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SHORT PAPER

In Silico Prediction of Molecular Interaction Within PmCBP-VP24 Complex to Understand Initial Instigation of WSSV into Shrimps

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Abstract

White Spot Disease is one of the most devastating diseases of shrimps. Molecular interaction between shrimp receptor protein PmCBP (Chitin binding protein of *Peneaus monodon*) and viral envelop protein VP24 is obligatory for binding of the White Spot Syndrome Virus to the shrimp digestive tract, and failure of this anchoring leads to an ineffectual infection. This is a first study that throws light on the molecular interaction of PmCBP-VP24 complex and provides important clues for initial steps of ingression of the virus into shrimps.

Introduction

White Spot Disease (WSD) is the deadliest viral diseases caused by the White Spot Syndrome Virus (WSSV) in shrimps. Several penaeid shrimps such as *Litopenaeus vannamei, Penaeus monodon, Marsupenaeus japonicus, and Fenneropenaeus indicus* are the most likely hosts infected by WSSV. The disease is transmittable and in its life threatening form it may completely obliterate the entire shrimp population within a week of the initiation of infection (Lightner, 1996). Despite of the diversified measures undertaken at present such as environmental control of WSSV, pre-exposure of shrimp to its pathogens, herbal treatments, DNA/RNA-based vaccines etc, no absolute drug/antiviral is available today that can obstruct the ingression of the virus into the host. The major drawback with the disease is that its mechanism starting from ingression, proliferation to dissemination of the virus inside the host, which is to be understood clearly (Verma, 2017).

A healthy shrimp is mostly infected by a WSSV infected shrimp because of the cannibalistic nature of shrimp. Hence, WSSV infection is triggered when a WSSV infected live/dead shrimp is engulfed by a normal shrimp. The lining of the digestive tube of shrimp serves as the main site from where the infection commences. The digestive tract is composed of oesophagus, stomach, midgut and hindgut (Felgenhauer, 1992). The oesophagus, stomach and hindgut portions are constituted by a chitinous lining while midgut

epithelium is generally lined by a semi-permeable peritrophic membrane (PM) (Hackman, 1987). This PM is constituted by chitin fibrils that get ingrained inside a collage of proteins, proteoglycans, and mucopolysaccharides. Chitin binding proteins (CBPs), Peritrophin-Like Protein (PTs), C Type lectins (CLs) are largely the most crucial receptors that form part of this PM. Among these receptors, a highly investigated protein is the chitin binding protein PmCBP (CBP in *Penaeus monodon*). An analogous type of CBP found in tissue of *Litopenaeus vannamei* is LvCBP (Chen *et al.,* 2009).

In a recent work, yeast two hybrid experiments were carried out to assess the proteins that interact in vitro with PmCBP. It was reported that PmCBP could get associated with a cluster of at least 11 viral envelope proteins and VP24 is one of the most important protein among this cluster (Huang *et al.,* 2014). Moreover, a novel multifaceted proteins aggregation (named 'infectome') got recognized, which was constituted by proteins namely VP24, VP28, VP31, VP32 VP39B, VP53A and VP56 (Huang *et al.,* 2014). This complex played pivotal role in mediating viral percolation across the basal membrane of the alimentary canal by getting associated with CBP. The chitin binding assays have further indicated that interaction between VP24 and PmCBP serves as a key association between the infectome and CBP (Li, 2015).

The viral envelop protein VP24 protrudes outside from the viral envelope (Sun *et al.,* 2016) and this feature additionally facilitates its interaction with CBP. Mutagenesis experiments have further revealed that amino acids starting from 186 up to 200 towards C-terminal portion of VP24 get in association with CBP (Li, 2015). Moreover, the ingested food takes a time of almost 4h to be ingested and absorbed from the digestive canal of the shrimp (Marte, 1980). Hence the interaction of chitin-VP24 within this stipulated time period is quintessential for anchoring of the viral particle onto the internal lining of the shrimp alimentary canal. Hindrance in this interaction may result into ineffective attachment progression leading to unsuccessful infection.

