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Research article

MODELLING AND LIGAND INTERACTION STUDIES OF ENDO-1,4-BETA-XYLANASE FROM BACILLUS SUBTILIS

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ABSTRACT: Xylanase is the name given to a class of enzymes which degrade the linear polysaccharide beta-1,4-xylan into xylose, thus breaking down hemicellulose, which is a major component of the cell wall of plants. The sequence of Xylanase from Bacillus subtilis was obtained from NCBI. The predicted domain was searched to find out the related protein structure to be used as a template by the Basic Local Alignment Search Tool (BLAST) program against Protein Databank (PDB). Sequence that showed maximum identity with high score and less e-value was aligned and used as a reference structure to build a 3D model for Xylanase. In order to understand the mechanisms of ligand binding and the interaction between the ligand and the Xylanase a three-dimensional (3D) model of the Xylanase is generated based on the crystal structure of the Template by using the Modeller. With the aid of the molecular mechanics and molecular dynamics methods, the final refined model is obtained and is further assessed by Profile-3D, which shows that the refined model is reliable. With this model, a flexible docking study is performed with the acetate ion as ligand. After the docking studies, important determined residues in binding are identified. The hydrogen bonds play an important role for the stability of the complex. These results may be helpful for further experimental investigations.

Key words: Acetate ion, Docking, Homology modelling, Modeller and Xylanase.

INTRODUCTION

Bacillus subtilis has many industrial applications and has been used in the food, beverage and detergent industries due to the production of various hydrolytic enzymes such as α-amylase and β-glucanase (1,2) , pectinases (3,1,4), chitinase, β-galactosidase (5,6), many cellulolytic enzymes such as lichenase (a type of β-glucanase), maltase, pectate lyase, endoglucanases with cellulose binding domains and several xylanases. Xyalnases are potentinal used in wood pulp industry, fruit juice industries, production of cheese, bread, modification of starch, saccharification of agricultural and forest wastes and improvement of the nutrional value of agricultural silage. Xylanases and cellulases have been classified into nine families namely based on the sequence similarity and hydrophobic cluster analysis, which are include A, B, C, D,E, F, G, H and I (7). Based on the classification xyalnases are grouped into F or G. (8; 9). Xylanases cleave linear polysacharide hydrolysis of xylopyranosyl linkages of β-(1,4)-xylan to xylo-oligosaccharides and xylose via an acid-base reaction mechanism, similar to that of lysozyme, involving two amino acid residues in their catalytic domains (10). The first residue is an acid catalyst that protonates the oxygen of the glycosidic bond, splitting two cellulose or hemicellulose subunits and forming an oxocarbonium intermediate (10). The second residue acts as a nucleophile, which binds with the oxocarbonium intermediate and promotes the formation of an OH- from a water molecule which converts the intermediate into a free xylobiose subunit (10). The present study to understand the mechanisms of acetae ion binding and the interaction between the acetae ion and the Xylanase a three-dimensional (3D) model of the Xylanase is generated based on the crystal structure of the Template by using the Modeller and these studies helpful for the further experimental investigations.

METHODS

TEMPLATE SEARCH AND SEQUENCE ALIGNMENT

The protein sequence of *B. subtilis* was retrieved from Uniprot database (accession ID E8VJS2) in FASTA format. The template structure for homology modelling was selected with the help of BLAST (11) search against PDB database. Based on maximum identity with high score and lower e-value, xylanase from glycoside hydrolase family 5 at 1.42 Å resolution (PDB code: 1NOF) was used as template. Query cover was 90% over the length of 281 residues starting from 36 amino acid to the 419 of query sequence.

THREE DIMENSIONAL STRUCTURE GENERATION AND VALIDATION

The academic version of MODELLER 9 v.6 (*http://www.salilab.org/modeller*) was used for 3D structure generation based on the information obtained from sequence alignment (12). The homology modelling method is based on the assumption that the structure of unknown protein will be similar to the known structure of some reference proteins. Out of the five models generated by MODELLER, the one with best G-score of PROCHECK (13) and with best VERIFY3D profile (14) was selected as final model.

