Mg^{2+}-dependency of the Helical Conformation of the P1 Duplex of the *Tetrahymena* Group I Ribozyme

Joon-Hwa Lee

Department of Chemistry, Research Institute of Natural Science, and Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju, Gyeongnam 660-701, Korea. E-mail: joonhwa@gnu.ac.kr
Received July 31, 2008

The P1 duplex of *Tetrahymena* group I ribozyme is the important system for studying the conformational changes in folding of ribozyme. The formation of the P1 duplex between IGS and substrate RNA and the catalytic activity of ribozyme require a variety of metal ions such as Mg^{2+} and Mn^{2+}. In order to investigate the effect of the Mg^{2+} concentration on the conformation of the P1 duplex, the NMR study was performed as a function of Mg^{2+} concentration. This study revealed that the less stable AU-rich region formed duplex at 50 °C under high Mg^{2+} concentration condition but melts out under low Mg^{2+} concentration condition. It was also found that in the active conformation under 10 mM $MgCl_2$ condition, the unstable central G·U wobble pair maintains the significant base pairing up to 50 °C. This study provides the information of the unique feature of the P1 duplex structure and the roll of Mg^{2+} ion on the formation of the active conformation.

Key Words : NMR, Mg^{2+}, RNA, Imino proton exchange, Group I ribozyme

Introduction

The *Tetrahymena* group I intron has served as a powerful model system for studying the conformational changes in large RNA and for understanding the catalytic mechanism of ribozyme.[1-3] The L-21 *ScaI* ribozyme catalyzes a trans-esterification reaction analogous to the first step of the self-splicing.[1] The internal guide sequence (IGS; 3'-GGAGGG-ribozyme) binds to a substrate RNA (5'-CCCUCUA) and then forms the P1 duplex. Metal ion plays a crucial role on ribozyme structure or activity. Global folding of *Tetrahymena* ribozyme can be supported by a variety of metal ions, however, only Mg^{2+} and Mn^{2+} activate ribozyme activity.[4] These results indicated that this ribozyme has two types of metal-ion binding sites; one contributed the formation of active conformation of ribozyme including RNA folding, the other involved in the chemical reaction.

The measurement of hydrogen exchange rates has also been one of good probe to study the macromolecular conformation or dynamics.[5,6] The exchange rate constants of the imino protons in nucleic acids can provide the information on thermal stability or the dynamics of each base pair.[5,6] The exchange rates of fast-exchanging imino protons can be determined by water magnetization transfer method of NMR.[5,6] Analysis of the hydrogen exchange of imino protons generally was based in the two-state (open/closed) model for the base pair where hydrogen exchange only occurs from the open state.[5-7] The apparent T_2 relaxation time, T_{2a}, can be derived from the line-width ($\Delta\nu_{1/2}$) of each proton resonance using Eq. (1):

$$\Delta\nu_{1/2} = \frac{1}{\pi T_{2a}} \quad (1)$$

The apparent R_2 relaxation rate constant ($R_{2a} = 1/T_{2a}$) for an exchangeable imino proton is the summation of the R_2 rate constant, which is the inverse of T_2 spin-spin relaxation time of this proton, and the exchange rate constant (k_{ex}) for the imino proton. Thus, the difference in the T_{2a} for the same imino proton provides the direct information on the change of exchange rate constant for this imino proton.

Here, we measured the line-widths of the imino proton resonances of the P1 duplex formed by an IGS-11mer, r[GGUUUGGAGGG] and a substrate 13mer, d[GGCCC-UCUAAACC] (Fig. 1A) at various temperatures. In order to investigate the effect of the Mg^{2+} concentration on the conformation of the P1 duplex, the R_{2a} values of the imino protons for three P1 duplex samples which were dissolved in 0.1, 1, and 10 mM $MgCl_2$-containing buffer, respectively, were compared in this study. We also determined the exchange rate constants of the imino proton for these P1 duplex samples by water magnetization transfer method to compare the base pair stabilities of these duplexes.

