

Comparative modeling and docking studies of β -galactosidase from *Aspergillus niger*

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Abstract In the present study we performed the comparative modeling, structural annotation, domain identification and the structural comparison of β -galactosidase enzyme from *Aspergillus niger*. Five domains were identified in the modeled structure at different residue regions. Two catalytic residues Glu200 and Glu298 were identified in the first domain of the modeled structure. The modeled structure showed highest similarity with crystal structure of *Penicillium* sp. β -galactosidase. The molecular docking of the structure with β -D-galactose was also performed.

Keywords β -Galactosidase · *Aspergillus niger* · Modeling · Docking and domains

1 Introduction

β -Galactosidase (*S*-D-galactosides galactohydrolase, EC 3.2.1.23), is the enzyme responsible for the catalytic hydrolysis of β -galactosides into monosaccharides. The enzyme is an exoglycosidase which hydrolyzes the β -glycosidic bond formed between a galactose and its organic moiety. The enzyme deficiencies in the human body result in galactosialidosis or Morquio B syndrome. The enzyme gene, *lacZ* in *Escherichia coli* is present as an integral part of the inducing *lac* operon system and is positively regulated by the incidences of low lactose levels in the organism (Dorland 1997).

The functionality of the enzyme relies on the two structural peptides, *lacZ* α and *lacZ* Ω , neither of which is

active by itself, but which are duly operative when coexisting in an aggregated entity. The presence or absence of an active β -galactosidase may be detected by *X-gal*, which produces a characteristic blue dye when cleaved by β -galactosidase, thereby providing an easy means of distinguishing the presence or absence of cloned product in a plasmid (Dimri et al. 1995).

The exclusive nature of the version of the enzyme found in *Aspergillus niger* is its thermostability at a wide tolerance range of 35–80 °C (Dimitris et al. 2005). The enzyme has significant role in *A. niger* in colony establishment, since it aids in breaking down complex sugars that can be readily assimilated by the fungi. Also, in large-scale industrial processes the fungi has been readily utilized for commercial production of β -galactosidase. The enzyme extracted from *A. niger* is useful in the commercial production of an array of sugars; glucose, galactose, heteropolysaccharides, galacto-oligosaccharides. β -Galactosidase based medical and industrial applications include cleavage of blood group A and B glycotypes, biosensor for specific lactose determination in milk and disease diagnosis (Staino et al. 2005), treatment of lactose malabsorption, production of lactose hydrolyzed milk (Mlichová and Rosenberg 2006). Immobilization of β -galactosidase through anion exchange resin, cellulose–gelatin carrier system, DEAE agarose, glyoxyl/epoxy/BrCN groups, glutaraldehyde, polyelectrolyte surfaces, silicon surface, sepharose-epoxy supports partially modified with boronate, iminodiacetic, metal chelates, and ethylenediamine improves its stability and re-usage (Klich 2002).

Considering the above facts, a greater insight into the properties of the β -galactosidase enzyme from *A. niger* would be more helpful in the working spectrum. In present study the comparative modeling, structural annotation, domain identification, and structural comparison of β -

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galactosidase enzyme from *A. niger* were performed. Further, the docking studies were carried out for catalytic sites, Glu200 and Glu298 with β -D-galactose (GAL) separately.

2 Materials and methods

2.1 Sequence retrieval

The amino acid sequence of β -galactosidase enzyme from *A. niger* was retrieved from GenPept database available at National Centre for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/protein/>).

2.2 Comparative modeling and structural annotation

The prediction of protein structure using comparative modeling is an active and promising area of research. The comparative modeling of the target protein was performed using 3D jigsaw server (<http://bmm.cancerresearchuk.org/~3djigsaw/>) (Bates et al. 2001), validated using PROCHECK (Morris et al. 1992), and the structural annotation was described using SAS-sequence annotated by structure server (<http://www.ebi.ac.uk/thornton-srv/databases/sas/>) (Milburn et al. 1998), ProFunc-protein function from structure server (<http://www.ebi.ac.uk/thornton-srv/databases/ProFunc/>) (Laskowski et al. 2005), and PDB-sum-pictorial database of 3D structures in the Protein Data Bank server (<http://www.ebi.ac.uk/pdbsum/>) (Laskowski 2001) available at European Bioinformatics Institute. Domain identification in the amino acid sequence of β -galactosidase enzyme from *A. niger* was performed using InterProScan tool (Bateman et al. 2004).

