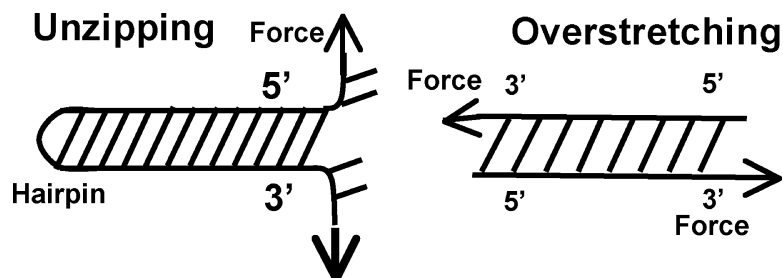


## Probing the Mechanical Stability of DNA in the Presence of Monovalent Cations

Julea Vlassakis, Jeremy Williams, Kristi Hatch, Claudia Danilowicz, Vincent W. Coljee, and Mara Prentiss

*J. Am. Chem. Soc.*, **2008**, 130 (15), 5004-5005 • DOI: 10.1021/ja0776576 • Publication Date (Web): 22 March 2008

Downloaded from <http://pubs.acs.org> on February 8, 2009



### More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

[View the Full Text HTML](#)

## Probing the Mechanical Stability of DNA in the Presence of Monovalent Cations

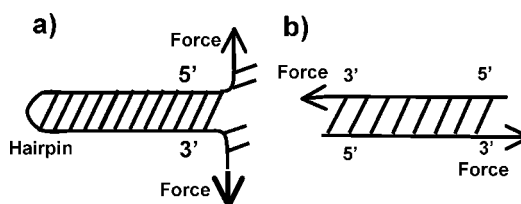
Julea Vlassakis, Jeremy Williams, Kristi Hatch, Claudia Danilowicz,  
Vincent W. Coljee, and Mara Prentiss\*

Department of Physics, Harvard University, 17 Oxford Street, Cambridge, Massachusetts 02138

Received October 4, 2007; E-mail: prentiss@fas.harvard.edu

Ions are known to significantly affect the structure of both single- and double-stranded DNA, as well as the sequence-dependent binding of complementary DNA strands. It is widely agreed that ion charge, size, and concentration are important variables that affect DNA, but there remains considerable disagreement about the detailed functional dependence.<sup>1–8</sup> Better understanding of the effect of ions on DNA may shed significant light on many biological processes that are affected by the stability of dsDNA. Experimental techniques including solution NMR,<sup>1</sup> X-ray crystallography<sup>2</sup> and scattering,<sup>3</sup> magnetic relaxation dispersion,<sup>4</sup> and electrophoresis<sup>5</sup> have been used to study the binding of ions to DNA. Much insight has also been contributed by molecular dynamics simulations.<sup>6–8</sup> The interaction between ions and water molecules is believed to play a significant role in the interaction between ions and DNA, but the complexity of a scenario that involves water, ions, and DNA makes separating effects extremely challenging both theoretically and experimentally. In this work, we use the response of DNA to an applied force to probe the influence of ions on the mechanical stability of DNA, where for simplicity we consider only monovalent salts at several concentrations in Tris buffer pH 7.5. In the absence of effects due to ion size, ion clustering, and interactions with water, all of the monovalent ions would be expected to have exactly the same effect on the DNA. We find that the melting temperature, the force required to separate double-stranded DNA into single strands,  $F_u$ , and the force at which the separated DNA strands recombine,  $F_z$ , are similar for LiCl, NaCl, RbCl, CsCl, KCl, and NaClO<sub>4</sub>. These DNA parameters are believed to be dominated by interactions between the two opposing phosphate backbones. In contrast, we have also considered the effect of ions on the relative stability of B-DNA and overstretched DNA and find significant differences. These results suggest that NaCl, RbCl, and LiCl interact more strongly than KCl, CsCl, and NaClO<sub>4</sub>.

