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Homology Modeling of Pyranose 2-oxidase from *Phanerochaete chrysosporium*

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Currently, most of the demand for energy relies on petroleum products. Nevertheless, the issues of energy security, economics and environment has led to the breakthrough of biofuel cells (BFCs) technology as one of the promising solution to the problems. However, the performance of BFCs need to be improved in order to compete with the existing technologies. One way to improve the efficiency of BFCs is to ensure that the enzyme used as the catalyst in BFCs, in this case, pyranose 2-oxidase (P2Ox) has better binding characteristic and more reactive. For this purpose, studies on three-dimensional (3-D) structure of P2Ox enzyme can offer insights on the structure-function correlations. Unfortunately, at present there is no available crystal structure of P2Ox from *Phanerochaete chrysosporium* (*Pc*P2Ox). Thus, in this study homology modelling was used as the reliable alternative method to predict the 3-D structure of *Pc*P2Ox enzyme and thus provide necessary information to improve the efficiency of the enzyme.

Key words: Homology modelling, Pyranose-2-oxidase, *Phanerochaete chrysosporium.*

The native pyranose 2-oxidase (P2Ox) enzyme has a molecular mass of about 250 kDa and is composed of four identical subunits of 65 kDa. It contains three isoforms of isoelectric point (pI) 5.0, 5.05 and 5.15 and does not appear to be a glycoprotein. Pyranose-2-oxidase (P2Ox; pyranose 2-oxidoreductase; glucose-2-oxidase; EC. 1.1.3.10) is a relatively large flavin adenine dinucleotide glycoprotein (ca. 300,000 kDa), widely distributed among wood-degrading basidiomycetous fungi[1]. It is presumably located in the hypal perisplasmic space, an outer compartment of the fungal hypae[2] and catalyze the C-2 oxidation of d-glucose with high affinity to its corresponding 2-keto sugars with concomitant generation[3] of H_2O_2.

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P2Ox catalyzes the oxidation of D-glucose (D-Glc) and several aldopyranoses by molecular oxygen at the C2 position to yield the corresponding 2-keto-aldoses and hydrogen peroxide[1]. The overall catalytic reaction can be divided into two half-reactions obeying a Ping-Pong-type mechanism at pH 7, as shown below:

The reaction mechanism catalyzed by P2Ox is the Ping Pong Bi Bi type[4] and involved two reactions which are reductive half-reaction and oxidative half-reaction.

$$FAD + D\text{-}Glucose \rightarrow FADH_2 + 2\text{-}keto\text{-}D\text{-}Glucose \quad ....(1)$$

$$FADH_2 + O_2 \rightarrow FAD + H_2O_2 \quad ...(2)$$

Firstly, a reductive half-reaction in which the protein-bound flavin receives a hydride equivalent from a sugar substrate, to produce the reduced FAD ($FADH_2$) and the 2-keto-sugar. Secondly, an oxidative half-reaction in which two hydrogens are transferred from the reduced flavin to O_2 to form H_2O_2.

Pyranose 2-oxidase from *Phanerochaete chrysosporium* (*Pc*P2Ox) is optimally stable at pH 8.0 and up to 60°C. It is active over a broad pH range (5.0 to 9.0) with maximum activity at pH 8.0±0.5 and at 55°C, and has broad substrate specificity. D-Glucose is the preferred substrate, but 1-β-auro-thioglucose, 6-deoxy-D-glucose, L-sorbose, D-xylose, 5-thioglucose, D-glucono-1, 5-lactone, maltose and 2-deoxy-D-glucose are also oxidized at relatively high rates[5].

This high range of sugar substrates and its excellent reactivity with alternative electron acceptors has led P2Ox to be extensively studied for applications in biofuel cells[6]. However, there is no computer modeling work done on this system. In this work we have carried out homology of modeling of 3-D structure of *Pc*P2Ox. This structure can be used to understand and to improve its ligand binding properties using computational methods.

Homology modeling or comparative protein modeling is a method of predicting 3-D structure of a protein by using an already solved structure within the same family as template. When compared to methods such as X-ray crystallography and NMR, this method is proven to have advantage of being a fast yet reliable technique in solving proteins' 3-D structures, starting from the target amino acid sequence[7].

