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Prediction of the Protein 3D Structure of Capsule Biosynthesis Protein capA of *Porphyromonas gingivalis* strain-ATCC 33277 using Homology Modeling and Structure Analysis

Dr. Priyanka K. Cholan,
Reader, SRM Dental college, Ramapuram, Chennai

Judith Grace Jenefa,
Intern, SRM Dental college, Ramapuram, Chennai

Abstract

Introduction:
Porphyromonas gingivalis, is a bacterium that is known to express a wide array of virulence factors, which attributes for its high degree of association with periodontal inflammation. Among the various virulence factors expressed by this periodontal pathogen, capsule is of particular interest as it forms an insulating envelope that not only protects it from the antibiotic penetration but also enables the bacteria to evade the host defense system, and sustain for a protracted time causing rapid progression of periodontitis. This study deals with the determination of the protein 3D structure of capsule biosynthesis protein capA of Porphyromonas gingivalis strain-ATCC 33277.

Materials Used:
FASTA protein sequence from NCBI Database, Homology modeling server Swiss-model workspace, CASTp server, Pro-Q and PROSESS.

Methodology:
The FASTA protein sequence of capsule biosynthesis protein capA of Porphyromonas gingivalis strain-ATCC 33277 was retrieved from NCBI database (NCBI- National Centre for Biotechnology Information). With the retrieved sequence the 3D structures of the protein were determined by homology modeling server Swiss-Model workspace. Three models were predicted, and the most relevant structure is estimated by passing various quality assessments steps like ProQ (Protein Quality Predictor) and validating test –PROSESS (Protein Structure Evaluation Suite and Server).

Results:
The protein 3D modeling of capsule biosynthesis protein capA of Porphyromonas gingivalis strain-ATCC 33277 were subjected to a series of quality check steps and the three most relevant models were prognosticated. In association with this model 1 was considered to be the most validated and likely structure.

Conclusion:
This study kick starts the long journey which enables us to target the active sites on proteins responsible for causing damage to the host. Further studies must be performed in this field including the recognition of the protein structure which would bind with the active sites, thus, helping in targeted drug therapy and thereby resulting in disease prevention.

Key Words: Capsule biosythesis protein capA, 3D protein structure, FASTA, Swiss model, CASTp, PROSESS.

1. INTRODUCTION

Periodontitis is a chronic oral inflammatory disease process that is triggered by Porphyromonas gingivalis, a predominant black-pigmented anaerobic rod of the red complex group residing in subgingival biofilms (1,2,3). It is widely recognized as a major contributor to the development of periodontal diseases and other systemic infections, including coronary artery disease, stroke, diabetes mellitus and preterm delivery of low birth weight infants (4,5,6). Porphyromonas gingivalis harbors many virulence factors such as capsule, fimbriae, haemagglutinin, lipopolysaccharide (LPS), outer membrane vesicles, organic metabolites such as butyric acid and various enzymes such Arg- and Lys-gingipains, collagenase, gelatinase, hyaluronidase and proteases and can also invade various cells including epithelial, endothelial and smooth muscle cells (7).

Among the multifarious virulence factors elicited the capsule plays a crucial role as it is a recognized way to protect the bacteria from the clearance mechanism of the host defenses. However, encapsulation also has a direct effect as they shield microbial surface components and being a heat-resistant factor (9),down regulates cytokine production promotes virulence through evasion of the host response and prevents antibiotic ingress leading to prolonged survival of the bacterium, sequentially resulting in a long-term inflammatory response. Greater heterogeneity is exhibited by P. gingivalis, as some strains are encapsulated and others are non-encapsulated.(8)

This study is based on the proposed plan of blocking the capsule biosynthesis protein in P.gingivalis, so that the host immune response mediators will directly enter into the microorganism leading to its destruction. Thus the cardinal step for the extensive plan of targeted drug delivery involves the identification of the structure of the protein. Many different types of biological experiments, including site-directed mutagenesis or structure-based discovery of specific inhibitors can be performed. Indeed, the number of known protein sequences is greater compared to the number of structurally characterized proteins.

