In Silico approach for therapeutic profiling and physiochemical findings of Serratiopeptidase an anti-inflammatory enzyme

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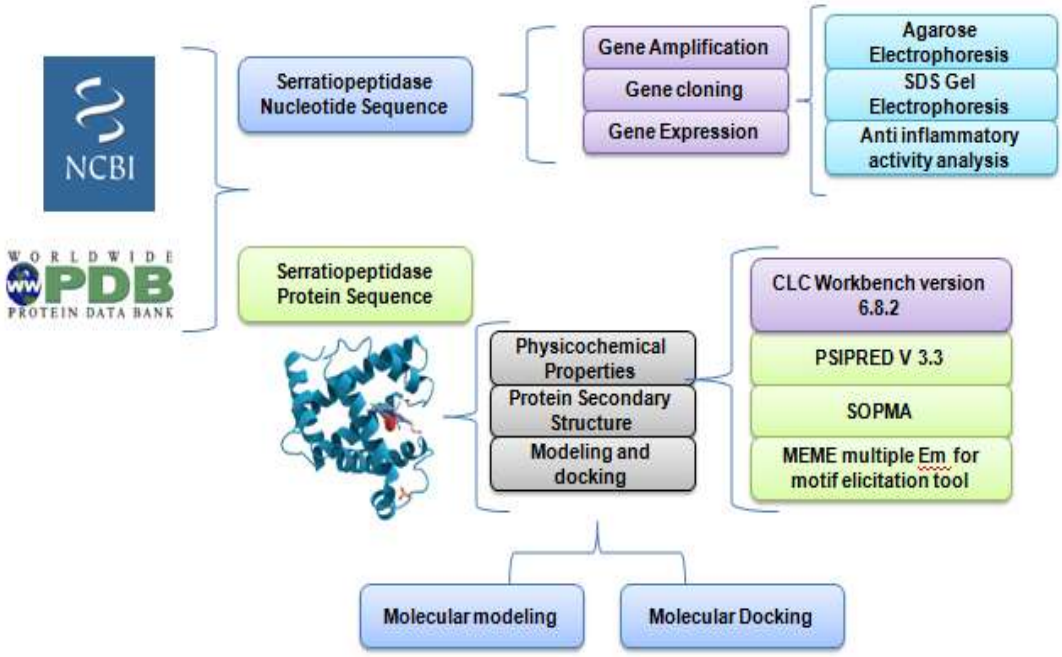
Abstract

Inflammation and inflammatory disorders are the leading cause of death and physical deformities worldwide. The management of inflammatory diseases entirely relies on mainly two classes of synthetic compounds i.e. non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal drugs. Often these drugs are associated with several side effects and adverse drug reactions. To combat inflammation, more efficiently enzyme-based medications emerged in last once decade. Several serine proteases were studied and characterized as anti-inflammatory in nature. Serratiopeptidase was reported as one potent anti-inflammatory enzyme-based drug and employed in the management of acute inflammation. Though the enzyme has a long history as anti-inflammatory therapeutic presently in sports medicine, however the critical mechanism remains unknown entirely. The major challenge associated with the serratiopeptidase is lack of molecular mechanism.

In this study, using in silico approaches, we have investigated structural and functional insights of serratiopeptidase associated with potent anti-inflammatory activity. The physicochemical properties were characterized to explore therapeutic potential. Secondary structure was predicted for stability of enzyme. Molecular modeling and docking studies were carried out to explore exact molecular mechanism. The different substrates were docked with the enzyme to investigate its affinity for its target. The affinity of enzyme with COX I (-458.36 kcal/mol), COX-II (-486.24 kcal/mol) and LOX (-462.24 kcal/mol) was determined via docking analysis. The anti-inflammatory activity of serratiopeptidase was determined as function of RBC cell membrane lysis with reference to diclofenac sodium as a standard anti-inflammatory drug. We report here a high degree of RBC cell membrane lysis (70.5%) at higher concentration of serratiopeptidase (2.5mg/ml).

Keywords: Serratiopeptidase, anti-inflammatory drugs, NSAIDs, *In Silico* profiling, modeling and docking studies.



Graphical Abstract of the study

Introduction

Inflammation is a double edge sword and essential physiological event in any high animal. The onset of inflammation, no matter whatever triggers, provides a line of defence to organism against noxious changes[1,2]. Both acute and chronic inflammation result in various kinds of inflammatory diseases and disorders. These inflammatory disorders are primarily associated with death and physical deformities[3-5]. In the present scenario, autoimmune disorders like arthritis, consequence of allergens like bronchitis and many more infection based inflammation are concerned as medical challenges.

Inflammation is complex physiological process associated with many endogenous and external triggers allowing activation of immune cells and inflammatory mediators[6]. There are several enzymes reported as potential candidate in controlling inflammation via binding with multiple targets[7,8]. The production of short peptides like interleukins, thromboxane and cytokines which possess immense pharmacological activity leads to beginning and progression of inflammation[9].

