Measurement of Hydrogen Exchange Times of the RNA Imino Protons Using by Phase-modulated CLEAN Chemical Exchange Spectroscopy

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NMR hydrogen exchange experiments are the important tools for studying the conformation or dynamics of the macro-molecules.[1] The imino proton resonances are good probes of the dynamic motions of the base pairs of nucleic acids.[1] Hydrogen-deuterium exchange method is widely used to determine the exchange rates of slowly exchanging imino protons.[1,2] The exchange rates of fast-exchanging imino protons could be measured by water magnetization transfer (WMT) method (Figure 1A).[1,2] This experiment have been used to determine the exchange rates of the imino protons in DNA,[3-5] RNA,[6,7] DNA-protein complex,[8] and RNA-protein complex.[7] However, this experiment, which needs long repetition time (d1 time in Figure 1) of 10 ~ 15 sec for complete relaxation of water signal, is prone to various sources of artifacts such as exchange-relayed NOE/ROE from rapidly exchanging protons in macro-molecules.[9]

To suppress efficiently this artifact, the application of phase-modulated CLEAN chemical exchange spectroscopy (CLEANEX-PM) to the mixing period of a water-selective 1D sequence has been reported.[9] The 1D WMT and CLEANEX-PM pulse sequences are outlined in Figure 1. In the CLEANEX-PM pulse sequence, to specifically detect the exchange of imino protons with water, the 90^{o}(hard)-gt$_1$-180° (selective)-gt$_1$ combination selectively excites water, while other signals are dephased by gradients. The spin-lock sequence for CLEANEX-PM is a repetitive windowless multiple-pulse sequence $135^{o}(x)$ - $120^{o}(-x)$ - $110^{o}(x)$ - $110^{o}(-x)$ - $120^{o}(x)$ - $135^{o}(-x)$ of radio-frequency pulsed during mixing time. To prevent radiation damping, a weak gradient (gzt$_m$) is applied through the whole mixing period. At the end of mixing period, the 3-9-19 WATERGATE sequence is applied to suppress water signal.

Tetrahymena group ribozyme is the valuable model for investigating the conformational changes in RNA and the catalytic potential of RNA.[10,11] The *Tetrahymena* group I ribozyme catalyze a transesterification reaction.[10] 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.[10] Recently, the thermodynamics and kinetics for base-pair opening of the P1 duplex of the *Tetrahymena* ribozyme were studied by NMR hydrogen exchange experiments, in which the WMT method was employed to measure exchange times of the imino protons.[6] Here, we have measured the hydrogen exchange times of the imino protons of the P1 duplex using by the CLEANEX-PM experiments and these results were compared with those of previous study[6] which was determined by WMT method.

The P1 RNA duplex was prepared as described in a previous report.[6] The RNA sample were dissolved in a 90% H$_2$O/10% D$_2$O NMR buffer that contained 10 mM sodium phosphate (pH 6.6), 10 mM MgCl$_2$, 100 mM NaCl, and 0.1 mM EDTA. All NMR experiments were performed on a Varian Inova 500 MHz spectrometer equipped with a triple-resonance probe. 1D data were processed with the program FELIX2004 (Accelrys, San Diego). CLEANEX-PM experiments were performed using mixing times ranging from 5 to 100 ms. The hydrogen exchange times of the imino protons (τ_{ex}) were determined by fitting to the following equation:
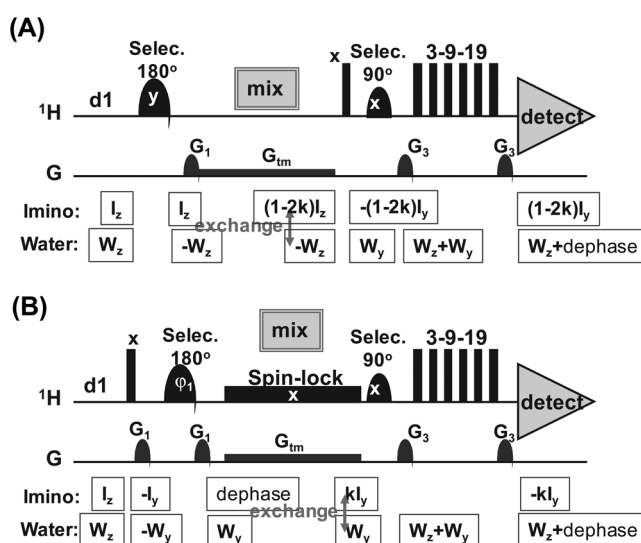

Figure 1. Timing diagrams of the (A) water magnetization transfer and (B) water-selective 1D CLEANEX-PM sequences. At 500 MHz, the selective 180° pulse is a 2.7 ms Gaussian. Phase cycle: ϕ_1 {*x, y, -x, -y*}, detect {*x, -x, x, -x*}.

