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MOLECULAR DOCKING OF SALIVARY CYSTATIN-D AND CATHEPSIN-S TO STUDY BINDING INTERACTION

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ABSTRACT

Cystatin D is a type II family proteinase inhibitor which is known to be playing a very vital role in the salivary system and ensuring oral health. Cystatin D is known to inhibit different types of proteinases among which cathepsin S is reported to be highly preferred. Orthologs and paralogs of cystatin D were found to understand their existence in other species and its isoforms. Using protein – protein molecular docking the binding affinity of cystatin D with cathepsin S was studied.

Keywords: Molecular Docking, Salivary Cystatin-D, Cathepsin-S

INTRODUCTION

The cystatin superfamily comprises a group of proteinase inhibitors which are widely distributed in human tissues and body fluids, and which form tight and reversible complexes with cysteine proteinases such as cathepsins B, H, L, and *S.* The cystatins are likely involved in the regulation of all normal or pathological processes in which these proteinases participate. Thus, cystatins may influence the intra- and extracellular catabolism of proteins and peptides **[1]**, regulate proteolytic processing of prohormones **[2, 3]** and proenzymes **[4]**, protect against penetration of normal tissues by malignant cells **[5]** or microorganisms **[6]** and modulate local inflammatory processes in rheumatoid arthritis **[7]** and purulent bronchiectasis **[8]**.

Salivary secretion

Saliva has manifold functions in maintaining the integrity of the oral tissues, in protecting teeth from caries, in the tasting and ingestion of food, in speech and in the tolerance of tenures, for example. Salivary secretion occurs in response to stimulation by neurotransmitters released from autonomic nerve endings. There are two secretory pathways: protein exocytosis and fluid secretion. Sympathetic stimulation leads to the activation of adenylate cyclase and accumulation of intracellular cAMP. The elevation of cAMP causes the secretion of proteins such as amylase and mucin. In contrast, parasympathetic stimulation activates phospholipase C and causes the elevation of intracellular Ca2+, which leads to fluid secretion; that is, water and ion transport. Ca2+ also induces amylase secretion, but the amount is smaller than that induced by cAMP. (KEGG source record: hsa04970)

The aim of this work is to provide readers with an overview of the benefits of salivary proteins along with up-to-date proteomic

approaches used for the identification and assessment of potential risk factors connected to dental caries.

MATERIALS AND METHODS

Data Retrieval and Pathway study

Cystatins are a diverse class of enzymes which play a very vital role in oral health. In order to understand cystatin and its pathway we downloaded human salivary pathway from the Kegg Encycloedia of Genes and Genomes (Reference). This schematic pathway gave us further insights into the roles of different salivary proteins. Further, using the keyword cystatin we tried to identify different paralogs of human cystatins. This was done using Kegg Orthology database (Reference). A phylognetic tree was also constructed using the data obtained from KEGG KO database.

Uniprot Database

Paralogs identified from the Kegg Orthology database helped us to dowload the protein sequences from the Uniprot database. Twelve paralogs seqeunces of human cystatin D were taken up for further study. Also, orthologs of cystatin D were also found from the NCBI database (reference). This was done to study the existence of this protein in other species other then homo sapiens.

Structural Data

Human cystatin D and Cathepsin S protein structrues were available in the PDB database holding a PDB IDs 1RN7 and 5QCD respectively. These structures were dowloaded in the pdb file format and stored onto the local computer for further structrual studies (Reference for PDB).

Protein – Protein Interaction HEX

Hex is a protein and DNA pairs docking software. It is a molecular graphics package that can be used to for docking studies that is performed by superimposing using knowledge of 3D shapes (Reference for HEX). Default parameters of Hex were used to implement protein-protein docking. Correlation between the proteins was based on shape and electrostatic criteria. Grid dimension (0.6), receptor and ligand range was set to 180 each with step size of 7.5. Best solution was chosen among 2000 different iterations restricted too distance range 40. Other parameters are shown in the **Figure 2**. Also, the best binding interaction mode between the two proteins was chosen with due importance to the energy of the complex.