Over many decades, the management of various kinds of inflammation relied on use of plant products and chemically synthesized drugs. Both steroidal and NSAIDs have different mechanisms and targets to combat inflammation[10,11]. The steroidal drugs primarily bind and block activity of phospholipase which regulate conversion of phospholipids to arachidonic acid. NSAIDs possess different targets including cyclooxygenase (COX I and COX II) and non-specifically lipoxygenase enzyme. However, lipoxygenase and COX I enzyme driven mediators are essential for physiological balance and their inhibition leads to side effects like gastric bleeding[12].

The class of drugs, non-steroidal anti-inflammatory (NSAIDs) and steroidal drugs remains first choice in all kinds of inflammation. These drugs were useful and provided only symptomatic relief rather than elimination of inflammatory triggers[13]. Both NSAIDs and steroidal drugs are associated with several severe side effects and adverse drug reactions. Further, long term use of steroidal drug leads to immune complication and affects cell-mediated immunity[14]. The NSAIDs are associated with gastric bleeding and physiological load on the kidney in long term use. To overcome these limitations caused by long term use of synthetic drugs, enzyme-based anti-inflammatory molecules emerged as novel tools in modern medicine[15]. Several enzymes basically protease and more likely serine protease have shown tremendous scope in dealing various kinds of inflammation.

However, many proteins are still in characterization phase as lack of molecular mechanism but their proteolytic nature and broad substrate affinity define a great significance in coming time[4]. These proteolytic enzymes are widely distributed in biological world including plants, animal and microbes. These enzymes are not only associated with delivering anti-inflammatory activity but also help in maintenance of physiological balance; homeostasis[5].

Serratiopeptidase is a leading commercial enzyme with a long history in medicine as an anti-inflammatory molecule. The protein is serine protease of average molecular weight 53 kDa with immense proteolytic activity[16,17]. The enzyme is naturally produced by Serratia species habitat in silkworm gut and provides a bottom line of defense in worm. The protein primarily acts on decades tissues and cell-free adhesion molecules which involve mostly in bringing immune cells at inflamed cite. Several earlier findings suggested that serratiopeptidase is as potential and other NSAIDs as diclofenac and salicylates[18]. The extended use of enzymes is designed in encapsulated formulation with several other NSAIDs and many more antibiotics. Despite its therapeutic potential and long history in medical, there is less evidence towards enzyme characterization[19].

This study designed to explore the physicochemical properties of enzyme and stability analysis via secondary structure prediction. The similarity search and phylogenetic tree analysis was carried out to investigate enzyme evolution. Three-dimensional structure of enzyme was predicted by molecular modeling using Swiss model tool. The protein was docked with cyclooxygenase and lipoxygenase enzyme for finding best affinity of drug.

Material and Methods

Retrieval of sequences, similarity search and phylogenetic tree analysis: The protein sequence of serratiopeptidase was retrieved from National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). The protein sequences was retrieved in FASTA format. Three-dimensional structures and coordinates (PDB) of serratiopeptidase were retrieved from the protein data bank (http://www.rcsb.org/pdb/home/home.do). A multiple sequence alignment (MSA) was carried out for finding similarity in the database for protein sequence (enzyme) retrieved from NCBI using ClustalW2 algorithm program, (http://www.ebi.ac.uk/Tools/msa/clustalw2/) an online tool used for multiple sequence alignment allowing prediction of similarities and differences of amino acids.

Construction of phylogenetic tree of serratiopeptidase was carried out by maximum likelihood method and the cladogram was constructed from the sequence retrieved from NCBI for enzyme.

The phylogenetic tree analysis provides a graphic interface for molecular evolution of proteins. There are several submissions of serratiopeptidase in NCBI and the sequence retrieved from NCBI was selected based on maximum and prime search hit in database. The *Serratia marcescens* and *Serratia sp. E-15* strains were purchased from MTCC Chandigarh for cloning and expression studies[20]. The

bacterial species were grown and maintained as per media and growth condition recommended by supplier. All the consumables used in present study were purchased of molecular biology grade.

Secondary structure prediction: The secondary structure of serratiopeptidase was predicted by online tool PSIPRED V 3.3 (http://bioinf.cs.ucl.ac.uk/psipred/?ffpred=1). The PSIPRED V 3.3 utilizes two-stage neural networks to predict secondary structure of the protein. Based on the result from PSIPRED V 3.3, presence of α helix, β sheet and random coil was calculated by SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html). The CYS_REC tool was used to identify presence of disulfide bonds in selected enzymes (http://manaslu.aecom.yu.edu).

Apart from these findings, presence of signal peptide cleavage sites in protein sequence was predicted and analyzed using the SingalP tool (http://www.cbs.dtu.dk/services/SignalP/). MEME multiple Em for motif elicitation is most reliable online tool to discover motif in given protein sequence. MEME Suite 4.10.1 (http://meme-suite.org/) was used for prediction of presence and motif position in enzyme. Another online tool Pfam (http://pfam.xfam.org/) was used to predict motif presence and location enzyme[21,22].

