AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Determination of three dimensional structure of scaA protein, a virulence factor of *Streptococcus gordonii* by homology modelling and design of inhibitors for scaA protein

Kannan I[1]*, Gomathi. GD[1], Sambandam Cecilia[1], Jayalakshmi M[1], Premavathy RK[1], Shantha S[1]

1.Department of Microbiology, Tagore Dental College and Hospital, Rathinamangalam, Chennai – 600 127, India

ABSTRACT

Streptococcus gordonii are one among the early colonisers of tooth surfaces of humans and are present in coronal plaque, gingival crevices and buccal and pharyngeal mucosal surfaces. One of the main virulence factor that is involved in *S. gordonii* adhesion and coaggregation is ScaA protein. Inactivation of scaA genes resulted in both impaired growth of cells and inhibition of Mn2+ intake. Thus the inhibitors of ScaA protein can act as an effective drug against *S. gordonii* especially in the prevention and treatment of infective endocarditis and dental caries. The three dimensional structure of ScaA protein is predicted by homology modelling. It is the method to determine 3D structure of protein with the help of 3D structure of homologous proteins. Softwares used are Modeller 9.11 and Easy modeller 2.0 GUI. A total of 500 ligands in 2D format were derived from myricetin with the help of software ACD chemsketch. Rapid virtual screenings of these compounds were performed in the docking tool iGEMDOCK v2.0. Based on the binding energy a total of three ligands were selected for the further study. Three ligands thus generated possessed very good docking energy. Thus three ligands with good inhibitory properties were generated among which 5-[3,4-dihydroxy-6-(pyridin-2-yl)-8a*H*-chromen-2-yl]benzene-1,2,3-triol, a novel compound is found to be very excellent drug candidate based on the molecular docking studies and its ADME properties.

Keywords: *Streptococcus gordonii*, ScaA protein, homology modelling, molecular docking, drug candidate

*Corresponding Author Email: kannan_iyan@hotmail.com
Received 11 January 2014, Accepted 16 January 2014

Please cite this article in press as: Kannan I *et al.*, Determination of three dimensional structure of scaA protein, a virulence factor of *Streptococcus gordonii* by homology modelling and design of inhibitors for scaA protein. American Journal of PharmTech Research 2014.

INTRODUCTION

Streptococcus gordonii are one among the early colonisers of tooth surfaces of humans and are present in coronal plaque, gingival crevices and buccal and pharyngeal mucosal surfaces[1]. They are also been identified in abscesses[2]. Around 50% of infective endocarditis is due to *S. gordonii*. Infection due to *S. gordonii* is due to its ability to adhere and colonise the host surface[3]. Interesting aspect of *S. gordonii* is its ability to colonise or infect various surfaces because of its property of adherence[4,5]. In addition, the streptococcal cells coaggregate with a variety of other oral bacteria such as the genera of Actinomyces, Fusobacterium, and Capnocytophaga[6, 7].

One of the main virulence factor that is involved in *S. gordonii* adhesion and coaggregation is ScaA protein[8]. ScaA lipoprotein in *S. gordonii*, is a member of Lral family of homologous polypeptides found among streptococci, pneumococci, enterococci[9, 10]. Inactivation of scaA genes resulted in both impaired growth of cells and inhibition of Mn2+ intake[11]. Thus the inhibitors of ScaA protein can act as an effective drug against *S. gordonii* especially in the prevention and treatment of infective endocarditis and dental caries. Main problem in drug discovery against *S. gordonii* is the lack of three dimensional structure of various virulence factors especially of ScaA protein.

Hence an attempt has been made in the present research study to determine the 3D structure of ScaA protein and to design an effective drug candidate that can inhibit ScaA protein.

