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Molecular modelling, docking and interaction studies of human-plasmogen and *salmonella*-enolase with enolase inhibitors

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Abstract:
Salmonella enteric serovar Typhi Ty2 is a human specific pathogen and an etiological agent for typhoid fever. Most of Salmonella serotypes produce glycogen which has a comparatively minor role in virulence and colonization, but has a more significant role in survival. Enzymes present in glycolytic pathway of bacteria help bacteria to survive by activating other factors inside host. Numerous pathogenic bacteria species intervene with the plasminogen system, and this plasminogen–enolase association may play a critical role in the virulence of *S. Typhi* by causing direct damage to the host cell extracellular matrix, possibly by enzymic degradation of extracellular matrix proteins or other protein constituents. In this study, molecular modelling of enolase of Salmonella has been accomplished *in silico* by comparative modelling; we have then analyzed Human alpha enolase which is a homodimer and serves on epithelial cells with our model. Both Structures were docked by D-tartronate semialdehyde phosphate (TSP) and 3-aminoenolpyruvate phosphate (AEP) enolase inhibitors. Our study shows that salmonella enolase and human enolase have different active sites in their structure. This will help in development of new ligands, more suitable for inhibiting bacterial survival inside host as vaccines for typhoid fever are not fully protective. The study also confirmed that enolase Salmonella and Human Plasminogen suggested direct physical interaction between both of them as the activation loop of plasminogen residues showed conformational changes similar to the tissue type plasminogen activator. Various computational biology tools were used for our present study such as Modeller, Molegro Virtual Docker, Grommacs.

Keywords: Salmonella Ty2, Enolase, TSP (D-tartronate semialdehyde phosphate), AEP (3-aminoenolpyruvate phosphate), Modelling, Docking.

Background:
Pathogenesis of *S. Typhi* is not completely understood. The treatment of typhoid fever is complicated by the emergence of drug resistance. Effectiveness of currently available vaccines is also limited. The major shortcomings of the live vaccine are the cost and requirement of multiple doses which do not enhance protection. Further, memory cells are not generated which also fails to induce intestinal secretory IgA response. Approximately 21 million cases are estimated, resulting in 216,519 deaths in the year 2000. More than half of all Salmonella enterica serovar Typhi genes still remain unannotated. Enolase is a ubiquitous enzyme that catalyzes the reversible conversion of 2-phosphoglycerate (2-PGE) to phosphoenolpyruvate (PEP). In addition to its metabolic role, **[1]** enolase has been implicated for its contribution to several biological and pathophysiological processes by acting as a heat

shock protein and in modulating gene transcription, as well as for its involvement in microbial diseases and autoimmunity gene. This implies that enolase is not a housekeeping gene since; its expression varies according to the pathophysciologically metabolic or development condition of cell **[2-5].** The presence of α-enolase on the surface of bacteria adds a new insight in the generation of antibodies against enolase, post infection. Numerous pathogenic bacterial species intervene with the plasminogen system and a hypothesis has emerged that bacteria use this system for migration across tissue barriers or for nutritional demands during infection. Cell-surface protein-mediated interactions are known to play a major role in disease-progression. In various pathogenic systems, including bacteria, fungi and protozoa, the invasive phenotype has been correlated with the ability of the organism to bind to laminin, an abundant extracellular matrix glycoprotein. For an in-silico validation of this hypothesis, a 3-D model of salmonella enolase has been constructed, considering enolase-palsminogen interaction between salmonella and human plasminogen. Structure based comparative analyses of Salmonella enolase and Human alpha enolase was performed in which different active residues and different active pockets in both structures were found. Both molecules were docked with enolase inhibitors, TSP (D-tartronate semialdehyde phosphate) and AEP (3-aminoenolpyruvate phosphate) **[6]** in order to inhibit salmonella's survival mechanism inside the host. This work will prove to be strategic for development of new inhibitors for Salmonella Tphi Ty2.

