Protein Dynamics Control the Progression and Efficiency of the Catalytic Reaction Cycle of AlkB

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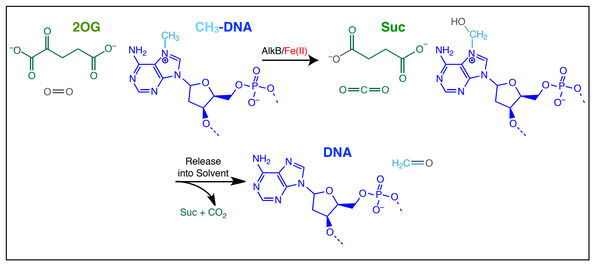
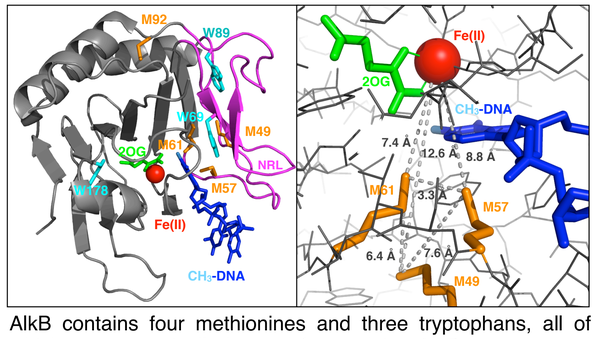
Abstract

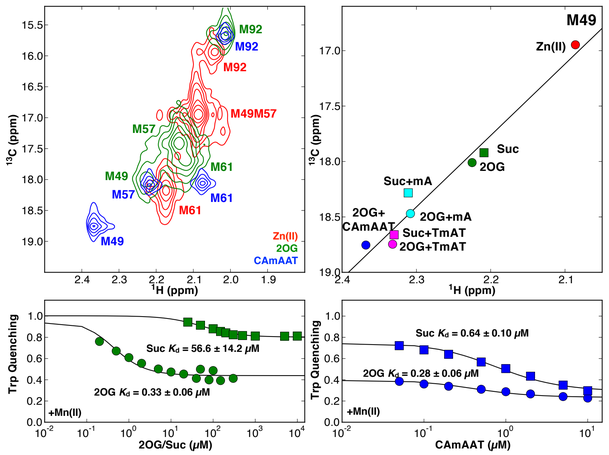
A central goal of enzymology is to understand how catalytic efficiency is controlled by the physical properties of proteins. A combination of enzymological and spectroscopic methods have been used to establish that a dynamic transition controls the progression and efficiency of the catalytic reaction cycle of the DNA/RNA-repair enzyme AlkB, which belongs to the iron/2-oxoglutarate (Fe(II)/2OG) dependent dioxygenase superfamily [1–4]. AlkB uses an Fe(II) cofactor and 2OG co-substrate to activate molecular oxygen (O_2) for the dealkylation of a methylated nucleobase [5–7]. The nucleobase substrate must bind after Fe(II)/2OG to avoid occlusion of their binding sites [8]. Reaction of O_2 with 2OG generates succinate (Suc) and an oxyferryl intermediate [9–13] that slowly hydroxylates the alkylated substrate [14, 15]. The substrate must remain bound during the oxidation reaction to prevent quenching of the oxyferryl intermediate. Fluorescence spectroscopy data and NMR chemical shift and relaxation data demonstrate that a microsecond-to-millisecond timescale conformational transition in the nucleotide-recognition lid (NRL) of AlkB is critical to the order of substrate/co-factor binding, progress of the multistep reaction, and timing of product release. Enzymological and spectroscopic data demonstrate mutations biasing the NRL towards the open state promote premature substrate release and uncoupled 2OG turnover. Therefore, the conformational properties and rate of a specific dynamic transition control the efficiency of the multistep catalytic reaction cycle of AlkB, providing a clear example of the importance of protein dynamics in enzyme function.



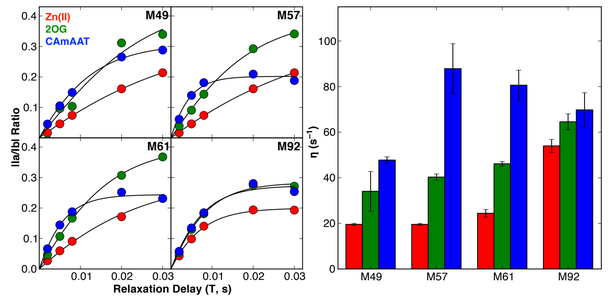


AlkB contains four methionines and three tryptophans, all of which are strategically located for studying AlkB structure and dynamics. Three of the methionines (M49, M57, and M61) and one of the tryptophans (W69) are located in the NRL. M92 and W89 are located in the hinge of the NRL, and W178 is in a cavity near the catalytic core.

