Computer Aided Design of Polygalacturonase II from *Aspergillus niger*

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Abstract— ***Pectin is a complex polysaccharide found in the cell walls of plants and consisting mainly of esterified D-galacturonic acid resides in α-(1-4) chain. In production of fruit juice, pectin contributes to fruit juice viscosity, thereby reducing the juice production and increasing the filtration time. Polygalacturonase improves the juice production process by rapid degradation of pectin. In this project we have designed a novel polygalacturonase enzyme using computer aided design approaches. The three dimension structure of polygalacturonase is first modeled on the basis of the known crystal structure. The active site in this enzyme is identified by manual and automated docking methods. Lamarckian genetic algorithm is used for automated docking and the active site is validated by comparing with existing experimental data. This is followed by in silico mutations of the enzymes and the automated docking process is repeated using the mutant enzymes. The strength of the binding of the ligands inside the active site is evaluated by computing the binding score using Potential Mean Force (PMF) method. The mutations R256Q, K258N and E252A show improvement of the binding score while N186E reduces the binding strength. The R256Q, K258N or E252A mutant enzymes can be used in the fruit juice industry to minimize the cost of juice production.***

Keywords - computer aided design; polygalacturonse; automated docking; in silico mutations

I. INTRODUCTION

Enzymes are natural catalyst which permits endogenous biological reaction to occur rapidly through well defined pathway. They occur in almost all creatures ranging from the simple microorganism to well defined complex human beings [1]. Most enzymes are much larger than the substrates that they are acting on and only a small portion of enzymes is directly involved in the catalysis. The region that contains these catalytic residues, binds the substrate, and then carries out the reaction is known as the active site. Enzyme's activity can be affected by reaction time, amount of enzyme, temperature, substrate concentration, pH, ionic strength, pressure, and inducers and inhibitors [2].

Pectins are very complex and heterogeneous molecules which are regular ingredient of all higher plant. Pectins build the cell walls together with cellulose and hemicelluloses; therefore contribute to cell wall functions [3]. Pectin molecules have a linear backbone composed of units of (1,4)-linked α-D-galacturonic acid and its methyl ester. Pectin contents are specific to fruit species. Pectins are primary cause of juice viscosity.

TABLE I. Pectin content of some fruits

Fruit	Pectin content, w%
Apple	0.5 – 1.6
Blackcurrent	1.0 – 1.2
Grape	0.1 – 0.4
Orange peel	3.5 – 5.5
Pear	0.7 – 0.9
Pineapple	0.04 – 0.1
Strawberry	0.5 – 0.7

Polygalacturonases are the enzymes that cleavage the α-1,4-glycosidic linkage of polygalacturonic acid chains by hydrolysis and release oligogalacturonides. Thus, they are involved in pectin degradation. They are classified as the family 28 of glycosyl hydrolase based on amino acid sequences similarity. Normally, the common source of producing these enzymes is the filamentous fungus *Aspergillus* sp. Sometimes, they also can be obtained from tomatoes and oranges. In addition, moulds are used frequently for commercial preparation of polygalacturonases because of the high pectolytic activities exhibited by member of the genera. Polygalacturonase is a major component of industrial pectinases [4]. It is widely used in fruit processing industry because it produces more rapid depolymerization. Polygalacturonases have heavy demand in fruit juice industry due to its activity that degraded the pectin and reduce the viscosity of juice produced. According to Nikolic and Mojovic [3], 50 % of overall viscosity is reduced for the initial 9% hydrolysis of substrate in degradation of apple pectin by polygalacturonases.

Molecular modeling is defined as a simplified or idealized description of a system to mimic the behavior of the molecule and molecular system [5]. Molecular modeling helps in understanding more about protein, enzyme and structures. The objectives of this project are to analyze the structure of polygalacturonase and model the active site that involved in

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enzyme activity. In addition, this model will help to suggest the modification to *A. niger* Polygalacturonase II to achieve the desired activity, that is better degrading of pectin.