$$\frac{I(t)}{I_0} = \frac{1}{1/T_{2a} - 1/T_{2w}} \frac{1}{\tau_{ex}} \left(e^{-t/T_{2a}} - e^{-t/T_{2a}} \right) \qquad \text{(Eq. 1)}$$

where I_0 and $I(t)$ are peak intensities of the imino proton in the CLEANEX-PM experiments at times zero and *t*, respectively

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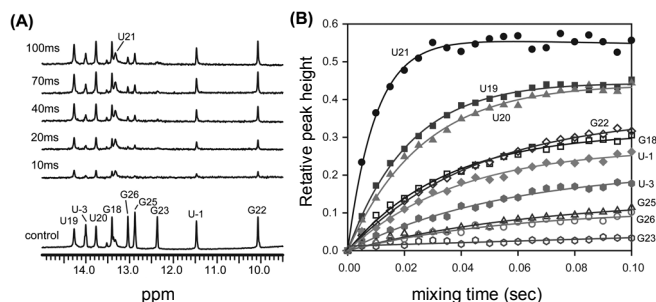**Figure 2.** (A) Imino proton region of CLEANEX-PM spectra acquired by varying the mixing time after water inversion for the P1 duplex at 35 $^{\circ}$C. The control spectrum is the same watergate solvent suppression experiment except that no water inversion was performed. (B) The relative peak intensities in the CLEANEX-PM spectra for various imino protons of the P1 duplex as a function of mixing time after water inversion. Solid lines indicate the best fit to Eq. 1.

Table 1. Hydrogen exchange times (sec) of the imino protons of the P1 duplex determined by WMT and CLEANEX-PM experiments.

Base pair	Imino	WMT[a]	CLEANEX-PM
G18·C5	G18	0.10	0.11
U19·A4	U19	0.036	0.048
U20·A3	U20	0.048	0.059
A21·U2	U21	0.025	0.018
G22·U-1	G22	0.079	0.13
	U-1	0.065	0.14
G23·C-2	G23	0.95	0.97
A24·U-3	U-3	0.21	0.26
G25·C-4	G25	0.31	0.48
G26·C-5	G26	0.15	0.52

[a]previous study.[6]

and T_{2a} and T_{2w} are the apparent relaxation times of the imino proton and water, respectively.

Figure 2A shows 1D spectra of the CLEANEX-PM experiment as a function of mixing time during spin-lock sequence for the P1 duplex at 35 $^{\circ}$C. Some imino protons show significant changes in peak intensities as a function of mixing time of spin-lock sequence (Figure 2A). For example, rapidly exchanging imino protons such as G18, U19, and U20 show strong peak intensities at short mixing times (40 ms in Figure 2A), whereas the G23 resonance, that is the slowest exchanging imino proton, could not be detected in the spectra of the mixing time up to 100 ms (Figure 2A). The relative peak intensities of the CLEANEX-PM spectra for the imino proton resonances of the P1 duplex at 35 $^{\circ}$C are plotted as a function of mixing time in Figure 2B and these data were used to determine τ_{ex} by fitting to Eq. 1. The τ_{ex} values for individual imino protons in the P1 duplex are given in Table 1. The G and U imino protons in the A·U-rich region (G18 to A21) have τ_{ex} from 0.01 to 0.1 sec, whereas the imino protons in the G·C-rich region (G23 to G26) all have $\tau_{ex} > 0.2$ sec. The U21 imino proton next to the central G22·U-1 base pair has the shortest exchange times within non-terminal base pairs (τ_{ex} of 0.018 sec). These exchange times of the imino protons of the P1 duplex determined by CLEANEX-PM experiment are very similar to the corresponding values determined by WMT in the previous study, except central G22·U-1 base pair.[6] The d1 time in the CLEANEX-PM method (1.5 sec) is just one-tenth of the WMT experiment (15 sec). Thus, this method can reduce the experiment times to determine the exchange times of the nucleic acids in compared to WMT methods. One interesting thing is that the exchange times of the two imino protons of central G22·U-1 base pair determined by the CLEANEX-PM are two-fold longer than those from the WMT method (Table 1). In the WMT experiment, the dipole-dipole interaction between two magnetically inverted imino protons (G22 and U-1) by exchange with solvent water significantly increases the spin-lattice ralxation rates of these resonance, wheareas this effect can not be occurred during the CLEANEX-PM experiment. The fast

relaxation of the imino proton resonances of the G·U base pair in the WMT experiment might be main reason for shorter exchange times of these imino protons than their actural exchange times.

In summary, we first measured the exchange times of the RNA imino protons using by the CLEANEX-PM method. This method has two advantages compared to the WMT method which is widely used: this method significantly reduce (i) the NMR experiment times to determine the exchange times and (ii) the spine-lattice relaxation effect caused by dipole-dipole interactions between very closed imino protons.

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