Insilco Homology Structure and Functional Site Prediction Studies on Collagenase Protein in *Fiddler* Crab

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Abstract

Enzymes are used in a variety of industrial processes to create an array of foods, cosmetics and pharmaceuticals. They offer advantages over chemical techniques including substrate specificity and elevated activity that allow better control of the production processes. However, the use of enzymes in industrial applications requires their large scale production. There are a few enzymes that breakdown collagen other than collagenase (cathepsin K and elastase), but collagenase enzymes (serine collagenase and metallocollagenase) are specific enzymes for collagen. They are particularly attractive because they do not require special conditions to break down the substrate. Collagenase enzyme can be isolated from digestive organs of different fish and invertebrates. They are secreted as latent form that can be activated with a member of different material that converts it to the active form. 4-Aminophenylmercuric Acetate (APMA) is the most commonly used but trypsin, Dithiothreitol (DTT) and other activators (potassium or sodium thiocyanate) have been used. On the other hand, Ethylenediaminetetraacetic Acid (EDTA), mercaptoethanol, O-phenanthroline and cysteine have similar mechanisms to inactivate collagenases. Collagenase enzymes are effective at physiological pH (6-8) and a wide range of temperature (20-40°C). Using pattern searching method, motif and domain region of collagenase proteins has been predicted. Studies on gene profiling using a review show the functional part of the target gene.3D structure of collagenase proteins were modeled by using CPH server.

Key words: Collagenase, Motif and domain analysis, Modeling, cph server.

INTRODUCTION

Fiddler crabs are named because of the extreme difference in the size of the claws of the male, with the larger claw resembling a fiddle. Several types of fiddler crabs are common to Rhode Island. All fiddler crabs are similar in shape, having a smooth carapace and a square-shaped body [1]. The eyes are located at the end of two long and slender, movable eyestalks located in the center of the carapace. The state also hosts a similar species called the marsh crab (*Sesarma spp.*). The marsh crab has two small, equal-sized claws and a square back with eyestalks on the outer corners of the carapace. Marsh crabs burrow with fiddler crabs, and although they are herbivores, they sometimes prey on the fiddlers. Fiddler crabs are the little crabs found living in burrows near the water's edge. The entrance to the burrow waving the larger claw in an effort to attract a female. Fiddler crabs are colonial, often living together in large clusters [2]. Territorial fighting occurs between the males, and they will go to extremes to defend their burrows.

The fiddler crab can stay out of the water in damp ground for months at a time. They have gills for breathing in the water, but they also have a primitive lung, which enables them to live on land. Collagenases are useful as reagents in pharmaceutical applications and can also assist in purification and truncation of collagen at a unique site. Collagenase itself has plastic surgery applications, such as enzymatic debridement. For example, particular applications include digesting connective tissues and releasing embedded cells large second claw of the male fiddler crab is known as a secondary sexual characteristic and is used to attract a mate during the breeding season as well as to protect territories. The male crab will stand by without destroying cell membrane and other essential structures [3]. The unique site for collagen is the 3/4, 1/4 site. Collagen is around 1000 amino acids long and is formed by three strands intertwining to form a triple helix. True collagenases cleave collagen at around 750 amino acids to produce a 3/4 and a 1/4 fragment (crab collagenase does this at a site slightly different than mammalian collagenases. Clostridium histolyticum does not provide such cleavage, as it is just a nonspecific protease that degrades collagen, but not specifically).Collagenases are also useful as laundry detergent additives, since protein stains are sensitive to collagenolytic activity.

Collagen is the specific collagenase substrate and is found in the connective tissues of animals, making up approximately 30% of the protein in the human body. It consists of three peptide chains wound in a triple helix structure that offer support to both cells and tissues. These peptide chains are in the sequence of Glycine -X-Y, with X and Y often proline and hydroxyproline and are usually stabilized by hydrogen bonding being in interand intramolecular cross-links. In mammals, some 21 collagen types have been identified while twenty eight distinct collagen types have been identified in the human body [4].

Collagens have been classified based on the expression of different genes during tissue construction [5]. Collagen type I is the most common type that is found in bone, tendon, skin and ligaments, while collagen type III is the second most common and is found in elastic tissues such as blood vessels and various internal organs . The abundance of types V and XI are low but they are found associated with the types I and II in bone and cartilage as well as in other tissues. Very few enzymes are capable of breaking down the complex triple helix structure of collagen. The enzymes that are capable of degrading collagen (including cathepsin and elastase) are known generally as collagenolytic enzymes. Cathepsin K cleaves collagen type I in an acidic medium .Elastase is the most well-studied collagenolytic enzyme and is considered a serine protease enzyme. It is principally responsible for the breakdown of elastin (a highly viscous insoluble protein found in connective tissue). Together with collagenase, they determine the mechanical properties of connective tissue by cleaving particular peptide bonds [6].

