

IN SILICO ANALYSIS OF INHIBITOR AND SUBSTRATE BINDING SITE OF SERRAPEPTIDASE FROM *SERRATIA MARCESCENS* MTCC 8708

N. S. KAVIYARASI^{a,b}, C. N. PRASHANTHA^c, V. S. SURYANARAYANA^{d*}

^aSchool of Natural Science, Bangalore University, Bangalore 560056, India, ^bResearch and Development Centre, Bharathiyar University, Coimbatore 641046, India, ^cDivision of Bioinformatics, Scientific Bio-Minds, Bangalore 560092, ^dIndian Veterinary Research Institute, Hebbal, Bangalore 560024, India
Email: veluvarthy@yahoo.com

Received: 14 Jan 2016 Revised and Accepted: 11 Feb 2016

ABSTRACT

Objective: Serrapeptidase is a therapeutic enzyme broadly used as an anti-inflammatory drug to treat inflammatory diseases like arthritis, bronchitis, fibrocystic breast disease and sinusitis. The objective of present study is *in silico* analyzes of the substrate and inhibitor binding sites of serratiopeptidase, expressed from a cloned gene.

Methods: The gene encoding Serrapeptidase was amplified from genomic DNA of *Serratia marcescens* MTCC 8707, an isolated from the flowers of summer squash plants. The gene was sequenced, the nucleotide sequence of 1464 nucleotides was submitted to Gen Bank nucleotide database and accession number GI: KP869847 obtained. The develop amino acid sequence was used to predict 3D structure using different bioinformatics tools and software's Further, CABS-dock and Swiss Dock, the docking servers were used for enzyme-substrate/inhibitor binding site analysis. The inflammatory mediators, bradykinin, and substance-P were used as substrates, whereas, EDTA and Lisinopril were used as an inhibitor for serrapeptidase. UCSF Chimera program was used for interactive visualization and analysis of docked results.

Results: The docking studies show substrates bradykinin and substance-P bind near zinc binding site with minimum RMSD value and the inhibitors EDTA and lisinopril showed favorable interaction at zinc binding site of serrapeptidase with minimum free energy.

Conclusion: The result of docking studies confirm that the substrate or inhibitor binds near zinc binding domain (HEXXH.) and the peptide bond of the substrate can be effectively cleaved by serrapeptidase.

Keywords: Serrapeptidase, Anti-inflammation, Arthritis, Molecular docking, Drug discovery, Protein-peptide interaction, Bradykinin, Substance-P

© 2016 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

INTRODUCTION

Serrapeptidase (SRP) is a metalloprotease protease that belongs to serralyisin family. It was first isolated in the late 1960s from *Serratia E15* [1], enterobacteria found in the intestine of silkworm, where it secretes Serratiopeptidase which helps in a dilating cocoon for emerging of mouth. The enzyme was tentatively named as Serratia peptidase based on its origin [2]. Subsequently, this enzyme was also isolated from *Serratia marcescens* ATCC 25419 [3]. The property of Serrapeptidase was exploited for the development of a drug to treat diseases caused by inflammation. It is usually prescribed for treating inflammatory, traumatic and post-operative swelling and pain. Serrapeptidase being a miracle enzyme, used to treat chronic inflammatory disease such as atherosclerosis, arthritis, bronchitis, fibrocystic breast disease and sinusitis [4] as well as Alzheimer's disease. Recent studies have even suggested the use of oral SPR to aid in the treatment or prevention of viral diseases, such as AIDS and hepatitis B and C [5].

It is available as a tablet or a capsule form in fixed dose combination with NSAIDs (Diclofenac sodium, Nimesulide, Paracetamol) and antibiotics (Amoxicillin, Roxithromycin). The generic Serratiopeptidase combination is manufactured by 710 companies. Medindia's database currently has 1012 Brands of Generics of Serratiopeptidase listed [6]. NSAIDs have potent anti-inflammatory, analgesic and antipyretic actions. It inhibits the enzyme, cyclooxygenase, thus resulting in reduced synthesis of prostaglandin precursors [7]. However as per literature Serrapeptidase appears to be hydrolyzing various peptides such as histamine, bradykinin, Substance P, serotonin, etc., and possesses proteolytic and fibrinolytic activities. Serratiopeptidase binds to α 2-macroglobulin in the blood in the ratio of 1:1 and the complex is internalized by fibroblasts culture and regenerates the protease activity in cells after 2-3 hr of incubation [8]. This helps to mask its antigenicity but retains its enzymatic activity. Generally, Serrapeptidase levels are

slowly transferred to the exudates at the site of infection and inflammation and act on inflammatory peptides. As a result, the cell debris is cleared and controlling the spreading of inflammation.

