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The Role of Bromelain and Papain Proteolytic Potential Binding to Crystalline P23T Γd Protein in Congenital Cataracts

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ABSTRACT

Background: Congenital cataracts can cause permanent vision loss. The etiology of congenital cataracts varies widely. The exact etiology can't be identified, but usually there an autosomal dominant inheritance.

Objective: To assess the proteolytic potential of Bromelain and Papain enzymes against Crystalline P23T γD.

Methods: This study is a pre-experimental study with a computer-based one shot case study approach. Papain enzyme data using 9PAP code and bromelain enzyme data using 1BI6 were obtained from the National Center of Biotechnology Information database (NCBI: www.ncbi.nlm.nih.gov/). Assessment of the proteolytic potential of Bromelain and Papain enzymes against Crystalline P23T D data using 2KFB code cluspro www.cluspro.bu.edu/home.php and data analysis.

Results: The binding affinity of bromelain is -735.1 KCal/Mol and papain is -730.4 KCal/Mol.

Conclusion: The bromelain enzyme has a lower binding affinity value than the papain enzyme, so it has the ability to form stable bond to the crystalline P23T D protein better than papain enzyme.

KEYWORDS: Crystalline P23T γD, Papain, Bromelain, Congenital Cataract

ARTICLE DETAILS

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INTRODUCTION

Congenital cataracts are the most common cause of visual abnormalities in children. This visual abnormalities can affect the visual system in children.[1] Congenital cataracts can cause permanent vision loss. The etiology of congenital cataracts varies widely. The exact etiology can't be identified, but usually there an autosomal dominant inheritance. [2] There are 200,000 children who suffer from blindness caused by congenital cataracts and 133,000 of them are residents of developing countries. There is 1-15 children out of 10,000 are blind due to congenital cataracts.[3] In 2010, the prevalence of cataracts in the United States was 17.1%. Cataracts mostly affect whites (80%) and women. Untreated congenital cataracts can lead to vision obstruction and blindness. Surgical of lens removal has several consequence including long wound healing due to the size of the incisions, the risk of astigmatism exists even though it is small, the equipment is not supportive, and its expensive cost. At present, the sole viable treatments for cataracts involve surgically removing the cloudy lens and substituting it with a synthetic intraocular lens.[4,5]

The eye lens is a biconvex, avascular, colorless and almost completely transparent structure. The lens has neither blood supply nor nervous innervation, and relies entirely on the aquatic humor for metabolism and disposal. The lens is located behind the iris and in front of the vitreus. The eye lens consists of three main parts, such as the lens capsule, lens epithelial cells and lens fibers. The normal eye lens is clear and transparent (transparent and avascular) and its being the one of the refractive media which functions to focus light to form a sharp image on the retina.The clarity of the eye lens is maintained by a dense and highly regular arrangement of lens fibers as well as a homogenity distribution and protein composition in the lens. [6] The lens of the eye contains the highest protein concentration in the human body to facilitate its function. As much as 60% of the total lens mass made of protein.[7]

Crystalline protein of the eye lens is the main component that makes up about 90% of the eye's water-soluble lens proteins, as well as cytoskeletal components,

including actin, myosin, vimentin, α-actinin, and microtubules.[7] The α- and βγ-crystalline families are the main crystalline proteins in the eye lens. α-Crystalline functions to recognize conformational features of proteins and separate misfolded / unfolded conformer proteins from one another. The role of βγ-crystalline is not very clear.[8,9] The occurrence of genetic mutations and degeneration cause structural changes in the crystalline protein, induce lens disorders such as congenital cataracts.[10] One of the crystalline proteins mutation is P23T γD. This Crystalline P23T γD protein forms fibrils and amorphous aggregates (figure 1). The Crystalline P23T γD proteins in CRYGD, CRYBA1, and CRYBA3 have been found in 38 family trees in Australia. Crystalline P23T γD protein is associated with the incidence of congenital cataracts. [11] So that the Crystalline P23T γD protein that undergoes aggregation must be cleaved using enzymes that can cleave proteins, such as the papain enzyme found in papaya *(Carica papaya L.)* and bromelain enzyme found in ginger *(Ananas comosus).* [12, 22]

