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The potential of papain and zingibain proteolytic against β-amyloid (Aβ) protein in senile cataracts

Nugraha Wahyu Cahyana
Department of Ophtahalmology, Faculty Medicine, Jember University, Jember, Indonesia

Abstract
Background: The protein aggregation process that forms senile cataracts is related to the pathway for making amyloid structures. With age the increase in β-secretase activity increases. β- and γ-secretase are enzymes that catalyze Amyloid-β Precursor Protein (APP) into β-amyloid (Aβ) structure. So that the β-amyloid (Aβ) formed must be cleaved using an enzyme containing protease to break down the proteins contained in the enzyme papain (Carica papaya L.) and zingibain enzymes (Zingiber officinale). So the In silico test is needed to see the binding ability between the proteolytic effects of the papain enzyme and the zingibain enzyme on β-amyloid (Aβ) protein. Determining the proteolytic potential of the papain enzyme and Zingibain enzyme against β-amyloid (Aβ) protein.
Methods: collecting data of papain, zingibain enzyme and protein from RSCB and NCBI predicting the proteolytic potential of papain and zingibain enzyme against β-*amyloid* (Aβ) with docking simulation by In Silico Study and data analysis was carried out.
Results: The binding interaction model is 1 for the papain enzyme and 0 for the zingibain enzyme with the binding affinity value for the papain enzyme is -697.2 and for the zingibain enzyme is -873.5.
Conclusion: The papain enzyme isolated from papaya latex (Carica papaya L.) and Zingibain found in ginger (Zingiber officinale) has the ability to form bonds with β-amyloid (Aβ) protein so it has the potential to be developed as a useful proteolytic as a preventive for senile cataracts.

Keywords: senile cataract, papain, zingibain

Introduction

Cataract is an eye disorder due to cloudiness in the lens of the eye which can reduce vision function. [1] Senile cataract is clouding of the lens in the eye caused by a degeneration process and is found after the age of 50 years. [2] This clouding disorder is influenced by age and is one of the causes of vision loss. The process of aggregation of proteins forming senile cataracts is related to the pathway for the formation of amyloid structures. With age the activity of β-secretase increases. [3] β- and γ-secretase are enzymes that catalyze Amyloid-β Precursor Protein (APP) into β-amyloid (Aβ) structures (Strooper and Annaert, 2000). β-amyloid (Aβ) has a strong association with oxidative stress [4]. Free radicals, including some Reactive Oxygen Species (ROS) such as SO2, H2O2, and SOH can cause structural damage to the crystalline lens and contribute to cataract formation [5]. The decrease in α-Crystalline with age causes the damaged βγ-Crystalline (misfolding or unfolding) continue to increase. α-Crystallin, a protein that is the main support system of adult eye lens fiber cells, recognizes the conformer features of proteins and separates misfolded / unfolded conformer proteins from one another. If the population of α-crystalline is limited then the eye lens will be filled with damaged βγ- Crystalline and not folded properly, this protein also contributes to the growing aggregates and eventually causes eye cloudiness [6].
Untreated senile cataracts can cause visual disturbances to blindness. The definitive treatment for cataracts is surgery, such as Intracapsular Cataract Extraction, Extracapsular Cataract Extraction, and Phacoemulsification, but this method is considered not free from complications, so other efforts are needed to prevent and treat further eye lens disorders [7]. There have been several studies using vitamin C and E to slow the growth of cataracts, but they have not been effective. Research also mentions the use of natural ingredients that have proteolytic potential in senile cataracts are papaya and ginger [8].
Papain is a protease class enzyme that is used in various industrial fields such as food and medicine. This protease enzyme comes from the papaya plant (Carica papaya L.). Papain can be obtained from papaya sap, from the fruit, stems and leaves. The part of the plant which are stems, leaves and young papaya contain a white sap containing papain [9]. Papain has proteolytic activity against proteins, acid esters, amide chains and short chain peptides such as lysine, phenylanine, and arginine. Previous studies have shown that the enzyme papain can reduce opacity in the eye [10].
Zingibain or ginger protease, which was first reported as a new protease source in 1973, exhibits remarkable proteolytic activity. Zingibain is a meat tenderizer that is very active against collagen and other connective tissue proteins. The good freezing activity of milk is also attributed to zingibain so it is used in the preparation of

ginger milk curd in southern China. Zingibain is an enzyme found in ginger, ginger is a plant that belongs to the zingiberaceae family. Zingibain was shown to have high activity against protein substrates [11].

The protease enzyme derived from the papaya plant (papain enzyme) and ginger (zingibain enzyme) has a proteolytic effect that has the potential to lyse β-amyloid (Aβ) protein so that it can be an alternative treatment for cataracts. Previous studies have shown that the use of plant-based proteases such as the enzyme papain can reduce the development of vitreous opacity in the eye [12]. However, there are no studies on the enzymes papain and zingibain related to proteolytic effects on β-amyloid protein. So that research is needed to assess the potential of active ingredients for the preventive effect in senile cataract therapy.