ACTIVE SITE IDENTIFICATION

Active site of xylanase (E8VJS2) was identified using CastP server (15). This program predicts automatically locating and measuring protein cavities, based on precise computational geometry methods. CAST identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings**.**

DOCKING METHOD

The ligands, including all hydrogen atoms, were built and optimsed with chemsketch software suite. Extremely Fast Rigid Exhaustive Docking (FRED) version 2.1 was used for docking studies (OpenEye Scientific Software, Santa Fe, NM). It is an implementation of multiconformer docking, meaning that a conformational search of the ligand is first carried out, and all relevant low-energy conformations are then rigidly placed in the binding site. The FRED process uses a series of shape-based filters, and the default scoring function is based on Gaussian shape fitting**.**

RESULTS

Homology Modelling of Xylan**ase** Domain

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the results of BLAST search against PDB, only the 1NOF which has a high level of sequence identity with the E8VJS2 domain. Structurally conserved regions (SCRs) for the model and the template were determined by superimposition of the two structures and multiple sequence alignment. In the following study, we have chosen 1NOF as a reference structure for modeling domain. Coordinates from the reference protein (1NOF) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. In the modeller we will get a 20 PDB out of which we select a least energy .The energy unit will be in kilo joule. All side chains of the model protein were set by rotamers. The final stable structure of the E8VJS2 protein obtained and the help of SPDBV it is evident that protein domain has 11 helices and 20 sheets and it is shown in the Figure 1.

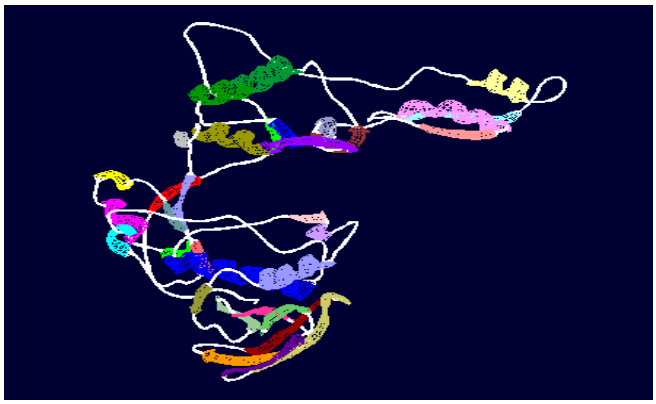

Figure 1: Final Refined Structure of xylanase

The final structure was further checked by verify3D graph and the results have been shown in Figure 2: The overall scores indicates acceptable protein environment.