2.3 Structural comparison

Protein structure comparisons are often used to highlight the similarities and differences among related homologous-3D structures. Homologous proteins are descended from a common ancestral protein, but have subsequently duplicated, evolved along separate paths, and thus changed over time (Mark and Jukka 2000; Dwivedi et al. 2013). Structural comparison of the modeled structure was performed using Dali server (http://ekhidna.biocenter.helsinki.fi/dali_server/start) (Holm and Rosenström 2010) in PDB database to find out the similar structures in another organisms.

2.4 Molecular docking

The catalytic residue and their ligand interaction for the modeled structure identified using SAS server was used for molecular docking to find out the protein–ligand interaction using ArgusLab 4.0.1 program (Thompson 2004).

3 Results

3.1 Sequence retrieval

The amino acid sequence of β -galactosidase enzyme from *A. niger* was retrieved in FASTA format using their accession number, AAC60538.1

3.2 Comparative modeling and structural annotation

The 3D structure of target protein was generated by 3D jigsaw server using comparative modeling approach. The predicted structure was visualized under PyMol program. (Fig. 1) The PROCHECK analysis of the modeled



Fig. 1 3D structure of β -galactosidase enzyme in *Aspergillus niger* from PyMol

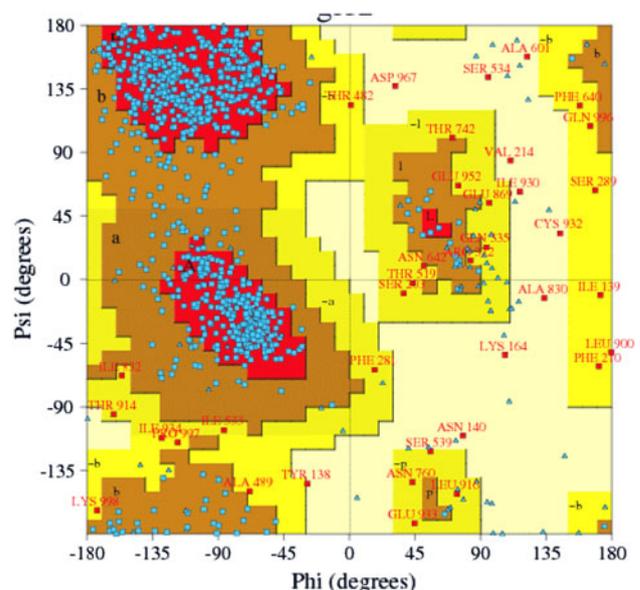


Fig. 2 Ramachandran plot of β -galactosidase enzyme from *Aspergillus niger* derived from PROCHECK

