JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Is DNA's Rigidity Dominated by Electrostatic or Nonelectrostatic Interactions?

Alexey Savelyev,[†,‡] Christopher K. Materese,[†] and Garegin A. Papoian*[,†,§]

[†]Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-3290, United States

[‡]Institute of Applied Physics, National Academy of Sciences of Ukraine, Petropavlivska st., 58, Sumy, Ukraine 40030

[§]Department of Chemistry and Biochemistry, Institute for Physical Science and Technology, University of Maryland, College Park, Maryland 20742, United States

Supporting Information

ABSTRACT: Double-stranded DNA is among the stiffest biopolymers, whose bending propensity crucially influences many vital biological processes. It is not fully understood which among the two most likely forces, electrostatic self-repulsion or the compressive base pair stacking, plays a dominant role in determining the DNA's unique rigidity. Different theoretical and experimental studies led so far to contradictory results on this issue. In this Communication, we address this important question by means of Molecular Dynamics (MD) simulations using both atomistic and coarse-grained force fields. Using two independent sets of calculations, we found that electrostatic and nonelectrostatic effects play a comparable role in maintaining DNA's stiffness. Our findings substantially differ from predictions of existing theories for DNA rigidity and may indicate that a new conceptual understanding needs to be developed.

Accurate descriptions of DNA flexibility both in vivo and in vitro remain a significant challenge for both experimentalists and theoreticians.[1] Surprisingly, despite active research and the significant progress made in this field during several past decades, there is still a debate as to which physical force, or forces play a dominant role in maintaining DNA's rigidity.[1] This issue is important because many vital biological processes are critically influenced by the bending propensity of the DNA molecule. For example, a meter-long DNA molecule compacts by many order of magnitudes and folds into a chromatin inside of a eukaryotic nucleus of several micrometers in diameter.[2] Because DNA chain is a highly charged semiflexible macromolecule with a persistence length of 50 nm at physiological conditions (see below), it is expected that the compaction is accompanied by neutralization of its charges and the optimal distribution of the incurred bending penalty along the DNA chain. These two processes are accomplished to a significant extent through the formation of the DNA−protein complex, a nucleosome, by association of the DNA with positive histone proteins. In addition to that, surrounding aqueous salt atmosphere (mobile ions) controls the neutralization of the remaining charge of the nucleosomal DNA and also the charge of the protein-free linker DNA connecting adjacent nucleosomes. Hence, better understanding of the physical mechanism behind DNA's rigidity, and the ways that it can be regulated by ions, small molecules, and other biological molecules, would help to gain deeper insights into the chromatin folding problem. Similarly, more thorough understanding of this issue may allow more controlled artificial manipulation of DNA stiffness, for example, via protonating the negatively charged phosphate groups and/or altering the base pair interactions, in applications such as gene therapy, where artificial condensation and packaging of DNA is required.[1]

From a physical perspective, DNA may be thought of as a highly charged polyelectrolyte carrying a charge of −2e per base-pair. Thus, it may be natural to assume that the main contribution to DNA's stiffness comes from the intramolecular repulsion among the negatively charged phosphates that are part of the molecule's backbone, resulting in a locally rigid but globally flexible structure. On the other hand, a double-stranded DNA molecule is stabilized by compressive base-pair stacking forces which act very strongly against even moderate variations in DNA shape. While these two effects, electrostatic charge repulsion and stacking compression, are commonly believed to be the most likely contributions to DNA stiffness, it is not understood which of them is the dominant one, or to what extent they balance each other. As briefly outlined below, various theoretical treatments of DNA rigidity, as well as related experimental works which study DNA shape and flexibility, lead to contradictory results, leaving this important issue unresolved. In the current Communication, we address this problem computationally by means of Molecular Dynamics (MD) simulations at two scales. As elaborated below, we utilize two related coarse-grained (CG) DNA models, one for the normally charged DNA, which was developed in a recent work[3] (see Figure 1), and another one for the hypothetical uncharged DNA, which was systematically derived here from the corresponding atomistic MD simulations. We take advantage of combining fully atomistic and coarse-grained MD simulations and obtain a new set of interesting results which challenge current understanding of the physical origin of DNA's rigidity.

