Journal of the Korean Magnetic Resonance Society **1998**, *2*, 33-40

An influence of the exchange rate on NOE intensities of a ligand: Application to 37kDa *trp*-holo-repressor/operator DNA complex

Donghan Lee and Weontae Lee*

Department of Biochemistry, College of Science, Yonsei University, Seoul, 120-749 Korea
Received April 10, 1998

Abstract : The cross peak intensities versus mixing times of 2D NOESY spectrum for a corepressor L-trp were simulated for the case of a ligand exchanging between free (AX) and bound (A' X') forms in protein/DNA complex. The direct NOE (I(AX)) of the free ligand exhibited a small positive intensity indicative of the strong dominant influence of the bound ligand. The exchange-mediated NOE peak (I(AX')) was very sensitive to corepressor exchange. However, both diagonal (I(A' A')) and direct NOE (I(A' X')) intensities of the bound ligand were not affected much at initial stage. Both peaks were severely influenced by exchange at mixing times of greater than 100 ms. In conclusion, since the NOE intensity is a function of exchange rate, the exchange effect should be considered to properly extract accurate distance information for bound ligand in the presence of conformational exchange.

INTRODUCTION

Escherichia coli trp apo-repressor consists of a homodimer of two 107-residue polypeptides and binds to an 18 base-pair consensus operator sequence with 1:1 stoichiometry in the presence of the corepressor, L-tryptophan.[1,2] The non cooperative binding of two L-tryptophan ligands to apo-repressor initiates an allosteric transition and the holo-repressor binds to operator sites in five operons involved in tryptophan metabolism and transport.[3-6] Recently, structural studies by NMR spectroscopy[7-9] and X-ray crystallography[10] have been reported for both holo-protein and holo-protein/DNA complex. Even though, the global fold and the overall orientation of *trp*-repressor molecules of both NMR and X-ray structures are in good agreement, the orientation and binding pockets of the L-tryptophan ligand in the NMR structure were different from those of X-ray structure.

The ligand, L-tryptophan is a key regulator of the stability and dynamics of *trp*-repressor/DNA complex. Equilibrium binding constants for holo-repressor binding to

operator DNA and tryptophan binding to apo-repressor have been determined to be 0.2 - 6 nM[11] and 15 - 18 μM,[12] respectively. Very recently, we have reported the dynamic properties of ligand L-tryptophan and the intermolecular interaction of the ligand with both protein and DNA in the repressor/operator DNA complex by the use of [ul-$^{13}C/^{15}N$]-ligand.[13] In addition, the exchange off-rate of the corepressor between the bound and free forms in holo-repressor/DNA complex was determined to be 3.4 Hz at 45 ℃ by heteronuclear $^{13}C/^{15}N$-edited NMR experiments. Moseley et al.[14] demonstrated a quantitative analysis of the ^{13}C-edited intermolecular transferred NOESY spectrum of the *trp*-repressor/operator complex using a complete relaxation and conformational exchange matrix methodology. In this study, we present the exchange effect on NOE intensities of both free and bound ligand in ternary complex of the ligand-protein-DNA complex.

MATERIALS AND METHODS

Sample preparation.

Trp apo-repressor protein was isolated from *E. coli* strain CY15070 and purified as previously described. The purified protein was dialyzed against 500 mM NaCl, 50mM sodium phosphate at pH 6.0 and concentrated for NMR sample. The palindromic concensus operator d(CGTACTAGAATTCTAGTACG) was synthesized on an Applied Biosystems 392 DNA/RNA synthesizer and was detritylated prior to uncoupling from the resin. The amount of DNA was determined at 260 nm with an extinction coefficient of 3.3×10^5 $M^{-1}cm^{-1}$. The operator DNA was finally checked by ^1H one-dimensional NMR spectrum. *Trp* holo-repressor/DNA complex was made by mixing *trp* holo-repressor with operator DNA in equimolar amounts into 50 mM sodium phosphate buffer at pH 6.0. The final concentration of the *trp* holo-repressor/DNA was 1.0 mM in *trp* apo-repressor dimer, 4.0 mM $^{13}C/^{15}N$-labelled L-tryptophan and 1.0 mM double-stranded DNA.