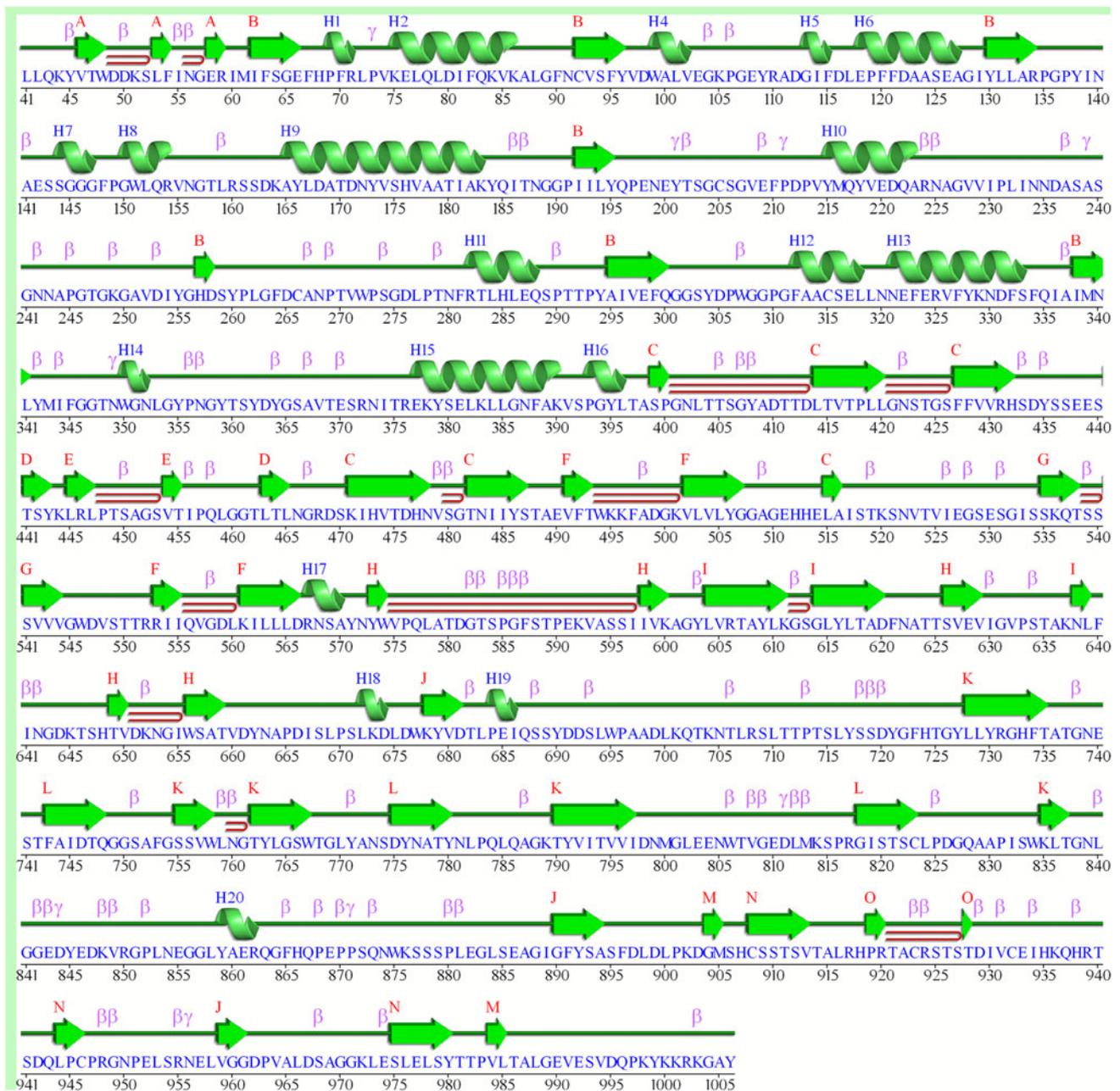


Fig. 3 The figure shows the wiring diagram of the β -galactosidase enzyme from *Aspergillus niger*, created via SAS server

structure yielded the corresponding Ramachandran plot for the predicted structure (Fig. 2), showing the following anomalies: the ϕ/ψ angles of 89.1 % residues are in the most favoured regions, 10 % in the additional allowed regions, 0.6 % in the generously allowed regions, 0.2 % in the disallowed regions. Based on an analysis of 118 structures of resolution of at least 2.0 Å and R-factor no greater than 20 %, a good quality model would be expected to have over 90 % in the most favored regions. The wiring diagram was generated by SAS server as depicted in Fig. 3. The ProMotif documentation of the enzyme via Profunc

server showed the results for the secondary structure summary as: the 966 residue span of the structure consisting of 226 residues (23.4 %), which were involved in the formation of the strands, 104 residues (10.8 %) for the alpha helices, 26 residues (2.7 %) for the 3–10 helix and 610 residues (63.1 %) for the several other structural moieties. Also, the result showed 15 β -sheets, 5 β - α - β motifs, 14 β -hairpins, 12 β -bulges, 52 strands, 20 helices, 12 helix–helix interactions, 109 β -turns, 9 γ -turns. Five distinct domains were found to compose the structural framework of the target protein. The first domain belonging

