Structure prediction of Stem Bromelain from pineapples (Ananas Comosus) using procaricain enzyme as a modelling template

Fatahiya Mohamed Tap
Chemical Energy Conversions and Applications, Malaysia Japan International Institute of Technology, Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 51310 Kuala Lumpur, Malaysia.

Fadzilah Adibah Abd Majid
Department of Bioprocess and Polymer Engineering, Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia.

Nurul Bahiyah Ahmad Khairudin
Chemical Energy Conversions and Applications, Malaysia Japan International Institute of Technology, Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 51310 Kuala Lumpur, Malaysia.

Abstract
Bromelain is a general name for a family of sulfhydryl that can be found in the proteolytic enzyme group from pineapples (Ananas Comosus). In this study focuses on the prediction of three dimensional structure (3D) of bromelain using homology modelling by MODELLER. The amino acid sequence of bromelain was obtained from the NCBI database and the analysis was done using various bioinformatics tools. 1PCI was used as the target templates for bromelain structure prediction. The 1PCI was found to be the most suitable template for the prediction of the bromelain structure.

Keywords: Bromelain, homology modellig, Sequence alignment.

Introduction
Bromelain can be categorized as a protease because it refers to the group of enzyme which is belongs to the catalytic function that hydrolyses the peptide bonds of proteins. In the presence of oxidizing agents the sulfhydryl group of cysteine will be oxidized and formed the disulphide bond. The effects of the oxidation process of the cysteine group will alter the important properties in substrate binding, structure stabilization, and thus cause changes in active site which results in the loss of its catalytic activity.

Bromelain can be derived from stem and fruit of pineapples. Stem bromelain (EC.3.4.22.32), ananain (EC.3.4.22.31) and comosain were isolated from the stem juice of pineapples by precipitation and centrifugation methods [1]. This stem bromelain considered as endopeptidase because it breaks the peptides bonds within the protein molecules. While fruit bromelain (EC.3.4.22.33) is mainly extracted from fruit juices of pineapples [1]. The fruit is used either naturally or as processed products such as juice, canned sliced, jam and ice cream. However, out of this fruit only 25% of it can be used as a marketable product while the remaining 75% (leaves, crown, stem and bark) is treated as agriculture wastes [2]. A large amount of the waste generated from the industrial processes contains an applicable amount of Bromelain that can act as a proteolytic enzyme with various applications in industrial and therapeutic purposes [3, 4].

Previous study claimed that some of the pineapple waste have highest proteolytic activity, protein content, and sources of bioactive compound [4]. The application of bromelain from the pineapple waste will decrease the excessive amount of waste from food industry and reduce waste management problems either from economics and environmental point of view. Bromelain draws high attention in industrial applications such as meat tenderization, baking industry, anti-browning agent, protein hydrolysate, alcohol production, textile industry and cosmetic industry because of its unique properties and rich with proteolytic activity [5]. Besides that, bromelain has also widely used in therapeutic applications for example platelet aggregation, fibrinolysis, anti-inflammatory activity, modulation of cell adhesion and antibodies [6]. Since bromelain has great commercial values in both industrial and pharmacology area, therefore it is essential to maintain and improve bromelain properties in conformation and catalytic activity in order to preserve its industrial and therapeutic values.

Even though researchers have discovered many methods to enhance the extraction, purification, optimum condition and the specific activity of bromelain for different substrates, the mechanism of this enzyme is still in question because of the unknown three dimensional structure. Therefore, this study was carried out to predict three-dimensional (3D) structure of Bromelain from stem pineapple by comparative modelling as well as to elucidate the unique features of predicted structure.

Methodology
Protein sequence retrieval. The amino acid sequence of bromelain was were retrieved from the NCBI’s protein
database [Gene Bank Accession Numbers: ADY68475]. This protein sequence that has 291 amino acid length was used to predict the bromelain tertiary structure.

Secondary structure prediction. The secondary structures of bromelain was predicted using PHYRE server [7] and visualized using UCSF Chimera [8].

