Salt effect on thermodynamics and kinetics of a single RNA base pair

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ABSTRACT

Due to the polyanionic nature of RNAs, the structural folding of RNAs are sensitive to solution salt conditions, while there is still lack of a deep understanding of the salt effect on the thermodynamics and kinetics of RNAs at a single base-pair level. In this work, the thermodynamic and the kinetic parameters for the base-pair AU closing/opening at different salt concentrations were calculated by 3- μ sec all-atom molecular dynamics (MD) simulations at different temperatures. It was found that for the base-pair formation, the enthalpy change ΔH is nearly independent of salt concentration, while the entropy change ΔS exhibits a linear dependence on the logarithm of salt concentration, verifying the empirical assumption based on thermodynamic experiments. Our analyses revealed that such salt concentration dependence of the entropy change mainly results from the dependence of ion translational entropy change for the base pair closing/opening on salt concentration. Furthermore, the closing rate increases with the increasing of salt concentration, while the opening rate is nearly independent of salt concentration. Additionally, our analyses revealed that the free energy surface for describing the base-pair opening and closing dynamics becomes more rugged with the decrease of salt concentration.

Keywords: RNA base pair; salt effect; thermodynamics; kinetics

INTRODUCTION

RNAs perform critical cellular functions at the level of transcription, translation, and the expression and regulation of genetic information, where the functions are generally mediated through simple Watson–Crick base-pairing (Huynen et al. 1996; Rauscher et al. 1997; Bevilacqua and Blose 2008). Also, the closing and opening of base pairs is the fundamental process in RNA structure folding (Giudice et al. 2003; Sarkar et al. 2010; Nayak et al. 2012; Steinert et al. 2012; Wang et al. 2019a), consequently, a full understanding of the thermodynamics and kinetics of this process is critical to understanding the biological functions of RNAs.

Because RNAs are highly charged polyanionic molecules, the structure and conformational change of RNAs are sensitive to their ionic environments. The electrostatic interactions between metal ions and RNAs make a significant contribution to the thermodynamic stability of RNA secondary structures and their flexibility (Brion and Westhof 1997; Ippolito and Steitz 1998; Tinoco and

Bustamante 1999; Heilman-Miller et al. 2001; Serra et al. 2002; Pyle 2002; Tan and Chen 2006, 2007, 2011; Draper 2008; Qiu et al. 2010, 2011; Kirmizialtin et al. 2012; Lipfert et al. 2014; Denesyuk and Thirumalai 2015; Drozdetski et al. 2016; Nakano et al. 2016; Sun et al. 2017; Fischer et al. 2018; Jin et al. 2018; Kolev et al. 2018; Chen and Pollack 2019). For instance, recent theoretical modeling (Tan and Chen 2006, 2007) on the salt-dependence of nucleic acid helix stability showed that nucleic acid helix stability can depend strongly on ion concentration, ion size and ion valence, and the ion-dependent loop stability made a significant contribution to the overall hairpin stability. Simultaneously, experimental measurements have found that the nucleic acid helix stability is affected by not only the sequence of helix but also apparently ion concentration (SantaLucia 1998; SantaLucia and Hicks 2004; Owczarzy et al. 2008; Chen and Znosko 2013). For example, thermodynamic and single-molecule measurements have both shown that an increase in salt concentration would

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apparently enhance the stability of secondary segments, and a logarithm salt-dependence of the folding free energy/entropy has been empirically derived at relatively high salt concentration (e.g., between ~ 0.1 – 1 M) (SantaLucia 1998; SantaLucia and Hicks 2004). In parallel, the influence of metal ions on the tertiary structure of RNA has been explored extensively (Woodson 2005; Draper et al. 2005; Koculi et al. 2007; Draper 2008; Tan and Chen 2011; Kim and Shapiro 2013; Li 2013; Wang et al. 2015; Wang and Xiao 2016; Shi et al. 2018; Xi et al. 2018; Hori et al. 2019; Jin et al. 2019). For example, Li (2013) studied the role of diffuse metal ions in stabilizing a 2-bp kissing complex by single-molecule techniques and found the significant contribution of diffuse cations to the stability of tertiary interaction.

