Abstract

This thesis investigates the structural and dynamical effects of glycosylation and dimerization on the catalytic domain of human carbonic anhydrase IX (HCA IX-c), a cancer-associated enzyme that plays a key role in pH regulation under hypoxic tumor conditions. Despite extensive studies on the HCAs, the molecular mechanisms by which glycosylation and oligomerization influence enzyme dynamics and catalysis remain incompletely understood. To address this, extensive classical molecular dynamics (MD) simulations combined with enhanced sampling techniques and kinetic modeling approaches were employed to explore the conformational landscape of glycosylated and un-glycosylated HCA IX-c in both monomeric and dimeric forms. The study characterizes the flexibility and conformational diversity of the N-linked glycan chains and examines their coupling with protein dynamics. Results reveal that glycosylation does not significantly alter the overall protein fold but modulates conformational fluctuations, active-site organization, and hydrogen-bonding networks. Free energy disconnectivity graphs were further utilized to map the underlying energy landscape, enabling identification of kinetically connected conformational basins and transition pathways of the glycan chains. Particular emphasis is placed on the proton-shuttling residue His-64 and the surrounding water network, which are critical for catalytic activity. The simulations demonstrate that glycosylation and dimerization influence the orientation dynamics of His-64 sidechain and the formation of the water-mediated proton-transfer pathways. Dimerization further enhances structural stability, introduces cooperative dynamics, and promotes the formation of structured hydration networks that may facilitate catalysis. These findings highlight glycosylation and dimerization as key regulators of enzyme dynamics, contributing to the modulation of active-site accessibility, conformational flexibility, and catalytic efficiency. This work provides atomistic insights into the structure-function relationship of HCA IX and offers a foundation for the rational design of isozyme-specific inhibitors targeting cancer-associated HCAs.

Keywords: Human Carbonic Anhydrase IX, Catalytic Domain, Markov State Model, Free Energy Disconnectivity Graphs, Molecular Dynamics Simulations.