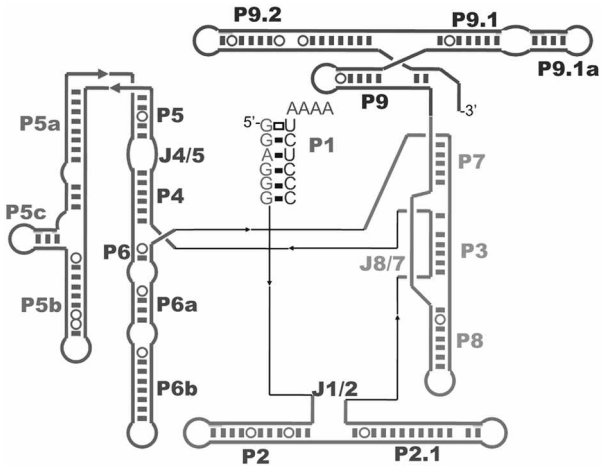

Figure 1. Secondary structure of *Tetrahymena* group I ribozyme.

Results and Discussion

Temperature dependence of imino proton spectra of the P1 duplex. The assignment of imino proton resonances of the P1 duplex was previously reported.[8] Figure 1B shows the temperature dependence of the imino proton spectra of the P1 duplex as a function of Mg^{2+} concentration. Under 10 mM $MgCl_2$ condition, all imino proton resonances (except U21) for non-terminal base pairs are still observed as sharp signal up to 45 °C (Fig. 1B). At 45 °C, when the concentration of $MgCl_2$ was changed to 1 or 0.1 mM, some imino proton resonances (for example, G18, U19, and U20) in the AU-rich region (Region I) became significantly line-broadened whereas the imino proton resonances in the GC-rich region (region II) were still remained as sharpened (Fig. 1B). The peak intensities of two imino resonances from the central G22·U-1 wobble pair also became slightly smaller compared to the signals at 35 °C (Fig. 1B). These results indicates that the region I and central G22·U-1 wobble pair in the buffer containing 0.1 or 1 mM $MgCl_2$ became destabilized at 45 °C but the Mg^{2+} ion with concentration of 10 mM stabilized the overall region of the P1 duplex. The most striking result is that all imino proton resonances of the P1 duplex in the buffer containing 10 mM $MgCl_2$ showed severely line-broadening at 15 °C whereas these phenomena were not observed when the $MgCl_2$ concentration was 0.1 or 1 mM (Fig. 1B). This means that high concentration (here 10 mM) of $MgCl_2$ in the buffer system may induce the unusual conformation of the P1 duplex contrast to the low $MgCl_2$ concentration.

R_{2a} of imino proton signals of the P1 duplex as a function of temperature. The line-widths of the exchangeable imino proton resonances of the P1 duplex under three different Mg^{2+} concentrations were determined at various temperatures. The R_{2a} for the imino protons were determined from the line-widths of their resonances by using Eq. (1). Figure 2 shows the R_{2a} values of four imino protons (U20, G22, G23, and U-3) as a function of temperature. Under 0.1 mM $MgCl_2$ condition, the all imino protons had the constant R_{2a} values smaller than 70 s^{-1} when temperature is lower than 35 °C (Fig. 2). As temperature is increased up to 50 °C, the R_{2a} values of some imino protons (U20 and G22) significantly became larger whereas the G23 imino proton still has similar R_{2a} value in this temperature range (Fig. 2). This increment of R_{2a} for the U20 and G22 imino protons at high temperature may be caused by rapid exchange of the imino proton indicating the instability of the P1 duplex at high temperature. The similar results of the R_{2a} patterns for all imino protons were observed under 1 mM $MgCl_2$ condition (Fig. 2).