Metal ions influence not only RNA structure stability but also the rate of conformational rearrangement and folding/unfolding pathways of RNAs (Bokinsky et al. 2003; Woodson 2005; Boerner et al. 2016; Vashishtha et al. 2016; Xu et al. 2016; Raper et al. 2018). For example, Bokinsky et al. (2003) probed the transition state for docking and undocking of the hairpin ribozyme using fluorescence resonance energy transfer (FRET) and found that the rate of docking increased with Mg^{2+} concentration, while the undocking rate remained constant. Recently, Shi et al. (2018) focused on the 3D structures and stability of RNA pseudoknots in monovalent and divalent ion solutions and found that the unfolding pathway of RNA pseudoknots can be significantly modulated by their sequences and solution ion conditions. However, these existing works mainly focused on the thermodynamics or kinetics of global RNA molecules and the salt effects on the closing and opening of single RNA base pair remain unclear.

Based on the extensive experimental data, the thermodynamic parameters of DNA and RNA base pairs at 1 M Na^+ concentration have been derived empirically (SantaLucia 1998; Xia et al. 1998; SantaLucia and Hicks 2004). These parameters form the basis for the predictions of nucleic acid structures, thermodynamics, and folding kinetics (Serra and Turner 1995; Zhang and Chen 2002; SantaLucia and Hicks 2004; Zhao et al. 2010). Moreover, some salt extensions of the thermodynamic parameters of DNAs and RNAs have been derived either by experiments (Blake and Delcourt 1998; SantaLucia 1998; SantaLucia and Hicks 2004; Owczarzy et al. 2004, 2008) or through theoretical modeling (Tan and Chen 2006, 2007). However, the ion-dependent kinetic parameters, such as the opening and closing rates of a single base pair, are still lacking until now. RNA folding kinetics is directly tied to RNA biological functions. The functions of ribozymes (Bartel and Szostak 1993), anti-HIV RNA aptamers (Ellington and Szostak 1990) and gene expression regulators, such as miRNA, siRNA, and riboswitches (Bartel 2004; Nudler and Mironov 2004), are often kinetically controlled. Experiments have demonstrated that helix formation is a zipping process

(Kuznetsov and Ansari 2012), and unraveling the kinetics of a single base pair is crucial to understand the overall kinetics of the full length RNA. Furthermore, the mechanism of how salt ions influence the opening and closing rate of a single base pair remains unclear. Therefore, understanding the salt effect on thermodynamics and kinetics of a single RNA base pair is important for understanding the biological functions of RNAs.

Recently, the thermodynamic and kinetic parameters of the AU base pair at high salt have been derived by 3- μ sec all-atom MD simulations at different temperatures (Wang et al. 2016); and furthermore, the effects of nearest neighbor and next nearest neighbor on the thermodynamic and kinetic properties have been explored (Wang et al. 2018).

In this work, the salt effects on the thermodynamic and kinetic parameters of the AU base pair were examined by our 3- μ sec all-atom MD simulations. Our calculations suggested that for the opening/closing of a base pair, the enthalpy change ΔH is almost unaffected by ion concentration, while the entropy change ΔS is strongly dependent on ion concentration. Our analyses revealed that such salt dependence of entropy change is mainly attributed to the change of ion translational entropy due to base-pair opening/closing. Furthermore, with the increase of ion concentration, the closing rate increases while the opening rate remains nearly invariant. In addition, the free energy surface for the opening and closing dynamics of the base pair was found to become more rugged with the decrease of salt concentration.

RESULTS AND DISCUSSION

Determination of closed state (cs), open state (os), and transition state (ts)

Similarly to our previous analyses (Wang et al. 2016, 2018), the terminal base-pair AU at different NaCl concentration would undergo the two-state closing–opening switch process (see Fig. 1) through a transition state. The corresponding structures can be divided into closed state, open state and transition state according to the time-dependent RMSD (root mean square deviation) of the two terminal nucleotides A and U relative to the initial structure, and the torsional angle ζ , which is the dihedral angle formed by the four atoms $C3'(i)-O3'(i)-P(i+1)-O5'(i+1)$ (Hershkovitz et al. 2003), where i represents the i -th nucleotide in a polynucleotide.

