Published in final edited form as:

J Am Chem Soc. 2018 December 12; 140(49): 16948–16951. doi:10.1021/jacs.8b11111.

Why does RNA collapse? The importance of water in a simulation study of helix-junction-helix systems

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Abstract

Using computer simulations we consider the balance of thermodynamic forces that collapse RNA. A model helix-junction-helix (HJH) construct is used to investigate the transition from an extended to a collapsed conformation. Conventional Molecular Dynamics and Milestoning simulations are used to study the free energy profile of the process for two ion concentrations. We illustrate that HJH folds to a collapsed state with two types of counter ions (Mg^{2+} and K^+). By dissecting the free energy landscape into energetic and entropic contributions we illustrate that the electrostatic forces between the RNA and the mobile ions do not drive the RNA to a collapsed state. Instead, entropy gains from water expulsion near the neighborhood of the RNA provide the stabilization free energy that tilt HJH into more compact structures. Further simulations of a three-helix hammerhead ribozyme show a similar behavior and support the idea of collapse due to increased gain in water entropy.

Graphical Abstract

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Supporting Information includes discussion about the entropy calculations, Milestoning, and simulation setups for HJH and HR systems. We examine the level of compactness of the HJH system and compare to the compactness determined from FRET experiments.¹⁶ We also include the calculation of the total energy with different distance cutoff schemes and give the CC theory parameters for the HJH system. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest

The importance of RNA inside biological systems cannot be stressed enough and interest in understanding both the structure and the function continue to rise. Catalyzing reactions, conveying genetic information, and regulating gene expression are just some of the impactful roles that this biomolecule performs.¹ These functions depend on the tertiary structure of the RNA and motivate further investigation.²⁻⁶ The RNA molecule itself is a polyelectrolyte where each monomer carries a negatively charged phosphate group. Electrostatic interactions play a significant role in determining feasible structures. The tertiary structure of RNA in aqueous solution is influenced by the concentration, charge, and activity of the counterions.⁷⁻⁹ Counterions like magnesium can bind at specific sites of RNA molecules and have been shown to help stabilize certain conformations.^{4,10,11} However, the systems studied in the present manuscript bind ions only diffusively.^{12,13}

The overall structure of RNA is hierarchical. Secondary structure elements like double-stranded A-form helices form early and are often linked by single-stranded junctions. The existence of helices can be predicted using bioinformatic and biophysics tools.^{5,14} The interplay between junction conformations and helix orientations have been the subject of numerous experimental, computational, and bioinformatic approaches.¹⁵⁻²⁰

The present manuscript is concerned with the physical forces that drive the RNA helices to collapse and to form a compact helix-junction-helix. We study two 12 bp double-stranded helices connected by a short 5 base junction called HJH with sequence 5'-CCCUAUACUCCCUUUUCCUCCUAAUCGC-3' that was investigated by FRET experiments.¹⁶ The two helices are relatively rigid and their orientation is constrained by the linker (junction) between the two helices. We also illustrate that our main conclusions for the HJH system hold in a more complex molecule, a hammerhead ribozyme (HR) (PDB 359D²¹). The ribozyme consists of two oligonucleotide chains that form a three-helix system and thermodynamic measurements are available for homologous systems.²²

We conducted simulations of HJH collapse at two ionic solutions: 60 mM KCl and 30 mM MgCl₂. Magnesium is known to be an efficient screener of coulombic interactions and has the

ability to locally bind in specific sites.²³⁻²⁵ As we illustrate below, even with these concentrations of ions we cannot qualitatively explain the HJH collapse using only counterions.