Invasive bacteria have evolved virulence strategies to interact with host hemostatic factors such as plasminogen and fibrinogen for infection. Different bacterial species gain access to the human body through different sites, such as the skin, nasopharynx, lungs, gastrointestinal, or urogenital tract. Bacterial invasion is generally mediated by bacterial surface and secreted products that can negate host innate and acquired defense systems.**[7]** Several gram-positive and gram-negative invasive bacterial pathogens have been found to express a plasminogen receptor (PlgR) function. These bacteria immobilize plasminogen on their cell surfaces and enhance the tPA catalyzed plasminogen activation. The bacterial plasminogen receptor functions to generate proteolytic activity on the bacterial surface by utilizing a host-derived proteolytic system**. [8]** *Salmonella enterica* have been identified as PlgRs Bacterial enzymes acting directly on mammalian extra cellular matrix (ECM) or activating on latent procollagenases. It is an established fact that plasmin degrades noncollagenous proteins of ECM, such as laminin, and activates latent procollagenases. It has also been proposed that one function of bacterial PlgRs is to potentiate bacterial damage to and bacterial spread through tissue barriers, such as basement membranes**. [9, 10**] *In vitro* tissue culture studies have identified some of the host cell responses that lead to Salmonella entry including actin rearrangement and polymerization at host cell membrane and accumulation of cytoskeleton protein at the site of bacterial entry **[11].** Most Salmonella serotypes produce glycogen playing a comparatively minor role in virulence and colonization, but having a more significant role in survival. Enzymes present in glycolytic pathway of bacteria helps in survival by activating other factors which leads deeper into tissues away from immune response.

Methodology:

Protein Modeling & Structure analysis:

Protein Sequence of Salmonella enolase was obtained from NCBI GeneBank (Accession number GI2938841). Molecular structures of Salmonella enolase were modeled by using restraint-based modeling implemented in the program Modeller9v8 **[12].** Further, the structure was refined and energy minimized with the insertion of Mg^{++} at the active site of our modeled structure. Energy minimization and structure refinement was done by Grommacs and Chimera program respectively. The modeled structure and Human alpha enolase structure (3B97), a homodimer structure were analysed for differences in their binding sites, active residues and active pockets by Castp and Pocket finder server respectively.

Protein-Protein Docking:

To study, enolase-plasminogen interaction the crystal structures of plasminogen 1B2I and 1DDJ were studied. Protein-protein docking was conducted on Grommacs server and VEGAZZ Program. For better results both docked structures were analyzed for conformational changes.

Protein Ligand Docking:

Both Salmonella and Human enolase were docked with enolase inhibitors CID: 151938 "TSP", CID: 3037152 "AEP" by proved enolase inhibitors using Molegro Virtual Docker.

Discussions:

The overall quality of our structure on the basis of the Ramachandran plot showed that 94.7% of residues were found in favored region (407 out of 432), 4% of residues in allowed regions (17 out of 432), while only 1.4% (6 out of 432) appeared in outlier regions. Human alpha enolase and salmonella enolase RSMD was found to be 2.36 by Superpose server. Enolase binds with 2-phosphoglycerate, where Gln167 and Lys396 are active residues, and are essential to hold and bind the divalent magnesium cation. This facilitates the rotation and neutralization of carboxyl group of PGA. Enolase catalyzes 11 different chemical reactions commonly as initial metal assisted abstraction of carboxylate group. A different active-site pocket catalyzes variety of chemical reactions including racemization, cycloisomerization and elimination of either water or ammonia. For a detailed study of interaction of the modeled structure with human plasminogen, Mg^{++} ion was inserted under the energy constraint of amber **[13]** force field implemented in the program Chimera.

Interaction of Salmonella enolase and Human Plasminogen

Human plasminogen is about 810 amino acid residues long containing different conserved domains such as ligand binding sites, putative domain-interaction sites, active sites and cleavage sites. We selected plasminogen structures which have a maximum coverage of ligand binding sites. 1B2I (residue position 183-263) and 1DDJ (residue position 564-810) were considered for the plasminogen activation studies. Based on rigid-body docking using Grommacs server and VEGAZZ, conformational changes were analyzed in both proteins to study the activation of plasminogen to plasmin **(Figure. 1 A).**

