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Homology Modeling of Coagulase in *Staphylococcus aureus*

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Abstract:
The close correlation between the ability of coagulase to clot blood plasma and their capacity to produce disease, and the corresponding absence of this property in nonpathogenic strains, have led to the assumption that the coagulase, plays important role in the pathogenesis of disease. Currently, crystal structure of coagulase in *Staphylococcus aureus* remains indefinable. Thus, the objectives of this research is to generate the three dimensional model of coagulase in *S. aureus* by using homology modeling approach. In this study, we used bioinformatics tools and databases such as BLAST (Basic Local Alignment Search Tool), GenBank, PDB (Protein Databank), and Discovery Studio to gain specific functional insights into coagulase. The model was validated using protein structure checking tools such as PROCHECK, Verify 3D and CE (Combinatorial Extension) for reliability. Therefore, structure prediction of coagulase in *S. aureus* can provide preliminary knowledge for understanding the function of the protein. The information from this finding will provide important information into the action and regulation mechanism of the coagulase protein in *S. aureus*.

Key Words: Structure prediction, coagulase, homology modeling, *Staphylococcus aureus*.

Background:
Staphylococcus aureus is a major human pathogen of increasing importance due to the spread of antibiotic resistance. *S. aureus* is causing a range of acute and pyogenic infections, including abscesses, central nervous system infections, endocarditis, osteomyelitis, pneumonia, urinary tract infections, chronic lung infections associated with cystic fibrosis, and several syndromes caused by exotoxins and enterotoxins, including food poisoning, scalded skin and toxic shock syndromes **[1]**. *S. aureus* is also a main cause of hospital acquired (nosocomial infections) of surgical wounds and infections related to indwelling medical devices **[2]**. This bacterium has a thick cell wall consisting of peptidoglycan, which is required for maintenance of cellular viability. *S. aureus* has three surface determinants which might mediate adherence to damaged valves and promote endocarditis, namely, (i) coagulase, which is mostly secreted in the medium **[3, 4]**, (ii) clumping factor (or fibrinogen-binding protein**) [4-6]**, and (iii) fibronectin-binding protein **[7]**. Coagulase positive Staphylococci from *S. aureus* are very well known pathogens causing a range of infections. Coagulase has been suspected to assist infection via its procoagulant and fibrinogen-binding activity **[3]**, it did not appear to promote either adherence to platelet-fibrin clots in vitro or experimental endocarditis in rats in the present experiments. By triggering coagulation, vegetation adherent bacteria might promote additional deposits of platelets and fibrin on top of the infection nidus and thus become protected from further mechanical detachment and/or cellular host defense mechanisms.

Today, the crystal structure of the coagulase in *Staphylococcus aureus* remains elusive. Hence, it is imperative to solve the tertiary structure of a protein or the quaternary structure of its complexes. Furthermore, information on molecular recognition provides information on how the protein functions. The function of an enzyme relies on the structure of its active site, a cavity in the protein with a shape and size that enables it to snugly fit the intended substrate. The active site also contains certain amino acids that are involved in the chemical reaction catalyzed by the enzyme. During host infection, coagulase conformationally activates the central coagulation zymogen, prothrombin, thereby triggering the cleavage of fibrinogen to fibrin. The association of the tetrameric complex enables fibrinogen binding at a new site with high infinity. This model can explain the coagulant properties and efficient fibrinogen by coagulase **[8]**. A study by Friedrich et al. (2006) presented the crystal structure of SC-(1–325) bound to bovine α- thrombin, along with a more detailed description and comparison of the atomic interactions in the SC-(1–325) human (pre) thrombin and SC-(1–325) bovine thrombin complexes to define structural differences responsible for the species specificity of ProT activation by SC **[9]**. The information from this finding will provide important information and insights into the action and regulation mechanism of the coagulase and the crystal structure of coagulase in *S. aureus* by using homology modeling approach.

Methodology:

Homology search

The sequence of coagulase in *S. aureus* (Accession Number: CAC 84776.1) was obtained from NCBI database **[10]**. The query sequence from coagulase in *S. aureus* was searched to find out the related protein structure to be used as a template(s) by the BLAST program **[11]** against Protein Data Bank database.

Model building

The 3D homology models were calculated using crystal structural coordinates of templates on the basis alignment of target and template sequence of coagulase. The procedures were performed by Discovery Studio by Accelerys (San Diego, CA, USA).

Model Evaluation and Refinement

Structural evaluations of coagulase model were performed by using two programs called PROCHECK and Verify 3D. The predicted model was submitted to the structure evaluation server (UCLA DOE) **[12]**. Model evaluation is important to check the correctness of the overall fold/structure, errors over localized regions and stereochemical parameters such as bond lengths and angles.

Discussion:

Homology modeling

Homology searches in PDB were performed using the program BLASTP **[11].** There were three templates had been identified (PDB id: 2AID (Staphylocoagulase bound to bovine thrombin); 1NU7 (Staphylocoagulase-Thrombin Complex) and 1NU9 (Staphylocoagulase-Prethrombin-2 complex)) by comparing and aligning the target sequence with each sequence in PDB. The sequence identity between the 2AID (Staphylocoagulase bound to bovine thrombin) is 54%, meanwhile 1NU7 (Staphylocoagulase-Thrombin Complex) 50% and 1NU9 (Staphylocoagulase-Prethrombin-2 complex) 51%. Then, followed by structural-based alignment of coagulase from *S. aureus* against the three selected templates. 1NU9- Chain C and F was selected for the model building because it hits the highest identity (27.1%) **(Figure 1a)**.

