COMPUTATIONAL CHARACTERISATION OF STRUCTURAL AND FUNCTIONAL ELEMENTS OF GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (GAPDH) OF *NISSERIA MENINGITIDIS*

Dr.V.Kabila[1]
Associate Professor
Department of Zoology
Sri Meenakshi Govt. Arts College for Women (Autonomous)
Madurai 625002

Abstract

Neisseria meningitidis is an obligate, gram negative, human commensal that is spread from person to person by droplet infection. This bacteria colonises the nasopharyngeal mucosa of human and in due course resulted in bacteraemia and finally fatal sepsis. Most of the metabolic enzymes which are involved in various biochemical cycles are found to have many secondary functions such as adhesions, regulators of transcription etc.

GAPDH is a glycolytic enzyme which is mainly located in the cytoplasm. In addition to its primary metabolic function, it is observed that GAPDH is present on the surface of several microbial pathogens and invasion of host tissues. Since no structural models are available for GAPDH of *N. meningitidis*, the present study was performed to explore its structural characteristics.

It was recorded that the selected protein GAPDH of *N. meningitidis* was cytoplasmic, stable and with secondary structural elements such as helix and coils, Ramachandran plot showed that it has 92% of aminoacids in the favourable region. The modeled 3D structure of the query protein was refined and validated for its structural features with Q mean, PROCHECK, ERRAT and PROTSAV servers. The scores given by all these server were in consistent with the fact that the 3D model predicted had good overall quality. Protein function prediction analysis (PFP) showed that the query protein C6S993 has enzymatic role in glycolysis. Binding site analysis revealed that the protein GAPDH of *N.meningitidis* has binding sites for NAD.

The structural model computed in the present study could be used for a comprehensive understanding of the structural characteristics and moonlighting role of GAPDH of *N. meningitidis*. Details regarding whether the primary and secondary functions are independent or related needs further investigation. This study will pave the way for further systematic analysis towards identification of targets and potential biomarkers to design specific targets

***Index Terms - Neisseria meningitidis*, GAPDH, moonlighting protein.**

I INTRODUCTION

With the overwhelming growth of genome sequence data produced by rapidly advancing sequence technologies, the challenge of correctly determining function of encoded protein become more evident. As the number of functionally characterised protein increases, it has been observed that there are proteins involved in more than one function. These were described as "moonlighting" protein (Jeffery, 2003).

The number of moonlighting proteins has been continuously growing and the phenomenon is a common occurrence because, the multifunctionality of moonlighting protein is an orchestral program by which the complexity of cells has been appropriately justified. (Jeffery, 2013).

Many proteins that moonlight are enzymes; others are receptors ion channel, or chaperones. The most common function of moonlighting protein is enzymatic catalysis, but these enzymes which include signal transduction, transcriptional, regulation adhesion, molecular chaperones etc. (Jeffery, 1999)

Glyceraldehyde 3- phosphate dehydorgenase (GAPDH) is a glycolytic enzyme It catalyzes the conversion of glyceraldehyde -3- phosphate to 1,3 diphosphoglycerate. The most common form is the NAD+ dependent enzyme found in all organisms studied so far and which is usually in the cytoplasm. In addition to its metabolic

function, studies have demonstrated that GAPDH is present on the surface of several microbial pathogens and may facilitate their colonization and invasion of host tissues by interacting directly with host soluble proteins and surface ligands (Tunio *et al*., 2010a and b).

Neisseria meningitidis is a gram –ve bacteria. It is an obligate human commensal that is spread from person to person by droplet infection .The organism colonises the nasopharyngeal mucosa in an asymptomatic manner, a condition known as carriage (Caugant and Maiden, 2009). Under certain circumstances, the bacteria can invade the epithelial layers to gain access to the blood and heam, which can result in a wide spectrum of clinical, symptoms ranging from transient bacteraemia to rapidly fatal sepsis. This bacteria may also interact with cerebrovascular endothelial cells and cross the blood –cerebrospinal fluid barrier to cause meningitidis (Stephen, 2009).