The most important large-scale characteristic of a semiflexible polymer is its persistence length.[4] Various experimental techniques indicate approximately 50 nm for the persistence length of the double-stranded DNA at physiological conditions.[1] The impact of the surrounding aqueous salt atmosphere on the DNA conformational flexibility was previously addressed by the celebrated theory of Odijk and Skolnick and Fixman[5]

Received: August 23, 2011
Published: October 31, 2011

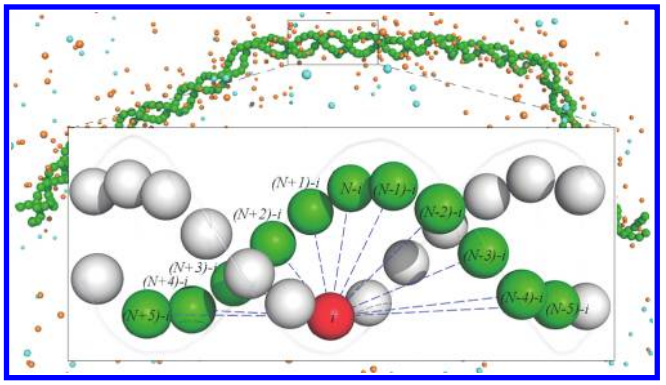

Figure 1. A chemically accurate two-bead coarse-grained model of the DNA with explicit mobile ions[3] was extensively used in this study. Blue dashed lines indicate effective interactions which represent a superposition of stacking and base pairing among two polynucleotides. To elucidate the role of electrostatics in mediating DNA rigidity, we continuously reduced residual DNA charges without change of solvation and measured DNA's persistence length. Another set of results for the fully uncharged DNA was obtained from the related all-atom MD simulations. The subsequent coarse-graining of the latter atomistic model is structurally identical to the depicted model. See the text and Supporting Information for details.

(OSF), which states that persistence length of the DNA (or other polyelectrolyte) consists of two additive contributions, inherent (elastic) one and the contribution caused exclusively by the electrostatic self-repulsion. OSF theory predicts that DNA's persistence length is nearly independent of the concentration of the surrounding mobile ions after ~0.05 M, which is consistent with some experimental observations.[6] Hence, OSF theory suggests a relatively small effect of DNA electrostatics in determining its rigidity in biologically relevant environments. Specifically, at physiological conditions, the 'electrostatic' persistence length constitutes less than ~10% (~5 nm) of the total DNA persistence length. This suggests that DNA's stiffness originates predominantly via the base-pair stacking interactions.

OSF theory has recently been challenged by conceptually different theory for DNA stiffness proposed by Manning,[7] which predicts that the persistence length of the '*null isomer of DNA*', a hypothetical structure of the DNA in the absence of DNA residual charges, constitutes only ~7 nm, or just ~14% of the persistence length of the normally charged DNA under physiological conditions. This suggests that the dominant contribution to the DNA stiffness comes from the repulsion of DNA charges, more precisely, from the 'electrostatic tension' within the DNA helix.[7] It must be noted, at the same time, that 'electrostatic' and 'nonelectrostatic' persistence lengths are not additive within Manning's approach, and numerical values for them (~7 and ~43 nm, respectively), which follow from such a nominal decomposition may not be applicable to arbitrary polyelectrolytes of the same linear charge density. Nevertheless, there are experimental studies which support Manning's theory and suggest that the dominant role in DNA stiffness and shape is played by electrostatic effects.[8,9]

Hence, there is an apparent controversy found within both theoretical predictions and experimental interpretations regarding the fundamental issue of whether electrostatic self-repulsion or base-pair stacking is primarily responsible for DNA's stiffness. MD simulations represent yet another approach which can be used to probe different aspects of DNA electrostatics and flexibility. For example, while experimental measurements of the persistence length of the Manning's 'null isomer' require novel sophisticated chemistry,[1] simulating a hypothetical neutral DNA chain is straightforward within an MD approach. Alternatively, one could reach the 'null isomer' by continuously reducing the charges along the DNA backbone. In this Communication, we model such charge-altered DNA systems with MD techniques, which provides new insights into the comparative strengths of DNA electrostatics and base-pair stacking forces.

Ideally, a DNA molecule immersed in a salty aqueous solution has to be studied on atomistic level. It has recently been demonstrated in our studies[10,11] that there are substantial differences between more exact all-atomistic (AA) MD simulations of DNA systems and the predictions from standard models of continuum electrostatics, such as Poisson−Boltzmann theory, whose concepts are used in coarser DNA models. However, AA MD simulations are only practical while dealing with a moderate number of particles, which is, at present, on the order of 100 000. At the same time, to faithfully measure persistence length of the DNA, a segment of at least ~150 base pairs (one persistence length) has to be simulated. This means that a simulation box of several tens of nanometers in linear size will possess a very large number of particles (tens of millions), mainly due to water and mobile ions. Thus, a simplified yet sufficiently accurate coarse-grained DNA model capable of capturing important aspects of system's electrostatics and conformational dynamics must be used.