Determination of physicochemical properties: The physiochemical parameters of the enzyme were analyzed using property values of residues as averaged over the whole sequence. The CLC free Workbench version 6.8.2 was used for predicting physicochemical properties of the protein (http://www.clcbio.com/products/clc-main-workbench/).
The properties were analyzed including average molecular weight (AMV), negative and positive charged residues (NCR and PCR), extinction coefficient (EC), isoelectric point *(pI*), instability index (II), aliphatic index (AI) and grand average of hydropathy (GRAVY) by using the ExPASy bioinformatics resource portal (http://www.expasy.org/).

Among these physicochemical parameters, instability index defines stability of given protein and an instability index (II value) less than 40 considered is stable. The GRAVY value is calculated as sum of hydrophobicity values of all the amino acids given in protein divided by the number of residues in the sequence[23]. The negative GRAVY values indicate the nonpolar character of the given protein.

Molecular modelling: Homology modeling allows developing a three-dimensional structure for the given protein sequence for docking and other studies. Swiss model, an online tool was used to model all enzymes (http://swissmodel.expasy.org/). Swiss model generates a comparative model for a given amino acid sequence based on close match of structure available in database. In this study, Swiss model allowed modeling of enzyme using a template PDB. The modeled structures were validated by using Ramachandran Plot analysis.

RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) is online tool for analysis of modeled structure and their validity. The tool allows understanding arrangement of amino acids in different regions like favourable, allowed and outlier. RAMPAGE provides a graphical interface showing location of amino acids of a protein sequence in the different areas of Ramachandran plot. The pre-requisite for a modeled structure to be valid three-dimensional protein structures is a score more than 95%[24,25].

Docking studies: There are many docking software stations for protein-protein docking study. In the current study, docking studies were carried out by Auto-Dock (http://autodock.scripps.edu/) which is free software and reliable. Both receptor and ligand were prepared according to docking manual. Docking simulations using the grid parameter file (Gpf) and a docking parameter file (Dpf) were prepared. The docking optimization was performed using the Lamarckian genetic algorithm (LGA) for 12, 000 run. The MGL tool 1.5.1 was used for the setting parameter of protein for docking study (http://mgltools.scripps.edu/).

MGL tool 1.5. is composite software providing an interface for docking studies and visualization of protein molecules. MGL is providing interface for AutoDock tool (ADT) and Python Molecule Viewer (PMV). An enzyme from selected organisms was docked with different proteins including cyclooxygenase and lipoxygenase. The docked structures were analyzed by calculating specific root mean square deviation (RMSD) values[26,27].

Molecular cloning and expression of serratiopeptidase: Total genomic DNA of *Serratia marcescens and Serratia sp. E-15* was isolated using Hi-Media HiPuraA (MB505) kit as per protocol recommended by the manufacturer. Gene-specific primers were designed and synthesized by Sigma Aldrich Pvt. Ltd. We used here EcoR1 and Hind III restriction sites in forward and reverse primer for gene cloning. The gene was amplified using polymerase chain reaction using reaction conditions: denaturation for 5 min for 95^0C (one cycle) followed by 30 cycles (denaturation for 20Sec at 95^0C, annealing temperature 56^0C for 45 Sec and extension for 90Sec for 72^0C) and final extension for 7 min at 72^0C.

Forward primer SFP 5` G**GAATTC**ATGCAATCTA CTAAAAAGGCAATTG and SRP 5` CCC**AAGCTT**TTA CACGATAAAGTCCGTGGC were used for amplification and cloning of serratiopeptidase. The amplified serratiopeptidase gene was cloned into pET28a expression vectors system and construct transformed into *E. coli* BL (DE 3) for expression studies. Here 5mM IPTG was used in different time intervals for expression of protein. The expressed protein was visualized in SDS-PAGE and quantified.

Anti Inflammatory activity Analysis: The anti-inflammatory activity of expressed serratiopeptidase analyzed via membrane stabilizing activity. Human red blood cell suspension was prepared from fresh blood. The blood samples were mixed with 2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water)[4]. The tubes containing human blood were subjected to brief spin at 3500rpm for 15 min and packed cells were rinsed with isosaline solution (0.85% pH 7.2) several times. The centrifuged blood was reconstituted as 15% iso-saline suspension. Here, we have used the concept of hemoglobin release via hypotonic solution containing expressed serratiopeptidase as driver for membrane lysis of human red blood cell membrane. We used as assay mixture of 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % V/V] with 0.5 ml of expressed protein (0.5 to 2.5 mg/ml) of serratiopeptidase.