MATERIALS AND METHOD

Prediction of three dimensional structures

The three dimensional structure of ScaA protein is predicted by homology modelling. It is the method to determine 3D structure of protein with the help of 3D structure of homologous proteins. Softwares used were Modeller 9.11 and Easy modeller 2.0 GUI[12]. First the primary structure of ScaA protein was retrieved from UniProtKB database (www.uniprot.org/help/uniprotkb) (The primary structure in FASTA format was submitted in BLASTp (*blast.ncbi.nlm.nih.gov/)* to find the homologous proteins. The proteins of low e value were selected for further study. The 3D structures of the homologous proteins were retrieved from RCSB database (*www.rcsb.org/*). The 3D structure of homologous proteins was submitted along with the primary structure of ScaA protein to Modeller software through GUI Easy Modeller. The predicted 3D structure was then validated with the Ramachandran plot. It was also further validated in ProQ online tool (*www.sbc.su.se/~bjornw/ProQ).*

Active site prediction

The possible binding sites of ScaA protein were searched using binding site prediction online tool Q site finder[13]. The binding sites which are more flexible were selected for this study.

Generation and optimization of Ligand

The structure of myricetin is obtained from PubChem database (*pubchem.ncbi.nlm.nih.gov/*(. A total of 500 ligands in 2D format were derived from myricetin with the help of software ACD chemsketch[14]. The ligands thus generated were saved in mol 2 format. The OPEN BABEL software (www.vcclab.org/lab/babel/start.html) was used to convert mol 2 format to pdb format. Rapid virtual screenings of these compounds were performed in the docking tool iGEMDOCK v2.0[15]. iGEMDOCK is an integrated virtual screening environment from preparations through post-screening analysis with pharmacological interactions . First, iGEMDOCK provides interactive interfaces to prepare both the binding site of the target protein and the screening compound library. Each compound in the library is then docked into the binding site by using the in-house docking tool GEMDOCK. Subsequently, iGEMDOCK generates protein-compound interaction profiles of electrostatic, hydrogen-bonding, and van der Waals interactions. Based on these profiles and compound structures, iGEMDOCK infers the pharmacological interactions and clusters the screening compounds for the post-screening analysis. Finally, iGEMDOCK ranks and visualizes the screening compounds by combining the pharmacological interactions and energy-based scoring function of GEMDOCK. A population size of 150 was set with 70 generation and one solution for quick docking. Based on the binding energy a total of three ligands were selected for the further study. The selected three ligands were then analyzed for drug- relevant properties based on "Lipinski's rule of five". Other drug like properties were analysed using OSIRIS Property Explorer (http://www.organicchemistry.org/prog/peo/) and Mol soft, the drug-likeness and molecular property explorer (http://www.molsoft.com/mprop/). On the basis of binding affinity and drug like properties, all these three ligands were taken for further molecular docking study.

Protein-ligand docking

The selected three ligands were subjected accurate docking (very slow docking) by setting population size of 800 with 80 generation and 10 solutions. After the completion of the docking the post docking analysis was performed to find the docking pose and its energy values.

RESULTS AND DISCUSSION

Three structure prediction

The primary structure of ScaA protein was retrieved from UniprotKB database. The ScaA protein is made of 310 aminoacid residues. The residues 1 to 19 is signal peptide and remaining

residues are involved in metal ABC transporter substrate-binding and Co aggregation-mediating adhesiveness. The primary structure of ScaA protein is shown in Figure 1.

>sp|P42364|MTSA_STRGN Metal ABC transporter substrate-binding lipoprotein OS=Streptococcus gordonii GN=scaA PE=3 SV=1

MKKCRFLVLLLLAFVGLAACSSQKSSTDSSSSKLNVVATNSIIADIT
KNIAGDKINLHSIVPVGQDPHKYEPLPEDVKKTSKADLIFYNG
INLETGGNAWFTKLVENAQKKENKDYYAVSEGVDVIYLEGQ
NEKGKEDPHAWLNLENGIIYAQNIAKRLIEKDPDNKATYEKN
LKAYIEKLTALDKEAKEKFNNIPEEKKMIVTSEGCPKYFSKAYN
VPSAYIWEINTEEEGTPDQIKSLVEKLRKTKVPSLFVESSVDDR
PMKTVSKDTNIPIYAKIFTDSIAEKGEDGDSYYSMMKYNLDKI
SEGLAK

Figure 1: The primary structure ScaA protein in FASTA format

The 3D structure of ScaA was successfully predicted by homology modelling. The predicted 3D structure of ScaA protein is given in Figure 2. Its 3D structure is viewed as PDB file with Rasmol structure colour scheme. Alpha helices are coloured magenta, beta sheets are coloured yellow, turns are coloured pale blue, and all other residues are coloured white.