Recently, we developed such a two-bead CG model for the double-stranded DNA with explicit mobile ions[3] (see Figure 1). It was systematically derived from fully atomistic AMBER10 MD simulations making use of the previously developed Molecular Renormalization Group Coarse Graining (MRG-CG) technique.[12,13] This approach ensures that complex local dynamics for both DNA and mobile ions, as well as the coupling between DNA conformational dynamics and ionic fluctuations, accurately match the corresponding motions in the underlying all-atom model. Particularly, many important aspects of ionic atmosphere around DNA, including hydration effects and long-range spatial correlations, were faithfully reproduced. The obtained CG model produced quantitative agreement with experimental data on the dependence of DNA persistence length on the ionic strength of the solution, which is very difficult to achieve with both atomistic and CG models of DNA.[3]

To address the problem of the present study, our CG model can readily serve as a starting point in the following numerical "*Gedankenexperiment*": charges of DNA nucleotides are continuously reduced from their normal values of −1 up to complete neutralization, which is the equivalent to morphing the regular DNA molecule into the Manning's 'null isomer', or fully uncharged DNA. We carry out such transformation without change of solvation, that is, at the same ionic concentration corresponding to physiological conditions. DNA's persistence length is then measured every incremental step of the residual charge reduction to find out what role is played by electrostatics in regulating DNA rigidity. It should be noted that this approach is rather different from the usual alternative of keeping DNA intact and changing the salt concentration. The new approach allows for the decomposition of the total DNA persistence length into electrostatic and inherent nonelectrostatic contributions. We test the numerical values for both contributions as predicted by OSF and Manning's theories against our MD simulation results. We refer

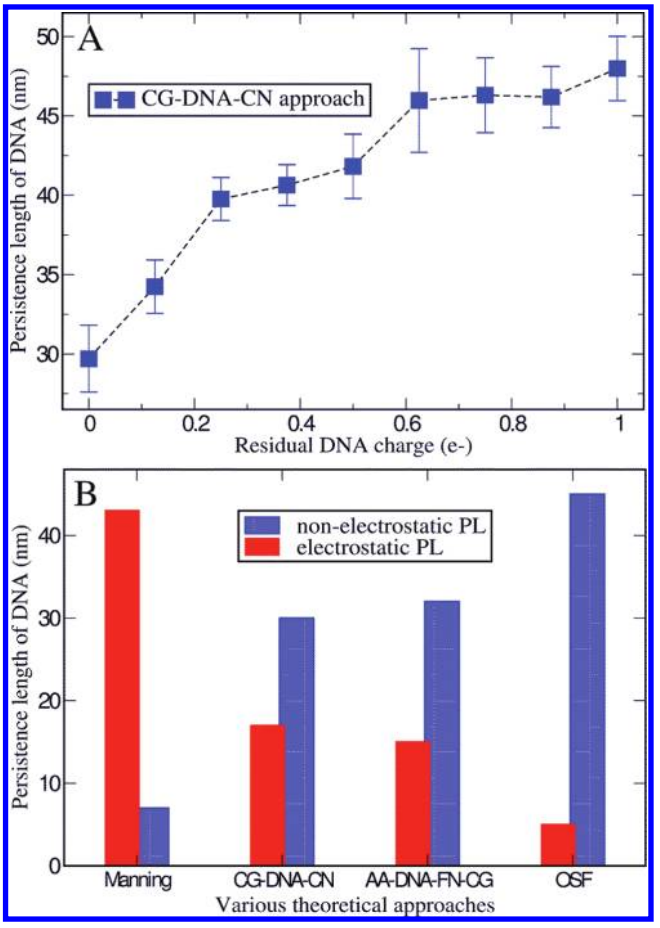

Figure 2. (A) Variation of persistence length (PL) as a function of DNA's residual charge, observed with our CG-DNA-CN approach. The error bars represent standard deviations. (B) Electrostatic and nonelectrostatic portions of the DNA's PL obtained from different theoretical approaches: Manning's theory,[6] CG-DNA-CN and AA-DNA-FN-CG computational approaches developed in this study, and OSF theory.[4] Electrostatic PL is given by a difference between PLs of the normally charged and fully neutralized DNA segments. Our results suggest that both electrostatic and nonelectrostatic effects play essential role in mediating DNA stiffness, contrary to predictions from OSF and Manning's theories (see panel B). CG-DNA-CN approach and a more sophisticated AA-DNA-FN-CG approach give virtually the same results, indicating that DNA electrostatic and nonelectrostatic interactions are not strongly coupled.

to this approach as CG-DNA-CN (Coarse-Grained DNA, Continuous Neutralization), to distinguish it from yet another technique used in this study (see below).

