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Research paper

In-silico structural investigation of *Neisseria meningitidis* (MC58) glutamate dehydrogenase

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ABSTRACT

Neisseria meningitidis is a commensal pathogen responsible for causing septicemia and meningitis worldwide. It has a mortality rate of 10% while the survivors demonstrate a high rate of sequelae case. The discovery of specific and effective therapeutics is necessary to subdue this lethal infection. Glutamate dehydrogenase is considered a crucial drug target as it is a fundamental enzyme in the metabolic pathway of *Neisseria.* It catalyzes the reversible reaction of 2-oxoglutarate into glutamate. During the present studies, the three-dimensional model of *N. meningitides* NADP-dependent glutamate dehydrogenase enzyme (NMB1710 gene product) was constructed using the crystal structure of *Corynebacterium glutamicum* glutamate dehydrogenase complexed with 2-iminoglutarate and NADP+ as a template using MODELLER software. According to the stereochemical analysis and energy profiling, the modeled structure was found to be valid. The model was constructed in monomeric form, comprising of two core domains including catalytic and nucleotide-binding domain with a characteristic Rossmann fold. The catalytically significant residues showed strict conservation with that of the template. Structural elucidation of the glutamate dehydrogenase enzyme will be helpful for protein-ligand docking studies in order to identify certain potent drugs against this deadly pathogen.

KEYWORDS *Neisseria meningitidis*; Homology Modeling; MODELLER; Glutamate dehydrogenase, Drug target.

INTRODUCTION

Neisseria meningitidis is a gram-negative diplococcus and an aerobic bacterium of Neisseriaceae family. *N. meningitidis* is responsible for the lethal human infection including meningitis and septicemia, worldwide. Meningococci usually inhabit the nasopharynx of the carriers [1]. The adhesion, invasion and survival of the bacteria in host is facilitated by various virulence factors, several immune escape mechanisms and exploitation of host metabolic pathways [2].

Based on capsular polysaccharide, *N. meningitidis* is divided into 13 different serogroups. Five serogroups including A, B, C, Y and W135 are pathogenic. Serogroup-specific vaccines have been developed against all serogroups, excluding serogroup B. Vaccine for serogroup B is poorly immunogenic due to high resemblance with human antigen [3]. Therefore, designing novel and potent drugs against serogroup B has become necessary to combat this lethal pathogen.

A genome-wide analysis of *N. meningitidis* serogroup B has revealed 73 genes

responsible for its pathogenesis. Half of these genes have been revealed to be responsible for encoding proteins involved in metabolic pathways whereas, eight genes were found to encode known pathogenic factors. Structural investigations of many of *N. meningitidis* pathogenic factors have been carried out [4-9]. The gene NMB1710 encodes NADP-dependent-glutamate dehydrogenase, one of the crucial protein involved in the pathogenicity of meningococcal infections[10].

Glutamate dehydrogenase (GDH) is a homohexameric protein, consisting of A, B, C, D, E and F chains. Depending on the cofactor specificity, GDHs are divided into three groups: NAD^+ specific, $NADP^+$ specific, and dual coenzyme-specific[11]. It is an essential enzyme as it plays an essential role in carbon and nitrogen metabolism by both catalyzing the reversible reductive amination of 2-oxoglutarate (2-OG) and the oxidative deamination of glutamate. However, NADP-dependent GDH is mostly involved in the reductive amination of 2-OG[12]. This reaction proceeds through the formation of an unstable intermediate, 2-iminoglutarate (2-IG) through a nucleophilic attack by ammonia, which is then reduced by NADPH resulting in the biosynthesis of glutamate[13].

GDH is involved in the adaptability of *Neisseria* to the host environment. The expression of gdhA is regulated by the levels of 2-oxoglutarate, a TCA cycle metabolite which represses GDH production. The levels of 2-oxoglutarate are affected by the carbon source, which inevitably affects GDH production. Consequently, in bloodstream and CSF environments with glucose as the predominant carbon source, producing lower levels of 2-oxoglutarate and lower energy, elevated levels of GDH have been recognized [14, 15]. This up-regulation of gdhA gene is responsible for nutritional virulence of the bacteria in the hyper-virulent strains [16].

MATERIALS AND METHODS

Sequence Retrieval

The sequence for the gene product of gdhA (NMB1710) was retrieved from UNIPROT[17] database in FASTA format, comprising of 444 amino acid residues. The sequence is registered on UniProt under the UniProt ID Q9JY71.

