poly(dA) is about 18nN and much higher than normal ssDNA. This indicates that poly(dA) in the overstretching region has unique elastic behavior, which suggests that poly(dA) may have a novel structure under high forces.

CONCLUSION

In summary, we used single-molecule manipulation technique to study the elastic behavior of poly(dA). We fit the force curve to different polymer elasticity models to determine the mechanical properties of poly(dA) under high force. The results show that poly(dA) has different elastic properties from dsDNA and ssDNA. This unique elastic properties of poly(dA) may have important biological implications. The results demonstrated that single-molecule manipulation technique is robust and can be used to study nanoscale biomaterials.

REFERENCES

[1] N. C. Harris, Y. Song, and C.-H. Kiang, Phys. Rev. Lett. **99**, 068101 (2007).
[2] E. M. Puchner and H. E. Gaub, Curr. Opin. Struct. Biol. **19**, 605 (2009).
[3] C. P. Calderon, W.-H. Chen, K.-J. Lin, N. C. Harris, and C.-H. Kiang, J. Phys.: Condens. Matter **21**, 034114 (2009).
[4] E. Botello, N. C. Harris, J. Sargent, W.-H. Chen, K.-J. Lin, and C.-H. Kiang, J. Phys. Chem. B **113**, 10845 (2009).
[5] C. Bustamante, Z. Bryant, and S. B. Smith, Nature **421**, 423 (2003).
[6] C. Ke, M. Humeniuk, H. S-Gracz, and P. E. Marszalek, Phys. Rev. Lett. **99**, 018302 (2007).
[7] W.-S. Chen, W.-H. Chen, Z. Chen, A. A. Gooding, K.-J. Lin, and C.-H. Kiang (2010).