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Structural and Biophysical Studies of β-Galactosidase from *Oryza sativa* (Osbg)

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Abstract: ***Beta-galactosidase has a very special space in the history of molecular biology. It proved to be a strong basis for the development of the Jacob and Monod model for gene expression. Here in this work, the enzyme has been isolated from Oryza sativa. A simple protocol for extraction and purification of the enzyme was chosen. Anion-exchange chromatography was performed to purify the enzyme. Circular Dichroism gave an important information about the α-helix (15.9%) and β-strands (30.4%) in the enzyme. In-silico methods have been implemented to attain the three-dimensional structure of β-galactosidase from Oryza sativa. To study the catalytic sites molecular docking have been performed. This work supports the structure-based drug designing for developing an analogue in place of non-functional lactase enzymes.***

Keywords: beta-galactosidase, gene, molecular biology, molecular docking

1. Introduction

Beta-galactosidase is an hydrolase enzyme that hydrolyses lactose into monosaccharides as glucose and galactose and therefore essential for the digestion of bovine milk [1,2]. The enzyme is secreted by the lacz-gene of the lac operon and deficiency for this gene leads to malabsorption of lactose and ultimately leads to its fermentation in the gut flora [3]. Lactose intolerant individuals suffer from abdominal pain, diarrhea, bloating, poor digestion and gastrointestinal symptoms after lactose intake [4]. Although the dairy products are the vital source of calcium and vitamin D, people with lactose intolerance have to avoid these dairy products and subsequently causing adverse health outcomes, especially reduced bone mineral density [5, 6].

Lactose is a disaccharide formed by the combination of glucose and galactose and its hydrolysis is possible in the presence of lactase, commonly used as β-galactosidase. Generating a lactose-free milk can be carried out introducing the enzyme directly into the milk [7]. In the past various research have been performed in this direction using microbial, animal, plant, fungi and yeast [8,9]. Although bacteria produce lactase enzyme very efficiently, they are not considered a safer source for prior use in food and dairy manufacturing industries due to the risk of microbial contamination. Fungal and yeast sources produce mesophilic lactase enzyme [10]. Different pH optima are required depending on the strains for example acidic and neutral pH for hydrolysis of lactose in whey and milk respectively [11]. Plant based lactases prove to be at low risk for contamination and also show high enzymic activity and probably concerned with the metabolism of galactolipids, such as β-D-galactosyl diacylglycerol, universally present in plants and algae [12]. To understand the concerned functions of the enzyme, catalytic domain of that particular enzyme is need to be explored. The catalytic site is responsible for the activity such as hydrolysis, trans-acetylation, transferase activity and etc [13, 14].

In this work, plant-based lactase enzyme has been extracted using a simple protocol and purified through anion-exchange chromatography. Purified enzyme has been obtained and further the activity of the enzyme was checked with ONPG, an analog of lactose. The absorbance of the released o-nitrophenol was recorded at 540 nm. This work reports the three-dimensional structure and catalytic site of the enzyme using molecular docking. There may be a possibility of producing non-functional lactase enzyme by the gene but catalytic sites could help to relate with some substitute that proves to be a novel target in designing any therapeutic treatment for lactose-intolerant people.

2. Materials and Methods

2.1 Extraction

Oryza sativa dried seeds were purchased from Etsy, online. Seeds were grounded in a fine powder using a blender. 100 gm of the powder was taken and added in 50 mL of 50mM Tris pH 8. The powder was mixed gently and kept for 20 minutes. Further, the solution was centrifuged at 10,000 rpm for about 20 minutes to collect the supernatant. The supernatant was further proceeded to perform ammonium-sulphate precipitation, initially 30% and then 80%. The overnight incubated solution with 80% salt (ammonium-sulphate) precipitation was centrifuged at 6000 rpm for 10 minutes and pellet was restored at -20˚C.

2.2 Purification

For purification, POROS^{TM} XQ (Thermofisher Scientific) resin were used to perform anion-exchange chromatography [15]. The stored pellet was resuspended in buffer of enzyme (50mM Tris pH 8) and spun down to obtain a clear supernatant. Meanwhile, the column was packed using the resin (POROS^{TM} XQ) and equilibrated with the buffer of protein. The supernatant collected loaded on to the column and allowed to bind with the resin, followed by the washing with the buffer to elute out all the non-specific proteins. As the absorbance at 280nm reaches 0, NaCl gradient was given starting from 0.1M to 0.5M.

