Persistence Length Changes Dramatically as RNA Folds

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(Dated: November 1, 2013)

We determine the persistence length, \( l_p \), for a bacterial group I ribozyme as a function of concentration of monovalent and divalent cations by fitting the distance distribution functions \( P(r) \) obtained from small angle X-ray scattering intensity data to the asymptotic form of the calculated \( P_{WLC}(r) \) for a worm-like chain (WLC). The \( l_p \) values change dramatically over a narrow range of \( Mg^{2+} \) concentration from \( \sim 21 \) Å in the unfolded state (U) to \( \sim 10 \) Å in the compact (Ic) and native states. Variations in \( l_p \) with increasing \( Na^+ \) concentration are more gradual. In accord with the predictions of polyelectrolyte theory we find \( l_p \propto 1/\kappa^2 \) where \( \kappa \) is the inverse Debye-screening length.

Elucidating the mechanisms by which RNA molecules self-assemble to form three dimensional structures is a challenging problem. Because the native state (N) cannot form without significantly neutralizing the negative charge on the phosphate group, RNA folding is sensitive to the valence, size, and shape of the counterions. At low counterion concentrations (C) RNA is unfolded (U) in the sense that it contains isolated stretches of base-paired stem loops that have large dynamical fluctuations. When \( C > C_m \), the midpoint of the transition from U to the N, RNA becomes compact as a result of formation of tertiary contacts. For many RNA molecules, such as the Tetrahymena ribozyme and RNase P, folding to the native state is preceded by the formation of multiple metastable kinetic intermediates (I).

The large dynamic conformational fluctuations in the U and I states make it difficult to characterize their structures. However, small angle scattering experiments can be used to determine the shape of RNA as it folds. The conformation of RNA in the U, N, and the I states is characterized by \( R_g \), the radius of gyration, and \( l_p \), the persistence length. Small Angle X-ray Scattering (SAXS) and Small Angle Neutron Scattering (SANS) experiments have been used to obtain \( R_g \) as a function of counterions for a number of RNA molecules. In contrast, \( l_p \), which is a function of \( C \) and valence and shape of counterions, is more difficult to obtain.

In this letter, we use SAXS data and theoretical results for the worm-like chain (WLC) to obtain \( l_p \) for a 195 nucleotide group I ribozyme from pre-trNA(Ile) of the Azorangus bacterium as a function of \( C \) for monovalent and divalent counterions. The major conclusions of the present study are: (i) The experimentally determined distance distribution functions \( P(r) \) can be accurately fit using the theoretical results for worm-like chains for \( r/R_g > 1 \) where \( R_g \) is the radius of gyration of RNA. The \( l_p \) values, which were calculated by fitting \( P(r) \) to \( P_{WLC}(r) \) for \( r > R_g \), change dramatically from \( l_p \approx 21 \) Å in the U state to \( l_p \approx 10 \) Å in the compact conformation. (ii) The large reduction in \( l_p \) occurs abruptly over a narrow concentration range in \( Mg^{2+} \) whereas the decrease of \( l_p \) in \( Na^+ \) is gradual. This result suggests that the compaction of RNA resembles a first order transition in the presence of multivalent counterions. (iii) For both \( Na^+ \) and \( Mg^{2+} \), the persistence length scales as \( l_p \approx l_D^2 \) where \( l_D \) is the Debye-screening length. From this finding, which is in accord with the predictions of polyelectrolyte theory, we find that the intrinsic persistence length of RNA is \( l_p^0 \approx 10 \) Å.

The Azorangus ribozyme was transcribed in vitro as described previously. We carried out SAXS measurements at Argonne National Lab Advanced Photon Source (BIOCAT) beamline using 1.05 Å X-rays that corresponds to 11.8keV in energy. A sample to detector distance of 1.89m allowed us to probe momentum transfer (Q) in the range from \( \sim 0.007 \) to \( \sim 0.266 \) Å\(^{-1}\). A quartz capillary flow cell was used to minimize the radiation damage due to X-ray exposure of a given RNA chain. The measurements at various flow rates showed that X-ray radiation damage is negligible. Each measurement was averaged from four separate exposures of two seconds each. The SAXS profiles were corrected for the background signal which was
cal expression has been derived \[16\] for the end-to-end force-extension curves can be fit using a worm-like chain (WLC) model. The square of the radius of gyration is given by
\[ \frac{g(R)}{P(r)} = \frac{g(R) \, P(r)}{g(R) \, P(r)} \]
where \( x = \frac{l_p}{R_g} \).