In this study, we used a drug design method using the chemical activity of a candidate drug molecule through computational methods. This method has advantages over laboratory scale studies, such as the ability to determine the amino acids associated with enzymatic reactions [13]. The method commonly used for drug design based on the bioinformatics approach is the In Silico docking simulation. This method is used to assist the virtual screening process in search of candidate drug molecules (ligands) based on the value of the interaction between ligands and receptors. Docking simulations can be used to assess interactions between candidate drug molecules and cell receptors [14].

Research related to papain and zingibain against β-amyloid (Aβ) protein has never been conducted before. So it is interesting for the authors to examine this and great hope that later this research will be continued with *In Vivo* and *In Vitro* research in the prevention of cataracts.

Methods

Papain and zingibain enzyme data material

Materials needed for this research were data of the papain and zingibain enzyme structure from literature reviews (scientific journals and articles). The structure of the papain and zingibain enzyme were obtained from https://www.rcsb.org/ with code 9PAP and Zingibain enzyme with code 1CQD.

Material data protein β-amyloid (Aβ)

Search for protein data using NCBI (National Center of Biotechnology Information) (www.ncbi.nlm.nih.gov/) with the appropriate keywords papain and zingibain enzymes and β-amyloid (Aβ) protein, through PDB (Protein Data Bank) with search on the site's search field http://www.rscb.org/. A search was made regarding binding affinity and binding interaction models. Binding affinity is the value of the binding interaction strength between the papain enzyme and the β-amyloid (Aβ) protein and the Binding Interaction Model is a three-dimensional structure image of the interaction between the papain enzyme and the zingibain enzyme with the β-amyloid (Aβ) protein which can be obtained from the website https: //cluspro.bu.edu/. The structure of the β-amyloid (Aβ) protein associated with senile cataracts with code 2BP4 which can be downloaded at https://www.rcsb.org/.

Docking simulation method

Opening the Cluspro site with the address http://cluspro.bu.edu/home.php. Enter the PDB code for papain and zingibain enzymes in the receptor column. Enter the code PDB for crystalline γD protein P23T and β-amyloid protein into the ligand column. Fill out an agreement that will not use Cluspro for commercial purposes. Clicking the dock button on Cluspro to start the docking process. Waiting for the docking process for approximately 4 hours. See the docking result interaction model in the results menu. Review the binding affinity value in the model score view menu. Analyze binding affinity values and binding interaction models.

Data analysis

Docking results were analyzed from the Cluspro application based on the binding affinity value. The molecule with the lowest binding affinity value shows stable interactions. Therefore, the interaction model with the lowest binding affinity is used.

Results

Research on in silico test of proteolytic potential of papain enzyme against senile cataract forming protein. After docking with Cluspro, the binding affinity results and the binding interaction model between papain and zingibain enzymes on β-amyloid (Aβ) protein were obtained. The binding affinity value and the enzyme binding interaction model to the docking target are shown in Table (Table 1) and the following figure (shown in Fig, 1).

Table 1: binding affinity value of papain enzyme

Docking Target	Papain enzyme		Zingibain enzyme	
	Binding Interaction Models	Binding Affinity Value	Binding Interaction Models	Binding Affinity Value
B-amyloid Protein	1	-697.2	0	-873.5

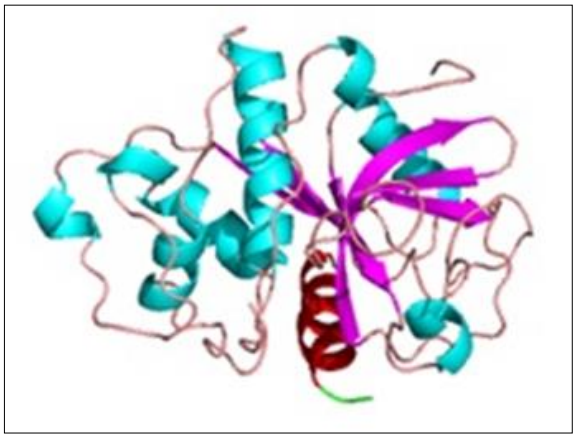

Fig 1: Binding Interaction Model 1 papain enzyme against *β-amyloid* protein (Aβ)

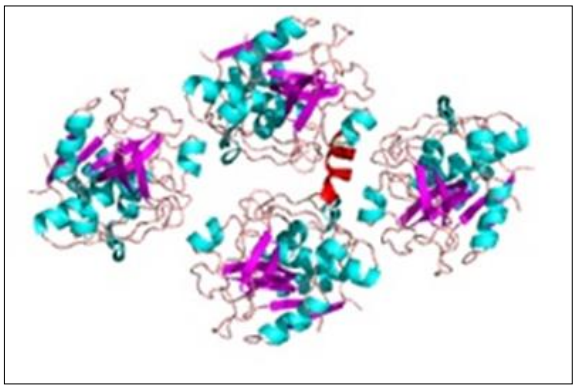

Fig 2: Binding Interaction Model 0 zingibain enzyme against *β-amyloid* protein (Aβ)

