Abstract

The primary aim of this thesis is to examine the effects of some structural and environmental factors on selected non-reactive steps in the catalysis by the enzyme human carbonic anhydrase II (HCA II). We have studied two important contributions to the rate determining intra-molecular proton transfer step in the catalysis by HCA II, including (a) reorganization of active site water molecules to form the proton transfer path and (b) sidechain rotation of His-64 that shuttles an excess proton out of the active site. Classical molecular dynamics (MD) simulation has been used in this thesis in combination with enhanced sampling methods and Markov state models to obtain the mechanism, free energies and kinetics of the processes investigated.

The first part of the thesis consists of two chapters where we have focused on the effects of partial unfolding and variable protonation states of titrable residues on the structure and functional dynamics of HCA II. In the second part, we focus on the interaction between HCA II and three known inhibitor molecules. First, the dynamics of repeated unbinding/rebinding events between the respective crystallographic binding location and other sites on the enzyme surface are investigated. The associated binding free energies are then estimated using MM/GBSA method to understand the thermodynamics of binding between these sites. Finally, a deep comparative analysis of the free energy and kinetics of the (un)binding events of three inhibitors has been undertaken using Markov state models to highlight how the structure of inhibitors and their interactions with the enzyme govern different mechanisms of inhibition of HCA II.

Keywords: Human Carbonic Anhydrase II, Unfolding, Multiple Protonation States, Ligand Unbinding, MM/GBSA, Markov State Model.