# Elucidation of Double Exponential Behavior in the $T_1$ Relaxation of the *Tetrahymena* Group I Ribozyme

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The *Tetrahymena* group ribozyme is the valuable model for investigating the principle of RNA folding, structure, and function.[1,2] The *Tetrahymena* ribozyme catalyze a phosphoryl transfer reaction with a rate enhancement of $10^{11}$-fold over the uncatalyzed reaction.[1] Binding of the *Tetrahymena* ribozyme's oligonucleotide substrate involves P1 duplex formation with the ribozyme's internal guide sequence (IGS) to give an open complex, followed by docking of the P1 duplex into the catalytic core via tertiary interactions to give a closed complex.[1] The monitoring on the structural or biophysical change between the docked and undocked states of the ribozyme is one of the good methods to investigate its dynamics and/or folding. The exchange of the imino protons with solvent water, which can be measured by NMR, implicates not only the base-pair opening rate but also the solvent accessibility.[3,4] The solvent accessibility provides the important information about the structure feature of nucleic acids.[3,4] Recently, the Hydrogen/Deuterium exchange study of ribozyme reported that some imino proton resonances near 12.8 and 13.4 ppm had the exchange times of 3.89 and 5.18 hours, respectively, indicating that these imino protons slowly exchanged to solvents.[5]

The water magnetization transfer experiment is a useful NMR method for the exchange time measurement of the imino protons in nucleic acids.[6-8] This approach required the measure the apparent $T_1$ ($T_{1a}$) time of the imino proton signals, which could be determined by the inversion-recovery experiment.[6-8] The $T_{1a}$ time is expressed by equation, $1/T_{1a} = R_{1a} = 1/T_1 + k_{ex}$, where $T_1$ is the dipolar relaxation time and $k_{ex}$ is the exchange rate constant. We performed the inversion-recovery experiment to measure the $T_{1a}$ time of the imino proton signals of the ribozyme at 35 °C. Surprisingly, these inversion-recovery data were not fitted by a single exponential equation but fitted well by a double exponential function (Fig. 1). This unusual double exponential relaxation has been reported in the NMR study of the short RNA duplex, which might result from partial aggregation of RNA duplex.[6] The imino proton resonances of the ribozyme are shown like the several broad peaks, but these resonances are the mixture of at least one hundred of imino resonances. Thus, this double exponential function of the inversion-recovery data is thought to be the summation of the $T_{1a}$ values of lots of imino protons. However, this hypothesis cannot explain the similar patterns of the non-exchangeable resonances (Fig. 1) because every base proton, except A-H2, has the similar $T_1$ (= $T_{1a}$) value. In order to explain this result, the Solomon equation, which is the best theory to explain the NOE effect by selective inversion, is introduced.

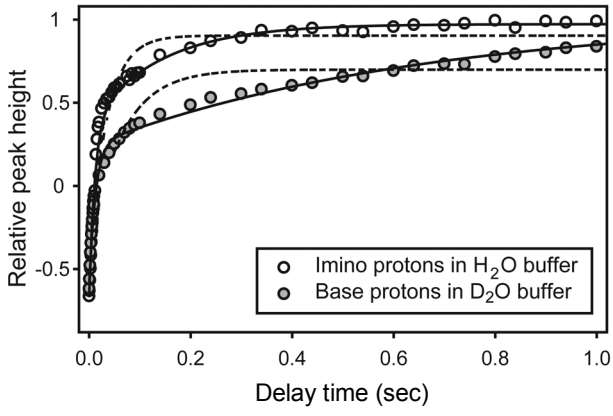


**Figure 1.** Relative peak intensities in the inversion recovery spectra for the imino proton (open circle) and base proton (gray circle) resonances as a function of delay time. Solid and dash lines indicate the best fit to single and double exponential functions, respectively.

First, we consider two-spin model, where spin 1 is selectively inverted at $t = 0$. This relaxation can be represented by Solomon equations.

$$\frac{d(I_{1z}(t)-I_{1z}^{\infty})}{dt} = -\rho_1(I_{1z}(t)-I_{1z}^{\infty})-\sigma_{12}(I_{2z}(t)-I_{2z}^{\infty}) \quad (1\text{-}1)$$

$$\frac{d(I_{2z}(t)-I_{2z}^{\infty})}{dt} = -\sigma_{12}(I_{1z}(t)-I_{1z}^{\infty})-\rho_2(I_{2z}(t)-I_{2z}^{\infty}) \quad (1\text{-}2)$$

