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Computational Mapping of Interaction Sites of Synthetic Antibody Clone P109d9on VP28 Protein

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ABSTRACT: Antibodies otherwise called immunoglobulins are the major proteins forming the primary line of defense during the pathogenic entry. The high specificity and affinity of the antibodies distinguish them from the other immune system components and it is achieved by the characteristic CDR (Complementarity Determining Regions) located on the variable arm. The single chain formats of antibody variable regions (ScFv) are used in the disease diagnostics and therapeutics development. Recently, antibody based techniques are developed for the detection and control of White Spot Syndrome Virus (WSSV), the catastrophic disease agent in shrimp farms. The ScFv clone targeting the VP28 protein component of WSSV named P109D9 was chosen and the sequence-structure information was used to locate the attachment sites of antibody. Theoretical model of the ScFV antibody interaction on VP28 protein was made using the computational resources and the analysis results were compared with the experimental information. The predictive interaction analysis can help in the development of antibodies with improved affinities for the detection and treatment of diseases in future.

I. INTRODUCTION

Antibodies are remarkable kind of glycoproteins produced by the immune system, are important in forming protective barrier during invasion of infectious agents (Kumagai and Tsumoto, 2001).. Structurally antibodies are made of two polypeptide chains designated as VH (heavy) and VL (light) chains whose N-terminal domains have variable sequences. The immunoglobulin (Ig) fold structure is characterized by the beta- barrel architecture made of the anti-parallel beta sheets flanked by loops (Braden and Poljak, 1995). The specificity of the immunoglobulins are achieved by the distinct domains called Complementarity determining regions (CDRs) which are formed of six characteristic hypervariable loops located within the variable domain (Kumagai and Tsumoto, 2001). The more constant amino acids outside the CDR regions also named frame work regions are important in bringing the CDR loop together to form surfacecomplimentarity to bind to the epitope of antigen eventhough do not directly take part in antigenic interactions (Sundberg and Mariuzza, 2003; Kumagai and Tsumoto, 2001).

The technology named Phage Display (Ph D) recombines the light and heavy chains of variable regions of immunoglobulins into a single chain called single chain Fv (ScFv) by a spacer and are expressed in phage based systems (Hoogenbomet.al, 1998). Single chain Fv antibody formats (ScFvs) are used effective tools in the disease diagnostics and in therapeutic developmentsdue to their high specificity.ScFv antibodies expressed in phage systems carry advantages that they are produced in large quantities in a relatively shorter time.

White Spot disease caused by White Spot Syndrome Virus (WSSV) is considered to be the major threat in shrimp farming industry (Sanchez-Paz, 2010). WSSV is a highly virulent pathogen and thatit rapidly spreads to the farms causing high mortalities. A fully protectivetherapeutic strategy such as drug or vaccine is not discovered so far to control the infection despite the fact that shrimps lack a true adaptive immune system. Several studies are made and many are underway to develop a therapeutic agent against the infection by passive protection mediated through antibodies. Antibodies targeting WSSV are also applied in the disease diagnostics development too. Single chain antibodies recognizing the VP28 of WSSV were synthetically made and their epitope specificities were validated



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experimentally(Wang *et.al*, 2008).The ScFv109D9nucleotide sequencewas chosen from the data bank and computational analyses were performed.

Computations help in the development and redesign of antibodies with high affinity or other improvements by protein modeling by exploiting the sequence to structural relationships (Kuroda *et.al*, 2012).In our study we attempted to model the ScFv antibody structure using the sequence entry and its antigenic interaction was analysed through the molecular docking simulations.

II. MATERIALS AND METHODS

ScFV sequence source

The nucleotide sequenceof the ScFVdesignated 109D9was downloaded from the Genbank sequence repository (Accession number: EU583429) and was translatedinto six reading frames using EXPASY 'Translate'tool.

Homology modeling of ScFv antibody

The translated protein sequence was modeled using the CPH models 3.2 server. The template was chosen by the server after the sequence identity analysis and was aligned with the query sequence. The sequence was then processed by the server for modeling.

Antibody Modevaluation

The model obtained was evaluated for dihedral angle analysis, associated energy, main chain and side chain properties. The structure was submitted for Ramachandran Plot analysis. The structure was then submitted to the respective programs to verify the associated energy, main chain and side chain propertiesand their statistics.

Molecular docking of ScFv and the VP28 protein

The crystal structure of VP28 (PDB identifier: 2ED6) was downloaded from the Protein Data Bank (PDB) and was visualized in Pymolsoftware. The monomeric chain of the antigen was selected for the docking analysis as the starting structure. The generated antibody structure and antigenic monomer were submitted to the Cluspro docking server for analysis. The docking protocol was performed with the default parameters and non-CDR regions of the antibody chains were automatically masked.

