Thermostability and Substrate Specificity of GH-11 Xylanase from *Thermomyces lanuginosus* VAPS24

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Abstract The three dimensional structure (3D structure) of GH-11 xylanase from *Thermomyces lanuginosus* was obtained through homology modeling. To study the enzyme interaction with an end product of enzyme catalysis, the xylanase two sugar molecules xylose and xylobiose has been docked into the active site of GH-11 xylanase through molecular docking. Based on the free binding energy and Inhibition constant, concluded xylose makes more stable complex than xylobiose. Further, the molecular dynamic simulation studies were carried out at different temperature, i.e. 323, 333, 343 and 353 K (i.e. 50, 60, 70 and 80 °C). It has been observed that there was minor structural modification in 3D-structure of xylanase at 323, 333, and 343 K. But the helix and sheets moved out of the initial structure when simulation carried out at during 353 K (80 °C).

Keywords Homology modeling · Molecular dynamic simulation · GH-11 xylanase · *Thermomyces lanuginosus*

Xylanases are hydrolytic enzymes which carry out breakdown of β -1, 4-xylosidic bonds of xylan straight chain [1]. Scientifically, xylanases also named as endo-1, 4- β -xylanase together with other synonyms such as endoxylanase, β -1,4-D-xylan-xylanohydrolase, xylanase, β -xylanase and β -1, 4-xylanase [2]. Xylanase bidegradation requires a combined action of several hydrolytic enzymes. Xylanase has been produced by various microbial communities such

as fungi, bacteria, actinomycetes and yeast [3]. Among filamentous fungi, *Thermomyces lanuginosus* a thermophilic fungus has known as potent producer of xylanase [4, 5]. Nowadays, the importance of xylanases has been increased due to its potential applications in several industries such as textile industry, bakery and breweries, pulp and paper industry, biofuel industry, food and feed industry [6]. Thermostability of xylanases play crucial role in industrial applications. In the current study, thermostability of xylanase has been predicted on the basis of their structural modification during molecular dynamic simulations at different temperatures. The xylanase 3D-protein structure interaction with an end product of xylanolytic hydrolysis also helps to predict the shift of enzymatic reactions leads towards the important assumptions.

Molecular docking of xylanase from *T. lanuginosus* VAPS24 with xylobiose and xylose, which were retrieved from zinc database, was carried out with AutoDock 4.2.5.1. Lamarckian genetic algorithm (LGA) has been employed to carry out docking using Autodock 4.2.5.1. A @ default value of 0.375 Å has been set for spacing between grid points. The central grid box has been set at 0.104 Å $\times$ 1.449 Å $\times$ 16.323 Å (x, y, and z axis) spacing to accommodate complete residues of amino acids associated with the protein molecule. A complete set of 50 autonomous runs has been carried out with translation step size of 0.2 Å and 50 for torsions and orientations. About thousand generations have been put as the highest number of generations and automatically survived top individuals maximum number the was set as to 1 and with other conditions as cluster tolerance: 0.5 Å, crossover rate: 0.8, external grid energy: 1000; mutation rate: 0.02.

GROMACS v. 4.5 packages with the force field was employed for xylanase protein simulation [7–9]. SPC water model in a cubic box (10.8 $\times$ 10.8 $\times$ 10.8 nm³) was used

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Table 1 Docking score and interaction of modeled thermophilic xylanase with xylose and xylobiose

Substrate	Binding energy	Inhibition constant	Interacting residue
Xylose	− 3.76	1.75 μ M	thr40
Xylobiose	− 2.88	7.75 μ M	gly113, his115

to perform protein–ligand solvation. To carry out neutralization of solvated system (1 NA+) as counter-ion was added. In order to check inappropriate geometry or steric

clashes in a solvated system of protein, the complex has been subjected to minimization of energy by the steepest algorithm up to 25,000 steps at the higher end. The 400

Fig. 1 Docking of modeled GH-11 Xylanase from *T. lanuginosus* VAPS24 with **a** xylose and **b** xylobiose **Fig. 2** Root mean squared fluctuation (RMSF) for each residue in protein complex at **a** 50 °C, **b** 60 °C, **c** 70 °C, **d** 80 °C

steps required to reach minimization conditions were scrutinized for the protein ligand complex. The present step was pursued by equilibration of the whole system by means of both NVT and NPT ensembles for 50,000 (100 ps) and 25,000 steps (50 ps) respectively at 300 K and 1 ATM. Primarily the system was equilibrated and then molecular dynamics simulation was executed for 2.5 ns. The analysis of the simulations RMSD, RMSF and Hydrogen bonds, plots were computed for the total time of simulations. Molecular dynamic simulation was carried out at different temperature, i.e. 323 K (50 °C), 333 K (60 °C), 343 K (70 °C) and 353 K (80 °C).