Various methods of identifying a protein structures are available. They include a. genetic methods– site-directed mutagenesis, conceptual translation -b. Protein purification: chromatography, protein assay, gel electrophoresis, electro-focusing; -c. Advanced studies such as x-ray crystallography, protein NMR, cryo-electron microscopy, small angle scattering, etc. These methods are extensive and very tedious. Hence computational methods for modeling 3D structures of protein have been developed to overcome these limitations. They include: molecular dynamics, protein structural alignment, protein ontology. Since the number of possible folds in nature appears to be limited and the 3D structure of proteins are better conserved than their sequences, there exists a possibility to identify a homologous protein with a known structure (template) for a given protein sequence (target). In these cases, homology modeling has proven to be the preferred method of choice to generate a dependable 3D model of a protein from its amino acid sequence as impressively shown in several meetings of the bi-annual CASP experiment. Hence this study was aimed at the identification of the protein3D structure of capsule biosynthesis protein capA of Porphyromonas gingivalis strain-ATCC 33277, by using homology modeling.

2. MATERIALS AND METHODOLOGY

2(i) Homology modeling

Homology modeling is routinely used in many applications. They include virtual screening, or rationalizing the effects of sequence variations. Building a homology model involves four elemental steps:

(1) Identification of the structural template(s),
(2) Alignment of the target sequence and template structure(s),
(3) Model building and
(4) Model quality evaluation.

These steps must be repeated until a satisfying modeling result is achieved. Each of the above steps require a specialized software as well as access to up-to-date protein sequences and structure databases (10).

Further research in homology modeling brings out the use of seven detailed steps. They include,

1. Template recognition and initial alignment
2. Alignment correction
3. Backbone generation
4. Loop modeling
5. Side-chain modeling
6. Model optimization
7. Model validation (11)

2(ii) FASTA- Sequence Alignment Program

To performing the first step in homology modeling, simple sequence alignment programs are used. In this case, modeling of P. gingivalis –capA protein is performed by FASTA, which is the Fast Adaptive Shrinkage Thresholding Algorithm, developed by Pearson and Lipman. This program compares the test sequence and the query sequence and helps in formatting a template, which in turn provides us with the required sequence.

2(iii) SWISS-MODEL Workspace

With the sequence obtained, further modeling is done using the SWISS-MODEL. There are various modeling modes in the SWISS-MODEL. The mode "My workspace" is used in this study. SWISS-MODEL workspace is an integrated Web-based modeling expert system. For a given target protein, a library of experimental protein structures is searched to identify relevant templates. On the basis of a sequence alignment between the target protein and the template structure, a three-dimensional model for the target protein is procured. The template structure database used by this workspace is derived from the Protein Data Bank (12). Thus homology modeling with SWISS-MODEL workspace has proved to be effective in determining the 3D protein structure capsule biosynthesis protein capA of Porphyromonas gingivalis strain-ATCC 33277 and the following models were obtained- (refer figure - 1)

2(iv) CASTp- Computed Atlas of Surface Topography of proteins

The active sites or pockets in the protein were identified by using CASTp and are highlighted with green in the models. (Refer figure - 2). The proteins function through certain sites and hence recognition and identification of these active sites is essential to understand the function of the proteins. These active sites can be inhibited, which will in-turn reduce the action of the bacteria.