III. RESULTS AND DISCUSSION

Translation of the VL and VH chains

The translated nucleotide products of light and heavy chains of ScFv are shown in Fig 1. The two chains connected by linker sequences are highlighted in different colors. BLAST similarity search was performed to identify and confirm the polypeptide chains.

MAEVLKQQSGPELVKPGASVKMSCKASGYTFTDYVISWVKQRTGQGLEWIGE
TYPGSGNTYYNEKFKGKATLTADKSSNTAYMQLSSLTSEDSAVYFCSRGLYT
MDFWGGQGTSTVSTGGGGSGGGGSAAGGSDVVMTQSHKFMSTSVGDRVSITCK
ASQDVSTAVAWYQQKPGQSPKLLIYSASYRYTGVPDRFTGSGSGTDFTLTISN
VQSEDLADYFCQQYYSYPYTFGGGTKLEIKRAAAGAPVPPDPLEPRAA

Fig1.The translated amino acids nucleotides representing the chains of variable region. VL chain(blue) and VH chain (red) and linker (green).

Homology Models of ScFv

The homology models were generated based on the templates and were visualized using the Discovery Studio visualiser. The query-template score was found to be 419 bits, with a sequence identity of 67.3 %.The associated energy of the model is found to be -5099.522 KJ/molimplies that the structure is stable.The structure with lesser energy was chosen for further analysis (Fig 2). The model was submitted for the Ramachandran Plot analysis of RAMPAGE program. The about 93 % of plotted dihedral angles were observed to fall in the most favored region in the

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Ramachandran plot (Fig 3) suggesting the quality of the model generated is good. The Z score value of the model was found to be -183 which indicates that the quality is good and the model is built with moderate accuracy.

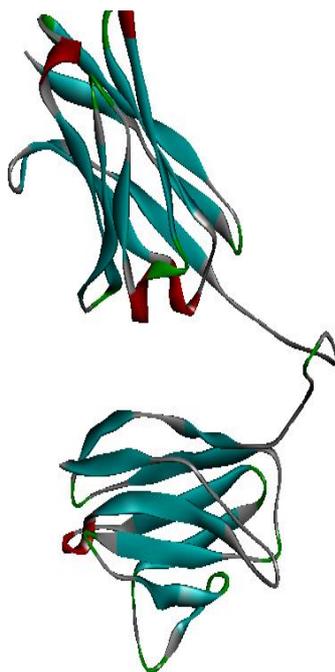


Fig 2. Homology based model generated by the CPH 3.2 modeling server. The antibody acquires beta barrel shape with the hypervariable loops connected represented in DS viewer.

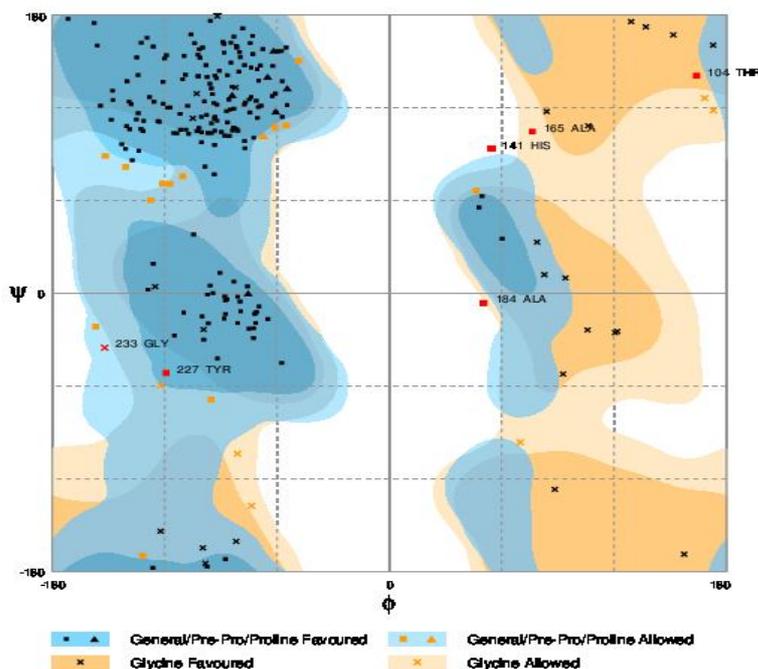


Fig 3. Ramachandran Plot showing the dihedral angle arrangement of the modeled structure. More than 93% of the residues are placed in most favoured regions.

