Opposing Effects of Multivalent Ions on the Flexibility of DNA and RNA

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Increasing the concentration of counterions (salt) is known to reduce the bending persistence length of DNA. Here we use atomistic molecular dynamics simulations to predict that multivalent counterions have the opposite effect on double-stranded RNA, increasing its bending rigidity by at least 30%. This counterintuitive effect is observed for various tri- and tetravalent ions alike, and is robust to methodological details and the RNA sequence. In contrast to DNA, multivalent counterions bind inside the RNA major groove, causing significant contraction of the molecule along its helical axis—as a result, its further deformation due to bending becomes energetically more expensive compared to bending without bound multivalent ions. Thus, the relationship between mechanical properties of a charged polymer and its ionic atmosphere may be richer than previously thought.

The mechanical properties of nucleic acids (NA) are known to be strongly affected by the ionic conditions of the solutions they are in [1–7]. Experiments show that increasing the concentration of monovalent salt reduced the DNA bending persistence length [1,3] from its classical value of ∼500 Å at a physiological NaCl concentration of ∼0.145 M. An even more dramatic decrease of the DNA persistence length with salt concentration has been observed when monovalent cations were replaced by multivalent ones [1]. The common explanation is that the counterion atmosphere around a NA molecule screens the repulsion between the negatively charged phosphates along the polymer, thus increasing its bending flexibility [8–12].

In contrast to DNA, the mechanical properties of double-stranded RNA (referred to simply as RNA for the remainder of the text) have not been investigated as extensively. From the similar charge density and overall structure of DNA and RNA double helices one might expect that they should exhibit similar elastic responses under applied forces. Indeed, recent single-molecule force and torque measurements of RNA bending flexibilities [13–15] have shown an analogous to DNA decrease of the bending persistence length of RNA with increasing monovalent salt concentration [14].

Here we compare the effects of multivalent counterions on RNA bending flexibility using atomistic molecular dynamics simulations; the study was motivated by a recent finding that binding patterns of multivalent counterions to DNA and RNA are drastically different [16–18]. We investigate how this difference in multivalent ion binding affects the RNA bending persistence length when monovalent counterions are replaced by ions with charges $+3e$ and $+4e$, and propose an explanation for the opposing effects on bending flexibilities of DNA and RNA.

DNA and RNA duplexes of 25 base pairs (bp) with the same mixed sequence described in Ref. [16] and a homopolymeric poly(rA) poly(rU) RNA fragment of the same length were generated in canonical *B* form for DNA and *A* form for RNA using Nucleic Acid Builder [19]. The NA molecules were then neutralized with three different combinations of monovalent Na^+ and trivalent Cobalt(III) Hexammine (CoHex): (i) 48 Na^+ ("no CoHex"); (ii) 8 $CoHex^{3+}$ and 24 Na^+ (0.17 $CoHex^{3+}$ per phosphate, indicated by P^-); and (iii) 16 $CoHex^{3+}$ (0.33 $CoHex^{3+}/P^-$), the latter corresponding to estimated bulk concentrations (measured 32 Å from the helical axis of the duplexes) of 4 mM for RNA and 6 mM for DNA. In addition, mixed sequence RNA molecules were also neutralized with two other counterion types: 16 hypothetical Na^{3+} ions and 12 spermine[4+] ions. "Na^{3+}" was simulated by increasing threefold the Na^+ charge in the Amber topology file. Each system was then solvated with ∼16 800 TIP3P water molecules in a periodic box. To account for monovalent salt background roughly equivalent to physiological conditions, 24 Na^+Cl^- ion pairs were added to all systems (low Na^+ concentration regimes were not explored for technical reasons). Unless otherwise stated, all MD simulations were carried out using AMBER 12 [20] and ff99bsc0 force field [21,22] at $T = 300$ K. Each system was first simulated for at least 100 ns while holding the duplex restrained to allow the ionic atmosphere to equilibrate around the molecule. Then, positional restraints were removed for production MD runs: 300 ns for

CoHex^{3+} and no CoHex systems, and 400 ns for spermine^{4+} and Na^{3+} systems.