For control study we used diclofenac sodium in increasing concentrations (50, 100, 250, 500, 750, 1000, 1500, 2500µg/ml) incubated at 37^0C for 30 min and centrifuged respectively. The content of hemoglobin present in test and control was determined by spectrophotometer at 560 nm. The percentage of hemolysis was human red blood cell membrane as determined by given equations:

For the % of haemolysis of human red blood cell membrane

% of Haemolysis = (OD of Test Sample / OD of Control) × 100

The percentage of HRBC membrane stabilization calculated using the formula

% of Membrane Stabilization = 100 - (OD of Test sample / OD of Control × 100)

Results

Retrieval of sequences, similarity search, and construction of phylogenetic tree: The protein sequences used in the current study were retrieved from NCBI are listed in the table (table 1). The multiple sequence alignment study among the sequences from Serratia species had shown a higher level of similarity. The phylogenetic tree was constructed based on information available on NCBI. The database contains only two sequences of serratiopeptidase from *Serratia marcescens* and *Serratia sp. E-15*. Both the sequences were analyzed using clustal W program for phylogenetic tree analysis[28].

Secondary structure prediction: The secondary structure of the enzyme was predicted using UCL (http://bioinf.cs.ucl.ac.uk/), an online tool for the determination of various forms in given amino acid. The serratiopeptidase from both the source analyzed and reported protein is primarily composed of alpha-helix and beta-sheet. There are traces of other structures like turn and coil which provide substantial evidence in enzyme stability and activity. The enzyme was also analyzed for different motifs using Pfam. The result shows proteins from both sources possessing eight different motifs. The detail of each motif is given in table 2[29].

Determination of physicochemical properties: ProtParam (http://web.expasy.org/protparam/) (ExPASy tool) provides an interface for calculating physicochemical properties of a given protein sequence. The results obtained from ProtParam are shown in table 3.

Table 1
The organism selected for current study and detail of protein sequence retrieved.

Organism	GenBank No	Length
Serratia marcescens	AKL80897	487
Serratia sp. E-15	P07268	487

Table 2
Table demonstrates here different location of functional motifs in serratiapeptidase, their E value.

Pfam	Motif detail (Position and E Value)	Description
Peptidase_M10_C	264..487 (1.7e-84)	PF08548, Peptidase M10 serralysin C terminal
Peptidase_M10	99..208 (2.5e-09)	PF00413, Matrixin
Hemolysin Cabind	348..365(0.0082), 358..374(0.0056) 367..383(0.0014), 375..392(0.087)	PF00353, Hemolysin-type calcium-binding repeat (2 copies)
Reprolysin 4	165..237 (7.3e-06)	PF13583, Metallo-peptidase family M12B Reprolysin-like
Reprolysin 5	165..237 (6.9e-06)	PF13688, Metallo-peptidase family M12
Reprolysin 3	173..202 (5.8e-05)	PF13582, Metallo-peptidase family M12B Reprolysin-like
Reprolysin 2	179..237 (0.0018)	PF13574, Metallo-peptidase family M12B Reprolysin-like
Peptidase_M43	177..223 (0.036)	PF05572, Pregnancy-associated plasma protein-A

The physicochemical properties of serratiopeptidase suggest enzyme possessing stability and very high probability of negatively charge as pI value lies near to 5.0 (4.6). The protein had shown weak disulfide bond percentage as less number of sulfur was reported. For a serine protease like serratiopeptidase, an average molecular weight (53kDa) and pI value close to 5.0 resemblances with other anti-inflammatory enzymes were reported earlier[30].

Molecular modeling: The three-dimensional models for serratiopeptidase were built using a template searched by Swiss model itself. Both the sequences modeled using the same template. We have found the modeled structure of serratiopeptidase as identical and one reason for identical model was at amino acid level; both sequences share 100% similarity. Despite different origin, both sequences of serratiopeptidase retrieved were similar and hence there was no difference in multiple sequence alignment, in cladogram and modeled designed. The modeled structure was analyzed for its valid nature using Ramachandran plot. Both the modeled structure scored more than 98% amino acid in favored region, 2% is allowed. The result shown here in figure 4 suggesting both the models are valid[31].

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CLUSTAL 2.1 multiple sequence alignment


gi|829625095|gb|AKL80897.1|     MQSTKKAIEITESSLAAATTGYDAVDDLLHYHERGNGIQINGKDSFSNEQ 50
gi|32172419|sp|P07268.2|PRZN_S  MQSTKKAIEITESNFAAATTGYDAVDDLLHYHERGNGIQINGKDSFSNEQ 50
                                ************.:**********************************

gi|829625095|gb|AKL80897.1|     AGLFITRENQTWNGYKVFGQPVKLTFSFPDYKFSSTNVAGDTGLSKFSAE 100
gi|32172419|sp|P07268.2|PRZN_S  AGLFITRENQTWNGYKVFGQPVKLTFSFPDYKFSSTNVAGDTGLSKFSAE 100
                                **************************************************

