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Molecular and Structural Characterization of GMP Reductase of *Streptococcus pneumonia* - A Potential Drug Target for Pneumonia

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Abstract

Streptococcus pneumoniae remains a major pathogen responsible for high morbidity and mortality in both the developed and developing countries. *S. pneumoniae* is estimated to kill annually close to one million children less than five years of age worldwide. Today antibiotic resistance in *S. pneumoniae* is common and increasing. Guanosine monophosphate reductase (GMPR) catalyzes the irreversible and NADPH- dependent reductive deamination of Guanosine Mono Phosphate (GMP) to Inosine Mono Phosphate (IMP) and plays a critical role in re-utilization of free intracellular bases and purine nucleosides. In this paper we had built the 3D structure of GMP reductase of *Streptococcus pneumonia-R6* by comparing the crystal structure of human guanosine monophosphate reductase 2 (hGMPr2, 2A7R). 3D Structure was built in modeler 9v8 by using information of alignment file developed in ClustalW 1.8.3. Developed GMP reductase *S. pneumonia-R6* model was submitted to PROCHECK and WHATIF programs for validation of the stereochemical quality and structure analysis. The Z-score was determined by ProSA web analysis. The developed model was deposited in PMDB and was accepted with <3% stereochemical failures. The present study would provide valuable insight information to search and rational drug design of a new generation of wide spectrum pneumonia drugs through a good understanding of structural characterization of GMP reductase.

Keywords: *Streptococcus pneumonia*, GMP reductase, Homology modeling, Modeller 9v8, Potential drug targets for Pneumonia.

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Introduction

Pneumonia is a serious infection and/or inflammation of our lungs. Pneumonia and influenza combined together were ranked as the seventh leading cause of death. Pneumonia was caused by a variety of bacteria including *Streptococcus, Staphylococcus, Pseudomonas, Haemophilus, Chlamydia, Mycoplasma,* several viruses, certain fungi and protozoans. Based on the available data, *S. pneumoniae* is estimated to kill annually close to one million children of less than five years of age worldwide. Pneumonia may cause up to 2.4 million deaths annually, mostly in the African, South-East Asian and Eastern Mediterranean regions of World Health Organization (WHO). This represents 1.5–2 times more child deaths than those from malaria and HIV infection together in these regions. In sub-Saharan Africa, HIV has resulted in a rapid increase in the incidence of pneumonia morbidity and mortality [WHO]. According to CDC Division of Bacterial and Mycotic Diseases, each year *S. pneumoniae* infections cause 100,000 to 135,000 hospitalizations for pneumonia, 6 million cases of otitis media and more than 60,000 cases of invasive disease, including 3,300 cases of meningitis. India accounts for almost 40% of worldwide childhood pneumonia cases [Center for Disease Control and Prevention]. Pneumococcal disease is more common in winter

months and when respiratory viruses such as influenza are circulating. In the United States, most deaths from pneumococcal disease occur in older adults, although in developing countries many children die of pneumococcal pneumonia.

Streptococcus pneumoniae (*S. pneumoniae*) is a common, spherical, gram-positive bacterium, normally found in the nasopharynx of 5-10% of healthy adults and 20-40% of healthy children [19]. Broad spectrums of diseases are caused by *S pneumoniae*, they are upper respiratory infections (sinusitis, acute otitis media, tracheobronchitis), lower respiratory infections (Bronchopneumonia, Pneumonia, with or without bacteremia or empyema), Invasive infections (primary bacteremia in children, meningitis, spontaneous bacterial peritonitis, sepsis with tissue seeding (septic arthritis, myositis, pericarditis, osteomyelitis, endocarditis), otitis media and acute sinusitis (main causes are infection with pneumococci species and *Haemophilus influenzae*) and pneumonia. Infections in other parts of the body such as in the bloodstream (bacteremia), lining of the brain and spinal cord (meningitis), bones (osteomyelitis), joints (arthritis), ears (otitis media) and sinuses (sinusitis). Some germs, particularly viruses, are breathed into the lungs because they are present in the air, especially if someone nearby has an infection and is coughing or sneezing.

Guanosine monophosphate reductase (GMPR) catalyzes the irreversible and NADPH- dependent reductive deamination of GMP to IMP and plays a critical role in re-utilization of free intracellular bases and purine nucleosides. It converts nucleobase, nucleoside and nucleotide derivatives of G to A nucleosides and maintains intracellular balance of A and G nucleotides. The important and simple reaction is shown in Figure 01.

At one time, disease caused by *S. pneumoniae* could be reliably treated with antibiotics. However, today antibiotic resistance in *S. pneumoniae* is common and increasing. ß-lactam resistance as a result of altered penicillin binding proteins was recognized [4]. Resistance to other non-ß-lactam drugs, such as the macrolides, clindamycin, tetracycline (13.2%), chloramphenicol (7.2%) and trimethoprim/ sulfamethoxazole (TMP/SMX 31.1%), began to increase [6]. Therapeutic failures in patients with pneumococcal infections treated with previously effective drugs were reported [8].

