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3D Structure Analysis of VhhP2 Protein in *Vibrio harveyi*

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Abstract: *Vibrio harveyi* is one of the important aquatic pathogens founded predominately in marine habitats. The gram-negative bacterium can cause infection in a broad range of marine vertebrates and invertebrates, involving fishsh, shrimp, lobster and mollusk. Until now, the traditional forms of vaccines against *V. harveyi* have been produced including killed whole bacterial cells (mostly) and extracellular products (in part). In addition to these vaccines, currently a number of protein-based (in fact recombinant subunit) vaccines have been manufactures and are shown to be effective against some *V. harveyi* starins.

Key words: *Vibrio harveyi*, vaccine, vhhP2, 3D structure, shrimp

INTRODUCTION

Vibrio harveyi is one of the important aquatic pathogens founded predominately in marine habitats. The gram-negative bacterium can cause infection in a broad range of marine vertebrates and invertebrates, involving fish, shrimp, lobster and mollusk (Austin, 1988; Austin and Zhang, 2006). Infection by the *Vibrio* sp. can lead to vibriosis. As in the control of a large number of other aquaculture diseases, the management of vibriosis resulted from *V. harveyi* infection relies largely on husbandry, vaccination and the use of antibiotics, immunostimulants and probiotics (Austin and Austin, 2007; Irianto and Austin, 2002). The use of the compounds which have antimicrobial properties, may lead to increasing antibiotic-resistance in bacteria and pollution in the environment. Thus, the use of vaccines has elevated for they are environmental-friendly and have long-term efficacy of protection (Crosbie and Nowak, 2004; Hastefnl *et al*., 2005). Until now, the traditional forms of vaccines against *V. harveyi* have been produced including killed whole bacterial cells (mostly) and extracellular products (in part) (Crosbie and Nowak, 2004; Hastein *et al*., 2005; Arijo *et al*., 2008, 2005; Zorrilla *et al*., 2003). In addition to these vaccines, currently a number of protein-based (in fact recombinant subunit) vaccines have been manufactures and are shown to be effective against some *V. harveyi* starins (Ningqiu *et al*., 2008; Zhang *et al*., 2008a, b).

In the technique called bacterial surface display, the interested protein is displayed as a fused passenger to the surface of bacterial cells via a carrier protein which is generally a protein with natural secretion capacity such as outer membrane proteins or for gram-positive bacteria, cell wall-anchoring proteins (Zhu *et al*., 2006). From its first report in 1986 untill now the surface display has been used in diverse forms in a broad range of areas, especially in the development of biocatalysts, biosensors, vaccine vectors and bioremediation systems (Freudl *et al*., 1986). The gene of vhhP2 belonged a pathogenic *V. harveyi* strain isolated from diseased fish has been identified and discovered that vhhP2 is widely distributed in *V. harveyi* strains of various geographical locations and sources (Charbit *et al*., 1986).

Now a days, bioinformatic tools are of interesting advantages for biologists. Prediction of 3D protein structure is one of the wide applications of these tools (Kafee and Sefid, 2016a, b; Payandeh *et al*., 2015). Several methods and algorithms are available for protein structure predictions, homology modeling being one of them. Homology modeling is an in silico method for prediction of 3D protein structures based on known homologous protein structures as a template. New genome analysis tools based on bioinformatics and immunoinformatics approaches help us select suitable antigens or epitopes directly from the genomes of pathogens in order to design a vaccine. These tools could be employed for epitope selection and vaccine design. Moreover, prediction of protein structures is one of their wide applications (Kofeiti *et al*., 2016; Masoumi and Sefid, 2016; Bastani and Sefid, 2016; Darbandian and Sefid, 2017; Sefid *et al*., 2013, 2015, 2016). In the present study we predicted 3D structure of vhhP2 protein in *V. harveyi* via in silico approaches and evaluated the functional properties of residues which involved in ligand binding site.

