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COMPUTATIONAL APPROACH IN DETERMINATION OF CURCUMIN AS AN ANTI BACTERIAL DRUG

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ABSTRACT: Turmeric has anti microbial activity due to Curcuminoid compounds. Staphylococcus aureus inhibition was done by using Curcumin, a principle compound present in Turmeric. Curcumin acts on Sortase, a membrane protein and inhibits its growth. The purpose of this work is to find the mode of action of Curcumin on Sortase using Bioinformatic tools. In the present study, we have selected Curcumin and docked to the Sortase of Staphylococcus aureus. Homology modeling of Sortase enzyme has been performed based on the crystal structure of the 1IJA A by using Modeller software. With the aid of the molecular mechanics and molecular dynamics methods, the final model is obtained and further assessed by procheck and verify 3D graph programs, which showed that the final refined model is reliable. With this model, a flexible docking study of Sortase enzyme with Curcumin was performed which showed best docking result. It was found that VAL 205, LYS206 of Sortase are the amino acids which play important role in strong hydrogen bonding interaction with Curcumin. To the best of our knowledge VAL 205, LYS 206 are conserved in the domain and may be important for structural integrity or maintaining the hydrophobicity of the inhibitor-binding pocket. Our results may be helpful for further experimental investigations.

Key Words: Curcumin, Docking, Modelling, Sortase and Turmeric

INTRODUCTION

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi and viruses. Technically, antibiotics are those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Turmeric (Curcuma longa L.) is a medicinal plant extensively used in Ayurvedha, Unani and Siddha medicine as home remedy for various diseases (Srimal, R. C et al 1997). Curcumina longa which is used as a food material in our daily usage consists of compounds such as Curcumin and these compounds act on several pathogenic bacteria such as Staphylococcus aureus (Ammon, H. P et al 1991). The inhibition of Staphylococcus aureus was done by using Curcumin, a principal curcuminoid of the popular Indian curry spice turmeric. Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has a-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%) (5). Curcumin (diferuloylmethane) (3–4%) is responsible for the yellow color, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%). Demethoxy and bisdemethoxy derivatives of curcumin have also been isolated (Sugiyama, Y et al 1996, Huang, M. T et al 1994 Hong, J et al 2004 Nishiyama, T et al 2005).

Staphylococcus aureus is the most common cause of staph infections such as pneumonia, meningitis, osteomyelitis endocarditis, Toxic shock syndrome (TSS), and septicemia. Gram-positive pathogenic bacteria display surface proteins that play important roles in the adhesion to specific organ tissues, the invasion of host cells, or the evasion of host-immune responses (Cossart, P et al 2000). These virulence-associated proteins are covalently anchored to bacterial cell wall peptidoglycan through a general sorting mechanism catalyzed by a super family of membrane associated transpeptidases termed sortases (Schneewind, O et al 1992). The inhibition of curcumin is due to the activity of curcumin on Sortase enzyme of Staphylococcus aureus. Sortase is an important protein involves in the membrane formation and shape determination in Staphylococcus aureus (Mazmanian, S. K et al 1999; 2002; 2003; Ton-That, H et al 1999). Antimicrobials include not just antibiotics, but synthetically formed compounds as well. Currently, bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant more quickly than new drugs are being found, thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means. Hence an attempt was made to identify some bioactive compounds which are having antibacterial properties from natural resources.

METHODOLOGY

The initial model of Sortase was built by using homology-modeling methods and the MODELLER software.; a program for comparative protein structure modeling optimally satisfying spatial restraints derived from the alignment and expressed as probability density functions (pdfs) for the features restrained. The pdfs restrain C^{α}-C^{α} distances, main-chain N-O distances, main-chain and side-chain dihedral angles. The 3D model of a protein is obtained by optimization of the molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf is derived as a combination of pdfs restraining individual spatial features of the whole molecule. The optimization procedure is a variable target function method that applies the conjugate gradients algorithm to positions of all non-hydrogen atoms (Sali, *et al*, 1991). The query sequence from Staphylococcus aureus was submitted to domain fishing server for Sortase domain prediction. The predicted domain *was* searched to find out the related protein structure to be used as a template by the BLAST (Basic Local Alignment Search Tool) program against PDB (Protein Data bank) (Altschul, *et al*, 1990; 1997). Sequence that showed maximum identity with high score and less e-value were aligned and was used as a reference structure to build a 3D model for Sortase. The co-ordinates for the structurally conserved regions (SCRs) for Sortase were assigned from the template using multiple sequence alignment, based on the Needleman-Wunsch algorithm. The structure having the least Modeller objective function, obtained from the Modeller was improved by molecular dynamics and equilibration methods using NAMD 2.5 software (Kale, *et al*, 1999) using CHARMM27 force field for lipids and proteins 5 along with the TIP3P model for water (Jorgensen, *et al*, 1983). Finally, the structure having the least energy with low RMSD (Root Mean Square Deviation) was used for further studies. In this step, the quality of the initial model was improved. The final structure obtained was analyzed by Ramachandran's map using PROCHECK (Programs to check the Stereo chemical Quality of Protein Structures) (Brunger, 1992) and environment profile using ERRAT graph (Structure Evaluation server) (Laskoswki, *et al*, 1993). This model was used for the identification of active site and for docking of the Curcumin with the enzyme.