Collagenase enzymes, as specific enzymes for the collagen substrate, have been isolated and characterized from both microbial cells and animal tissues. Microbial collagenases have been recovered from pathogenic microorganisms, principally Clostridium histolyticum. These collagenases split each polypeptide chain of collagen at multiple sites. They are thought to function as an exotoxin, causing hydrolysis of collagen in the host cells and disrupting metabolism in connective tissues. Bacterial collagenases are quite versatile, being capable of hydrolyzing both water-insoluble native collagens and water-soluble denatured collagens[7] .While much of the research with microbial collagenases has focused on a single species, tissue collagenases have been isolated and characterized from a number of different tissues in many

animals. Since tissue collagenases are digestive enzymes, they are commonly isolated from the digestive tracts of various fish and invertebrates including: tadpole tailfin, rabbit skin, rat uterus, rheumatoid synovial tissue, mouse bones, crabs. Electrophoresis is used to characterize collagenase, principally by estimating molecular weight. Reported molecular weights vary significantly based on the enzyme type (serine or metallocollagenase) and the source (microbial or animal tissue). Harper et al. (1965) isolated two collagenases from Clostridium histolyticum with molecular weights of 105 and 57 kDa. Bond and Van Wart (1984) isolated six different collagenases from the same species with molecular weights ranging from 68-128 kDa [8].

Collagenases are secreted as zymogens or inactive enzyme precursors. These latent enzymes require a change in structure (or activation) to achieve collagenolytic activity. Inactive forms of collagenase may, also, be present due to interaction with other molecules or inhibitors and cleaving the linkages between inhibitors and collagenases may be necessary to generate collagenolytic activity [9]. Thus, addition of activators and control of inhibitors during extraction and purification of collagenase becomes critical. In any process used to isolate collagenases, it is crucial to avoid denaturation of the enzyme and maintain its activity. While a number of structures serve as activators or inhibitors, temperature and pH are considered to be the most important factors in retaining collagenolytic activity [10-11].

MATERIALS AND METHODS

Sequence Retrieval system

The Collagenase protein sequence was retrieved from NCBI in order to perform motif and protein modeling prediction.

Motif prediction

The retrieved protein sequence was applied in to advanced HTH (Helix –Trun-Helix) motif sequence regions in order to find out the functional part of the sequences.

RESULTS

Collagenase

>gi|2982083|pdb|1AZZ|B Chain B, Fiddler Crab Collagenase

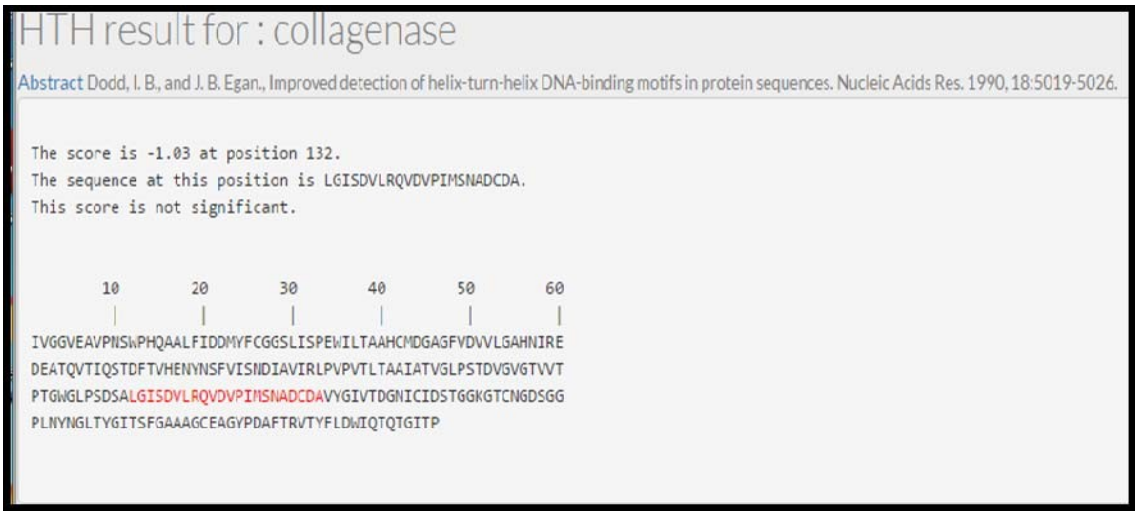
Protein modelling

The Identified motif sequence was applied into the Protein Modelling Server. (The amino acids Protein sequence was converted into 3D structure). Protein structure prediction The predicted 3 Dimensional structure was viewed with the help of advanced molecular visualization tools such as Discovery Studio Software.

