Hydrogen-Deuterium Exchange of *Tetrahymena* Group I Ribozyme

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Tetrahymena group I intron has served as good model system for conformation or dynamics study of large RNA.[1,2] The T*etrahymena* L-21 ScaI ribozyme is derived from the self-splicing group I intron.[1] The internal guide sequence (IGS) in the ribozyme forms the P1 duplex with a substrate and then specific site of substrate was cleaved by attack of an exogenous guanosine.[1] X-ray crystal structures of group I intron from several species have been solved and useful in the understanding of previous biochemical and biophysical study.[3,4] The dynamics study of ribozyme is motivated by the desire to understand the conformational states and flexibility of ribozyme relating RNA function.

The measurement of hydrogen exchange rates has been an important tool for studying the macromolecular conformation or dynamics.[5,6] The imino proton resonances of nucleic acids are good probes of the hydrogen exchange study for studying the dynamics of each base pair.[5,6] The exchange rates of imino protons depend on not only the base pair stability but also solvent accessibility.[6] Hydrogen-deuterium (H/D) exchange method is widely used to measure the exchange rates of slowly exchanging imino protons whereas the exchange rates of fast-exchanging imino protons can be determined by water magnetization transfer method of NMR.[5-7]

It is very difficult to study the dynamics or conformational change of the *Tetrahymena* ribozyme by using NMR spectroscopy because whole ribozyme is too large (greater than 120 kDa). The NMR study on the dynamics or flexibility of the ribozyme can provide lots of information about the long-range molecular interaction, tertiary folding, or enzymatic kinetics in the large-sized ribozyme. The problem caused from resonance assignment may be solved by incorporation of the site-specific isotope-labeling method. However, it is still unclear which NMR method successfully provides the answer for the question on the dynamics or structure of the 120-kDa ribozyme. Here, we first challenged the H/D exchange study of 120-kDa ribozyme by NMR in order to investigate the dynamics property of very large RNA molecule. The success of this experiment suggests that the *Tetrahymena* ribozyme can be studied on its dynamics or conformational change in the molecular level when the site-specific isotope-labeled ribozyme is prepared.

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 or 600 MHz spectrometer equipped a triple resonance or cold probe. The NMR data were processed using FELIX2004 (Accelrys) as described previously.[8] 1D proton spectra were collected using a gradient 11 echo pulse sequence for water suppression. The ribozyme sample in 90% H_2O/10% D_2O NMR buffer was exchanged into100% D_2O NMR buffer by using a 1mL bed volume G-25 size exclusion spin column. 1D imino proton spectra were acquired as function of time after deuterium exchange. The intensities of some slowly exchanging imino proton resonances were fit to the simple exponential decay $I(t) = I_0 e^{-t/\tau_{ex}} + c$ in Sigma plot, where $I(t)$ and I_0 are peak intensities at time t and zero, respectively, and τ_{ex} is the exchange time.[9]

Figure 1 shows 1D imino proton spectra of *Tetrahymena* group I ribozyme acquired on 500 MHz NMR spectrometer as function of temperature. Ribozyme has lots of base-paired imino protons and then shows a cluster of imino protons resonances in the range of 11-15 ppm which become a little sharper as temperature increased (Fig. 1).

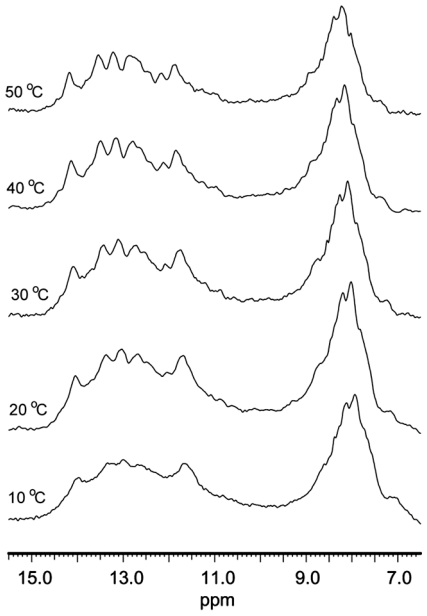

Figure 1. Temperature-dependent imino proton spectra of the Tetrahymena group I ribozyme in 90% H_2O/10% D_2O NMR buffer (10 mM $MgCl_2$, 100 mM NaCl, 10 mM sodium phosphate (pH 6.6), and 0.1 mM EDTA) acquired on 500 MHz NMR with triple resonance probe.