NA helical axis representations for each frame were generated from MD trajectories using Curves+ [23]. We then calculate, for each MD snapshot, the bending angle θ between average vectors of consecutive helical repeats (10 bp in our case), which should reduce the possibility of error from the nonsymmetric nature of the double helix [24]. The result of the calculation is the angular probability distribution $P(\theta, l)$, where l is the average contour length of the 10 bp helical repeat (calculated for each NA-ion system individually by summing all base-pair rise parameters). For $l \ll L_p$ we can use the inextensible wormlike chain [25–28] approximation for the bending energy:

$$E(\theta, l) = \frac{1}{2} k_B T \frac{L_p}{l} \theta^2. \qquad (1)$$

For spherically isotropic distribution in 3D space [27]

$$P(\theta, l) = \sin\theta \exp(-E(\theta, l)/k_B T), \qquad (2)$$

which leads to

$$-\ln\left(\frac{P(\theta, l)}{\sin\theta}\right) = \frac{L_p}{2l} \theta^2 \qquad (3)$$

used to estimate L_p, Fig. 1.

For all calculated values of L_p in this work the relative statistical error never exceeded 2%, and is not reported below. For mixed sequence DNA the addition of a neutralizing amount of CoHex yields the predicted relative change $\Delta L_p/L_p = -37\%$ (Table I, bottom row), which is within the reported error margin of the corresponding experimental estimate [1] of $-44 \pm 8\%$. Thus, despite unavoidable methodological limitations, including known imperfections of modern force fields [29], our calculations
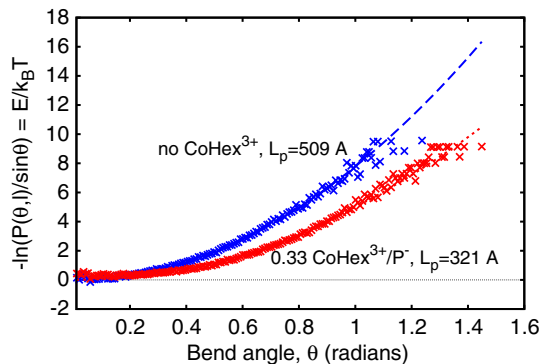

FIG. 1. Estimation of the bending persistence length L_p of the DNA duplex with (red) and without (blue) CoHex^{3+} counterions. L_p is estimated by fitting (dashed lines) the data points to Eq. (3); each point represents a value of $-\ln[P(\theta, l)/\sin\theta]$ averaged over the $\sim$0.008 interval of θ.

predict the main quantity of interest—the relative change of persistence length $\Delta L_p/L_p$ upon addition of multivalent ions—in acceptable agreement with experiment. Furthermore, recent WAXS [30] and CD spectra [16] measurements have not detected any significant changes in DNA structure upon addition of CoHex, consistent with our results that the molecule contracts by no more than 2%; see Table I. Previous all-atom [31] and even coarse-grained models derived from the same atomistic potentials [32,33] also reproduce correct quantitative dependence of DNA L_p on ionic strength.

Unexpectedly, the effect of CoHex on RNA is the opposite of its effect on the DNA: the addition of CoHex^{3+} results in a significantly higher L_p of the RNA, Table II. The counterintuitive increase in RNA bending rigidity caused by CoHex does not appear to be sequence dependent: the same strong effect of similar magnitude is seen when we replace the mixed sequence RNA with a homopolymeric poly(rA) poly(rU) fragment (first row of Table III). This effect on the RNA persistence length is not limited to CoHex^{3+} counterions: tetravalent spermine^{4+} (a linear polyamine ion) and hypothetical trivalent Na^{3+} also stiffen the RNA; see Table III.

We propose the following qualitative explanation for this novel effect. The persistence length of a charged polymer in the presence of counterions can be conceptually decomposed into two distinct contributions: $L_p \simeq L_{int} + \Delta L_{scr}$ [34]. The "base" contribution L_{int} depends on the internal structure (short-range interactions) and charge distribution of the polymer, while ΔL_{scr} represents a correction to L_{int} due to the screening by the counterions of the long-range charge-charge interactions along the polymer. Both of these contributions can significantly affect the total persistence length L_p [35]. In the case of NA, ΔL_{scr} is always negative because the screening results in a reduced effective electrostatic repulsion between the negatively charged phosphate groups. In general, DNA and RNA double helices have very similar overall structure and electrostatic properties, including linear charge density and other parameters relevant to L_p according to existing models [34,36]. Thus, given the same amount of reduction in the charge-charge repulsion along the NA polymer due to partial neutralization by the counterions, the ΔL_{scr} term is expected to be roughly equal for DNA and RNA

TABLE I. Effect of CoHex^{3+} counterions on DNA flexibility. Shown are relative changes in bending persistence length (L_p) and length of the helical repeat (10 bp) segment (l), relative to the same system with no CoHex^{3+} ions (multivalent ions replaced by Na$^+$ to maintain overall neutrality).

