

Poster Presentation

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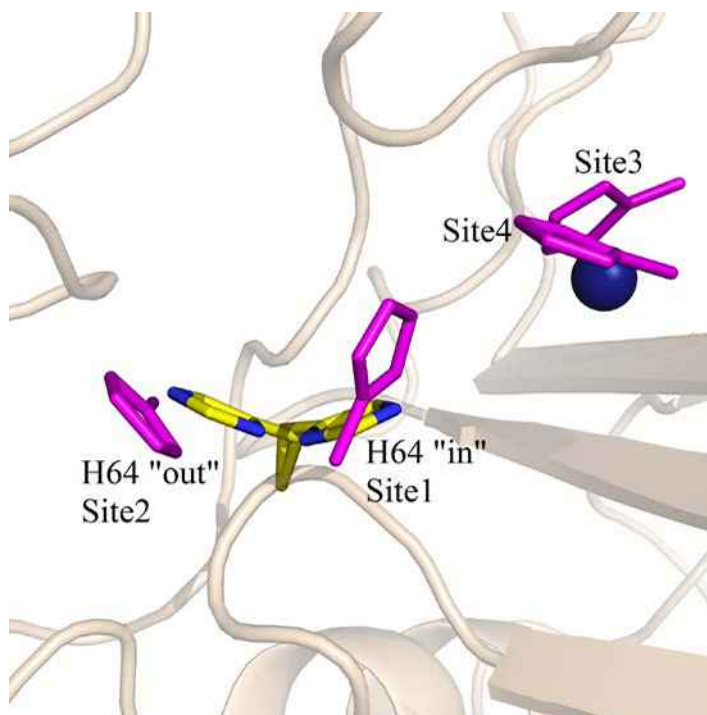
Insights into Activity Enhancement of H64A Carbonic Anhydrase by Imidazoles

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Human carbonic anhydrases (CAs) are zinc metalloenzymes that catalyze the hydration and dehydration of CO₂ and HCO₃⁻, respectively. The reaction follows a ping-pong mechanism, where the rate limiting step is the transfer of a proton from the zinc-bound solvent out of the active site, via His64 which is widely believed to be the proton shuttling residue. Being involved in a number of physiological processes such as respiration, pH regulation, ureagenesis etc., CAs are therapeutic targets for inhibition to treat various diseases. However, the physiologically dominant isoform is CA II, which is catalytically highly efficient and is easily crystallizable. Thus, most of our knowledge in the design of CA inhibitors with pharmacological applications is based on detailed CA II crystallographic studies. The catalytic activity of a variant of CA II in which His64 is replaced with Ala (H64A CA II) can be enhanced by exogenous proton donors/acceptors, usually derivatives of imidazoles and pyridines. This article examines the mechanism through which this activity enhancement might occur. X-ray crystal structures of H64A CA II in complex with four imidazole derivatives have been determined and reveal multiple binding sites. We have identified two molecules of imidazoles that bind in region that is otherwise occupied by the "in" and "out" dual conformation of the side chain of His64 in wild-type CA II. The data presented here not only corroborates the importance of imidazole side chain of His64 in proton transfer during CA catalysis, but also provides a complete structural understanding of the mechanism by which imidazoles enhance (and inhibit when used in higher concentrations) the activity of H64A CA II. In addition to inhibition of CA by these imidazoles, the presence of a large number of binding sites also gives insights and preliminary data required to fragment addition approach of drug design against CA.

[1] Silverman, D. N. & McKenna, R. (2007). *Acc. Chem. Res.* 40, 669–675., [2] Duda, D., Tu, C., Qian, M., Laipis, P., Agbandje-McKenna, M., Silverman, D. N., & McKenna, R. (2001). *Biochemistry.* 40, 1741–1748., [3] Elder, I., Tu, C., Ming, L.-J., McKenna, R., & Silverman, D. N. (2005). *Arch. Biochem. Biophys.* 437, 106–114.



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