Folding Pathways of RNA by Cation Condensation

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Introduction

RNA molecules, much like proteins, have to fold into a 3-D compact state in order to function. Proteins collapse into this dense form as a result of the hydrophobic interactions in their cores in the presence of water. RNAs, on the other hand, which are highly charged polyanions, need to neutralize their charges with cations. The charge condensation screens the repulsion between the negatively charged phosphate groups, allowing the unfolded (U) RNA to collapse. This compact state, however, does not necessarily imply a folded and functional RNA. Many monovalent and multivalent cations such as Na^+, $Ca2^+$, and Co^{3+} are capable of compacting the RNA into a non-native, meta-stable intermediate state (I_C). Mg^{2+} is exclusively required to put the RNA in the functional native form (N).

A deep physical understanding of the U, I_C, and N states in solution will provide fundamental insight into the folding process of RNA molecules. Comprehending and controlling RNA folding will have an impact in areas such as gene therapy or inhibition of viral replication.

In this series of experiments, we studied the size and shape of the *Azoarcus* group I ribozyme in the presence of different counter ions. This allowed us to locate the collapse transitions from U to I_C states in different salts and to compare the sizes of the collapsed and extended states under different conditions. We are planning to compare our results to models of polyelectrolytes.

Methods and Materials

The 195 nucleotide *Azoarcus* ribozyme [1] was transcribed *in vitro*, as previously described [2]. RNA was diluted to 0.4 mg/mL in 25 mM Na-Hepes (pH 7.0). This solution was titrated with solutions of Na^+, Mg^{2+}, Ca^{2+}, and Co^{3+} from zero to different final concentrations. The titration solutions contained 0.4 mg/mL RNA to maintain the sample at the same concentration.

Small-angle x-ray scattering (SAXS) experiments were carried out at the SAXS instrument on BESSRC beamline 12-ID at the APS [3]. X-rays with a wavelength of 1.03 Å were used. Sample-to-detector distances were 2 m and 1 m in order to have access to a momentum transfer range from ~0.07 to ~0.4 Å-1. To prevent possible radiation damage on the samples, we used a quartz capillary continuous-flow cell with a wall thickness of 0.01 to 0.02 Å. Solutions without RNA were also measured in the same experimental configuration to account for the background signal.

Results

In the low q region where qR_g is ≤~1, the scattering can be approximated by the following expression:

$$I(q) \cong I(0)\exp\left(-\frac{R_g^2 q^2}{3}\right). \qquad (1)$$

This is the so-called Guinier approximation [4]. One can determine the R_g of the RNA particles in different salt conditions by linear fits to ln(I) versus q^2. The linear fits to RNA data in several Mg^{2+} concentrations are shown in Fig. 1. Our fitting range was limited from the high q with the validity of the Guinier approximation and from the low q with the experimental restrictions.

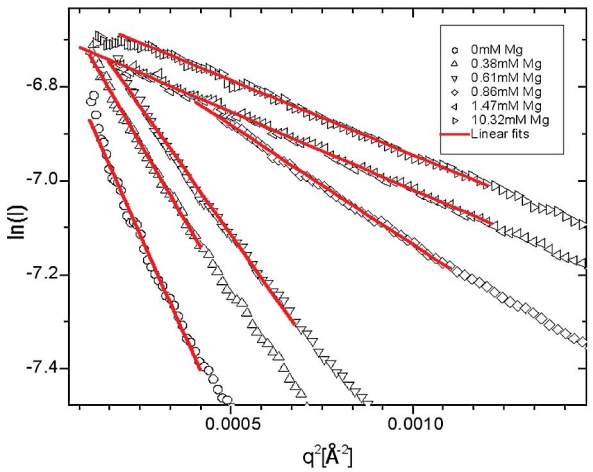

FIG. 1. ln(I) plotted against q^2 for Guinier fits.