DNA w/CoHex^{3+}	$\Delta L_p/L_p$ (%)	$\Delta l/l$ (%)
0.17 CoHex^{3+}/P$^-$	-31	-1.8
0.33 CoHex^{3+}/P$^-$	-37	-1.8

TABLE II.　Effect of CoHex^{3+} on RNA flexibility. Persistence length (L_p) of RNA increases with CoHex^{3+} concentration—an effect that is opposite to the DNA response to CoHex^{3+}. Bound CoHex^{3+} also significantly shrinks the RNA duplex along its helical axis.

RNA w/CoHex^{3+}	$\Delta L_p/L_p$ (%)	$\Delta l/l$ (%)
0.17 CoHex^{3+}/P$^-$	+68	−12
0.33 CoHex^{3+}/P$^-$	+90	−14

surrounded by the same type and amount of counterions. Indeed, increasing the concentration of monovalent ions (which are known to form loose distributions around NA molecules [37–39]) leads to similar changes in observed L_p of both DNA and RNA. However, the situation is very different with multivalent counterions. For DNA, these ions mostly bind to the externally exposed surface of the phosphate groups [16], Fig. 2 (left panel), leading to their partial neutralization, which mainly affects ΔL_{scr} in the expected way. In contrast, CoHex binds preferentially inside the RNA molecule, closer to the helical axis [16,17], Fig. 2 (right panel). This striking difference in the binding pattern can be rationalized as follows. Structural differences between DNA and RNA helices (B vs A form) lead to a much more negative electrostatic potential in the major groove of RNA [16,40], causing the preferential CoHex binding. While qualitative, this picture is consistent with experimental differences in the CoHex binding constants: the ion binds much stronger to the RNA (binding constant $\sim 10^4$ M^{-1}) [41] compared to the DNA ($\sim 10^2$ M^{-1}) [42] at near physiological NaCl concentrations. This specific binding preference of trivalent CoHex is expected to be robust: RNA is always in A form, and the fraction of bound ions is insensitive to water model and force-field choice; simulations with TIP4P water and the χ_{OL3} modifications for RNA to ff99bsc0 force-field [43] result in a negligible 3% difference in the number of CoHex ions bound to the RNA. The corresponding L_p/L_p of +76% is comparable to the values shown in Table II. The internally and strongly bound CoHex ions can be considered as part of the internal structure of RNA itself, mainly affecting L_{int}.

TABLE III.　CoHex^{3+} increases persistence length (L_p) of homopolymeric poly(rA)poly(rU) RNA molecules. Other tri- and tetravalent ions have the same qualitative effect: spermine^{4+} and hypothetical Na^{3+} increase L_p and decrease the fragment length (l) of RNA duplexes.

RNA w/ + 3 and +4-valent ions	$\Delta L_p/L_p$ (%)	$\Delta l/l$ (%)
0.33 CoHex^{3+}/P$^-$ [poly(rA)·poly(rU)]	+56	−14
0.33 Na^{3+}/P$^-$ (mixed RNA)	+85	−16
0.25 spermine^{4+}/P$^-$ (mixed RNA)	+29	−14