NMR experiments

All ^{13}C-edited NOESY experiments were performed in D_2O solution on a Varian Unity600 spectrometer in quadrature detection mode at 45 ℃. Low-power GARP decoupling for aliphatic and aromatic carbons was used during the detection period. Mixing times of 50 - 800 ms were employed for ^{13}C-edited 2D-NOESY experiments. All NMR data were transferred to SGI Indigo2 workstation and processed using NMRPipe program.

Theory and Calculations

The modified Solomon equation for considering both cross relaxation and exchange

rate between two sites were expressed as

$$-d\mathbf{M}/dt = [\mathbf{R}+\mathbf{K}]\mathbf{M} - [\mathbf{R}+\mathbf{K}]\mathbf{M}_0 \qquad [1]$$

where, matrix $\mathbf{M_0}$ is a thermal equilibrium magnetization and **R** represents a complete relaxation matrix for two sites. In addition, **K** is a diagonal exchange matrix.

The general solution for Eq.[1] can be written as

$$\mathbf{M}(t) = \exp\{-[\mathbf{R}+\mathbf{K}]t\}\{\mathbf{M}(t=0) - \mathbf{M}_0\} + \mathbf{M}_0 \qquad [2]$$

Lee et al.[15] has already shown that the expression of NOE intensity I(kl) of a peak between spins k and l is

$$\mathbf{I}(kl) = m_{l0}\sum_p \underline{\mathbf{U}}_{kp}\exp\{-\lambda_p\tau\}\underline{\mathbf{U}}^{-1}_{pl} \qquad [3]$$

where τ is a mixing time and $\underline{\mathbf{U}}$ is a transformation matrix which can diagonalize the matrix [**R** + **K**].

In order to consider the influence of NOE peak intensities, we can approximate a simple two-spin-1/2 system. By labeling AX and A′ X′ at the two conformations for the case of conformational exchange, we have driven the intensities of the diagonal [I(AA)], [I(A'A')] and the cross peaks [I(AA'), I(AX) , I(AX') and I(A'X')] as a function of mixing time.

RESULTS AND DISCUSSION

NMR measurements and simulation parameters of corepressor:

The ring proton chemical shifts of both free and bound ligands have been assigned based on ^{13}C-edited 2D NOESY spectra. Fig. 1 shows that NMR resonance peaks of both free and bound corepressor were completely assigned including exchange cross peaks between two forms.[13] We have already determined the exchange off rate of the corepressor (k_{off}) between the bound and free states in *trp*-repressor/operator DNA complex. The inter-proton distance between $C^{\alpha}H$ and H4(R) for bound ligand was measured from NMR structure of *trp*-repressor/DNA complex (Fig. 2). The free ligand structure was generated by molecular modeling and energy minimization procedure and served for inter proton distance information of the free corepressor. All of the parameters used for simulations are listed in Table I.

Corepressor exchange and NOESY cross peaks in trp-repressor/operator complex:

Fig. 3 shows a panel of simulation curves demonstrating cross peak intensity versus mixing times of 2D NOESY spectrum for both free and bound forms of corepressor. The simulations were performed for the case of a ligand exchanging between free (AX) and bound (A'X') forms in a solution containing two times of excess ligand concentration than protein. Intensities were calculated by parameters[14] of $\tau_C = 1.5 \times 10^{-10}$s, $\tau_C' = 1.3 \times 10^{-8}$ s, k_{off} = 3.40 Hz, r' = 3.1Å, r' = 1.98Å, $K_D = 10^{-5}$ μM as listed in Table I. From fig. 3-C, the I(AX) exhibits a small positive intensity indicative of the strong dominant influence of the bound ligand.