Surprisingly, it was observed that the two interesting features for the R_{2a} pattern for the imino protons of the P1 duplex in the 10 mM $MgCl_2$-containing buffer, which is completely different from those of the P1 duplex under low $MgCl_2$ concentration condition. First, when temperature is lower than 20 °C, the R_{2a} of all imino protons in the 10 mM $MgCl_2$-containing buffer are 3~4-fold larger than those of corresponding signal in the low concentration of $MgCl_2$ (Fig. 2). Line-broadening in NMR strongly correlates with the size of macromolecules. This severe line-broadening of the P1 duplex in 10 mM $MgCl_2$ at low temperature was caused by not rapid exchange process of the imino protons but their slow tumbling motions compared to data under low Mg^{2+} concentration. This result indicates that the high concentration of Mg^{2+} ion produces partial aggregation (or as multimer formation) which increases the molecular weight of the RNA sample and then reduces the rate of tumbling motion even though the same P1 duplex sample was studied here. Second, contrast to data under 0.1 mM $MgCl_2$ buffer condition, there was no change in the R_{2a} of all

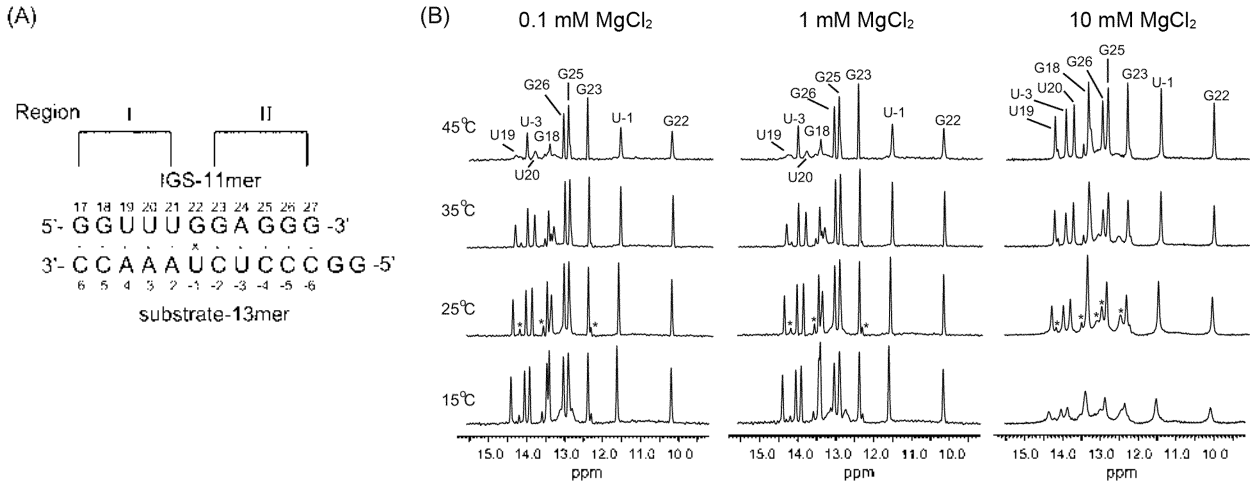

Figure 2. (A) RNA sequence context of the P1 duplex. The P1 duplex can be divided into an AU-rich region (denoted region I) and a GC-rich region (denoted region II) around the central G·U base pair. (B) Temperature dependence of the imino proton resonances of the ^{1}H-NMR spectra for the P1 duplex in a NMR buffer containing sodium phosphate (pH 6.6) and 100 mM NaCl with various $MgCl_2$ concentration. The experimental temperatures are shown on the left of the spectra. The $MgCl_2$ concentrations are shown on the top of the spectra. The resonance assignments for the imino protons are shown on the spectra acquired at 45 °C under each $MgCl_2$ concentration condition. The asterisk symbols on the spectra at 25 °C indicate the minor conformation of the P1 duplex.

imino protons of the P1 duplex in 10 mM $MgCl_2$ buffer even though the temperature is up to 50 °C (Fig. 2). These results demonstrate that the unique conformation of the P1 duplex is still stable at high temperature when the Mg^{2+} concentration is 10 mM, whereas the region 1 and central G·U wobble pair is seriously destabilized at high temperature under low Mg^{2+} concentration condition.