And the solution of these equations, which can explain the double exponential relaxation, is followed:

$$\begin{aligned}\frac{I_{1z}(t)}{I_{1z}^{\infty}} &= 1-\left\{\frac{R+\rho_1-\rho_2}{R}e^{-\frac{(\rho_1+\rho_2)+R}{2}t}\right. \\ &\quad \left.+\frac{R-\rho_1+\rho_2}{R}e^{-\frac{(\rho_1+\rho_2)-R}{2}t}\right\} \\ &= 1-\{\kappa_1 e^{-\lambda_1 t}+\kappa_2 e^{-\lambda_1 t}\}\end{aligned} \quad (2\text{-}1)$$

$$\frac{I_{2z}(\tau)}{I_{2z}^{\infty}} = 1-\frac{2\sigma_{12}}{R}\left\{e^{-\frac{(\rho_1+\rho_2)+R}{2}\tau}-e^{-\frac{(\rho_1+\rho_2)-R}{2}\tau}\right\} \quad (2\text{-}2)$$

where $R=\sqrt{(\rho_1-\rho_2)^2+4{\sigma_{12}}^2}$, $\rho_1$ and $\rho_2$ are self relaxation con stants of spin 1 and 2, respectively, and $\sigma_{12}$ is cross relaxation constant. In the isolated two-spin system, self relaxation ($\rho_1$, $\rho_2$) and cross relaxation ($\sigma_{12}$) constants are expressed by following equations:

$$\rho_1 = \frac{K}{r_{12}^6}\{3J(\omega_1)+6J(2\omega_1)+J(0)\} \quad (3\text{-}1)$$

$$\rho_2 = \frac{K}{r_{12}^6}\{3J(\omega_2)+6J(2\omega_2)+J(0)\} \quad (3\text{-}2)$$

$$\sigma_{12} = \frac{K}{r_{12}^6}\{6J(\omega_1+\omega_2)-J(\omega_1-\omega_2)\} \quad (3\text{-}3)$$

where $r_{12}$ is distance between two spins, $\omega_i$ is precession frequency of $i$-spin, $\tau_c$ is correlation time of molecule, $K = 58 \times 10^9$(s$^{-2}$Å$^6$) and $J(\omega) = 1/[1+(\omega\tau_c)^2]$. The values of $J$ function and maximum NOE ($\eta = \sigma_{12}/\rho_1$) as a function of correlation time ($\tau_c$) of molecule are shown in Supporting Information Table S1. When the spin 1 is G/A-H8 proton which is base-paired and well-stacked in A-form helix and spin 2 is the H2' proton of ($n$ −1) residue which is the nearest proton from H8 proton ($r_{12}$ = 2.0 Å), the exponential coefficients of the double exponential function as function of correlation time were calculated and shown in Supporting Information Table S2. The $T_1$ [= $1/(\rho_1+\sigma_{12})$] relaxation time of the H8 proton as a function of correlation time is shown in Fig. 2A. However, the simulated $T_1$ relaxation time did not fit the experimental data well (Fig. 2B). Thus, new model system, such as three-spin system, is required to explain the double exponential relaxation behavior of the base protons.

In the three-spin system, the spin 2 is close to the spin 3 and there exists dipolar relaxation between two spins. The self relaxation ($\rho_1$, $\rho_2$) and cross relaxation ($\sigma_{12}$) constants are represented by following equations:

$$\rho_1 = \sum_i \rho_{1i} = KA\sum_i\left(\frac{1}{r_{1i}^6}\right) = KA\left(\frac{1}{r_{12}^6}+\frac{1}{r_{13}^6}\right) \approx \frac{KA}{r_{12}^6} \quad (4\text{-}1)$$

$$\rho_2 = \sum_i \rho_{2i} = KA\sum_i\left(\frac{1}{r_{2i}^6}\right) = KA\left(\frac{1}{r_{12}^6}+\frac{1}{r_{23}^6}\right) = \frac{KA}{r_{12}^6}\left(\frac{r_{12}^6+r_{23}^6}{r_{23}^6}\right) = \alpha\rho_1 \quad (4\text{-}2)$$

$$\sigma_{12} = \frac{KB}{r_{12}^6} \approx \eta\rho_1 \quad (4\text{-}3)$$

where $A = 3J(\omega) + 6J(2\omega) + J(0)$, $B = 6J(2\omega) - J(0)$, $\eta = B/A$, $\alpha = ({r_{12}}^6 + {r_{23}}^6)/{r_{23}}^6$. Thus the coefficients of double exponential relaxation are expressed by the following equations:

$$\kappa_1 = \frac{\beta+1-\alpha}{\beta} \quad (5\text{-}1)$$

$$\kappa_2 = \frac{\beta-1+\alpha}{\beta} \quad (5\text{-}2)$$

$$\lambda_1 = \rho_1\frac{(1+\alpha)+\beta}{2} \quad (5\text{-}3)$$

$$\lambda_2 = \rho_1\frac{(1+\alpha)-\beta}{2} \quad (5\text{-}4)$$

where $\beta = [(1-\alpha)^2+4\eta^2]^{-1/2}$. When the spin 3 is H3' proton of ($n$ −1) residue which is the nearest proton from H2' ($r_{23}$ = 2.37 Å), the exponential coefficients of the double exponential function as function of correlation time were calculated and shown in Supporting Information Table S3. Fig. 2C shows the simulated relaxation curves at various correlation times, in

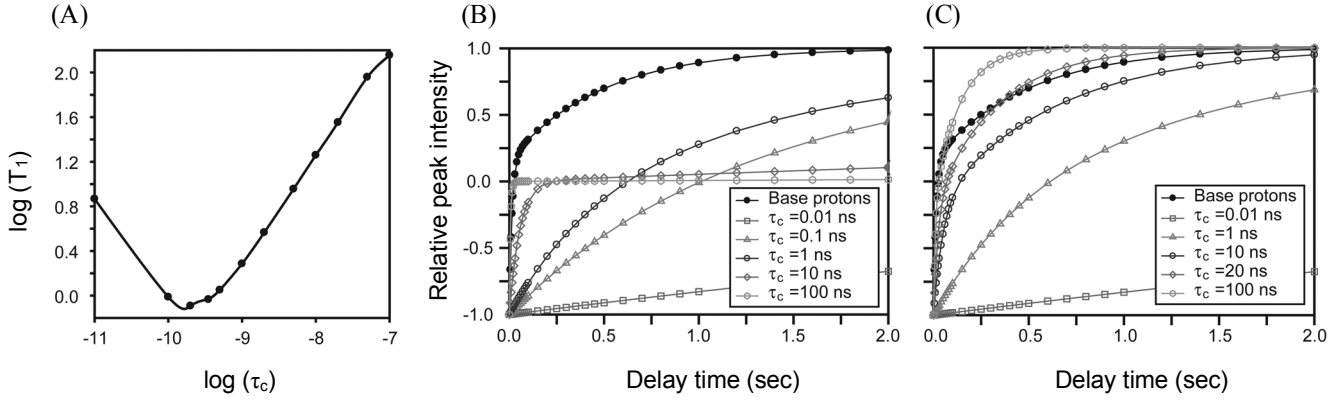


**Figure 2.** (A) $T_1$ relaxation time of the G/A H8 proton based on two spin model as a function of the correlation time at 500 MHz field. (B) Simulated $T_1$ relaxation of the G/A H8 proton at various correlation times based on the two-spin model and (C) modified three-spin model.

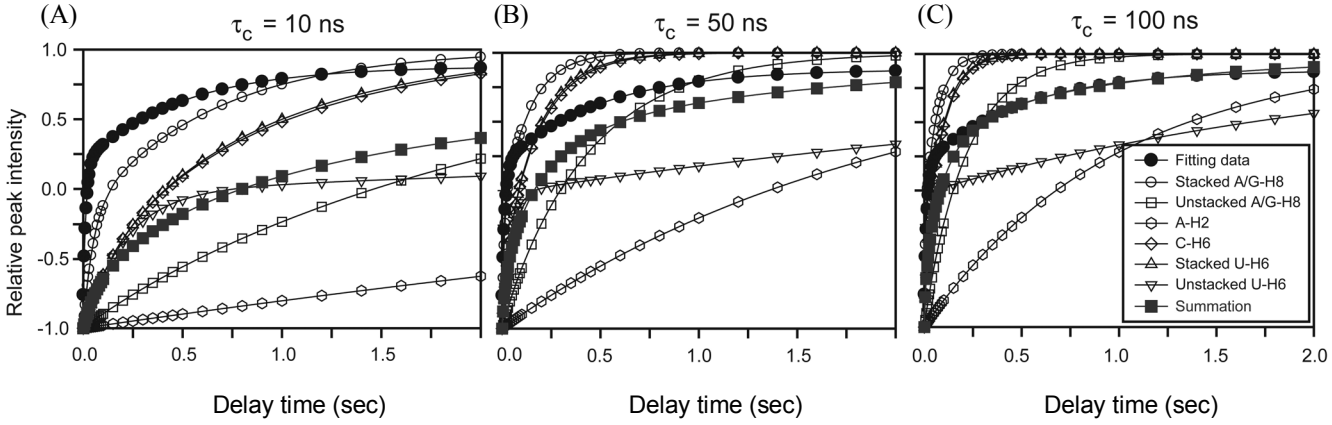


**Figure 3.** (A) Simulated $T_1$ relaxation of the various kinds of base protons at the correlation times of 10 ns, (B) 50 ns, and (C) 100 ns based on the modified three-spin model.

which the experimental data is similar to the exponential curve at $\tau_c = 20$ ns.