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Docking analysis of the ScFv antibody-VP28 complex

Molecular docking analysis unravels the antibody interaction specificity to the epitope regions on VP28 protein. The various docking solutions obtained were analyzed thoroughly and compared (Fig 4). Complementarity was observed at the binding interface of the antibody-epitope complexes. In all the docked complexes the CDR regions of the variable domain was found to be interacting each other and were clubbed together in order to attain the epitope shape complimentarity and the best fit. The three docking solutions were screened and selected based on the best fit formed across the complexes. The experimental data of ScFv 109D9 shows that it reacts with multiple epitope regions on the VP28 protein. Hence the antibody is thought to be having discontinuous epitope specificity. Our docking interaction study also reveals the attachment of the antibody on the multiple sites on the VP28 protein.

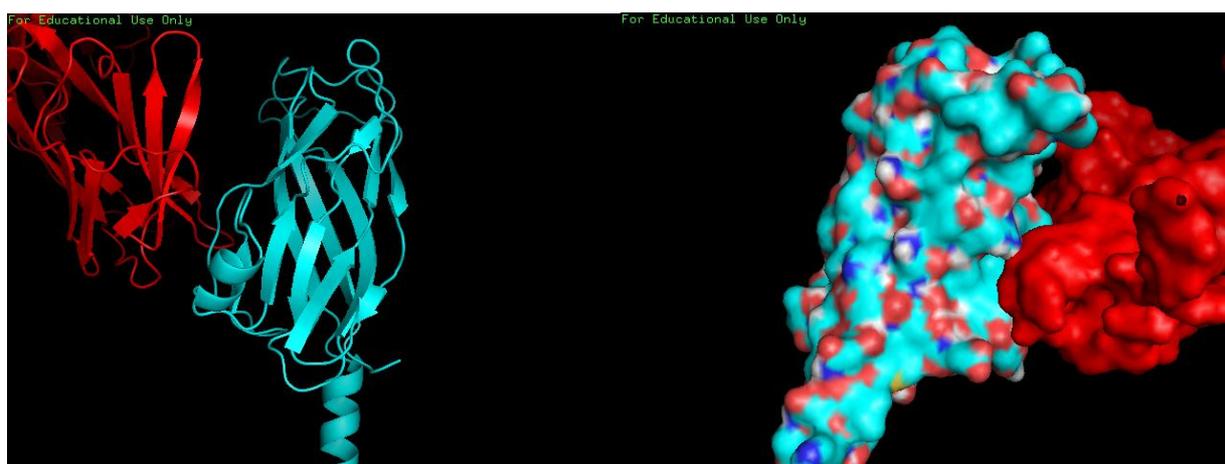
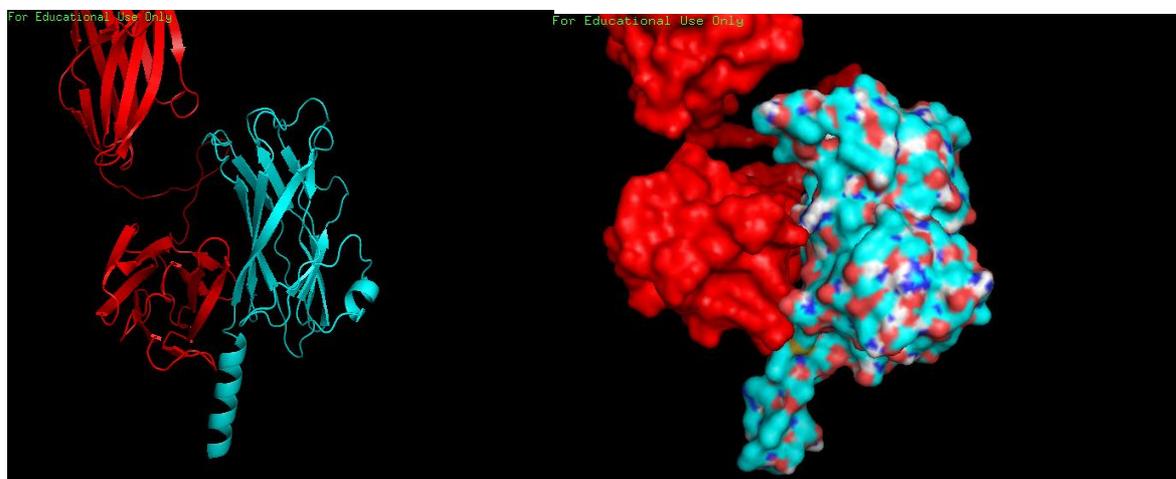


Fig 4. The docking solutions of ScFv antibody binding on the VP28 protein represented in Pymol. A and B; The two different docking orientations and attachment sites of ScFv P109D9 antibody on the VP28 protein represented in cartoon and space fill models.

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