Australian Journal of Basic and Applied Sciences, 6(1): 89-92, 2012
ISSN 1991-8178

Structural And Dynamics Behavior Of Native Endoglucanase From Fusarium Oxysporum

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Abstract: Molecular dynamics methods are very useful tool in understanding the behavior of the enzymes at higher temperatures. In this work we employ molecular dynamics simulation of an endoglucanase from *Fusarium oxysporum* to examine its structural and dynamics behavior at 80°C, by analyzing the root mean square derivation (RMSD) from the initial structure. The RMSD values of coil and turn regions are found to be higher compared to helix and β-sheet regions. The surface area of the structure is found to have larger RMSD compared to the buried part of the enzyme, due the β-jelly roll nature of the enzyme. For the same reasons, the number of hydrogen bonds between among residues in β-sheet is found to larger compared to those in the coil regions. However, the number of hydrogen bonds between water and proteins is highest in turn regions and lowest in helix regions. The turn regions connecting the 3_{10}-helix are found to fluctuate more rapidly compared to the other parts of the enzyme. These factors can explain the loss of the activity of the enzyme at high temperatures.

Key word: Endoglucanase, Molecular dynamics simulation, turn regions, Cel7B, hydrogen bonds.

INTRODUCTION

Molecular dynamics simulation has becomes an important tool for studying and understanding the dynamical behavior of macromolecules (Leach, 2001; Noorbatcha, *et al.,* 2010). Enhancing enzyme properties and functionalities via molecular dynamics studies is one of the tools available in computational protein engineering methods.

Endoglucanase (EG) is a key component of cellulase which degrades the cellulose material polymer to be smaller monomers and have been used in various industries such as in textiles, fruits and vegetable processing, and recently in bioethanol production (Reily, *et al.,* 2004; Kumar, *et al.,* 2009). Due to these essential applications of endoglucanase, cellulase and xylanase, various protein engineering approaches have been employed to enhance the functionality and properties of these enzymes to achieve better performance.

Fusarium oxysporum is a good source of endoglucanase but the EG thus secreted lacks activity at high temperatures. One of the experimental studies has shown this enzyme becomes inactive at 80°C (Vlasenko *et al.,* 2010). We have investigated the dynamical behavior of endoglucanase from *Fusarium oxysporum* (EGFO) at 80°C by carrying out the molecular dynamics simulation of EGFO in water as the solvent medium. It is has been shown that the different parts of the enzymes responds differently at higher temperatures and these contrasting effects can be useful in understanding the changes in the activity of the enzymes at higher temperatures.

MATERIAL AND METHODS

Three dimensional structure of native endoglucanase from Fusarium Oxysporum (PDB code: 3OVW) (Sulzenbacher *et al.,* 1997) was placed into 72576 water molecules in a box of dimension 102Å *x*91Å*x*90Å. The system is made neutral by adding 10 Na and 12 Cl ions in place of 22 water molecules. The structure was first minimized by using velocity quenching method followed by conjugate gradient method. The minimized structure was heated up to 353K by using reassignment of velocities at periodical steps.

The equilibration and dynamics production steps were performed using Langevin dynamics to maintain target temperature. All dynamics simulation was carried out by NAMD (Phillips, *et al.,* 2005) with CHARMM parameter force field (MacKerel, *et al.,* 1998; Mackerel, *et al.,* 2004) while VMD (Humphrey, *et al.,* 1996) were used in the analysis dynamics results. Root mean square deviation (RMSD) of each region (helix, β-sheet, coil and turn), number of hydrogen bonds at different segments of the structure and secondary structure during dynamics simulation were analyzed to observe structural and dynamics behavior of this enzyme at 80°C.

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RESULTS AND DISCUSSION

The dynamic behavior of the enzymes are analyzing by calculating the root mean square derivation (RMSD) from the initial structure. There are significant differences in the RMSD values of each region as shown in Fig.1. RMSD values in the coil and turn regions found to be higher than in helix and β-sheet, implying that compared to the helical or β-sheet regions of the enzyme structure, coil and turn regions on EGFO structure shows much larger fluctuations at 80°C. The surface area of endoglucanase from *Fusarium oxysporum* is found to show larger fluctuation compare to that in the buried area as shown in Fig. 2.