We have performed four different types of experiments: unzipping, melting, reziping, and overstretching. In the first two cases, the dsDNA molecules are forced to convert to ssDNA by mechanical force or temperature, respectively, providing independent estimates of the interstrand binding energy. Additional information on the interstrand binding energy is provided by measurements of the force at which the unzipped DNA rezip. In the unzipping experiment, represented schematically by Figure 1 a, the force pulls the two ends of the double-stranded DNA apart, creating two single strands connected by a fork. On the other hand, in the overstretching experiment, we applied force to opposite ends of opposite strands (see Figure 1b). It is well-known that, at forces above 70 pN, B-form DNA undergoes a phase transition to a new DNA form with a length per base pair that is 1.7 times the spacing for the B-form. Some have suggested that overstretching is a melting transition<sup>9</sup> where

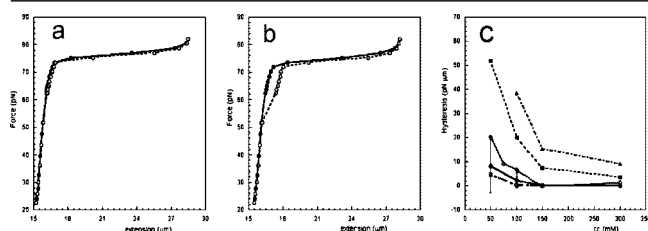


**Figure 1.** Schematic representation of (a) unzipping and (b) overstretching experiments.

long single-stranded regions are interspersed with double-stranded regions. Others assert that the overstretched form is a new form of dsDNA: S-DNA<sup>10–12</sup> as described initially by Cluzel et al.<sup>10</sup> In these measurements, we have focused on the salt dependence of  $F_R$ , the force at which the overstretched state relaxes back to the B-DNA form. If the overstretching is a melting transition, then  $F_R$  will correlate with the interstrand stability that is measured by melting, unzipping, and reziping of the dsDNA. In contrast, if the overstretched form is S-DNA, then  $F_R$  may not correlate with the interstrand stability. We find that the interstrand stability is similar for all salts; however,  $F_R$  is salt-dependent, suggesting that the overstretched state is not melted DNA.

In both the unzipping and the overstretching experiments, the applied force was provided by a magnetic tweezers apparatus that has been described in detail elsewhere.<sup>13</sup> In the overstretching experiment, single molecules were attached by one single bond at each of the 3'3' ends, as shown in Figure 1b. One single biotin label was present at one end and one single digoxigenin label at the other end (see the Supporting Information for more details about the sample preparation and protocol). In order to be able to apply forces as high as 250 pN, we used 4.5  $\mu$ m Dynal beads.

We have found that the relaxation from the overstretched state to the original B-form is strongly dependent on the type of monovalent cation in solution. Figure 2 a and b shows the typical curves for different dsDNA single molecules in the presence of 150 mM NaCl and 150 mM CsCl, respectively, both in Tris 10 mM pH 7.5. The curves were obtained by applying a constant force for 1 s, and then increasing or decreasing the force by a fixed step of 0.2 pN at lower forces or 0.05 pN around the transition, after which the force was then left constant for another dwell time of 1 s. It is evident from the figure that, for NaCl, the curves for increasing and decreasing forces are very similar, whereas in CsCl, there is a clear hysteresis and a significant decrease in  $F_R$ . We quantified the extent of the hysteresis by calculating the difference in the integral of the force versus extension curve for increasing force and decreasing force for many different single molecules (at least 10 for each salt). The averages and standard deviations for 150 mM LiCl, NaCl, KCl, RbCl, CsCl, and NaClO<sub>4</sub> are shown in Table 1. Table 1 also



**Figure 2.** (a and b) Typical overstretching curves: force versus extension, for dsDNA in 150 mM salt and Tris 10 mM pH 7.5. The extension measured while increasing force is shown by the black circles; the open gray circles show the extension obtained for decreasing force: (a) NaCl; (b) CsCl. (c) Hysteresis versus concentration for LiCl (black and white squares and dashed line), NaCl (circles and solid line), RbCl (diamonds and dotted line), KCl (squares and dashed line), and CsCl (triangles and dashed line). Standard deviations are shown only for NaCl data, where, at high NaCl concentrations, the standard deviations are smaller than the symbols. For all cations, the standard deviations are approximately equal to the hysteresis for all points shown.