II. METHODOLOGY

A. Acqcuiring Crystal Structure

The crystal polygalacturonase from *Aspergillus niger* (PGII) by accession number 1CZF was obtained from Protein Data Bank (PDB: www.rcsb.org/pdb) that is maintained by the Research Collaboratory for Structural Bioinformatics (RCSB).

B. Cleaning the structure

The bonding in HET groups were checked and corrected, then the hydrogen atoms are added and the atom hybridization is defined.

C. Visualizing Protein

The process started with atom definition, proceeds with hydrogen bonds displaying, protein sequences viewing, coloring and ends with labeling. All the mentioned steps are very crucial and foundation steps for enzyme modification. By following these steps, a clear picture about the structure of the enzyme can be obtained.

D. Analyzing the Active Site

Conceptually, active site is the lowest energy region in the structure which contains catalytic residues that will react with the ligand. The active site region of PGII-1CZF was identified according to the information given in the literature. All the residues around that particular region can be recognized. The active site location is studied through accessible surfaces to observe the nature of the active site in terms of hyrophobicity and hydrophilicity areas and crevice surface able to pin point ideal location on the active site where potential binding was possible.

For confirmation of the active site, the comparison has been made between the PGII and other structure with the ligand in the structure. The docked ligand into the PGII structure and the location of ligand was compared.

Before enzyme modification, the possible candidates was examined or screened to make sure they pose the similar traits of physical and chemical properties that are near the current ligand. The study of currently available ligand leads the information in choosing the best candidates for modification.

E. Docking with Hexagalactironide 1 (HGA1)

The docking process was conducted by genetic algorithm docking. An identified ligand was docked onto the active site region. Then, the docked position was adjusted to fit well the enzyme structure and the evaluation process started. The binding energy depends upon the number of hydrogen bonds and other interaction between the ligand and protein.

Docking model used in this study assumes that the protein and ligand dock non-covalently. It is assumed that bonds are not formed between the ligand and protein because bond formation would cause changes in the atom types and possible substantial changes in the shape of the ligand and the active site. The active bound site was specific to 35Å to ensure the enough space for ligand to be docked.

F. Single Point Mutation

Simulated single point mutation was done on the amino acid residues in the conserved region and other residues of the enzyme by substituted amino acids randomly within 5Å from the ligand. It was done to determine the significance of each residue in altering binding affinity.

G. Validation

In this study, the validation was done through docking scores and the number of hydrogen bonds. The better binding affinity was indicated by more negative value of docking score and increased in the number of hydrogen bonds.

III. RESULT AND DISCUSSION

A. Crystal Structures Selection and Cleaning

The crystal structure of polygalacturonase from *Aspergillus niger* (PGII) was selected from The Protein Data Bank (PDB) website with the access number of 1CZF. The selection was done based on the major applications in the fruit juice industry as reported in the literature.

Hydrogen atoms were added to the structure and the position of the hydrogen atom was optimized by molecular mechanics calculation to ensure their placement at the most favorable potential energy configuration. Hybridization of the whole structure was balanced by molecular mechanics calculations. The resultant structure is shown in Fig. (1).

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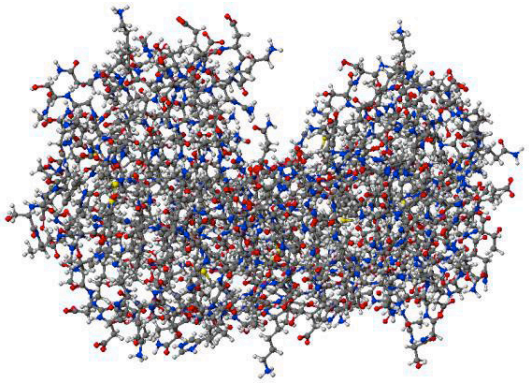

Figure 1: Cleaned structure of polygalacturonase (PGII-1CZF)

B. Analyzing PGII-1CZF Structure

PGII folds into a right-handed parallel β-helical structure comprising 10 complete turns (Fig. 2) with overall dimensions of approximately 65Å × 35Å × 35Å. The number of amino acids per turn varies from 22 to 39, averaging 29 residues per turn. This variation is due to the diversity of lengths of the loops connecting the β-strands. The average rise per turn is 4.8Å, a typical value for parallel β-helical structure and it is formed by four parallel β-sheets, named PB1, PB2a, PB2b, and PB3. In addition, the β-helix has a small α-helix near the N-terminus, which shields the enzyme's hydrophobic core [8].

