BIORESEARCH BULLETIN

Homology modelling and in-silico analysis of multidrug resistant protein, pmpm of Pseudomonas aeruginosa

April 7, 2014 · Volume 3; Issue 2

Tarali Chowdhury[1], Handique P J[1]
1 Gauhati University

Chowdhury T, P J H. Homology modelling and in-silico analysis of multidrug resistant protein, pmpm of Pseudomonas aeruginosa. Bioresearch Bulletin. 2014 Apr 7 [last modified: 2014 Apr 7]. Edition 1.

Abstract

Comparative modeling remains the most dependable and routinely used method for protein structure predictions. The pmpM gene belonging to MATE family of transporters of Pseudomonas aeruginosa, an opportunistic nosocomial pathogen in humans, is a prime target sequence responsible for conferring multidrug resistance through H(+)/drug antiporter efflux pumps. Its structure elucidation is necessary to analyze its functional characteristic which makes it resistant to many known antibiotics. In this study we have reported a 3D-structure predicted using homology modelling software Modeller 9v8. The structural validation was done in RAMPAGE, energy minimization was performed in ANOLEA and the model was finally verified in PROCHECK. The secondary structure, clefts and domain analysis was done using different bioinformatics tools. Thus various computational analyses will help in uncovering its possible activity within the target sites in human and design novel drugs based on their active site analysis.

Introductiion

In humans, *P. aeruginosa* can cause infection in almost every part of the human body including urinary tract, blood, lung (pneumonia) and is also associated with intra-abdominal sepsis and wound infections.*P. aeruginosa* has been found to be one of the most prevalent causes of hospital-acquired (nosocomial) infections resulting in 11-13.8% of these infections when a microbiological isolate is identifiable[1]. In burn wounds it is considered to be the major cause of gram-negative infections world-wide. Moreover its multi-drug resistant properties have contributed to high morbidity and mortality rates among the immunocompromised patients.

P. aeruginosa shows intrinsic and acquired resistance against many antibiotics and disinfectants. Many mechanisms such as active efflux, especially multidrug efflux have been known to confer multidrug resistance in this bacterium.

MATE transporters are one of the family which consist of 3 out of 34 efflux pumps that mediate multiple-drug resistance (MDR) in bacteria and mammals, modulating the efficacy of many pharmaceutical drugs used in the treatment of a variety of diseases[2]. MATE transporters couple substrate transport to electrochemical gradients of Na and are the only remaining class of MDR transporters whose structure has not been determined. This includes the gene PA1361 designated as gene pmpM that encode a multidrug efflux pump of *P. aeruginosa*.[3] Gui-Xin He et al[2] have reported PmpM (a product of the PA1361 gene) of *P. aeruginosa* as a unique multidrug efflux pump or an H_-drug antiporter belonging to the MATE family[4] conferring multi-drug resistance properties to the bacterium. Its alternative gene name has been given as norM and the ordered locus number is PA1361.

Comparative modeling remains the most dependable and routinely used method for protein structure prediction for further structural and functional analysis .Here we have reported the theoretical model of the pmpM gene of*P. aeruginosa* produced through Modeller 9v8[5] based on the Cation-bound Multidrug and Toxin Compound Extrusion (MATE) transporter of Vibrio cholorae. The bio-computational analyses were performed in silico using web-based software and servers.

In a Ramachandran plot, the core or allowed regions are the areas in the plot show the preferred regions for psi/phi angle pairs for residues in a protein. The Ramachandran plot using RAMPAGE software[6], helps to validate a theoretical model by showing the various residues falling under allowed, favoured and in disallowed regions within a modelled protein.

One of the primary factors determining how proteins interact with other molecules is the size of clefts and presence of domains in the protein's surface. Analysis of the clefts and domains were done because of their role in protein function or structure, In enzymes, for example, the active site is often characterized by a particularly large and deep cleft, while interactions between the molecules of a protein dimer tend to involve approximately planar surfaces. A large cleft provides an increased surface area and, hence, increased opportunity for the protein to form interactions with other molecules, particularly small ligands. Smaller sites may also be important in some cases as in the binding of allosteric effectors.

Materials and Methodology

Sequence alignment

The protein sequence of *Pseudomonas aeruginosa*, pmpM was downloaded from Uniprot Protein knowledgebase (http://www.uniprot.org/uniprot/Q9I3Y3) with accession number Q9I3Y3 (PMPM_PSEAE)[2]. Using PSI-BLAST search engine against Protein Data Bank (PDB) the crystal structure of Vibrio cholerae (pdb code: 3MKT.pdb, chain A) was selected and used as template for comparative modelling.