Active site Identification

Active site of Sortase was identified using CastP server (Carpena, et *al*, 2003). A new program, CAST, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CAST identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings.

Docking method

The ligand, including all hydrogen atoms, were built and optimsed with chemsketch software suite. Extremely Fast Rigid Exhaustive Docking (FRED) version 2.1 was used for docking studies (OpenEye Scientific Software, Santa Fe, NM). It is an implementation of multi conformer docking, meaning that a conformational search of the ligand is first carried out, and all relevant low-energy conformations are then rigidly placed in the binding site. This two-step process allows only the remaining six rotational and translational degrees of freedom for the rigid conformer to be considered. The FRED process uses a series of shape-based filters, and the default scoring function is based on Gaussian shape fitting.

RESULTS AND DISCUSSION

Homology Modeling of Sortase

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the results of BLAST search against PDB, only the 1IJA which has a high level of sequence identity with the sortase domain. Structurally conserved regions (SCRs) for the model and the template were determined by superimposition of the two structures and multiple sequence alignment. In the following study, we have chosen 1IJA as a reference structure for modeling Sortase domain. Coordinates from the reference protein (1IJA) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. In the modeller we will get a 20 PDB out of which we select a least energy. All side chains of the model protein were set by rotamers. The final stable structure of the Sortase protein obtained is shown in Figure I.
By the help of SPDBV it is evident that Sortase domain has 2 helices and 11sheets and it is shown in the Figure II.

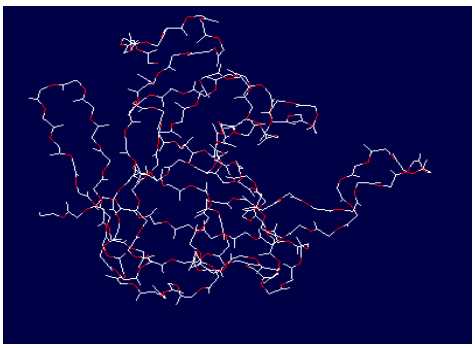

Figure I: Modeller result for Sortase

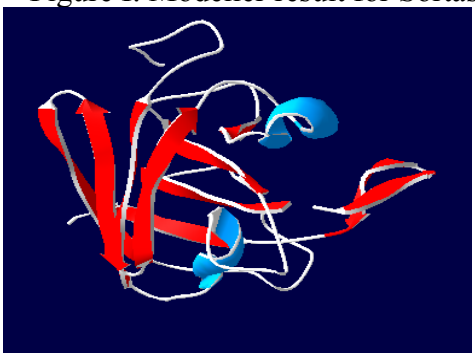

Figure II: Final Refined Structure of Sortase

The final structure was further checked by verify3D graph and the results have been shown in Figure III. The overall scores indicates acceptable protein environment.