Serrapeptidase resembles alkaline protease of *Pseudomonas aeruginosa* rather than the *Bacillus subtilis* enzyme, which specifically acts on peptide bonds of gly-hydrophobic amino acid. The enzyme cleaved the gly-leu bond in eledoisin related peptide, the gly-phe bond in bradykinin and split oxidized insulin B-chain at twelve different peptide bonds [9]. The metalloprotease of *Serratia proteamaculans*, Arazyme, similar to Serrapeptidase possesses high hydrolytic activity on substance P and peptides related to bradykinin [10]. Although the substrate specificity and cleavage site of Serrapeptidase were analyzed thoroughly, the enzyme-substrate binding model is not given in detail. Therefore, *in silico* analysis and receptor-ligand docking studies may help in understanding the binding of substrate and inhibitor to the enzyme.

Hence, the present study deals with the structure modeling of Serrapeptidase and docking studies of the specific substrate and inhibitors with the enzyme. CABS-dock web server was used for substrate (Bradykinin, Substance-P) docking. It provides an interface for modeling protein-peptide interactions using a highly efficient protocol for the flexible docking of peptides to proteins [11]. Serrapeptidase and metalloprotease inhibitor (EDTA and Lisinopril) was docked using Swiss Dock web server. It is a web server dedicated to the docking of small molecules on target proteins [12]. Finally, UCSF Chimera program was used for interactive visualization and analysis of molecular structures.

MATERIALS AND METHODS

Sequence selection

The deduced amino acid sequence of Serrapeptidase gene was used in this study. The Serrapeptidase gene was amplified from genomic

DNA of *Serratia marcescens* MTCC 8707, isolated from the flowers of summer squash plants, sequenced and uploaded in GenBank nucleotide database (GI: KP869847) (fig: 1) [13]. The derived amino acid sequence of the gene id is separated from UniProt database (UniProt ID: A0A0G4AMK3) having 487 amino acids was further used for structural analysis.

Comparative modeling

The amino acid sequence of Serrapeptidase was further used for template search using PSI-BLAST program from NCBI database. Further, the protein structures were built and modeled based on template sequences of Serrapeptidase. The sequences of conserved regions were efficiently employed to study the functional and structural relationships. The three-dimensional structures of conserved regions were predicted using Swiss-Model workspace and aligned models were observed using the Swiss-PDB viewer. The resultant 3D structure (template) mapping exhibited significant interactive sites on amino acid sequences against targets. During modeling studies, the factors such as positive target and template alignment, the building of model and quality evaluation were achieved. The quality of modeled structures in the course of stereochemistry was analyzed by using residue-by-residue geometry and overall structure geometry method. The modeled 3D structures were verified by Molprobit server. The quality was determined based on stereochemical models of predicted ERRAT, PROCHECK, and Ramachandran Plot. Most of the structural misalignment errors in rotamers occurred due to the side chain packing and folding. The values of Structural deviation measure (SDM), Root mean square deviation (RMSD), quality (Q)-factor and B-factor were quite efficient to verify the quality structures of Serrapeptidase by using Chimera 1.6

Ligand selection and molecular docking

In the present in-silico pharmacological investigation of Serrapeptidase inhibition, we have selected Bradykinin and

Substance-P, the key anti-inflammatory mediators, as substrates and EDTA and Lisinopril as inhibitors (Fig: 4) and studied. The CABS-dock is used for protein-peptide docking in two different inputs; first amino acid sequence of the peptide and second 3D structure of the protein receptor. The docking is progressed by molecular simulation using Monte Carlo simulation with 10 replicates is uniformly spread on the temperature scale to generate random docking structures. About 10000 models were generated during simulation of which 1000 models were selected for further steps in the following procedure: 1) all unbound states (where interaction energy between the peptide and the receptor is zero) were rejected 2) from the remaining models up to 100 from each of the 10 trajectories are picked by the lowest interaction energy.

