NMR Dynamics Studies of Low-Affinity Drug Interaction with Proteins: Experimental Validation of New Protein Theory of General Anesthesia

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Introduction: We recently proposed a new theory of low-affinity drug action on proteins by considering the drug effects on protein global dynamics at the tertiary or quaternary structure level without necessarily involving drug binding to any fixed protein pockets (Tang & Xu, PNAS, 99:16035, 2002). Here, we provide direct evidence in support of the theory from the experimental and computational studies of a structurally well characterized dimeric enzyme, ketosteroid isomerase (KSI). The structure of KSI has been solved to the atomic resolution by NMR. We have found that its enzymatic activity is sensitive to several volatile anesthetics. High-resolution NMR and molecular dynamics simulations were performed on KSI in the presence and the absence of halothane to characterize the KSI-halothane interaction, the interaction-induced structural and dynamical changes, and the potential impacts that these changes have on the enzymatic activity of KSI.

Methods: ^{15}N labeled KSI was expressed in the BL21(DE3) stain of bacteria *Escherichia coli* using M9 minimal medium and purified using the DEAE-sephacel anion exchange column. Its enzymatic activity was measured with the substrate 5-androsten-3,17-dione (Steraloids Inc, Newport RI) using a standard protocol. ^{1}H-^{15}N HSQC experiments and dynamics measurements (R1, R2, and NOE of backbone ^{15}N) of KSI were conducted on 400 MHz and 600 MHz NMR spectrometers. The dynamics data were analyzed using Modelfree program. For molecular dynamics (MD) simulations, four halothane molecules were docked in a fully solvated dimeric KSI (1BUQ) using the program AUTODOCK3.0. MD simulations were carried out on two parallel systems (with and without halothane) using the NAMD program on *Jonas* at the Pittsburgh Supper Computer Center.

Results and Discussion: Significant chemical shift changes in ^{1}H-^{15}N HSQC spectra due to the presence of halothane were observed predominately for those residues that reside on the dimer interface (Figure 1), indicating the preference of halothane to the amphipathic sites in KSI. Unbiased halothane docking in KSI yielded the consistent results in agreement with the NMR finding. Of 300 separate and random dockings with Lamarckian genetic algorithm, 96% showed that halothane was situated in three well defined amphipathic sites at the dimer interface. One of these sites at the center of the interface is highly preferred (Figure 1), accounting for up to 86% of docking appearances. The effects of halothane on KSI dynamics were determined by both the KSI backbone RMSF from MD simulations and from the KSI order parameters measured by NMR experiments (Figure 2). It was clear that the presence of halothane altered the KSI flexibility at the interfacing β strands at the dimer interface without affecting the KSI structure. The dynamics changes are allosterically transmitted to the substrate binding site on the opposite face of the β strand network, altering the enzymatic activity of KSI.

Conclusion: Anesthetics such as halothane can alter KSI's enzymatic activity without directly binding to, or competing with the substrate for, the active sites in the enzyme. Because the dimerization of KSI is essential for its stability in the aqueous environment, the action of halothane on KSI is apparently via the allosteric modulation of the KSI stability at the dimer interface. Such anesthetic effects on protein global dynamics at the tertiary or quaternary structure level may be important and universal as an underlying mechanism of general anesthesia.

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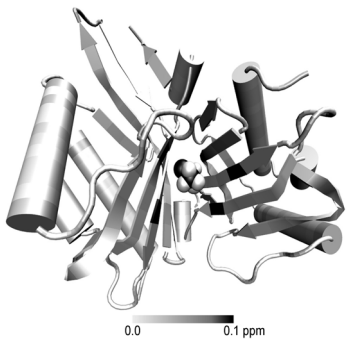

Fig.1 Halothane-induced changes in KSI backbone amide ^{1}H and ^{15}N NMR chemical shifts are highlighted using the gray scale as indicated. The darker color represents greater changes in chemical shifts. A halothane molecule is shown in the middle of dimer interface, where majority of auto-docking results are found. NMR and computational results are consistent and indicate that anesthetics favor the amphipathic sites at the dimer interface instead of the enzymatic active sites.

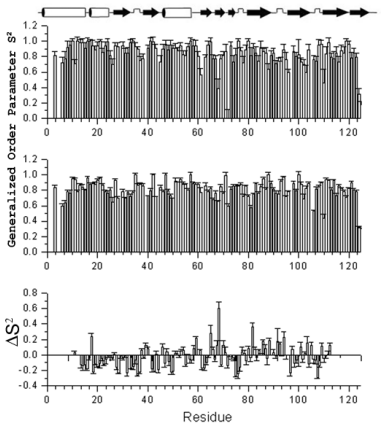

Fig. 2 NMR-determined general order parameter S^2 in the absence (top panel) and presence (middle panel) of halothane are plotted as a function of residue number. The S^2 differences are shown in the bottom panel.