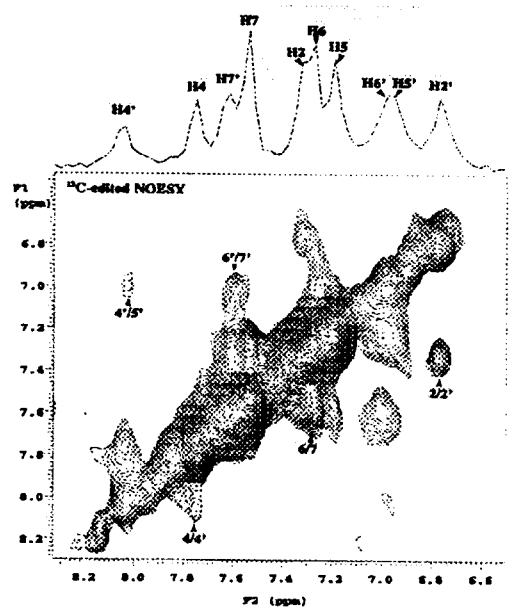

Fig. 1. A 2D slice of ^{13}C-edited 3D-NOESY spectrum of *trp*-repressor/operator/[^{13}C/^{15}N]-L-*trp* complex with a mixing time of 150 ms in 99.99 % ^2H_2O solution. The NMR resonances of the free and bound (primed) corepressor are indicated.

Table I. Parameters for calculation of NOESY cross peaks.

	Free	Bound
Correlation Time(τ_c)	1.5×10^{-10} s	1.3×10^{-8} s[16]
Intramolecular Distance * (r)	3.1 Å	1.98 Å
Exchange off Rate(k_{off})	3.4 s^{-1}	3.4 s^{-1}
Dipolar Relaxation Rate (ρ ,σ)	0.056857, 0.015718	12.312673, -12.281981

* Inter-proton distances were measured between $C^{\alpha}H$ and H4(R) for both free and bound ligands.

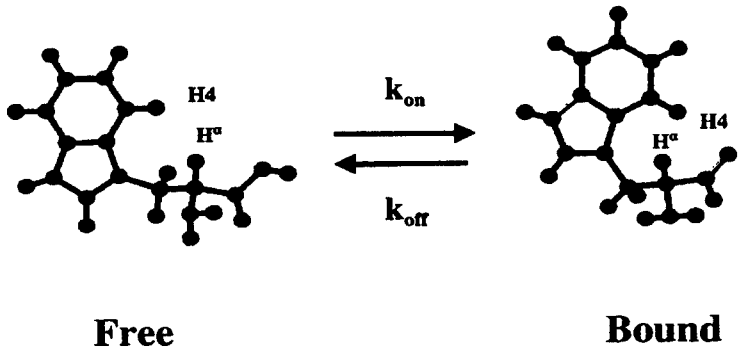

Fig. 2. Structures of the free and bound corepressor L-Trp . Bound corepressor is taken from the NMR structure of *trp*-holo-repressor/operator DNA complex.

This is easily explained from a build-up curve of the direct exchange peak (I(AA′)) in Fig. 3-E, indicating that the fast growing exchange dominates the direct NOE of the free ligand. The exchange-mediated NOE peak (I(AX')) is also influenced by the exchange rate. As the exchange rate increases, intensities of both I(AA') and I(AX') grow rapidly even for small mixing times. The diagonal (I(A'A')) and direct NOE (I(A'X')) of bound ligand are not affected much at the initial stages. However, both peaks are severely influenced at mixing times of greater than 100 ms, demonstrating a slow decay of intensity.