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MATERIALS AND METHODS

Sequence availability and homology search: The VhhP2 protein sequence with accession No. ACM68726.1 and GI. 222862145 acquired from NCBI at http://www.ncbi.nlm.nih.gov/protein was saved in FASTA format for further analyses. The sequences served as a query for protein BLAST at http://blast.ncbi.nlm.nih.gov/Blast.cgi against non redundant protein database. Probable putative conserved domains of the query protein were also searched for at the above address.

Primary sequence analysis: Protparam online software at http://expasy.org/tools/protparam.html was employed for estimation and determination of properties such as molecular weight, theoretical pI, amino acid composition, total number of negatively and positively charged residues, instability index and aliphatic index.

3D structure prediction: The SWISS-MODEL workspace at http://swissmodel.expasy.org/ is a web-based integrated service dedicated to protein structure homology modelling. It assists and guides the user in building protein homology models at different levels of complexity. Building a homology model comprises four main steps: identification of structural template(s) alignment of target sequence and template structure(s) model building and model quality evaluation. These steps can be repeated until a satisfying modelling result is achieved. Each of the four steps requires specialized software and access to up-to-date protein sequence and structure databases.

Ligand binding site predictions: Cofactor at http://zhanglab.ccmb.med.umich.edu/COFACTOR/ is a structure-based method for biological function annotation of protein molecules. Important amino acid involved in ligand binding site is predicted by this server.

Pocket detection: Dog site scorer at http://dogsite.zbh.uni-hamburg.de/ is an automated pocket detection and analysis tool which can be used for protein drugability assessment. Predictions with DoG Site Scorer are based on calculated size, shape and chemical features of automatically predicted pockets, incorporated into a support vector machine for druggability estimation.

Identification of functionally and structurally important residues: InterProSurf a http://curie.utmb.edu/pattest9.html predicting functional sites on protein surface using patch analysis was employed. VhhP2 3D structure determined in a previous study, served as an input file for this server.

RESULTS AND DISCUSSION

Sequence availability and homology search: The protein sequence with 195 residue obtained from NCBI and saved in FASTA format. Protein sequence serving as query for BLAST produced a set of sequences as the highest similar sequence.

BLAST search revealed numerous hits to the VhhP2 subunit sequence. Most of hits were of vibrio. Putative conserved domains were detected within this sequence and are shown in Fig. 1.

Primary sequence analysis: The protein sequence served as input for the computation of various physical and chemical parameters. The computed parameters included the molecular weight, theoretical pI, instability index, aliphatic index and grand average of hydropathicity (indicates the solubility of the proteins: positive GRAVY (hydrophobic), negative GRAVY (hydrophilic)) are summarized:

- Number of amino acids = 195
- Molecular weight = 21669.2
- Theoretical pI = 6.59

Amino acid composition:

- Ala (A) (12) = 6.2 (%)
- Arg (R) (11) = 5.6
- Asn (N) (17) = 8.7
- Asp (D) (12) = 6.2
- Cys (C) (2) = 1.0
- Gln (Q) (12) = 6.2
- Glu (E) (8) = 4.1
- Gly (G) (20) = 10.3
- His (H) (1) = 0.5
- Ile (I))(12) = 6.2
- Leu (L) 15 = 7.7
- Lys (K) 9 = 4.6
- Leu (L) (15) = 7.7 Lys
- Met (M) (2) = 1.0
- Phe (F) (9) = 4.6
- Pro (P) (6) = 3.1
- Ser (S) (11) = 5.6
- Thr (T) (13) = 6.7
- Trp (W) (3) = 1.5
- Tyr (Y) (8) = 4.1
- Val (V) (12) = 6.2
- Pyl (O) (0) = 0.0
- Sec (U) (0) = 0.0

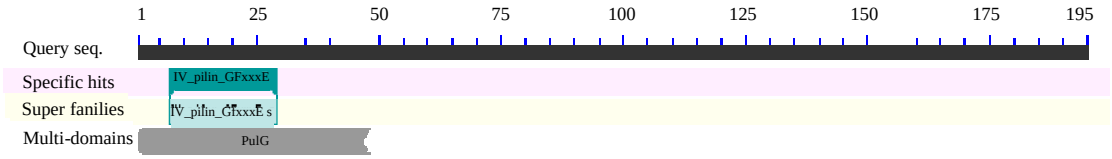

Fig. 1: Putative conserved domains have been detected

Total number of negatively charged residues (ASP+GLU) = 20. Total number of positively charged residues (Arg+Lys) = 20.