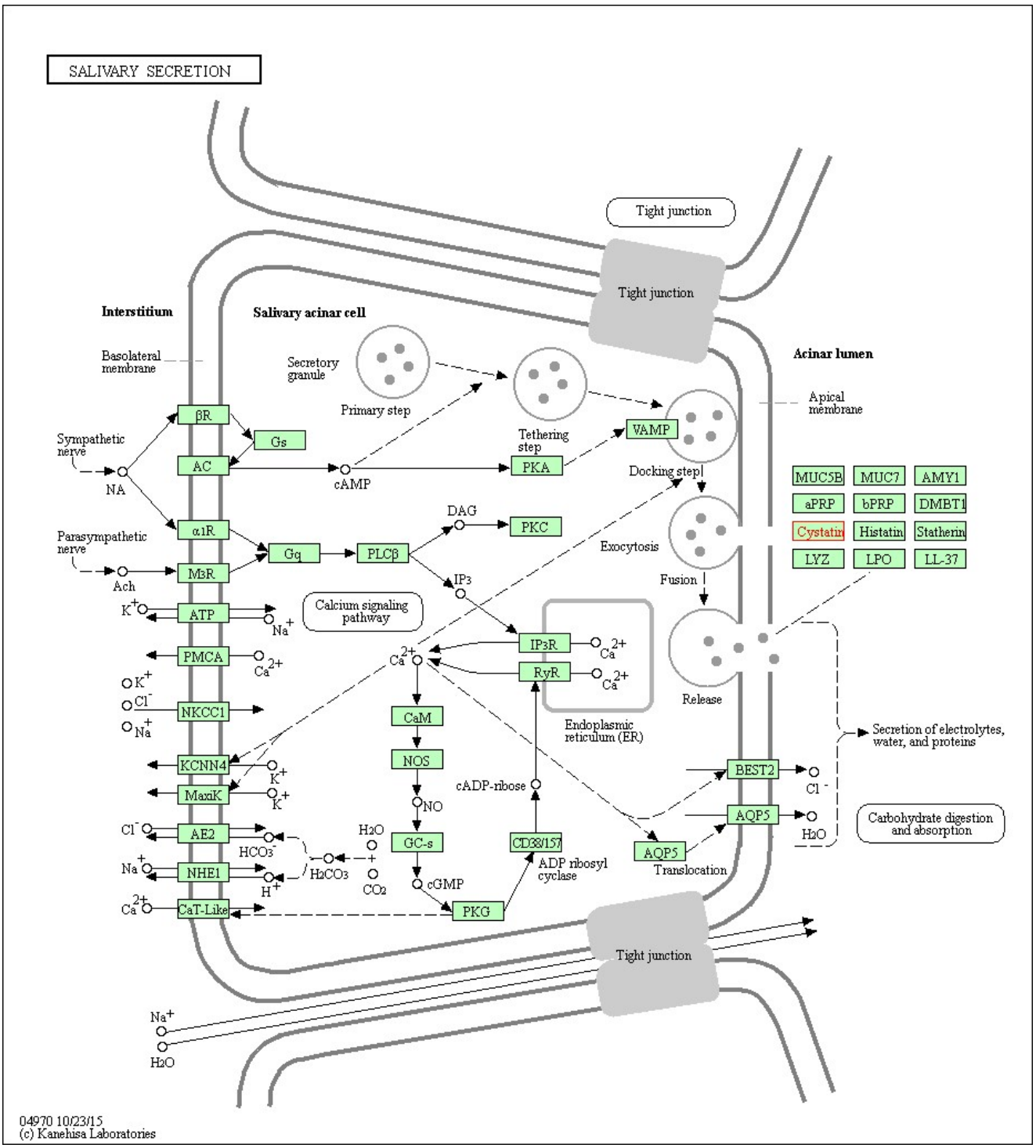


Figure 1: Homo sapiens salivary pathway and role of several salivary proteins

Tax_id	Org_name	GeneID
9606	Homo sapiens	1473
9544	Macaca mulatta	704408
9565	Theropithecus gelada	112633442
259920	Rhincodon typus	109910571
1737458	Cebus capucinus imitator	108313148
9598	Pan troglodytes	107969900
9531	Cercocebus atys	105581972
9568	Mandrillus leucophaeus	105530812
336983	Colobus angolensis palliatus	105519015
61622	Rhinopithecus roxellana	104672214
60711	Chlorocebus sabaeus	103215512
9541	Macaca fascicularis	102146503
9593	Gorilla gorilla	101152045
27679	Saimiri boliviensis	101033770
9555	Papio Anubis	101024005
9597	Pan paniscus	100992748
61853	Nomascus leucogenys	100601918
9601	Pongo abelii	100445936
9483	Callithrix jacchus	100404749

