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Role of Water and Ions on the Dynamical Transition of RNA

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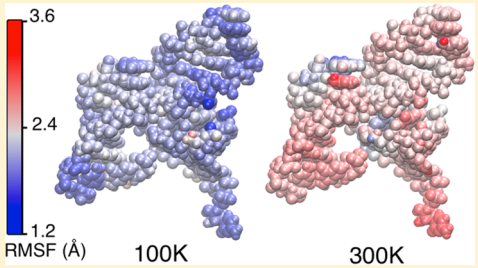
Supporting Information

ABSTRACT: We have performed molecular dynamics simulations of hydrated polycrystalline powder hammerhead ribozyme at various temperatures from 50 to 300 K to determine the role of water and ions on the dynamical transition of RNA. Calculated mean-square displacement as a function of temperature is in agreement with existing neutron scattering experiments. Using this model, we were able to investigate the role of water and monovalent ion structure and dynamics on the appearance of anharmonic motions in RNA as a function of temperature. Compared to experimental and simulation results of proteins under similar environmental conditions, the amplitude of motions in RNA are smaller. While the structure and dynamics of monovalent ions interacting with RNA infer a stronger coupling than that of water, it is found that the relaxation of water from the surface of RNA is sufficient for the increase in anharmonic motions above the dynamical transition temperature. The nature and temperature dependence of fast and slow hydrogen bond dynamics between proteins and RNA were found to be similar, thus indicating that the dynamical transition of RNA and proteins are governed by relaxation of surface hydration water.



SECTION: Biophysical Chemistry and Biomolecules

The dynamics of macromolecules play a critical role in various biological processes such as ligand recognition and binding, enzymatic catalysis, and folding pathways.[1] The nature of the dynamics of proteins, DNA, and RNA changes with temperature in a manner that is similar to that observed for molecular glasses. The change of dynamics from low-temperature harmonic motions to large-scale anharmonic motions has been called the dynamical transition[2] (DT). The observed increase of anharmonic dynamics above the dynamical transition temperature, T_d, has been shown to be due to the presence of hydration water by experimental and computational studies.[3−7] Recently, the temperature and hydration dependence of the dynamics of nucleic acids has been the focus of several experimental studies of RNA[8−10] and DNA[11] systems. It was proposed that water acts as a "lubricant" in facilitating enhanced motion in solvated RNA molecules,[9] and recent research suggested that hydration and electrostatic environment contribute collectively to the local dynamics of the RNA molecules.[12] However, the molecular mechanism and the relative importance of hydration and electrostatic interactions in RNA dynamics remain unclear. In this work, we present seminal molecular simulations of a hydrated polycrystalline powder of RNA in order to extract atomistic determinants of the temperature dependence of the underlying dynamics. Realistic structural models and environments are essential starting points for dynamical studies in the condensed phase as shown by Tarek and Tobias.[13] Incoherent neutron scattering has proven to be a useful method to validate simulation methodologies in liquid, amorphous, and crystalline environments.[13−16] MD simulations and incoherent neutron scattering can access very similar time (∼ns) and length (<100 Å) scales. Macromolecular dynamics obtained experimentally or via computer simulation are required to understand interactions, stability, and the function of these complex systems. This has allowed for the study of protein glassy dynamics[15,17−21] and structural relaxation[22] in a variety of environments.

The initial coordinates for hammerhead RNA that were used to construct the hydrated polycrystalline RNA powder system presented in this study were taken from the Protein Data Bank (299D).[23] The snapshot of the periodic cell of the final system is shown in Figure 1. Simulations were performed with periodic boundary conditions using the CHARMM-27 protein-nucleic acid force-field[24] and the TIP3P water model[25] using the molecular dynamics program NAMD.[26] A control computation based on the most recent CHARMM-36 force field[27] yielded identical results. Briefly, to build the system, a single hammerhead molecule was placed into a pre-equilibrated box of water and overlapping water molecules were removed. The system was energy minimized and a neutralizing number of sodium ions were added at positions in the periodic cell at minimum values of the calculated electrostatic potential,[28] with the concurrent removal of overlapping water molecules. The

