Loss of Hydrogen Bond in the Transition State of NtrC Leads to Altered Dynamics

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Introduction

Molecular Dynamics simulations were previously used to determine interactions that might play important rate-limiting steps in the interconversion between active and inactive forms of $NtrC^R$. One of the interactions that were found involves a hydrogen bond formed only in the transition state. Mutation of the hydrogen bond donor (serine) to either glycine or aspartate should stop formation of the bond, leading to a reduction in the rate of interconversion compared to wildtype if this hydrogen bond is a rate-limiting step in the transition.

Experimental

^{15}N-CPMG experiments performed on the NHMFL 600 MHz solution magnet were used to determine the rate of exchange between the active and inactive forms of $NtrC^R$. Dispersion curves were fit to the fast form of the Carver-Richards equation:

$$R_2^{eff}(\nu_{CPMG}) = R_{20}(\nu_{CPMG\rightarrow\infty}) + (p_A p_B \Delta\omega^2/k_{ex})[1-(4\nu_{CPMG}/k_{ex})\tanh(k_{ex}/4\nu_{CPMG})] \quad [1]$$

Results and Discussion

Dynamics of both the SG and SD mutants show a decrease in the rate of exchange, fit globally to 3200 +/- 900 sec^{-1} and 2900 +/- 200 sec^{-1} respectively, down from the 13,500 +/- 900 sec^{-1} seen in wildtype $NtrC^R$. (Fig. 1)

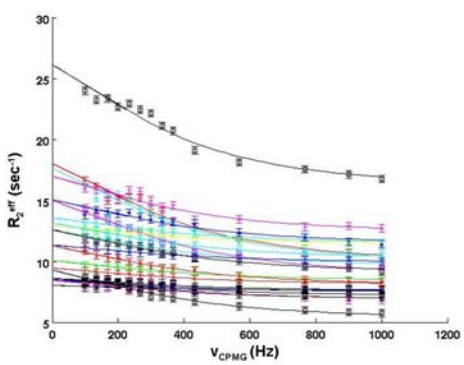

Fig. 1- ^{15}N-CPMG dispersion curves of SD $NtrC^R$

Conclusions

This hydrogen bond discovered through Molecular Dynamics simulations and tested with ^{15}N-CPMG NMR experiments seems to be an important rate-limiting step in the transition between inactive and active $NtrC^R$.

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References

[1] Volkman, B.F. *et al*., Science, **291**, 2429-2433 (2001).
[2] Luz, Z. *et al*., J Chem Phys, **39**, 366- (1963).
[3] Gardino, A.K. *et al*., Methods Enzymol, **423**,149-165 (2007).