PROPERTIES OF HISTATIN 5

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ABSTRACT

The structure of histatin 5 can be found in the first 24-amino acids of histatin 3 from the N-terminal, indicating that histatin 5 is derived from histatin 3. Histatin 5 is a short 24-amino acid long peptide chain that is known to exhibit antimicrobial abilities. Recent studies had shown that enolase is able to bind to histatin. Therefore, we intend to bind histatin 5 to enolase to study the binding properties of histatin 5. We chose to bind the enolase from *S. cerevisiae* to histatin as histatin is secreted into the oral cavity. A 3-dimensional structure of histatin 5 was modelled based on the amino acid sequence of histatin 5 (5). Histatin 5 was docked into the putative binding site of the enolase.

The docking results show that histatin 5 binds to all the predicted ligand binding sites that were highlighted in the predicted active site. Histatin 5's ability to cover all the ligand binding sites which occurred in the active site show that histatin is an effective inhibitor. A BLAST on histatin 3 returned proteins which had similar amino-acid sequences to histatin 3. Results suggest that all primates produce histatin. Yet, further experimental confirmation is necessary.

1. INTRODUCTION

Histatins comprise a group of related neutral and basic histidine-rich peptides present in human salivary secretions and in serum (1). Histatin 1 and histatin 3 have been shown to arise from structural genes while the other histatins are derived from posttranslational proteolysis. The structure of histatin 5 can be found in the first 24-amino acids of histatin 3, showing that histatin 5 is derived from histatin 3. Histatins possess antimicrobial activity against strains of *Streptococcus mutans and Porphyromonas. gigivalis,* and inhibit hemagglutination of *P. gingivalis* and co-aggregation between *P. gingivalis* and *Streptococcus mitis.* Histatins have also shown inhibitory effects on *Candida albicans*, an oral opportunistic pathogen (1).

Histatin 5 is the most potent form of histatins, with respect to its fungicidal activity against *Candida albicans* (2). Its fungicidal mechanism is not one of classical pore-forming but works by binding with a yeast cell envelope protein, followed by intracellular translocation and efflux of ions (3). It is speculated that the only condition in which histatin 5 exhibits its antimicrobial abilities is with saliva as the medium. Its unusual inhibition of the growth of *Candida albicans* suggests that membrane damage or a change in membrane permeability of the target microbes may be the cause of the growth inhibition (1). Zinc and copper ions are known to bind to histatin. Furthermore, zinc has healing properties of its own, suggesting metal binding might improve antimicrobial activities of histatin 5 (12). Whether other metal ions can bind to histatin 5 and the specific metal binding processes are not yet known. Yeast Ssa1/2 proteins have been shown to function as a cell envelope binding receptor for Histatin 5 in mediating

fungicidal activity, suggesting that other proteins might function as a mediator, but do not directly function in the antimicrobial action of histatin 5.

Histatin 5 is a short 24-amino acid long peptide chain. Its length limits the number and type of interactions with other substances. Histatin 5 is also known to exhibit therapeutic abilities, making its study of protein-protein interactions all the more essential in understanding its mechanism for other medical applications.

We intend to bind histatin 5 to enolase to study the binding properties of histatin 5. Enolase is a metalloenzyme that catalyses the dehydration of 2-Phospho-D-Glycerate Hydrolase (PGA) to phosphoenolpyruvate (PEP) and the reverse reaction (anabolic pathway) (16). Enolase can be found in *Candida krusei, Candida albicans, Saccharomyces cerevisiae* and humans (4). Enolase is known to cause cancer, systemic fungal diseases, dental diseases and autoimmune disorders

This study aims to find out putative protein binders of histatin 5 and how histatin 5 binds to the proteins with the aid of bioinformatic tools and biomolecular modeling methods. We are also interested in finding out under what circumstances histatin 5 would be secreted and in which organisms would it be secreted. Recently, research has shown that enolase is able to bind to histatin. Therefore, we would like to further investigate that enolase is able to bind to histatin and study how the two proteins bind.