The Ramachandran plot was generated from the predicted 3D structure of ScaA protein to validate it. The Ramachandran plot is shown in Figure 3. From the figure it is seen that most of the residues clustered tightly in the most-favoured regions with very few outliers showing that the predicted structure is good.

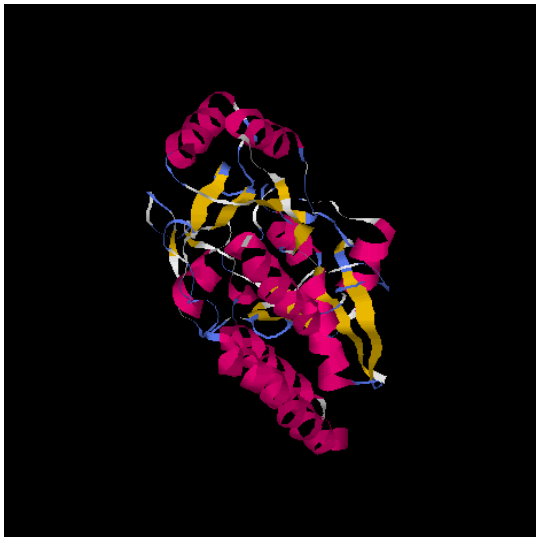

Figure 2: The 3D structure of ScaA viewed with Rasmol structure colour scheme

The predicted structure was further validated by ProQ online tool. The predicted LGscore and Maxsub are 6.566 and 0.520 respectively. The values obtained shows that the predicted structure is extremely good model.

A total of 500 ligands were derived from the myricetin using ACD chemsketch software. It was converted to pdb format using OPEN BABEL software. All the 500 ligands were then subjected to virtual rapid screening with iGEMDOCK software and three compounds were found to have good fit with a low binding energy. The structure and the IUPAC name of the three ligands were shown in the Figure 4. The selected three ligands were then studied for its drug relevant properties.