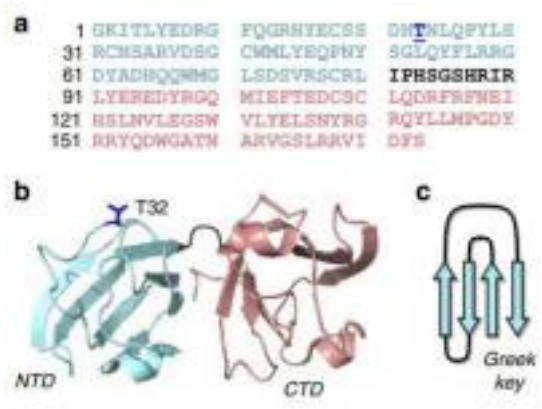

Figure 1. Fibrils and amorphous aggregates formed by Crystalline protein.[11].

Bromelain enzyme derived from pineapple extract (Ananas comosus) is a strong molecule that can destabilize protein aggregates and can inhibit the formation of these aggregates from monomer and oligomeric states. Therefore bromelain is an important and useful element of pineapple, one of which is in the pharmaceutical field.[20] Papain enzyme is a protease that comes from plants. It's a cysteine endolytic protease enzyme derived from papaya latex (Carica papaya L.). Papain, part of the papain superfamily, is a crucial proteolytic enzyme involved in vital biological processes across living organisms. Its proteolytic action extends to proteins, short-chain peptides, amino acid esters, and amide links, finding widespread applications in food and medicine. Papain particularly targets peptide bonds linked to basic amino acids, especially arginine, lysine, and residues following phenylalanine. Prior research indicates that utilizing plant-based proteases like papain has demonstrated a potential reduction in the development of cloudy areas in the eye's vitreous.[22]

Research to assess the potential of an active ingredient in affecting the desired medicinal effect is needed. One of the low-cost and fast research methods to prove the effect is using in silico method. In silico predicts the biological effect of an active ingredient through computerized technology with the aim of finding new drugs.[15] The method used in this research is molecular docking which can predict the ability of an active ingredient (ligand) to form bonds with target proteins (such as receptors). This interaction is based on binding affinity or binding energy. An application or software that can be used to perform molecular docking is Cluspro. [16] Based on the background described above, the authors would like to further investigate the in silico test for the proteolytic potential of bromelain and papain against the Crystalline protein P23T γD induce congenital cataracts.

Questions for Assessment: The purpose of this article is to identify information about in silico test for the proteolytic potential of bromelain and papain against the crystalline protein P23T γD induce congenital cataracts.

MATERIALS AND METHODS

2.1. Experimental Design

This study is a pre-experimental study with a computer-based one shot case study approach.

2.2. Materials

Papain enzyme data using 9PAP code and bromelain enzyme data using 1BI6 were obtained from the Research Collaboratory for Structural Bioinformatics database (RCSB: www.rscb.org/). [13]

Crystalline P23T γD protein data using 2KFB code were obtained from the National Center of Biotechnology Information database (NCBI: www.ncbi.nlm.nih.gov/). [17]

2.3. Methods

1. Search for papain enzymes, bromelain enzymes, and Crystalline P23T γD protein data on the NCBI (National Center of Biotechnology Information) website (www.ncbi.nlm.nih.gov/) using relevant keywords.
2. Collect data on the specific identity or protein code of papain and Crystalline P23T γD protein via the Protein Data Bank (PDB) by conducting a search on the website http://www.rcsb.org/.
3. Access the Cluspro website at http://cluspro.bu.edu/home.php.
4. Input the PDB codes for papain and bromelain in the receptors column. 5. Input the PDB code for the Crystalline P23T γD protein in the ligand column. 6. Agree not to utilize Cluspro for commercial purposes.
7. Initiate the docking process by clicking on the dock button within Cluspro. 8. Allow approximately 4 hours for the docking process to complete. 9. View the docking interaction model in the results menu.
10. Review the binding affinity value in the view model score menu.

11. Analyze the binding affinity value and the binding interaction model. Lower binding affinity values indicate more stable interactions, suggesting better proteolytic potential in samples with the lowest binding affinity.[16]

2.4. Analysis

1. The binding affinity represents the strength of the interaction between the bromelain and papain enzymes with the crystalline protein, acquired from https://cluspro.bu.edu/.[16] 2. The Binding Interaction Model refers to a three-dimensional structural depiction

Illustrating the interactions between bromelain and papain enzymes with the crystalline protein, accessible from https://cluspro.bu.edu/.[16]

RESULTS

An in silico research experiment evaluating the proteolytic capabilities of papain and bromelain enzymes against Crystalline P23T γD protein has been conducted. Post-docking with Cluspro, the outcomes depicting binding affinity values are presented in Table 1, while the corresponding binding interaction models for papain and bromelain against Crystalline P23T γD protein are illustrated in Figures 2 and 3, respectively.