Entry	KO	len	SW-score	(margin)	bits	identity
hsa:1470 cystatin SA	K13898	141	555	(-)	132	0.560
hsa:1472 cystatin S	K13900	141	545	(-)	130	0.553
hsa:1469 cystatin SN	K13897	141	529	(-)	126	0.553
hsa:1471 cystatin C	K13899	146	488	(-)	117	0.510
hsa:1474 cystatin E/M	K13902	149	210	(-)	54	0.268
hsa:10047 cystatin 8	K13904	142	205	(-)	53	0.289
hsa:140880 cystatin 11	K13906	138	202	(-)	52	0.281
hsa:128817 cystatin like 1		145	201	(-)	52	0.292
hsa:8530 cystatin F	K13903	145	201	(-)	52	0.303
hsa:128821 cystatin 9 like	K13905	147	181	(-)	47	0.290

Figure 2: Paralogs of CystatinD in homo sapiens

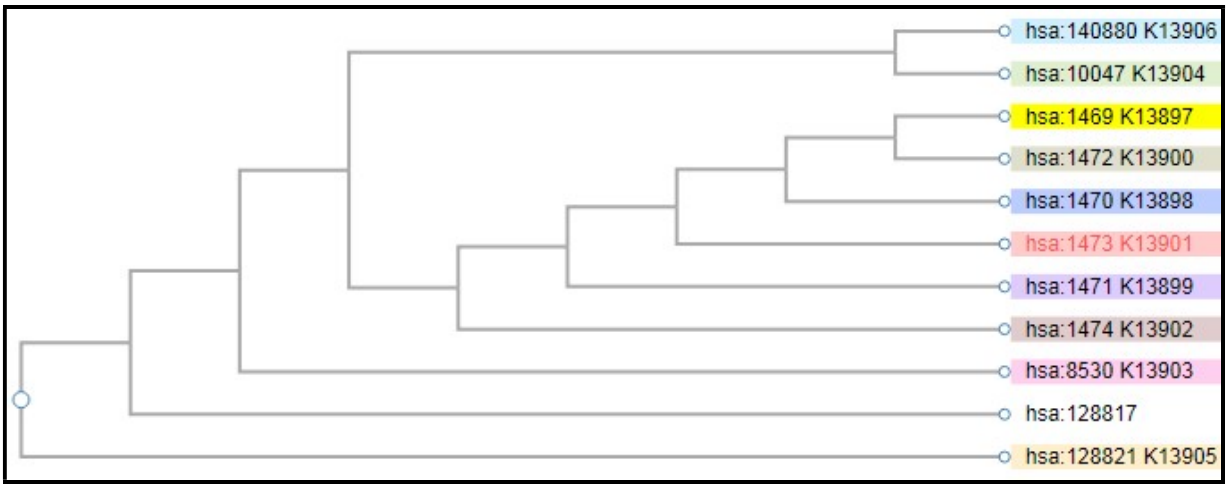


Figure 3: Relationship of homo sapiens cystatin family paralogs

Figure 4: Structure of CystatinD (PDBID:1ROA_A & Swissprot ID: P28325)

Figure 5: Cartoon model orientations of Cystatin-D (left) and Cathepsin-S (right) prior to protein-protein docking

RESULTS AND DISCUSSION

The idea of this study was to understand the interaction between salivary proteins cystatin-D and cathepsin-S using insilico approaches. Cystatin protein family consists of multiple cystatin like sequences few of which include active inhibitors of proteases. Cystatins constitute a single evolutionary protein superfamily of which type I consists of 100 amino acid residues and also may lack disulfide bridges. CST5 a class of type II family comprise of 120 amino acids and do have 2 intrachain disulfide bonds. Cystatin-D is a cystein proteinase inhibitor playing a protective role against proteinases present in the oral cavity. They do have a preference for inhibition in the order in which cathepsin S over cathepsin H and L and cathepsin B

being relatively less preferred among the lot. This type of cystatin consists of 142 amino acids.

In our study the salivary pathway from the kegg database gave us insights into the interaction between other molecules in the saliva. From the kegg orthology database we identified both paralogous and orthologous sequences. With respect to paralogs we were able to identify 10 different members of cystatins with which cystatin D had the pairwise sequence identity score ranging from 0.560 to 0.290 for cystatin SA and cystatin 9 like proteins respectively. Smith wateerman based local alignment score was in the range of 555 yo 181 as well. Further the orthologs of human cystatin D were also identified. As it can be seen from the table totally 19 cystatin D orthologs were identfied from so many species. A phylogenetic tree constructed as shown in figure using these paralogous sequences resulted in two major bigger clades in which one had cystatin 11 and cystatin 8 and the other had cystatin SN, cystatin S, cystatin SA, cystatin C, cystatin E/M and cystatin D was part of this clade as shown in the figure in pink colour.