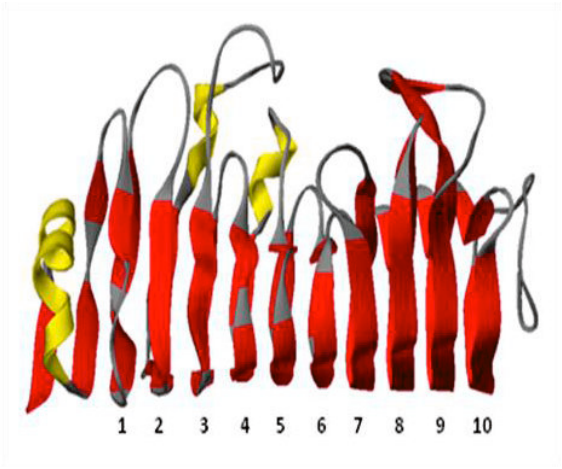

Figure 2: Three dimensional structure of PGII-1CZF. Complete ten rung (number 1-10)

C. Active Site Identification and Analysis

The active site of PGII was characterized based on the information given in the literature because there is no ligand bound to this structure. The active residues are Asp180, Asp201, Asp202, Asn178, His223, Gly224, Gly229, Ser229, Arg256, Lys258 and Tyr291. They are conserved among polygalacturonase. The catalytic residues of this enzyme are Aspartate group (Asp180, Asp201 and Asp202).

For confirmation purpose of the PGII active site, it was compared with the polygalacturonase from *Stereum purpureum* (1KCC) associated with a ligand binds to the structure (Fig. 3). This method of confirmation is valid because both structures are almost same. Three basic residues of 1KCC are His195, Arg226, and Lys228 will interact with carboxyl group for enzymatic activity.

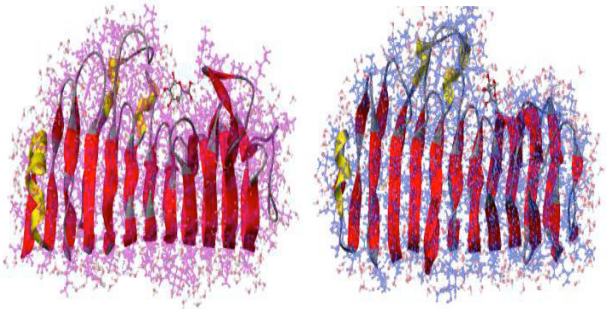

Figure 3: Location of the ligand (GTR) in the structure; (a) GTR is docked in structure from *Aspergillus niger* (1CZF) (b) location of GTR in the structure from *Stereum purpureum* (1KCC).

The active site was also analyzed by creating an accessible surface (Fig. 4) of its region. The accessible surface was generated from the defined active site. Areas colored with mauve (purple-red) and blues are hydrophilic while the cream areas are hydrophobic. Mauve indicates hydrogen acceptor and blue indicates areas of hydrogen donors.

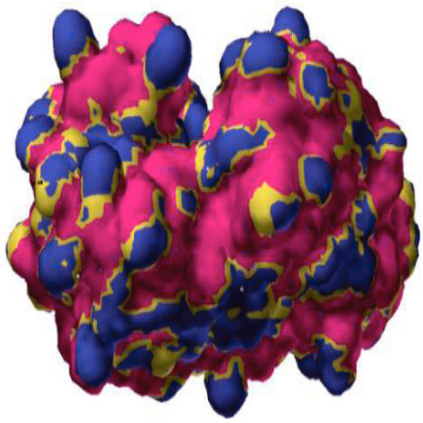

Figure 4: Accessible surface of active site

D. Mutation Analysis- Docking Score and hydrogen bonds analysis

Single point mutation was done to the structure of PGII-1CZF. The mutations were done to the residues at positions of 256, 258, 252 and 186 with R256Q, K258N, E252A and N186E respectively.