At all concentrations of Na\(^+\) and Mg\(^{2+}\), Eq. (3) fits the data extremely accurately as long as \( r/R_g > 1 \) (Fig. (2)). The excellent fits in Fig. (2) allows us to determine \( l_p \) as a function of the counterion concentration. When RNA is unfolded at low Na\(^+\) or Mg\(^{2+}\) concentration, \( l_p \approx 21 \AA \) with \( R_g \approx 65 \AA \). As the concentration of Na\(^+\) increases from about (20 - 200) mM, \( l_p \) gradually decreases. There is a sharp decrease in \( R_g \) when [Na\(^+\)] \( \approx 250 \) mM that is accompanied by a large reduction in the persistence length to \( l_p \approx 10 \AA \). The changes in \( l_p \) are even more dramatic in Mg\(^{2+}\) (Fig. (2B)). As Mg\(^{2+}\) increases from 0.01 mM to 0.26 mM the persistence length changes only by about 3 Å from \( l_p \approx 21 \AA \) (0.01 mM) to \( l_p \approx 18.3 \AA \) (0.26 mM). In this concentration range \( R_g \) decreases from 65 Å to 60 Å. A further increase in Mg\(^{2+}\) to 4.26 mM leads to a reduction in \( R_g \) to about 31 Å with a dramatic decrease in \( l_p \) to about 10 Å. The near discontinuous change in \( R_g \) in Mg\(^{2+}\) (Fig. (1B)) suggests a first-order coil-globule transition in Mg\(^{2+}\).

While less common in neutral homopolymers, a discontinuous coil-globule transition has been predicted to occur in strongly charged polyelectrolytes \[17\].

To complement the experimental studies we calculated the \( P(r) \) functions for the native three dimensional structure of Azoarcus ribozyme using the coordinates from X-ray crystallography crystal structure \[13\] (PDB id: 1U6B) and a model based on sequence comparison \[13\]. The computations were done using the coordinates of only the heavy atoms (C, O, P, and N). To compare the results obtained from crystal structures and SAXS data, we used only the heavy atom coordinates for chain B (excluding nucleotides 1 and 197) from 1U6B structure to compute \( P(r) \). Similarly, the exon fragments were excluded from the Westhof model.

The \( P(r) \) function from the SAXS data for the N state and those obtained using the X-ray structure and the Westhof model are in good agreement with each other and the SAXS data(Fig. 3A). The radii of gyration for the native state calculated using \( \langle R_g^N \rangle^2 = \frac{1}{2N} \sum_i \sum_j \langle r_i - r_j \rangle^2 \) for the X-ray structure and the Westhof model are 31.1 Å and 30.7 Å respectively. These values agree well with the results from the SAXS data (\( R_g^N = 30.9 \AA \)). The \( l_p \) for the native state obtained by fitting the crystal structure \( P(r) \) to Eq. (3) is 11 Å, while for the Westhof model we obtain \( l_p \approx 10.8 \AA \). The good agreement between the crystal
From the linear fits of experimental data confirm the predictions of polyelectrolyte persistence length and the electrostatic contribution is unribosome undergoes the Na\textsuperscript{+} is intrinsically stiff. Surprisingly, over the range of concentrations in which the Na\textsuperscript{+} and Mg\textsuperscript{2+} it has been shown that both Na\textsuperscript{+} and Mg\textsuperscript{2+} (Fig. (3B)). For both flexible and stiff polyelectrolytes \cite{21, 22} it has been shown that \( l_p = l_p^I + l_p^\rho \), where \( l_p^I \) is the intrinsic persistence length and the electrostatic contribution is \( l_p^\rho \propto 1/\kappa^2 \). Deviation from the OSF predictions can occur for finite-sized flexible polyelectrolytes. However, we do not expect such deviations because RNA is intrinsically stiff. Surprisingly, over the range of Na\textsuperscript{+} and Mg\textsuperscript{2+} concentrations in which the Azoarcus ribozyme undergoes the U \( \rightarrow \) I\textsubscript{C} transition, the experimental data confirm the predictions of polyelectrolyte theory. From the linear fits of \( l_p \) to \( \kappa^{-2} \) (Fig. (3B)) we obtain \( l_p \propto 10 \text{ Å} \) which is similar to those found for single stranded DNA \cite{23, 24}.

To assess if \( P(r) \) for WLC can be used to fit scattering measurements on other RNA molecules we used Eq. (3) and SAXS data for RNase P \cite{9} as a function of Mg\textsuperscript{2+} concentration. Unlike the Azoarcus ribozyme, folding of RNase P is best described using three states, namely, U, an intermediate I, and the native state, N \cite{3}. The I state is populated in the Mg\textsuperscript{2+} range 0.02 < Mg\textsuperscript{2+} < 0.2. From the accurate fit of the SAXS data using Eq. (3) for \( r/R_g > 1 \), the \( l_p \) values are found to be 24.5 Å, 14.1 Å, and 11.6 Å in the U, I, and N states respectively. The largest decrease in \( l_p \) and the associated \( R_g \) occurs in the U \( \rightleftharpoons \) I transition, which is consistent with the notion that the early event in RNA collapse is initiated by counterion condensation \cite{3}.
The present work shows that the size and flexibility of RNA molecules as a function of counterion concentration can be obtained using scattering experiments and the WLC model. Given that RNA is a highly branched and charged polymer, it is surprising that the distance distribution functions can be described using elasticity-based polymer models for the distance distribution functions can be described using elasticity-based polymer models for $r/R_g > 1$. Although the structural basis for such a behavior is not obvious, the demonstration that single stranded DNA, double stranded DNA, and polypeptide chains also behave like WLC suggests that for compatible interactions between biomolecules the local flexibility should be similar.

We are grateful to T. R. Sosnick for providing the $P(r)$ data for RNase P in tabular form. This work was supported in part by a grant from the National Science Foundation to DT (grant number 05-14056) and the National Institutes of Health to SAW.

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