The Swiss-Dock server used for docking with Serrapeptidase and inorganic inhibitors such as EDTA and Lisinopril were selected based on ZINC database. Docking-type was exact and rigid. Each docking experiment was the result of 250 different consecutive runs. Those binding models possessing the most favorable energies were estimated by fast analytical continuum treatment of solvation (FACTS), and clustered. Binding modes were scored via their Full Fitness and clustered. Then clusters were classified based on the average Full Fitness of their elements. The results obtained from CABS-Dock and Swiss Dock was pictured by UCSF Chimera.

RESULTS AND DISCUSSION

We have predicted the Serrapeptidase template sequences using PSI-BLAST with PDB structural analysis. The sequence having >30% homologous were selected as the best templates. The templates selected were shown in table 1. Using PSI-BLAST target sequences were compared to align with human reference genome databases which showed 395 templates as matched with Serrapeptidase sequence and they were used to predict 3D structures.

Table 1: Predicted serrapeptidase template sequences based on homology search using PSI-Blast

Template	Sequence identity	Oligo state	Description
1srp.1. A	99.58	Monomer	Serralyisin
1smp.1. A	99.58	Hetero-oligomer	Serratia metallo proteinase
1srp.1. A	99.57	Monomer	Serralyisin
1smp.1. A	99.57	Hetero-oligomer	Serratia metallo proteinase
1af0.1. A	98.94	Monomer	Serratia protease

The 3D structure of Serra peptidase was built by highest sequence identity using homology models (fig: 1). The protein structures were validated based on Z-score less than 1, GMQE: 0.99 and QMEAN4: 0.20, which predicted the best quality model structures. The 1SRP template with 99 % quality on the stereochemical activity of amino acids was observed in Ramachandran plot and the resultant protein structure was used to identify zinc binding region using chimera 1.6.

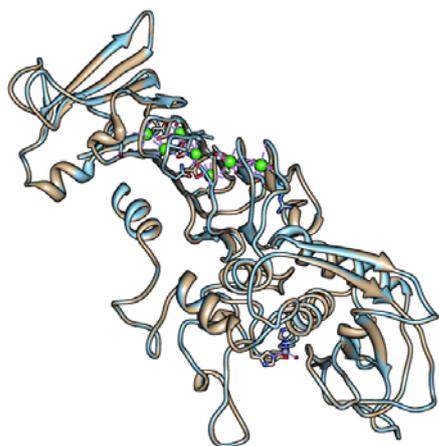


Fig. 1: Model of serrapeptidase 3D structure with 1SRP template

As seen in the fig, the 3D model structure exhibits N-terminal zinc-binding domain and the zinc ion is bound by His 192, His 196, His 202, Tyr 232 and along with the water molecule in a distorted trigonal bipyramidal manner (Fig: 2) because it belongs to Met-zincins- 'zincins superfamily' with topologies of M-turn (HExx Hxx GxxH...xxM).

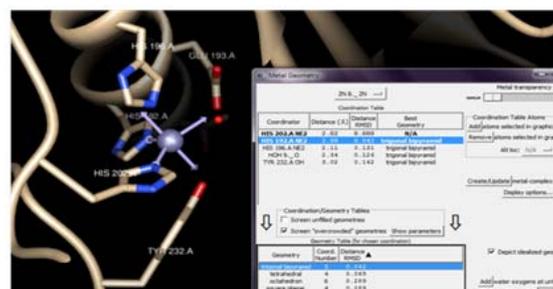


Fig. 2: Trigonal bipyramidal structure of serrapeptidase with zinc binding site of metal geometry

The C-terminal domain is a β strand-rich domain containing eighteen β strands, a short α -helix and seven Ca^{2+} ions bound to calcium binding loops. It contains multiple tandem repeats of nine-

residue motif including the pattern GCXGD, which forms a corkscrew (fig. 3). This pattern is similar to haemolysin and hence thought to be important for secretion of the protein as well as for the folding and stability. Serratia protease has several bound calcium ions which are not essential for its catalytic function but are required for protection from autolysis [17].