**Table 1.** Summary of the Experimental Results for the Different Cations in 150 mM Salt and Tris 10 mM pH 7.5

salt	$F_u$ (pN)	$F_z$ (pN)	$T_{\text{melt}}$ (°C)	$H$ (pN μm)	$F_0$ (pN)
LiCl	$19.1 \pm 1.9$	$13.6 \pm 1.6$	$95.3 \pm 2$	$0.11 \pm 0.16$	$83 \pm 2$
NaCl	$16.6 \pm 2.0$	$11.9 \pm 1.3$	$93.4 \pm 2$	$0.23 \pm 0.31$	$82 \pm 4.3$
KCl	$18.4 \pm 3.7$	$13.6 \pm 2.7$	$93.2 \pm 2$	$7.3 \pm 5.0$	$79 \pm 4.9$
RbCl	$17.8 \pm 0.8$	$12.5 \pm 0.5$	$93.2 \pm 2$	$0.07 \pm 0.14$	$83 \pm 3.9$
CsCl	$17.5 \pm 2.9$	$12.7 \pm 2.7$	$92.6 \pm 2$	$15.4 \pm 12.3$	$79 \pm 4.3$
NaClO <sub>4</sub>	$17.2 \pm 3.1$	$12.7 \pm 2.5$	$93.5 \pm 2$	$8.6 \pm 6.3$	$79 \pm 3.3$

includes the unzipping force, the reziping force, the melting temperature, and the overstretching force for 150 mM salt concentration. The measured melting temperatures are consistent with other experiments that have shown almost the same melting temperature for all of the salts except LiCl, which has a slightly higher melting temperature.<sup>14</sup> Additional work using all of the cations except Rb shows the concentration dependence of the melting temperature is the same for all ions.<sup>15</sup>

Figure 2c shows the hysteresis as a function of salt concentration for LiCl, NaCl, RbCl, KCl, and CsCl. Data at lower salt concentrations are not shown because the molecules become too unstable to provide reliable statistics. For all ions, the hysteresis increases with decreasing ion concentration, suggesting that the hysteresis is reduced by improved screening. At a given ion concentration where all ions are hysteretic, the list of ions in order of increasing hysteresis is LiCl, NaCl, RbCl, KCl, and CsCl. Ions with lower hysteresis such as LiCl, NaCl, and RbCl exhibit hysteresis versus concentration curves that are shifted to lower concentrations. For most ions, the data show that higher charge densities allow more efficient relaxation from the overstretched state to the B-form; however, rubidium seems to be an exception.

Previous experimental results have shown that Na<sup>+</sup> displaces multivalent cations more effectively than K<sup>+</sup>.<sup>16</sup> NMR solution measurements have also unambiguously demonstrated differential effects of Na<sup>+</sup> versus K<sup>+</sup> on DNA structure and dynamics.<sup>7</sup> The unusual behavior of rubidium is supported by additional experimental evidence that has shown that Na<sup>+</sup> and Rb<sup>+</sup> compete for the same sites, with the latter having slightly higher affinity. In addition, both can be displaced by the minor groove binder: netropsin.<sup>4</sup>

Simulations also showed more efficient DNA charge neutralization by NaCl than KCl. Such behavior has been attributed to several effects: better fit in the DNA sites and closer approach to the DNA by sodium ions and/or clustering of the cation with the surrounding chloride anions in the case of KCl due to smaller dehydration penalty.<sup>6</sup>

Finally, earlier work predicted that ion clustering should play a role, so using the same cation with a different anion may have an effect. Again, we found that NaCl and NaClO<sub>4</sub> have similar values for the unzipping force, the reziping force, the overstretching force, and the melting temperature; however, in NaCl, we find a much smaller hysteresis than in NaClO<sub>4</sub>. This result can be compared with the destabilizing effect of perchlorate on proteins as well as on nucleic acid triplexes.<sup>17</sup> This anion has been described as a water structure breaker or chaotrope; however, recent work suggests that anions do not change the bulk water dynamics beyond the solvation shell, contradicting the classical description of ions as bulk water structure “breaking” or “making”.<sup>18</sup>

In summary, we observe that the DNA melting temperature, unzipping force, reziping force, and overstretching force are similar for monovalent ions. In contrast, the hysteresis in the force versus extension curves for molecules that undergo the overstretched transition differs for different ions, suggesting that the overstretched state is not melted DNA. For all ions, the force versus extension curves for the transition between the overstretched form and the B-form become more hysteretic as the concentration of ions decreases, implying that the hysteresis decreases with increased screening; therefore, the hysteresis is a measure of screening efficiency that shows that NaCl, RbCl, and LiCl screen more effectively than KCl, CsCl, and NaClO<sub>4</sub>.