Template Selection

The template for th**e** target protein was searched by subjecting the protein to PSI-BLAST[18] to identify a sequence homolog as potential template. The template for homology modeling was selected on the basis of highest sequence identity, maximum sequence coverage, less number of gaps, minimum E-value, and complexed structure.

Secondary Structure Prediction and Multiple Sequence Alignment

For the prediction of secondary structure, the web server PSIPRED[19] was used for the target protein; however, the secondary structural information of the template was obtained from PDBsum[20]. To record sequence conservation, multiple sequence alignment was carried out using CLUSTALX by taking 20 homologous protein sequences from different species including the target and the template sequence [21].

Homology Modeling Studies

Structure-based sequence alignment of target and template was made and utilized to construct a homology model with confidence. Homology modeling was carried out by MODELLER 10.1[22] program. The constructed model was evaluated based on stereochemistry and energy profile by PROCHECK[23]and PROSA[24], respectively. Discovery Studio Visualizer (DSV)[25] was used to examine the constructed three-dimensional model of the protein and its active site.

RESULTS

Sequence Alignment

For the homology modeling of *N. meningitidis*glutamate dehydrogenase (*Nm*GDH), the crystal structure of *Corynebacteriumglutamicum*glutamate dehydrogenase (*Cg*GDH), complexed with NADP and 2-IG was used as a template, having a sequence identity of 60.27%. Significant similarity was observed between the structure-based sequence alignment of *Nm*GDH and *Cg*GDH including the conservation of the catalytically essential residues (Figure 1).

Homology Modeling

The model was constructed with subunit A in monomeric form, comprising of two core domains, i.e., catalytic domain and nucleotide-binding domain (Figure 2A). The catalytic domain consists of 10 alpha helices and 4 beta strands. Meanwhile, the nucleotide-binding domain consists of 7 alpha helices and 8 beta strands.

*Nm*GDH protein structure was predicted complexed with 2-IG and NADP+. The ligands were observed, wedged in the binding pocket of the protein. (Figure 2B) The comparison between the overall fold of *Nm*GDH and *Cg*GDH showed significant structure similarity as evident by lower RMSD value of 0.2 Å.

Evaluation of the Model

The stereochemistry of the model was validated by the Ramachandran plot generated by PROCHECK which showed 347 (92.3%) residues in allowed region, 25 (6.6%)residues in additionally allowed region, 4 (1.1%) residues in generously allowed region, and 0% in disallowed region(Figure3A).

The z-score generated by PROSA for the model was -11.10, whereas the z-score generated for the template was -12.64. The energy plot showed no unnecessary peaks, confirming the stability of the model (Figure 3B).

Analysis of 2-IG Binding Residues

Important residues in the binding site of *Cg*GDH are K92, G93, G94, Q113, K116, K128, A166, G167, D168, R208, N347, V376, and S379. These residues are involved in interaction with carboxyl and 2-imino group of 2-IG by forming hydrogen bonds, salt bridge interactions and other non-covalent interactions. All the residues involved in the binding of 2-IG in crystal structure of *Cg*GDH showed conservation in *N. meningitidis*homology model (Figure 4A). Therefore, it can be speculated that 2-IG will bind *Nm*GDHwith the same mechanism as it does in *Cg*GDH.

Analysis of NADP Binding Residues

NADP is a coenzyme that accepts electrons in hydride (H^-) form. NADP is recognized by the residues from domain II, the nucleotide binding domain. The residues involved in binding with NADP in the crystal structure of *C. glutamicum*GDH include R96, H98, L109, K116, K128, K136, G167, D168, I169, G170, R208, T212, G240, S241, G242, N243, V244, A245, S263, D264, S265, S266, K284, R290, C320, A321, T322, Q323, G345, A346, N347, N372, G375. In *Nm*GDH model, all the residues involved in NADP binding showed conservation excluding S266 residue, which was replaced by N266 (Figure 4B).