2(v) ProQ – Protein Quality Predictor

ProQ is a software to check the quality of the obtained model. If the predicted structure satisfies the validation parameters of ProQ then the structure was taken for further analysis. The following results were obtained for the various models. (Refer figure - 3)

FIGURE – 1-Showing SWISS model workspace

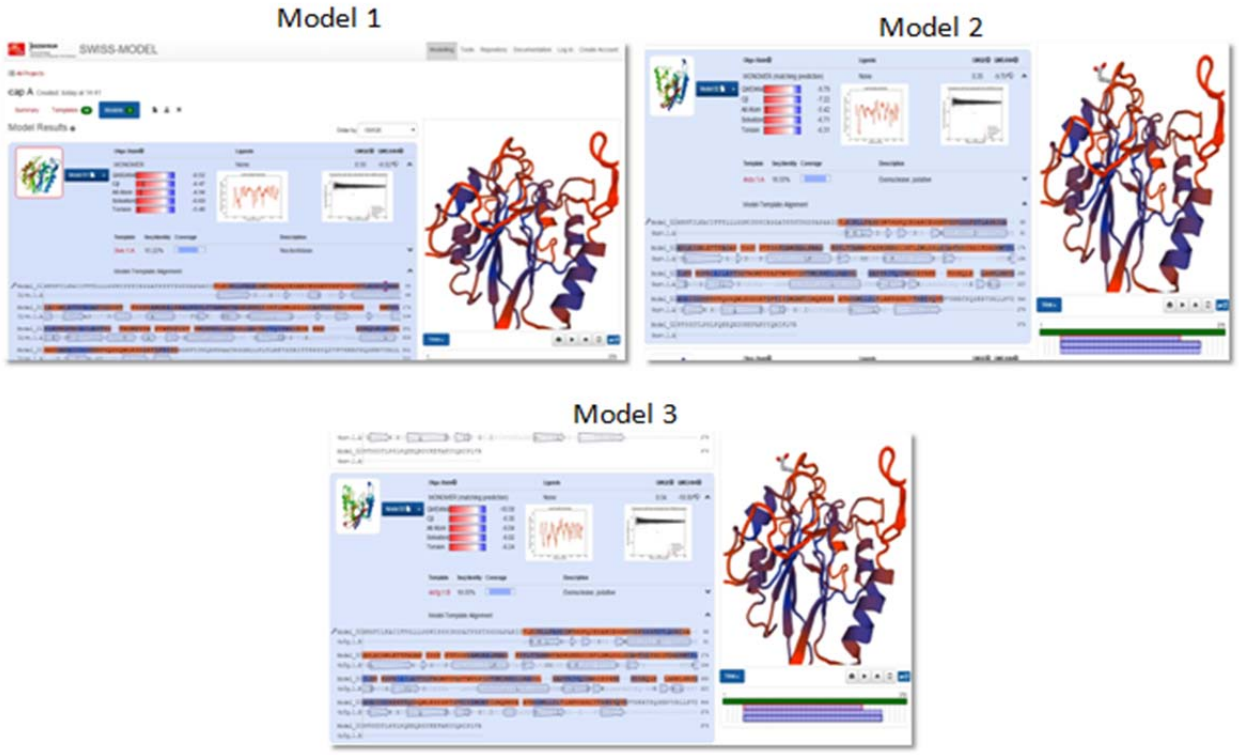


FIGURE – 2- Showing Computed Atlas of Surface Topography of proteins (CASTp)