R_g can then be plotted against the salt concentrations to locate the collapse transition of RNA in different cations. R_g, however, only gives clues about the relative dimensions of the molecule studied, leaving out the details on shape and conformation. SAXS data can be used to assess this type of information by an indirect inversion of the data from the inverse space to the real space. This can be done by using the following equation:

$$P(r) \cong \frac{1}{2\pi^2}\int_{Q_{\min}}^{Q_{\max}} I(Q)Qr\sin(Qr)dQ \qquad (2)$$

P(r) in the above equation is the probability of the distance distribution function within a particle. This distance distribution gives an insight into the shape of the RNA. *P(r)* will be calculated by using an indirect inversion algorithm, GNOM, available online at http://www.embl-hamburg.de/ExternalInfo/Research/Sax/ [5].

As can be seen from Eq. (2), the larger the limits of the integration over *Q* are, the more accurate the *P(r)* that is calculated will be. Therefore, for a few salt concentrations, experiments were set up so that a larger momentum transfer range could be accessed. This was done by merging two sets of data measured at different sample-to-detector distances. Data measured at two different instrumental setups and glued together are shown in Fig. 2.

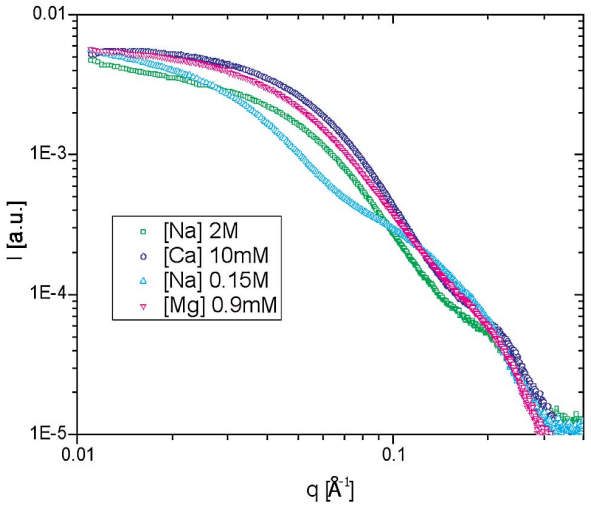

*FIG. 2. SAXS spectra of **Azoarcus** ribozyme at selected salt conditions measured at a large momentum transfer range.*

Another advantage of *P(r)* is that one can compare the RNA structure to standard physical models for single polymer chains (random walk, freely jointed, Gaussian chain, etc.) in order to have a physical picture of different conformations of the RNA chain and in order to see whether it can be interpreted in terms of these simplistic models. This analysis remains to be done.

Discussion

SAXS spectra of *Azoarcus* group I ribozyme in different salts and salt concentrations were measured. Preliminary analysis demonstrated that SAXS is a viable method for analyzing the size of these biopolymers and changes therein.

The collapse transition between U and IC states can be probed by measuring R_g as function of salt concentration. Titration analysis showed that the collapse transition depends strongly on the valence of the counterions. To have a better insight into the shape of the molecules in U, I_C, and I forms, *P(r)* will be calculated from the SAXS data. *P(r)* gives an initial estimate about the shape and the dimensions of the RNA molecule.

We are planning to compare computer simulations to our SAXS data in order to have a molecular-scale picture of the conformation of the U state.

Acknowledgments

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References

[1] P. Rangan, B. Masquida, E. Westhof, and S.A. Woodson, Proc. Natl. Acad. Sci. U.S.A. **100**, 1574-1579 (2003).

[2] P. Davanloo, A.H. Rosenberg, J.J. Dunn, and F.W. Studier, Proc. Natl. Acad. Sci. U.S.A. **81**, 2035-2039 (1984).

[3] S. Seifert, R.E. Winans, D.M. Tiede, P. Thiyagarajan, J. Appl. Crystallogr. **33**, 782-784 (2000).

[4] L.A. Feigin and D.I. Svergun, Structure Analysis by Small-Angle X-Ray and Neutron Scattering, (Plenum Press, New York, NY, 1987).

[5] A.V. Semenyuk and D.I. Svergun, J. Appl. Crystallogr. **24**, 537-540 (1991).