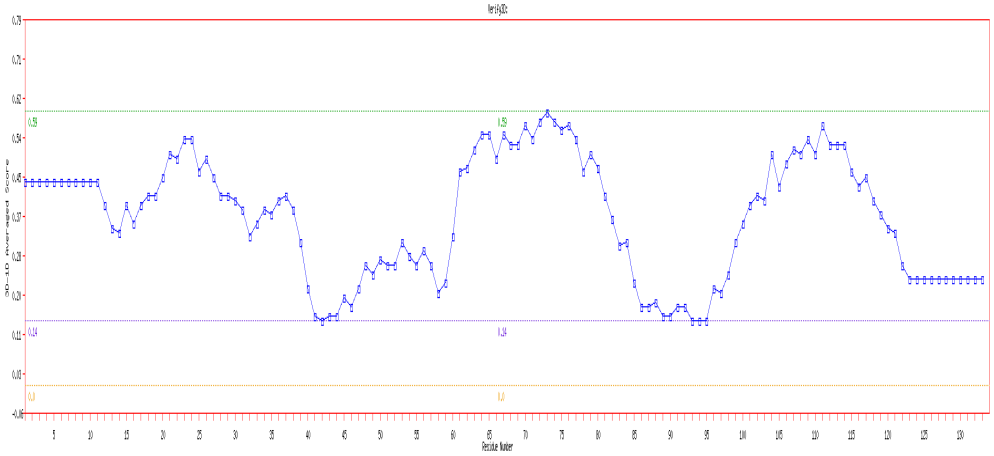

Figure III: Graphical Representation for Verify3D

Validation of Sortase Domain

After the refinement process, validation of the model was carried out using Ramachandran plot calculations computed with the RAMPAGE Server. The distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Table I. The RMSD (Root Mean Square deviation) deviation for covalent bonds and covalent angles relative to the standard dictionary of Sortase was -4.27 and -0.65 Å. Altogether 96.2 % of the residues of sortase was in favored and allowed regions. The overall PROCHECK G-factor of Sortase was – 1.32 and verify3D environment profile was good (Fig IV).

Table I: % of residue falling in the core region of the Ramachandran's plot

% of residue in most favored regions	83.6
% of residue in the additionally allowed zones	15.9
% of residue in the generously regions	0.9
% of residue in disallowed regions	0.0
% of non-glycine and non-proline residues	100.0

Active Site Identification of Sortase Domain

After the final model was built, the possible binding sites of Sortase was searched based on the structural comparison of template and the model build and also with CASTP server and was shown in Figure V. Since, Sortase staphylococcus aureus and the 1IJA are well conserved in both sequence and structure; their biological function should be identical. It was found that secondary structures are highly conserved and the residues, APG151, LYS 152, TYP153, LYS154, MET141, LYS177, GLU204, LYS206, VAL 205.
The inhibitor Curcumin used for docking (Fig VI):