2.3 Enzyme Activity

The enzyme assay was carried out using the substrate ONPG (o-nitrophenyl-β-D-galactopyranoside) [16]. The reaction was incubated for 30 minutes in a 1.5 mL Eppendorf with a mixture containing 390µL of phosphate buffer (pH 7.2), 100µL of 5mM ONPG and 10µL of enzyme. After incubation of 30 minutes 500µL of 1M sodium carbonate was added to the mixture to stop the reaction and absorbance was recorded at 420nm using PerkinElmer UV-vis spectrophotometer.

2.4 Circular Dichroism spectroscopic (CDS) studies

CDS measurements of purified β-galactosidase were observed on a JASCO CD Spectrometer. 0.5mg/ml concentration of the enzyme in 50mM Tris pH 8 buffer was used to monitor the structural changes. Absorbance was recorded in far-UV region (190-240nm) using a quartz cuvette with a path length of 1mm.

2.5 3-dimensional structure prediction

The sequence of the enzyme **(Q10NX8 · BGAL6_ORYSJ**) in a FASTA format was obtained from UniProtKB database and used for template-based structure prediction [17]. The 3-Dimensional structure of beta-galatctosidase from Oryza sativa has been predicted using SWISS-MODEL, tool offered by Expasy: Swiss Institute of Bioinformatics (SIB). Further, PyMOL was used to visualize and analyse the structure. The enzyme has 55.7% similarity with beta-galactosidase from Solanum lycopersicum (tomato).

2.6 Molecular Docking

To study the binding sites and the amino acid interaction, molecular docking of beta-galactosidase with NAG (2-acetamido-2-deoxy-beta-D-glucopyranose) was carried out using AutoDockVina. To process the docking, the grid within a spacing of 40 Å × 40 Å × 40 Å centered on the selected flexible residues present in the active site of the enzyme had been chosen. Optimum conformational space had been provided by the grid box to observe rotation to achieve best binding conformation. The complex was visualized on PyMOL [18].

3. Results and Discussions

3.1 Purification

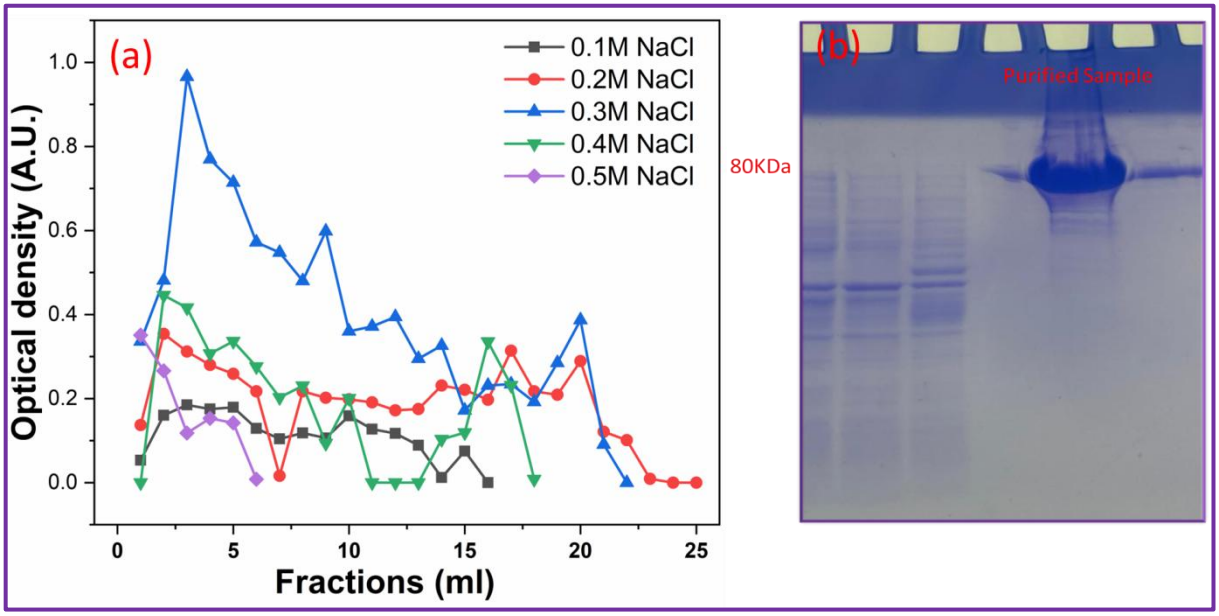

Figure 1: (a) chromatogram for the anion-exchange chromatography. 1(b) purified sample on SDS-PAGE with molecular weight as 80 kDa.