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TABLE II. Docking Score

Mutation	Docking scores (kcal/mole)
Non-mutated	-252.784
R256Q	-273.845
K258N	-266.261
E252A	-263.672
N186E	-241.073

Mutation affects the formation and disruption of hydrogen bond between residues in the active site and ligand as well as the residues within the active site which has significant consequences on the ligand binding.

TABLE III. H-bonds: before mutation and after mutation

Mutation	Total hydrogen bond at active site	
	Before mutation	After mutation
R256Q	3	5
K258N	3	6
E252A	3	5
N186E	3	3

E. Binding Analysis

The purpose of single point mutation in this project is to increase the binding strength of PGII. The suggested mutation was done based on the experimental data that have been reported in literature. In this process, the lower docking score indicates the increase in binding strength. Moreover, the increasing number of hydrogen bonds shows that the stronger binding strength.

Based on Table II, the docking score for all mutations gave the more negative values except for the mutation of N186E with score value of -241.073 kcal/mol. The scores which improve the binding are -273.845 kcal/mol, 266.261 kcal/mole, -263.672 kcal/mole for the mutation of R256Q, K258N and E252A respectively compared to the non-mutated with 252.274 kcal/mole docking score.

The numbers of hydrogen bonds (H-bonds) also increase with respect to the very negative value docking scores. Compared to the non-mutated structure with 3 H-bonds, the H-bonds of other mutations have been recorded in Table III. For mutation of N186E, the hydrogen is remained as non-mutated.

In fruit juice industry, polygalacturonase is used as a catalyst to degrade the pectin contained in the fruits. In pectin degradation, minimum activation energy is required for reaction to occur. Thus, the catalyst will lower the activation energy of the reaction by having more reaction and increasing the number of hydrogen bonds. This will stabilized the reaction and reaction will be faster.

F. Validation

It is mutation affects the formation and disruption of hydrogen bond between residues in the active site and ligand as well as the residues within the active site which has significant consequences on the ligand binding. It is important to note that, Arg256 (R256) and Lys258 (K258) are very crucial for substrate binding. Based on the experimental mutation study, these residues displayed the highest specific activity and highest *Km* values on polygalacturonic acid [7] as stated in Table IV.

TABLE IV. Experimental Data of PGII mutations

Enzyme	***Km* value (mg/ml)**
Wild type	< 0.15
R256Q	1.7
K258N	2.8

In this project, the substrate used for docking was partially methyl-esterified hexagalacturonides 1 (HGA1).

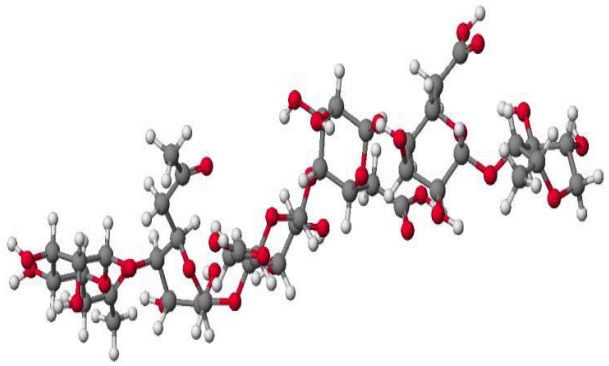

Figure 4: Partially methyl-esterified hexagalacturonides 1 (HGA1) is a substrate used for docking

Mutant E252A showed the increase affinity for partially methyl-esterified substrate. In addition, N186E was the second mutant for methyl-esterified but this mutant displayed opposite characterictics to the E252A [8].

IV. CONCLUSION

Based on the presented data, the binding strength of polygalacturonase can be improved through mutation of residues in the active site region. Three out of four suggested mutations which are R256Q, K258N and E252A give assurance that can improve the binding strength of polygalacturonase. This is because the result from the simulated data shows better binding score and increase the number of hydrogen bonds. The active site and the trends in binding strengths compare very well with the available experimental results. Thus, a new enzyme of polygalacturonase with an improved binding strength can be obtained by computer aided site directed mutation

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