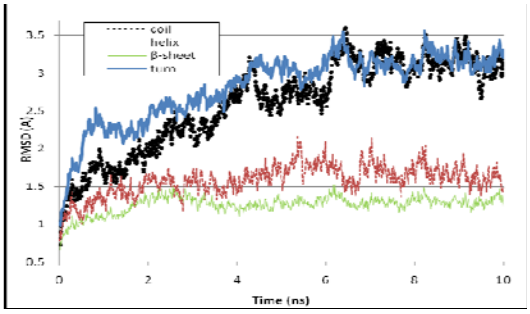

Fig. 1: Root mean square derivation (RMSD) as a function of time for different structural regions of EGFO at 353K.

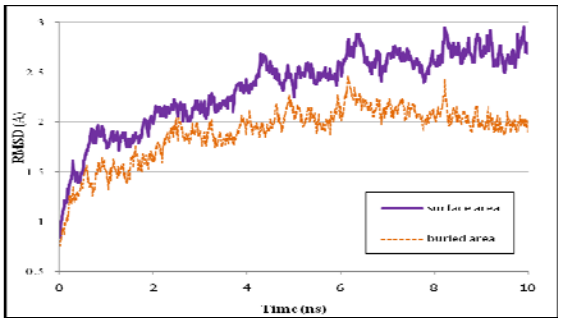

Fig. 2: Root mean square derivation (RMSD) as a function of time, for surface and buried areas of EGFO at 353K,

An examination of the total number of hydrogen bonds shows that number of hydrogen bonds between amino acid residues and water molecules is highest in turn regions and lowest in helix regions, compare to that in other regions. However, the number of hydrogen bonds between amino acid residues within the β-sheet regions of EGFO is highest and those within the coil region are the lowest (Fig.3). The larger surface and the large number of hydrogen bonds is consistent with the β-jelly roll structure of the enzyme.

(A) (B)

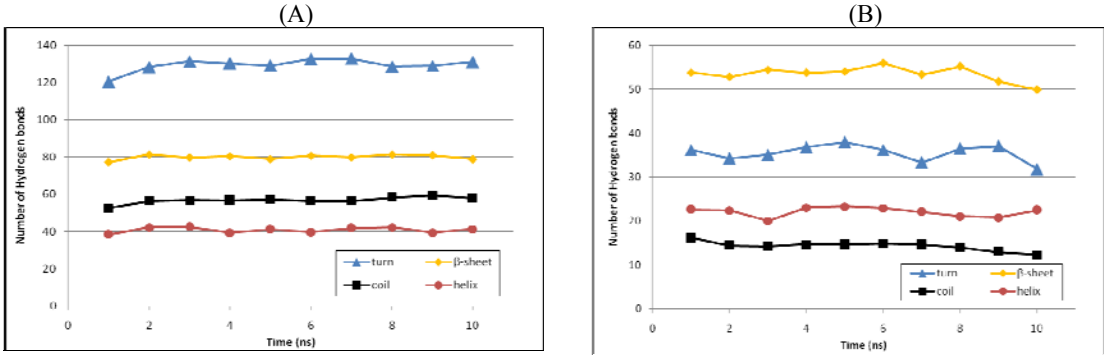

Fig. 3: (A) Number of hydrogen bonds between amino acid residues in each region of the enzyme and water molecules as a function of time for EGFO at 353K; (B) Number of hydrogen bonds between amino acid residues within each region of EGFO at 353K.

The snap shots of the enzyme at various time intervals during dynamics simulation at 80°C are shown in Fig 4. The turn region connecting the 3_{10}-helix in the enzyme is found to show significant changes as a function of time. The existence of large fluctuations in the turn regions has been already demonstrated in Fig. 1. A careful analysis of the snap shots of the enzyme dynamics reveals that a major contribution of the large fluctuations originates from the turn regions connecting the β-sheet and the 3_{10}-helix. These rapid changes of the turn region linking the β-sheet and the 3_{10}-helix can change the active conformation necessary for the activity of the enzyme leading to decreased enzyme activity at higher temperatures.