For the closed state, the RMSD is ~ 0.7 Å and ζ is centered in the region around $\sim -75^\circ$ (-50° to -100°), as shown in Figure 2. The corresponding conformations are that the terminal two nucleotides vibrate slightly only around their starting positions, and the interactions of terminal base-pairing and base stacking are not disrupted. As A-RNA helix has a narrow deep groove and higher negative charge density, Na^+ ions are mainly concentrated near the

FIGURE 1. The illustration for the simulation system in the work, where an RNA hairpin is in salt solution. In the simulations, the hairpin (PDB code, 1ZIH) was fixed with strong harmonic constraint except for the two free terminal nucleotides. The *left* and *right* panels show the two typical states of the hairpin with the closed and open terminal base pair: *left*, closed state; *right*, open state. The three-dimensional structures are shown with VMD software (Humphrey et al. 1996), and the brown balls denote monovalent sodium ions.

negatively charged phosphate group and in the deep groove binding to the N7 atom of terminal nucleotide A.

For the open state, the RMSD is larger than 2 Å and ζ is centered in the region around $\sim 50^\circ$ (30° – 100°), as shown in Figure 2. The corresponding conformations are that the two terminal nucleotides flip out into the solvent, and the

interactions of terminal base-pairing and base-stacking are both disrupted, some of the Na^+ ions in the deep groove move out. However, there are still some Na^+ ions binding to the phosphate group.

For the transition state, the RMSD values fluctuate beyond 2 Å with a very short residence time; however, the torsion angle is still in the region of closed state, and the bases are flipped out into the solvent; see Figure 3. It was found that all the transition paths from the closed to open states or from the open to closed state go through the transition state. The actual transition paths from the closed to open state and from the open to

closed state take roughly the same amount of time as the residence time of an *oto* and *ctc* configuration, respectively. According to the location of the transition state that appeared, the transition states (Dokholyan et al. 2000; Zhang et al. 2006) can be divided into *ctc*, which transits from the closed state and subsequently returns to the closed state

FIGURE 2. The RMSD values and torsional angles ζ for the AU base pair at different ion concentrations during the whole simulation times (0–3000 nsec): (A,B) 0.1 M NaCl at 390 K, 400 K, 410 K, and 420 K; (C,D) 0.05 M NaCl at 390 K, 400 K, 410 K, and 420 K.

FIGURE 3. (A) The RMSD values and (B) torsional angles ζ for base-pair AU at 0.1 M NaCl among the simulation times of 1012–1041 nsec at 420 K. (C) The RMSD and torsional angles ζ near the transition states.

(see Fig. 3A,C), and oto, which transits from the open state and subsequently returns to the open state (see Fig. 3B,C). The difference of transition states ctc and oto is nominal; the structures of the two states are similar. But the transition rates from the transition states to the open state and closed state are different, so the lifetimes of the ctc and oto are different. When the conformations are in the transition states, since the twist angle does not flip, some Na^+ ions can still be trapped around the released negatively charged phosphate group. However, the flipped bases of two terminal nucleotides A and U swing constantly in solvent, sometimes individual Na^+ ion binds to the O4 atom of terminal nucleotide U, but the time is relatively short.

The thermodynamic parameters of the terminal AU base pair at different ion concentrations

The population distribution of the closed, open and transition states at a given temperature can be calculated as:

$$\begin{aligned} p_c &= \sum_{i=1}^{N_c} \tau_i^c / \tau, \\ p_o &= \sum_{i=1}^{N_o} \tau_i^o / \tau, \\ p_t &= \sum_{i=1}^{N_t} \tau_i^t / \tau, \end{aligned} \quad (1)$$

where τ is the total simulation time, τ_i^c , τ_i^o , and τ_i^t are the i -th dwelling period of the conformations in the closed, open, and transition states, respectively, and N_c , N_o , and N_t are the total number of snapshots of those conformations in the closed, open and transition states, respectively. As shown in Figure 4A,B, when the simulation time exceeds $\sim 2 \mu\text{sec}$, the occupied probability of the closed state approximately reaches a stable value. Therefore, the simulation times were all set to be 3000 nsec at each temperature to ensure that all the simulations can remain in the

closing-opening two-state equilibrium for enough time. The probabilities of the closed, open and transition states at different temperatures are listed in Table 1.

