Research Article

IDENTIFICATION OF SUITABLE DRUGS FOR ORAL CANDIDIASIS BY MOLECULAR DOCKING STUDIES

Karthikeyan*.K, Menakha.M and C. Manoharan
PG and Research Department of Botany and Microbiology, A.V.V.M Sri Pushpam College Poondi, Thanjavur, Tamilnadu, India.
***Corresponding author's E-mail:** menagamuniraj@yahoo.com

Accepted on: 24-06-2011; **Finalized on:** 25-09-2011.

ABSTRACT

Proteolytic activity is an important virulence factor for *Candida albicans*. Secreted aspartyl proteinases (SAP) isoenzymes can be responsible for adherence and tissue damage of local infections, while others cause systemic diseases. Determination of the structures of isoenzymes can contribute to the development of new SAP-specific ligands for the treatment of superficial infections with a structure-based drug design program. In the present study, structure based docking of SAP family was targeted as receptors and the antifungal drugs, such as fluconazole, caspofungin, micafungin and anidulafungin, and antibodies such as sIgA, IgG and IgM were used to act as ligands. Out of ten SAP enzymes of *C.albicans*, PDB structures are available, only for SAPs 1-8 and hence docking studies were carried out for SAPs 1-8. From the docking study, it is concluded that antibodies are more effective over antifungal drugs with SAPs. Among the eight SAPs, SAPs 1-4 & 6-8 showed a strong binding affinity towards sIgA immunoglobulin whereas remaining SAP5 showed strong binding affinity with IgM.

Keywords: Antibodies, antifungal drugs, *Cadida albicans*, docking, oral candidiasis, secreted aspartyl proteinases, SAP.

INTRODUCTION

Candida infections are a problem of growing clinical importance. *C. albicans* is the most common fungal pathogen of humans and has become the fourth leading cause of nosocomial infections[1]. Majority of patients, notably immuno suppressed individuals with human immunodeficiency virus (HIV) infection, experience some form of superficial mucosal candidiasis. Species of *Candida* usually reside as commensal organisms as part of an individual's normal micro flora and can be detected in approximately 50% of the population in this form. However, if the balance of the normal flora is disrupted or the immune defenses are compromised, *Candida* species often become pathogenic. Determining exactly how this transformation from commensal to pathogen takes place and how it can be prevented is a continuing challenge for the medical mycology field.

C. albicans possesses a repertoire of virulence attributes. In particular, the secreted aspartic proteinases (SAPs), encoded by the SAP gene family, with ten members, appear to play a major role in *C. albicans* virulence. Different SAP genes appear to be crucial for mucosal and systemic infections. In terms of virulence, SAP activity can therefore directly induce damage to host cells, facilitate hyphal growth for invasion of tissue, increase adherence following exposure of receptor sites, and also degrade host immunoglobulins and other defense proteins[2] causing oral candidiasis. Currently, in the treatment of oral candidiasis, antifungal agents like fluconazole, caspofungin, micafungin, anidulafungin are administered by the physicians. However, these antifungal drugs are resistance to *C.albicans*. Therefore, many laboratories use computer-assisted design methods to develop new drugs to inhibit proteinases of *C.albicans*. One among them is docking, involving protein-ligand interaction, playing a significant role in structure based drug design. Recently, computational techniques for docking potential ligands, based on the shape of protein receptors, have been developed. Mapping the interaction surface between receptor and ligand interaction that bind each other is of outstanding interest in molecular immunology. Given the limited number of suitable and effective antifungal drugs, the continuing increase in the incidence of *Candida* infections, together with increasing drug resistance, highlights the need to discover new and better agents that target fundamental pathogenic determinants of *C. albicans*.[3] Therefore in the present study, in order to prevent virulent enzyme activity of *C.albicans* effectively, molecular docking between SAP enzymes and, antifungal agents and antibodies, was carried out.

MATERIALS AND METHODS

The organism selected for the study is *Candida albicans***.** For the present study, bioinformatics online databases and softwares were used. The databases and softwares used were as follows:

PubMed (www.ncbi.nlm.nih.gov/pubmed/) for retrieving literature informations. The structure of SAP receptor, antibody and antifungal drugs were retrieved from following database: PDB (http://www.rcsb.org/), SWISSPROT (http://www.expasy.org/sprot/) and Drug databank (www.redpoll.pharmacy.ualberta.ca/ drugbank/). Docking of receptor and ligand were carried out using Hex software. Q-SiteFinder (http://www.modelling.leeds.ac.uk/qsitefinder/) locates ligand binding sites by clustering favorable regions for vander Waals (CH3) probes on the protein surface.