The binding energy of modeled structure and xylose was observed as $-3.76 \text{ kcal mol}^{-1}$ whereas the binding energy of modeled structure and xylobiose was observed as $-2.88 \text{ kcal mol}^{-1}$ showed in Table 1 reveals that xylose shows higher binding affinity as compared to xylobiose. Hydrogen bonds are formed during the interaction between Thy40 of the modeled structure with xylose (Fig. 1a). The amino acids involved in the interaction and the formation of hydrogen bonds between modeled structure and xylobiose were Gly113, His115 (Fig. 1b). The binding energy here refers towards the stability of the enzyme–substrate

interactions. Three-dimensional (3D) protein structure of GH-11 xylanase from *Bacillus firmus* K-1 were docked with various oligos such as xylobiose, xylotriose, xylotetrose, xypentose, xylohexose xyloheptaose by the Jommuengboub et al. [10] and free energy binding reveals that xylobiose and xylotriose acted as the end product inhibitors. The xylanase from *Bacillus pumilus* has been sequenced and tertiary structure of protein has been docked to observe catalytic interactions by Lin et al. [11]. The information collected from molecular docking were analyzed and found involvement of various amino acid residues towards its vital role in catalytic reaction which instructs to incorporate a further modification in xylanase for site directed evolution of xylanase [11].

The ligand bound protein complex was analyzed after simulation for minimization of energy to stabilize the complex. During the first ns (nanosecond) 1.5 Å deviations in protein complex has been observed and the deviation continued for 0–2.5 ns, after that it acquired a steady conformation for the remaining trajectory. Hydrogen bonds and RMSF per residue has been calculated for all through simulations among ligand and protein (Fig. 2). The root mean squared fluctuation (RMSF) and root mean squared

Fig. 3 Root mean squared deviation (RMSD) for all residue in protein complex at **a** 50 °C, **b** 60 °C, **c** 70 °C, **d** 80 °C

Fig. 4 **a** Structures of Xylanase GH-11 from *T. lanuginosus* VAPS24 orange color presents the structure at 300 K (27 °C) and yellow color 323 K (50 °C). **b** Structures of Xylanase GH-11 from *T. lanuginosus* VAPS24 orange color presents the structure at 300 K (27 °C) and yellow color 333 K (60 °C). **c** Structures of Xylanase GH-11 from *T. lanuginosus* VAPS24 orange color presents the structure at 300 K (27 °C) and yellow color 343 K (70 °C). **d** Structures of Xylanase GH-11 from *T. lanuginosus* VAPS24 orange color presents the structure at 300 K (27 °C) and yellow color 353 K (80 °C). Shift in the position of helix is shown in red circle

deviation (RMSD) for each residue has been calculated and depicted in Figs. 2 and 3. Generally, amino acid residues of representing loops have elevated RMSF values and the residues residing in the interior regions of protein have low RMSF. Molecular dynamic simulation was carried out at different temperatures as explained in Fig. 4. There was very little change in the structure of the xylanase from *Thermomyces lanuginosus* at 323, 333, 343 K (50, 60, 70 °C) but during the 353 K (80 °C) the helix and sheets moved out of the initial structure Fig. 4. Thus the simulation could lead to point the stability of the enzyme at higher temperature. The similar report were given by Purmonen et al. [12] to compare mesophilic and thermophilic xylanase of the GH-11 family and showed higher thermostability of thermophilic proteins rather than mesophilic one. The in silico results are fairly agreed with the wet lab experimental results as wet lab data showed that, the xylanase from *T. lanuginosus* VAPS-24 showed thermo stability at temperature ranges from 50 to 70 °C and temperature optima for xylanase activity was found 65 °C which is already reported by Kumar et al. [5]. However, it was found stable only for an hour at 80 °C which strengthens the in silico results.

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