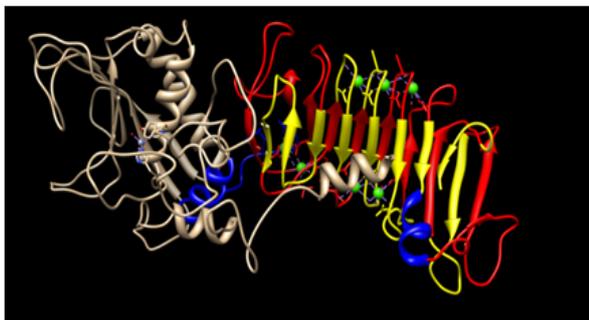


Fig. 3: Final predicted model of Serrapeptidase enzyme structure with N and C-terminal regions

Ligand preparation and molecular docking

The structural elucidation of Serrapeptidase belongs to Serralyisin family members and may have a broad spectrum of substrates such as bradykinin (RPPGFSPFR), substance-P (RPKPQQFFGLM) and inorganic substances such as EDTA and Lisinopril. These features were used for pharmacological analysis (fig: 4). Using molinspiration

online server pharmacophore were predicted, and the results are shown in table: 2. The EDTA structure has highest TPSA of >140 indicating the highest precipitation and interaction with receptor protein. Similarly, Lisinipril has very good pharmacophores, and the results were used for molecular docking.

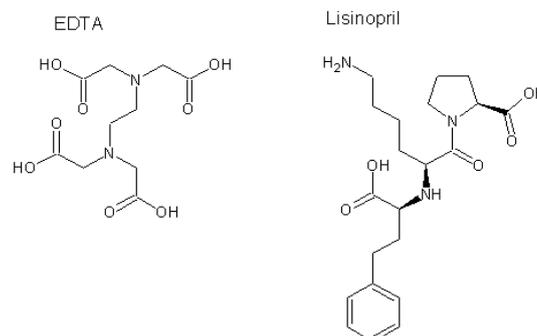


Fig. 4: Predicted molecular docking pictures with Inorganic substances of EDTA and Lisinopril

Cabs-Dock online server was used for protein-peptide docking based on Monte Carlo simulation and the resultant cluster details and contact maps were presented in table 3. In this selection Serrapeptidase-Bradykinin has average RMSD of 7.68 by forming 157 elements interacts with active site amino acids His196, His202 of Serrapeptidase with Ser474 amino acids of Bradykinin (Fig: 5a-b). Similarly, substance-P also interacts within the active site amino acids His196, Glu 197 of Serrapeptidase (fig: 6a-b).

Table 2: Pharmacophore analysis of chemical substance as predicted using molinspiration, online server

Substrate name	Cluster density	Average RMSD	max RMSD	Number of elements
Bradykinin	20.4248	7.68674	30.7166	157
Substance-P	111.804	0.894423	2.87267	100

Table 3: Clustering details of Bradykinin and substance P with serrapeptidase

Substrate name	Cluster density	Average RMSD	max RMSD	Number of elements	Receptor amino acid	Peptide amino acid					
Bradykinin	20.4248	7.68674	30.7166	157	Tyr A 213	PRO B 471					
					His A 183	PHE B 476					
					His A 177	PHE B 476					
					His A 173	PHE B 473					
					Gln A 169	GLY B 472					
					Asn A 157	PHE B 473					
					Leu A 135	PHE B 476					
					Tyr A 132	PRO B 475					
					Ala A 131	PHE B 473					
					Gln A 130	PHE B 473					
					Substance-P	111.804	0.894423	2.87267	100	TYR A 227	LEU B 478
										TYR A 213	LEU B 478
										TYR A 213	GLN B 474
										TYR A 213	LYS B 471
										LEU A 210	PRO B 472
										THR A 195	PHE B 476
										HIS A 177	PRO B 472
GLU A 174	LYS B 471										
HIS A 173	LYS B 471										
GLN A 169	GLN B 473										
LEU A 135	PRO B 470										
ALA A 133	LYS B 471										
TYR A 132	LYS B 471										
ALA A 131	GLN B 474										
GLN A 130	GLN B 474										
THR A 129	ARG B 469										