**Acknowledgment.** We would like to acknowledge contributions by Alyson Conover and Duncan Eddy. This research was funded by grants ONR DARPA N00014-01-1-0782; Materials Research Science and Engineering Center (MRSEC), NSF # DMR 0213805, and Army Research Office Grant W911NF-04-1-0170.

**Supporting Information Available:** Experimental details, including sample preparation and apparatus information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) (a) Hud, N. V.; Sklenar, V.; Feigon, J. *J. Mol. Biol.* **1999**, *286*, 651–660. (b) Heddi, B.; Foloppe, N.; Hantz, E.; Hartmann, B. *J. Mol. Biol.* **2007**, *368*, 1403–1411.
- (2) Chiu, T. K.; Dickerson, R. E. *J. Mol. Biol.* **2000**, *301*, 915–945. (b) Minasov, G.; Tereshko, V.; Egli, M. *J. Mol. Biol.* **1999**, *291*, 83–99.
- (3) Das, R.; Mills, T. T.; Kwok, L. W.; Maskel, G. S.; Millett, I. S.; Doniach, S.; Finkelstein, K. D.; Herschlag, D.; Pollack, L. *Phys. Rev. Lett.* **2003**, *90*, 188103.
- (4) Marincola, F. C.; Denisov, V. P.; Halle, B. *J. Am. Chem. Soc.* **2004**, *126*, 6739–6750.
- (5) Stellwagen, E.; Dong, Q.; Stellwagen, N. C. *Biopolymers* **2005**, *78*, 62–68.
- (6) Savelyev, A.; Papoian, G. A. *J. Am. Chem. Soc.* **2006**, *128*, 14506–14518.
- (7) Cheng, Y.; Korolev, N.; Nordenskiöld, L. *Nucleic Acids Res.* **2006**, *34*, 686–696.
- (8) Ponomarev, S. Y.; Thayer, K. M.; Beveridge, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14771–14775.
- (9) (a) Williams, M. C.; Wenner, J. R.; Rouzina, I.; Bloomfield, V. A. *Biophys. J.* **2001**, *80*, 874–881. (b) Williams, M. C.; Wenner, J. R.; Rouzina, I.; Bloomfield, V. A. *Biophys. J.* **2002**, *82*, 3160–3169.
- (10) Cluzel, P.; Lebrun, A.; Heller, C.; Lavery, R.; Viovy, J.-L.; Chatenay, D.; Caron, F. *Science* **1996**, *271*, 792–794.
- (11) Smith, S. B.; Cui, Y.; Bustamante, C. *Science* **1996**, *271*, 795–799.
- (12) Kosikov, K. M.; Gorin, A. A.; Zhurkin, V. B.; Olson, W. K. *J. Mol. Biol.* **1999**, *289*, 1301–1326.
- (13) Danilowicz, C.; Coljee, V. W.; Bouzigues, C.; Lubensky, D. K.; Nelson, D. R.; Prentiss, M. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 1964–1969.
- (14) Dix, D. E.; Straus, D. B. *Arch. Biochem. Biophys.* **1972**, *152*, 299–310.
- (15) Nakano, S.; Fujimoto, M.; Hara, H.; Sugimoto, N. *Nucleic Acids Res.* **1999**, *27*, 2957–2965.
- (16) Hibino, K.; Yoshikawa, Y.; Murata, S.; Saito, T.; Zinchenko, A. A.; Yoshikawa, K. *ChemPhysChem* **2006**, *426*, 405–409.
- (17) (a) Broering, J. M.; Bommaris, A. S. *J. Phys. Chem. B* **2005**, *109*, 20612–20619. (b) Lavelle, L.; Fresco, J. R. *Biophys. Chem.* **2003**, *105*, 681–699.
- (18) Omta, A. W.; Kropman, M. F.; Woutersen, S.; Bakker, H. J. *Science* **2003**, *301*, 347–349.

JA0776576