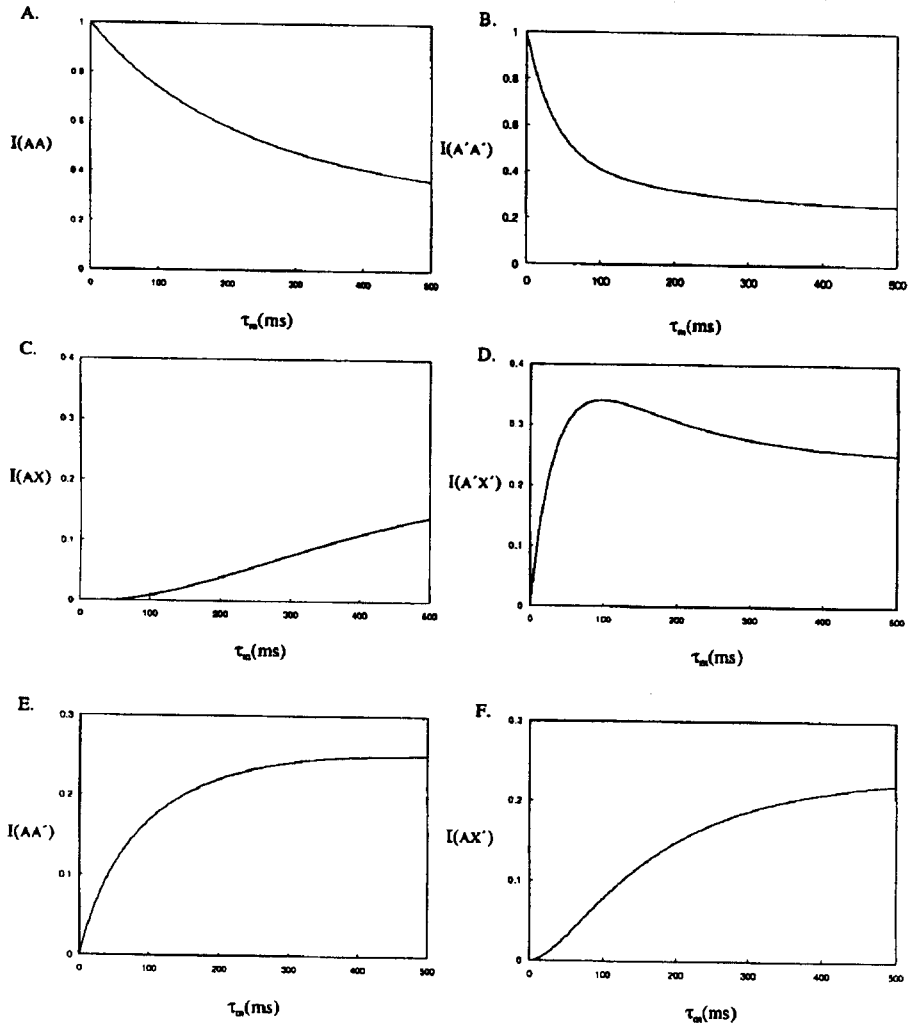

Fig. 3. Cross peak intensity versus mixing times of 2D NOESY spectrum for a corepressor L-trp undergoing exchange between free and bound forms.

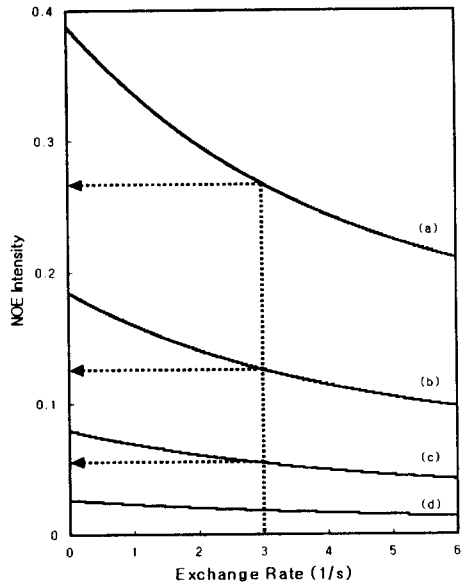

Fig. 4. A plot of NOE intensity versus exchange rate at mixing time of 150 ms. Four distances which are r=2.3 Å(a), 2.8 Å(b), 3.3 Å(c) and 4.0 Å(d) were used for calculations.