The 3D structure of cystatin D consisted of only one helix and there were plenty of beta sheets where as it was not the same in case of cathepsin S where we can see as many as five helices and again many beta sheets. Using the Hex software to the find the binging affinity between these two proteins, we can see from the figure that these two were able to interact with each other mostly with the help of electrostatic interactions. As it can also be seen from the figure the point of interaction between these two proteins was the sheet dominated loop region. Total energy of these interaction was -698.23 kj/moles. Therefore, from this study we have studied the interaction and binding affinity of cystatin D with cathepsin S. This study can further be extended to study the importance of residues in facilitating the binding of these two proteins using molecular dynamics approaches. As we had already listed above that there are only two disulfide bridges in the cystatin D and hence residue based simulation studies will be a good option.

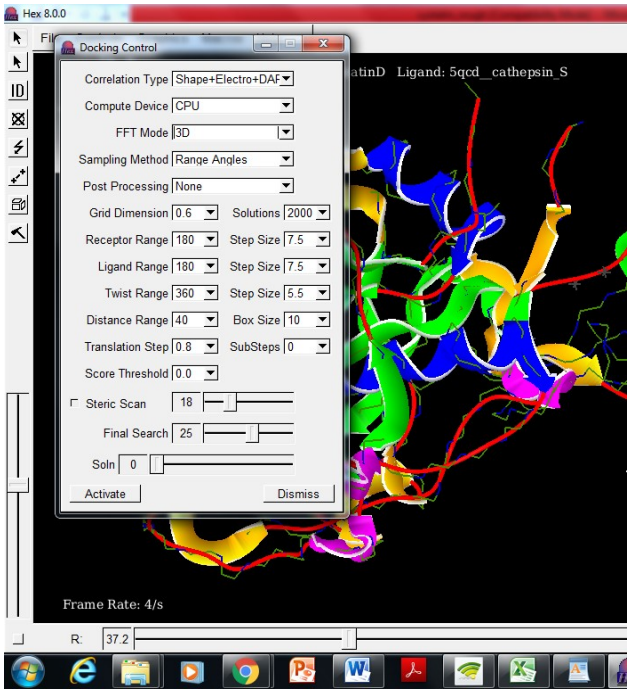
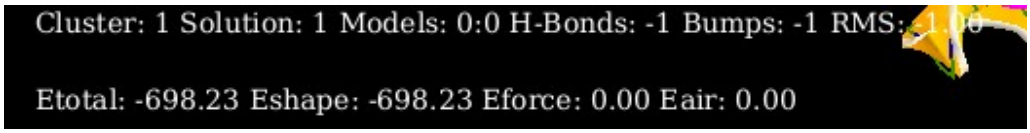


Figure 6: Algorithmic parameters set for docking (default)



DISCUSSION

The cystatin superfamily has been subdivided into families I, 11, and I11 (also called the stefin, cystatin, and kininogen families, respectively), each with members differing from those of the other families in structural organization and biological distribution. The family I cystatins **A** and B are small proteins consisting of single polypeptide chains of about 100 amino acids residues without disulfide bridges. The family I1 cystatins consist of polypeptide chains of approximately 120 amino acid residues with two intrachain disulfide bonds. Finally, the family I11 cystatins, the kininogens, display a higher degree of structural complexity characterized by the presence of three family I1 cystatin-like domains, each with two disulfide bridges at positions homologous to those in family I1 cystatins **[9]**. Family I and I1 cystatins are mainly present intracellularly and in secretory fluids whereas kininogens are highly concentrated in blood plasma. The cystatin superfamily encompasses proteins that contain multiple cystatin-like sequences. Some of the members are active cysteine protease inhibitors, while others have lost or perhaps never acquired this inhibitory

activity. There are three inhibitory families in the superfamily, including the type 1 cystatins (stefins), type 2 cystatins and the kininogens. The type 2 cystatin proteins are a class of cysteine proteinase inhibitors found in a variety of human fluids and secretions. The cystatin locus on chromosome 20 contains the majority of the type 2 cystatin genes and pseudogenes. This gene is located in the cystatin locus and encodes a protein found in saliva and tears. The encoded protein may play a protective role against proteinases present in the oral cavity. The cystatin superfamily encompasses proteins that contain multiple cystatin-like sequences. Some of the members are active cysteine protease inhibitors, while others have lost or perhaps never acquired this inhibitory activity. Cystatin SN and cystatin SA are also members of family II; they have been isolated from saliva and show about 90% identical residues in pairwise comparisons. These are the so-called secretory gland cystatins, which share about 50% identical residues with cystatin C. Furthermore, salivary cystatins contribute with statherin to the regulation of calcium and phosphorous metabolism. The preservation of the supersaturation of calcium phosphate mineral in saliva and plaque fluid is a condition for preventing the formation of enamel lesions or the progression of dental caries.

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