Studies regarding the structural and functional analysis of GAPDH in this gram negative bacteria was not recorded so far. Moreover, it is understood that this highly conserved glycolytic enzyme might play secondary roles in various organisms (Matta et al., 2010; Jeffery, 2011).

Keeping these views in mind, the present study was designed to explore various structural and functional aspects of this key enzyme GAPDH in *N.meningitidis.* Analysis of secondary moonlighting functions was also performed.

II MATERIALS AND METHODS

To perform the structural and functional analysis of Glyceraldehyde -3- Phosphate Dehydrogenase of *Neisseria meningitidis,* various tools and softwares are used. The protein sequence of the gram negative bacteria *Neisseria meningitidis* with the Genbank Acc No: WP_015815952 and Uniprot Acc No: C6S993 was retrieved from Uniprot of NCBI database with the Accession No:C6S993. The FASTA format of GAPDH of *N. meningitidis* was downloaded and used for further structure analysis.

2.1 Structural Prediction:

The primary sequence of the selected protein was submitted to Protparam tool to analyse the primary structure . The secondary structural elements of C6S993 was predicted by GOR4 . For the selected GAPDH of *N. meningitidis*, only the primary sequence was available. The data on 3D structural information was not available in any of the structural databases .Hence homology modeling was done to deduce the three dimensional structure the protein.

Swissmodel server was used to select the suitable template in automated mode. The refined model was submitted to ERRAT server to know about the overall quality factor of the protein This was subjected to a series of tools to check its functional annotation quality such as PROCHECK, and PROTSAV .The generated model was also evaluated by Verify3D and visualized by RASWIN.Protein Function Prediction was done by the method of Khan *et. al*.(2012}.

III RESULTS

3.1 Prediction of Primary Structure

Various physicochemical parameters computed using Expasy's Protparam tool is represented Table 1.

Table 1: Biophysical parameters of GAPDH of *N.meningitidis*

Ala (A)	33	9.6 %
Arg (R)	14	4.6 %
Asn (N)	18	5.2 %
ASP (D)	22	6.4 %
Cys (C)	4	1.2 %
Gln (Q)	8	2.3 %
Glu (E)	16	4.7 %
Gly (G)	28	8.2 %
His (H)	8	2.3 %
Ile (I)	17	5.0 %
Leu (L)	31	9.0 %
Lys (K)	21	6.1 %
Met (M)	10	2.9 %

Phe (F)	11	3.2 %
Pro (P)	10	2.9 %
Ser (S)	20	5.8 %
Thr (T)	27	7.9 %
Trp (w)	3	0.9 %
Tyr (Y)	6	1.7 %
Val (V)	36	10.5 %
Pyl (O)	0	0.0 %
Sec (U)	0	0.0 %

Total number of Aminoacids:343
Aliphatic index: 94.64
Grand average of hydropathicity (GRAVY): -0.007

3.2Prediction of secondary structure:

The secondary structure of proteins were predicted by GOR4 . It indicates whether the given aminoacid sequence lies in a helix, strand or coil. The results reveal that the alpha helix was to be 30.03%, followed by extended strand (26.44%) and random coil (43.44%) (Fig 1)

GOR4: Sequence length: 343
Alpha helix (Hh) : 103 is 30.03%
Extended strand (Ee) : 91 Is 26.53%
Random coil (Cc) : 149 is 43.44%