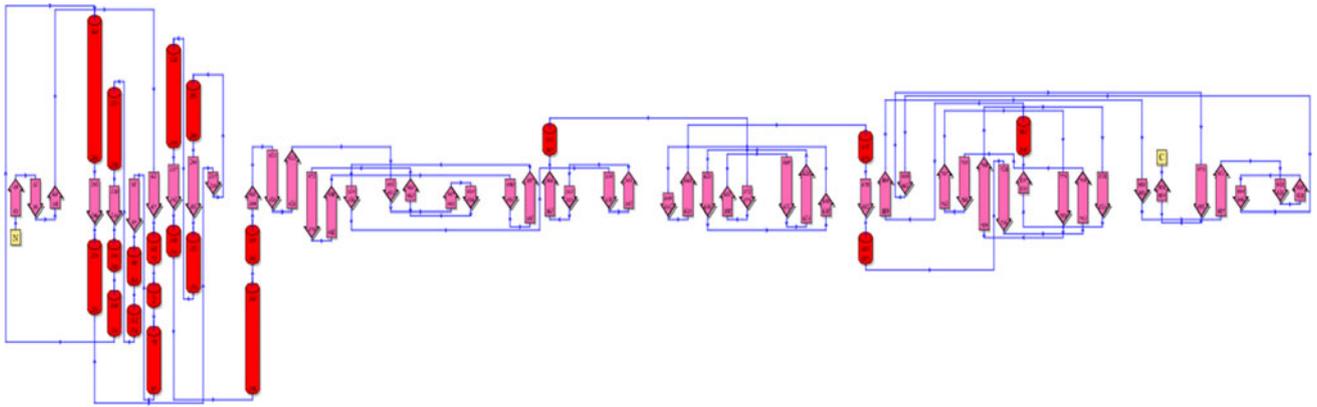


Fig. 4 Topology of the enzyme β -galactosidase showing the pattern alignment of the five constituent domains

Fig. 5 The ball and stick representation of the molecular interaction between β -galactosidase active site Glu200 (dark green) and the ligand β -D-galactose (yellow) (color figure online)

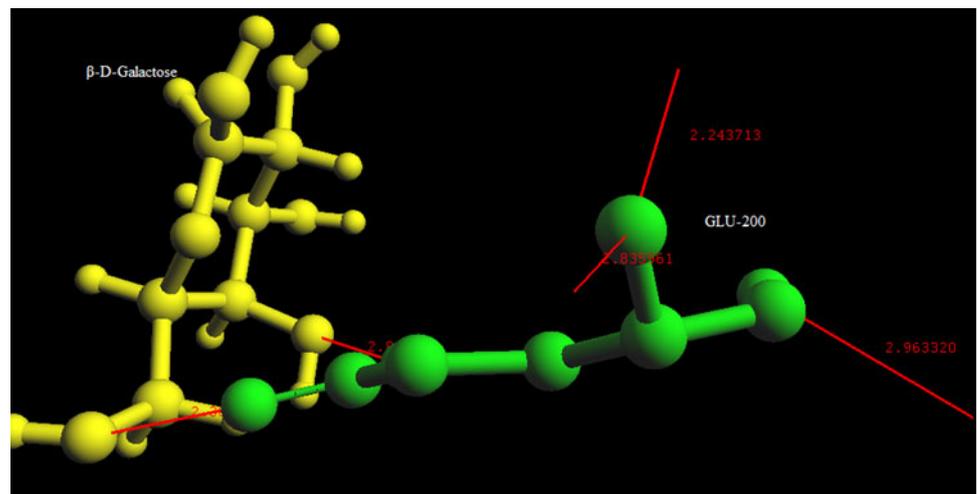


Fig. 6 The binding of β -D-galactose (red solid sphere) at the enzyme active site Glu200 of β -galactosidase (gray solid surface) (color figure online)