Comparative modelling

MODELLER 9v8 software was used for generation of the theoretical model by comparative modelling of protein structure prediction approaches. The 3-dimensional structure prediction was carried out by generating the alignment file of target sequences with template structures of the Cation-bound Multidrug and Toxin Compound Extrusion (MATE) transporter of Vibrio cholorae (3MKT.pdb chain A)[4] in CLUSTALX (http://www.ncbi.nlm.nih.gov/clustalX) . Loop refinement was done multiple times for refining the structure and finally out of the five predicted iterative models, the best model having lowest value of MODELLER objective function was obtained.

Representation and structure validation

Graphic representation was performed by using USCF-Chimera program[7] and model evaluation and validation was carried out on the RAMPAGE server, according to the Ramachandran Plot for model validation (http://www-cryst.bioc.cam.ac.uk/rampage). The energy minimization was realized using Anolea server[8], and the model was finally verified in PROCHECK[9].

Structural analysis of clefts and domains

The cleft regions within the protein were predicted by PDBSUM (http:/www.ebi.ac.uk/ pdbsum)[10] and transmembrane protein helix probability curve was analyzed by using TMHMM server V.2.0 (http://www/cbs.dtu.dk/ services/ TMHMM. 2.0)[11]. Secondary structure prediction and tertiary fold-recognition were carried out using Phyre server[12]. A domain and motif identification search was done using InterproScan version 4.8 (www.ebi.ac.uk/ Tools/ InterProScan)[13] against various domain databases.

Secondary structure prediction and detection of cavity

TMpred (http://www.ch.embnet.org/ software/ TMPRED_form.html) was used for prediction of Transmembrane Regions and orientation of the target sequence. Detection of protein cavities within the modelled protein was done using Molegro Docker trial version which was compared and validated with Pocket finder (http:// www.modelling.leeds.ac.uk/ pocketfinder/).

Results and Discussions

We have developed a three dimensional model for the multidrug resistant protein pmpM of *Pseudomonas aeruginosa* (Accession No. Q9I3Y3) . For modeling the template protein were obtained by PSI-BLAST search in EBI(http://www.ebi.ac.uk) with PDB databases and the template Cation-bound Multidrug and Toxin Compound Extrusion (MATE) transporter of *Vibrio Cholerae* (3MKT_A) was selected with an X -ray resolution (3.65Å), the sequence identity 95.699% and E-value is 8.61 e-77.

The TMHMM server V.2.0 and PHYRE server predicted 12 transmembrane helices with 262.85049 amino acids within them. It has also predicted one of the transmembrane helix to be a signal peptide in the N-terminal region having an expectation number of 26.85049. Helices and coil region on the consensus were found to be true for the modeled protein produced by us on comparison of the PHYRE analysis of fold regions with the modelled protein. The 3d structure rendered in Chimera (Fig.1) shows it mainly as a helical protein with prominent loop regions.

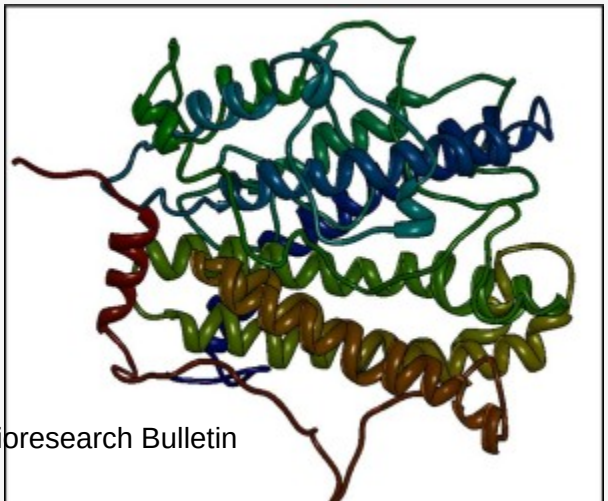


Fig. 1: The structure of theoritical model for pmpM of P. aeruginosa.

The Ramachandran plot indicated that most (85.5 %) of residues have ? and ? angle in the core and allowed regions the bond angle, bond length and torsion angles were in the range of value expected for a naturally folded protein, additional allowed regions 13.9 %, generally allowed regions 2.8.The PROCHECK validation was found to be similar to this RAMPAGE validation of the theoretical model.

InterProScan detected two MATE domains (Fig. 2), from Pro27-Tyr186 and Pro254-Tyr414 within the protein identifying it as an antimicrobial extrusion protein with a eukaryotic signal peptide at the N-terminal region spanning from Met1 to 39Gly amino acid residues.