2. MATERIALS AND METHODS

Molecular Modelling. The PDB file of *S. cerevisiae* enolase was downloaded from the RCSB Protein Data bank (PDBID: 2ONE) and uploaded onto biomolecular modelling programme Sybyl 8.0. We chose to bind the enolase from *S. cerevisiae* to histatin as S. cerevisiae is found in the oral cavity. Firstly, 7 possible ligand binding sites (what are these 7 sites) were highlighted. Next, Ser36, Gly37, Ala38, Ser39, Thr40, Ser293, Cys247, Asp246, Asp210 (4, 10), constituents of the predicted active site of enolase, were highlighted as well.

In Sybyl 8.0, a 3-dimensional structure of histatin 5 (Fig 3) was modelled using the based on the amino acid sequence of histatin 5 (5). The modelled histatin 5 was engetically minimised by steep-descent method.The molecular surface of histatin 5 had been dotted and the transparency of the histatin5 structure had been increased to 62%.

An entry had also been submitted to PDBSUM for analysis. A modelling showing histatin 5's predicted clefts had been produced by PDBSUM and operated through Jmol.

Molecular Docking. The software utilized for docking was GOLD 3.1. Histatin 5 was docked into the putative binding site of the enolase. The binding site was defined based on a 20 angstrom radius from the amino acid residue Ala38. Histatin 5 was docked to generate 10 conformations. Following docking process, all the conformations were visually inspected for selection of the best-fit binding pose of the histatin 5-enolase complex.

Scanning for Protein Domains. A search on histatin 5 was done through the Interpro Scan database. Under the NCBI database, a search on histatin 3, which is homologous to histatin 5, was carried out in the Entrez Gene database. Using the data available on the database, the relative distances of the genes regulating the proteins which have been proven to bind to histatin 3 were noted.

FASTA sequence of Histatin 3 was entered into BLAST, returning sequences of which showed a high degree of homology to histatin 3.

3. RESULTS

3.1 Molecular Modelling

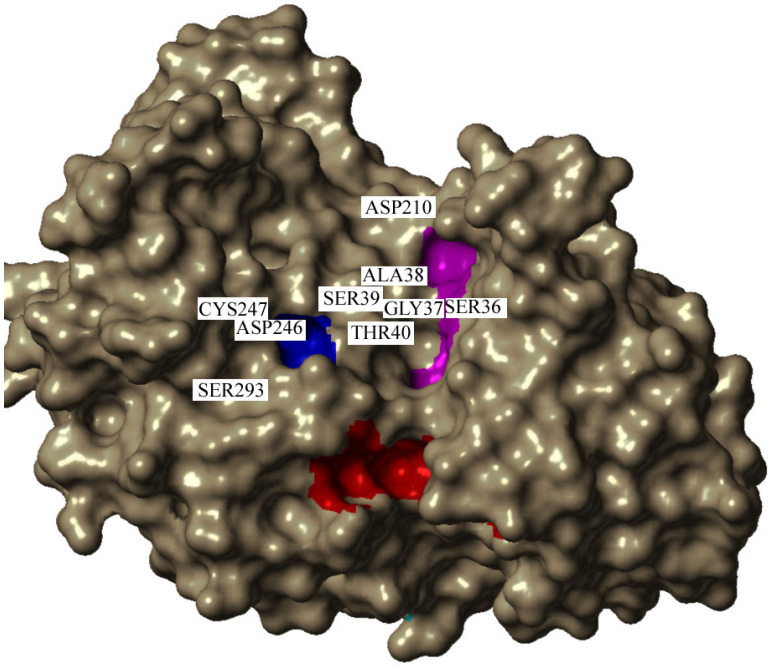

Figure 1. Molecular Structure of *S. cerevisiae* enolase with 8 amino acids and 3 possible ligand binding sites highlighted.