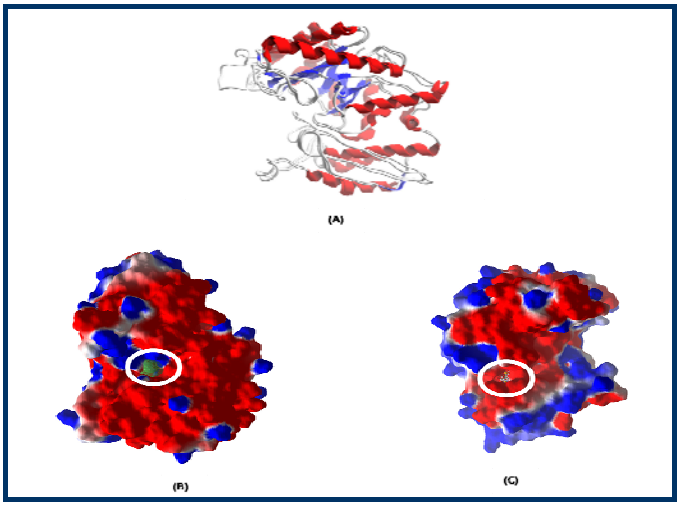

Figure 1: Salmonell and human enolase overlapping complex **(A),** docked regions being highlighted in circles in Human **(B)** and Salmonella **(C)** enolase respectively

1B2I (Kringle 2 Domain of Human Plasminogen) 83aa

Tissue-type plasminogen activator kringle-2 domain has two ligand binding sites one at C-terminal (lysyl residue), Tyr-Leu-Leu-Lys (YLLK) and one at N-terminal, Ala-Phe-Gln-Tyr-His-Ser-Lys (AFQYHSK). The sequence AFQYHSK is found within the plasminogen activation peptide whereas tetrapeptide YLLK corresponds to fibrinogen-B beta chain. Ligand-free and ligand-bound kringle 2 samples leads to the conclusion that all the small ligands as well as the C-terminal interact with a common binding site in kringle-2. Aromatic rigs of Tyr36, Trp62, His64, Trp72, Tyr74 and aliphatic side chains of Val35 and Asp55 participate in the common ligand binding sites of kringle-2 domain. Interaction between refined 1B2I Structure of Human Plasminogen with the modeled structure on common ligand binding sites shows conformational change in reported binding sites and different representation showing interaction of Plasminogen binding sites with Modeled Structure with conformational changes **(Figure 2)[14, 15, 16].**