Table 1. Binding affinity value of papain and bromelain enzymes to the docking target.

Target Docking	Crystalline P23T γD Protein Binding Affinity Value	Binding Interaction Models
Bromelain Enzyme	-735.1 KCal/Mol	4
Papain Enzyme	-730.4 KCal/Mol	1

The table above shows the bromelain enzyme has a lower binding affinity value than the papain enzyme, so it has the ability to form stable bond to the crystalline P23T D protein better than papain enzyme. The following is a picture of the binding:

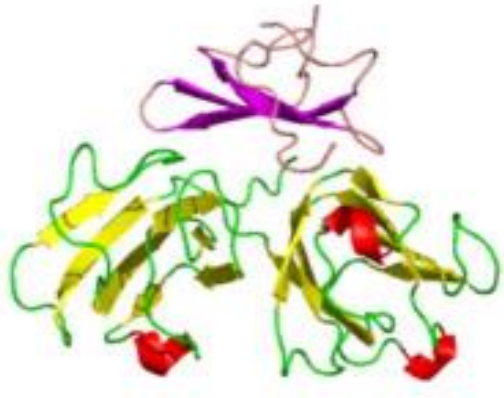

Figure 2. Bromelain enzyme binding interaction model to the crystalline γD P23T protein

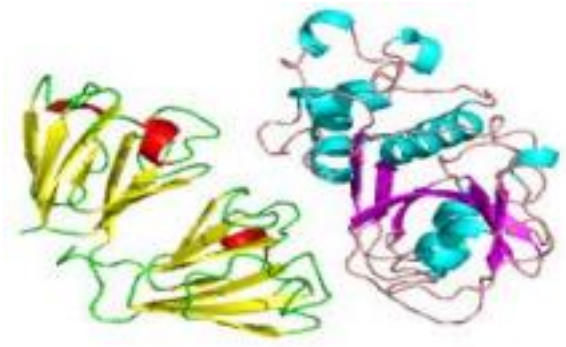

Figure 3. Papain enzyme binding interaction model to the crystalline γD P23T protein.

DISCUSSION

Congenital cataracts are eye disorders that can cause lifelong vision loss. Amount of 200,000 children worldwide who suffer from blindness caused by congenital cataracts and 133,000 of them are residents of developing countries.[2,3]Currently, surgical removal of the cloudy eye lens and replacement with a synthetic intraocular eye lens are the only cataract treatments available. Untreated congenital cataracts can cause visual disturbances to blindness. The lens of the eye contains the highest protein concentration in the human body to facilitate this function. As much as 60% of the total lens mass consists of protein. [4, 7]

Proteins are compounds that carry out biological functions, have a size on the nanometer scale, and are a molecular compound.All cells of the human body are composed of proteins, and all body cells of organism things are also composed of proteins. Proteins also play a role in the process of replication, reproduction of life, and defense.[7] Proteins are the most extensive and complex macromolecules in the body of organism. Protein is very interesting to review because one protein and another protein has a different amino acid sequence, so that their biological function is also different. [21] The occurrence of genetic mutations and degeneration can cause structural changes in proteins. Mutations in crystalline proteins in the eye can cause lens disorders such as congenital cataracts. [10] One of the mutated crystalline proteins is Crystalline P23T γD protein. Apart from crystalline proteins, there are other types of proteins such as enzymes. Enzymes, a specific type of proteins, act as catalysts for chemical reactions. They exhibit specificity towards the molecules they interact with, known as substrates, facilitating their conversion. Factors such as substrate concentration, temperature, and pH levels significantly impact enzyme activity. [17]One of the enzymes that are proteolytic is papain which is found in papaya. Papain is a cysteine endolytic plant protease enzyme isolated from papaya latex (Carica papaya L.). [14, 22]