Conclusions:

We have examined the changes in the dynamical behavior of the EGFO at 80°C. It has been found that different regions of the enzymes behave differently. Hence a more detailed analysis, rather than the overall backbone behavior of the enzyme is necessary to understand the dynamical factors influencing the activity and the stability of the enzymes. The number of the hydrogen bonds between the amino acid residues and water molecules in the turn regions of EGFO is found to be highest. Turn regions show larger fluctuation than helix and β-sheet regions. A major contribution for these fluctuations arises from the rapid changes the turn regions linking the β-sheet and the 3_{10}-helix. These effects could potentially disrupt the active conformation of the enzyme, leading to the loss of enzyme activity at higher temperatures.

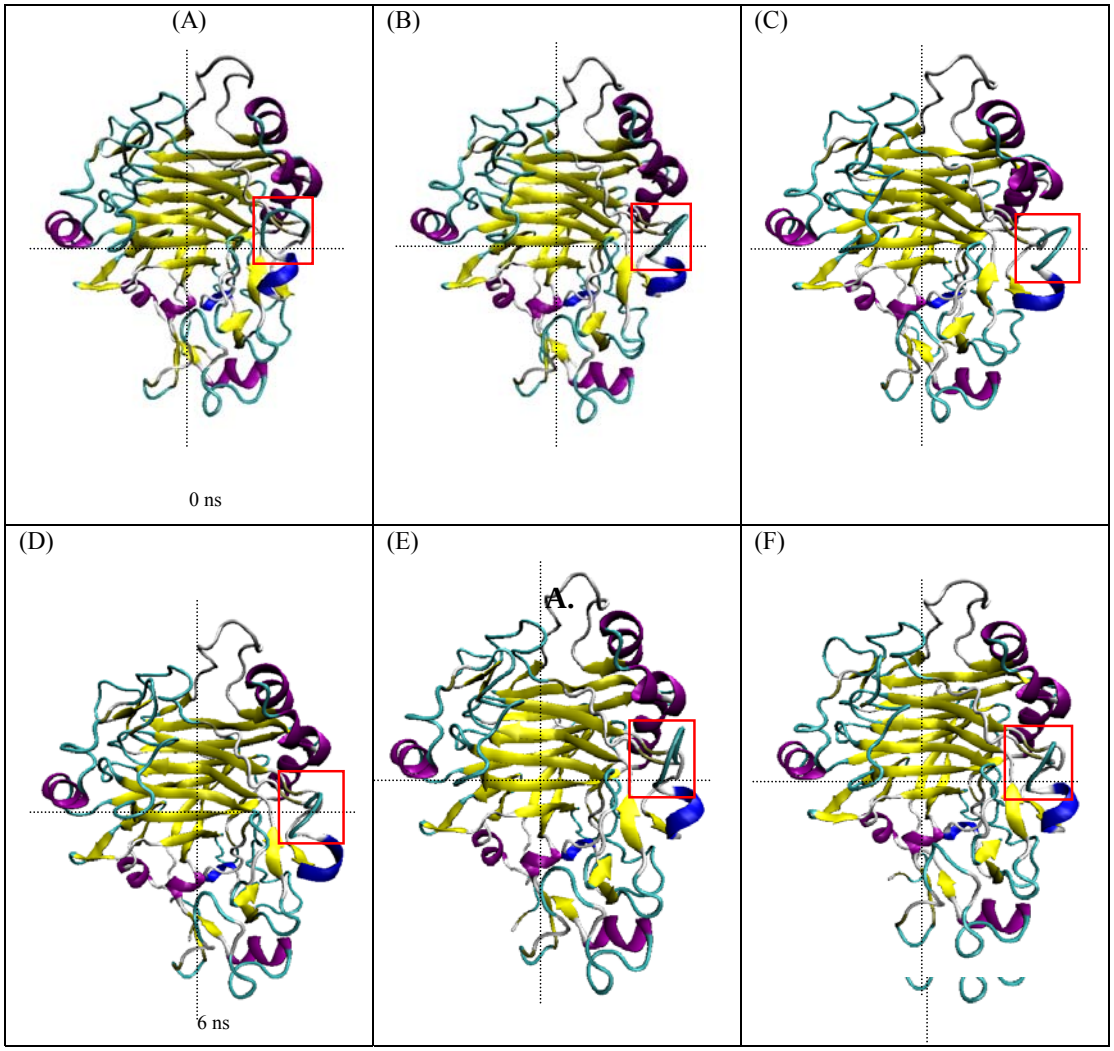

Fig. 4: Snap shots of the cartoon diagrams of the 3D-structure of EGFO at (A) 0 ns (B) 2 ns, (C) 4 ns (D) 6 ns, (E) 8 ns and (F) 10 ns of dynamics simulation. β-sheet regions = yellow, Coil regions = white, Turn regions = cyan, 3_{10}-helix regions = blue and α-helix = purple.

ACKNOWLEDGEMENTS

The authors thank Research Computational Unit (RCU) in Research Management Office (RMO) for providing high performance computing (cluster) to run all dynamics simulations. This research was supported by research grants from International Islamic University Endowment Fund (EDW Type B) and Fundamental Research Grant Scheme (FRGS No. 0510-121) from Ministry of Higher Education Malaysia.

REFERENCES

Humphrey, W., A. Dalke, and K. Schulten, 1996. VMD: Visual molecular dynamics. J. Mol. Graph., 14: 33-38.

Kumar, S., S.P. Singh, I.M. Mishra and D.K. Adhikari, 2009. Recent Advances in Production of Bioethanol from Lignocellulosic Biomass. Chem. Eng. Technol., 32: 517-526.