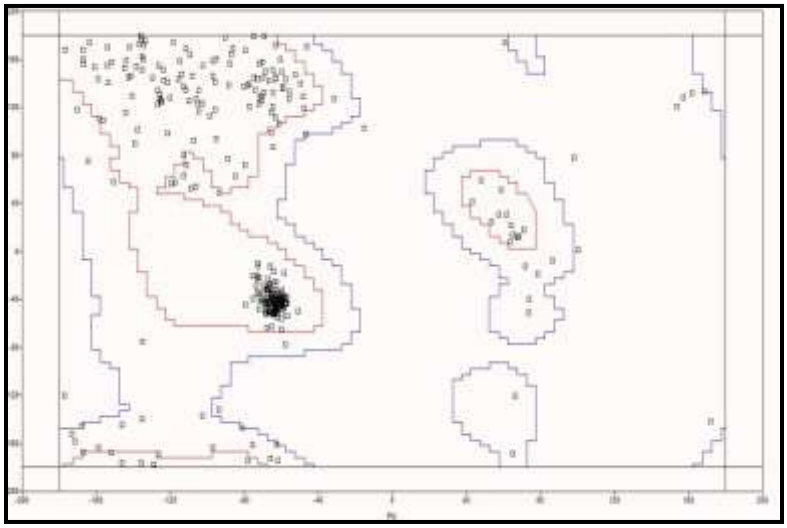

Figure 3: Ramachandran plot of ScaA protein

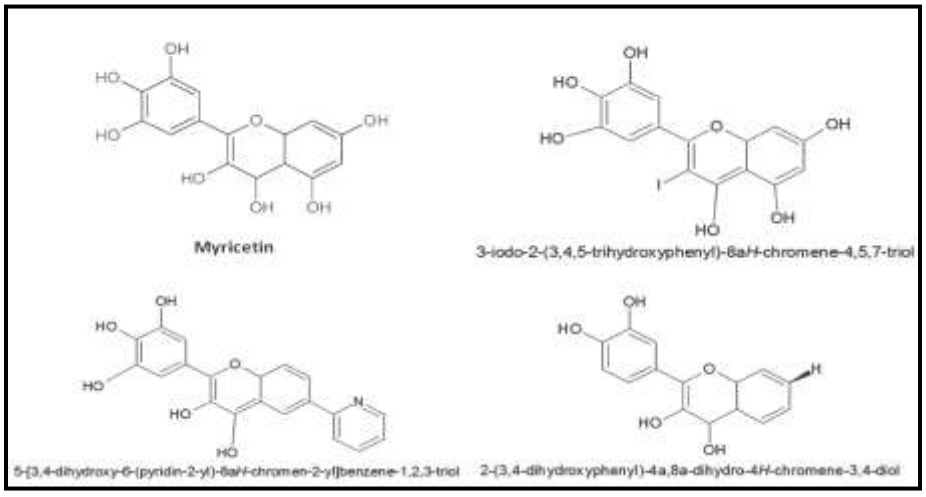

Figure 4: Structure of myricetin and its derivatives

The Table 1 depicts the values related to the Lipinski's rule of Five. From the table it is evident that all the three selected ligands obey the rule. The Table 2 shows the drug relevant properties of the three ligands. They all possess good drug score and drug likeness.

Table 1: The Lipinski's properties of the selected three ligands

S. No.	Ligand	Molecular weight	Xlog p	H bond donor	H bond acceptor
1.	3-iodo-2-(3,4,5-trihydroxyphenyl)-8a*H*-chromene-4,5,7-triol	188.14	-0.847	5	6
2.	5-[3,4-dihydroxy-6-(pyridin-2-yl)-8a*H*-chromen-2-yl]benzene-1,2,3-triol	186.16	0.034	4	5
3.	2-(3,4-dihydroxyphenyl)-4a,8a-dihydro-4*H*-chromene-3,4-diol	170.16	0.368	3	4

Table 2: The drug relevant properties of selected three ligands

S. No.	Ligand	Drug likeness	Drug score	Mutagenic	Tumorigenic	Irritant
1.	3-iodo-2-(3,4,5-trihydroxyphenyl)-8a*H*-chromene-4,5,7-triol	0.41	0.79	No	No	No
2.	5-[3,4-dihydroxy-6-(pyridin-2-yl)-8a*H*-chromen-2-yl]benzene-1,2,3-triol	1.6	0.9	No	No	No
3.	2-(3,4-dihydroxyphenyl)-4a,8a-dihydro-4*H*-chromene-3,4-diol	0.51	0.8	No	No	No

After the confirmation of ADME properties, the three ligands were then subjected to further molecular docking with iGEMDOCK subjecting to accurate docking (very slow docking) by setting population size of 800 is set with 80 generation and 10 solutions. The results were projected in the Table 3.

Table 3: The results of iGEMDOCK showing binding energies of three selected ligands

S.No.	Ligand	Total binding Energy (kcal/mol)	Vanderwaals force	H Bond	Electrostatic bond
1.	3-iodo-2-(3,4,5-trihydroxyphenyl)-8a*H*-chromene-4,5,7-triol	-79.0902	-79.0902	0	0
2.	5-[3,4-dihydroxy-6-(pyridin-2-yl)-8a*H*-chromen-2-yl]benzene-1,2,3-triol	-75.2362	-75.2362	0	0
3.	2-(3,4-dihydroxyphenyl)-4a,8a-dihydro-4*H*-chromene-3,4-diol	-71.1688	-71.1688	0	0

From the table it is clear that all the ligands have low binding energy showing its possibility being competitive inhibitors for ScaA protein. Further the ligand 5-[3,4-dihydroxy-6-(pyridin-2-yl)-8a*H*-chromen-2-yl]benzene-1,2,3-triol shows a very good drug likeness and drug score of 1.6 and 0.9 respectively. Its docking pose is shown in Figure 5.