We also carried out independent simulations at very detailed atomistic level, with explicit water, where we neutralized DNA in a single step (mimicking the 'null isomer of DNA'), followed by coarse-graining to measure the resulting persistence length. This CG model is structurally analogous to the 2-bead model depicted in the Figure 1. Again, our MRG-CG technique was used for this purpose. We call this second approach the AA-DNA-FN-CG (Atomistic DNA, Full Neutralization, followed by Coarse-Graining). Additional atomistic simulations allow us to (1) find out whether there is an agreement with the results from CG-DNA-CN, and (2) investigate potential coupling between electrostatic and nonelectrostatic interactions, for example mediated through solvent.

By combining CG-DNA-CN and AA-DNA-FN-CG approaches, we can answer the following questions: (1) what is the character of the dependence for the DNA persistence length on the DNA residual charge (monotonous, smooth, etc.)? (2) how do electrostatic and nonelectrostatic forces balance each other in maintaining DNA rigidity, that is, what are the *quantitative* measures for the electrostatic and nonelectrostatic portions of the DNA persistence length and how they agree with the predictions from OSF and Manning's theories?

Details on preparation and MD simulation of the atomistic DNA systems are elaborated in the Supporting Information (SI). In short, neutral DNA was prepared by adding a proton to the negatively charged phosphate group of each nucleic acid base of the all-atom 32 base-pair DNA segment, resulting in a zero total net charge for each base (see SI-1). Modified nucleic acid residues were constructed using the antechamber package[14] from the AMBER suite[19] with the Restricted Electrostatic Potential (RESP) technique.[15] Quantum calculations were performed in the Gaussian Suite.[20] The refined AMBER Parmbsc0 force field for nucleic acids[16] and the TIP3P model[17] for the water, as well as the recently developed force field for alkali and halide monovalent ions,[18] were used to set up AA MD simulations. After sufficient equilibration of the all-atom system, MRG-CG technique was utilized to derive an accurate CG model. Additional description of the MRG-CG technique, as well as the MD simulation protocol of CG systems is provided in the Supporting Information.

The central result of the present study is shown in Figure 2. The upper panel demonstrates how the persistence length of DNA varies with the change of the DNA's residual charge, as follows from the CG-DNA-CN approach. As expected, DNA becomes softer when the residual charge drops to zero. What is particularly interesting is that the persistence lengths of the normal and uncharged DNA molecules differ by ~17 nm, the result which supports predictions from neither OSF nor Manning's theories and suggests a "*crossover*" regime of the DNA conformational dynamics with respect to these two opposite limiting cases. Specifically, electrostatic persistence length of the DNA constitutes ~35% of the total value (contrary to the ~10% and ~86% predicted by OSF and Manning's theories, respectively). Our MD simulation findings indicate that both electrostatic and nonelectrostatic interactions play important, comparable roles in maintaining DNA rigidity. Thus, there is no overwhelmingly dominant contribution to the DNA rigidity but rather a fine balance of electrostatic and nonelectrostatic forces.

The lower panel of Figure 2 represents an illustrative comparison of predictions from different theories for the interplay between DNA's electrostatic and nonelectrostatic effects, including that from the second, AA-DNA-FN-CG approach. Following the preceding discussion, the latter approach, which is more laborious compared to CG-DNA-CN, is expected to produce a more reliable quantitative estimate for electrostatic and nonelectrostatic contributions to the DNA persistence length. The persistence length of the CG "null isomer of DNA" devised from AA-DNA-FN-CG approach appeared to be smaller by ~15 nm than that of the normally charged DNA. The value obtained is close to the previously mentioned difference of ~17 nm which suggests that DNA electrostatic and nonelectrostatic interactions are not strongly coupled.