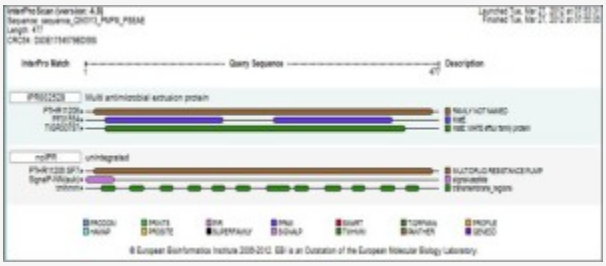


Fig. 2: InterProScan results showing presence of two MATE domains within the modelled protein.

It was noted that both the domain has proline and tyrosine as the first and the terminal residue respectively. The region functioning as multidrug resistant pump, span from amino acid Lys12-Asp468. Among the 10 clefts found by PDBsum (given pdb code: ho33), 4 were predicted to be of considerable size to be considered as functional active sites for ligand interactions given in Table 1. It predicted 1 anti-parallel beta-sheet, 1 beta-hairpin at Ser264-Ile 265belonging to 1:3 hairpin class, 2 strands, 16 helices,18 helix-helix interactions,40 beta turns and 7 gamma turns present within the theoretical model (Fig 3). Ten cavities were detected within the protein which may function as putative binding site for ligands (Table 2).

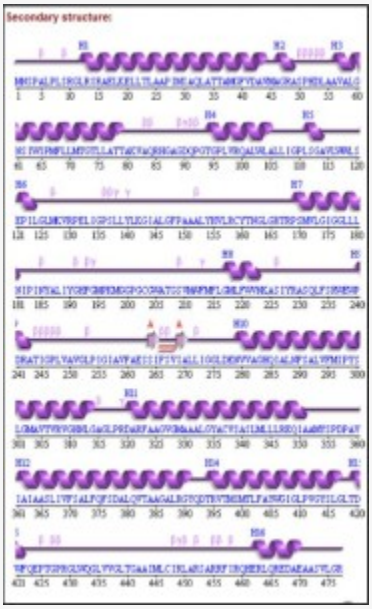


Fig. 3: Various Secondary structure of pmpM protein detected by PDBsum.

Clefts	Volume (a^{o3})	Accessible vertices	Buried vertices	Average depth
1.	7632.98	78.09	16.23	20.65
2.	4621.64	76.07	15.98	12.88
3.	3642.47	61.83	10.90	13.58
4.	2497.08	67.28	12.34	10.43

Table 1: The four major clefts detected by PDBsum within the modeled protein of pmpM.

Cavity	Volume (A^3)	Surface (A^2)	Amino acid residues	Cavity	Volume (A^3)	Surface (A^2)	Amino acid residues
1	[illegible]	[illegible]	[illegible]	6	[illegible]	[illegible]	[illegible]
2	[illegible]	[illegible]	[illegible]	7	[illegible]	[illegible]	[illegible]
3	[illegible]	[illegible]	[illegible]	8	[illegible]	[illegible]	[illegible]
4	[illegible]	[illegible]	[illegible]	9	[illegible]	[illegible]	[illegible]
5	[illegible]	[illegible]	[illegible]	10	[illegible]	[illegible]	[illegible]

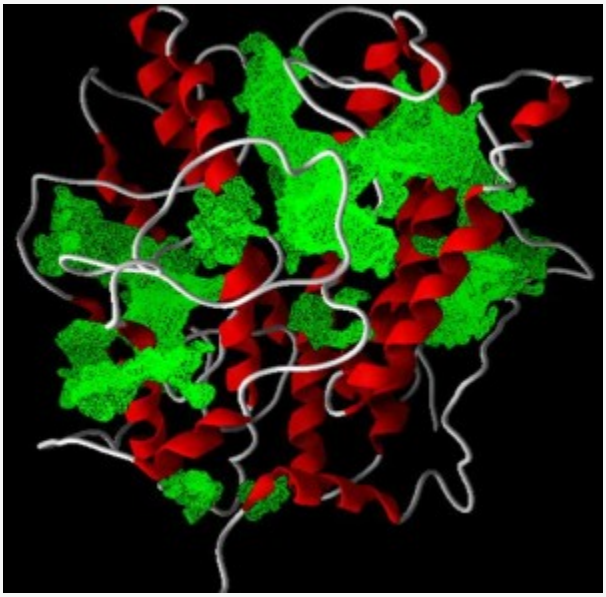
Table 2: 10 cavities with volume and surfaces detected within the modelled protein by Pocket finder.