As the probabilities of the transition states are much smaller than those of the closed and open states, we would first treat the conformations as the (closed and open) two-state model. According to the equilibrium population distribution, the free energy difference ΔG between the two states (closed and open state) for the AU base pair can be calculated through:

$$\Delta G = -k_B T \ln \left(\frac{p_o}{p_c} \right), \quad (2)$$

where k_B is the Boltzmann constant, T is the absolute temperature, and p_o and p_c are the occupied populations of the open and closed states, respectively.

As shown in Figure 4C, the free energy difference ΔG and the temperature T have a linear relationship. According to $\Delta G = \Delta H - T\Delta S$, where ΔH and ΔS are the enthalpy and entropy changes for the transition between the closed and open states, the thermodynamic parameters of the terminal AU base pair are found to be $\Delta H = -7.31 \text{ kcal/mol}$ and $\Delta S = -18.86 \text{ eu}$ at 0.1 M NaCl, and $\Delta H = -7.30 \text{ kcal/mol}$ and $\Delta S = -18.98 \text{ eu}$ at 0.05 M NaCl. At 0.5 M NaCl, the thermodynamic parameters of terminal base-pair AU are $\Delta H = -7.30 \text{ kcal/mol}$ and $\Delta S = -18.50 \text{ eu}$ (Wang et al. 2016). As shown in Figure 4D, it can be seen that the enthalpy changes ΔH of the base-pair AU remain nearly invariant for 0.5, 0.1, and 0.05 M NaCl, and the difference of ΔH for different $[\text{Na}^+]$ is very small ($< \sim 0.01 \text{ kcal/mol}$). However, the entropy changes ΔS are apparently different for 0.5, 0.1, and 0.05 M NaCl, and the entropy change gradually decreases with the increase of salt concentration in a linear logarithm-dependence. It is encouraging that such salt-dependence of enthalpy and entropy changes is in good accordance with the assumption made in the experimental and theoretical salt-extensions of thermodynamic parameters for DNA/RNA base-pair closing/opening in the salt range of [0.05–1 M] (SantaLucia 1998; SantaLucia and Hicks 2004; Tan and Chen 2006, 2007; Owczarzy et al. 2008; Chen and Znosko 2013). In the following, we examine the contribution of ion entropy to the linear logarithm-dependence of entropy change ΔS on salt.

According to the counterion condensation theory (Manning 1978, 2001, 2007; Lin et al. 2019), the ion condensation entropy change for a base-pair closing (i.e.,

FIGURE 4. The occupied population of the closed state versus the simulation time at (A) 0.1 M NaCl, and (B) 0.05 M NaCl. (C) The temperature dependence of $\ln(p_o/p_c)$. (Symbols) Full square for 0.5 M NaCl; full circle for 0.1 M NaCl; full triangle for 0.05 M NaCl; from MD simulations; (lines) from linear fitting. (D) The NaCl concentration dependence of ΔH and ΔS . (Symbols) Full square for ΔH ; full triangle for ΔS ; from MD simulations; (lines) from linear fitting.

open state $\rightarrow$ closed state) can be given by

$$\Delta S_{ion}(\text{Na}^+) = -R \left[N_b^c \ln \left(\frac{C_b^c}{C_0} \right) - N_b^o \ln \left(\frac{C_b^o}{C_0} \right) \right], \quad (3)$$

where R is the gas constant ($R = 1.987$ cal/K/mol), N_b^c and N_b^o are the numbers of Na^+ binding to the RNA when the terminal base-pair AU is in the closed (c) state and open (o) state, respectively. C_b^c and C_b^o are the corresponding concentration of binding ions, respectively. C_0 is the bulk salt concentration.

