Detection of Chemical Exchange in Methyl Groups

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Introduction

The identification of residues subject to conformational exchange is an important first step in the characterization of dynamic processes in proteins and other biological macromolecules. For backbone ^{15}N spins, the ^{15}N TROSY Hahn-echo experiment enables the detection of this exchange. However, for side-chain conformational dynamics, which are frequently studied by methyl spin relaxation techniques, a similar approach does not exist.

We demonstrate the detection of methyl conformational exchange can be accomplished using $^1H_3{}^{13}C$ zero- and double-quantum methyl TROSY Hahn echo experiments that we recently developed [1] in conjunction with a ^1H-^1H dipole-dipole cross correlation experiment [2]. The combination of these two experiments is used to detect conformational exchange in the DNA repair enzyme AlkB and in *apo* ribonuclease H (RNase H), both from *E. coli*.

For AlkB, side-chain conformational exchange is examined for four methionines (M49, M57, M61, and M92) with different substrate complexes: Zn^{2+} (an Fe^{2+} surrogate) alone; Zn^{2+} and α-ketoglutarate (2OG); or Zn^{2+}, 2OG, and methylated DNA substrate (DNA). The results are consistent with those recently determined by biochemical and NMR spectroscopic studies [3].

For RNase H, side-chains from isoleucine, leucine, and valine are studied as a function of temperature, enabling the examination of thermodynamic constants, including activation energies.

Theory

Multiple Quantum Hahn-Echo

$$\Delta R_{MQ} = (R_{DQ} - R_{ZQ})/2 = \Delta R^0 + \Delta R_{ex}/2 \qquad \bar{R}_{MQ} = (R_{DQ} + R_{ZQ})/2 \qquad (1)$$

$$\Delta R^0 = \frac{2}{5}\left(\frac{\mu_0}{4\pi}\right)^2 \hbar^2 S_{axis}^2 \tau_c \left\{ \frac{8}{3}\gamma_C\gamma_H\gamma_D^2 \sum_{D^E} \left\langle \frac{1}{(r_{CD^E} r_{HD^E})^3} \right\rangle + \gamma_C\gamma_H^3 \sum_{H^E} \left\langle \frac{1}{(r_{CH^E} r_{HH^E})^3} \right\rangle \right\} \qquad (2)$$

$$\Delta R_{ex} = 4 p_1 p_2 \Delta\omega_C \Delta\omega_H / (k_1 + k_{-1}) \qquad (3)$$

^1H-^1H Dipole-Dipole Cross-Correlated Relaxation

$$\eta = \frac{9}{10}\left(\frac{\mu_0}{4\pi}\right)^2 \hbar^2 \gamma_H^4 P_2(\cos\theta_{HH})^2 S_{axis}^2 \tau_c \left\langle \frac{1}{r_{HH}^6} \right\rangle \qquad (4)$$

Combination of R_{MQ} and η for Detection of Conformational Exchange

$$\Delta R^0 = \eta \left[\frac{4}{9} P_2(\cos\theta_{HH})^{-2} \left\langle r_{HH}^6 \right\rangle \left\{ \frac{8}{3}\frac{\gamma_C\gamma_D^2}{\gamma_H^3} \sum_{D^E} \left\langle \frac{1}{(r_{CD^E} r_{HD^E})^3} \right\rangle + \frac{\gamma_C}{\gamma_H} \sum_{H^E} \left\langle \frac{1}{(r_{CH^E} r_{HH^E})^3} \right\rangle \right\} \right] \qquad (5)$$

Figure 1. The difference between the zero- and double-quantum relaxation rates (R_{DQ} and R_{ZQ}) measured by the Hahn Echo **(1)** contains terms corresponding to the intrinsic relaxation rate **(2)** and conformational exchange **(3)**. Equation (2) can be combined with that for the ^1H-^1H dipole-dipole cross-correlated relaxation rate **(4)** to produce **(5)**, which is a linear equation with slope defined by the term in outer brackets.

Enzyme Structure

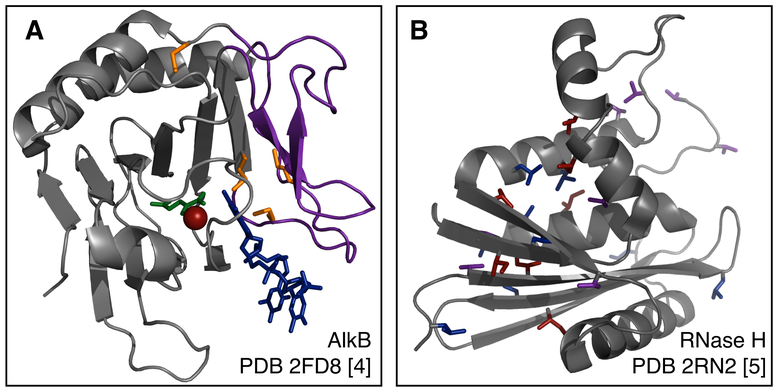

Figure 2. (A) AlkB shown with Fe^{2+} co-factor (red), 2OG co-substrate (green), and DNA substrate (blue). Methionines (49, 57, 61, and 92) are shown in orange and the dynamic lid region is purple. **(B)** RNase H is shown with isoleucines, leucines, and valines colored red, blue, and purple, respectively.