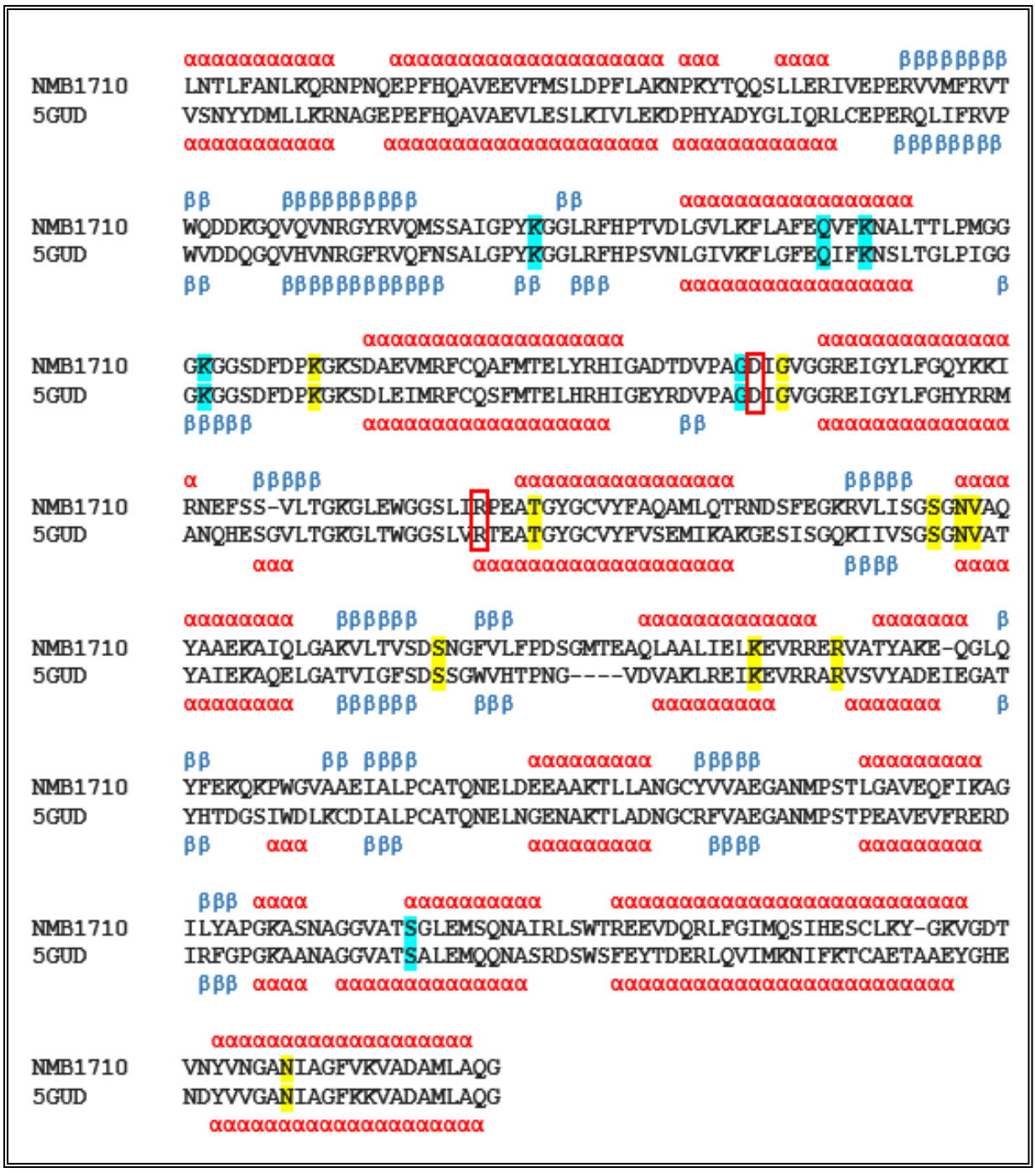

Figure 1: Structure-based pairwise sequence alignment of Glutamate Dehydrogenase from *C. glutamicum*and *N. meningitidis.* Alpha helices and beta sheets are indicated using α and β characters, respectively. 2-IG binding residues are highlighted by cyan color, $NADP^+$ binding residues are highlighted by yellow color, and residues which interact with both are represented in red box.