Figure 2. Molecular structure of *histatin 5* at active site, modelled by Jmol under PDBSUM. Clefts of the protein are highlighted in colour.

The modelling of histatin 5's predicted clefts shows 4 possible binding sites, a promising sign to histatin's ability to bind to other proteins

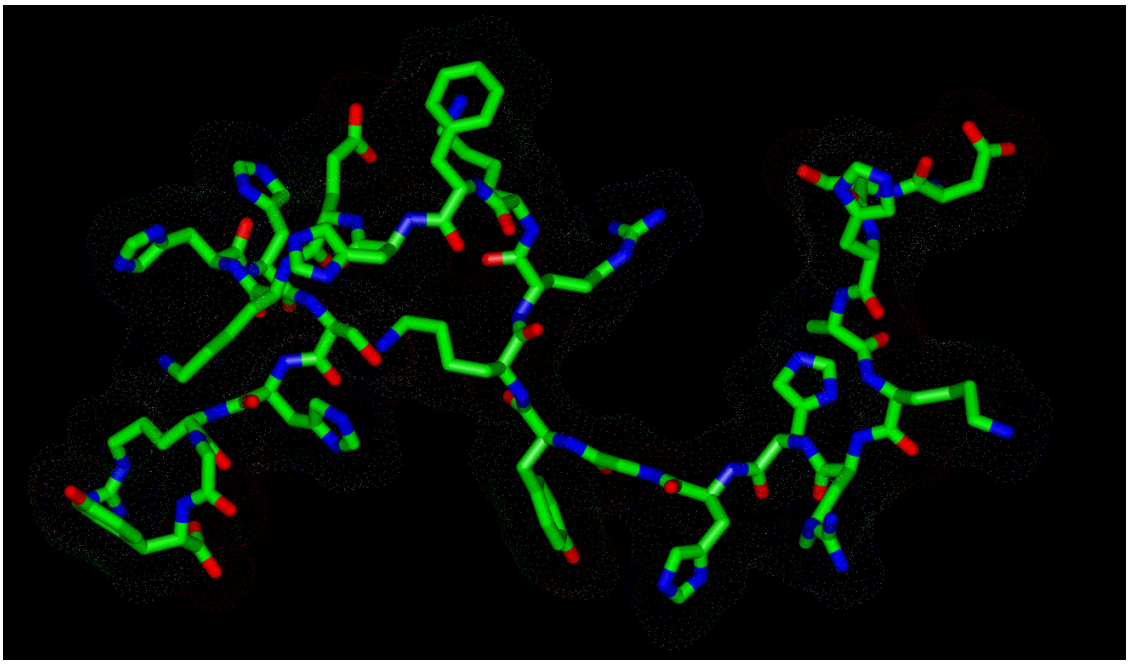

Figure 3. Molecular structure of histatin 5 with dotted molecular surface.

3.2 Docking

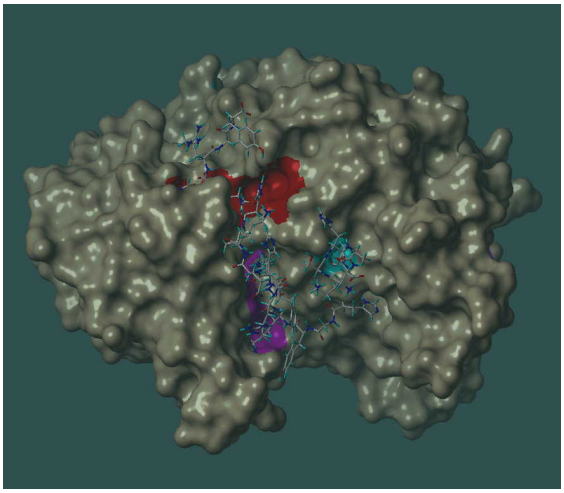

Figure 4. Enolase-Histatin 5 complex (structure of histatin 5 can be clearly seen).