Homology modeling involves multiple sequence alignment between target protein sequence and the template's sequence in order to determine the score of the two sequences. The multiple sequence alignment will give percentage on the sequences similarity, and only templates with ≤30% sequence similarity of the target sequence were selected as templates with lower percentage of sequence similarity are usually considered unreliable[8,9].

In this study, the 3-D structure of P2Ox from *Phanerochate chrysosporium* (*Pc*P2Ox) was developed using homology modeling since there is no crystal structure available for P2Ox from this source. The P2Ox structure from other sources which are from *Trametes multicolor* and *Peniophora* sp. were used as the template to achieve this goal. The structure obtained was then validated using PROCHECK[10] and WHAT IF[11] programs.

MATERIALS AND METHODS

The protein sequence in FASTA format for P2Ox from *Phanerochaete chrysosporium* (*Pc*P2Ox), *Trametes multicolor* (*Tm*P2Ox), and *Peniophora* sp. (*Ps*P2Ox) were obtained from GenBank with accession number of Q6QWR1, 3BLY, and 1TZL_H_A respectively. The sequence of target protein (*Pc*P2Ox) and possible templates (*Tm*P2Ox and *Ps*P2Ox) were aligned together to determine the sequence similarity. The structural data file of the template obtained from protein data bank (PDB; http://www.rcsb.org/pdb/)), having higher similarity with target sequence was then used for developing 3-D structure of target protein (*Pc*P2Ox). Using automated mode option in Swiss-Model, the target protein sequence in FASTA format and suitable template in pdb format were provided in order to generate possible 3-D structure in pdb format of *Pc*P2Ox. 3-D structure developed in Swiss-Model was then validated using PROCHECK and WHAT IF to determine how well the 3-D structure.

RESULTS AND DISCUSSION

The multiple sequence alignment was done in http://www.clustal.org, using EBI web server. Based on the multiple sequence alignment, both templates (*Ps*P2Ox and *Tm*P2Ox) have > 30% of sequence similarity with the target protein (*Pc*P2Ox) thus make them acceptable[10] to be used in homology modeling of *Pc*P2Ox. Since *Tm*P2Ox has higher sequence similarity to *Pc*P2Ox (40%) than *Ps*P2Ox (39%), its template was chosen to be used to develop the 3-D structure. Automated mode

Fig. 1. Modelled 3-D structure of *Pc*P2Ox

		No. of residues	%-tage
Most favoured regions	[A,B,L]	441	85.5%*
Additional allowed regions	[a,b,l,p]	65	12.6%
Generously allowed regions	[~a,~b,~l,~p]	6	1.2%
Disallowed regions	[XX]	4	0.8%*
Non-glycine and non-proline residues		516	100.0%
End-residues (excl. Gly and Pro)		1	
Glycine residues		41	
Proline residues		50	
Total number of residues		608	

Fig. 2. Ramachandran plot statistics

was used in Swiss-Model by providing protein sequence of *Pc*P2Ox in FASTA format, and PDB template of *Tm*P2Ox (PDB ID: 3BLY), downloaded from Protein Data Bank. The target protein was then aligned with the template to generate possible 3-D structure of *Pc*P2Ox. The 3-D structure of *Pc*P2Ox was modeled in the residue range of 13 to 620 based on *Tm*P2Ox template (Figure 1). The sequence identity is 36.3% which is though not very high, but still in acceptable range[12].

FAD molecule

The stereochemical validation of model structures of proteins is an important part of the comparative molecular modeling process. This assessment checks the stereochemical quality of a protein structure by analyzing residue by residue geometry and overall structure geometry. There is some agreement about which measurements are good indicators of stereochemical quality. These include planarity, chirality, phi/psi preferences, chi angles, non-bonded contact distances, unsatisfied donors and acceptors. The 3-D structure of *Pc*P2Ox was validated using PROCHECK program.

In the generated 3-D structure of PcP2Ox, the percentage of residues in the favored regions is 85.5% (Figure 2), and the percentage of residues in disallowed region is less than 1% (0.8%) indicating the good quality of the 3-D structure. This information is shown graphically using Ramachandran plot[13] in Figure 3.