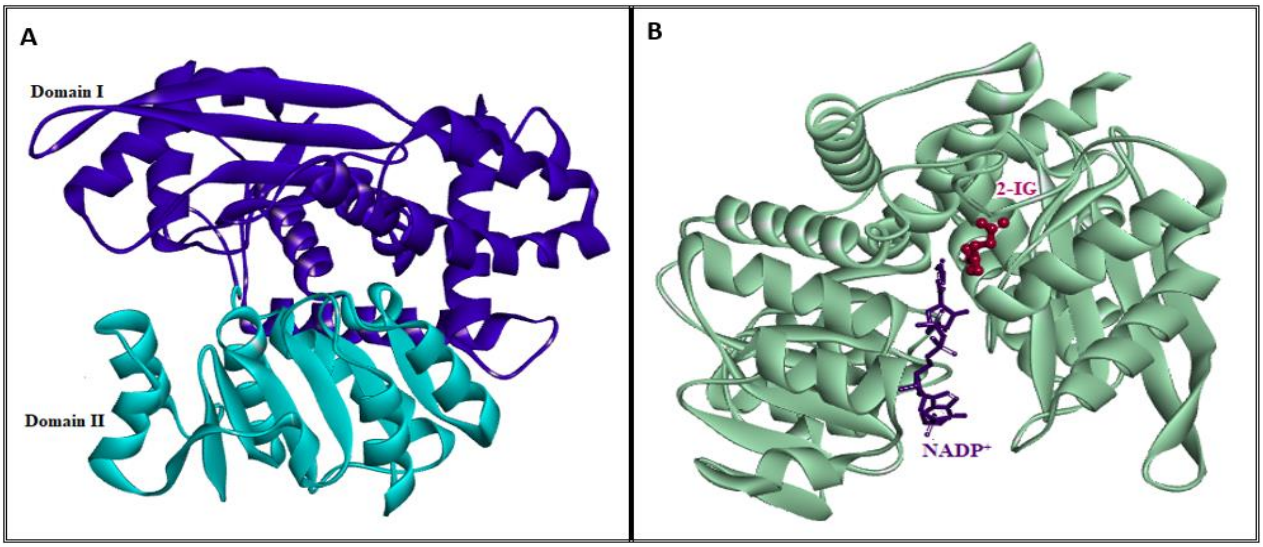

Figure 2: Domains of NmGDH. Domain I (Catalytic domain) is represented by purple color and domain II (Nucleotide-binding domain) is represented by cyan color (A), Monomeric structure of NmGDH shown in solid ribbon representation. Cofactor $NADP^+$ bound in cleft were represented by sticks in purple color and the ligand 2-IG is demonstrated in scaled balls and stick in pink color (B).

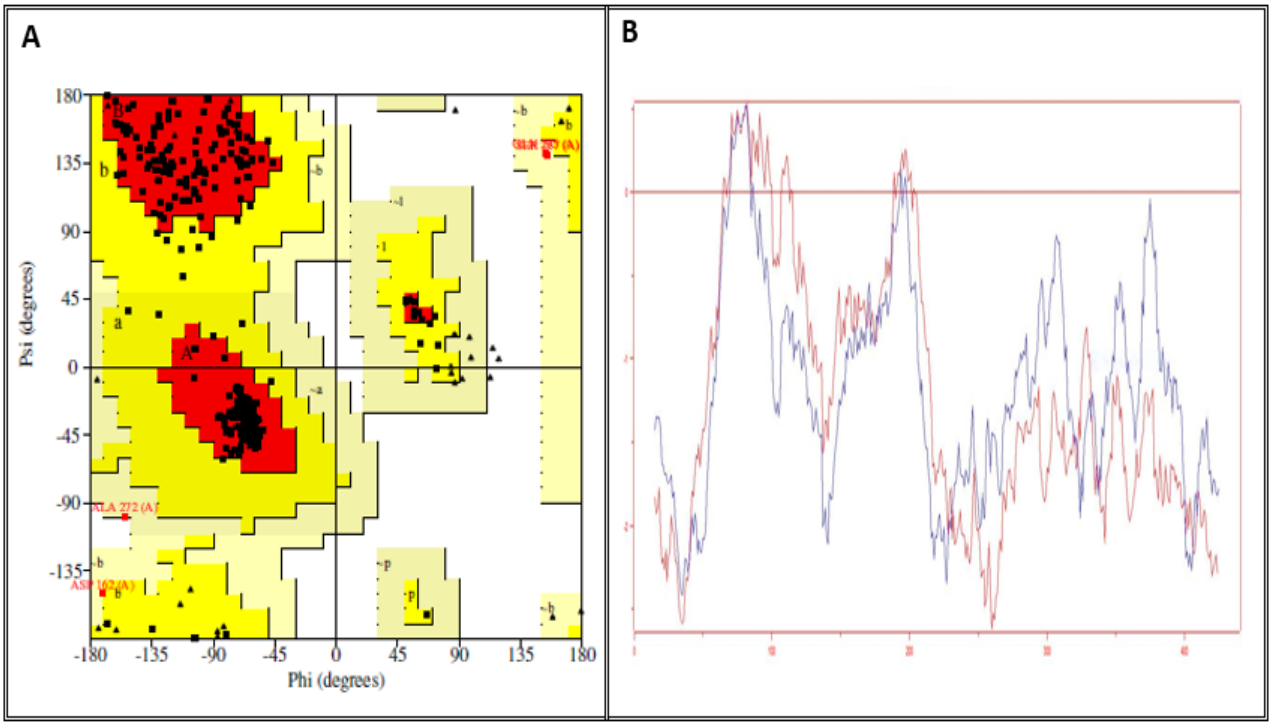

Figure 3: Ramachandran plot of the model of *Nm*GDH (A). Energy profile of the model of *Nm*GDH. Energy profile of *Cg*GDH and by red color and *Nm*GDH is shown in red and blue color, respectively (B).