RESULTS AND DISCUSSION

In the present computer aided docking studies, enzymes of SAP family (secreted aspartyl proteinases) were targeted as receptors and the antifungal drugs, such as fluconazole, caspofungin, micafungin and anidulafungin, and antibodies such as sIgA, IgG and IgM were used to act as ligands. Out of ten SAP enzymes of *C.albicans*, PDB structures are available, only for SAPs 1-8 and hence molecular docking studies were carried out for SAP 1-8.

SAP enzymes are polypeptides formed by more than 340 amino acid residues. Each SAP enzyme is a single polypeptide with two chanins, A and B (Fig.1). Among the 8 SAP isoenzymes studied, the structure of the enzymes, 1&5 are larger in size having more than 62,000 cubic angstroms while others are smaller and less than 32,000 cubic angstroms.

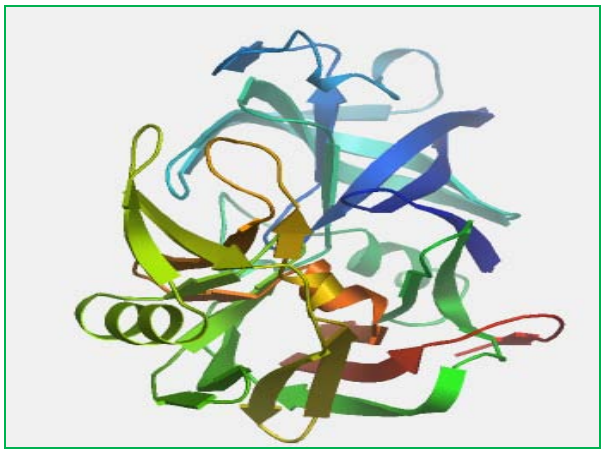

Figure 1: 3D structure of SAP1 from *C.albicans* using Rasmol.

Smallest enzyme is the SAP8 (28789 cubic angstroms). On the surface and interior of each of the SAP enzymes, active sites for docking with inhibitors are available in various sizes. Among the various binding sites available, larger site was predicted as the major active binding site. The size, number of amino acids on the surface of the binding site and type of amino acids vary from SAP to SAP. Among the potential binding sites of SAPs studied, the largest potential site (Fig.2) was found in SAP1 (574 cubic angstroms).

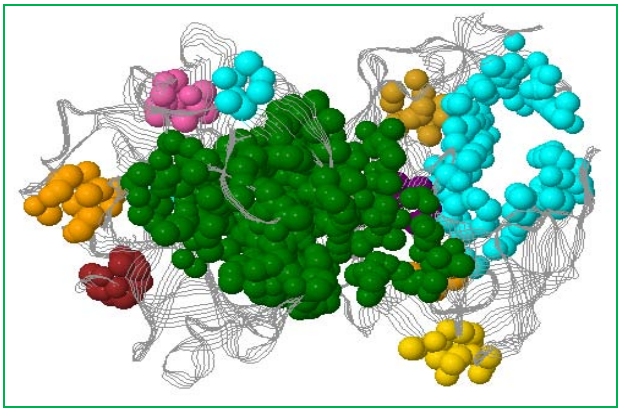

Figure 2: Binding sites in SAP1 using Q site finder.
(Larger binding site is in green. See also other smaller binding sites in various colours)

SAPs 1, 7 & 8 showed potential binding sites of larger size (more than 500 cubic angstroms) in others, they were smaller ranging from 215 to 289 cubic angstroms. The active sites of SAPs 1, 7 & 8 are formed by 25 to 27 amino acid residues while others are formed by 15 to 20 amino acids. Among the various amino acids found in the binding sites, no specific amino acid was dominant in the active site (Table.1). The active sites were also observed on the surfaces of antibodies (Fig.3). Given the three dimensional structure of the target receptor, the protein (Fig.1), the antifungal drug molecules and antibodies (Fig.3) having potential affinity towards the receptors, made to dock with each other (Fig.4). The target protein SAP and ligands were geometrically optimized. All the ligand molecules were docked against the active site of the target protein using Hex software.