We show in Figure 1 the free energy profile of HJH as a function of the radius of gyration (R_g). Small R_g corresponds to a collapsed state (top left structure in Fig. 1) and larger R_g to an extended conformation. We use Milestoning^{26,27} to compute the free energy profile. The explicit solvent simulations were run with the program NAMD.²⁸ In brief (more details are in SI), we initiate an ensemble of short trajectories with initial conditions sampled from the canonical distribution at different discretized R_g positions (milestones). A trajectory that reaches a new milestone is terminated and its final structure and arrival time, t_i , are recorded. We estimate the probability, p_{ij} , that the trajectory will transition from milestone i to a nearby milestone $j \pm 1$. Let $\mathbf{q}^i$ be the eigenvector with eigenvalue 1 of the matrix $\mathbf{p}$. The free energy F_i is given by $F_i = -kT \log(q_i t_i)$.²⁹

The minimum in the free energy profile is a collapsed stable state. To search for driving forces, we decompose the free energy as a function of R_g into a sum of internal energy and entropic contributions: $F(R_g) = U(R_g) - TS(R_g)$.

First, we consider the internal energies. The empirical energies of the final terminated structures are analyzed with the CHARMM 36 force field.³¹ In Fig. 2 we examine the Lennard Jones (LJ) and the electrostatic energies of the entire system averaged over the set of structures from the Milestoning trajectories as a function of R_g . Even with the contribution of the counterions the electrostatic energy profile (Fig 2b) is monotonically decreasing as the R_g increases. Hence, the repulsion between the helices charges leads to a preference of the extended state. The counterions reduce the energy of the collapsed state relative to the extended state. However, there remains enough electrostatic repulsion between similar charges to destabilize the collapsed state.

In contrast to the electrostatic energy, the LJ interactions support the collapse state (Fig. 2a). However, their absolute magnitude is significantly smaller than the electrostatic repulsion. Once the two terms are added up, the total non-bonded energy disfavors the collapsed state (Fig. 2C). As the entire system is considered, the energy scale is in thousands of kcal/mol. The subtraction and addition of large numbers are subject to considerable numerical errors. However, the trends we mentioned remain even when the calculations are conducted with different distance cutoff schemes (see SI).

CCT (Counterion Condensation Theory)¹² showed that the interactions of linear polyelectrolytes and mobile ions is a competition between electrostatic attraction between opposing charges and entropic changes of the mobile ions upon binding. We consider ion condensation by examining the net charge of the system (ions and HJH) as a function of the distance from the RNA (Fig. 3). As the radius of gyration decreases, the number of ions trapped near the collapsed RNA structure increases. This is a consequence of the higher charge density of the collapsed RNA molecule.

Figure 3 suggests that the entropy of the ions at the collapsed state is lower than the extended state. Hence, the mobile ion entropy prefers the extended state and resists collapse.

To examine the entropic contribution in more detail we have used correlation functions to compute solvent and solute entropies as outlined in Laird et al.³² following the density expansion by Kirkwood³³ and entropy analysis by Green.³⁴ We approximate the entropy by three leading terms: $S \cong S_0 + S_1 + S_2$ where S_0 is the direct contribution of the density, S_1 is the entropy of the pair correlation function and S_2 of the triple correlation function. More details about formulas and the calculations are provided in the SI.

The entropies of the ions are inaccurate using the Green's expansion due to the long-range interactions. However, we illustrate below that the ions' contribution to the total entropy is small and negative (hence changes in ion entropy resist collapse). The entropy changes associated with the water molecules are significantly larger than the entropy changes of the counterions. We therefore believe that our approximate entropy calculations for the aqueous solution do not affect the conclusions of this manuscript.

Fig. 4 makes two important observations. The first is that the water entropy increases as the R_g decreases and therefore supports the formation of a compact structure. The second is the large magnitude of the free energy change associated with the water entropy. The entropy gain upon HJH collapse is much larger than the entropy loss due to capturing more counterions at the RNA. The TS term is comparable in magnitude to the repulsive energy estimated for the non-bonded interactions (Fig. 2).