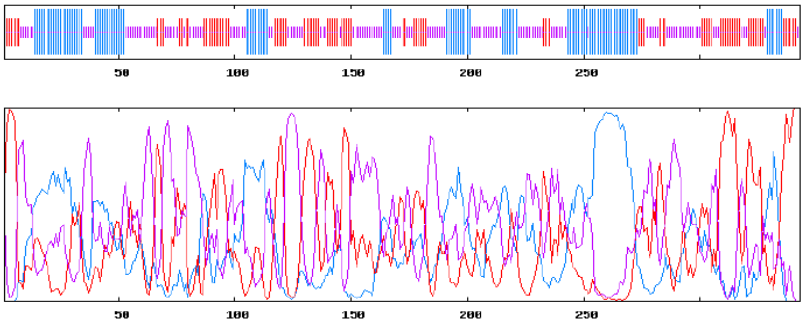


Fig 2: GOR4 Results of GADPH of *N. meningitidis*

3.3 Prediction of tertiary structure:

Three dimensional structures are predicted computationally for proteins where such data is not available in the database. In the present study, no predicted 3D structure was found for GAPDH of *N. meningitidis* in structural databases such as Swiss Model repository . Hence homology modeling of the three dimensional structure of the selected protein C6S993 was performed by the modeling server Swiss Model . The 3D structure predicted has 10bf1A as template with the sequence identify of 69.13 % The computed model was visualised using RASWIN. (Fig 2). The modeled query was compared with the non-redundant structures of PDB. Results show that the query protein lies well within the good scoring areas (Fig3) .The overall quality factor of this model was found to be 96.785 by ERRAT server (Fig 4).

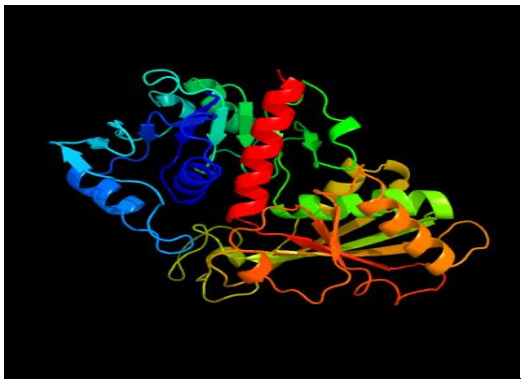


Fig 2: Homology model of GADPH

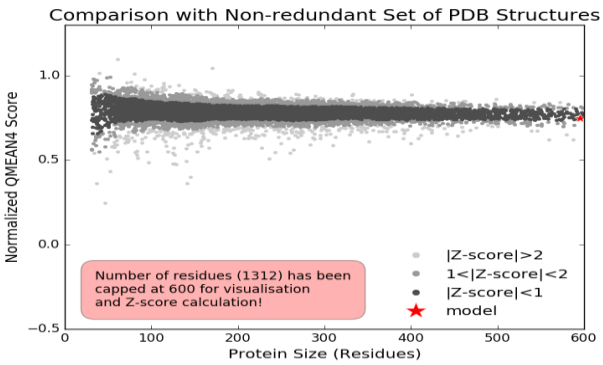


Fig3 Showing the the modeled query (in red colour) with its Z score.