In context to this, the objective of the present study is to understand molecular interaction between viral envelop protein VP24 and shrimp receptor protein PmCBP by applying the *insilico* approaches. To achieve this, molecular modelling was done to design the 3D structure of PmCBP. After designing the 3D structure of PmCBP, molecular docking and dynamics approaches were applied to docked 3D structure of PmCBP to VP24 in order to understand the amino acid interactions occurring within the VP24-PmCBP complex. The present study provides a glimpse on initial infection and ingression of the virus within the shrimp body because PmCBP-VP24 complex is a vital complex that takes part in assisting virus to enter shrimp body. This study can further be used to search appropriate inhibitors that can interfere in PmCBP-VP24 complex formation thereby impeding the entry of the virus into the shrimp body.

Material and Methods

Sequence Analysis of PmCBP Protein

The protein sequence of PmCBP was retrieved (Chen, 2007). Primary structure specifications like molecular weight, theoretical pI, atomic composition, extinction coefficient, estimated half-life, aliphatic index and grand average of hydropathicity (GRAVY) of PmCBP protein were computed using ProtParam tool (Gasteiger *et al* 2005). In order to have a detail insight into the secondary structure of the protein, DiANNA tool was used. DiANNA tool is a neural network application that provides information of disulfide connectivity and fold stabilization within the protein. The tool was used to access the disulphide linkages in PmCBP protein (Ferre and Clote, 2005).

Preparation of Molecules PmCBP and VP24

In order to predict 3D structure of PmCBP, homology modelling was done by using BlastP and Protein Data Bank. Since an appropriate template was not obtained using BlastP, therefore, we performed de novo modelling approach. We used iTASSER server (Roy *et al.,* 2010) for de novo modelling of the protein PmCBP. When the protein sequence of PmCBP is used as input to I-TASSER server, then the server starts searching for appropriate template for PmCBP. These templates are identified by LOMETS (a multiple threading approach) from Protein Databank (PDB). LOMETS uses 10 threading programs namely 1. PROSPECT2 2. MUSTER 3. Neff-PPAS 4. SPARKS-X 5. PROSPECT2 6. HHSEARCH2 7. PROSPECT2 8. HHSEARCH I 9. SPARKS-X and 10. Neff-PPAS. All these programs generate thousands of template alignments, and I-TASSER selects only the most significant alignments. The ten most significant templates are selected by I-TASSER and the output is revealed by the parameters *v.i.z* Iden1, Iden2, Coverage, and normalized Z-score. The most important parameter that determines best template is the Z-score. The value of Z-score >1 suggests that the template is showing good homology with the target. After assessment of a reasonable template, I-TASSER generated a large assembly of structural conformations known as decoys. Further, I-TASSER used SPICKER program to cluster all the decoys on the basis of similarity to generate 5 structural models of PmCBP. The best model among the 5 generated models was tabulated on the basis of C-score and Verify3D score (Eisenberg *et al.,* 1997). Since amino acid 186-200 of VP24 interact with PmCBP, therefore, the corresponding peptide sequence was traced and secondary structure prediction of VP24 was also performed using PSIPRED server. The models generated were visually analysed using Pymol (Lill and Danielson, 2011).

Molecular Docking and Simulation Studies

The molecular docking and simulation studies were carried out using CABS-dock web server. The algorithm running behind CABS-dock server is very robust and does not require any prior information regarding either template or binding site between the protein and peptide. The online server can be accessed at (http://biocomp.chem.uw.edu.pl/CABSdock/). CABS-dock provides a user friendly interface for understanding flexible docking between a protein and peptide (Kurcinski *et al.,* 2020). A detailed insight into docking procedure is provided by the server whilst providing complete flexibility to the peptide sequence as well as providing permissible flexibility to the protein receptor sequence. CABSdock performs a blind search of the binding site while performing docking. The docking involved in our study was performed using three steps: (i) Random structure of VP24 encompassing region between residues 186-200 were generated and positioned on the surface of PmCBP. (ii) Replica exchange Monte Carlo dynamics was employed to dock PmCBP to V24(residue 186-200) and 10,000 models of complex PmCBP-V24(residue186-200) were generated. The most appropriate docked complex was selected in following steps: (i) all the unbound conformations were rejected; (ii) 100 lowest binding energy models of PmCBP-VP24 complex were selected and further screened; (iii) the screened model of the docked complex was clustered on the basis of root-mean-square deviation (RMSD) value (iv) the clusters were categorized according to size and the ten most populated clusters were finally selected. Finally, the docking prediction result, clustering details and contact maps generated by CABS-dock were analyzed. The final docked PmCBP-VP24 complex was visualized in Pymol. Further, the amino acid interactions occurring between protein PmCBP and peptide VP24 were tabulated using Ligplot (Wallace *et al.,* 1995). The binding energy of PmCBP-VP24 complex was evaluated using HawkDock server (Weng *et al.,* 2019). The server employs molecular mechanics Poisson–Boltzmann surface area (MM-PBSA) to estimate interaction free energies between various protein-protein complexes (Weng *et al.,* 2019).