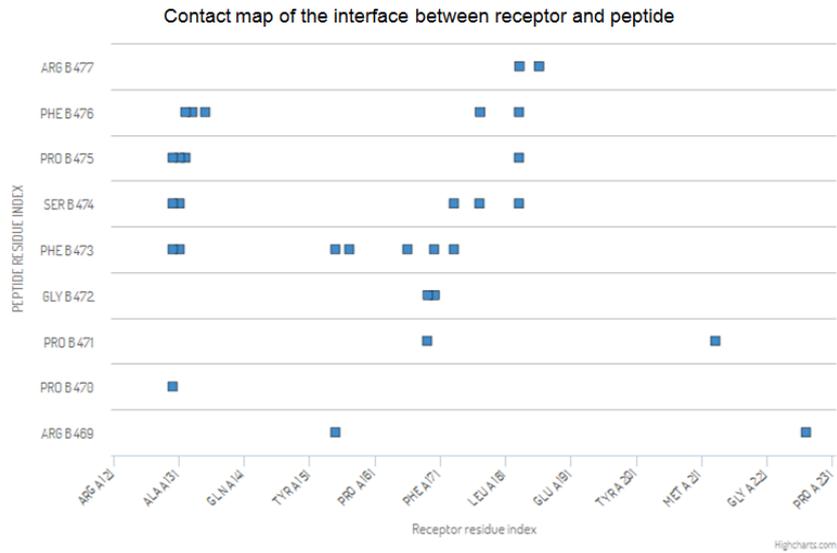


Fig. 5a: Contact map of Bradykinin with serrapeptidase

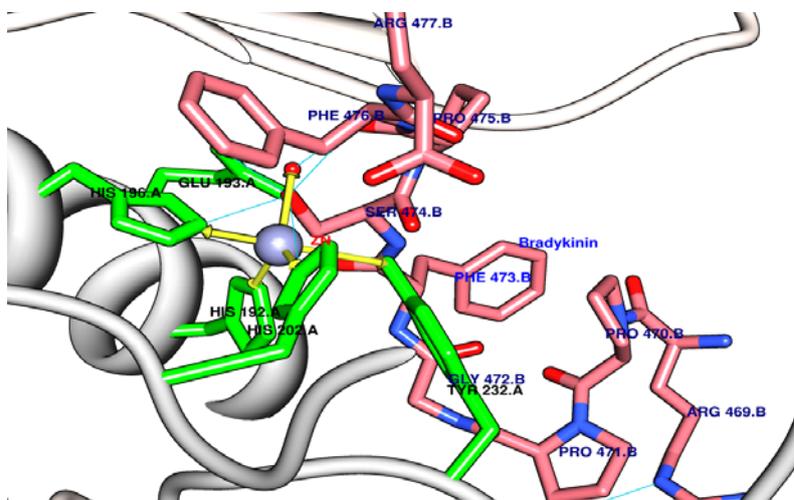


Fig. 5b: Bradykinin binding with serrapeptidase

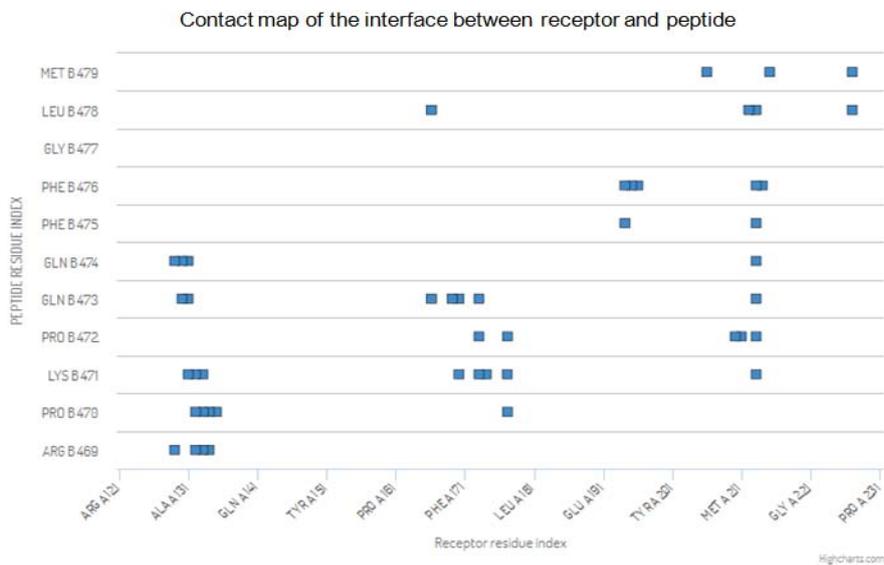


Fig. 6a: Contact map of Substance-P with serrapeptidase

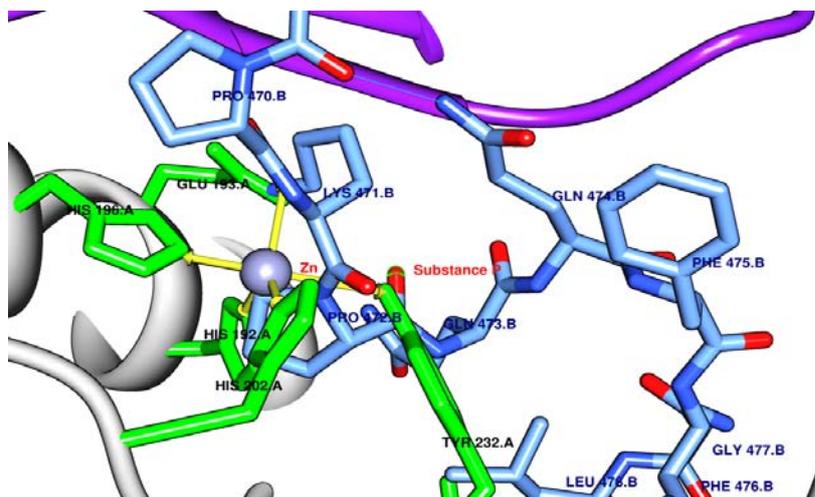


Fig. 6b: Substance-P binding with Serrapeptidase

The other inorganic substances such as EDTA and Lisinopril were docked with Serrapeptidase enzyme using Swiss dock. The results of Swiss Dock showed full fitness and Gibbs free energy predicts docking interactions (table: 4). The EDTA showed full fitness of-1735.25 kcal/mol and estimated ΔG of-9.74 kcal/mol within active site amino acids His 192, His196, His202 of

Serrapeptidase (fig: 7). The Lisinopril showed Full Fitness of-1830.91 kcal/mol and estimated ΔG of-10.50 kcal/mol within active site amino acids His192, His196, and His202 of Serrapeptidase (fig: 8). Inhibition of Serrapeptidase using EDTA and Lisinopril, the metal chelating agent helps in understanding that it is a metalloproteinase.