Exchange rate constants of the imino protons of the P1 duplex. The inversion-recovery and water magnetization transfer experiments for the imino protons of the P1 duplex dissolved in 10 mM $MgCl_2$-containing NMR buffer at 35 °C were previously reported.[8] The same experiments for the P1 duplex dissolved in the 0.1 mM $MgCl_2$ NMR buffer were performed in order to compare the Mg^{2+} effect on the imino proton exchange. At 15 °C, the k_{ex} for the imino protons of the P1 duplex in 10 mM $MgCl_2$ buffer could not be determined because the apparent spin-diffusion R_1 relaxation rate constant (R_{1a}) data for the imino proton resonances did not fit well to a single exponential and showed unusual double exponential relaxation.[8] This result may result from partial aggregation of the P1 duplex at low temperature, which supports the explanation of the severe line-broadening described above. However, inversion recovery relaxation data for all imino protons fit well to single exponentials when the concentration of $MgCl_2$ is 0.1 or 1 mM (*data not shown*). This means that the partial aggregation of the P1 duplex is induced by only high Mg^{2+} concentration as well as 10 mM. This may result from the effective protection of charge-charge repulsion between phosphate backbones of RNA duplex molecules.

At high temperature such as 35 °C, the double exponential relaxation of the imino proton resonances was not observed any more in any range of Mg^{2+} concentration (*data not shown*). The exchange rate constants (k_{ex}) of the imino protons for the P1 duplex in the 0.1 mM $MgCl_2$ buffer were determined by the curve fitting of the intensity data of the water magnetization transfer experiment to Eq. (2). The k_{ex} values for the P1 duplex in the 0.1 mM and 10 mM $MgCl_2$ buffer at 35 °C are compared each other in Figure 3. The U19 and U20 imino protons in the region I have $k_{ex} \geq 20\ s^{-1}$, whereas the imino protons in the region II all have $k_{ex} \leq 10\ s^{-1}$ (Fig. 3). This exchange pattern of the imino protons is independent on the concentration of the $MgCl_2$ (Fig. 3). However, the significant effect of the Mg^{2+} concentration was observed in the k_{ex} of the two imino protons of central G22·U-1 base pair at 35 °C. The k_{ex} values of the G22 and U-1 imino proton of the P1 duplex in 10 mM $MgCl_2$ buffer 2--3-fold larger than those dissolved in 0.1 mM $MgCl_2$ buffer (Fig. 3). This result demonstrates that the central G22·U-1 base pair is unusually destabilized by high concentration of the Mg^{2+} ion.

Biological implication. The P1 duplex of *Tetrahymena* group I ribozyme is the important system for studying the conformational changes in folding of ribozyme.[1-3] The formation of the P1 duplex between IGS and substrate and the catalytic activity of ribozyme require a variety of metal ions such as Mg^{2+} and Mn^{2+}.[4] This NMR study for the P1