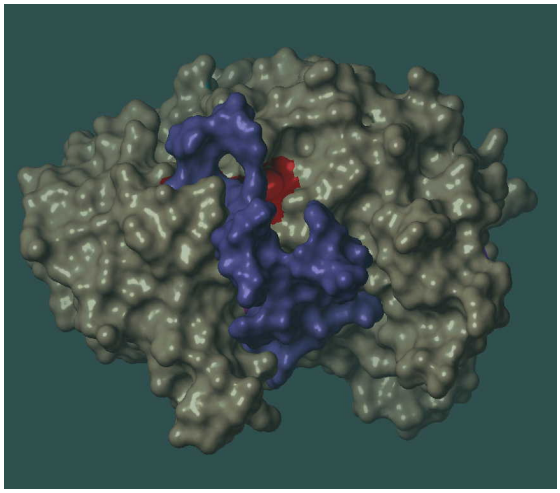

Figure 5. Enolase-Histatin 5 complex, molecular surface version.

Histatin 5 fully covers the blue-highlighted ligand binding sites and covers half of the red-highlighted ligand binding sites and almost fully covers the pink ligand binding sites of enolase.

3.3 Domain Scan

A search of Histatin 3 on the NCBI database shows that the gene HTN3 is located on chromosome 4 (4p13). HTN1 (4q13), STATH (4q11-q13), CSN (4q21.1/ 4q13.3) and its variations and MUC7 (4q13-q21), genes which are involved in the production of histatin 1 and 2, statherin, casein and mucin 7 respectively, are also found to be located on the same chromosome. In total, 5 variations of CSN are located before and after HTN3. STATH is located one gene before HTN3, while MUC7 is located 12 genes after HTN3.

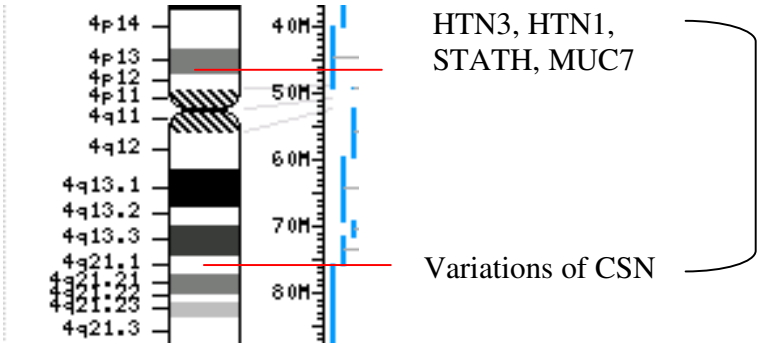

Figure 6. A segment of chromosome 4, where genes of common salivary peptides are found to be located.

3.4 Sequence alignment for histatin 3

Protein	Organism		E-values
	Scientific name	Common name	
Histatin 3	*Gorilla gorilla*	Common gorilla	2e-12
Histatin 3	*Nomascus Leucogenys*	White-cheeked gibbon	1e-10
Histatin 1	*Gorilla gorilla*	Common gorilla	2e-10
Histatin 3	*Macaca fascicularis*	Crab-eating macaque	5e-08
Histatin 3	*Presbytis cristata*	Silvered leaf monkey	9e-06
Histatin 1	*Presbytis cristata*	Silvered leaf monkey	0.001
Histatin 1	*Macaca fascicularis*	Crab-eating macaque	2e-05
Histatin 3	*Chlorocebus aethiops*	Grivet (green monkey)	2e-09
Histatin 1	*Chlorocebus aethiops*	Grivet (green monkey)	0.006

Figure 7. Table for sequence alignment of histatin 3. Data retrieved from NCBI BLAST database.

Histatin 1 and 3 of the gorilla, white-cheeked gibbon, crab-eating macaque, silvered leaf monkey, as well as the grivet are shown to have similar protein sequences as that of histatin 3 of homo sapiens. All of the aforementioned species are primates.