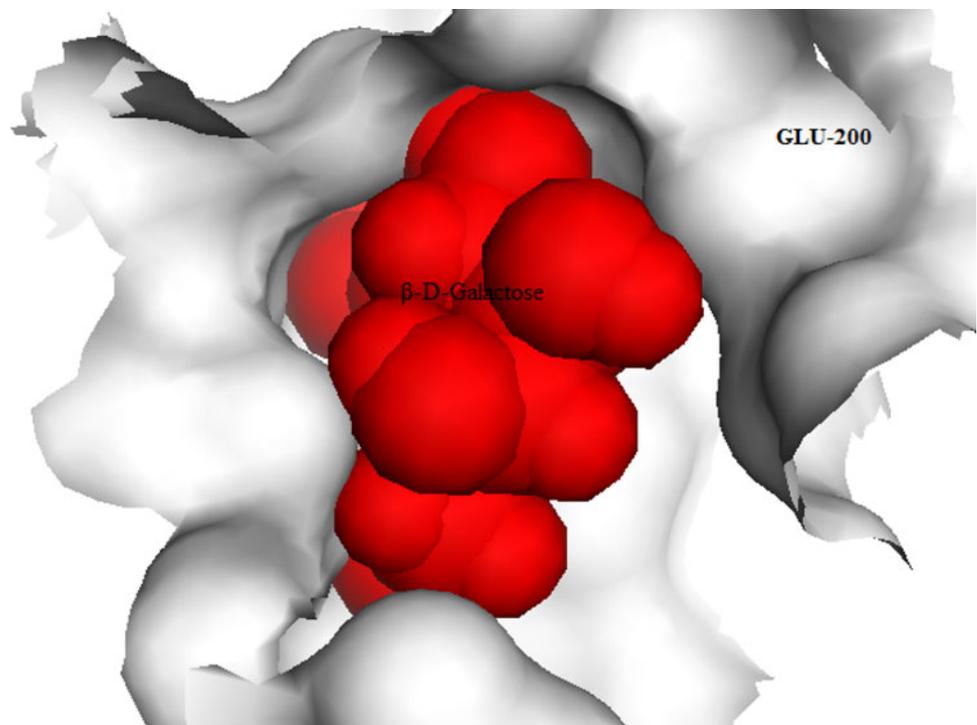


Fig. 7 The figure shows the Ball and stick representation of the molecular docking between β -galactosidase active site Glu298 (bicolor) and the ligand beta-D-galactose (*green*) (color figure online)

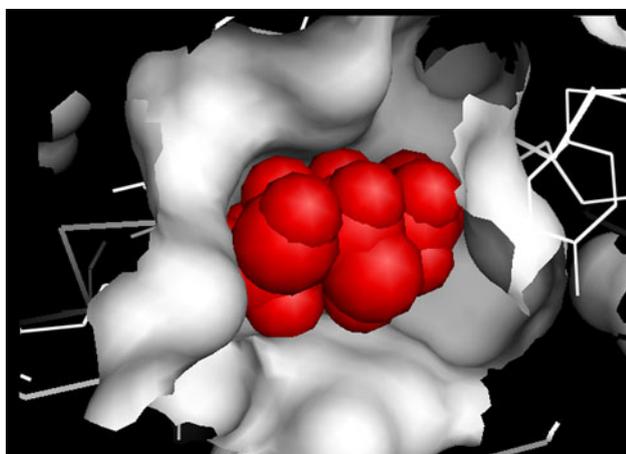
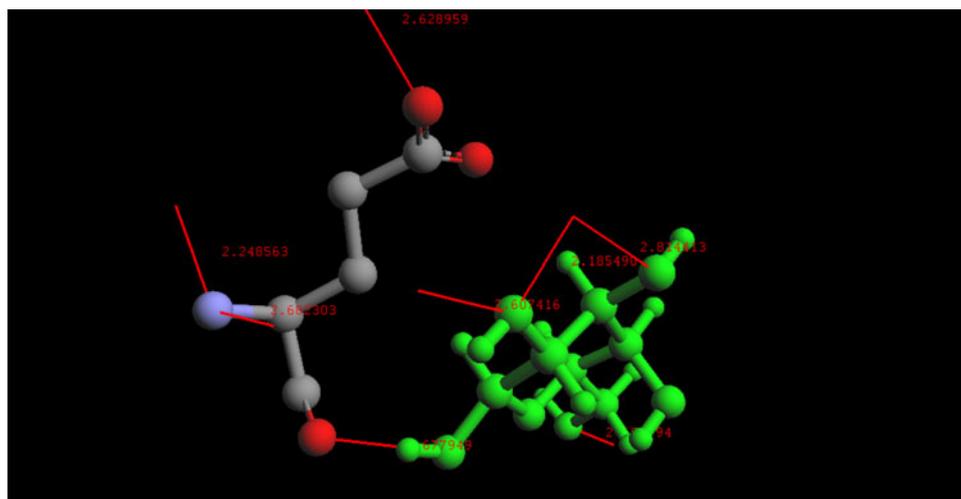