Results

Sequence Analysis of Protein PmCBP

In the study, we performed sequence analysis of PmCBP using ProtParam, which revealed that PmCBP was made of 168 amino acids. The estimated molecular weight of PmCBP is 17471.11 daltons. The total negatively charged residues and positively charged residues are 8 and 18 respectively. The Aliphatic index of the protein is 56.43 suggesting that it is a soluble protein. The Grand average of hydropathy (GRAVY) is -0.114 suggesting that protein is hydrophilic in nature. DiANNA tool predicted that the protein contained 3 disulphide linkages, which provided extracellular stability to the PmCBP (Table1).

Preparation of Molecules PmCBP and VP24

The secondary structure prediction of PmCBP revealed that the protein is mainly comprised of loops (83%) while sheets and helix make 11% and 6% respectively (Figure 1). iTASSER fetches reasonable template proteins based on the similarity between folds obtained from the PDB library. I-TASSER calculates precise folds with much descent accuracy rate and with a lower computational time. The structure having PDB ID: 6bn0 is selected as a suitable template as it shows most favourable values for all the four parameters (i.e. Iden1, Iden2, Coverage and normalized Z-score) cumulatively.

The final 3D structure of PmCBP was designed by I-TASSER on the basis of 6bn0 template. I-TASSER generated numerous decoys that were clustered on the basis of similarity by using SPICKER program to generate 5 models. The relevance of the generated models can be assessed by C-score. C-score typically ranges between -5 and 2. Higher the value of C-score, the more significant

Table 1. Predicted disulphide bonds as suggested by DiANNA tool

Predicted bonds	Amino acids
86-126	STSFACLDRPY-QYSFLCGEGSR
101-139	DEENSCHIFHI- QKELTCVAESE
107-148	HIFHICYPALF-SEAIPCQESSN

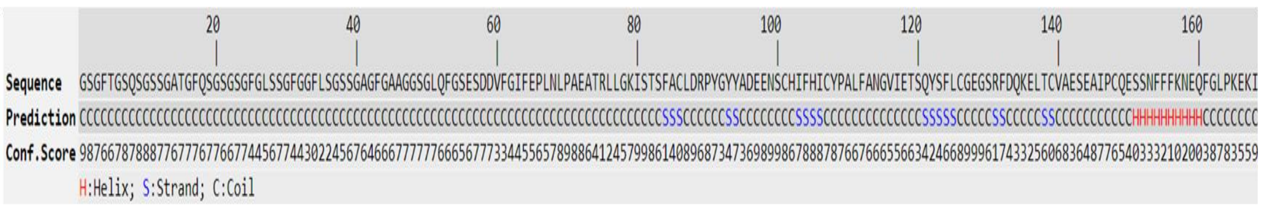

Figure 1. Secondary structure prediction of PmCBP showing presence of helix, strands and coils

model is considered (Yang 2008). Since model 1 depicted maximum C score, and 90.48% of the PmCBP residues show average 3D-1D score ≥ 0.2 as per Verify3D results therefore, it was finally selected as the best model for PmCBP (Figure 2). VP24 secondary structure prediction revealed that the amino acid 186-200 correspond to the strand portion (Figure 3).