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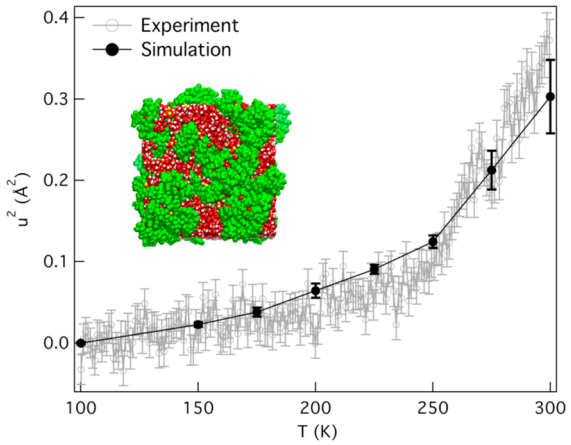

Figure 1. Mean-square displacement of nonexchangeable hydrogen atoms calculated from simulation of hammerhead RNA and experimental neutron measurements of torula yeast t-RNA.[10] Experimental error bars represent ±1 standard deviation. Inset: snapshot of periodic box of hammerhead RNA system.

system was energy minimized and then equilibrated for 1 ns. This single hammerhead system was duplicated to obtain 15 RNA/water/ion systems, and each was placed on a separate lattice point for a face centered cubic unit cell (a = 120 Å). The number of waters was reduced at this stage such that the corresponding hydration level of the final system was 0.55 g water/g RNA. Each hammerhead molecule and surrounding water and ions was then rotated by a random angle about a random principle axis. The final system, comprising 19935 RNA atoms, 615 sodium ions, and 6079 water molecules, was then simulated in the isothermal−isobaric ensemble at 300 K and 101325 Pa to reduce the volume of the system to the equilibrium density. The system was then equilibrated for 5 ns at a series of lower temperature configurations (250 K, 225 K, 200 K, 175 K, 150 K, 100 K, 50 K). After this final equilibration, each system was simulated for an additional 50 ns at its given temperature.

We validated our methodology by comparing the mean-square displacement, $\langle u^2 \rangle$, obtained from simulation to that obtained from incoherent neutron scattering measurements of tRNA at the same hydration level.[10] $\langle u^2 \rangle$ was calculated as previously reported.[13] Briefly, the incoherent intermediate scattering function, $I(\mathbf{Q},t)$, was calculated for nonexchangeable RNA hydrogen atoms over the course of the trajectories (where $\mathbf{Q}$ is momentum transfer). Calculations were averaged over 32 random $\mathbf{Q}$ vectors in the range $0.6 \leq |\mathbf{Q}| \leq 1.6$ Å^{-1} using multiple time origins.[29] The incoherent dynamic structure factor $S(Q, \omega = 2\pi/\text{period})$ was calculated from the Fourier transform of $I(Q,t) \times R(t)$, where $R(t)$ is the experimental resolution function (0.8 μeV), and applying the Debye−Waller factor.[13,14] Finally, $\langle u^2 \rangle$ was determined from the slope of plotting $\ln(S(Q,0))$ versus $|\mathbf{Q}|^2$, with $\langle u^2 \rangle = -3\, d(\ln(S(Q,0)))/d(|\mathbf{Q}|^2)$. Representative plots are shown in Figure S1. The agreement with experiment is satisfactory at all temperatures and within the relative statistical uncertainty of both experiment and simulation as shown in Figure 1. The apparent T_d from MD, noted by the inflection of $\langle u^2 \rangle$, agrees with the experimental result.

Structural and dynamical properties of water and sodium ions were studied to characterize the role of each on the temperature-dependent dynamics of RNA. In Figure 2a, we