Actually, the base proton peak of the *Tetrahymena* ribozyme is the mixture of the A/G-H8, A-H2, C/U-H6 protons. The spin systems for the flanking G/A-H8, A-H2, C-H6, stacked/flanking U-H6 are considered by the similar way to the stacked G/A-H8. First, in the case of flaking G/A-H8, the spin 2 is *n*-H3' ($r_{12}$ = 3.2 Å) and spin 3 is *n*-H2' ($r_{23}$ = 2.37 Å). Second, in the case of A-H2 proton, the spin 2 is *n*-H2' ($r_{12}$ = 4.2 Å) and spin 3 is *n*-H3' ($r_{23}$ = 2.37 Å). Third, in the case of C-H6 proton, the spin 2 is *n*-H5 ($r_{12}$ = 2.5 Å) and spin 3 is *n*-H41 ($r_{23}$ = 2.4 Å). Forth, in the case of stacked U-H6 proton, the spin 2 is (*n*-1)-H2' ($r_{12}$ = 2.5 Å) and spin 3 is (*n*-1)-H3' ($r_{23}$ = 2.37 Å). Fifth, in the case of flanking U-H6 proton, the spin 2 is *n*-H5 ($r_{12}$ = 2.5 Å) and spin 3 is *n*-H3' ($r_{23}$ = 4.35 Å). Supporting Information Table S4 shows the exponential coefficients of inversion recovery data for each base proton at various correlation times are shown in Supporting Information Table S4 and the relaxation curves of these base protons at $\tau_c$ = 10, 50 and 100 ns are shown in Fig. 3.

When we assume that *i*) four kinds of nucleotides exist as the same amounts; and *ii*) 50% of base are base-paired and stacked, the exponential function of the base proton signals is expressed by summation of function of each base (*see* Eq. 6).

$$I_{all} = 0.25 \times \sum I(A) + 0.25 \times \sum I(G) + 0.25 \times \sum I(C) + 0.25 \times \sum I(U) \quad (6)$$

$$= 0.25 \sum_{A} I_{H2} + 0.25 \sum_{A,G}^{stacked} I_{H8} + 0.25 \sum_{A,G}^{unstacked} I_{H8} + 0.25 \sum_{C} I_{H6} + 0.125 \sum_{U}^{satcked} I_{H6} + 0.125 \sum_{U}^{unstacked} I_{H6}$$

Fig. 3 shows the summation of exponential function of each base proton at $\tau_c$ = 10, 50 and 100 ns. The experimental data are matched with the summation of exponential functions at correlation time of 100-ns. This analysis is consistent with the fact that the molecular weight of the *Tetrahymena* ribozyme is about 120 kDa. Thus, our theory can explain the double exponential character in the inversion-recovery experiment of large size molecule.

In summary, in order to understand the double exponential relaxation of proton signal of the *Tetrahymena* ribozyme, we derived the coefficients of the double exponential functions based on the modified three-spin system. This derivation shows the similar exponential curve with actual inversion recovery data.

## Experimental Section

The purified *Tetrahymena* group I ribozyme was kindly gifted by Prof. Daniel Herschlag (Stanford University). Ribozyme was buffer exchanged into 10 mM $Mg^{2+}$ NMR buffer (10 mM sodium phosphate pH 6.6, 100 mM NaCl, 10 mM $MgCl_2$, 0.1 mM EDTA) in 90% $H_2O$/10% $D_2O$. All NMR experiments were performed on Varian Inova 500 (equipped a triple resonance probe) or 600 MHz (equipped a cold probe) spectrometer. The NMR data were processed using FELIX2004 (Accelrys) as described previously.[9] The apparent longitudinal relaxation rate constants ($R_{1a} = 1/T_{1a}$) of the imino protons were determined by semi-selective inversion recovery 1D NMR experiments, where a selective 180° inversion pulse was applied to imino proton region (9 - 15.5 ppm) before the jump-return-echo water suppression pulse.[6]

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**Supporting Information.** The supporting Information Tables are available on request from the correspondence author (Fax: +82-55-761-0244, E-mail: joonhwa@gnu.ac.kr).

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