Molecular Docking and Simulation Studies

The docking prediction performed by CABS dock server result showed 10 models for the docked complex. Model 1 (Figure 4) is considered to be the most probable model because it depicted RMSD (root-mean-square deviation) value 2.45 Å. The model showing RMSD value <3 denotes a high quality model; RMSD value 3-5.5 Å denotes a medium quality model; RMSD value >5.5 Å denotes a low quality model. After selection of an appropriate model, a detailed investigation was done into the amino acid sequences that interact between the protein PmCBP and peptide sequence of VP24. The amino acid interactions revealed that the complex is stabilized by 4 hydrogen bonds (Table 2). Within the PmCBP-VP24 complex, Gly1, Thr5, Asp88 and Cys86 of PmCBP respectively interact with Tyr5, His4, Asn2, Leu7 of VP24 through H-bonds (Figure 5). These H-bonds contribute towards the stability of the complex. The binding free energy of complex PmCBP-VP24 peptide was evaluated by HawkDock server and found to be -35.04 (kcal/mol) proving that the complex is stable. Additionally, some lipohilic interactions also contribute towards the stability of PmCBP-VP24 complex (Table 3).

Discussion

WSSV infection commences when a healthy shrimp engulfs a WSSV infected shrimp. The virion particles from the engulfed diseased shrimp get dispersed inside the shrimp alimentary canal lumen till they come in direct contact with the alimentary canal membrane. The primary component of WSSV is its envelop and the constituent envelop protein that come in direct contact with shrimp digestive membrane thereby playing a crucial role in instigation of the disease. The most abundant viral envelop proteins of WSSV are VP28, VP24, VP26, and VP19. The proper anchorage of the viral particles from the dead or infected shrimp to the inner membrane of shrimp alimentary canal is mandatory for the commencement of the WSSV infection. After an accurate binding between the viral proteins with the shrimp receptor proteins that line alimentary canal of shrimp, the virus traverses across the alimentary canal of shrimp in order to reach at the basal membrane (Verma *et al.,* 2017). Once the virus reaches the basal membrane, the virion particles ooze through the alimentary canal to circulate in the plasma and finally

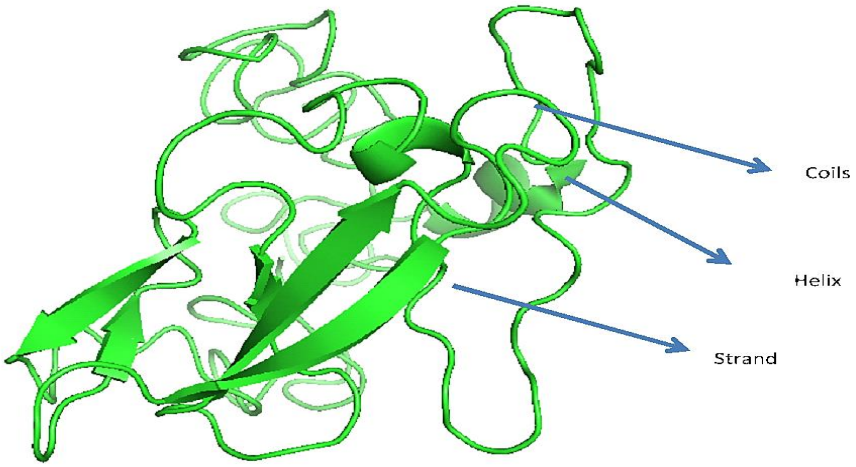

Figure 2. 3D structure of PmCBP showing helix, coils and beta sheets as predicted by iTASSER server.

