Hinge-bending motion in S-adenosyl-L-homocysteine hydrolase: mutagenesis, fluorescence and modeling studies

Mengmeng Wang[1], Chen Hu[1], Jay Unruh[2], Carey K. Johnson[2], Richard L. Schowen[3], Ronald T. Borchadt[3] and Krzysztof Kuczera[1,2]

Departments of [1]Molecular Biosciences, [2]Chemistry and [3]Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045

S-adenosyl-L-homocysteine hydrolase (SAHH) is a crucial enzyme in transmethylation reactions. This homotetrameric protein exists in the open conformer in absence of susbstrate, while enzyme:inhibitor complexes populate the closed structure, with the ligand engulfed by an 18^o domain reorientation. To study the nature and function of these domain reorientations, we have characterized 8 singe site-directed point mutants in the SAHH hinge region. For three mutants, M351P, H353A, and P354A, which retained the quaternary structure and catalytic activity of the wild type, reorientational motions were characterized by time-resolved fluorescence anisotropy measurements. In wild type, substrate-free SAHH, domain reorientations were detected on a time scale of 10-20 ns. Fluorescence studies with several ligands showed that the enzyme exists in an equilibrium of open and closed structures, and that both binding and oxidation of the ligand are needed to shift the equlibrium toward the immobile closed form. A similar pattern was observed for M351P and P354A mutants, while the H353A protein appeared to favor the closed form of the enzyme. Normal mode analysis of the tetrameric protein showed markedly different mechanical properties of the two conformers, with the open form exhibiting vibrations along the open-to-closed direction, while the closed form vibrations coupled different domains and subunits, suggesting a possible mechanism for enzyme cooperativity. Finally, our 15 ns molecular dynamics trajectory of unliganded SAHH showed domain reorientations by up to 12^o on a time scale of 8-16 ns, in excellent agreement with experiments. Interestingly, the trajectory motions have components of comparable magnitude parallel and perpendicular to the open-to-closed conformational transition. Overall, our studies have identified a crucial hinge region in the SAHH enzyme and characterized the physical nature and function of its large-amplitude domain motions. While normal mode analysis predicted high frequency (20 ps) and low amplitude (1^o) domain motions, the molecular dynamics results were more realistic, showing presence of both pico- and nanosecond reorientations, with the time scales and amplitudes of the latter in accord with our fluorescence measurements.