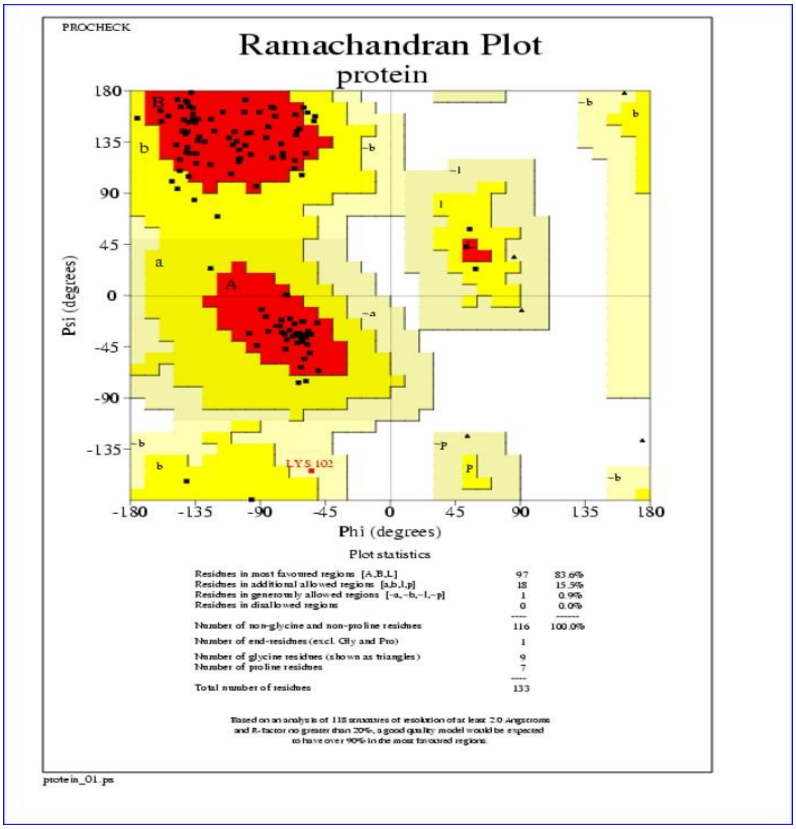

Figure IV: Ramachandran Plot Using RAMPAGE Server

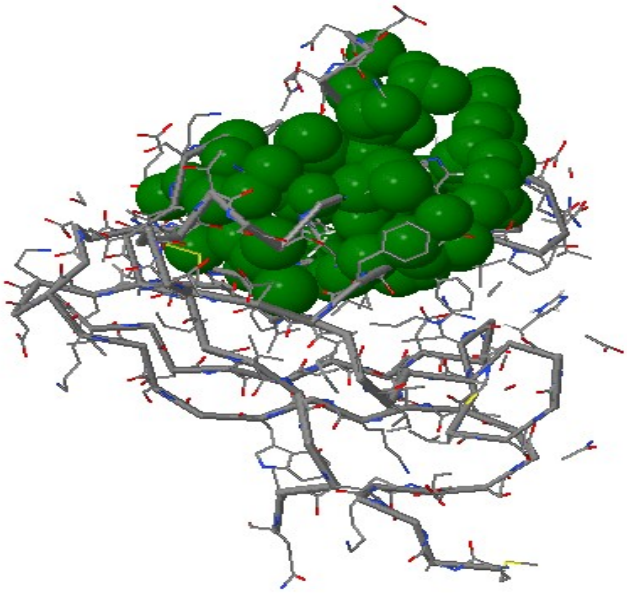

Figure V: Active site of Sortase (Green color indicates active Site Regions)

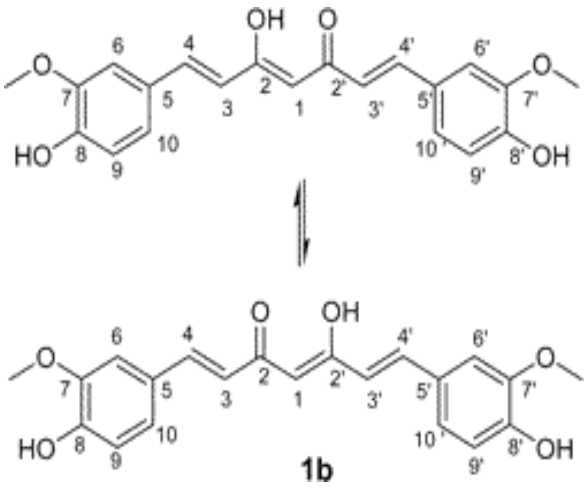

Figure VI: Curcumin structure

Docking of inhibitors with the active site of Sortase

Docking of the Curcumin with Sortase was performed using FRED v 2.1, which is based on Rigid Body Shape-Fitting (Open Eye Scientific Software, Santa Fe, NM). This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site. This box was defined by extending the size of a co crystalized ligand by 4 Å (add box parameter of FRED). This dimension was considered here appropriate to allow, for instance, compounds larger than the co crystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was converted in 3D with OMEGA (same protocol as above) (Open Eye Scientific Software, Santa Fe, NM). To this set, the substrate (generation of multi conformer with Omega) corresponding to the modeled protein were added. The docking result showed the interactions between Curcumin and Sortase (Fig VII) and Table II indicates the docking value.

Table II: The total energies of Chemguass score, Chemscore, PLP score and shapeguass score of the best-docked conformations of sortase

Molecule Name	Chemgauss	Chem Score	PLP	Screen Score	Shapegauss	Total
Curcumin	-41.18	-0.48	-11.72	-37.85	-314.33	-405.56

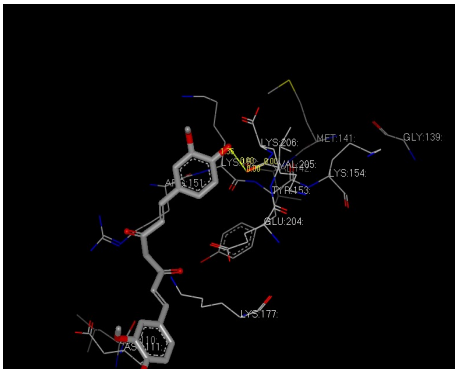

Figure VII: Curcumin docked with Sortase

CONCLUSION

Sortase A universal target for therapeutic agents against gram positive bacteria. In this work, we have constructed a 3D model of domain, from staphylococcus aureus using the MODELLER software and obtained a refined model after energy minimization. The final refined model was further assessed by ERRAT & PROCHECK program, and the results show that this model is reliable. The stable structure is further used for docking of substrate with the Curcumin compound. Docking results indicate that conserved amino-acid residues in Sortase domain play an important role in maintaining a functional conformation and are directly involved in donor substrate binding. The interaction between the domain and the inhibitor proposed in this study are useful for understanding the potential mechanism of domain and the inhibitor binding. As is well known, hydrogen bonds play important role for the structure and function of biological molecules. In this study it was found that VAL 205, LYS206 of Sortase are important for strong hydrogen bonding interaction with Curcumin. To the best of our knowledge VAL 205, LYS 206 are conserved in this domain and may be important for structural integrity or maintaining the hydrophobicity of the inhibitor-binding pocket. The molecule Curcumin showed best docking results with target protein.

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