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Role of Functional Divergence in Specific Inhibitor Design: Using γ-Glutamyltranspeptidase (GGT) as a Model Protein

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Abstract : γ-glutamyltranspeptidase (GGT: EC 2.3.2.2) is an N-terminal nucleophile hydrolase conserved in all three domains of life. GGT plays a key role in glutathione metabolism where it catalyzes the breakage of the γ-glutamyl bonds and transfer of γ-glutamyl group to water (hydrolytic activity) or amino acids or short peptides (transpeptidase activity). GGTs from bacteria, archaea, and eukaryotes (human, rat and mouse) are homologous proteins sharing >50% sequence similarity and conserved four layered αββα sandwich like three dimensional structural fold. These proteins though similar in their structure to each other, are quite diverse in their enzyme activity: some GGTs are better at hydrolysis reactions but poor in transpeptidase activity, whereas many others may show opposite behaviour. GGT is known to be involved in various diseases like asthma, parkinson, arthritis, and gastric cancer. Its inhibition prior to chemotherapy treatments has been shown to sensitize tumours to the treatment. Microbial GGT is known to be a virulence factor too, important for the colonization of bacteria in host. However, all known inhibitors (mimics of its native substrate, glutamate) are highly toxic because they interfere with other enzyme pathways. However, a few successful efforts have been reported previously in designing species specific inhibitors. We aim to leverage the diversity seen in GGT family (pathogen vs. eukaryotes) for designing specific inhibitors. Thus, in the present study, we have used DIVERGE software to identify sites in GGT proteins, which are crucial for the functional and structural divergence of these proteins. Since, type II divergence sites vary in clade specific manner, so type II divergent sites were our focus of interest throughout the study. Type II divergent sites were identified for pathogen vs. eukaryotes clusters and sites were marked on clade specific representative structures HpGGT (2QM6) and HmGGT (4ZCG) of pathogen and eukaryotes clade respectively. The crucial divergent sites within 15 A radii of the binding cavity were highlighted, and in-silico mutations were performed on these sites to delineate the role of these sites on the mechanism of catalysis and protein folding. Further, the amino acid network (AAN) analysis was also performed by Cytoscape to delineate assortative mixing for cavity divergent sites which could strengthen our hypothesis. Additionally, molecular dynamics simulations were performed for wild complexes and mutant complexes close to physiological conditions (pH 7.0, 0.1 M ionic strength and 1 atm pressure) and the role of putative divergence sites and structural integrities of the homologous proteins have been analysed. The dynamics data were scrutinized in terms of RMSD, RMSF, non-native H-bonds and salt bridges. The RMSD, RMSF fluctuations of proteins complexes are compared, and the changes at protein ligand binding sites were highlighted. The outcomes of our study highlighted some crucial divergent sites which could be used for novel inhibitors designing in a species-specific manner. Since, for drug development, it is challenging to design novel drug by targeting similar protein which exists in eukaryotes, so this study could set up an initial platform to overcome this challenge and help to deduce the more effective targets for novel drug discovery.

Keywords : γ-glutamyltranspeptidase, divergence, species-specific, drug design
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