AlkB Methyl Relaxation

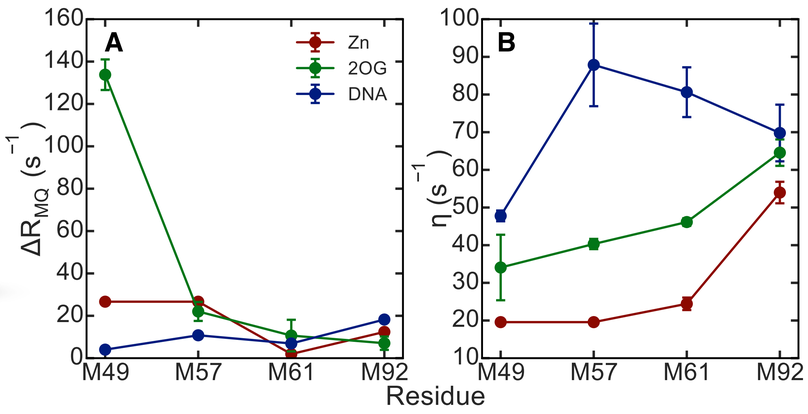

Figure 3. Methyl relaxation for 13Cε-Met AlkB acquired at 21.1 T. **(A)** Multiple quantum relaxation rates, ΔR_{MQ}, calculated from a zero- and double-quantum Hahn echo and **(B)** the ^1H-^1H cross-correlated relaxation rate constant (η). Relaxation rates are measured with successive addition of Zn^{2+} (red), 2OG (green) and DNA (blue).

RNase H Methyl Relaxation

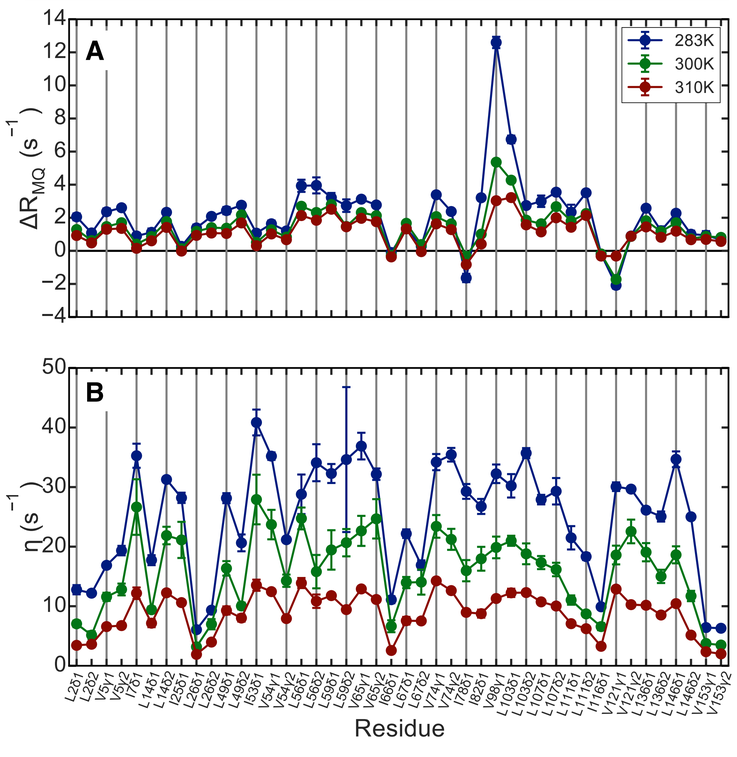

Figure 4. Methyl relaxation for ^{13}C-ILV RNase H acquired at 14.1 T. **(A)** The multiple quantum relaxation rate, ΔR_{MQ}, calculated from a zero- and double-quantum Hahn echo and **(B)** the ^1H-^1H cross-correlated relaxation rate constant (η). Relaxation rates are measured at 283, 300, and 310 K (blue, green, and red, respectively).