File: /home/saves/Jobs/4938805/pdb_errat.logf
Overall quality factor**: 96.785

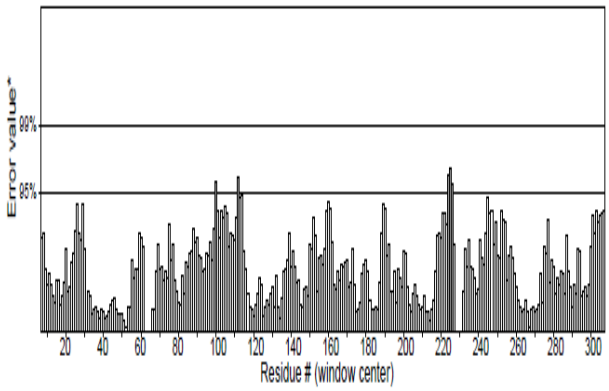


Fig 4: ERRAT analysis of GAPDH of *N.meningitidis*

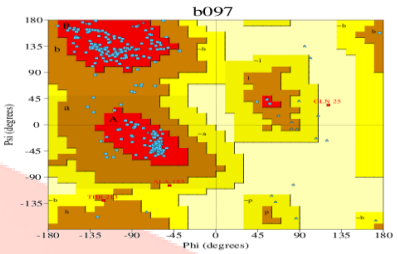


Fig.5 Ramachandran plot of Query Protein

The Ramachandran Plot showed that model structure has more aminoacid residues in the most favoured region (Fig 5). ProTSAV server predicted the model structure (Black dot in yellow color band) with RMSD lies between 2.0 to 5 Å (Fig 6). Verify 3D shows that the model has good quality (Fig 7) .

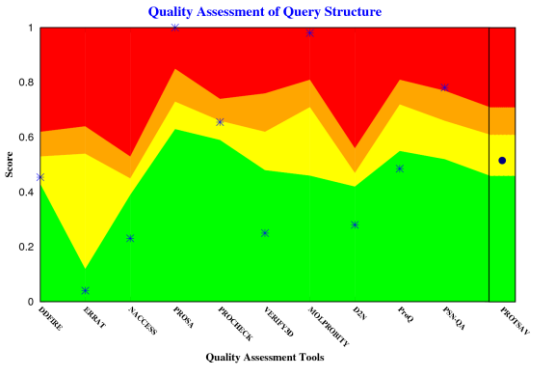


Fig.6 ProtSAV result of query protein

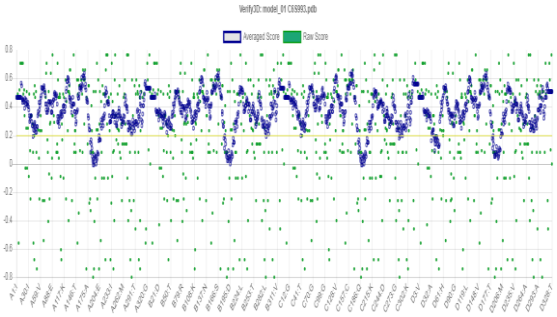


Fig 7: Results of Verify 3D of C6S993 *of N.meningitidis*

Presence of various domains might give insights of moonlighting functions of proteins. The CDD BLAST tools were used to find out various domains present in the selected protein C6S993 . Results revealed that the sequence under present study has GAPDH domain and NAD binding domain (Fig 8 **).**
NAD binding domain (Fig 8 **).**