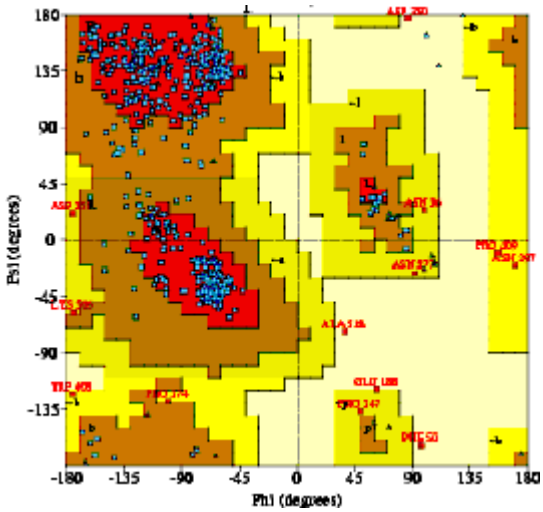

Fig. 3. Ramachandran plot showing residues in favorable regions (small circles) and unfavorable regions (small square with residue names)

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and *R*-factor not greater than 20.0, a good quality model would be expected to have over 90% in the most favored regions [A, B, L].

As seen in Figure 4, the secondary structure contains 9 beta sheets, 1 beta-alpha-beta unit, 10 beta hairpins, 7 beta bulges, 28 beta strands, 19 helices, 12 helix-helix interactions, 62 beta turns, and 6 gamma turns.

Next, the WHAT IF program was used to further validate the developed 3-D structure, in which the calculated Z-score values for different structural elements, provides an indication of the quality of the structure. Analysis shows that the structure has RMS Z-score of 0.655 for bond length. The RMS Z-score is expected to be near 1.0 for a normally restrained data set and values lower than 0.667 might indicate that too-strong restraints have been used in the refinement.

The RMS Z-score for bond angles for the structure is 1.240 shows that the structure has normal bond angle variability since the value is around 1.0. For the backbone conformation Z-score, the backbone conformation analysis gives a score that is normal for well refined protein structures which is -1.428. The value of -1.970 for Ramachandran Z-score shows that the backbone conformations of all residues correspond to the known allowed areas in the Ramachandran plot.

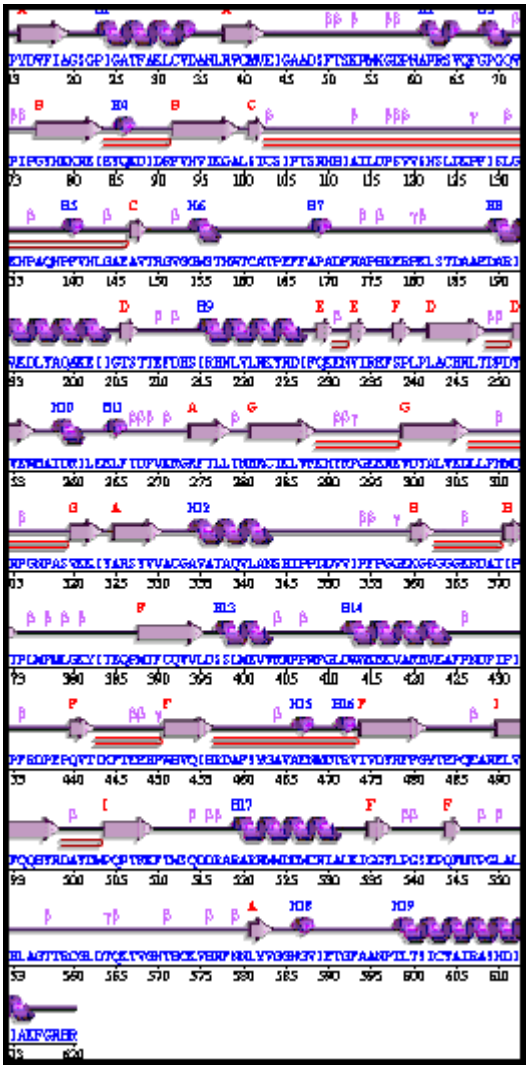

Fig. 4. Wiring diagram of PcP2Ox secondary structure

CONCLUSIONS

In this study, homology model of P2Ox from *Phanerochaete chrysoporium* was obtained. From the validations and assessments done using PROCHECK and WHAT IF program, the modeled 3-D structure is found to be of good quality. This structure can be used for future computational protein design studies, whereby the active site can be modeled, computational docking performed to evaluate the characteristics of the interactions involved in the ligand binding process, and the point of mutation can be suggested to improve the binding capabilities of the enzyme.

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