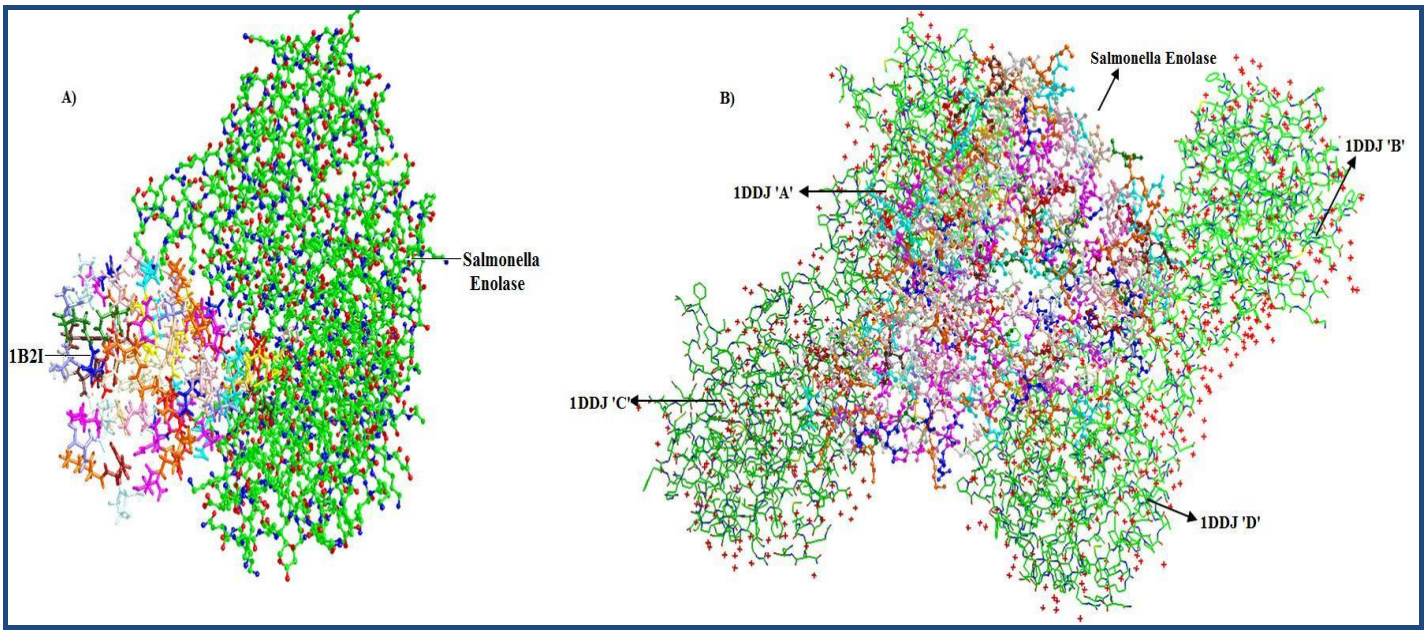

Figure 2: Plasminogen-enolase interaction with 1B2I **(A)** and 1DDJ **(B)** domains respectively

1DDJ (crystal structure of human plasminogen catalytic domain) 247aa:

In this Structure N domain, consist of an activation loop (550-570aa), Ca binding loop (620-630aa), autolysis loop (670-690aa), methonine loop(710-729aa), oxyanion stabilizing loop(730-760aa), with two, six and seven-stranded beta-barrel. Each Beta-barrel forms the core of another separate subdomain. The N domain and C domain are connected by three trans-segments as well as the A-chain peptide (residues 542-557). The molecular surface of mPLG (micro plasminogen) consists of various loops and two Alpha-helices. Functionally active loop (558-566) is involved in plasminogen activation by tissue type plasminogen activator (tPA) and urokinase **[17].**

Interaction between four chains of 1DDJ with Modeled Structure and interaction with different representations of the activation loop in chain `B` confirms the direct interaction of modeled enolase in Plasminogen activation. Plasmin production and Proteolysis in order to penetrate the host extracellular matrix by Salmonella enolase involves plasminogen activation which can be observed by conformational changes in the activation loop site. The proteolytic target bonds of Plasminogen (Arg 561-Val 562) are completely accessible to the solvent. Upon activation the proteolysis- released alpha- amide group of Val562 moves in order to enter the activation pocket **(Figure. 2).**

Molecular Docking

Enolase inhibitors were downloaded from PubChem as TSP (D-tartronate semialdehyde phosphate) and AEP (3-aminoenolpyruvate phosphate). These were docked with human enolase and salmonella enolase on the basis of best binding score and ligand stable conformation. We hypothesize that both structures have a different expression scenario with different electrostatic potential on structure surface **(Figure 1 B & C).**

Conclusion:
In the present study, it has been concluded that α-enolase could mediate the binding of *S. Typhi* to laminin, a major component of the basal membrane of the vasculature. It can therefore be postulated that the plasminogen–enolase association may play a critical role in the virulence of *S. Typhi* by causing direct damage to the host cell extracellular matrix, possibly by enzymic degradation of extracellular matrix proteins or other protein constituents. This might function as a guidance mechanism, first allowing *S. Typhi* adherence to the extracellular matrix, initiating tissue colonization, followed by plasminogen activation and laminin degradation in restricted areas. Hence, expression of α-enolase at the cell surface seems to be a common mechanism by which *S. Typhi* could induce destruction of the extracellular matrix, hence favoring their invasion and dissemination. Attempts to block this interaction could be of clinical relevance. Structural differences between enolase salmonella and human can provide a better understanding of bacterial survival inside host. Specifically, the bacterial metabolic enzyme could prove to be a potential drug target or could increase the efficiency of exiting drugs. Autoimmune responses need to be further answered with *in vitro* or *in vivo* validations.

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