In summary, MD simulations of accurate CG DNA models, which were systematically derived from fully atomistic MD simulations, enabled an estimation of the relative contributions

of electrostatic and nonelectrostatic (elastic) forces to DNA's stiffness at physiological conditions. Contrary to predictions from both the OSF theory and Manning's approach for description of the DNA rigidity, the importance of these effects appeared to be on similar footing. Our findings suggest that the current theoretical view of DNA flexibility may be in need of a major overhaul and, furthermore, additional experiments may be necessary to quantitatively investigate the extent of electrostatic and nonelectrostatic contributions to DNA's persistent length. Furthermore, it would be interesting to explore this question when DNA sequence is varied, since it is possible that certain sequences may favor nonelectrostatic contribution to larger or smaller degrees. Our current CG model of DNA is averaged over DNA's sequence; hence, sequence specific models need to be developed to investigate this question. At the same time, the model used in the present study is appropriate when making comparisons to well-recognized theories for DNA rigidity (such as OSF, Manning and others), which consider no sequence-dependent effects. Some of these sequence-independent theories are widely used to interpret the experimental data on flexibility of various DNA chains.

■ ASSOCIATED CONTENT

Supporting Information. Preparation details and MD simulation protocols for both atomistic and coarse-grained systems, as well as the analysis description. The full list of authors for the reference [20] is provided in the reference [4] of the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
gpapoian@umd.edu

■ ACKNOWLEDGMENT

We were inspired to investigate the origin of the large stiffness of DNA chains by long-term efforts of Dr. L. James Maher, III, whose laboratory chemically modified DNA in various ways to tease out the importance of the electrostatic contribution. We are grateful to Dr. Michael Rubinstein for helpful discussions. This work was supported by the Beckman Young Investigator Award. All calculations were carried out using the University of North Carolina Topsail supercomputer and the research computer cluster from the Institute of Applied Physics, Sumy, Ukraine.

■ REFERENCES

(1) Peters, P. J.; Maher, L. J. *Q. Rev. Biophys.* **2010**, *43* (I), 23–63.
(2) Albertz, B. et al. *Molecular Biology of the Cell*; Garland: New York,2002.
(3) Savelyev, A.; Papoian, G. A. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107* (47), 20340–20345.
(4) Doi, M; Edwards, S.; *The Theory of Polymer Dynamics*; Oxford Univ. Press: New York, 1986.
(5) (a) Odijk, T. *J. Polym. Sci., Polym. Phys. Ed.* **1977**, *15*, 477–483. (b) Skolnick, J; Fixman, M. *Macromolecules* **1977**, *10*, 944–948.
(6) Baumann, C. G.; Smith, S. B.; Bloomfield, V. A.; Bustamante, C. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6185–6190.
(7) Manning, G. S. *Biophys. J.* **2006**, *91*, 3607–3616.
(8) Bloomfield, V. A. *Biopolymers* **1997**, *44*, 269–282.
(9) Podesta, A.; Indrieri, M.; Brogioli, D.; Manning, G. S.; Milani, P.; Guerra, R.; Finzi, L.; Dunlap, D. *Biophys. J.* **2005**, *89*, 2558–2563.
(10) Savelyev, A.; Papoian, G. A. *J. Am. Chem. Soc.* **2006**, *128*, 14506–14518.
(11) Savelyev, A.; Papoian, G. A. *J. Am. Chem. Soc.* **2007**, *129*, 6060–6061.
(12) Savelyev, A.; Papoian, G. A. *Biophys. J.* **2009**, *96*, 4044–4052.
(13) Savelyev, A.; Papoian, G. A. *J. Phys. Chem. B* **2009**, *113*, 7785–7793.
(14) Wang, J.; Wang, W.; Kollman, P. A.; Case, D. A. *J. Mol. Graphics Modell.* **2006**, *25*, 247260.
(15) Bayly, C. I.; Cieplak, P.; Cornell, W. D.; Kollman, P. A. *J. Phys. Chem.* **1993**, *97*, 10269–10280.
(16) Perez, A.; Marchan, I.; Svozil, D.; Sponer, J.; Cheatham, T. E.; Laughton, C. A.; Orozco, M. *Biophys. J.* **2007**, *92*, 3817–3829.
(17) Miyamoto, S.; Kollman, P. A. *J. Comput. Chem.* **1992**, *13*, 952–962.
(18) Joung, I. S.; Cheatham, T. E. *J. Phys. Chem. B* **2008**, *112*, 9020–9041.
(19) Case, D. A., et al. ; *AMBER 11*; University of California: San Francisco, CA, 2010.
(20) Frisch, M. J. et al. ; *Gaussian 03*, Revision C.02, Gaussian, Inc.: Wallingford, CT, 2004.