Estimation of Conformational Exchange (R_{ex})

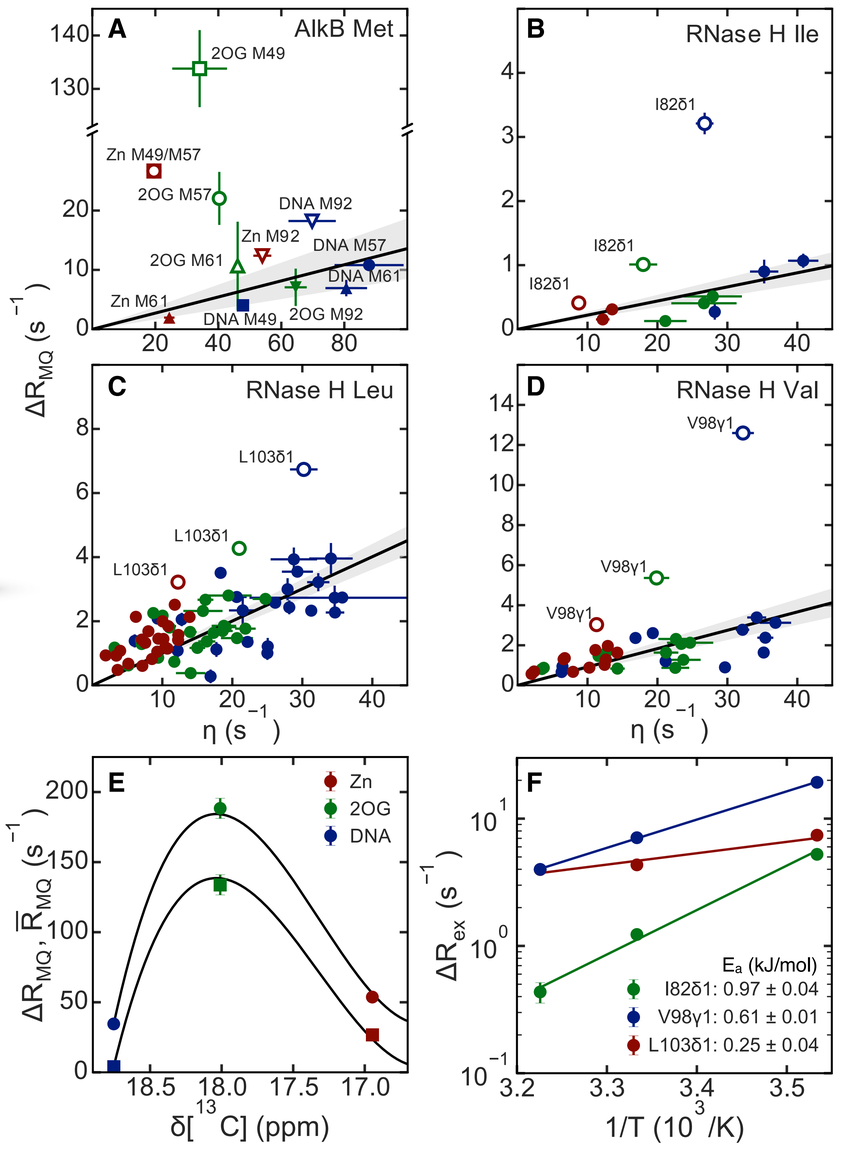

Figure 5. The ^1H-^1H dipole-dipole cross-correlated relaxation rate constant (η) is plotted relative to the multiple quantum relaxation rate, ΔR_{MQ}, for **(A)** AlkB methionines with successive co-factor and substrate complexes (Zn, 2OG and DNA), and RNase H **(B)** Ile, **(C)** Leu, **(D)** and Val at three temperatures (283, 300, and 310 K). Colors for (A–D) are as in Figures 3 and 4, respectively. Residues excluded from the regression are plotted with open symbols. For parts (B–D), only excluded residues are labeled. **(E)** Determination of populations and exchange rates for AlkB co-factor and substrate complexes [3]. **(F)** Activation energies (E_a) determined from the slope of $\ln[\Delta R_{ex}]$ *vs* 1/T for RNase H residues I82δ1, V98γ1, and L103δ1.

Conclusions

- Conformational exchange can be detected on a per-residue basis by comparison of relaxation rates from a zero- and double-quantum Hahn echo, (ΔR_{MQ}), and ^1H-^1H dipole-dipole cross correlated relaxation rates (η) for methionine residues in AlkB in complex with co-factor and substrates
- For RNase H, exchange can be detected across a range of temperatures for isoleucine, leucine, and valine residues
- It is anticipated this method will be a valuable first step in the detection of conformational exchange in methyl sidechain residues

References
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