To further explore the determinants of the collapse process in another RNA molecule we investigate a hammerhead ribozyme system.²¹ We simulated two RNA configurations, one restrained to a low R_g (a collapsed state) and the other to high R_g value (an extended state), while retaining the overall secondary structure. The details of the simulations are in the SI. We report the total internal energy (Electrostatic and Lennard Jones terms) and the entropy of water molecules and ions computed from the pair correlation functions (Table 1). The internal energy changes favor the extended state, while the water entropy is more favorable at the collapsed state. This is the same qualitative result that we obtained for the HJH system and agrees with what was observed experimentally.²²

What is the molecular origin of the entropy changes shown in Fig. 4 and Table 1? The collapsed state of RNA has a smaller water-exposed surface area compared to the extended state and it therefore binds a smaller number of water molecules. A water molecule is assumed to bind diffusely to the RNA if its oxygen is within 7.5Å distance from any RNA atom. 7.5Å is the calculated CCT radius (shown in SI). The average number of water molecules bound to the extended state is estimated as ~7160 for $MgCl_2$ and ~7090 for KCl. In contrast, the number of water molecules bound to the collapsed state is only 6470 and 6500 for $MgCl_2$ and KCl respectively. The release of ~10% of the bound water molecules to the bulk solution leads to a significant entropy gain, which according to our analysis is the main force supporting the collapse of the HJH and HR molecules.

Site-specific magnesium binding is a significant factor in the selection of a dominant structure.³⁶ However, for the model systems we considered there is no tight binding of magnesium and the RNA still collapses. A concern is the quality of the force field.³⁷ It has been shown though that ionic solutions near the polyelectrolyte, when the secondary

structure of RNA remains unchanged are described well with existing force fields.³⁸ In both HJH and HR the secondary structure remains unchanged and we only examine tertiary structure changes.

This gain of structure upon transfer of water molecules at the interface to the bulk is similar to hydrophobic interactions. Apolar interfaces during protein folding order water molecules and cause entropy loss compared to aqueous solution.³⁹ Here, the high charges of the RNA molecule order the nearby water molecules and upon collapse release them into the bulk solution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This research was supported by grants from the NIH GM059796 and GMGM085062. It was also supported by a grant from the Welch foundation F-1896.

ABBREVIATIONS

R_g	Radius of Gyration
HJH	Helix Junction Helix
HR	hammerhead ribozyme
CCT	Counterion Condensation Theory

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Figure 1.

Free Energy of HJH as a function of R_g . Error bars were calculated according to ref ³⁰. The images on the lower right and upper left corners illustrate an extended and a collapsed structure respectively. The impact of ionic concentrations on the free energy are discussed in the SI.

Figure 2.

The non-bonded energy of the entire system (electrostatic and Lennard Jones energies) as a function of the radius of gyration. (a) LJ energy. (b) Electrostatic energy. (c) total non-bonded energy.

Figure 3.

The net charge of the polyelectrolyte-counter-ion system as a function of the distance from any heavy atom of the RNA molecule and R_g . The left and right panels are for (a) Mg^{2+} 30mM, and (b) K^+ 60mM respectively.

Figure 4.

The entropy of the RNA system in two ionic solutions as a function of R_g . Panels (a) and (b) show the water and ion entropies respectively. The changes in the water entropy upon RNA collapse are two orders of magnitude larger than the entropy changes of the ions. The total entropy of the solution (panel (c)) decreases as a function of the radius of gyration.

Table 1.

Energy and entropy differences for the extended and the collapsed states of HR at 30mM MgCl₂ solution.

Results compare qualitatively with a similar ribozyme examined by Mikulecky et al.²² in which the collapsed state is energetically unfavorable, but entropically favorable. The error bars are large. However, the averages follow the same trends observed for HJH.

Energy (kcal/mole)	Collapsed-extended
Lennard Jones	-28.67 ±25.63
Electrostatic	37.2 ±41.8
Total non-bonded energy	8.1 ±28.36
Entropy (kcal/mole/K)	Collapsed-extended
Water-water correlations	0.46 ±0.18
Ion – ion correlations	-0.010 ±0.002