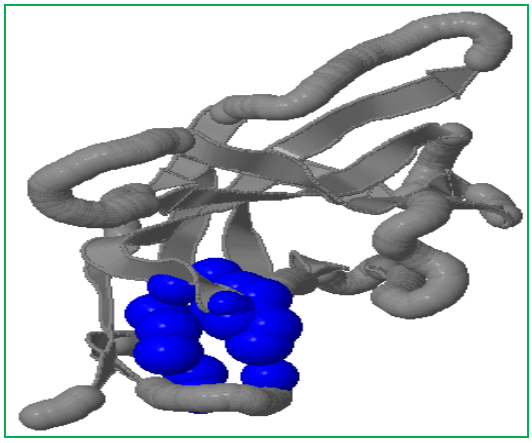

Figure 3: Structure of antibody showing active binding site (blue) using Q site finder.

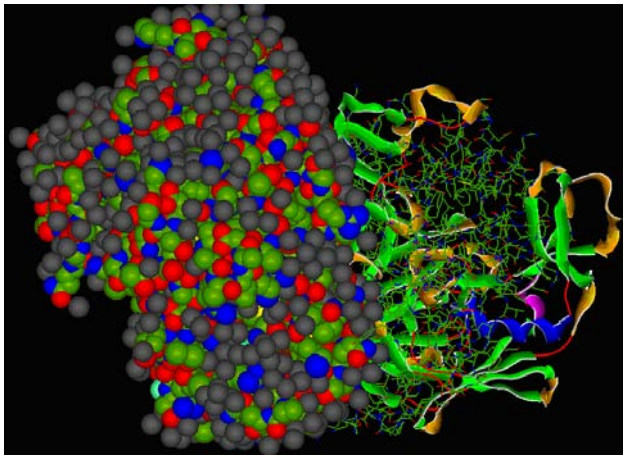

Figure 4: Docking of SAP (right) with sIgA (left) antibody using Hex.

The SAPs were docked with the drugs and antibodies, and the energy values obtained through docking were tabulated (Table.2, Fig.4). SAP1, on docking with drugs like fluconazole, caspofungin, micafungin and anidulafungin produced energy value of -169.94, -381.42, -442.44 and -368.66 respectively. Among the four drugs, micafungin showed an increased energy value of -442.44.

Table 1: Details of active sites in SAP isoenzymes from *C. albicans*

Name of the enzymes	Volume of enzymes (in Cubic Angstroms)	Volume of Major active sites (in Cubic Angstroms)	Number of amino acids in active sites	Amino acid residues in active site
SAP1	62106	574	27	ILE ASP GLY SER ASP ILE GLY TYR GLY ASP SER ILE ASN GLU ALA ARG GLU ARG LEU ASP GLY THR TYR SER ALA ILE
SAP2	31107	215	15	THR ILE ASP GLY SER TYR GLY ASP SER ILE LEU ASP GLY THR ILE
SAP3	31020	248	17	ASP SER ASP LEU TRP PHE ILE TYR SER ILE TYR LYS THR HIS GLU ASN GLU
SAP4	31003	269	17	THR ILE ASP GLY SER LYS TYR ALA ASP SER ILE LEU ASP GLY THR ASP ILE
SAP5	63454	289	20	ALA ILE THR TYR ALATHR ILE SER GLU PHE LEU PHE ARG ASP ASN ARG CYS LYS THR SER
SAP6	31284	253	17	THR ILE ASP GLY SER LYS TYR ALA ASP SER ILE LEU ASP GLY THR ASP ILE
SAP7	31064	510	25	THR SER VAL SER ASN LYS VAL GLY VAL ILE TYR GLY THR ASP SER PHE ASP LYS LEU GLU PHE ASN TYR ILE LYS
SAP8	28789	525	27	PRO ILE ASP GLY SER ASP ILE ARG TYR GLU ASP SER VAL ILE ASP GLU SER GLU ARG VAL ASP GLY THR LEU TYR ALA ILE

On the other hand, among the antibodies used, sIgA showed an increased energy value of -615.73 over the IgM (-335.79) and IgG (-400). Similarly, SAPs 2-8 when docked with various ligands (antifungal drugs and Igs), immunoglobulins exhibited maximum e-values when compared to antifungal agents. Among the antibodies, IgA showed higher e-values such as -615.73, -805.73, -848.07, -807.75, -700.51, -721.25 and -746 for SAPs 2-4 & 6-8 respectively. On the other hand, IgM recorded higher e-value of -602.39 when docked with SAP5 (Table.2).