Figure 5: Docking pose of 5-[3,4-dihydroxy-6-(pyridin-2-yl)-8a*H*-chromen-2-yl]benzene-1,2,3-triol with ScaA protein

CONCLUSION

The ScaA protein of *S. gordonii* is found to be one of the major virulence factor involved in the causation of dental caries and infective endocarditis. Hence the inhibitors of the ScaA protein can be an effective drug in the prevention of dental caries and infective endocarditis caused by *S. gordonii*. In the present study the ligands were generated and were studied for its ability to inhbit the ScaA protein by molecular docking method. Three ligands with good inhibitory properties were generated among which 5-[3,4-dihydroxy-6-(pyridin-2-yl)-8a*H*-chromen-2-yl]benzene-1,2,3-triol, a novel compound is found to be very excellent drug candidate based on the molecular docking studies and its ADME properties.

ACKNOWLEDGEMENTS

We thank Prof. J. Mala, Chairperson, Tagore group of Colleges, for providing necessary facilities for the present study. We are thankful to Dr. T. N. Swaminathan, Advisor and Dr. Chitraa R. Chandran, Principal, Tagore Dental College and Hospital for their kind support and encouragement.

REFERENCES

1. Frandsen, EVG, Pedrazzoli V, Kilian M. Ecology of viridans streptococci in the oral cavity and pharynx. Oral Microbiol. Immunol. 1991; 6:129-133.
2. Herzberg MC, Brintzenhofe KL, Clawson CC. Aggregation of human platelets and adhesion of Streptococcus sanguis. Infect. Immun. 1983; 39:1457-1469.
3. Xiong YQ, Bensing BA, BayerAS, Chambers HF, FM Sullam FM. Role of the serine-rich surface glycoprotein GspB of *Streptococcus gordonii* in the pathogenesis of infective endocarditis. Microb Pathog 2008; 45: 297.
4. Ligtenberg AJM, Walgreen-Weterings E, Veerman ECI, De Soet JJ, De Graaff J, Amerongen AVN. Influence of saliva on aggregation and adherence of Streptococcus

gordonii HG 222. Infect. Immun. 1992; 60:3878-3884.

5. Gibbons RJ, Hay DI, Schlesinger DH. Delineation of a segment of adsorbed salivary acidic proline-rich proteins which promotes adhesion of Streptococcus gordonii to apatitic surfaces. Infect. Immun. 1991; 59:2948-2954.
6. Kolenbrander, PE, Inouye Y, Holdeman LV. New Actinomyces and Streptococcus co aggregation groups among human oral isolates from the same site. Infect. Immun. 1983; 41:501-506.
7. Kolenbrander PE, Ganeshkumar N, Cassels FJ, Hughes CV. Coaggregation: specific adherence among human oral plaque bacteria. FASEB J. 1993; 7:406-413.
8. Kolenbrander PE, Andersen RN, Baker RA, Jenkinson HF. The adhesion-associated *sca* operon in *Streptococcus gordonii* encodes an inducible high-affinity ABC transporter for Mn21 uptake. J. Bacteriol. 1998; 180:290–295.
9. Andersen RN, Ganeshkumar N, Kolenbrander PE. Cloning of the *Streptococcus gordonii* PK488 gene, encoding an adhesin which mediates co aggregation with *Actinomyces naeslundii* PK606. Infect. Immun. 1993; 61: 981–987.
10. Jenkinson HF. Cell surface protein receptors in oral streptococci. FEMS Microbiol. Lett. 1994; 121:133–140.
11. Kolenbrander PE, Andersen RN. Characterization of *Streptococcus gordonii* (*S. sanguis*) PK488 adhesin-mediated co aggregation with *Actinomyces naeslundii* PK606. Infect. Immun. 1990; 58:3064-3072.
12. Eswar N, Marti-Renom MA, Webb B, Madhusudhan MS, Eramian D, Shen M, Pieper U, Sali A. Comparative Protein Structure Modelling With MODELLER. Current Protocols in Bioinformatics, John Wiley & Sons, Inc., Supplement 15, 5.6.1-5.6.30, 2006.
13. Laurie AT, Jackson RM, Q-SiteFinder: an energy-based method for the prediction of protein-ligand binding sites, Bioinformatics. 2005; 21:1908.
14. ACD/ChemSketch Freeware, version 10.00, Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2012.
15. Yang JM, Chen CC, "GEMDOCK: A generic evolutionary method for molecular Docking." Proteins: Structure, Function, and Bioinformatics, 2004; 55: 288.