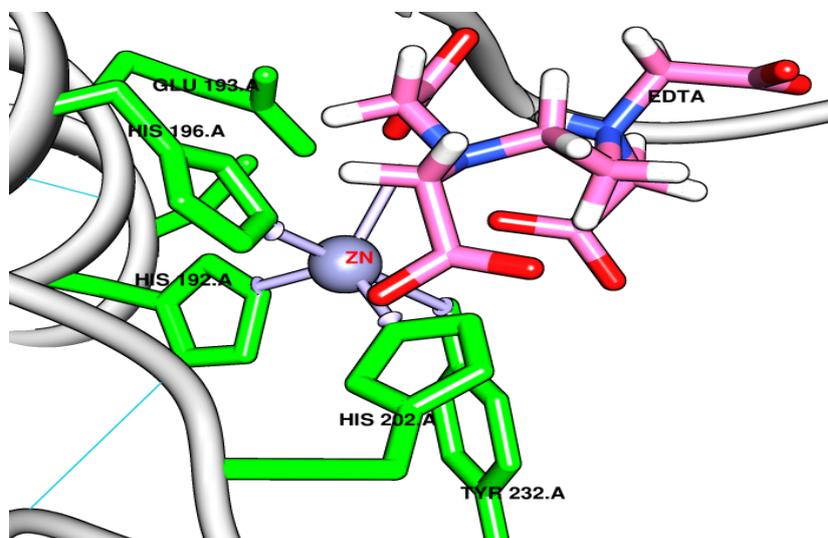


Fig. 7: EDTA binding with serrapeptidase

Table 4: Clustering results obtained from docking of inhibitors with serrapeptidase

S. No.	Ligands	Receptor	No. of Swiss dock clusters	Cluster rank	Full fitness (Kcal/mol) Cluster	Estimated dG (Kcal/mol)	Remarks	
1.	Ethylenediaminetetraacetic acid dipotassium salt dihydrate EDTA ZINC 19364242	SRP	30 (250)	0	0	-1742.31	-11.19	Binds near Zn binding site.
				0	1	-1742.31	-11.19	
				0	2	-1742.18	-11.18	
				0	3	-1742.11	-11.17	
				1	0	-1740.43	-10.70	
2.	Lisinopril ZINC 3812863	SRP	30 (250)	1	1	-1735.25	-9.74	Binds near Zn binding site.232.
				0	1	-1830.91	-10.50	
				0	2	-1830.66	-10.47	
				0	3	-1830.66	-10.47	
				0	4	-1829.39	-10.39	
				0	5	-1829.39	-10.39	

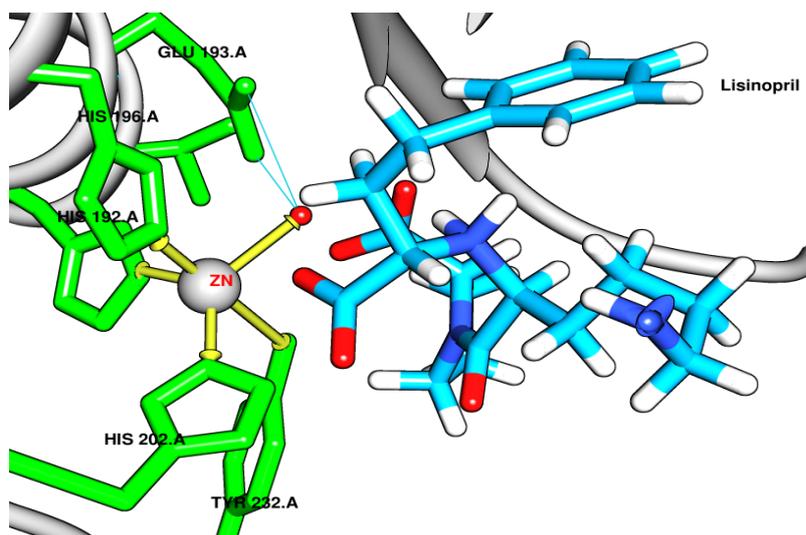


Fig. 8: Lisinopril binding with serrapeptidase

According to Schechter and Berger model (1967,1968), S₁ subsite of Serrapeptidase is found to have a broad specificity, being Gly the preferred amino acid for this subsite followed by positively charged residues (Arg and His). Then, S₂ and S'₁ subsites accommodate better hydrophobic residues with aliphatic or aromatic side chains like Leu, Phe [10]. Serralyisin family preferentially cleaves bonds with hydrophobic residues in P1'. Through the docking studies, it was found that the preferred cleavage site Gly-Phe of bradykinin docked near zinc binding domain of SRP and hydrogen bond formed between His 196 (SRP) and Serine residue of bradykinin. Similarly, Substance-P preferred cleavage site Gly-Leu binds near zinc binding domain and a hydrogen bond between His 196 (SRP) and Lys residue (Substance-P). Inhibitor binding studies also support the fact that zinc binding site may be involved in the mechanism of enzyme catalysis. Hence, it is hypothesized that as like the metalloendopeptidase, which catalysis the hydrolysis of internal, α -peptide bonds in a polypeptide chain by a mechanism in which water acts as a nucleophile and charged amino acids side chains are ligands for the metal ions. Thus, the docking studies uphold the fact.