4. DISCUSSION

The possible ligand binding sites that were highlighted in Figure 1 were in the same region as the 8 amino acids, which were constituents of the proposed active site of enolase. This supports our hypothesis that histatin is likely to bind at the proposed active site. The docking results show that histatin 5 binds to all the predicted ligand binding sites that were highlighted in the predicted active site. Histatin 5's ability to cover all the ligand binding sites which occurred in the active site show that histatin is an effective inhibitor. Its small 24-amino acid structure also ensures more flexibility and movement of the peptide, thereby aiding in its enzyme-inhibitory function. The successful binding of histatin and enolase confirms the findings by Heidelberger (2003) (17).

It has been proven that the selective binding that takes place between MUC5B and histatins are where the active site of MUC5B is enriched with cysteine and contained both hydrophobic and charged residues (11). However, the amino-acid sequence of enolase contains only one cysteine residue, suggesting that, while it is possible that the presence of cysteine in the target protein, for example, enolase, could help with the binding mechanism of histatin, it might not be the only possible binding mechanism histatin could possess. Yet, the binding between enolase and histatin as demonstrated in the docking results shows that Cys247 (Figure 1) is located near the fully-covered blue-highlighted ligand binding site. Still, further experimental confirmation is necessary.

A previous study by Inotcheva (2000) (11) has shown that mucin 7, caesin and statherin, all of which are salivary peptides, can bind to histatins. Their gene location on the same chromosome within very close vicinity (Figure 6) suggests that histatin 5 might have secretion mechanisms similar to that of mucin 7, casein and statherin, given that genes located within the same area are harder to be segregated and likelier to be secreted under similar circumstances. Therefore, studies on the aforementioned salivary peptides might

eventually be viable leads to either support or discover new secretion mechanisms of histatins.

Given that mammals such as dogs and cats are known to lick their bodies, it seems plausible theorized that histatins might be found in most mammals. A BLAST on histatin 3 returned proteins which had similar amino-acid sequences to histatin 3. Surprisingly, all of the similar proteins which were reviewed by NCBI were found in primates. Primates have been shown to have histatin in their mRNA in the salivary glands (9). This might partly be due to the fact that species under the same order share a high degree of sequence homology. However, histatin was also found in mice mRNA (6) and in rodents' salivary glands (7), both of which belong to the same subfamily, suggesting that the similarity between species under the same order is not a factor in determining the presence of histatin in species. A NCBI search on other classes of the animal kingdom gave no returns, suggesting that, while not all mammals have histatins, the peptide is exclusive to mammals. A recent genome mapping of the red jungle fowl, *Gallus gallus*, also shows that genes encoding salivary-associated proteins, including histatins, are not present in chickens (8). Since birds, like chickens, do not have teeth, their salivary glands are also underdeveloped, as compared to that of humans. The absence of teeth means that the organism is unable to chew food at the mouth, thereby reducing the need for antimicrobial peptides, such as histatin, to be secreted at the mouth (8). This is in comparison to primates and rodents, which have teeth to chew food at the mouth, therefore the need to develop a non-immune defence system at the mouth. Applying this concept to insects would mean insects which adopt chewing and piercing and sucking as modes of feeding would secrete antimicrobial peptides, and possibly histatin. Similarly, reptiles are quite unlikely to secrete salivary antimicrobial peptides, given that their mode of feeding is swallowing their prey wholesale. Therefore, we suggest that all primates would be able to secrete histatins, while certain insects and all birds would not secrete histatins due to their dentition and mode of feeding.

5. EXTENSIONS

We would like to find out if histatin 5 will function as per normal outside of its usual environment, taking into consideration temperature changes, and whether it is in a nonaqueous and aqueous environment. From this, we would be able to tell if histatin would still be able to display its antimicrobial properties outside of the mouth, such as the skin and may be developed into medical skin products.

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