Docking analysis investigates stable interactions between ligand and receptor proteins, aiming to identify the lowest energy model interaction. This study focuses on evaluating the interaction between the papain and bromelain

enzymes and the crystalline P23T γD protein, a cataract-forming protein, utilizing binding affinity and interaction models. Papain and bromelain enzymes are recognized for their proteolytic activity against P23T crystalline protein γD, capable of reducing insoluble, high molecular weight protein prone to aggregation. This highlights their potential in congenital cataract treatment. In silico studies reveal that papain exhibits a binding affinity of -730.4 KCal/Mol towards PT23T γD crystalline protein. Previous research demonstrates higher binding affinity values for papain when interacting with other docking targets. For instance, papain's in silico test against α amylase showed a binding affinity value of -799.4, while its interaction with α-glucosidase exhibited a binding affinity value of -934.7.[16]

This shows that the enzyme papain and bromelain enzyme have good potential when used as drug therapy in congenital cataracts. Papain enzyme binding interaction modelvalued 1 means the interaction model for binding of 1 papain enzyme to the crystalline γD P23T protein. Docking analysis helps identify the most stable interaction between receptor and ligand proteins. As per the findings, it's evident that the papain enzyme exhibits a lower binding affinity value, indicating a more stable bond compared to the bromelain enzyme when interacting with the crystalline γD P23T protein. This determination relies on binding affinity, where a smaller energy value signifies easier bonding. [16]

Binding affinity represents the strength of interaction between molecules that bind reversibly, playing a crucial role in the pharmaceutical realm. It significantly expedites and streamlines the assessment of affinity, aiding immensely in drug design and discovery processes. [18] The binding affinity is impacted by non-covalent intermolecular interactions like hydrogen bonding, electrostatic forces, hydrophobic interactions, and Van der Waals forces occurring between two molecules. Furthermore, the presence of other molecules can also influence the binding affinity between a ligand and its target molecule. The degree of binding between protein and ligand depends on the binding affinity. Binding affinity is influenced by interactions between molecules. The interaction of proteins with ligands requires energy to be released. Energy released during the interaction between proteins and ligands is known as binding energy. Negative energy indicates that the bond formation process does not require energy from the environment, but releases energy from the bonds to the environment. So the negative binding energy value means that the binding process between protein and ligand will be better, easier, and more stable. [19] Based on the research that has been done, it shows that the papain enzyme has the ability to form a bond to the P23T crystalline γD protein better than the bromelain enzyme.

The results of docking through the Cluspro application between the bromelain enzyme and P23T crystalline γD protein showed 24 models of binding interactions with various binding energy values. The binding interaction model 4 (figure 2) has the lowest binding energy, which is -735.1 KCal/Mol compared to other interaction models. This shows that interaction model 0 has the most stable bond strength and the most potential to interact in real conditions. Meanwhile, the papain enzyme and P23T crystalline γD protein showed 30 models of binding interactions with various binding energy values. The binding interaction model 1 (figure 3) has the lowest binding energy, which is -730.4 KCal/Mol compared to other interaction models. This shows that interaction model 1 has the most stable bond strength and the most potential to interact in real conditions. Research for docking papain and bromelain to P23T crystalline D protein has never existed before, but research on papain has been carried out in diabetics by Jong-Anurakkun (2007) explained that inhibition of -glucosidase will delay the digestion and absorption of carbohydrates, thereby suppressing postprandial hyper-glycemia. [23] The difference in the
Value of binding energy is due to differences in the structure of the P23T γD-crystallin mutation protein and amyloid protein with IE1 protein. Differences in molecular structure can cause differences in the number of hydrogen bonds, electrostatic interactions, hydrophobic forces, and Van der Waals formed so that it will affect the value of binding energy. In addition, there are studies comparing the ability of various plant protease enzymes to hydrolyze proteins in meat. Of the various enzymes studied, actinidin is the most effective enzyme for hydrolyzing meat myofibril proteins. [24]

CONCLUSIONS

It was concluded that the bromelain enzyme which derived from the Ananas comosus has ability to form more stable bonds against the crystalline P23T γD protein compared to the papain enzyme derived from the Carica papaya plant. Therefore, bromelain enzyme has the potential to be developed as a proteolytic agents which are useful in the treatment of congenital cataracts.

Further research is needed, both in vivo as well as in vitro to ensure the ability proteolytic bromelain enzyme interacts with proteins P23T crystalline γD. Other than that, research needs to be done using enzymes proteolytics derived from other plants for determine the comparison of the proteolytic potential of each the enzyme.

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