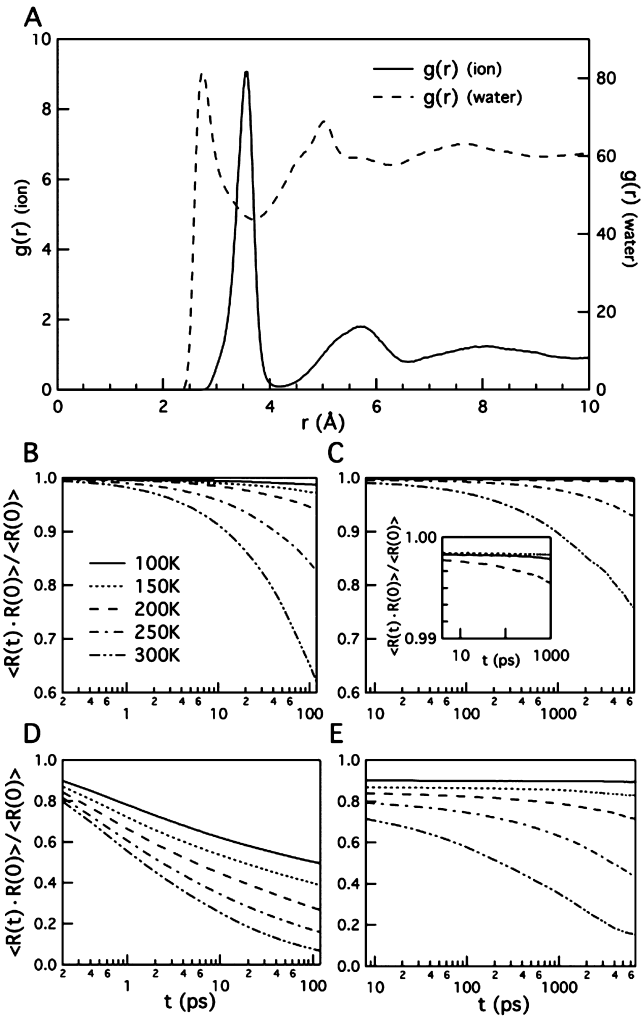

Figure 2. Water and sodium ion structure and dynamics. (A) Water O-RNA and Na^+-RNA:$PO4^-$ pair distribution functions at 300 K. Residence time correlation functions: (B) "fast" Na^+-RNA:$PO4^-$, (C) "network" Na^+-RNA:$PO4^-$, (D) "fast" water O-RNA, and (E) "network" water O-RNA. Legend for B−E is shown in panel B. The inset in C is provided for clarification.

present the structure of water and sodium ions from the RNA surface via pair distribution functions. These were calculated for water oxygen atoms and hydrogen bond donors and acceptors on the RNA and for sodium ions to backbone phosphate atoms on RNA, respectively. We find that sodium ions have a much larger relative density near RNA than water.

In the following we will describe residence and hydrogen bond correlation functions. Analogous to methods from previous studies,[5] we delineate dynamics via correlation functions of two types: "fast" describes the lifetime of a single occupancy, and therefore occupancy is not counted if the initial criterion is broken, and "network" where occupancy is counted whenever satisfied. Thus the relaxation of the "network" correlation function indicates that the atom/molecule has diffused from the initial site. In Figure 2b−e we characterize the dynamics through residence correlation functions. Residence was calculated for those atoms within the first peak of $g(r)$, which was taken to be r < 4.1 Å for sodium ions and r < 3.7 Å for water oxygen atoms. In terms of both their "fast" and "network" residence, one can see that ions are less mobile at the RNA surface. Characteristic relaxation times, τ, were

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determined by fitting the correlation functions to the form $(1 - A)\ \exp[-(t/\tau)^{\beta}] + A$, where β accounts for multiexponential decay[30] and A is a nonzero offset. This stretched exponential form was used to be consistent with similar analyses reported for ribonuclease A,[5] although other fitting functions to probe protein and RNA dynamics have been used.[31,32] Fitting parameters and sample fits are shown in Tables S1, S2 and Figure S2, and τ values are shown in Figure 4. We find that the sodium ion residence times are significantly longer (10^3–10^4-fold) than that for water oxygen atoms at all temperatures.

We further characterized water dynamics by calculating hydrogen bond correlation functions shown in Figure 3a,b.

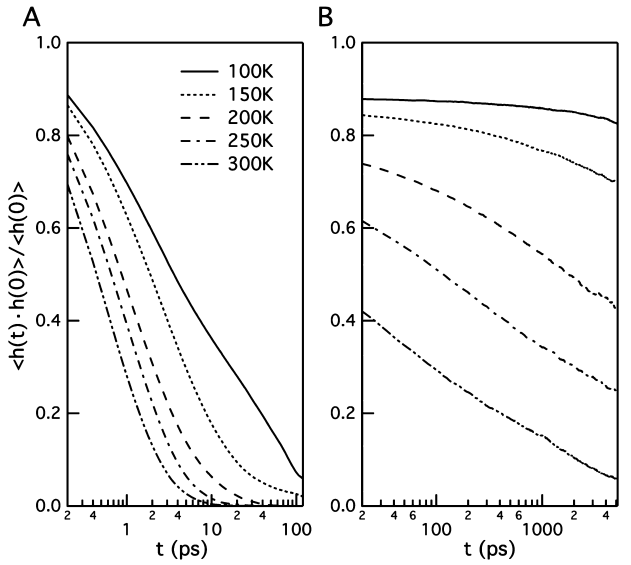

Figure 3. Water hydrogen bond dynamics. (A) "Fast" hydrogen bond time correlation function,; (B) "network" hydrogen bond time correlation function. Legend is shown in panel A.