IVGGVEAVPNSWPHQAALFIDDMYFCGGSLISPEWILTAAHCMDGAGFVDVVLGAHNIREDEATQVTIQS
TDFTVHENYNSFVISNDIAVIRLPVPVTLTAAIATVGLPSTDVGVGTVVTPTGWGLPSDSALGISDVLRQ
VDVPIMSNADCDAVYGIVTDGNICIDSTGGKGTCNGDSGGPLNYNGLTYGITSFGAAAGCEAGYPDAFTR
VTYFLDWIQTQTGITP

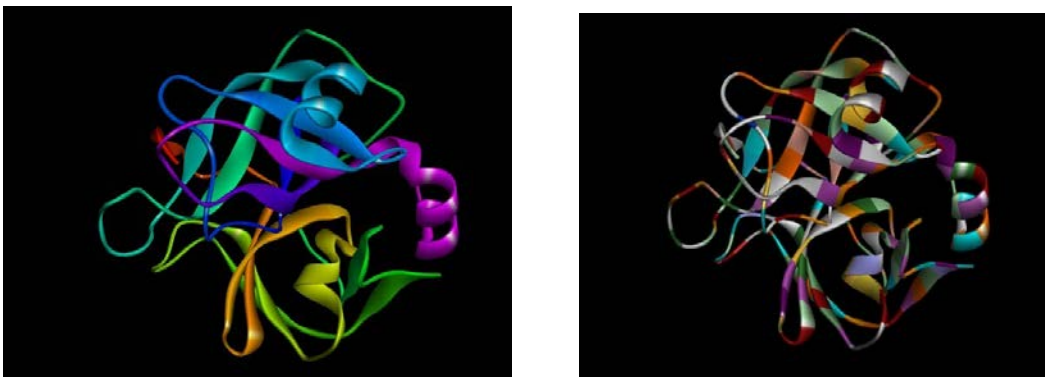
Motif Prediction Server

Fig 1. The motif predictions of collagenase protein form fiddler crab



Protein modeling

Fig 2. Discovery studio software 3D structure of protein–collagenase



Hydrophobic yellow1 ala,val,phe,pro,met,ile,leu polar pink ser, thr, tyr, his, cys, cyss, asn, gln, trp,gly charged (+) blue lys,arg charged(-) red asp,glu

DISCUSSION

In this research work we focus on protein motif based structure prediction using Bioinformatics tools. We retrieved the collagenase protein sequence and applied it into GYM motif server in order to identify the functional part of the protein sequence. The Helix-Turn-Helix (HTH) motif is one of the best studied motifs in proteins. Proteins with such motifs are usually transcription factors. They bind to DNA and affect the function of RNA polymerase, thus regulating the gene expression. It is found that the HTH motifs of these proteins are responsible for binding with DNA. Fig (1) shows the GYM motif prediction sequence. Detecting motifs including HTH motif has become an important issue in biochemistry. The most widely used methods are statistically based profile methods, with Dodd & Egan (DE) method being the most popular used for detecting HTH motif. The program also gives some comparisons on these two methods. The 3D structure was predicted using Cph model server. The collagenase protein sequence was converted into 3D structure. Fig (2) here we have given notes on the applications of CPh model server. CPHmodels-3.0 is a web-server predicting protein 3D-structure by use of single template homology modeling. The server employs a hybrid of the scoring functions of CPHmodels-2.0 and a novel remote homology-modeling algorithm. A query sequence is first attempted modeled using the fast CPHmodels-2.0 profile-profile scoring function suitable for close homology modeling. The new computational costly remote homology modeling algorithm is only engaged provided that no suitable PDB template is identified in the initial search. CPHmodels-3.0 was benchmarked in the CASP8 competition and produced models for 94% of the targets (117 out of 128), 74% were predicted as high reliability models (87 out of 117). These achieved an average RMSD of 4.6. When superimposed to the 3D-structure. The remaining 26% low reliably models (30 out of 117) could superimpose to the true 3D-structure with an average RMSD of 9.3. These performance values place the CPHmodels-3.0 method in the group of high performing 3D-prediction tools. Beside its accuracy, one of the important features of the method is its speed. For most queries, the response time of the server is less than 20 minutes. After the prediction of the protein structure, we find out the molecular protein binding sites using Discovery studio software and Mol soft software. All the above results were discussed.

CONCLUSION

Comparative ("homology") modeling approximates the 3D structure of a target protein for which only the sequence is available, provided an empirical 3D "template" structure is available with >30% sequence identity. Homology modeling can produce high-quality structural models when the target and template are closely related, which has inspired the formation of a structural genomics consortium dedicated to the production of representative experimental structures for all classes of protein folds. We perform motif sequence based structure prediction in the collagenase protein (fiddler crab). A comprehensive study of gene may be further used in research.

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None.

CONFLICT OF INTERESET

No conflict of interest.

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