Leach, AR., 2001. Molecular Modelling: principles and applications. Pearson Education Ltd.

MacKerel, Jr.A.D., D. Bashford, M. Bellott, Jr.R.L. Dunbrack, J.D. Evanseck, M.J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F.T.K. Lau, C. Mattos, S. Michnick, T. Ngo, D.T. Nguyen, B. Prodhom, W.E. Reiher III., B. Roux, M. Schlenkrich, J.C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiorkiewicz-Kuczera, D. Yin, M. Karplus, 1998. All-atom empirical potential for molecular modeling and dynamics Studies of proteins. J. Phys. Chem. B., 102: 3586-3616.

MacKerel, Jr.A.D., M. Feig and C.L. Brooks III, 2004. Extending the treatment of backbone energetics in protein force fields: limitations of gas-phase quantum mechanics in reproducing protein conformational distributions in molecular dynamics simulations. J. Comput. Chem., 25: 1400-1415.

Noorbatcha, I.A., A.M. Khan and H.M. Salleh, 2010. Molecular Dynamics Studies of Human ß-Glucuronidase. Am J Appl Sci., 7: 823-828.

Phillips, J.C., R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R.D. Skeel, L. Kale and K. Schulten, 2005. Scalable molecular dynamics with NAMD. J. Comput. Chem., 26: 1781-1802.

Reily, P., A. Suurnakki and L. Viikiari, 2004. Enzymes in textile production. In W. Aehle, Enzymes in industry. Leiden: Wiley-VCH Verlag GmbH and Co., pp: 155-244.

Sulzenbacher, G., M. Schulein and G.J. Davies, 1997. Structure of the Endoglucanase I from Fusarium oxysporum: Native, Cellobiose, and 3,4-Epoxybutyl ß-D-Cellobioside-Inhibited Forms, at 2.3 Å Resolution. Biochemistry, 36: 5902-5911.

Vlasenko, E., M. Schülein, J. Cherry and F. Xu, 2010. Substrate specificity of family 5, 6, 7, 9, 12, and 45 endoglucanases. Bioresour. Technol., 101: 2405-2411.