As sufficient number of CoHex counterions bind into the major grove of RNA, they pull the oppositely charged phosphate groups closer together. The strong pull results in the contraction of the duplex along its helical axis (Tables II, III). Changes in experimental WAXS profile of RNA (which correlate well with simulated profiles from MD) [30], specifically the "sharpening" of the features observed in the WAXS regime, are consistent with better defined structures when CoHex is present. This suggests a stiffer (less flexible) RNA duplex. In contrast, virtually no structural change occurs in DNA upon binding of the same multivalent counterions (see Table I). Note that even though Na^{3+} is sterically about 3 times smaller than CoHex^{3+}, the relative contraction of the RNA double helix caused by Na^{3+} remains almost the same, Tables II and III, suggesting that any further contraction of the RNA double helix becomes highly unfavorable energetically. The strong electrostatic pull between phosphates and buried counterions is balanced by the short-range interactions that maintain the internal structure of the double helix. Bending of a polymer implies some combination of contraction and stretching (of the opposite side of the chain), and both of these deformations require more energy in the already contracted state of the RNA compared to the original "relaxed" conformation, which ultimately results in higher bending modulus of the polymer. Thus, the
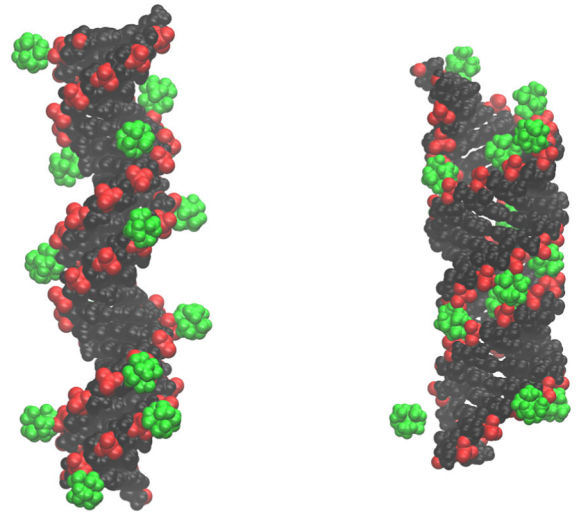

FIG. 2.　In DNA (left), multivalent counterions (illustrated for CoHex^{3+}, green) bind mostly externally [16] onto the negatively charged phosphates (red), with little effect on the DNA structure. The binding reduces the effective electrostatic repulsion along the helix, which in turn decreases the helix bending rigidity. In contrast, the same ions bind deep inside the RNA major groove (right) [16], causing the double helix to contract and significantly stiffen its internal structure. This leads to an overall increase of the RNA bending rigidity—the pull of the ions works as taut bicycle spokes that tighten the wheel. The distributions (not shown) of bound spermine^{4+} around DNA and RNA are similar to the above.

binding of multivalent counterions to the RNA helix increases L_{int} significantly, resulting in a net increase of its L_p, while in the DNA the ions mostly reduce L_p via negative ΔL_{scr}.

Our main result—that multivalent counterions can significantly increase RNA bending persistence length—is unexpected from the perspective of the completely opposite and well-known effect of increasing salt concentration on RNA's closest cousin, DNA. The physics behind the salt effect on bending rigidity of long charged polymers appeared well understood: the charge-charge repulsion along the polymer is screened out by the counterions, making it easier to bend. But apparently, this is only one side of the story: small differences in structure between RNA and DNA can make dominant a previously unexplored consequence of counterion binding, changing the sign of the overall salt effect on the polymer's persistence length. While the magnitude of the predicted effect might have some dependence on details of the methodology, it is clear that the effect is strong, persists over a range of ion concentrations, and is robust to ion type and sequence details, all of which should facilitate direct experimental verification. We believe that single-molecule measurements such as magnetic or optical tweezers experiments [1,14] are best suited for studying this phenomenon. AFM studies [26] are another possible way to observe this effect, provided the interactions of the surface with the NA duplexes have minimal influence on the binding of ions to the molecule. The new effect may manifest itself in other scenarios: mechanical properties of DNA with sequences and/or structure that have relatively stronger affinity for multivalent ions compared to canonical B-form DNA considered here may also show unexpected, or even counterintuitive response. For example, it is known that under low hydration [44,45], as well as with increasing concentrations of CoHex [46,47], DNA can spontaneously transition from the B to A form, which in turn may alter mechanical properties of the double helix, either directly (the A form is expected to be stiffer) or via the mechanism proposed here for the RNA. In reality, the net change in the stiffness maybe a combination of both effects; dehydration may become particularly important in living cells. Given the importance of nucleic acid stiffness in genome packing, these effects may have significant biological consequences. The influence of divalent ions such as Mg^{2+}, known to bind to nucleic acids, is also worth exploring. Perhaps most importantly, the physics of the relationship between mechanical properties of charged polymers and counterion binding is worth revisiting.

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