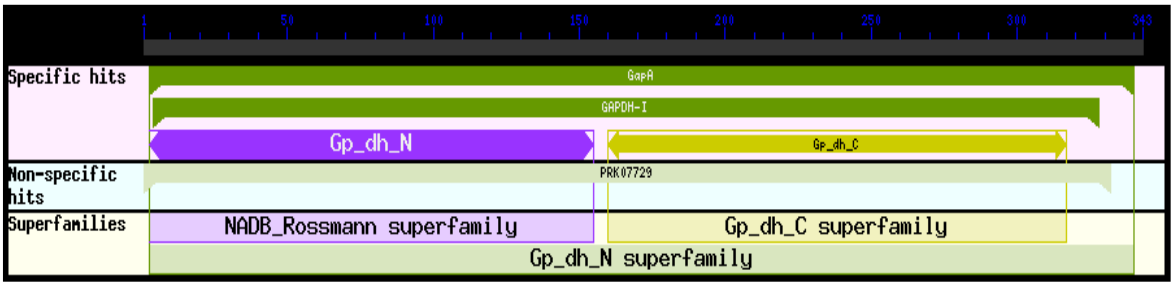
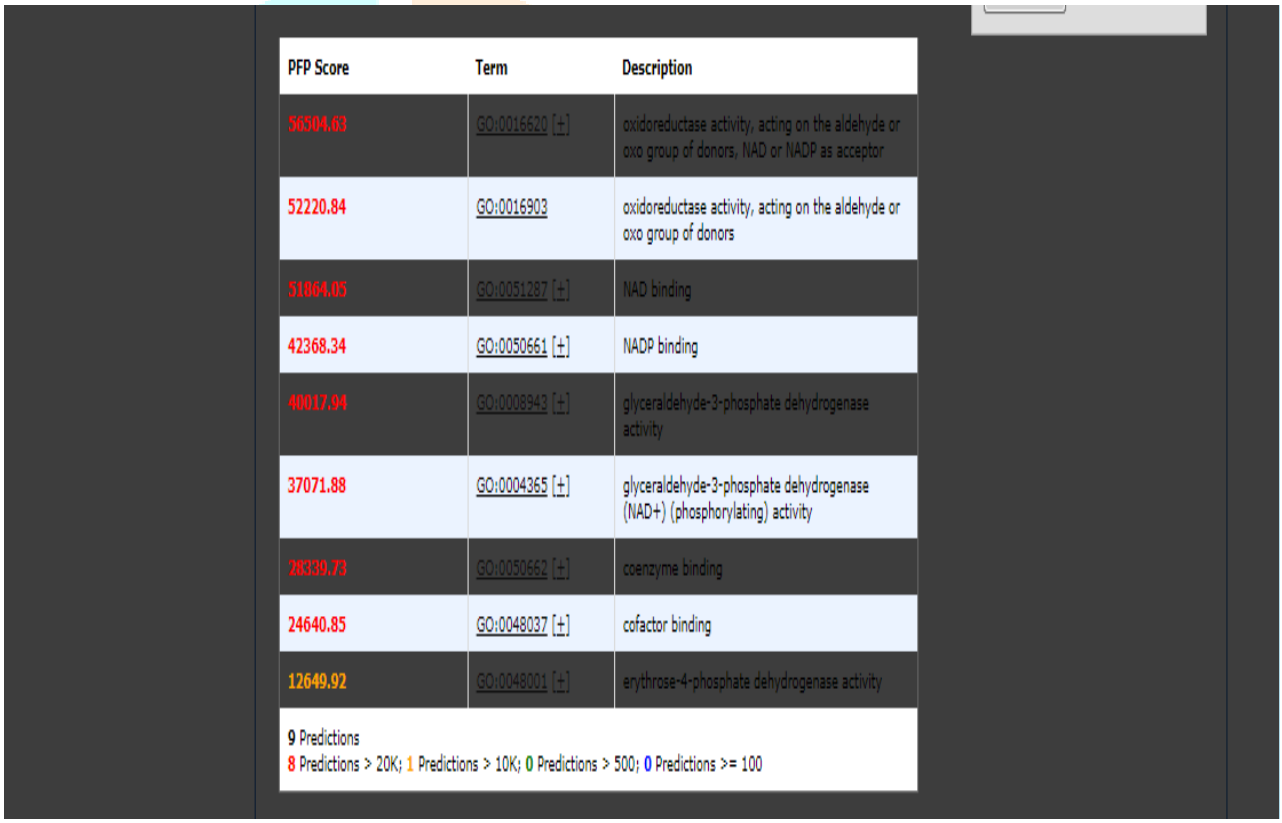


Fig.8 CDD BLAST results

Gene Ontology analysis:

The Gene Ontology (GO) terms annotated in the Uniprot database predominantly described the intracellular functions of the protein. Moreover in any of the protein, even if they have secondary moonlighting functions, they are found to have original function in the central metabolic pathways. PFP analysis showed that the GAPDH of *N.meningitidis* has Glyceraldehyde 3-phosphate dehydrogenase activity in carbohydrate metabolism as primary function . In addition to this, that it has NAD & NADP binding function (Fig 9).