Atomic composition:

- Carbon (C) = 957
- Hydrogen (H) = 1487
- Nitrogen (N) = 271
- Oxygen (O) = 297
- Sulfur (S) = 4

Formula:

$C_{957}H_{1487}N_{271}O_{297}S_4$

Total number of atoms = 3016

Extinction coefficients: Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water. Ext. coefficient 28545. Abs 0.1% (= 1 g L^{-1}) 1.317 assuming all pairs of Cys residues form cystines. Ext. coefficient 28420. Abs 0.1% (= 1 g L^{-1}) 1.312 assuming all Cys residues are reduced.

Estimated half-life: The N-terminal of the sequence considered is M (Met). The estimated half-life is: 30 h (mammalian reticulocytes *in vitro*) >20 h (yeast *in vivo*) >10 h (*Escherichia coli in vivo*).

Instability index: The instability index (2) is computed to be 35.71. This classifies the protein as stable. Aliphatic index = 78.00. Grand average of hydropathicity (GRAVY) = -0.466

3D structure prediction: Building a homology model comprises four main steps: identification of structural template(s) alignment of target sequence and template structure(s) model building and model quality evaluation. These steps can be repeated until a satisfying modelling result is achieved. Each of the four steps requires specialized software and access to up-to-date protein sequence and structure databases. Swiss model software recruited for homology modeling introduced 1 model. Predicted model is shown in Fig. 2.

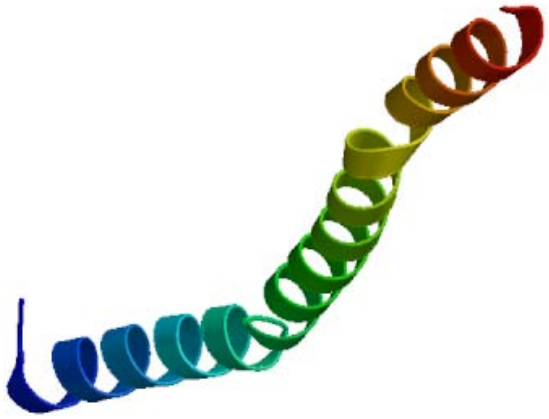

Fig. 2: 3D structure of VhhP2

Ligand binding site predictions: Ligand binding sites determined using Cofactor Software, indicate involvement of conserved residues include 10, 11, 13, 14, 17 in binding site with the highest Cscore LB (the confidence score of predicted binding site) (Fig. 3).

The calculated BS-score for this predicted binding site was 1.35. BS-score is a measure of local similarity (sequence and structure) between template binding site and predicted binding site in the query structure. Based on large scale benchmarking analysis, observed that a BS-score >1 reflects a significant local match between the predicted and template binding site. Template proteins with similar binding site are listed in Table 1.

Pocket detection: Pockets and descriptors have been calculated for VhhP2 structure with DoGSiteScorer: active site prediction and analysis serveris sumerized (Fig. 4 and 5). Identification of functionally and structurally important residues: Interprosurf annotated functional residues on the 3D structure of VhhP2. Residues predicted by auto patch analysis are: 50, 51, 52, 53, 54, 55, 56, 57, 32, 33, 34, 35, 36, 37, 38, 39, 40, 16, 17, 18, 19, 20, 21, 22, 23, 24, 14, 15, 13, 27, 28, 29, 30, 31, 25, 26, 11, 12, 10 (Fig. 5).