The treatment and management of patients with oral candidiasis involving only antifungal medications alone is difficult to achieve or it may not be possible in patients with chronic diseases. Successful treatment of candidiasis could be hampered when there is an established biofilm. *Candida* biofilms exhibit significantly higher tolerance to traditional antifungal agents. As a consequence, alternative strategies like promotion of local immune system will ensure the eradication of oral candidiasis. This involves screening of various drugs commercially used for candidiasis and antibodies so for tried. The protein-ligands interaction plays a significant role in determining the suitable drugs or antibodies for the treatment. A variety of computational methods, to identify the suitable drug are available. One such method is docking of drug molecule with receptors. The energy value obtained through docking is used as a criteria for the selection of drugs. From the energy values, lead molecules, due to the high energy value, are identified. This infers that the lead molecules are one with maximum interaction having high negative e-value[4]. Thus the concept of protein-ligand docking helps in finding the suitable drugs for oral candidiasis. The interaction of SAP enzymes with antifungal drugs and antibodies were examined by docking study. In this process, SAP enzymes are acting as receptors and the antifungal drugs and antibodies act as ligands. Eight SAPs when docked with various ligands, antibodies showed maximum energy values, when compared to antifungal drugs. Out of eight SAPs docked, seven SAPs showed increased high negative e-values on sIgA while only one SAP, SAP5 alone showed high negative e-value, when docked with the IgM antibody molecules. Thus the overall results clearly indicate that sIgA is more effective in treating SAP enzymes over the antifungal drugs in the treatment and management of oral candidiasis. From the above docking studies, it is clear that SAP enzymes could be neutralized with specific antibodies for specific SAPs. Studies using polyclonal antibodies raised against SAP1-6 demonstrated that the antibodies were not able to differentiate between the individual SAP proteins[5].

Numerous studies have described the presence of SAP proteins during *C.albicans* infection, but only a few studies have described antibody response to proteinase of *C.albicans*. Proteinase specific IgG antibodies were first detected by Macdonald & Odds[6]. Few studies have investigated the sIgA response and particular secretary sIgA, to SAP proteins during mucosal *Candida* infections, such as those in the oral cavity and vaginal lumen. This could clearly be more relevant than IgG response, since sIgA is the predominant antibody present at mucosal surface and is known to prevent the attachment of *C.albicans* to the mucosal epithelium.

Table 2: Docking results of SAP isoenzymes with Antifungal agents and Antibodies
(Higher negative values are best docking score)

Name of the enzyme	Name of the Ligands (Antifungal drugs and Antibody)	E-values
SAP1	IgA	**-615.73**
	IgM	-335.79
	IgG heavy chain V-II region	-400
	Fluconazole	-169.94
	Caspofungin	-381.42
	Micafungin	-442.44
	Anidulafungin	-368.66
SAP2	IgA	**-805.65**
	IgM	-527.46
	IgG heavy chain V-II region	-578.55
	Fluconazole	-107.25
	Caspofungin	-184.31
	Micafungin	-241.74
	Anidulafungin	-290.34
SAP3	IgA	**-848.07**
	IgM	-584.29
	IgG heavy chain V-II region	-382..11
	Fluconazole	-96.48
	Caspofungin	-263.63
	Micafungin	-256.60
	Anidulafungin	-268.03
SAP4	IgA	**-807.75**
	IgM	-518.65
	IgG heavy chain V-II region	-616.33
	Fluconazole	-101.67
	Caspofungin	-199.49
	Micafungin	-274.08
	Anidulafungin	-282.99
SAP5	IgA	-534.74
	IgM	**-602.39**
	IgG heavy chain V-II region	-378.84
	Fluconazole	-79.13
	Caspofungin	-175.43
	Micafungin	-219.12
	Anidulafungin	-233.01
SAP6	IgA	**-700.51**
	IgM	-538.95
	IgG heavy chain V-II region	-666.29
	Fluconazole	-102.24
	Caspofungin	-182..01
	Micafungin	-295.06
	Anidulafungin	-292.47
SAP7	IgA	**-721.25**
	IgM	-376.71
	IgG heavy chain V-II region	-354.42
	Fluconazole	-120.48
	Caspofungin	-254.67
	Micafungin	-295.88
	Anidulafungin	-248.14
SAP8	IgA	**-746.90**
	IgM	-570.61
	IgG heavy chain V-II region	-524.46
	Fluconazole	-185.58
	Caspofungin	-312.81
	Micafungin	-373.89
	Anidulafungin	-320.68