gi|829625095|gb|AKL80897.1|     QQQQAKLSLQSWADVANITFTEVAAGQKANITFGNYSQDRPGHYDYGTQA 150
gi|32172419|sp|P07268.2|PRZN_S  QQQQAKLSLQSWADVANITFTEVAAGQKANITFGNYSQDRPGHYDYGTQA 150
                                **************************************************

gi|829625095|gb|AKL80897.1|     YAFLPNTIWQGQDLGGQTWYNVNQSNVKHPATEDYGRQTFTHEIGHALGL 200
gi|32172419|sp|P07268.2|PRZN_S  YAFLPNTIWQGQDLGGQTWYNVNQSNVKHPATEDYGRQTFTHEIGHALGL 200
                                **************************************************

gi|829625095|gb|AKL80897.1|     SHPGDYNAGEGNPTYRDVTYAEDTRQFSLMSYWSETNTGGDNGGHYAAAP 250
gi|32172419|sp|P07268.2|PRZN_S  SHPGDYNAGEGNPTYRDVTYAEDTRQFSLMSYWSETNTGGDNGGHYAAAP 250
                                **************************************************

gi|829625095|gb|AKL80897.1|     LLDDIAAIQHLYGANLSTRTGDTVYGFNSNTGRDFLSTTSNSQKVIFAAW 300
gi|32172419|sp|P07268.2|PRZN_S  LLDDIAAIQHLYGANLSTRTGDTVYGFNSNTGRDFLSTTSNSQKVIFAAW 300
                                **************************************************

gi|829625095|gb|AKL80897.1|     DAGGNDTFDFSGYTANQRINLNEKWFSDVGGLKGNVSIAAGVTIENAIGG 350
gi|32172419|sp|P07268.2|PRZN_S  DAGGNDTFDFSGYTANQRINLNEKSFSDVGGLKGNVSIAAGVTIENAIGG 350
                                ********************** ************************

gi|829625095|gb|AKL80897.1|     SGNDVIVGNAANNVLKGGAGNDVLFGGGGADELWGGAGKDIFVFSAASDS 400
gi|32172419|sp|P07268.2|PRZN_S  SGNDVIVGNAANNVLKGGAGNDVLFGGGGADELWGGAGKDIFVFSAASDS 400
                                **************************************************

gi|829625095|gb|AKL80897.1|     APGASDWIRDFQKGIDKIDLSFFNKEAQSSDFIHFVDHFSGTAGEALLSY 450
gi|32172419|sp|P07268.2|PRZN_S  APGASDWIRDFQKGIDKIDLSFFNKEAQSSDFIHFVDHFSGAAGEALLSY 450
                                ****************************************:*********

gi|829625095|gb|AKL80897.1|     NASSNVTDLSVNIGGHQAPDFLVKIVGQVDVATDFIV 487
gi|32172419|sp|P07268.2|PRZN_S  NASNNVTDLSVNIGGHQAPDFLVKIVGQVDVATDFIV 487
                                ***.*********************************
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Fig. 1: Multiple sequence alignment of serratiapeptidase enzymes sequence retrieved from NCBI from *Serratia marcescens* and *Serratia sp. E-15.* The multiple sequence alignment resulted identical amino acid in both the organism

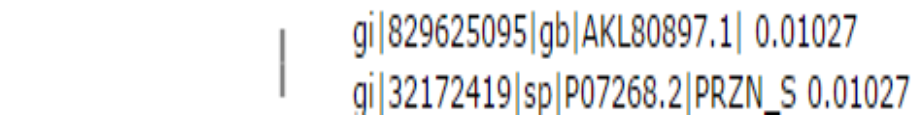

Fig. 2: A claudogram of serratiapeptidase among information available in database. The serratiapeptidase claudogram suggest both the enzyme are identical and does not possess any variation in molecular evolution.