According to the ion concentration distributions around the phosphorus atoms shown in Figure 5A, we calculated the numbers of binding Na^+ for the RNA in the closed

and open states of the terminal base pair. Our calculations show that the changes in the average number of binding ions due to the formation of the terminal base pair are approximately invariant for 0.5, 0.1, and 0.05 M NaCl. Generally, in thermodynamic measurements and modeling, 1 M Na^+ is used as the standard ionic condition, and thus, the salt-extension of the entropy change due to the formation of the terminal base pair can be approximately expressed as based on Equation 3:

$$\Delta S_{ion}([\text{Na}^+]) = \Delta S_{ion}(1\text{M}) + R(N_b^c - N_b^o) \ln([\text{Na}^+]), \quad (4)$$

where the difference in $[N_b^c \ln(C_b^c) - N_b^o \ln(C_b^o)]$ from 1 M Na^+ is ignored due to the relatively small value (e.g., for

TABLE 1. The average lifetime τ_{ave} (ns), the occupied probability p , and the total number N of occurrences of conformations at closed, open and transition states for base-pair AU at different NaCl concentrations and temperatures from the 3- μ sec simulations

Concentration Na^+ (M)	Temperature T (K)	Closed state (cs)			Open state (os)			Transition state (ctc)			Transition state (oto)		
		τ_{ave}^c	N_c	p_c	τ_{ave}^o	N_o	p_o	τ_{ave}^{ctc}	N_{ctc}	p_{ctc}	τ_{ave}^{oto}	N_{oto}	p_{oto}
0.1	390	26.67	55	0.49	28.39	54	0.51	0.299	314	0.031	0.296	114	0.011
	400	20.46	63	0.43	27.59	62	0.57	0.289	336	0.032	0.290	158	0.015
	410	16.21	70	0.38	27.03	69	0.62	0.285	410	0.038	0.284	188	0.017
	420	12.89	76	0.33	26.58	76	0.67	0.281	357	0.033	0.282	221	0.020
0.05	390	27.01	52	0.47	31.27	51	0.53	0.301	346	0.034	0.299	127	0.012
	400	20.95	59	0.41	30.41	58	0.59	0.291	364	0.035	0.289	176	0.016
	410	16.54	65	0.36	29.61	65	0.64	0.284	459	0.043	0.285	204	0.019
	420	12.94	72	0.31	29.13	71	0.69	0.280	383	0.035	0.282	245	0.023

FIGURE 5. (A) The average concentration of Na⁺ around phosphorus atoms of terminal base pair over the conformations of the closed state (full symbols) and open state (open symbols) for 0.5, 0.1, and 0.05 M NaCl (from top to bottom). Here, $T = 410$ K. (Symbol) Triangle, 0.5 M; circle, 0.1 M; square, 0.05 M. (B) The ion concentration dependence of $\Delta = \Delta S_{Na^+} - \Delta S_{1M}$. (Symbol) Square, from Equation 2; circle, from Equation 4; triangle, from the empirical formula of SantaLucia et al. (SantaLucia 1998).

0.1 M Na⁺, such difference is ~ 0.047 eu, while the second term in Equation 4 is ~ 0.59 eu). We would like to compare the slope of the linear logarithm-dependence in Equation 4 based on the information of ion binding from the MD data, with that in the empirical formula from SantaLucia (1998). As shown in Figure 5B, the slope of the logarithm-dependence in Equation 4 is ~ 0.26 , a close value to that (~ 0.21) from the above thermodynamic calculations. However, such slope of the dependence (~ 0.26) is slightly smaller than that (~ 0.368) from the empirical salt-extension from SantaLucia (1998). Such deviation between our calculations and the empirical formula may be partially attributed to the end effect and the small RNA employed in the MD simulations, because the formation of a base pair at ends or a base pair in a smaller RNA would generally cause a smaller increase in the number of binding ions. Thus, our analyses also suggest that for a base-pair formation, ion translational entropy may make the major contribution to the logarithm-dependence of entropy change on salt concentration.

