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Title of PhD thesis: Structural Studies on Carbonic Anhydrases: Comparisons of Stability in Extremes of pH and Chemical Denaturants

The carbonic anhydrases (CAs, EC 4.2.1.1) are zinc containing metallo-enzyme, which efficiently catalyze the reversible conversion of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and release the proton. These enzymes are essentially important for biological system and play important physiological functions. CAs are involved in pH regulation, ion transport, bone resorption, electrolyte secretion, CO_2 fixation and biosynthetic reactions such as gluconeogenesis, lipogenesis and ureagenesis. CAs are present in all form of life kingdoms from unicellular to multicellular (Bacteria, Archaea, and Eukarya). . In human, there are 16 different CAs are reported and all belongs to the α-family, among them 13 are enzymatically active (CAI, CAII, CAIII, CAIV, CAVA, CAVB, CAVI, CAVII, CAIX, CAXII, CAXIII, CAXIV, and CAXV). While three isoforms (CAVIII, CAX and CAXI) which are named as carbonic anhydrase related proteins (CARPs) found to be enzymatically inactive.

For structural, biophysical characterization and structure-based drug design, three different human CAs were selected. These enzymes are mitochondrial carbonic anhydrase VA (CAVA) carbonic anhydrase IX (CAIX) and carbonic anhydrase II (CAII). All three CAs are important enzymes involved in various critical physiological functions. But little structural and biophysical information know about two CAVA and CAIX enzymes. Therefore, protein folding studies have been carried on these two important enzymes. Protein folding studies have been performed using biophysical techniques, like circular dichroism (CD), fluorescence spectroscopy and UV-VIS spectroscopy. Further, molecular dynamic (MD) simulations have also been done to characterize the folding behavior of CAs.

The CAs enzymes are attractive and validated drug targets for several diseases, since numerous symptoms are directly related to an abnormal activity of these proteins. CAII, CAVA and CAIX are involved in various diseases such as glaucoma, cancer, obesity, osteopetrosis, epilepsy, retinitis pigmentosa, edema and etc. Thus, further structure based drug designing and biological

evaluation of designed drugs studies have been carried out to find potential ligands which can be a possible inhibitor of CAs. Binding affinity of designed drugs has been calculated by fluorescence spectroscopy. CAs inhibition assay studies also carried out.

CAVA has been cloned, expressed and purified in bacterial system for biophysical studies. CAVA showed aggregation in the acidic pH range However, it was stable and active in neutral and alkaline pH range. A reversible guanidinium chloride (GdmCl) and urea induced isothermal denaturation of CAVA carried and estimated ΔG_D^0, Cm, and m. A coincidence of normalized transition curves of all optical properties suggests that unfolding/refolding of CAVA in urea is a two-state process. However, in the presence of GdmCl unfolding/refolding of CAVA is tri phasic. An intermediate (PMG) was observed and well characterized.

A reversible GdmCl and urea-induced isothermal denaturation of CAIX have been also carried out. CAIX also has been cloned, expressed and purified. The GdmCl and urea-induced reversible denaturation curves were used to estimate ΔG_D^0, *C*m and m. A coincidence of normalized transition curves of all optical properties suggests that unfolding/refolding of CAIX is a two-state process against both GdmCl and urea.

Further structure based drugs were designed and synthesized against CAII and CAIX. For chemical evaluation of designed drugs, CAII also has been cloned, expressed and purified. The evaluation of designed drugs has been carried out to find potential ligands which can be a possible inhibitor of CAs. Binding affinity of designed drugs has been calculated by and fluorescence spectroscopy.

Structure-based drug design studies suggest that synthesised 1-(3-(phenyl/4-fluorophenyl)-7-imino-3H-[1,2,3] triazolo[4,5d] pyrimidin6(7H)yl) urea (TPUI) appeared as novel class of CAIX inhibitor, and may be used as a lead molecule for the development of potent and selective CAIX inhibitor for the hypoxia-induced cancer therapy.

Another a series of novel 2-(4-(4-substituted piperazin-1-yl)benzylidene) hydrazinecarboxamide derivatives has also synthesized and evaluated their potential as CAII inhibitors.