In this paper we predicted the 3D structure of GMP reductase of *Streptococcus pneumoniae* based on the Crystal Structure of Human Guanosine Monophosphate Reductase 2 (Gmpr2). The template (2A7R) sequence taken from PDB and the model was developed in modeller 9v8.

Materials and Methods

Fasta sequence of GMP reductase of *S. pneumoniae- R6* is obtained from NCBI BLASTp program and for good template selection the sequence compared with structurally similar sequences in NCBI. Crystal structure of human guanosine monophosphate reductase 2 (hGmpr2) (PDB ID: 2A7R) taken as a template for pneumonia [5]. The GMP reductase sequences were aligned with template carried out by using the online server of Clustal*W* [15]. Model was built using modeler 9v8 (http://www.salilab.org/modeller /9v8). Hundred models were generated and inspected. SWISS-PDB Viewer (spdbv) [2] was used for verification of RMS values all the models.

The stereochemical quality checking of the developed structure was carried out by Ramachandran plot. Online server PDB Sum was used for the prediction of secondary structure of target and template sequences [9]. Active site of template structure was obtained by submitting of PDB ID 2A7R to PDB Sum. PROCHECK and WHATIF program was used for determining stereochemical quality and structural analysis of built GMP reductase structure [11]. Z-score of developed GMP reductase was obtained by submitting its PDB structure (target) to ProSa (Protein Structure Analysis). [13]. Pymol and VMD (visual molecular dynamics) software's were used for representation of developed structure [http://pymol.sourceforge.net/, 3].

Results and Discussion

GMP reductase (EC: 1.7.1.7) (spr1128 CDS) is involved in the Purine metabolism of *S. pneumoniae- R6*. The FASTA sequence of target sequence was obtained from NCBI and this sequence was subjected to BLASTp analysis. From BLASTp results the protein having highest bit score was 2A7R hGMPR2 chosen as the template. The template sequence of PDB ID: 2A7R downloaded from RCSB PDB by comparing with FASTA sequence in NCBI BLASTp program. hGMPr2 shows 34% identities, bit score and expected e-value 175, 2e-44 respectively. The template has the following parameters such as 3.00 A^{o} resolutions, 0.228(obs) R-value and 0.276 R-free value. Multiple sequence alignment between the GMPR of *S. pneumoniae- R6* and

GMPR2 of human accomplished by using online tool Clustal*W* 1.83 (Figure 02a). The conserved regions showed in red color boxes. Using modeler 9v8 hundred models of GMP reductase were developed, the best one taken among the developed hundred models based on procheck analysis. Pymol and VMD (visual molecular dynamics) software's were used for representation of developed structure of GMP reductase of *S. pneumoniae_R6* (Figure 02b).

The developed GMP reductase of *S. pneumoniae- R6* was submitted to PROCHECK, SWISS-PDB viewer of spdbv, WHAT IF and ProSA for stereochemical quality checking. According to Ramachandran Plot, statistics of the developed model are 94.9% residues in most favoured region, 4.4% residues in additionally allowed region and only 0.3% of residues in generously and disallowed regions (Figure 03a). The main chain and side chain parameters statistics also show that there are no considerable bad contacts. It explains that the proposed model is of good quality.

SWISS-PDB viewer (spdbv) version 3.7 used for determining the root mean square deviation (RMSD) of the experimentally determined structure of GMPR of *S. pneumoniae- R6*. The structural superimposition of C_α crystal structure (2A7R) structures on GMPR of *S. pneumoniae- R6* model, revealed the RMSD value of $0.32A^0$ and 1336 number of atoms involved, which is characterized as good theoretical model. The data of WHATIF explains the evaluation parameters (quality conformation) of the structural model of GMPR of *S. pneumoniae- R6*. It shows average package quality 1.168 for target and 1.707 for template, rotamer normality 0.778 for target and 2.618 for template, backbone conformation 0.954 for target and 0.850 for template, bond length 0.924 for target and 0.366 for template and bond angles 1.244 for target and0.660 for template. Z-score of GMPR of *S. pneumoniae- R6* was calculated by using of online software ProSA web analysis. The built model shows overall model quality value-8.49 which is well inside the range of a typical native structure (Figure 03b) and local model quality the residue energies are negatively large. All these analyses show that the developed model of GMPR of *S. pneumoniae- R6* is of good quality.