The formation of a base pair from the open state results in entropy reduction, which comes from the RNA bases' entropy loss due to the conformation restriction for the pairing, the ion's entropy loss due to the binding with the RNA base pair and the solvent's entropy loss due to hydration. As the salt concentration increases, the entropy loss of ion binding and hydration will decrease. Our analyses revealed that such salt concentration dependence of the entropy change mainly results from the dependence of ion translational entropy change for the base-pair closing/opening on salt concentration. As our calculated results are slightly

smaller than that from the empirical salt-extension, it is likely that the hydration effects on the terminal base pair are less than that in the groove. The enthalpy change and the opening barrier, which mainly comes from the stacking interaction and the hydrogen bond between the base pairs, may not be affected by the salt concentration.

The kinetic parameters of the terminal AU base pair at different ion concentrations

The average lifetimes τ_{ave} of the closed, open and transition states

were calculated through $\tau_{ave} = \sum_{i=1}^N \tau_i / N$, where N is the total number of times the base pair resides in each state and τ_i is the i -th lifetime of the conformation in the corresponding state. The average times of the states are all listed in Table 1. It can be seen that the average lifetime of the closed and open states is much longer than those of the transition states.

For the closing-opening two-state model (Wang et al. 2016, 2018), the closing rate from the open state to the closed state k_+ and the opening rate from the closed state to the open state k_- can be calculated using the formulas $k_+ = 1/\tau_{ave}^c$ and $k_- = 1/\tau_{ave}^o$, where τ_{ave}^o is the average lifetime of the open state and τ_{ave}^c is the average time of the closed state. Figure 6A shows the temperature dependence of the closing rate and the opening rate at 0.5, 0.1, and 0.05 M NaCl. It can be seen that the opening rate at 0.5, 0.1, and 0.05 M NaCl exhibits a strong dependence on temperature, whereas the closing rate shows only weak temperature-dependent behavior. Furthermore, the opening rate is invisibly affected by ion

FIGURE 6. (A) Temperature dependence of the closing rate k_+ (full symbols) and the opening rate k_- (open symbols) at different ion concentrations. (B) Temperature dependence of the ratios of $t_c/t_{bp}^{o \rightarrow c}$ (open symbols) and $t_o/t_{bp}^{c \rightarrow o}$ (full symbols) at different ion concentrations. (Symbols) From the MD simulations (square for 0.5 M NaCl; circle for 0.1 M NaCl; triangle for 0.05 M NaCl); (Lines) Fitted with Equations 5 and 6.

concentration, while the closing rate is apparently dependent on ion concentration. The barrier for the formation of a single base pair from the open state is the reduction in entropy, which results from the RNA bases' entropy loss due to the conformation restriction for the pairing with each other, the ion's entropy loss due to the binding with the RNA base pair and the solvent's entropy loss due to hydration. As the salt concentration increases, the entropy loss of ion binding and hydration will decrease, and then, at a given temperature, the closing rate increases visibly as ion concentration is increased.

Based on the three-state (closed, open and transition state) model, the transition rates from the transition state to the closed state $k_{t \rightarrow c}$ and to the open state $k_{t \rightarrow o}$ can be calculated as $k_{t \rightarrow c} = 1/\tau_{ave}^{ctc}$ and $k_{t \rightarrow o} = 1/\tau_{ave}^{oto}$, where τ_{ave}^{ctc} is the average lifetime of transition state ctc and τ_{ave}^{oto} is the average lifetime of the transition state oto. The transition path time $t_{tp}^{c \rightarrow o}$ from the closed state to the open state and that from the open state to the closed state $t_{tp}^{o \rightarrow c}$ are: $t_{tp}^{c \rightarrow o} = \tau_{ave}^{oto}$ and $t_{tp}^{o \rightarrow c} = \tau_{ave}^{ctc}$, respectively. According to the transition-state theory (Bruce et al. 1988; Hänggi et al. 1990; Hummer 2004; Chung et al. 2009; Chung and Eaton 2013):