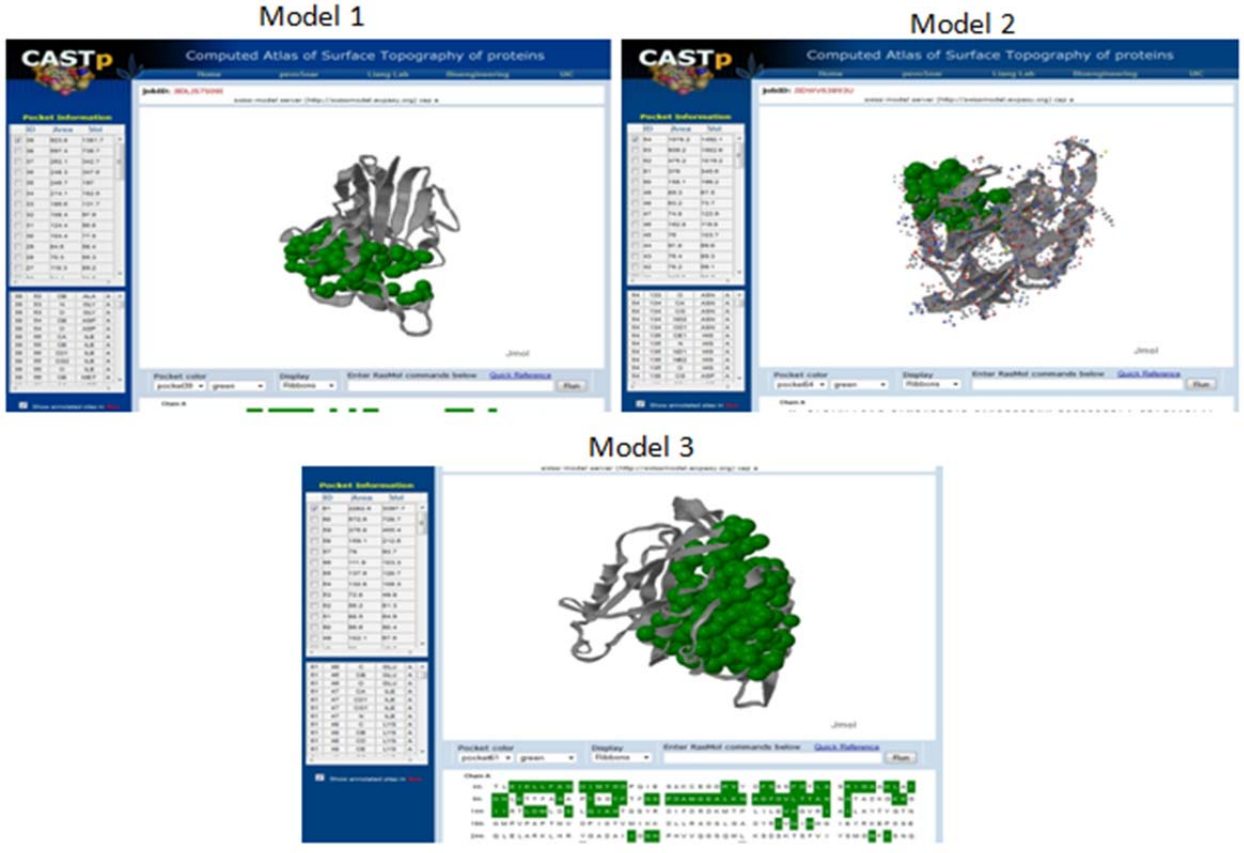


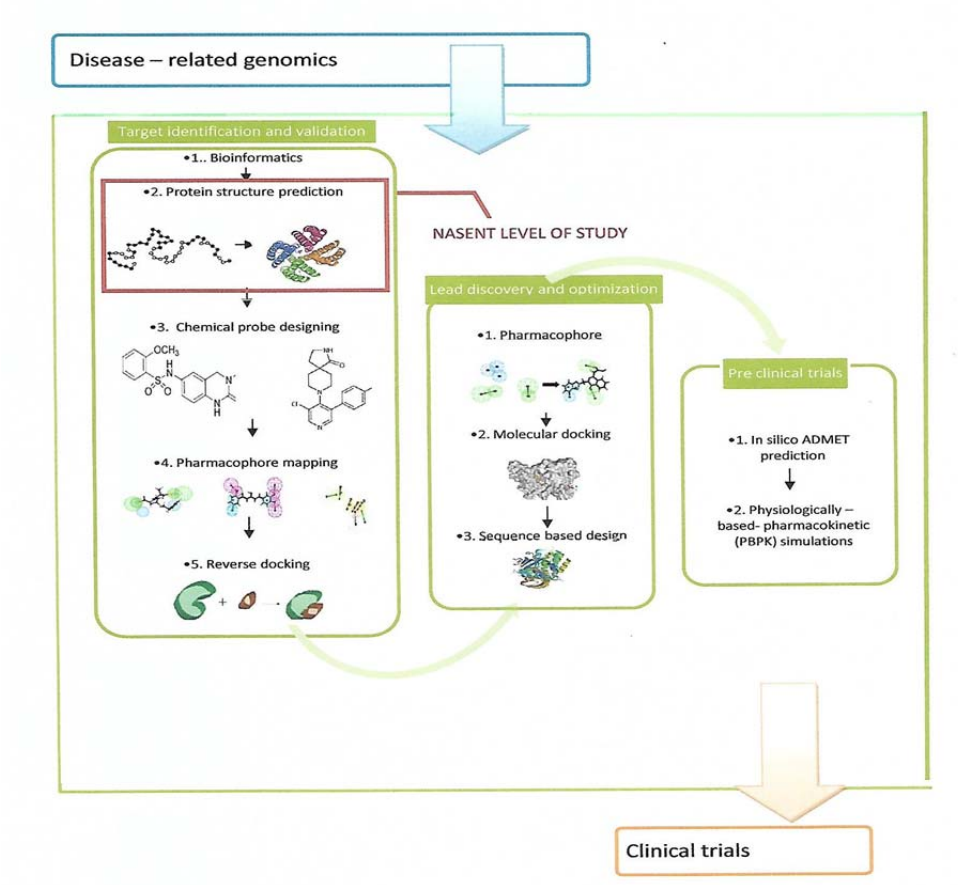
FIGURE – 3- Showing protein quality predictor analysis

Model 1

Model 2

Model 3

FIGURE – 4- Showing basics of Targeted drug delivery

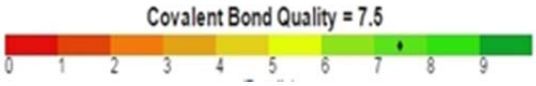


2(vi) PROSESS- Protein Structure Evaluation Suite and Server

The three models were further assessed by PROSSESS, to validate the best model.

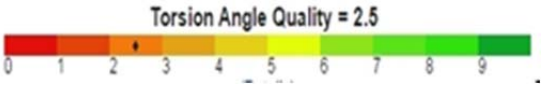
Table 1 showing the comparison of ProQ, CASTp and PROSESS values between the 3 models

	Model 1	Model 2	Model 3
LG score	2.685	3.275	2.178
Max sub	0.247	0.321	0.208
Overall quality	3.5	2.5	2.5
Covalent bond Quality	7.5	6.5	3.5
Non-Covalent bond Quality	3.5	2	2.5
Torsion angle Quality	2.5	1.5	1.5
Pocket area	923.8	1078.2	2282.6
Pocket volume	1381.7	1492.1	3397.7



The covalent bonds/peptide bonds play an important role in determining the shape of the protein that is very important for its function. The bond links the adjacent amino acid residues in a protein formed by condensation reaction between the amine group of one amino acid and the carboxyl group of another with the release of a water molecule. These bonds are highly specific, thus they are important in determining the structure of the protein. (13)

The non-covalent bonds also referred to as interactions are weak bonds and maintains the 3D structure of the large protein molecules. Their existence is transient and multiple bonds act together to produce highly stable and specific associations between different parts of a large molecule. (14)



These angles are important local structural parameters that control protein folding and provide the flexibility required for the polypeptide backbone to adopt a certain fold. Thus they provide insights into the function of the protein.