Table 1: Template proteins with similar binding site

Ranks	Cscore[LB]	PDB hit	TM-score	RMSD[a]	IDEN[a]	Cov.	BS-score	Lig. name	Predicted binding site residues
1	0.03	2r03A	0.772	2.05	0.057	1.000	0.50	Peptide	10, 13, 14, 17, 20
2	0.03	1hvvC	0.678	2.07	0.057	1.000	1.35	Tar	10, 11, 13, 14, 17
3	0.02	1w7i0	0.680	2.33	0.059	0.943	1.02	Peptide	12, 15, 17, 19, 20, 21, 23, 24, 25, 26, 28, 29, 32, 35
4	0.02	2xs8A	0.764	2.11	0.057	1.000	0.49	Peptide	27, 30, 31, 34, 35

Cscore[LB] is the confidence score of predicted binding site. Cscore[LB] values range in between [0-1]; where a higher score indicates a more reliable ligand-binding site prediction. BS-score is a measure of local similarity (sequence and structure) between template binding site and predicted binding site in the query structure. Based on large scale benchmarking analysis we have observed that a BS-score >1 reflects a significant local match between the predicted and template binding site. TM-score is a measure of global structural similarity between query and template protein. RMSD[a] the RMSD between residues that are structurally aligned by TM-align. IDEN[a] is the percentage sequence identity in the structurally aligned region. Cov. represents the coverage of global structural alignment and is equal to the number of structurally aligned residues divided by length of the query protein

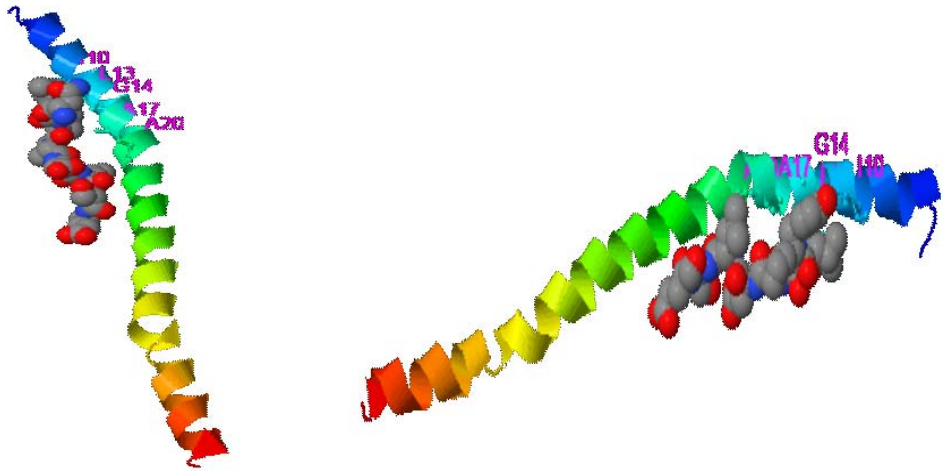

Fig. 3: VhhP2 ligand binding site predictions

Name	Volume [A^3]	Surface [A^2]	Lipo surface [A^2]	Depth [A]	Drug score [A^2]
PO	64.32	253.84	180.91	10.08	0.23

Undruggable=>druggable

0 1

Fig. 4: Pockets and descriptors calculated for VhhP2

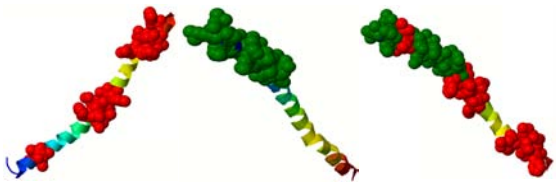

Fig. 5: Functional residues on VhhP2 3D structure

CONCLUSION

The gene of vhhP2 belonged a pathogenic *V. harveyi* strain isolated from diseased fish has been identified and discovered that vhhP2 is widely distributed in *V. harveyi* strains of various geographical locations and sources. The present study was designed to in silico resolving the major obstacles in the control or in prevention of *Vibrio harveyi* infections. We exploited bioinformatic tools to better understanding and characterizing the vhh P2 3D structure and select appropriate regions as effective B cell epitops.

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