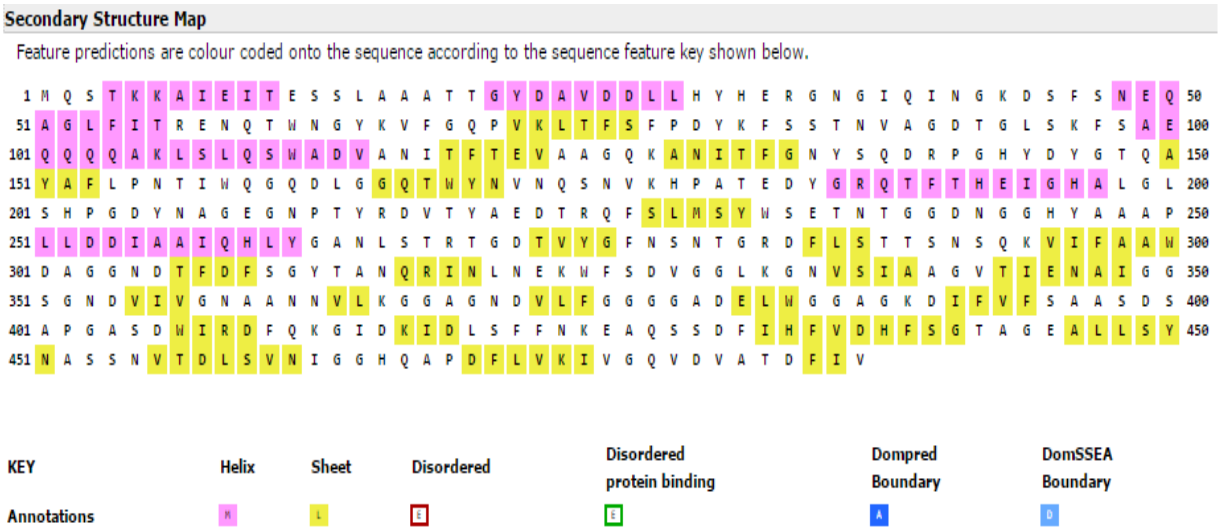

Fig. 3: The presence of various secondary structures forms in serratiapeptidase. The enzyme is largely composed of helix and sheets responsible for activity and stability.

Table 3
The details of physiochemical properties of serratiapeptidase and predicted properties. The enzyme share many properties of typical trypsin like protein in activity and stability.

Organism	Physiochemical Properties	Values
Serratia marcescens **AKL80897 Amino Acid 487**	Molecular weight	52275.8 Da
	Isolectric point (*pI)*	4.6
	Total Negative Charge Residues	55
	Total Negative Charge Residues	29
	Total No of atom	7161
	Carbon	2318
	Hydrogen	3457
	Nitrogen	631
	Oxygen	753
	Sulphur	2
	Ext. coefficient	77810
	instability index (II)	25.23
	GRAVY	-0.392

Docking studies: The validated three-dimensional structure of serratiopeptidase was docked with cyclooxygenase I, cyclooxygenase II and lipoxygenase. The binding energy of all three clusters was calculated to ensure the perfect binding of serratiopeptidase and its target. The serratiopeptidase had shown tremendous affinity for cyclooxygenase (much higher to COX II) and least for lipoxygenase. These docking results are shown in figure and binding energy of docked cluster is given in table 4[32].

Molecular cloning and expression of serratiopeptidase: Serratiopeptidase gene encoding an active protein for anti-inflammatory activity is 1460 bp and gene encodes a 487 amino acid protein. The total genomic DNA was isolated from both Serratia species used in present work (fig. 6a).

The gene-specific primers containing restriction sites successfully amplified the serratiopeptidase gene (fig. 6b). The gene was successfully loaded on expression vector system pET28a and transformed into *E coli BL 21 (DE3*) (fig. 6c). At zero time we reported minimal expression of protein; however, IPTG induction (5mM) resulted in high expression profile (fig. 6d).

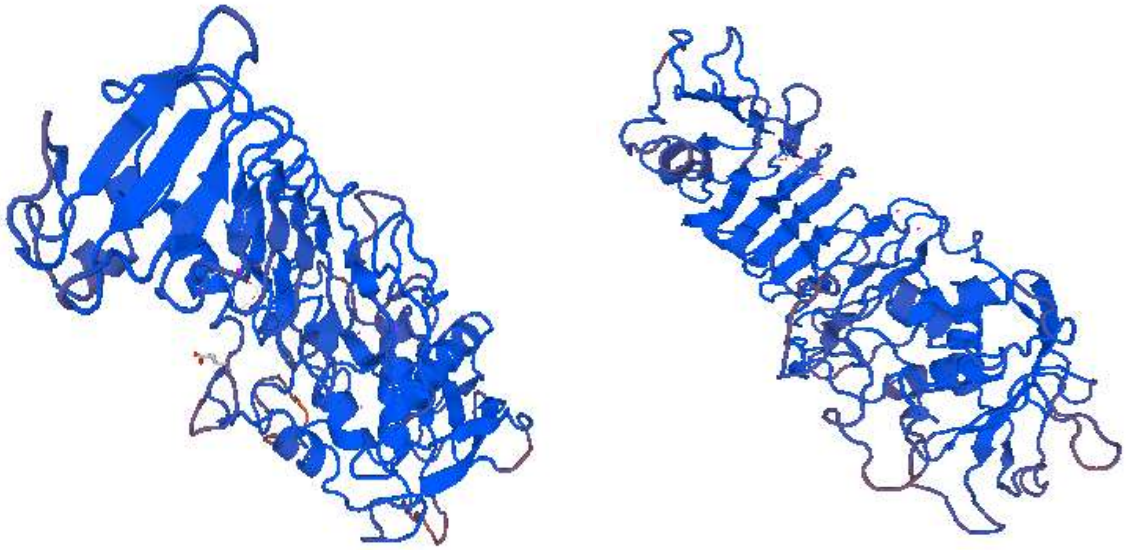

Fig. 4: The modelled serratiapeptidase three dimensional structures using Swiss Model Station. The first model is from *Serratia marcescens* while second one *Serratia sp. E-15* under similar modelling conditions.