Exchange rate effect on NOE intensities for bound corepressor:

In order to consider the influence of the conformational exchange, four distances which are strong (2.3 Å), medium-strong (2.8 Å), medium (3.3 Å)and medium-weak (4.0 Å), have been used for calculations. Fig. 4 shows that the NOE intensities decrease as exchange rate increases for all cases. Especially, the strong NOEs are greatly modulated by exchange effects (Fig. 4-A,B). For the case of r = 2.3 Å and k_{off} = 3.0 Hz, the NOE intensity can be observed to be about 30% less. For a weak NOE, the intensity loss is relatively small, expecting no dramatic change in NOE intensity. However, it is unlikely that we could detect weak or very weak NOEs in the presence of exchange. Therefore, we would have fewer NOEs for the case of conformational exchange.

In conclusion, the exchange effect should be considered properly to extract an accurate distance information of the bound ligand in the presence of conformational exchange.

Acknowledgements

This work was supported by a research grant from Yonsei University of 1997 (to W.T.L). The authors thank TMSI Korea for the use of the molecular simulation programs

(Molecular Modeling Tools, Molecular Simulations Inc.) and Drs. M. Kainosho, N. Utsunomiya-Tate, Y. Miyake and A. Nakamura for the gift of $^{15}N/\ ^{13}C$-L-tryptophan. We also thank Dr. Cheryl H. Arrowsmith for helpful discussions and critical reading of the manuscript.

REFERENCES

1. J. Carey, *Proc. Natl. Acad. Sci. USA* **85**, 975 (1988).
2. L.S. Klig, I. P. Crawford, and C. Yanofsky, *Nucleic Acids Res.* 15, 5339 (1987).
3. C. L. Squires, F. D. Lee, and C. Yanofsky, *J. Mol. Biol.* **92**, 93 (1975).
4. R. P. Gunsalus, and C. Yanofsky, *Proc. Natl. Acad. Sci. USA* 77, 7117 (1980).
5. P. Sarsero, P. J. Wookey, and A. J. Pittard, *J. Bacteriol.* **173**, 4133 (1991).
6. V. M. Heatwole, and R. L. Somerville, *J. Bacteriol.* **173**, 108 (1991).
7. C. H. Arrowsmith, R. Pachter, R. B. Altman, S. Iyer, and O. Jardetzky, *Biochemistry* **29**, 6332 (1990).
8. Zhao, C. H. Arrowsmith, X. Jia, and O. Jardezky, *J. Mol. Biol.* **229**, 735 (1993).
9. H. Zhang, D. Zhao, M. Revington, W. Lee, X. Jia, C. H. Arrowsmith, and O. Jardetzky, *J. Mol. Biol.* **238**, 592 (1994).
10. R. –G. Zhang, A. Joachimiak, C. L. Lawson, R. W. Schevitz, Z. Otwinowski, and P. B. Siger, *Nature* **327**, 591 (1987).
11. B. Hurlburt, and C. Yanofsky, *J. Biol. Chem.* **267**, 16783 (1992).
12. A. N. Lane, J.-f. Lefevre, and O. Jardetzky, *Biochim. Biophys. Acta* **867**, 45 (1986).
13. W. Lee, M. Revington, N. A. Farrow, A. Nakamura, N. Utsunomiyatate, Y. Miyake, M. Kainosho, and C. H. Arrowsmith, *J. Biomol. NMR* **5**, 367 (1995).
14. H. N. B. Moseley, W. Lee, C. H. Arrowsmith, and N. R. Krishna, *Biochemistry* **36**, 5293 (1997).
15. W. Lee, and N. R. Krishna, *J. Magn. Reson.* **98**, 36 (1992).
16. X. Shan, K. Gardner, R. Muhandiram, N. Rao, C.H. Arrowsmith, and L. E. Kay, *J. Am. Chem. Soc.* **118**, 6570 (1996).