Extracting the enzyme followed by the purification using anion exchange chromatography. The chromatogram was plotted for the fractions collected during the chromatography and the 3rd collected with 0.3M NaCl gradient had the highest absorbance as shown in figure 1(a). The purity of the sample was depicted on Coomassie blue stained 15% SDS-PAGE and molecular weight was estimated as 80 KDa (figure 1(b)). After concentrating the sample using Amicon filter (30,000 MW cut-off) the concentration was found to be 5mg/ml.

3.2 Activity and secondary structural studies (CD)

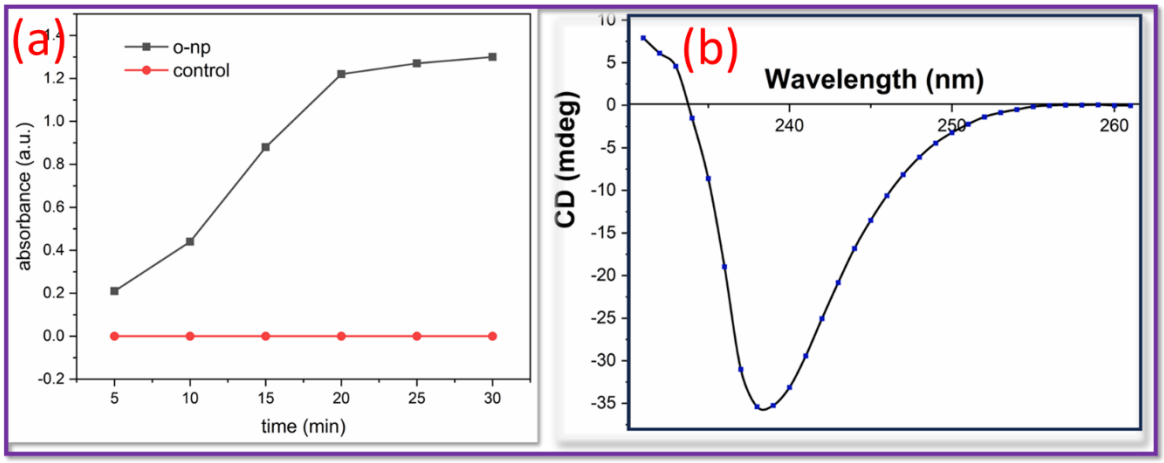

Figure 2: (a) enzyme-substrate catalysis depicting the maximum activity of the enzyme. (b) CD spectra of the enzyme in far-UV region

The hydrolysing activity of the enzyme was measured using colorimetric essay. Thec enzyme was found catalytically active which hydrolyzes ONPG into o-nitrophenol. The rate of catalytic reaction increased linearly with time but did not reach V_{max} in 30 minutes (figure2(a)). CD spectroscopy far-UV measurements of the sample depicted 15.9% α-helices and 30.4% β-strands as analyzed through K2D3 online server for data analysis (figure2(b)) [19].