Results

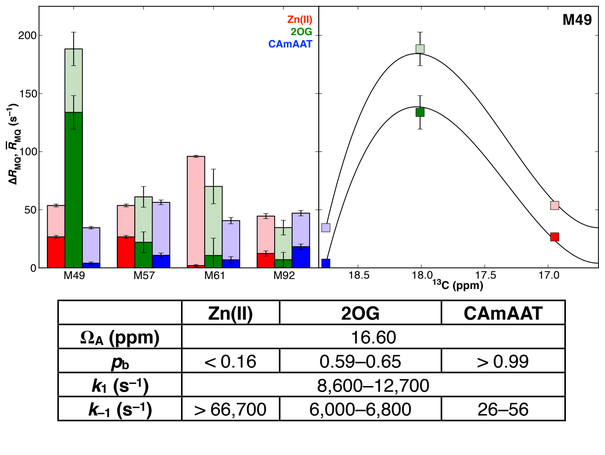


AlkB $^{13}C^{\varepsilon}$-methionine chemical shifts are consistent with conformational exchange and stabilization of the closed form by cofactor and substrate addition. Tryptophan quenching confirms AlkB has a greater affinity for 2OG than Suc and for methylated substrate in the presence of 2OG relative to Suc. The DNA oligos dC-dA-(1-me-dA)-dA-dT, dT-(1-me-dA)-dT, and the monomer N1-methyladenosine-5'-triphosphate are abbreviated as CAmAAT, TmAT, and mA, respectively. Mn(II) and Zn(II) are used as catalytically inactive, and diamagnetic in the case of Zn(II), substitutes for Fe(II). All titrations are successive with metal, 2OG/Suc and then DNA substrate being added subsequently to the previous complex.



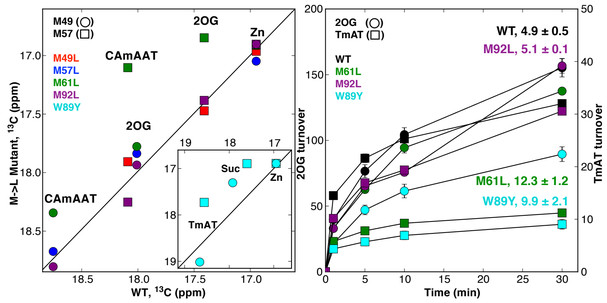
$$\left|\frac{I_a}{I_b}\right| = \frac{-0.5\eta\tanh(\sqrt{\eta^2+\delta^2}T)}{\sqrt{\eta^2+\delta^2}-\delta\tanh(\sqrt{\eta^2+\delta^2}T)} \qquad \eta \approx \frac{9}{10}[P_2(\cos\theta_{axis,HH})]^2 \frac{S^2_{axis}\gamma^4_H\hbar^2\tau_C}{r^6_{HH}}$$

Fast timescale (ps—ns) dynamics of $^{13}C^{\varepsilon}$-methionine were measured using a ^1H–^1H cross-correlated relaxation experiment (CCR) [16]. The ^1H–^1H CCR interference rate constant, η, is proportional to $S^2_{axis} \times \tau_C$, with S^2_{axis} being the generalized order parameter of the methyl symmetry axis and τ_C the protein rotational correlation time. For M49, M57, and M61, the value of η increases throughout the titration, which is consistent with rigidification of the methionine sidechains as 2OG and then CAmAAT are added.



	Zn(II)	2OG	CAmAAT
Ω_A (ppm)		16.60	
p_b	< 0.16	0.59–0.65	> 0.99
k_1 (s^{-1})		8,600–12,700	
k_{-1} (s^{-1})	> 66,700	6,000–6,800	26–56

A Hahn echo experiment [17] was used to measure the zero- and double-quantum relaxation rate constants, R_{ZQ} and R_{DQ}, which were used to calculate $\bar{R}_{MQ} = (R_{DQ} + R_{ZQ})/2$ (translucent) and $\Delta R_{MQ} = (R_{DQ} - R_{ZQ})/2$ (solid). The open and closed populations (p_a and p_b, respectively), opening rate (k_{-1}), closing rate (k_1), and chemical shift of the open state (Ω_A) were estimated by fitting the parameters of the conformational exchange equation, $R_2 = R_{20} + p_a p_b \Delta\omega^2/k_{ex}$, where $k_{ex} = k_1/p_b$, to $\bar{R}_{MQ}$ and ΔR_{MQ} for M49. The DNA-bound form was assumed to represent the closed form, i.e. p_b(DNA) = 1.0 and Ω_B = 18.8 ppm were constant in the regression. It was assumed k_1 remains unchanged throughout the titration.



Mutations affecting enzyme dynamics promote catalytic uncoupling. The ^{13}C chemical shifts of M49 (left panel, circles) and M57 (left panel, squares) in AlkB deviate from those of WT for mutations (M61L, green and W89Y, cyan) that affect the ratio of 2OG decarboxylation (right panel, circles) to TmAT dealkylation (right panel, squares). For M49L (red), M57L (blue), and M92L (purple), where the turnover ratio is similar to WT, the ^{13}C chemical shifts are linearly correlated with those of WT. For W89Y, Suc and TmAT were used for the chemical shift correlation and data are compared to chemical shifts of the identical WT complexes.

Conclusions

AlkB undergoes a conformational exchange involving opening and closing of the NRL that is fast on the NMR chemical shift timescale. Chemical shift and spin relaxation data demonstrate the closed conformer is favored by the successive binding of metal cofactor and substrates. Mutations which affect the ratios of the two populations, as determined by ^{13}C chemical shift perturbation, also lead to decoupling of 2OG and DNA turnover rates. These results indicate the kinetics of the conformational transition that gates cofactor and substrate binding are also important for the efficiency of the enzyme's multistep catalytic reaction cycle. Dynamic transitions of this kind are likely to control the catalytic efficiency of other Fe(II)/2OG dioxygenases as well as many other enzyme families performing complex biomolecular reactions.

Acknowledgements

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