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

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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. The L-21 Scal ribozyme catalyzes a transesterification reaction analogous to the first step of the self-splicing. 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. 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. 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. The exchange rates of fast-exchanging imino protons can be determined by water magnetization transfer method of NMR. 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. 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):

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

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[GGUUUGGGAGGG] 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.](image-url)
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. 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.

$R_2$ 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 $R_2$ for the imino protons were determined from the line-widths of their resonances by using Eq. (1). Figure 2 shows the $R_2$ 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 $R_2$ values smaller than 70 s$^{-1}$ when temperature is lower than 35 °C (Fig. 2). As temperature is increased up to 50 °C, the $R_2$ values of some imino protons (U20 and G22) significantly became larger whereas the G23 imino proton still has similar $R_2$ value in this temperature range (Fig. 2). This increment of $R_2$ 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 $R_2$ pattern 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 $R_2$ 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 MgCl₂ 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 Mg²⁺ concentration is 10 mM, whereas the region I and central G·U wobble pair is seriously destabilized at high temperature under low Mg²⁺ 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 MgCl₂-containing NMR buffer at 35 °C were previously reported. The same experiments for the P1 duplex dissolved in the 0.1 mM MgCl₂ NMR buffer were performed in order to compare the Mg²⁺ effect on the imino proton exchange. At 15 °C, the k_{ex} for the imino protons of the P1 duplex in 10 mM MgCl₂ buffer could not be determined because the apparent spin-diffusion R₂ relaxation rate constant (R₂o) data for the imino proton resonances did not fit well to a single exponential and showed unusual double exponential relaxation. 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 MgCl₂ is 0.1 or 1 mM (data not shown). This means that the partial aggregation of the P1 duplex is induced by only high Mg²⁺ 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 Mg²⁺ concentration (data not shown). The exchange rate constants (k_{ex}) of the imino protons for the P1 duplex in the 0.1 mM MgCl₂ 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 MgCl₂ buffer at 35 °C are compared each other in Figure 3. The U19 and U20 imino protons in the region I have k_{ex} ≥ 20 s⁻¹, whereas the imino protons in the region II all have k_{ex} ≤ 10 s⁻¹ (Fig. 3). This exchange pattern of the imino protons is independent on the concentration of the MgCl₂ (Fig. 3). However, the significant effect of the Mg²⁺ 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 MgCl₂ buffer 2–3-fold larger than those dissolved in 0.1 mM MgCl₂ buffer (Fig. 3). This result demonstrates that the central G22-U-1 base pair is unusually destabilized by high concentration of the Mg²⁺ ion.

**Biological implication.** 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 and the catalytic activity of ribozyme require a variety of metal ions such as Mg²⁺ and Mn²⁺. This NMR study for the P1 duplex as a function of Mg²⁺ concentration provides the information of the unique features of the P1 duplex structure and the rolls of metal ion (Mg²⁺ 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 Mg²⁺ concentration condition. Third, the less stable AU-rich region forms duplex at 50 °C under high Mg²⁺ concentration condition but melts out under low Mg²⁺ concentration condition. Forth, the GC-rich region is very stable up to 50 °C and this character is independent on the Mg²⁺ concentration. Finally, in the active conformation under 10 mM MgCl₂ 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 3.** The apparent spin-spin R₂ relaxation rate constants (R₂o) 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 MgCl₂ (black closed circle) determined by line-width of each resonance by using Eq. 1.

**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 MgCl₂ (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. The similar effects of the Cd$^{2+}$ and Mg$^{2+}$ were also previously observed. 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. 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. 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$_2$O/10% D$_2$O NMR buffer (10 mM sodium phosphate, 100 mM NaCl, pH 6.6), respectively. The concentrations of EDTA of the all NMR samples are 1%

The hydrogen exchange rates of the imino protons were measured by water magnetization transfer experiments. The $R_{1w}$ was determined by water selective inversion recovery experiment. The imino hydrogen exchange rate constants ($k_{ex}$) were determined by fitting the data to Eq. (2):

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

where $R_{1w}$ and $R_{1a}$ 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.

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**References**