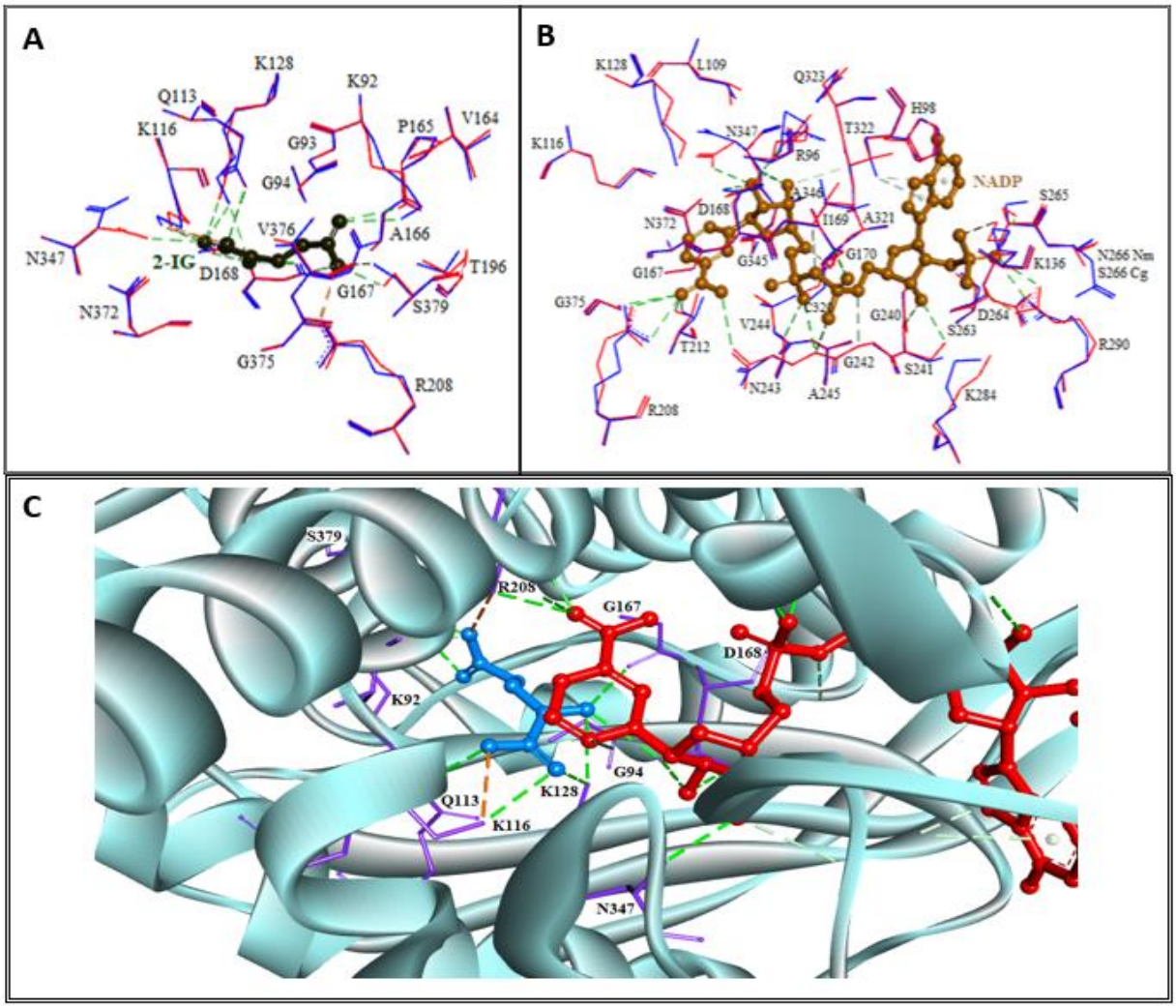

Figure 4: Superposition of the ligand-binding residues*of* *Nm*GDH(blue) and *Cg*GDH(red) structures. Superposed 2-IG binding-pockets of NmGDHand *Cg*GDH showing 2-IG ligand in green ball-and-stick representation (A), Superposed NADP binding-pockets of *Nm*GDHand CgGDH showing NADP in brown ball-and-stick representation (B). Active site residues are represented by lines in purple color, ball-and-stick representations are depicted for 2-IG (blue) and NADP (red) (C).