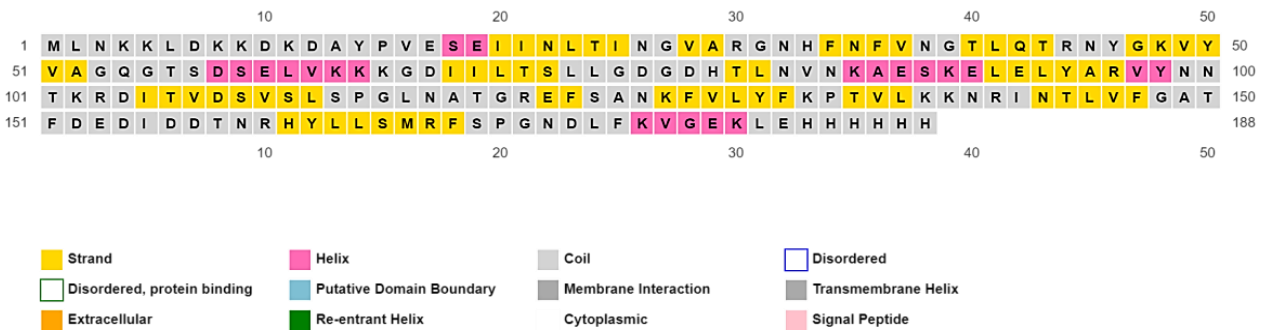

Figure 3. Secondary structure prediction of VP24

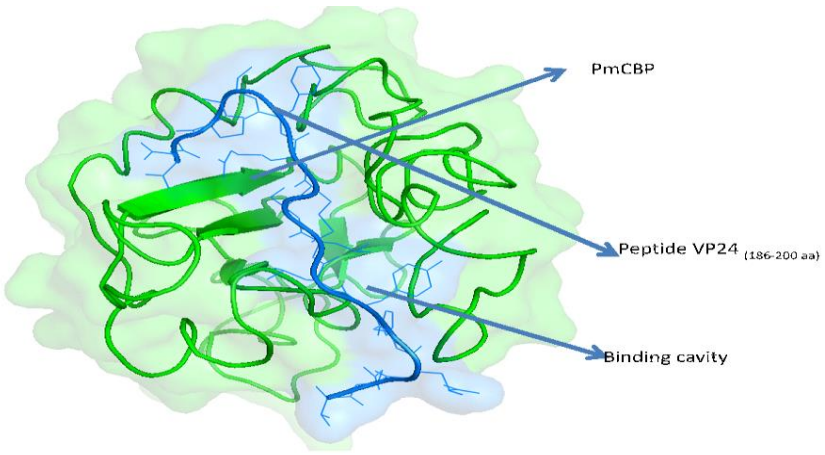

Figure 4. 3D structure of PmCBP-VP24 complex as generated by CABSdock server. The binding cavity and binding pose are shown in light blue colour. The shrimp receptor protein PmCBP is in green colour while amino acid residues 186-200 of VP24 are depicted with dark blue colour.

Table 2. Amino acid interactions between the receptor (PmCBP) protein and peptide (VP24 186-200 amino acid)

Receptor residue	Peptide residue	Receptor residue	Peptide residue
GLY1	TYR5	THR5	HIS4
ASP88	ASN2	CYS86	LEU7

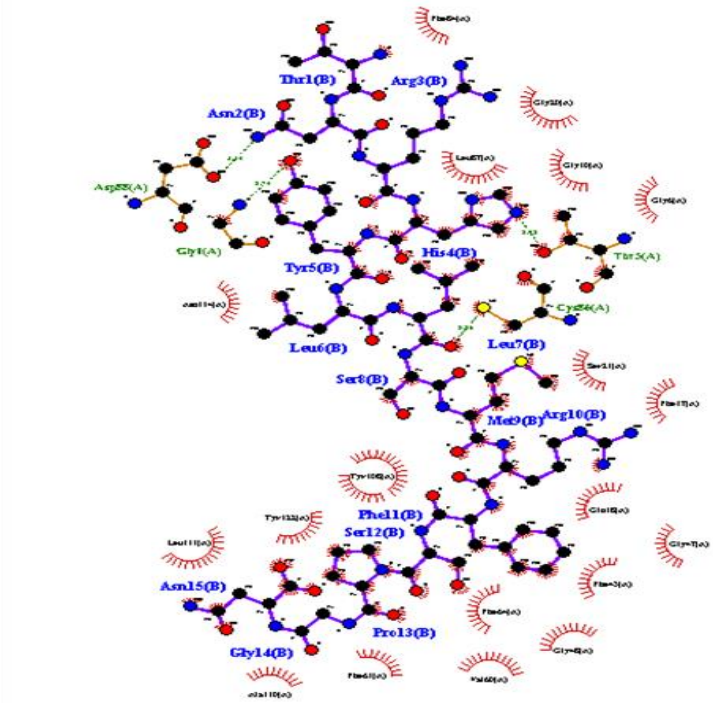

Figure 5. Detailed amino acid interactions between the PmCBP-VP24 cavity showing the amino acids that interact between PmCBP and VP24 peptide