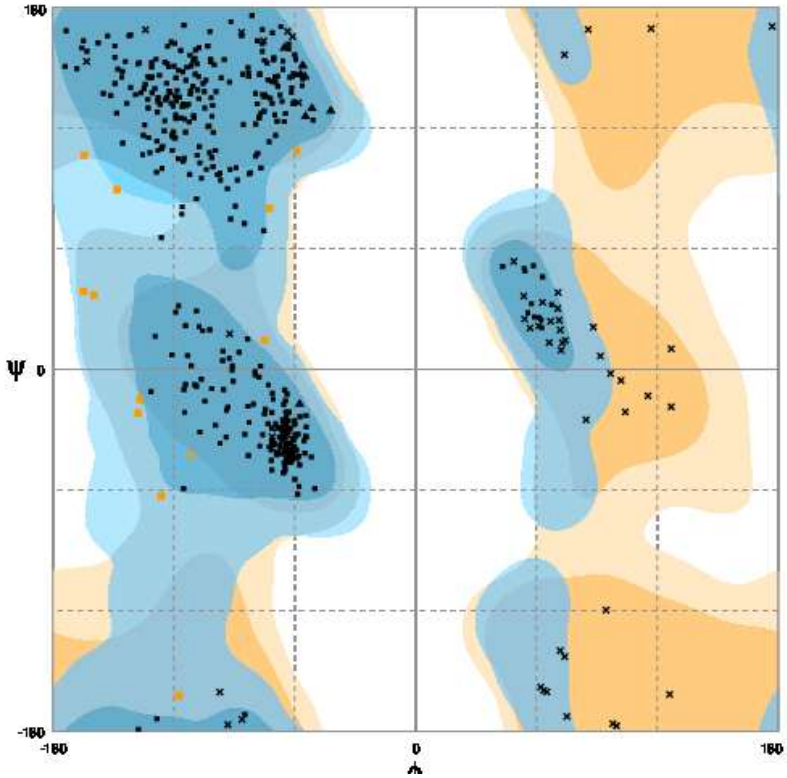

Fig. 5: Validation of modelled serratiapeptidase structure using Ramachandran plot analysis. The enzyme passed validation and scored 98% amino acid in favoured region

The expressed protein was purified using His Tag affinity antibodies via immunoprecipitation. The expressed protein was evaluated for its protease activity by plate diffusion assay using casein as substrate. The SDS-PAGE analysis showed level of expression in time-dependent manner.

Anti Inflammatory activity Analysis: For anti-inflammatory activity analysis here, we used different concentrations of expressed anti-inflammatory protein as test and diclofenac sodium as positive control. Human red blood cell membrane hemolysis was reported as function of hemoglobin released from the membrane. The hyosaline buffers were used in pre-treatment of human red blood cells before examination of human red blood cell membrane hemolysis. We reported here human red blood cell membrane showing a high level of resistance at initial concentration of serratiopeptidase (up to 2.5 mg/ml).

However, at higher concentration of a significantly higher percentage of human red blood cell membrane lysis was

reported (up to 70.50%) with expressed serratiopeptidase and up to 78.0% with reference anti-inflammatory drug Diclofenac Sodium (fig. 7 and table 5). The finding based on in vitro studies confirms a potent anti-inflammatory activity of serratiopeptidase enzyme[33].

Discussion

There is minimal information about serratiopeptidase available at the NCBI database. Only two protein sequences are available retrieved in FASTA format. The sequence alignment and phylogenetic tree analysis suggested that both protein sequences are identical with 100% similarity in amino acid.

Table 4
The affinity of Serratiopeptidase with cyclooxigenase (COX) and lipoxygenase (LOX). The affinity was measured as binding energy for each docked cluster.

Docked cluster	Binding Energy (kcal/mol)
Serratiopeptidase with COX I	-458.36
Serratiopeptidase with COX II	-486.24
Serratiopeptidase with LOX	-462.24