Characteristic relaxation times are shown in Figure 4 with fitting parameters shown in Table S3, and representative fits are shown in Figure S2. The lifetime of hydrogen bonds is lower than the water oxygen residence and continuous with decreasing temperature analogous to that found for a hydrated protein system.[5] We find that the "network" relaxation of hydrogen bonds is essentially arrested near the T_d of RNA. One should note that τ values are smaller than what would be obtained using longer simulations as apparent in the value of A obtained at lower temperatures (see Table S1–S3). The scalar offset A is larger for the lower temperature systems since the relaxation mechanisms are not well characterized by a τ value in the limited simulation time, thus implying that a larger value of A reflects an inherently longer τ is present in the data. In previous studies of protein hydration water,[5] it was concluded that the network relaxation time appears to diverge at T_d, which we depict as a dashed line at 200 K in Figure 4. Our results using 50-fold longer simulation time indicate that for RNA, the hydrogen bond relaxation times do not diverge at any temperature, yet they become increasingly large with decreasing temperature. The net effect is that structural arrest of "network" water/RNA hydrogen bonds play a larger role than the relaxation of "fast" water/RNA hydrogen bonds at lower temperatures, which has been reported for proteins.[5] Since we find that sodium ions have a higher relative density near the RNA surface and the "network" relaxation of ions is far less

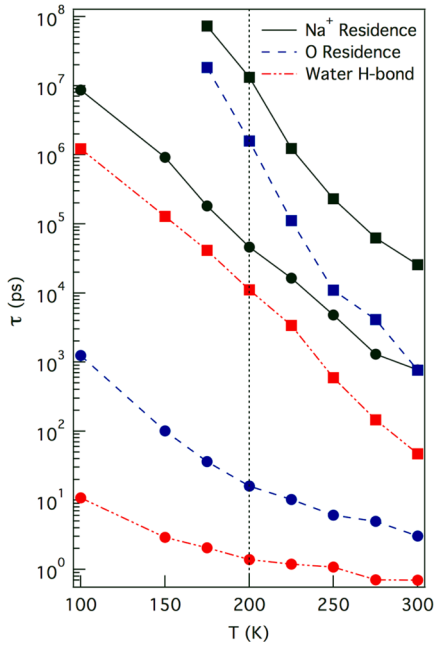

Figure 4. Na^+-RNA:$PO4^-$, water O-RNA residence and water-RNA hydrogen bond relaxation lifetimes. Circles represent "fast" relaxation, and squares represent "network" relaxation.

than that observed for water one could speculate that ions may play an equally essential role in the appearance of anharmonic dynamics with increasing temperature.

To test this hypothesis we carried out an additional series of simulations at 300 K where the water, sodium ions, or water and sodium ions were restrained in harmonic potential (0.6 kJ mole^{-1} Å^{-2}) to restrict the diffusion of the restrained entities and thus artificially affect the associated "network" relaxation. The results of these simulations alongside the original unrestrained simulation are shown in Figure 5. Mean-square displacement (MSD) results of water (Figure 5a) and sodium ions (Figure 5b) indicate that the restraints restricted the diffusion adequately. By comparing Figure 5a and b, it is obvious that restraining sodium ion had little effect on water mobility, whereas restraining water reduced the MSD of sodium ion by a factor of ~5. This is consistent with a previous simulation result that water molecules prefer to cluster around counterions,[9] and it indicates that restraining water molecules lock the sodium ions on the RNA surface.