$$t_c = \frac{1}{k_-} = \frac{2\pi}{\beta D^* \omega^* \omega_c} \exp(\beta \Delta G_c),$$

$$t_o = \frac{1}{k_+} = \frac{2\pi}{\beta D^* \omega^* \omega_o} \exp(\beta \Delta G_o), \quad (5)$$

$$t_{tp}^{o \rightarrow c} = \frac{1}{\beta D^* (\omega^*)^2} \ln(2e^\gamma \beta \Delta G_o),$$

$$t_{tp}^{c \rightarrow o} = \frac{1}{\beta D^* (\omega^*)^2} \ln(2e^\gamma \beta \Delta G_c), \quad (6)$$

where $\beta = 1/k_B T$, k_B is the Boltzmann constant, T is the absolute temperature, D^* is the diffusion coefficient at the free energy barrier top, $(\omega^*)^2$, $(\omega_c)^2$ and $(\omega_o)^2$ are the curvatures of the free energy surface at the barrier top and the closed and open states, respectively. γ is the Euler's constant, and ΔG_c and ΔG_o are the free energy barrier heights of the closed and open states, respectively.

According to Equations 5 and 6, the ratios of $t_c/t_{tp}^{c \rightarrow o}$ and $t_o/t_{tp}^{o \rightarrow c}$ depend only on the free energy barrier, ω^*/ω_c and ω^*/ω_o , but are independent of D^* . As shown in Figure 6B, $t_c/t_{tp}^{c \rightarrow o}$ shows a strong temperature-dependence, whereas $t_o/t_{tp}^{o \rightarrow c}$ is nearly independent of temperature. Furthermore, $t_c/t_{tp}^{c \rightarrow o}$ is invisibly affected by ion concentration, while $t_o/t_{tp}^{o \rightarrow c}$ is strongly dependent on ion concentration. Considering that ω^*/ω_o is a constant and was generally defined as a constant in modeling protein folding kinetics (Chung and Eaton 2013), the free energy barrier of the base pair from the open state to the closed state should be proportional to temperature T , $\Delta G_o \propto T$. The free energy changes of the closed and open states were: $\Delta G = \Delta G_c - \Delta G_o = \Delta H - T\Delta S$. By fitting the curves, the free energy barriers from the closed state to the open state for the

terminal AU base pair at 0.1 M and 0.05 M NaCl are equal to ~ -7.31 kcal/mol, which corresponds to the enthalpy changes ΔH between the closed state and open state. The free energy barrier from the open state to the closed state is $\Delta G_o = T\Delta S$, where ΔS is the entropy change between the open and closed states.

It has been suggested that the diffusion coefficient D^* should exhibit super-Arrhenius behavior for a diffusion in a rough energy landscape (Bryngelson and Wolynes 1989), that is, $D^* \propto \exp[-(\Delta E/k_B T)^2]$, where $(\Delta E)^2$ is the local mean-squared fluctuation in energy and is a measure of the underlying landscape roughness. Afterwards, according to $\Delta G_o = T\Delta S$, $\Delta G_c = \Delta H$, and Equation 5, ΔE is found to be 0.78 kcal/mol and 0.80 kcal/mol for the AU base pair at 0.1 M NaCl and 0.05 M NaCl by fitting the temperature-dependent opening and closing times, respectively. Additionally, ΔE for the AU base pair at 0.5 M NaCl is 0.76 kcal/mol (Wang et al. 2016). Therefore, the analyses indicate that the free energy landscape of the terminal base-pair AU becomes more rugged as salt concentration decreases, that is, local mean-squared fluctuation becomes more apparent.

Conclusions

In summary, the thermodynamic and kinetic parameters of the AU base pair at different ion concentrations were derived by 3- μ sec all-atom molecular dynamics (MD) simulations at different temperatures. Our calculations showed that the enthalpy change ΔH for the closing/opening of a base pair is nearly unaffected by ion concentration, whereas the entropy change ΔS is strongly dependent on ion concentration, which indicated that Na^+ binding to the RNA group can promote the folding of an open base pair by reducing the disorder of the unfolded conformations. The ion concentration dependence of the entropy change mainly results from the differences of ion translational entropy changes for the closing/opening of the base pair at different ion concentrations. The closing and opening rates show different temperature-dependent behaviors. The closing rate is greatly affected by salt concentration and the closing rate increases with the increase of salt concentration, whereas the opening rate is almost unaffected by salt. Our analyses show that the free energy landscape of the AU base pair becomes rougher as ion concentration is decreased.