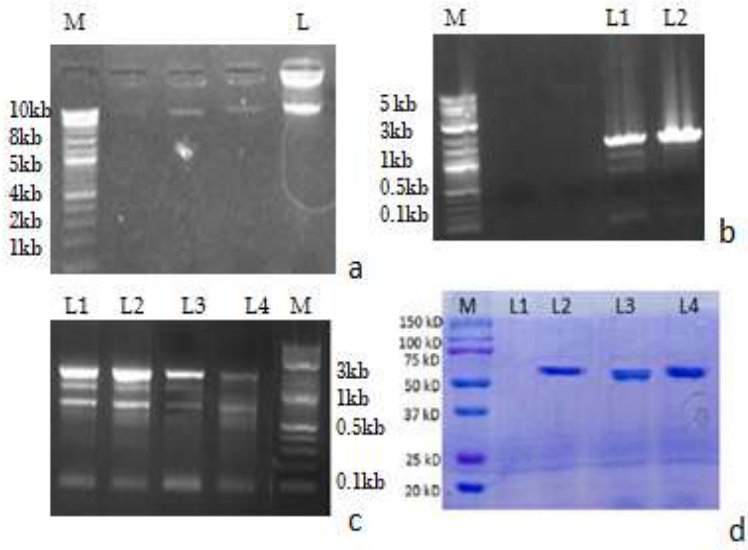

Fig. 6: The molecular cloning and expression studies of serratiapeptidase enzyme; (a) genomic DNA isolation, (b) amplification of serratiapeptidase gene with specific primers via polymerase chain reaction; (c) confirmation of cloned serratiapeptidase gene and (d) SDSPAGE analysis of expressed serratiapeptidase protein.

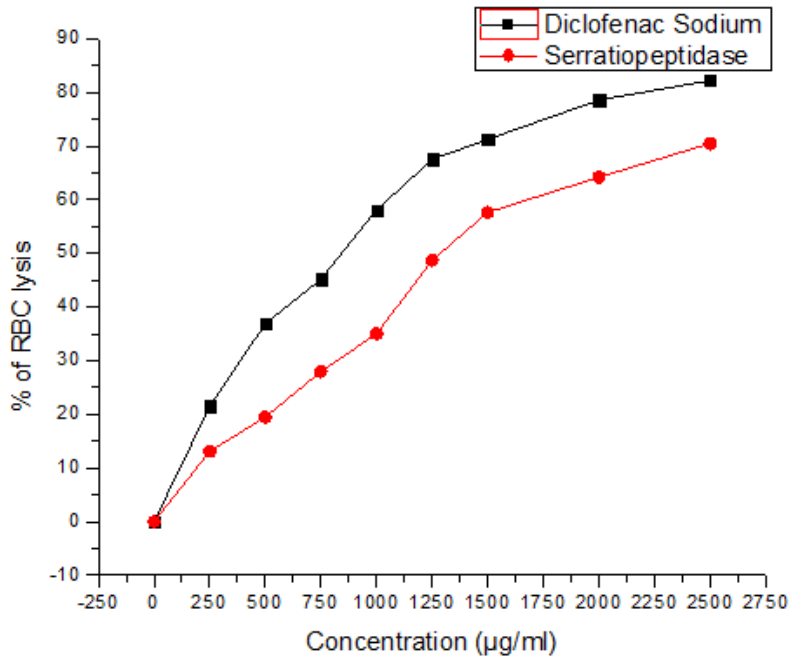

Fig. 7: A comparative study of anti inflammatory activity of expressed serratiapeptidase protein with diclofenac sodium as positive control.

Table 5
Evaluation of anti inflammatory activity of Serratiopeptidase enzyme with reference drug diclofenac sodium.

Concentration (µg/ml)	% of RBC lysis	
	Diclofenac Sodium	Serratiopeptidase
0	0	0
250	21.54	13.12
500	36.88	19.5
750	45.2	28
1000	58.1	35.05
1250	67.55	48.71
1500	71.25	57.63
2000	78.6	64.2
2500	82.2	70.5

Figure 2 which a cladogram of both sequences fails to provide any substantial evidence toward protein evolution and its close relation in organic evolution. The serratiopeptidase is primarily composed of α helix and β sheets. There is very less percentage of other forms of secondary structures. The presence of helix and sheet defines enzyme stability and its broad substrate affinity. The pattern of both the type of secondary structure also illustrates the close relativeness of enzyme with trypsin family.

The protein possesses eight different motifs located widely. Among all these motifs, two motifs Peptidase_M10_C and Peptidase_M10 are necessary for enzymatic activity based on E value interpretation. The physicochemical properties of enzymes illustrate that enzymes are critical members of trypsin family based on isoelectric point (*pI*) and arrangement of amino acids. The average molecular weight of protein nearly was 52.025 kDa related to other anti-inflammatory proteases reported earlier.

Conclusion

The enzyme does not possess many sulphur residues and hence there is lack of sulphide bond in mature structure. However, protein maintains high stability based on the instability index value. The total negative charge on protein provides another clue for its security, further supported by *pI* values of 4.6. The modeled three-dimensional structure of serratiopeptidase was valid to suggest enzyme capacity in aligning week interaction for its catalytic triad. The modeled structure was docked with COXI, COX-II and LOX and result obtained from study suggest that enzyme had maximum affinity for CoX II which is ideal for an anti-inflammatory drug molecule.

The stability of docked cluster ensured by binding energy and least energy was reported in case of serratiopeptidase and COX-II. *In silico* studies suggest enzyme does possess strong anti-inflammatory activity and is potential to become ideal drug for the management of inflammation. LD_{50} and SD_{50} analysis are essential for making enzyme approval for clinical application.

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