It has been suggested that the active site usually lies in the largest of all the protein's clefts or cavities as they are usually the locus of protein-protein interactions and host the enzymes' active site. Identification of potential ligand-binding pockets is an initial step in receptor-based drug design to determine the pocket's ability to bind small drug-like molecules. In the case of protein domains, they can also be the locus of domain-domain interactions that lead to the structure of the whole protein. Ultimately domains, cavities and active site prediction go a long way in determining the ability of the target protein to take part in different types of interaction to perform their various metabolic functions. Though our model is predictive and needs to be confirmed experimentally, this model can be used to docking and inhibition analysis of MATE family with small molecules to create a novel combinatorial library of drug-like compounds.

Conclusions

Due to low resolution and high R factors of many crystal structures, homology modelling is now regarded as an important technique for not only in obtaining the 3D-structure of a putative protein but also for refining the existing low accuracy experimental structures.

The above analysis points out that homology models may provide an insight to simulations and related computational studies such drug docking calculations and protein-drug or enzyme-target interactions to obtain useful insights into structure–function relationships in transmembrane microbial proteins with mammalian targets. This approach also provides a potential route to understanding the function and toxicity of bacteria membrane transport proteins on their target sites. It is hoped that the prediction model of such multidrug resistant targets will help it studying its functional activities and pathways to develop novel drugs to inhibit their pathogenicity by specially emphasizing studies on their domains and active sites.



References

1. Pittet D, Harbarth S, Ruef C, Francioli P, Sudre P, Petignat C, Trampuz A, Widmer A. 1999. Prevalence and risk factors for nosocomial infections in four university hospitals in Switzerland. Infect Control Hosp Epidemiol., 20(1):37-42.[PMID:9927264].

2. Gui-Xin He. 2004. An H+-Coupled Multidrug Efflux Pump, PmpM, a Member of the MATE Family of Transporters, from Pseudomonas aeruginosa. Journal of bacteriology 186(1):262-265.[PMID-14679249].

3. Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Hufnagle WO, Kowalik DJ, Lagrou M, Garber RL, Goltry L, Tolentino E, Westbrock-Wadman S, Yuan Y, Brody LL, Coulter SN, Folger KR, Kas A, Larbig K, Lim R, Smith K, Spencer D, Wong GK, Wu Z, Paulsen IT, Reizer J, Saier MH, Hancock RE, Lory S, Olson MV. 2000. Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. Nature 406:959-964.[PMID:10984043].

4. He Gui-Xin, Szewczyk P, Karyakin A, Evin, M, Hong WX, Zhang Q, Chang G. 2010. Structure of a cation-bound multidrug and toxic compound extrusion transporter. Nature 467:991-994. [PMID:20861838].

5. Fiser A, Sali A. 2003. Modeller: generation and refinement of homology-based protein structure models. Methods Enzymol., 374:461-91. [PMID:14696385].

6. Lovell SC, Davis IW, Arendall III WB, De Bakker PIW, Word JM, Prisant MG, Richardson JS and Richardson DC. 2002. Structure validation by Calpha geometry: phi,psi and Cbeta deviation. Proteins: Structure, Function & Genetics 50:437-450.[PMID:12557186].

7. Laskowski RA, Luscombe NM, Swindells MB, Thornton JM. 1996. Protein clefts in molecular recognition and function. Protein Science 5(12): 2438-2452. [PMID:8976552].

8. Melo F, Devos D, Depiereux E, Feytmans E. 1997. ANOLEA a www server to assess protein structures. Proc Int Conf Intell Syst Mol Biol., 5:187-90.

9. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. 1993. PROCHECK - a program to check the stereochemical quality of protein structures. J. App. Cryst., 26:283-291. [PMID:9322034].

10. Laskowski RA, Hutchinson EG, Michie AD, Wallace AC, Jones ML, Thornton JM. 1997. PDBsum: a Web-based database of summaries and analyses of all PDB structures.Trends Biochem. Sci.., 22(12):488-90. [PMID:9433130].

11. Krogh A, Larsson B, Von Heijne G and Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes.J Mol Biol.19:305(3):567-80. [PMID:11152613].

12. Kelley LA and Sternberg MJE. 2009. Protein structure prediction on the web: a case study using the Phyre server.Nature Protocols 4:363-371.[PMID:19247286].

13. Quevillon E. 2005. InterProScan: protein domains identifier. Nucleic Acids Res., 33:W116-20. PMID:15980438