Title: Biophysical studies of β-gal from Oryza sativa

Biophysical studies of β-gal from Oryza sativa: an alternative for lactose free milk

Category: Biochemistry (Bchm)

Introduction and Objectives:
Beta-galactosidase has a very special space in the history of molecular biology. It has 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 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 has been performed. This work supports the structure-based drug designing for developing an analogue in place of non-functional lactase enzymes.

In this work, plant-based lactase enzymes have 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 enzymes 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.

Innovation
This project involves extracting and purifying β-Galactosidase from Oryza sativa, a plant source. While β-Galactosidase is commonly obtained from microbial or animal sources, using a plant-based source, like Oryza sativa, is a unique approach. This innovation can potentially offer a safer and more efficient alternative for lactose digestion and lactose-free milk production. The project identifies the active site of the enzyme, which could serve as a novel target for designing therapeutic treatments for lactose-intolerant people. This has the potential to open new avenues for drug development to help individuals with lactose intolerance.

The project's innovation lies in the use of a plant-based source for β-Galactosidase extraction, comprehensive structural analysis, and the identification of active sites for potential drug development. These findings contribute to a deeper understanding of this enzyme and its practical applications, especially in addressing lactose intolerance.

Method:
Oryza sativa dried 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. The solution was centrifuged at 10,000 rpm 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 and was restored at -20˚C.

For purification, POROSTM XQ resins were used to perform anion-exchange chromatography. The stored pellet was resuspended in a buffer of enzymes and spun down to obtain a clear supernatant. The column was packed using the resin and equilibrated with the buffer of protein. As the absorbance at 280 nm reaches 0, NaCl gradient was given starting from 0.1M to 0.5M.

The enzyme assay was carried out using the substrate ONPG. The reaction was incubated for 30 minutes in a 1.5 mL Eppendorf with a mixture containing 390µL of phosphate buffer. After incubation, 500µL of 1M sodium carbonate was added to the mixture to stop the reaction and absorbance was recorded at 420 nm using the PerkinElmer UV-vis spectrophotometer.
CDS measurements of purified β-galactosidase were observed on a JASCO CD Spectrometer. 0.5mg/ml concentration of the enzyme in the 50mM Tris pH 8 buffer was used. Absorbance was recorded in the far-UV region (190-240 nm) using a quartz cuvette with a path length of 1mm.

Results and Conclusions
Osbg enzyme was extracted from oryza sativa and purified to homogeneity using chromatographic technique. The CD measurements depict the stable three-dimensional structure, predominantly with the presence of 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 a 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.

Acknowledgements and Reference Links

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