3.3 Structure of Osbg

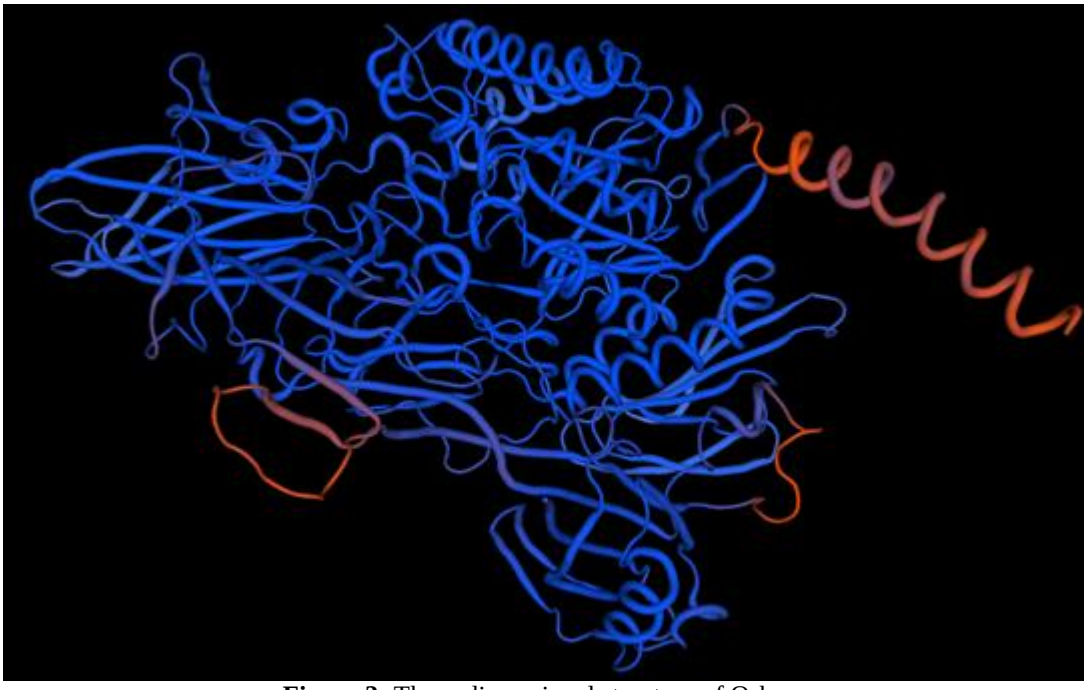

Figure 3: Three-dimensional structure of Osbg .

The bioinformatics studies suggested that the Osbg depicts highest similarity with the crystal structure of tbg (PDB ID: 6ik5) with sequence identity of 55.5%. The secondary structure contains 40 strands, 20 helices and 59 coiled regions (figure 3) as estimated by PDBsum and verifiable with the data obtained from CD analysis. The LG score of the model was found to be 4.877 which brings it in the list of good quality models. The results suggest that the 3-D model for Osbg is reliable. On the basis Ramachandran plot, 87.9% of the residues were found in the most favored region, 11.6 % in the additionally allowed region and 0.3%residues in the disallowed region.

3.3 NAG binding site

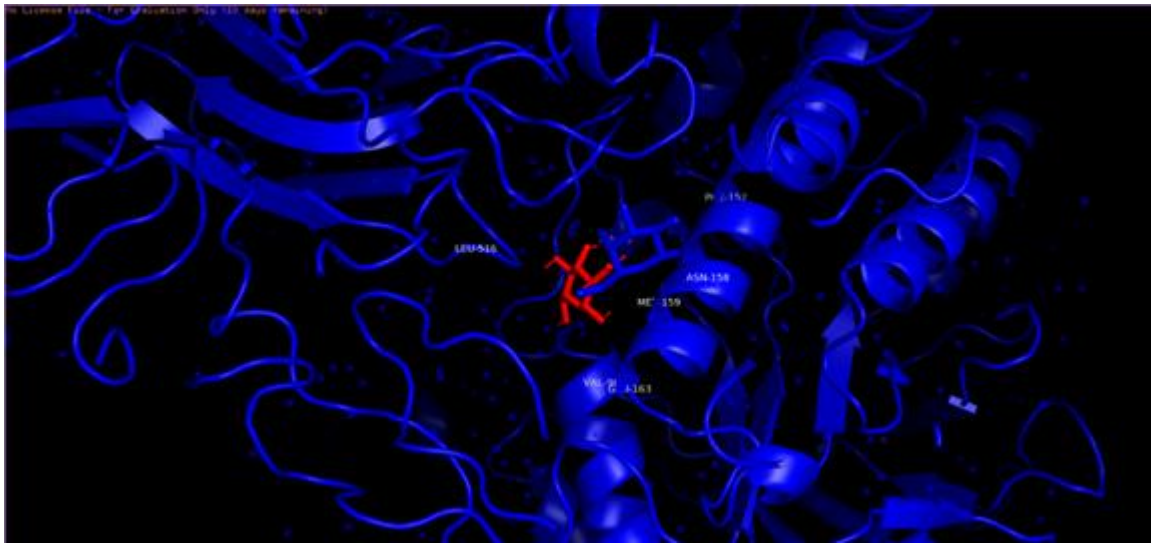

Figure 4: Active participation of the residues with the ligand(NAG) through molecular docking.

NAG was docked on Osbg in order to obtain the recognition and position of catalytic site of the enzyme. As shown in figure 4, the ligand was located and stabilized by attractive interactions with LEU 516, ASN 158, MET 159, GLU 163, VAL 96, and PHE 152 residues in the substrate binding site of the enzyme.

4. Conclusion

Osbg enzyme was extracted from oryza sativa and purified to homogeneity using chromatographic technique. The CD measurements depict about the stable three-dimensional structure, predominantly with the presence β-strands. Osbg forms a very stable complex with NAG and describes the active participation of the catalytic residues of the enzyme. The in-silico studies suggested that the structure is similar to the B-gal from S. lycopersium. The whole study proves to be an unique possibility for the people where the enzyme is produced non-functionally. The active site of the enzyme provides the information about the target for futuristic drug developments.

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