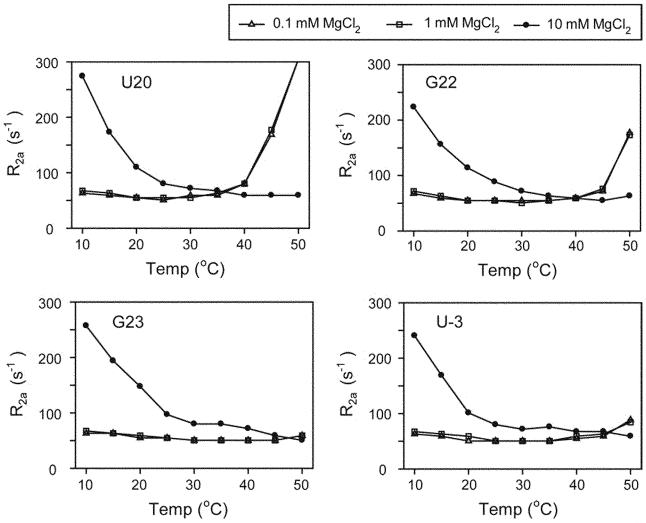

Figure 3. The apparent spin-spin R_2 relaxation rate constants (R_{2a}) of the imino protons (U20, G22, G23, and U-3) of the P1 duplex dissolved in NMR buffer containing 0.1 mM (grey closed triangle), 1 mM (open square), or 10 mM $MgCl_2$ (black closed circle) determined from line-width of each resonance by using Eq. 1.

duplex as a function of Mg^{2+} concentration provides the information of the unique features of the P1 duplex structure and the rolls of metal ion (Mg^{2+} here) on the formation of the active conformation. First, the P1 duplex is divided into three parts, the central G·U wobble pair, the unstable AU-rich region, and the very stable GC-rich region. Second, the P1 duplex can be partially aggregated at low temperature under high Mg^{2+} concentration condition. Third, the less stable AU-rich region forms duplex at 50 °C under high Mg^{2+} concentration condition but melts out under low Mg^{2+} concentration condition. Forth, the GC-rich region is very stable up to 50 °C and this character is independent on the Mg^{2+} concentration. Finally, in the active conformation under 10 mM $MgCl_2$ condition, the unstable central G·U wobble pair maintains the significant base pairing up to 50 °C. The reaction rate constants of thio-modified substrate at

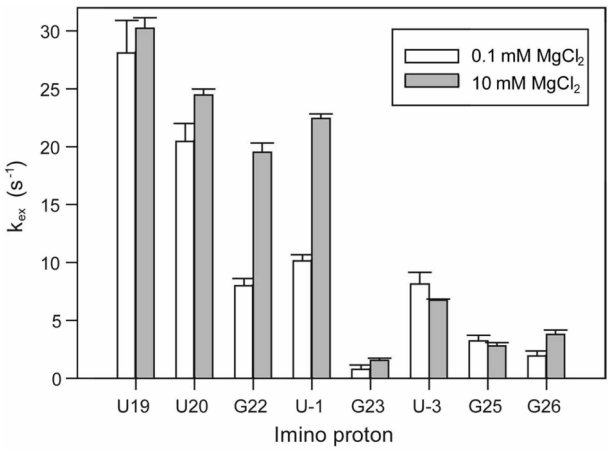

Figure 4. The exchange rate constants of the imino protons for the P1 duplex dissolved in NMR buffer containing 0.1 mM (open bar) or 10 mM $MgCl_2$ (grey closed bar) at 35 °C determined by water magnetization transfer experiment.

the phosphate between U-1 and A2 became 100-fold larger as the Mn^{2+} concentration was increased from 0.1 to 10 mM.[4] The similar effects of the Cd^{2+} and Mg^{2+} were also previously observed.[9] Three metal ions involved in the transition state of the *Tetrahymena* ribozyme reaction; the first interacts with 3'-O of U-1 and phosphate oxygen between U-1 and A2, the second interacts with 3'-O of incoming G and the above phosphate oxygen, the last interacts with 2'-OH of incoming G.[4,9] The unique structural feature, studied here, of the P1 duplex dependent on Mg^{2+} concentration might be correlated with metal coordination at phosphate group between U-1 and A2. Thus, we suggested that one of three metal ions was involved in the formation of P1 duplex but the other two metal ions acted in the formation of the transition state in the catalytic core.