Table 3. Lipophilic interactions between the receptor (PmCBP) protein and peptide (VP24 186-200 amino acid)

Receptor residue	Peptide residue	Receptor residue	Peptide residue
Gly10	Gly6	Leu111	Tyr122
Phe84	Gly20	Phe61	Ala110
Phe84	Leu87	Phe64	Val60
Ser21	Leu87	Phe43	Gly48
Tyr108	Asn 114	Gln18	Gly47

reach to the target organs such as eye-stalk, cells of circulatory system, brain, and reproductive organs such as gonads (Lo *et al.,* 1997; Rajendran *et al.,* 1999; Kou *et al.,* 1998). Here the virus enters in the nucleus of individual host cell and replicates itself to get disseminated throughout the shrimp body resulting into death of individual shrimp (Escobedo *et al.,* 2008).

Hence, ingression of the virus into shrimp body initiates by the proper anchorage between virus and shrimp that is assisted by formation of complexes between shrimp receptor proteins and viral envelop protein. PmCBP-VP24 is one of such important complex that plays pivotal role in process of disease commencement in shrimps. Since, the structure of PmCBP was not available therefore; we designed PmCBP by *insilico* approaches. Further, VP24 has been postulated to form the hub of the envelop protein complex 'infectome' that plays critical role in recognition of the host cells, anchoring to the host cell as well as guiding WSSV into the host cell (Huang *et al.,* 2014). Recently VP24 was reported to show interaction with shrimp chitin receptors but the exact role and molecular mechanism involved remained unknown (Huang *et al.,* 2014; Li *et al.,* 2015). Furthermore, VP24 has been suggested to depict a possible monomer-trimer transition during this ingression process of WSSV into the shrimp (Sun *et al.,* 2016). Hence, when the virus intrudes into the shrimp body, the protein VP24 transits to its monomeric form and presents its β9 to surface of chitin binding protein PmCBP. The amino acids spanning within this β9, namely amino acid 186-200 show protein-protein interaction with PmCBP (Sun *et al.,* 2016, Li *et al.,* 2015). For the first time, we have found that this PmCBP-VP24 complex is stabilized by means of H-bonds and lipophilic interactions. The H bonds located between Gly1, Thr5, Asp88 and Cys86 of PmCBP respectively interact with Tyr5, His4, Asn2, Leu7 of VP24 and these bonds might play crucial role in adhering VP24 to PmCBP but they have to be further validated experimentally. After the formation of PmCBP-VP24, VP24 promotes anchoring of WSSV to the alimentary canal of the shrimp by facilitating the attachment of VP28 to the host cell, thereby promoting membrane fusion in order to initiate the viral infection (Sun *et al.,* 2016).

The infective amino acid residues that were sandwiched within the complex PmCBP-VP24 traced in the present study might be imperative for shrimp-viral interaction at molecular level. Hence, disruption of bonding between these amino acids might result in an ineffective formation of PmCBP-VP24 complex thereby stopping the cascade of molecular events involving various viral structural proteins especially VP24 and VP28. Thereby, the virus might probably not percolate seamlessly across the digestive tract hence causing a hindrance in dissemination of the virus throughout the body. Since PmCBP-VP24 complex is vital for the ingression of WSSV into the shrimp therefore, we would try to predict a potential inhibitor in our future studies that can disrupts the amino acid interaction occurring within the complex. After wet lab validation, such inhibitor/s will can be presumed to be a plausible antiviral that can be introduced as a shrimp feed in order to disrupt the initial instigation process of the virus into shrimp thereby resulting into disease mitigation.

Ethical Statement

Not applicable.

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Author Contribution

Conceptualization: AA, AKV Data Curation: KY Formal Analysis: KY, AKV Investigation: KY Methodology: KY, AKV, Resources: AA, Supervision: AA, AKP Visualization: KY, AKV, Writing -original draft: KY, AKV Writing -review and editing: AA, AKV

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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