To judge the effect of the restraints on the relaxation dynamics of RNA, we calculated $I(\mathbf{Q},t)$ for nonexchangeable hydrogen atoms in RNA as shown in Figure 5c. Restraining water molecules had a much greater effect on the relaxation dynamics of RNA than the restriction of the sodium ions, thus indicating that the ability of water to couple to RNA has a greater effect on the underlying RNA dynamics than does that of sodium ions. Previous studies indicated that water acts as a "lubricant" in facilitating the conformational motions of the hydrated RNA.[9] Our work indicates that restraining the water molecules deprived RNA of this "lubricating" ingredient and consequently hindered its conformational motions and anharmonic motions above T_d. However, restraining sodium ions had little effect on the relaxation dynamics of RNA; this indicates that the sodium ion mobility is not a major contributor to the RNA relaxation dynamics, although the presence of counterion was suggested to play a critical role in

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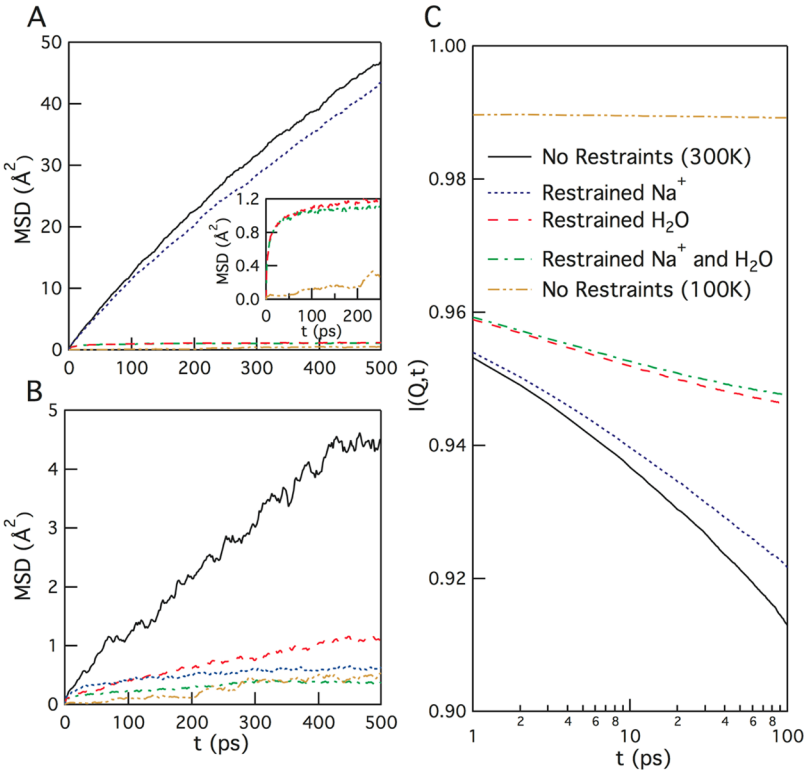

Figure 5. Effect of restrained solvent on RNA dynamics. (A) Mean-square displacement of water. (B) Mean-square displacement of Na^+. (C) Intermediate scattering function of nonexchangeable hydrogen atoms of hammerhead RNA for $|\mathbf{Q}| = 1.0$ Å^{-1}. Unrestrained data are shown for both 100 and 300 K, while restrained data is from simulations at 300 K. Legend is shown in panel C.

stabilizing the structure and altering the electrostatic environment around RNA.[12] Given that the molar concentration of water is much higher than the concentration of counterion the native inherent contribution of each solvent component to the DT of RNA is unclear. Practically, in hydrated powders, the concentration of water will be higher than that of the counterion, thus water has a larger influence on the dynamics of RNA.

While there is a plentitude of structural data for proteins, there are fewer experimentally determined structures of RNA molecules.[33] RNA is a highly charged molecule, and thus it presents particular challenges to the simulation community. Ions are known to affect both the structure[34−36] and dynamics[12] of RNA, and therefore the simulation of such systems requires the use of robust methodologies to represent the electrostatic forces in periodic systems.[37] MD simulation studies of RNA have shown that the simulation of such systems can lead to important atomic insight.[38,39] Our study has shown that one can obtain quantitative agreement of the amplitude of motions between simulation and quasi-elastic neutron scattering experiments. While we find that monovalent ions are structurally and dynamically restrained to the surface of the RNA molecule, it is the diffusion of water away from the surface that is essential for the increased anharmonic motions of RNA with increased temperature. We do not see a structural or dynamical role of monovalent ions that lead to a higher T_d in RNA than that of proteins. While the motions of specific residues in protein and RNA differ due to the inherent potential energy of each residue type, we conclude that the relaxation of surface hydration water is essential for the DT in RNA as has been shown for proteins.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Further details regarding data fitting, simulations, and error analysis are in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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