Experimental Section

The substrate strand was prepared by *in vitro* transcription as previously described.[10] The IGS-11mer was purchased from Dharmacon, Inc. (Lafayette, CO). The oligomers were desalted using NAP-5 columns (Amersham Biosciences, NJ). The three different RNA duplex samples with concentration of about 0.5 mM were prepared by dissolving molar equivalents of IGS and the substrate in 0.1, 1, and 10 mM $MgCl_2$-containing 90% H_2O/10% D_2O NMR buffer (10 mM sodium phosphate, 100 mM NaCl, pH 6.6), respectively. The concentrations of EDTA of the all NMR samples are 1% of the $MgCl_2$ concentration. All NMR experiments were carried out on a Varian Inova 500-MHz spectrometer (University of Colorado, CO) equipped with z-axis pulsed-field gradient triple resonance probe. 1D NMR data were processed and analyzed with the program FELIX (Accelrys) or VNMR J as previously described.[8,11,12] 2D NMR data were processed with the program NMRPIPE and analyzed with the program Sparky as previously described.[8,11,12] The exchange rates of the imino protons were determined as previously described.[8] The apparent longitudinal relaxation rate constants, R_{1a}, of the imino protons were determined by semi-selective inversion recovery 1D NMR experiments.[8] The hydrogen exchange rates of the imino protons were measured by water magnetization transfer experiments.[8] The R_{1w} was determined by water selective inversion recovery experiment.[8]

The imino hydrogen exchange rate constants (k_{ex}) were determined by fitting the data to Eq. (2):

$$\frac{I_0 - I(t)}{I_0} = 2\frac{k_{ex}}{(R_{1w} - R_{1a})}(e^{-R_{1a}t} - e^{-R_{1w}t}) \quad (2)$$

where R_{1a} and R_{1w} were the independently measured and are the apparent longitudinal relaxation rates of the imino proton and water, respectively, and I_0 and $I(t)$ are the peak intensities of the imino proton in the water magnetization transfer experiments at times zero and t, respectively.[7]

Acknowledgments. I thank Prof. A. Pardi (University of Colorado) for helpful support in whose laboratory the NMR experiment was performed. This work was supported by grant to the EB-NCRC (15-2003-012-01001-0) and the KOSEF grant (R01-2007-000-10691-0) (to J.-H.L.) funded by the Korea Government (MEST).

References

1. Cech, T. R. In *The RNA World*; Gesteland, R. F., Atkins, J. F., Eds.; Cold Spring Harbor Laboratory Press: New York, 1993; pp 239-269.
2. Shan, S. O.; Herschlag, D. *RNA* **2002**, *8*, 861-872.
3. Karbstein, K.; Carroll, K. S.; Herschlag, D. *Biochemistry* **2002**, *41*, 11171-11183.
4. Shan, S. O.; Herschlag, D. *RNA* **2000**, *6*, 795-813.
5. Leroy, J. L.; Bolo, N.; Figueroa, N.; Plateau, P.; Gueron, M. *J. Biomol. Struct. Dyn.* **1985**, *2*, 915-939.
6. Gueron, M.; Leroy, J. L. *Methods Enzymol.* **1995**, *261*, 383-413.
7. Snoussi, K.; Leroy, J. L. *Biochemistry* **2001**, *40*, 8898-8904.
8. Lee, J.-H.; Pardi, A. *Nucleic Acids Res.* **2007**, *35*, 2965-2974.
9. Shan, S.; Kravchuk, A. V.; Piccirilli, J. A.; Herschlag, D. *Biochemistry* **2001**, *40*, 5161-5171.
10. Milligan, J. F.; Groebe, D. R.; Witherell, G. W.; Uhlenbeck, O. C. *Nucleic Acids Res.* **1987**, *15*, 8783-8798.
11. Lee, J.-H.; Park, C.-J.; Choi, B.-S. *Bull. Korean Chem. Soc.* **2006**, *27*, 1731-1732.
12. Lee, J.-H. *Bull. Korean Chem. Soc.* **2007**, *28*, 1643-1644.