Online server PDB sum was used for the generation of secondary structure of GMPR of *S. pneumoniae- R6* and structure of hGMPr2 (PDB ID: 2A7R). Template structure (PDB ID: 2A7R) shows one disulphide bond in between 68-95 residues and catalytic residue ASN. Developed structure of GMPR of *S. pneumoniae- R6* shows highly conserved regions in between 176 to 188, it contain 66 strands (19.0%), alpha helix 111 (32.0%), 3-10 helix11 (3.2%), others 159 (45.8%) and total 347 residues. Based on the alignment of the templates, catalytic residue (active site) are determined in built model of GMPR of *S. pneumoniae- R6*. The template structure (hGMPr2) (PDB ID: 2A7R) submitted to online server PDBSUM, it shows that the catalytic residue (active site) ASN -158 was conserved in hGMPR2. Active site region showed in thick red color box in Multiple Sequence Alignment (Figure 02a). Catalytic residues (active Amino acids) showed in colors red, yellow and they are also involved in the secondary structural conformation (Figure 03c). The developed GMPR of *S. pneumoniae- R6* model was submitted to PMDB (Protein Data Model Base), with a model ID as: PM-0075465. The built model was accepted as less than 3% stereochemical check failures [17].

http://www.cdc.gov/ncidod/dbmd/diseaseinfo/streppneum_t.html (CDC)

http://www.salilab.org/modeller /9v8 (Modeller)

http://pymol.sourceforge.net/ (Pymol)

http://www.who.int/en/ (World Health Organization)

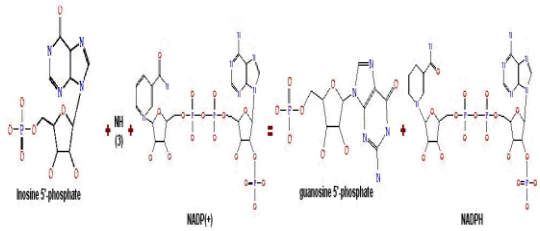

Figure 01: The reaction carried out by the enzyme involvement of GMP reductase, catalyzes the irreversible and NADPH- dependent reductive deamination of GMP to IMP.

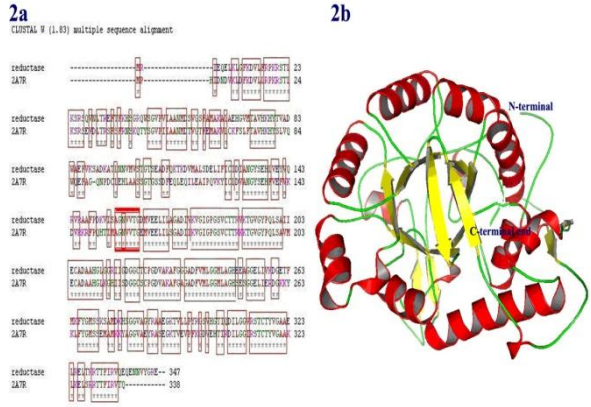

Figure 02a) GMP reductase of *S. pneumoniae*-R6 aligned with template human GMPr2 (PDB ID: 2A7R) conserved regions showed in red color boxes, and catalytic residue showed in thick red color box. 2b). The Developed 3D

structure of GMP reductase of *S. pneumoniae*_R6, in which α-helicals showed in red color, β-turns showed in yellow color and loops showed in green color.

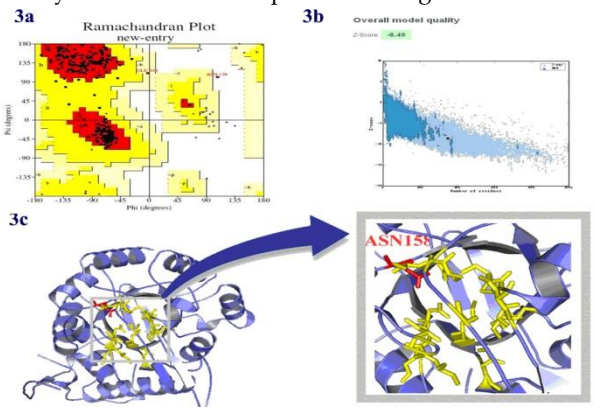

Figure 03a) PROCHEK plot statistics of GMP reductase of *S. pneumoniae*-R6 shows 94.9% residues in most favored region, 4.4% residues in additionally allowed region and only 0.3% of residues in generously and disallowed regions. 03b). The generated model GMP reductase of *S. pneumoniae-R6* Z-score value -8.49 well inside the range of a typical native structure. 03c). Developed 3D structure of GMP reductase of *S. pneumoniae*-R6 catalytic residue (active site region) ASPARAGINE-158 showed in red color.

Conclusion

Generation of 3D structure of GMP reductase of *S. pneumoniae-R6* using X-ray diffraction information and NMR data is very difficult. Computational studies are widely accepted for prediction of 3D structure of GMPR of *S. pneumoniae- R6* and can aid the structure-based drug design. The developed structure GMPR of *S. pneumoniae- R6* shows 94.9% residues in most favoured region. The present study would provide valuable insight information for research and rational drug design of new generation of wide spectrum pneumonia drugs.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Ethical Approval

Ethical approval was not required for this study because it was an in-silico computational study and did not involve human participants, animals, or human/animal biological specimens.

Informed Consent

Informed consent was not applicable because this study did not involve human participants or identifiable human data.

Institutional Review Board Statement

Institutional Review Board approval was not required because the study was entirely computational and did not involve human participants, animals, or human/animal biological specimens.

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