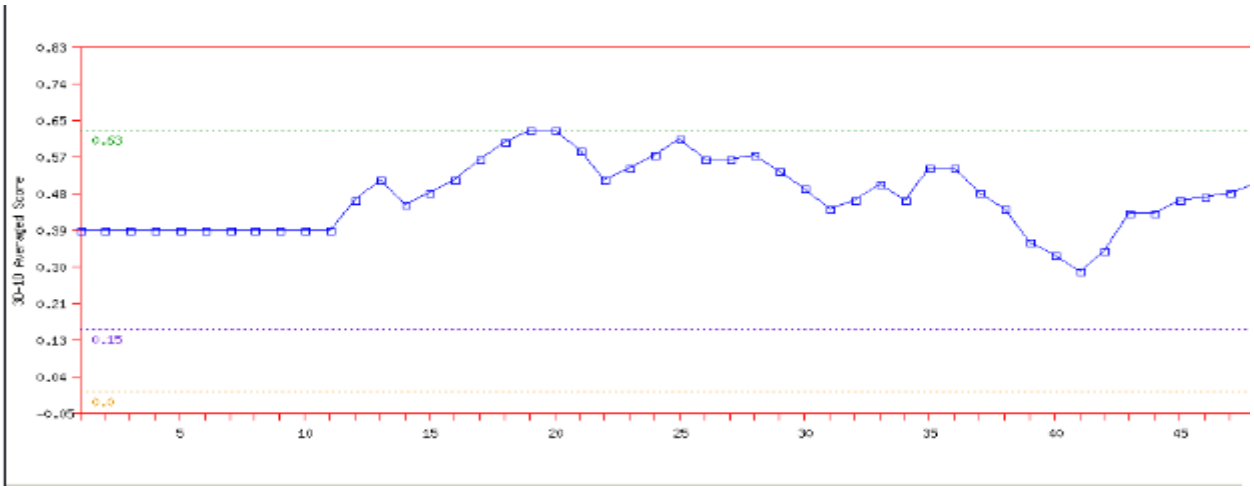

Figure 2: Graphical Representation For Verify3D

Validation of xylanase Domain

After the refinement process, validation of the model was carried out using Ramachandran plot calculations computed with the RAMPAGE Server. The ψ and φ distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Table 1. The RMSD (Root Mean Square deviation) deviation for covalent bonds and covalent angles relative to the standard dictionary of xylanase (E8VJS2) was -5.27 and -0.55 Å. Altogether 96.2 % of the residues of xylanase (E8VJS2) was in favored and allowed regions. The overall PROCHECK G-factor of xylanase (E8VJS2) was – 2.32 and verify3D environment profile was good.

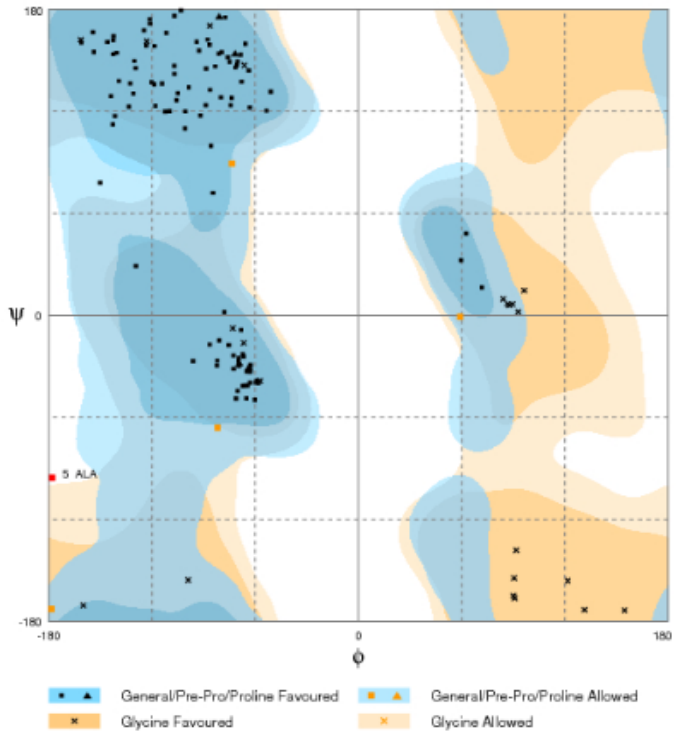

Figure 3: Ramachandran Plot Using RAMPAGE Server

Table 1: % of residue falling in the core region of the Ramachandran's plot

% of residue in most favored regions	96.2
% of residue in the additionally allowed zones	3.0
% of residue in the generously regions	0.0
% of residue in disallowed regions	1.1
% of non-glycine and non-proline residues	100.0

Active site Identification of E8VJS2 domain

After the final model was built, the possible binding sites of E8VJS2 was searched based on the structural comparison of template and the model build and also with CASTP server and was shown in Figure 4. Since, E8VJS2 from Bacillus substilis and the 1NOF are well conserved in both sequence and structure; their biological function should be identical. It was found that secondary structures are highly conserved and the residues, GLY 26, PHE 27,VAL 29,GLU 31,ALA 42,GLY 43,PRO 44,PHE 46,ASN 47, ARG 51, LYS 52,GLY 54, GLY 55,PRO 56,GLU 60,ARG 61,VAL 63, GLY 64,ASP 65,GLY 67,THR 98 ,VAL 100,VAL 101,GLU 103,LYS 104,ASP 106,GLY 109,GLY 112,SER 116,SER 117,LYS 118,THR 119,GLY 120,ALA 122,GLY 123 ,SER 124, ARG 125, ASN-207, PHE-206, GLY-229, GLY-228, ILE-227, SER-203, GLU-202, ALA-201, VAL-200.

