

Molecular Dynamics Approach in Designing Thermostable *Bacillus circulans* Xylanase

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Abstract — We have applied molecular dynamics methods as a tool in designing thermostable *Bacillus circulans* Xylanase, by examining Root Mean Square Deviation (RMSD) of enzymes structure at its optimum temperature and compare with its high temperature behavior. As RMSD represents structural fluctuation at a particular temperature, a better understanding of this factor will suggest approaches to bioengineer these enzymes to enhance their thermostability. In this work molecular dynamic simulations of *Bacillus circulans* xylanase (BcX) have been carried at 318K (optimum catalytic temperature) and 343K (BcX reported inactive temperature). Structural analysis revealed that the fluctuations of the β -sheet regions are larger at higher temperatures compared to the fluctuations at optimum temperature.

Keywordst; *thermostable xylanase; in silico design; bacillus circulans xylanase*

I. INTRODUCTION

Enzyme applications in industrial processes have become very important as it is safe and environmental friendly, besides having cost advantages compared to the chemical reagents. However, the enzymes have their characteristic temperature and pH ranges as well as the substrate specificity. These properties make it unique and at the same time, limit the function of enzyme itself.

In particular, xylanases have been proposed for the pulp and paper industry as an effective bio-reagent to achieve biobleaching in place of environmentally hazardous chlorine compounds which have been conventionally used to achieve pulp brightness in the manufacture of high-quality paper products. But xylanases have to be functional at 60-70° C, which is the temperature of the incoming pulp for the bleaching operation

In this research *Bacillus circulans* xylanase (BcX) was selected and it is a 20.4-kDa endo-(1,4)- β -xylanase belonging to the Family 11 of glycosyl hydrolase [1]. This enzyme catalyzes the hydrolysis of the polysaccharide xylan, a major constituent of plant biomass. BcX and several homologous xylanases from *Aspergillus*, *Bacillus*, *Thermomyces*, and *Trichoderma sp.* have been characterized extensively in terms of structure and enzymology [2-3].

Selected system which is from Family 11 xylanases has several advantages over other xylanases in pulp bleaching applications. Most of the Family 11 xylanases are smaller than xylanases in other families. The small size relative to other xylanases is beneficial in penetrating the pulp fibers to release xylan from the pulp and enhance the bleaching. The Family 11 xylanases are also "pure" xylanases in terms of their catalytic

activity. Unlike the xylanase enzymes in other families, these enzymes hydrolyze only xylan and do not hydrolyze cellulose. Cellulose hydrolysis damages the pulp and is unacceptable in a commercial mill.

Bacillus circulans xylanase has all the advantages in term of size and pH optimum which is reported to be 7.0-8.0 that meet the bleaching requirement. But the optimum temperature of this enzyme is 45°C which hampers its potential use in pulp bleaching industry. Thus there is a great need to design a new xylanase enzyme from *Bacillus circulans* that has the optimum temperature of 60 - 70° C, to take advantage of its ideal size and existing state of pH optimum.

Much work has been done to engineer enzymes to achieve the desirable state through mutations [4-5]. During past years many research groups have identified specific mutations that improve enzyme properties such as selectivity, activity, alternate catalytic route and thermal stability [6]. However, these random mutation approaches are too costly and time consuming. Computer aided design approaches offer an inexpensive means to narrow the set of possible solutions quickly, and then follow them up with experimental testing.

In this research we have carried out molecular dynamics [7-8] simulations and identified the region that has potential to be mutated by analyzing the root mean square deviation (RMSD) of backbone, helical, sheets, turn and coil of BcX at its optimum temperature (318K) in comparison with its inactive temperature (343K).

II. METHODOLOGY

The initial atomic coordinates of BcX were obtained from Protein Data Bank [9] file 1BVV. Explicit solvent water box added to the structure and the protein charge was neutralized by inserting NaCl salt. All dynamics simulation was carried out using NAMD software package with CHARMM parameter force field [10]. The structure was minimized using conjugate gradient iteration method [11] and heated using velocity reassigning method, before equilibration. The simulation were carried out for the total duration of 2ns at 318K and 343K

III. RESULTS AND DISCUSSION

Fig. 1 shows the root mean square deviation (RMSD) of BcX during 2ns simulation at 318K and 343K as a function of time. There is no significant change in the backbone fluctuations of the whole enzyme.