From the above, our molecular dynamic simulations provide an atomistic-level picture for the closing-opening transition of base-pair AU, which is difficult to be observed experimentally, and reveal the microscopic mechanism for the effects of ions on thermodynamic and kinetic parameters of a single RNA base pair. Opening the terminal base pair would disrupt the stacking interactions of the terminal base pair with the next base pair, which would increase the probability of the next base pair to be destabilized.

Although the accuracies of the simulation-based calculations and analyses may be limited by the force field and simulation time, the all-atom MD simulation can be still an effective tool for exploring more complex kinetic processes, such as RNA pseudoknot unfolding kinetics (Zhang et al. 2011), the effect of argonaute protein on miRNA's seed base (Wang et al. 2019b), and catalytic interactions between ions and RNA (Casalino and Magistrato 2016). A very recent work has shown that an MD-based treatment with the combination of other methods such as master equation and kinetic Monte Carlo technique is effective for analyzing RNA helix-terminal base-pairing (Wang et al. 2019a). Nevertheless, the present work provides a microscopic understanding on the salt dependence of RNA thermodynamics and kinetics at single base-pair level.

MATERIALS AND METHODS

Following our preceding works (Wang et al. 2016, 2018), the initial structure of sequence 5' – (AAGCGCAAGCUU) – 3', which includes four base pairs and a tetraloop, was obtained from the crystal structure (PDB ID: 1ZIH). In our simulations, the model molecule was solvated in a triclinic box with TIP3P (Jorgensen et al. 1983; Mahoney and Jorgensen 2000) water molecules as solvent. The counterions of Na⁺ and the salt of NaCl were added to ensure that our simulated systems are fully neutralized.

Our MD simulations were carried out using the GROMACS 4.5.6 simulation package (Hess et al. 2008) with the Amber10 all-atom force field (Wang and Kollman 2001; Wang et al. 2000; Pérez et al. 2007; Zgarbová et al. 2011), and the periodic boundary condition was employed in the simulations. The velocity rescaling (Bussi et al. 2007) and the Parrinello–Rahman barostat algorithm (Parrinello and Rahman 1981) were used to achieve constant temperature and pressure, respectively. The Particle-Mesh Ewald method (Darden et al. 1993; Essmann et al. 1995) was used to treat long-range interactions with a 10 Å cutoff, and Lennard-Jones interactions (Lennard-Jones 1931; Dzyaloshinskii et al. 1961) were truncated at 10 Å. The bond lengths of the solute were constrained by the LINCS algorithm (Hess et al. 1997), and the geometry of water molecules was kept completely rigid with the SETTLE algorithm (Miyamoto and Kollman 1992). The neighboring grid search method (Hess et al. 2008), which was updated every ten steps, was also employed. In the simulations, a time step of 2 fsec was used in the calculations and the coordinates of atoms were saved every 2 psec.

The energy of the system was minimized using the steepest descent method for 20 nsec at 290 K without any position restraints. Afterwards, one of the equilibrium structures was chosen as the starting structure for further MD simulations at higher (different) temperatures.

In our simulations, in order to characterize the opening/closing of a single base pair, we fixed all other nucleotides except for the two terminal nucleotides (A and U) of the RNA hairpin with harmonic constraints, by which the base pairs except the terminal base pair stay paired and could vibrate around their initial positions. So when the terminal base pair is in the closed state as

the RMSD is <2 Å, the stacking interaction of the terminal base pair with the nearest-neighbor base pair will not be affected. Each of our simulations lasted 3 μsec for each salt concentration and temperature for our analyses on thermodynamic and kinetic parameters. The details of our MD simulations have been described previously in Wang et al. (2016, 2018).

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