DISCUSSION

Each year, an estimated 2.5 million cases of meningitis are reported worldwide, with around 250,000 fatalities. It leads to the death of one in every ten infected persons and causes disabilities in one in every five infected persons[1]. Although the pathogenicity of *N. meningitidis* enabled by a number of virulence factors which facilitate the adhesion and invasion of hosts and evasion of their immune system, there is enough evidence to support the hypothesis of nutritional virulence. In order to survive the unfavorable conditions in a host, a harmless commensal bacteria may adapt by exploiting the metabolic pathways of the host [26].

Among the 73 genes which are essential for the meningococcal infections, eleven encode proteins that are involved in amino acid biosynthesis[10].The protein encoded by gene NMB1710 from *Neisseria meningitidis*serogroup B (MC58)is Glutamate dehydrogenase. This protein is a fundamental enzyme involved in the metabolic pathway and essential for energy metabolism. It is also crucial for the production of glutamate, an essential metabolite which is further converted into other amino acids by the action of transaminases. Therefore, it is essential for the synthesis of a nitrogen source for the bacteria[12].GDH facilitates the adaptability of *Neisseria* in different host environments under bacterial stress conditions. The finding that gdhA (NMB1710) is highly up-regulated in human blood and hyper-invasive strains (Serogroup B) indicates the importance of the enzyme in meningococcal infections[16]. Therefore, this protein may serve as a potent target for antibacterial drug designing.

Structure-based drug designing utilizes techniques of structural bioinformatics to construct three-dimensional models to analyze protein drug targets. Based on which, large-scale virtual screening is performed to identify hit lead compounds which can be used to inhibit the target and can be further utilized in therapeutics.

Since the structure of *N. meningitidis* glutamate dehydrogenase had not been previously solved, homology modeling of this essential drug target was carried out to determine its three-dimensional structure. *Corynebacterium glutamicum* NADP-dependent glutamate dehydrogenase complexed with 2-iminoglutarate and NADP was selected as a template based on 60.27% sequence identity with the query sequence. The selection of this template was favored over other homologs based on the presence of ligand which facilitated the analysis of important binding site residues.

Structure-based pairwise alignment between *Nm*GDH and secondary structure of *Cg*GDH showed significant similarity. Multiple sequence alignment also demonstrated conservation for all the catalytically essential residues in the glutamate dehydrogenase proteins across different species. The overall predicted three-dimensional fold of *Nm*GDH was similar to that of *Cg*GDH. Stereochemistry and energy analysis validated the quality of the model. The Ramachandran plot of the protein showed 92.3% residues in allowed region which indicates an overall good stereochemical quality. The stability of the model was also confirmed by the energy plot of PROSA and z-score which was well within the allowed range. The model was constructed with subunit A in monomeric form. It consists of two core domains, catalytic domain, and cofactor binding domain. The catalytic domain is comprised of 10 alpha helices (α1-α7, α15-α 17) and 4 beta strands (β1- β4),

which form a beta sheet. The cofactorbinding domain consists of 7 alpha helices (α8-α14) and 8 beta strands (β8- β12), forming an alpha/beta/alpha sandwich like Rossmann fold.

The amino acid residues involved in catalytic reaction; K92, G93, G94, Q113, K116, K128, A166, G167, D168, R208, N347, V376, and S379 remained conserved in *Nm*GDH with *Cg*GDH. NADP binding residues also remained conserved excluding S266 which binds NADP by Van der Waal forces. It was substituted by N266 in *Nm*GDH. Since both the residues are polar neutral amino acids, the substitution was conservative. Therefore, we believe that *Nm*GDH will bind with NADP in a similar manner.

CONCLUSION

The current study provided a reliable three-dimensional model of one of the most crucial enzymes of *Neisseria meningitides* that is involved in protein biosynthetic pathway and serves as a potential drug target. Almost all catalytically important residues showed conservation with the template residues except one NADP binding residue. Since the substitution was conservative, the interaction between the ligand and the protein remained unchanged. This model can be utilized to achieve the designing of effective and safe drugs against the lethal infections caused by this pathogen.

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