PFP Score	Term	Description
56504.63	GO:0016620 [+]	oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor
52220.84	GO:0016903	oxidoreductase activity, acting on the aldehyde or oxo group of donors
51864.05	GO:0051287 [+]	NAD binding
42368.34	GO:0050661 [+]	NADP binding
40017.94	GO:0008943 [+]	glyceraldehyde-3-phosphate dehydrogenase activity
37071.88	GO:0004365 [+]	glyceraldehyde-3-phosphate dehydrogenase (NAD+) (phosphorylating) activity
28339.73	GO:0050662 [+]	coenzyme binding
24640.85	GO:0048037 [+]	cofactor binding
12649.92	GO:0048001 [+]	erythrose-4-phosphate dehydrogenase activity

9 Predictions
8 Predictions > 20K; 1 Predictions > 10K; 0 Predictions > 500; 0 Predictions >= 100

Fig. 9 Results of PFP analysis of the query protein

IV DISCUSSION

Proteins have been described as "the most versatile class of Biomolecules". An unexpected facet of this versatility is that one protein could be used by cells to perform completely different unrelated functions. These moonlighting proteins have the capability to execute two or more unique biological functions (Hernandez et al.,2011).

Many species of bacteria were identified with secondary function for GAPDH such as transferring, plasminogen binding, mucin etc (Jeffery, 2011). But studies regarding the structure and functional analysis of GAPDH in the human pathogen *N .meningitidis* were not recorded in view of its moonlighting capability. Hence, the present study was aimed to bring out the structural and functional characteristics of GAPDH in *N.meningitidis.*

4.1 Primary structure characters:

The selected protein C6S993 has 343 amino acids and a has aliphatic index of 94.64. A higher aliphatic index is an indicator of high thermostability and also an indicator of its solubility. In the present study, the value recorded was 94.64 which reveal that the protein is stable and GRAVY score (-0.007) confirms that the protein is soluble one.

4.2 Secondary structure analysis:

GOR4 server predicted the secondary structure as α helix (30.03%) random coil (43.44%) and extended strand (26.53%) , all essential secondary structure elements.(Fig1).

4.3 Tertiary structure characteristics:

Protein sequence or structure homology of a protein may be used to infer both primary functions as well as secondary moonlighting functions of a protein.

The tertiary structure of GAPDH was predicted by homology modeling and was visualized using RASWIN (Fig3). Thus, the results computed by various *Insilico* tools for GAPDH of *N. meningitidis* showed that the structure of the predicted protein was stable , with good quality and with good Z-score Fig.4,5 , 6 and 7).

4.4 Prediction of moonlighting functions:

Many of the moonlighting proteins are enzymes being conserved protein with original functions in the central metabolism (Moreno et al.,2012). The number of aminoacids present in them was within the range of 200-600. The present study also shows that the GAPDH of *N.meningitidis* has primary function as enzyme in the carbohydrate metabolism to convert glucose 3 phosphate to glucose 1, 3 diphosphate

The query protein in the present study has GP_dh C domain, GP_dh N domain and NAD binding domains which states it has enzymatic role as primary function. From structure point of the view, existence of multiple motfis and domains in the protein GAPDH of *N. meningitidis* shows its binding activity (Fig.8).

PFP analysis of C6S993 of *N.meningitidis* revealed that this enzyme GAPDH might have acquired secondary moonlighting function as evidenced by the GO predictions (Fig. 9).

This non- enzymatic roles exhibited by GAPDH of *N.meningitidis* reveals that it might also have acquired moonlighting functions in this gram negative bacteria(Knaust et al.,2007; Tumio et al.,2010a). This needs an integrated approach to confirm the location of this protein and its various secondary functions.

Structural analysis can provide a physical concrete picture of proteins, especially moonlighting proteins. Even though it is only applicable to proteins that have large experimentally solved structures, computationally modeled structures could also be used. In the present study, since no structural model was available in the structure databases, the 3D model computed in the present study could be used for a comprehensive understanding of the structure characteristics and the moonlighting role of GAPDH of *N .meningitidis*. The role played by this enzyme during pathogenecity in human needs further study. Details regarding whether the primary and secondary functions are independent or related needs further investigation

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