On compiling all the elaborate testing methodology datas, model 1 retrieved is the most pertinent protein structure of the capsule biosynthesis protein cap A of P.gingivalis

3. DISCUSSION

The structure of the protein influences its function and this depends on the various physical and chemical parameters. Although the information needed for life is encoded by the DNA molecule, the dynamic process of life in maintenance, replication, defense and reproduction are carried out by these proteins. Thus obtaining the three dimensional protein structure provides us with the information about medically relevant receptors, small ligands, etc and targeting this protein in the pathogen for therapy is essential with many benefits, including, decreasing the dosage of the drug, reduced adverse effects, low rates of drug toxicity, faster rates of action and definitive outcomes. Drugs are ligands that not only fit onto the binding pocket of the target protein, but are also absorbed, transported, distributed to the right compartment. Finding a lead compound, optimizing its properties and obtaining a drug takes enormous time and money and this in turn is simplified by multifarious molecular modeling tools (15) (refer figure 4). The current study which is at its nascent level dealing with targeted drug delivery has promising results down the road.

Any protein structure has four levels including primary, secondary, tertiary and quaternary structures respectively. Primary structure is the linear sequence of amino acids. Secondary structure is the local con-formation of α-helixes, β-sheets and random coils. The angle between two adjacent amino acids is called torsion angle, which deter-mines the twists/turns of the sequences resulting in secondary structure. The three dimensional tertiary structure is an outcome of the combination of one or more subunits or chains. (15). Obtaining an accurate model through the conventional techniques such as NMR (nuclear magnetic resonance spectroscopy) analysis or X-ray diffraction techniques is time consuming and elaborate. Thus homology modeling proves to be one of the most valid and efficient means of obtaining an authentic model of protein structure.

And so, in this study, the protein 3D structure of capsule biosynthesis protein capA of P. gingivalis was found by using the aforesaid technique using the SWISS MODEL workspace. This included obtaining the FASTA sequence by using the amino-acid sequence, which was then used for homology modeling from which the three models were obtained (refer figure 1). Model quality assessment tools are used to evaluate the reliability of the resulting models. With the help of CASTp, the active sites were predicted for the three protein models (refer figure 2). These structures obtained were validated by ProQ and the LG scores were 2.685, 3.275 and 2.178respectively and the Max Sub scores were 0.247, 0.321 and 0.208 respectively (refer figure 3).PROSESS was done to assess the quality of the structure obtained and the results of PROSESS, CASTp

and ProQ were formulated (refer table 1). Subsequently, model 1 was considered the finest model and the values of the covalent bond quality showed a high score of 7.5. But certain values related to the non-covalent bond quality and the torsion angle quality however, showed lower values of about 3.5 and 2.5. This becomes a limitation of the protein modeling of capsule biosynthesis protein capA of P.gingivalis and further laborious studies are required to validate the above results and predict the appropriate structure making use of our analysis.

This homology modeling technique is currently the most meticulous and time saving computational method to generate reliable structural models and is frequently used in many biological scenarios. Normally, the computational effort for a modeling project is fairly less and lasts only for a few hours. However, this does not include the time required for visualization, clarification and comprehension of the model, which may vary depending on personal experience working with protein structures.

Thus this study has resulted in the identification of the three dimensional protein model of capsule biosynthesis protein capA of P.gingivalis –strain ATCC 33277, which is an extremely good model – a model verified and validated by many tests. This study is only the beginning of an elaborate and extensive research work that must be carried out to aid in the discovery of targeted drugs and other substances that can inactivate the periodontal pathogen at the very germinal stage of evasion of host defences.

4. CONCLUSION

Thus, from the above modeling performed and after being subjected through a series of analysis, MODEL-1 was found to be more accurate when compared to the other models obtained. Thus a validated three dimensional model or a protein structure of the capsule biosynthesis protein cap-A of P.gingivalis is obtained through homology modeling. Identifying this protein structure is only the initial step for manifold meticulous time-consuming procedures including identifying and proving the functions of the protein and its pathogenic role in periodontitis and finally targeted drug delivery. Even though targeted drug delivery seems to be one of the premier ways in therapy for multitudinous diseases, its potential role in periodontal inflammation still remains an unanswered question.

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