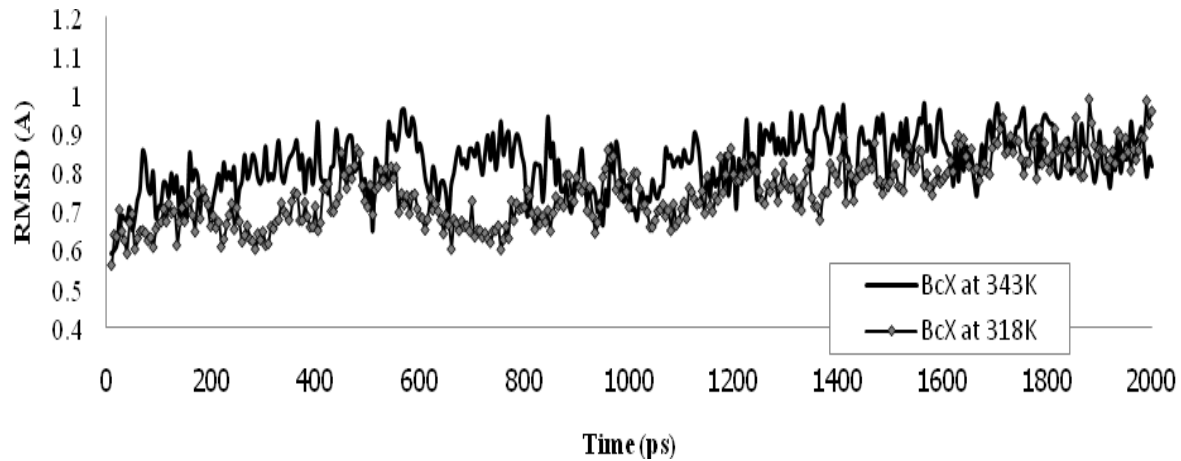


Figure 1: RMSD backbone at 318K and 343K of *Bacillus circulans* xylanase

However, a closer examination of the RMSD of the helical (Fig. 2), β -sheet (Fig. 3) and the coil regions revealed that β -sheet shows larger fluctuations at higher temperatures compared to any other region in the molecule.

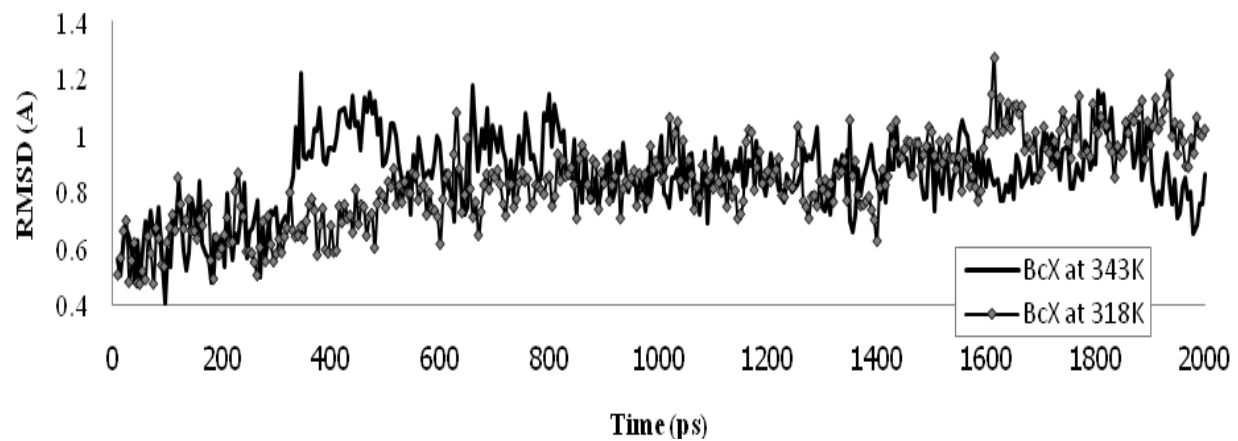


Figure 2: RMSD for α -helix at 318K and 343K of *Bacillus circulans* xylanase

While still there is a need for detailed investigation of the reasons for this difference in the behavior of the fluctuations in the β -sheet region compared to the other region of the enzyme, we suggest that any attempt to design thermostable BcX should include mutation that could retain the β -sheet

fluctuation of BcX at the similar level as that of fluctuations at the optimum temperature. We are currently undertaking *in silico* mutational studies to identify the mutations that will bring about this effect.

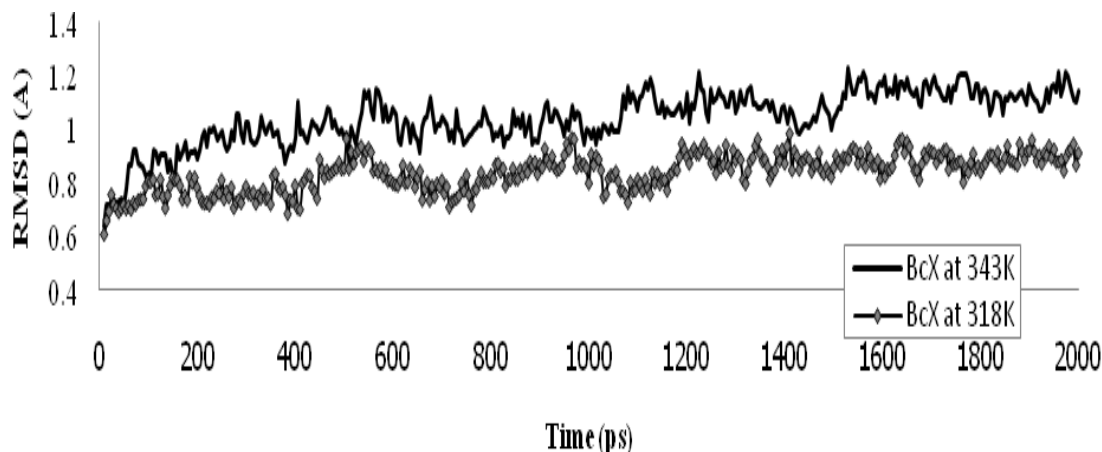


Figure 3: RMSD for β -sheets at 318K and 343K of *Bacillus circulans* xylanase

As RMSD of β -sheets showed significant difference at higher temperatures compared other regions of the enzyme, we conclude that β -sheets region contribute significantly to the thermostability of BcX. Mutagenesis should be targeted to amino acid residues within β -sheets in BcX structure with the objective of maintaining the β -sheet regional fluctuation to the similar level as that of fluctuations at 318K. If that can be achieved, we propose that such mutant BcX will be the thermostable and suitable for use in the pulp industry. Further work in this direction is in progress.

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