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Towards enhancing L-Asparaginase stability by computational-structural modeling

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Abstract

L-asparaginase enzymes are widely used for the treatment of lymphoblastic leukemia. The extracellular and glutaminase-free asparaginases have been used for leukemia treatment [1, 2]. Asparaginase folds as homotetramer with four active sites. The enzyme active sites contain five residues, Thr12, Tyr25, Thr89, Asp90, and Lys162 (according to *E.coli* numbering) [1, 3]. Two therapeutically important sources are *Escherichia coli* and *Erwinia chrysanthemi* [1]. To enhance stability, the enzymes need to be optimized by protein engineering [2]. In this study, structural alignment and electrostatic potential maps were used to examine structural features of the L-asparaginase active site in the two most important medicinal species to enhance enzymatic stability.

Material & Methods

The structures of *E.coli* and *Erwinia chrysanthemi* enzymes (PDB ID: 3ECA, 5F52 respectively) were obtained from Protein Data Bank (PDB) [4, 5]. Structural alignment has been done with PyMol Molecular Graphics. Molecular preparation for Poisson-Boltzmann calculations was obtained from the pdb2pqr server [6, 7]. For APBS analysis, the PyMol APSB plugin determined the electrostatic properties of the enzyme.

Results

APBS results show the negative overall electrostatic potential of the *E.coli* active site, while it shows a positive trend in *Erwinia chrysanthemi*. The negatively charged pockets of *E.coli* contain six Asp and one Glu, and *Erwinia* positively charged ones have two His, eight Arg, and three Lys. However, the main interactions of the active sites remain intact in both enzymes that occur through polar interactions. Ligand has 11 polar interactions in the active site with residues; Thr12, Tyr25, Ser58, Gln59, His87, Thr89, Asp90, Thr91, Lys162, Arg248, and Glu283.

Conclusions

Although the essential interactions in these two medical enzymes are the same, the differently charged binding pockets could be a promising target for further enzyme optimization designs.

Key Words: L-Asparaginase, Leukemia, Binding pocket, Protein engineering, Structural modeling

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