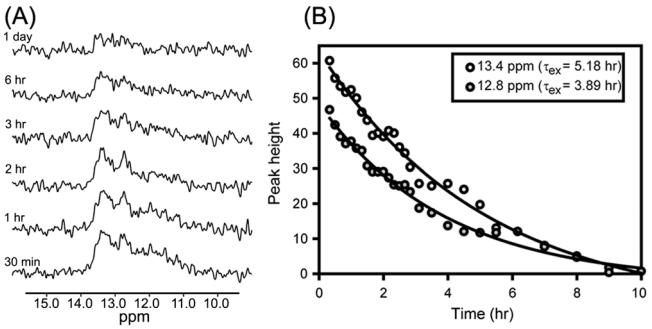

Figure 2. (A) The H/D exchange spectra of the *Tetrahymena* ribozyme on the 600 MHz with cold probe at 5 °C. The RNA sample dissolved in H_2O buffer (10 mM $MgCl_2$, 100 mM NaCl, 10 mM sodium phosphate (pH 6.6), and 0.1 mM EDTA) was exchanged to D_2O buffer containing the same salts. The times after H/D exchanging are shown on the left of each spectrum. (B) Exponential curve fitting of the H/D exchange data or two resonances at 12.8 (grey) and 13.4 ppm (white) by using equation of decay $I(t) = I_0 e^{-t/\tau ex} + c$. The determined exchange times are shown in right corner of figure.

The H/D exchange experiment is the useful NMR tool to study the kinetics/dynamics and folding of the protein as well as large nucleic acids such as tRNA. The large sized molecule (ribozyme: MW ~120 kDa) has very small T_2 relaxation time in NMR and then the signal-to-noise (S/N) of the spectrum is very low. So, the quality of the H/D exchange spectra which were acquired on 500 MHz at 35 °C is very poor. All imino signals look completely disappeared at 10 minute after buffer exchange into D_2O (Fig. 3S). Signals from amino and base (H2, H6, and H8) protons also decreased significantly which may be caused by exchange of amino protons and dilution of ribozyme sample during H/D exchange. When the threshold of the exchange spectra is increased, some imino protons resonances are observed until 1 hour after buffer exchange (Fig. 3S). However, we couldn't analyze anymore this data because of very poor resolution of spectra.

We performed again the H/D experiment of ribozyme at 5 °C on 600 MHz NMR spectrometer equipped cold probe in order to increase the S/N of these spectra. Figure 2A shows the H/D exchange spectra of the ribozyme as function of time. Some imino proton resonances near 12.8 and 13.4 ppm, which are mixture of many imino proton resonances still observed until 6 hours after H/D exchange, indicating that these imino protons slowly exchanged to solvents. The exchange times of two resonances at 12.8 and 13.4 ppm are 3.89 and 5.18 hours, respectively, which were determined by the exponential curve fitting of the peak height as function of the time (Fig. 2B).

The exchange rates of imino protons in nucleic acids are very sensitive to hydrogen bonding interactions, base pair opening, and secondary and tertiary structures.[6] The poor S/N of the H/D exchange spectra of ribozyme might be improved by increment of sample concentration and high field of NMR spectrophotometer. Although the S/N of the spectra is very low, we first measured the H/D exchange time of the about 120 kDa RNA by NMR. Generally, the slow exchange of imino protons in nucleic acids results from protection of imino proton from solvent water by tertiary structure rather than base pairing or secondary structure. The extremely slow exchange of some imino protons of the *Tetrahymena* group I ribozyme indicates the unique tertiary fold of large ribozyme. Thus it is suggested that the H/D exchange spectra of large nucleic acids without any assignment might be a good probe to determine their correct folding or not.

This study is the direct evidence for that it is possible to study for 120 kDa ribozyme molecule by NMR spectroscopy. The ribozyme binds to sequence specific substrates by its IGS to form the P1 duplex and then the substrate is cleaved by external guanosine. If the complex between ribozyme and isotope-labeled substrate is prepared, the NMR signal from substrate can be selected by isotope-selection method. The NMR study of this system might provide the important information about the intermolecular interaction in the complex or the catalytic core. This study is a direct evidence for that it is possible to extend to study for 120 kDa ribozyme molecule by NMR spectroscopy.

Supporting Information. The Supporting Information Fig. 3S (the H/D exchange spectra of the *Tetrahymena* ribozyme on the 500MHz with triple resonance probe at 35°C) is available at BKCS online (www.kcsnet.or.kr/bkcs).

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