CONCLUSION

The structural elucidation shows that Serrapeptidase belongs to Serralyisin family member. In general, a serralyisin family has more influence on inflammatory peptides, therefore Serrapeptidase also expected to act on inflammatory peptides. Also, the present study has given a new insight to the enzyme-substrate binding model of Serrapeptidase. Hence, the knowledge on the mechanism of Serrapeptidase activity on its substrate supports its pharmaceutical application as an anti-inflammatory and analgesic agent.

CONFLICT OF INTERESTS

All authors have nothing to declare.

REFERENCES

- Miyata K, Maejima K, Tomoda K, Isono M. Serratia protease part I, Purification and general properties of the enzyme. *Agric Biol Chem* 1970;34:310-9.
- Miyata K, Tomoda K, Isono M. Serratia protease part II, the substrate specificity of the enzyme. *Agric Biol Chem* 1970;34:1457-5.
- Decedue CJ, Broussard EA, Larsan AD, Braymer HD. Purification and characterization of the extracellular proteinase of *Serratia marcescens*. *Biochim Biophys Acta* 1979;569:293-8.
- Klein G, Kullich W. Short-term treatment of painful osteoarthritis of the knee with oral enzymes. A randomized, double-blind study versus diclofenac. *Clin Drug Invest* 2000;19:15-8.
- Hanaa HA, Nevein NF, Karima AE, Amal HH. Miracle enzymes serrapeptase and nattokinase mitigate neuroinflammation and apoptosis associated with alzheimer's disease in an experimental model. *World J Pharm Pharm Sci* 2014;3:876-15.
- www.medindia.net/drug-price/serratiopeptidase-combination. [Last accessed on 10 Dec 2015].
- Bennett G, Villa. Nimesulide: an NSAID that preferentially inhibits COX-2, and has various unique pharmacological activities. *Expert Opin Pharmacother* 2000;1:277-86.
- Maeda H, Molla A, Oda T, Katsuki T. Internalization of serratia1 protease into cells as an enzyme-inhibitor complex with α_2 macroglobulin and regeneration of protease activity and cytotoxicity. *J Biol Chem* 1987;23:10946-4.
- Miyata K, Tomoda K, Isono M. Serratia protease part II, the substrate specificity of the enzyme. *Agric Biol Chem* 1970;34:1457-5.
- Bersanetti PA, Park HY, Bae KS, Son KH, Shin DH, Hirata IY, *et al.*, Characterization of arazyme, an exocellular metalloprotease isolated from *Serratia proteamaculans* culture medium. *Enzyme Microb Technol* 2005;37:574-6.
- Kurcinski M, Jamroz M, Blaszczyk M, Kolinski A, Kmiecik S. CABS-dock web server for the flexible docking of peptides to proteins without prior knowledge of the binding site. *Nucl Acids Res* 2015;43:W419-24.
- Grosdidier A, Zoete V, Michielin O. Swiss Dock, a protein-small molecule docking web service based on EADock DSS. *Nucl Acids Res* 2011;39:W270-7.
- Kaviyarasi NS, Swaroop S, Suryanarayana VVS. Characterization of a gene encoding serrapeptidase from *Serratia marcescens* strain (SRM) MTCC 8708, a plant isolate. *Int J Curr Microbiol Appl Sci* 2015;4:206-14.
- Benkert P, Biasini M, Schwede T. Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics* 2011;27:343-7.
- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl Acids Res* 1997;25:3389-13.
- Remmert M, Biegert A, Hauser A, Soding J. HHblits: lightning-fast iterative protein sequence searching by HMM-HMM alignment. *Nat Methods* 2012;9:173-2.
- Hamada K, Yasuo H, Katsuya Y, Hiramatsu H, Fujiwara T, Katsube Y. Crystal structure of serratia protease, a zinc-dependent proteinase from serratia sp. E-15, containing a β -sheet coil motif at 2.0 \AA resolution. *J Biochem* 1996;119:844-7.