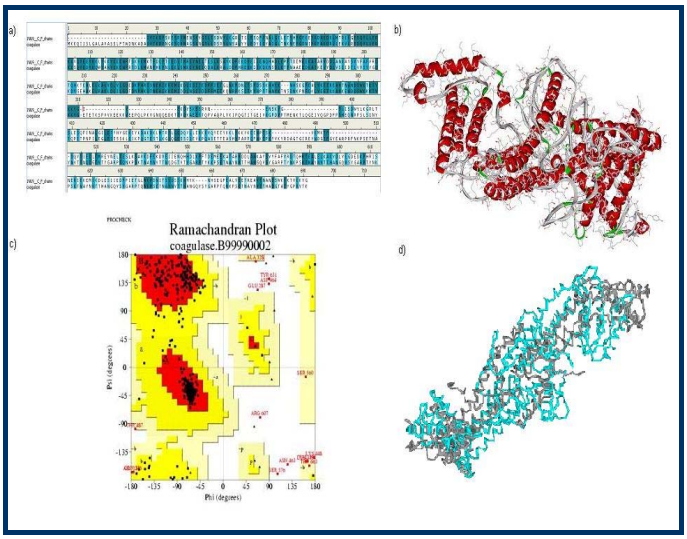

Figure 1: (a) Structural-based alignment of coagulase from *S. aureus* and the template PDB ID: 1NU9 (Staphylocoagulase-Prethrombin-2 complex). The sequence shaded in cyan represents sequence similarity; **(b)** The best predicted model of coagulase (coagulase.B99990002); **(c)** Ramachandran Plot of the coagulase model calculated by PROCHECK; **(d)** Superposition of the predicted model of coagulase from *S. aureus* onto to the template PDB ID: 1NU9.

Model building and Refinement

The 3D homology model of coagulase in *S. aureus* (Accession No. CAC84776.1) was predicted using the crystal structure coordinates set of Staphylocoagulase **[13]** (1NU9) obtained from homology search and completed with sequence-structure alignment **(Figure 1a).** The rest of the steps in homology modeling were performed by the program Discovery Studio by Accelerys, build homology model protocol. It used MODELER automodel to build homology models. An accurate sequence alignment between the model and the template protein are essential to achieve high quality models. There were three annotated model structures of coagulase predicted. They were coagulase.B99990001, coagulase.B99990002 and coagulase.B99990003. Hence, the best model structure of coagulase superimpose to the template (1NU9) also generated (coagulase) in order to predict the best models among all of the models. Model of coagulase.B99990002 **(Figure 1b)** shown the lowest value in PDF (Probability Density Functions) Total Energy (5700.54), PDF Physical Energy (1849.15) and DOPE (Discrete Optimized Protein Energy) score (-500029.3) which indicate the best model of coagulase compare to the others models.

Model Evaluation

Structural evaluations of coagulase model were performed by using two programs called PROCHECK and Verify 3D. PROCHECK check the stereochemical quality of a protein structure, producing a number of PostScript plots analyzing

its overall and residue-by-residue geometry. It includes PROCHECK-NMR for checking the quality of structures solved by NMR. PROCHECK have been expended to evaluate NMR structure quantitatively through a package called PROCHECK NMR **[14]**. The evaluation of coagulase model revealed that sterochemical and geometrical parameter implemented in PROCHECK was satisfied in this model. **(Figure 1c)** shows Ramachandran Plot of the coagulase model calculated by PROCHECK. It shows a good distribution of 570 amino acid residues of coagulase. About, 99.4% residues are most favored and additionally allowed region, and only five residues are generously allowed region.

As methods for determining protein three-dimensional structure, a continuing problem is how to verify that the final protein model is correct. The revision of several protein models to correct errors has prompted the development of new criteria. For judging the validity of X-ray and NMR structures, as well as the formation of energetic and empirical methods to evaluate the correctness of protein models **[12]**. Verify3D analyzes the compatibility of an atomic model (3D) with its own amino acid sequence (1D). The scores of a sliding 21-residue window (from -10 to +10) are added and plotted for individual residues **[15, 16]**. The coagulase protein seems to be consistent with the respective amino acid with 60.30% of the residues had an averaged 3D-1D.

The model evaluation of coagulase then was carried out by referring to the quality of the model built; coagulase. B99990002. The quality of a model can be approximately predicted from the sequence similarity of both structure; coagulase.B99990002 and 1NU9_C_F_chains **(Figure 1d).** Alignment of the model is available at CE for protein structure comparison **[17]**. CE is a method for calculating pairwise structure alignments. CE aligns two polypeptide chains using characteristics of their local geometry as defined by vectors between C alpha positions. Matches are termed AFPs (Aligned Fragment Pairs). Heuristics are used in defining a set of optimal paths joining AFPs with gaps as needed. The path with the best RMSD is subject to dynamic programming to achieve an optimal alignment. For specific families of proteins additional characteristics are used to weight the alignment **[18]**.

The RMSD of the models is 1.8Å, which indicates 95% model accuracy referring to similar structure **(Figure 1d).** Hence, the z-score value is 6.9. Frequently, z-score is the measure of the statistical significance of the result relative to an alignment of random structures. Typically proteins with a similar fold will have a z-score of 3.5 or better **[14]**. The z-score can be used to filter less significant results or alternatively look for weak similarities In spite of that, the relationship between backbone RMSD in Å and structure quality for NMR structure ensemble and for protein structure comparison show that they were barely acceptable and have a very closely related to each other**.** Therefore, the model predicted was acceptable.

Conclusion:

The knowledge of the three-dimensional structure of coagulase is fundamental in understanding its native conformation hence the mechanism of the coagulase action. Furthermore, structure prediction via homology modeling offers an alternative way to obtain structural information well before the structure of the new protein is determined by X-ray crystallography or NMR. Then, this will assist in searching the answer to design a suitable inhibitor for *S. aureus* in effort to explore secreted proteins for vaccine development.

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