Template selection and 3D structure prediction. BLASTP [9] was used to search for the possible templates and the best template was selected based on E-value and percentage of sequence identity. The 3D structure was predicted using comparative modelling or homology modelling approaches MODELLER [10]. In homology modelling, the template was identified based on position-specific profile search method which improves the accuracy of sequence alignments and also extends the boundaries of detectable sequence similarity. After templates identification, global alignment was carried out between the query sequence and the identified templates. The crystal structure of the template was obtained from protein data bank (PDB) [11].

Results and Discussions
The Amino acid sequence of bromelain was retrieved and the secondary structures were predicted using PHYREP2 server [7]. The secondary structure of bromelain enzyme was made up mostly of helices (40%), coils (39%) and beta-strands (11%). Figure 1 shows the predicted secondary structure of bromelain from stem pineapple (Ananas Comosus). The 1PCI templates was used for the protein structure prediction by considering the lowest E value (5e-85), highest query coverage (86%) and the highest percent identity (56%) calculated by protein-protein BLAST server [9]. Both of the amino acid sequences for the model and template were aligned in order to identify the conservation among the amino acids sequence. The active sites of the model and template were conserved (Gln142 and Cys148) and was shown as in figure 2. The sequence alignment between model and 1PCI template was shown at figure 3. The 3D model structure of Bromelain-1PCI was produced by homology modelling using MODELLER as shown in figure 4. The energy calculation comparison was implemented in order to evaluate the accuracy of bromelain -1PCI model and template. Figure 5 shows the DOPE score between the Bromelain-1PCI model and 1PCI template. From figure 5 it shows that residues Ser25 to Asp50 of the model have high energy compared to the template. Therefore this region needs to be further analysed. However from figure 3 and 4 showing that bromelain have similarity with papain structure and this was supported by previous the work done by Shekar and co-workers [12]. The energy analysis shows that 1PCI is the most suitable to use as the template structure for bromelain because the energy calculation between these two structures is highly similar. Therefore in predicting this bromelain structure 1PCI was choose as the best template and recommended that bromelain-1PCI probably near to the real structure. However further experimental is needed in order to validate this predicted structure.
A. The helices, beta strands and coils was identify by green, purple and red colours, respectively. The ball and stick represents the side chain at the beta strand. B) The superimposed between the bromelain-1PCI model (Cyan) and 1PCI template (yellow). The purple and red sticks representation refer to the side chains of unfavourable region.

Figure 4. The structure of bromelain-1PCI predicted by modeller. A) The helices, beta strands and coils was identify by green, purple and red colours, respectively. The ball and stick represents the side chain at the beta strand. B) The superimposed between the bromelain-1PCI model (Cyan) and 1PCI template (yellow). The purple and red sticks representation refer to the side chains of unfavourable region.

Figure 5. The DOPE energy comparison between the bromelain-1PCI model and 1PCI template. The oval shape indicated the amino acid region from Ser20 to Asp50 and shows the problematic area in the model sequence. The blue region is bromelain-1PCI model and pink region is 1PCI template structure

Conclusion
The 3D structure of bromelain was predicted using homology models with 1PCI (procaricain) as the modelling template. Helices was found to be the major element of secondary structure. The region of the sequence that covers from Ser25 to Asp50 need to be further refined in order to get more stable conformation. Further research and quality assessments are required to refine and validate this structure.

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References

Biographical Sketch:
Fatahiya Mohamed Tap is a Ph.D student at Universiti Teknologi Malaysia. She is currently doing a research in the area of structural bioinformatics. Fadzilah Adibah Abd Majid, Ph.D is a senior lecturer at Universiti Teknologi Malaysia. Her area of specialization are on Nutraceutical and cosmeceutical products formulation and pre-clinical study. Nurul Bahiyah Ahmad Khairudin, Ph.D is a senior lecturer at Universiti Teknologi Malaysia. Her main research interest are molecular simulation and structural bioinformatics.