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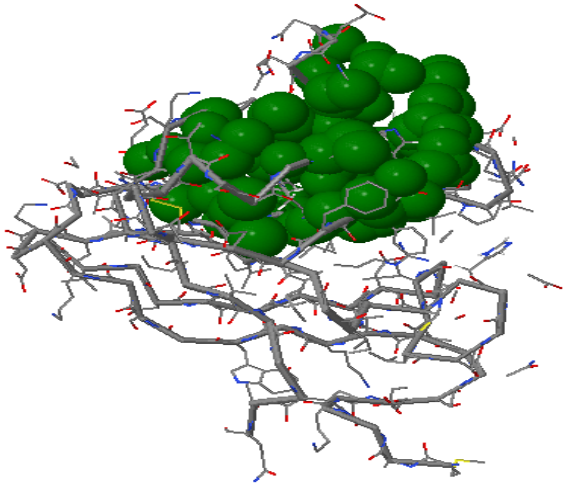

Figure 4: Active site of E8VJS2 (Green color indicates active Site Regions)

Ligand used for docking:

The interaction between active site lining residues and substrates plays an important role in any catalytic reaction. Such interactions can be studied by calculating the number of hydrogen bonds and hydrophobic interactions between substrate molecules and enzyme residues within the active site. Fig. 6 shows acetone molecule docked in active site of enzyme. VAL 200, ALA 201, GLU 202, SER 203, PHE 206, ASN 207, LEU 228 GLY 228 and GLY 229 amino acids were involved in the catalytic site.

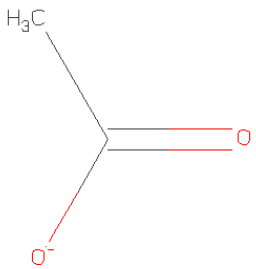

Fig 5: Acetate ion

Docking results:

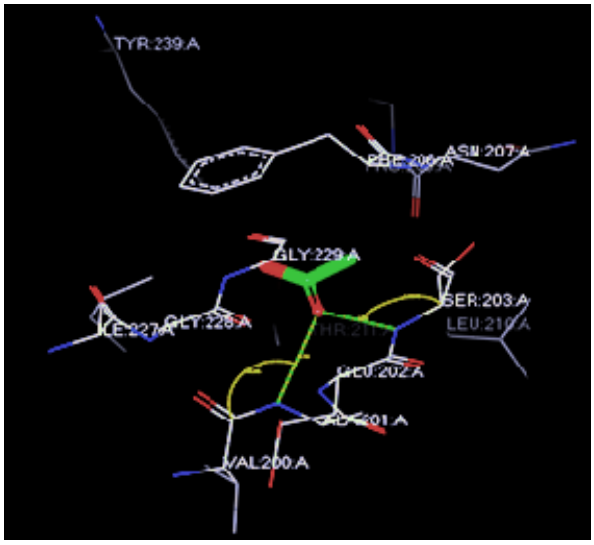

Fig 6: Docking of Acetate ion to the Xylanase

CONCLUSION

Xylanase (E8VJS2) hydrolyzes xylans, indigestible components of plant fibers. In this work, we have constructed a 3D model of Xylanase domain, from Bacillus substilis using the MODELLER software and obtained a refined model after energy minimization. The final refined model was further assessed by ERRAT & PROCHECK program, and the results show that this model is reliable. The stable structure is further used for docking of substrate with Acetate ion. Docking results indicate that conserved amino-acid residues in Xylanase main play an important role in maintaining a functional conformation and are directly involved in donor substrate binding. The interaction between the domain and the inhibitors proposed in this study are useful for understanding the potential mechanism of domain and the inhibitor binding. As is well known, hydrogen bonds play important role for the structure and function of biological molecules. In this study it was found that ALA-201 and SER-203 of Xylanase are important for strong hydrogen bonding interaction with the inhibitors. To the best of our knowledge ASN-207, PHE-206, GLY-229, GLY-228, ILE-227, SER-203, GLU-202, ALA-201, VAL-200 are conserved in this domain and may be important for structural integrity or maintaining the hydrophobicity of the inhibitor-binding pocket. The Acetate ion showed best docking results with target protein.

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