Discussion

Drug design is done to determine the chemical activity of a drug molecule candidate through computational methods. Bioinformatics helps to make it easier to calculate complex molecular characters with certain algorithms through programming languages to determine the design of drug molecules. [13] The method commonly used for drug design based on the bioinformatics approach is docking simulation. Docking analysis was performed to determine the most stable interaction between ligand protein and receptor protein which is processed through docking. The interaction parameters that can be used are the bond between the receptor and the ligand, the ligand shape conformation when interacting with the receptor, and the evaluation of ligand affinity with the receptor in terms of Gibbs free energy (ΔG). Docking simulations can be used to assess interactions between candidate drug molecules and cell receptors. Candidates for drug molecules selected through docking simulations are continued through laboratory trials (Rao and Srinivas, 2011). This interaction is based on binding affinity, the smaller the energy value, the easier it will be to bond. The results of docking analysis are presented in various interaction models, to determine which interaction model to use is the one with the lowest energy [15].

Binding affinity is the strength of the interaction between two (or more) molecules that bind reversibly [16]. Binding affinity is influenced by non-covalent intermolecular interactions such as hydrogen bonds, electrostatic interactions, hydrophobic forces and Van der Waals between the two molecules. In addition, the binding affinity between a ligand and its target molecule can be affected by the presence of other molecules [17]. The degree of binding of a ligand to a protein refers to the binding affinity, whereas the energy released due to the formation of bonds, or more precisely, the interaction of ligands and proteins is called the binding energy [18].

Proteins are polymers composed of amino acid units that are bonded to each other through amide bonds. The arrangement of shorter amino acid units or oligomers is called a peptide and plays a role in various biological processes in the body [19]. Enzymes are a type of protein that have the function of catalysing chemical reactions. Enzymes have the specificity of molecules that become the substrate for catalysts. Substrate concentration, temperature and pH are factors that influence enzyme activity [20]. Papain is a non-glycosylated polypeptide that has a single chain of 212 amino acids containing three disulfide bonds. Structures with an initial resolution of 2.8A˚ are enhanced to 1.65A˚. Several structures are available for ligands and inhibitors of the papain complex. The polypeptide chain is folded to form a globular protein, two domains interact and limit the gaps in the enzyme surface or the substrate side to bind. The active form of papain is formed by the active site residues of His159 and Cys25, consisting of a thiolate-imidazolium ion pair. Papain can be accessed by code 9PAP on PDB [21].

The zingibain enzyme is one of the glycoproteins containing 221 amino acids. The zingibain enzyme has Asn99 and Asn156, which are N-linked or 8% glycosylated oligosaccharide chains by weight. The structure of the glycosyl chain can be seen from the available chain sequence, the effect of the glycosyl chain on protein conformation can also be observed from the chain sequence. The three-dimensional structure of the GP-II can be determined using X-ray crystallography (overall R-factor = 0.214, free R = 0.248) with a resolution of up to 2.1A. Homologous papain, actinidine, and glycyl endopeptidase homologs have a similar structure to that of GP-II, folding into two distinct domains of approximately the same size divided by a gap. Zingibain can be accessed by code 1CQD on PDB. In this case, the interaction between the enzyme papain and zingibain against β-amyloid (Aβ) protein as a protein forming senile cataracts can be known binding affinity and its interaction model [22]. In this *In silico* study, the binding interaction model 1 describes the interaction of the enzyme papain against the B-amyloid protein. This binding interaction model shows a binding affinity value of -697.2, which is higher than the zingibain enzyme, which shows a value of -873.5 with the 0 binding interaction model. Negative energy shows that during the bond formation process energy is not required from the environment but bonds that release energy into the environment. Therefore, the smaller or negative the binding energy, the better the binding of ligands and proteins. So that it is known to be proteolytic to β-amyloid (Aβ) protein so that it can reduce the amount of protein with low solubility, high molecular weight, and susceptibility to aggregation [18]. This shows the potential use of the enzyme papain and zingibain enzymes as a therapy for senile cataracts. Based on the results mentioned, it can be seen that the zingibain enzyme has a lower binding affinity value or a more stable bond than the papain enzyme when interacted with β-amyloid protein.

Conclusion

It was concluded that the papain enzyme isolated from papaya latex (Carica papaya L.) and Zingibain found in ginger (Zingiber officinale) has the ability to form bonds with β-amyloid (Aβ) protein so it has the potential to be developed as a useful proteolytic as a preventive for senile cataracts.

Declaration

Abbreviations

Not Applicable

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Yes.

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article or its supplementary materials.

Competing interests

Not Applicable.

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Not Applicable.

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