Studies which have also been addressed this issue, described that total levels of sIgA against the SAPs 1, 2 & 6 were found to be higher in saliva from HIV positive patients[7,8]. Majority of patients with mucosal *Candida* infections have normal or elevated levels of both serum and mucosal anti *Candida* antibodies[9,10]. The secretory IgA antibodies are able to bind *Candida* and reduce the adherence of pathogen *(Candida)* to epithelial cells and thereby preventing the colonization of *C. albicans.*

CONCLUSION

The Protein-Ligand interaction in the present study significantly helped to identify the best drug molecule. From the study, it is concluded that antibodies are more effective over antifungal drugs with SAPs. Among the eight SAPs, SAPs 1-4 & 6-8 showed a strong binding affinity towards sIgA whereas SAP 5 alone exhibited strong binding affinity with IgM.

Acknowledgement: The authors are thankful to the Director Dr. Selvasurabi, INI Centre for Research and Cognizance (ICRAC)**,** Chennai for the technical support and healthy discussion.

REFERENCES

1. Pfaller, M. A., R. N. Jones, S. A. Messer, M. B. Edmond, and R. P. Wenzel. National surveillance of nosocomial blood stream infection due to *Candida albicans*: frequency of occurrence and antifungal susceptibility in the SCOPE Program. Diagn. *Microbiol. Infect. Dis*. 31: 1998.327–332.
2. Ruchel R. On the role of proteinases from *Candida albicans* in the pathogenesis of acronecrosis. *Zentralbl Bakteriol Mikrobiol Hyg A* 255: 1983;524_36.
3. Naglik Julian R., Stephen J. Challacombe, and Bernhard Hube. Candida albicans secreted sspartyl proteinases in virulence and pathogenesis. Microbiology and molecular biology reviews, 2003,p. 400–428.
4. Virupakshaiah DBM, Chandrakanth Kelmani, Rachanagouda Patil, and Prasad Hegade, Computer Aided Docking Studies on Antiviral Drugs for SARS World Academy of Science, Engineering and Technology 30:2007;297-299
5. Schaller, M., W. Scha¨fer, H. C. Korting, and B. Hube. Differential expression of secreted aspartyl proteinases in a model of human oral candidosis and in patient samples from the oral cavity. Mol. Microbiol. 29:1998;605– 615.
6. Macdonald, F., and F. C.Odds. Inducible proteinase of Candida albicans in diagnostic serology and in the pathogenesis of systemic candidosis. J. Med. Microbiol. 13: 1980. 423–435.
7. Drobacheff, C., L. Millon, M. Monod, R. Piarroux, E. Robinet, R. Laurent, and D. Meillet. Increased serum and salivary immunoglobulins against Candida albicans in HIV-infected patients with oral candidiasis. Clin. Chem. Lab Med. 39: 2001.519–526.
8. Millon, L.,C. Drobacheff, R. Piarroux, M. Monod, G. Reboux, R. Laurent, and D. Meillet. Longitudinal study of anti-Candida albicans mucosal immunity against aspartic proteinases in HIV-infected patients. J. Acquir. Immune Defic. Syndr. 26: 2001. 137–144.
9. Coogan, M. M., S. P. Sweet, and S. J. Challacombe. Immunoglobulin A (IgA), IgA1, and IgA2 antibodies to Candida albicans in whole and parotid saliva in human immunodeficiency virus infection and AIDS. Infect. Immun. 62: 1994.892–896.
10. Sweet, S. P., S. J. Challacombe, and J. R. Naglik. Whole and parotid saliva IgA and IgA-subclass responses to Candida albicans in HIV infection. Adv. Exp. Med. Biol. 371B: 1995.1031–1034.

****