Fig. 8 The figure shows the binding of the ligand (*red solid sphere*) at the enzyme active site of β -galactosidase (*grey solid surface*) (color figure online)

to glycosyl hydrolases family 35 was found from Ser52 to Phe387 residue regions containing the catalytic site Glu200 (Rojas et al. 2004) and Glu298 (Maksimainen et al. 2011). The second domain belonging to β -galactosidase, domain 2 family was found from Tyr395 to Tyr573 residue regions. The third domain belonging to β -galactosidase, domain 3 family was found from Trp574 to Ala658 positions. The fourth and fifth β -galactosidase jelly roll domains belonging to β -galactosidase domain 4 and 5 families were found

from Tyr690 to Leu802 residue regions. The topology of the enzyme structure is illustrated in Fig. 4.

3.3 Structural comparison

The structural comparison of the modeled structure showed highest similarity with crystal structure of *Penicillium* sp. β -galactosidase in PDB database.

3.4 Molecular docking

The catalytic residues (Glu200 and Glu298) and the interacting ligand (GAL) were identified using SAS server. The molecular docking of both the catalytic residues executed separately with GAL showed the best docking score in both the cases (Figs. 5, 6, 7, 8). The bond interactions between the catalytic sites and the ligand are shown in Table 1. Though the bond interactions are efficient at both the sites, the bond formed between the catalytic site Glu200 was found stronger due to two H-bonds in comparison to the other catalytic site Glu298.

4 Discussion

The modeled structure of β -galactosidase from *A. niger* resembled the high-resolution experimental structure of the

Table 1 The table shows the order of H-bonding formed between the two catalytic sites as observed under ArgusLab 4.0.1 Software tool

Enzyme	Ligand (H-bonding atom)		Distance (of the order 10^{-10} m)
Residue	H-bonding atom		
Glu200	Oxygen (no. 7431)	Oxygen (no. 1271)	2.305053
Glu200	Oxygen (no. 7424)	Oxygen (no. 1270)	2.897954
Glu298	Oxygen (no. 7426)	Oxygen (no. 2000)	2.677949

same enzyme from *Penicillium* sp. Hence it could be evidently concluded that the range of functionality of the enzyme from *A. niger* could be enhanced. Also, β -D-galactose was found to behave as a ligand for the enzyme in *A. niger*. β -D-galactose was reported with strong bonding affinities for two different catalytic sites Glu200 and Glu298 of the enzyme, forming a greater association with Glu200 catalytic site. The molecular docking of β -D-galactose with β -galactosidase from *A. niger* was carried out to throw light into the stereotypic behavior of the ligand toward the enzyme binding sites. It was found that β -D-galactose behaved as a potent inhibitor of the enzyme. In cellular pool, where β -galactosidase is not available in its native form, this conclusion was quite far-fetched. Analytically, if β -D-galactose can be made to accumulate in the enzyme activity area, it would compete with the substrate and irreversibly bind with the active site of the enzyme and rendering it inactive for functioning, thereby, causing a feedback inhibition.

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