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Molecular Dynamics Simulation of Esterase (EstA) from *Aspergillus niger*

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ABSTRACT

Molecular dynamic simulation was done, in neutral and acidic conditions mimicking through protonation of the surface accessible residues, on *Aspergillus niger* esterase (EstA) to get an insight of the domain movement of the protein. The structure was taken from protein databank under pdb Id 1UKC. It has áâ hydrolase superfold with catalytic triad as Ser210, Glu338 and His440. Simulation studies revealed larger fluctuations of EstA residues in acidic environment as compared to neutral environment, the conformation of protein changed from closed to open form at acidic conditions whereas at neutral conditions enzyme was in closed conformation. Movements in residues of regulatory domain were found responsible for the correct conformation and proper orientation of active site residues. Absence of lid domain was also seen during simulation. Simulation of *Rhizomucor mehei* lipase was also performed at an acidic pH for comparative analysis.

Keywords: Aspergillus niger, molecular dynamic simulation, catalytic triad, á/â domain, regulatory domain, Lid domain.

INTRODUCTION

Carboxylesterase (EC 3.1.1.1) catalyse the hydrolysis of ester bonds, both the alcohol moiety and the acid moiety may be aliphatic as well as aromatic (1-3). They are restricted to water-soluble esters of shortchain carboxylic acids (4). They are very diverse in their enzymatic properties and substrate specificities, useful for industrial applications (5). Esterases belong to the family of á/â-hydrolases. The active sites of EstA are composed of serine, aspartate or glutamate and histidine residues, which form a catalytic triad similar manner to those of serine proteases (6-7).

Aspergillus niger have a wide spectrum of polysaccharides like cellulose, hemicellulase, pectinase and carboxylesterase required for the degradation of plant cell wall (8). These polysaccharides act as accessory enzymes that modify the side chains of polysaccharides **(9, 10).** EstA from *Aspergillus niger* has been reported as a novel class of fungal esterase within the áâ hydrolase superfamily. (11). It has close homologs with genome of other fungi (*Aspergillus nidulans*, *Aspergillus fumigatus*) some of

which are broad host range pathogens. EstA comprises of 538 amino acids, where initial 16 amino acids forms the signal peptide (12). Crystal structure of EstA (pdb id 1UKC) has a cannonical ser/glu/his catalytic triad (Ser210, Glu338, His440) which present at the bottom of large, solvent accessible, and bowl shaped cavity. The EstA molecule consists of a 12-stranded central â sheet surrounded by 13 á helices, with overall dimensions of 55 ×53 ×43 A (13**).**

Molecular dynamics (MD) studies have been used extensively in the study of protein folding and enzyme catalysis (14, 15). Molecular dynamic simulation was done under neutral and acidic conditions to get an insight of the domain movement of the protein. Structural changes were recorded during simulation, under acidic and aqueous environment. The conformation changed from closed to open form at acidic conditions whereas at neutral conditions enzyme was in closed conformation. EstA has three domains that forms it's structure: catalytic domain,áâ domain and regulatory domain. Movements in residues of regulatory domain were found responsible for the correct conformation and proper orientation of active site residues.

METHODOLOGY

Crystal structure of *Aspergillus niger* EstA (pdb id: 1UKC) and *Rhizomucor mehei* lipase (pdb id: 1TGL) was obtained from Protein Data Bank. (www.rcsb.org/pdb/), multiple sequence analysis of 1UKC and other hydrolase from different fungal strains were performed using CLUSTALW. Catalytic Site Atlas (CSA) (<http://www.ebi.ac.uk/thornton-srv/databases/CSA/>) was used to identify active sites in EstA.

The non specific interactions that can interfere with simulation were removed from the crystal structure of 1UKC and 1TGL using CHIMERA (<http://www.cgl.ucsf.edu/chimera/>). GROMACS 4.5.5 (<http://www.gromacs.org>) software was used for molecular dynamic simulation in aqueous and acidic environments. The acidic condition was mimicked by protonating titratable residues like Lys, Gln, Arg, Asp, Glu, His. EstA (1UKC) was solvated with explicit solvent SPC water in a cubic box of 1.5nm. 22 sodium ions were added to neutralise the system. Reference temperature was kept at 300 °K. G43a1 force field in GROMACS was used for energy calculations. A complete MD run was performed for 1000ps. Co-ordinate files were stored at every 200ps. GROMACS built-in tools were used to compute Root Mean Square deviations (RMSD), Root Mean square Fluctuations (RMSF) and Radius of Gyration. All graphs were visualised using XMGRACE (<http://www.plasma-gate.weizmann.ac.il/Grace/>). Accessible molecular surface was computed using WHAT-IF software (<http://swift.cmbi.ru.nl/servers/html/index.html>). Images of superimposed structure were obtained using VMD software.

RESULTS AND DISCUSSION

Multiple sequence alignment

The multiple sequence alignment of lipases/esterase from fungal species found that EstA (1UKC) from *Aspergillus niger* has a continuous stretch of amino acid residues from Phe228-Glu254 and Tyr270-Asp292, The stretch is also present in lipases from *Candida rugosa* (ACN78942.1), *Candida cylindracea* (AAR24090.1), and *Geotricum cadidum* (P22394.2) The continuous stretch may favour the activity of EstA in acidic environment while the absence of this stretch favours the activity in alkaline environment.

Fig 1: MSA of lipase/ esterase from Candida rugosa, Candida cylindracea, Galatomyces geotricum, Rhizomucor mehei, Aspergillus niger, Fusarium solani, Candida antartica and Thermomyces lanuginose

Motif

In our study Motif with regular expression as [DM]EF[LQ][FG][EM]R is found at 2 sites starting from 30-36 (DEFLGMR) and 234-240 (EMEFQFER) in EstA respectively. More importantly this motif was found in continuous stretch of amino acid (228-254), in close proximity to the deleted in the other sequences. Another motif [PK][LV][PT][VMD][WH][YL]F[IL] was also present between the residues 270-292 which were found deleted close proximity to other lipases. It can be inferred that the presence of motif may have important role in structural and functional properties of EstA.

4.3. Molecular dynamics simulation studies:

Molecular dynamics Simulation studies were performed on EstA (pdb id: 1UKC) in

aqueous and acidic environment for 1000 picosecond (ps). It was observed that EstA structure before simulation was highly compact and active site was found buried in the core of protein and the constant fluctuations in all residues were recorded during simulation in aqueous environment. Hence the active site was not exposed due to unsuitable aqueous environment for catalytic activity.

The RMSD were recorded to assess the degree of deviation in EstA (1UKC) structure from *A. niger* during course of simulation in all environment. RMSD values at acidic environment reach up to a range of 0.15nm and was stabilised after 650ps (Figure .

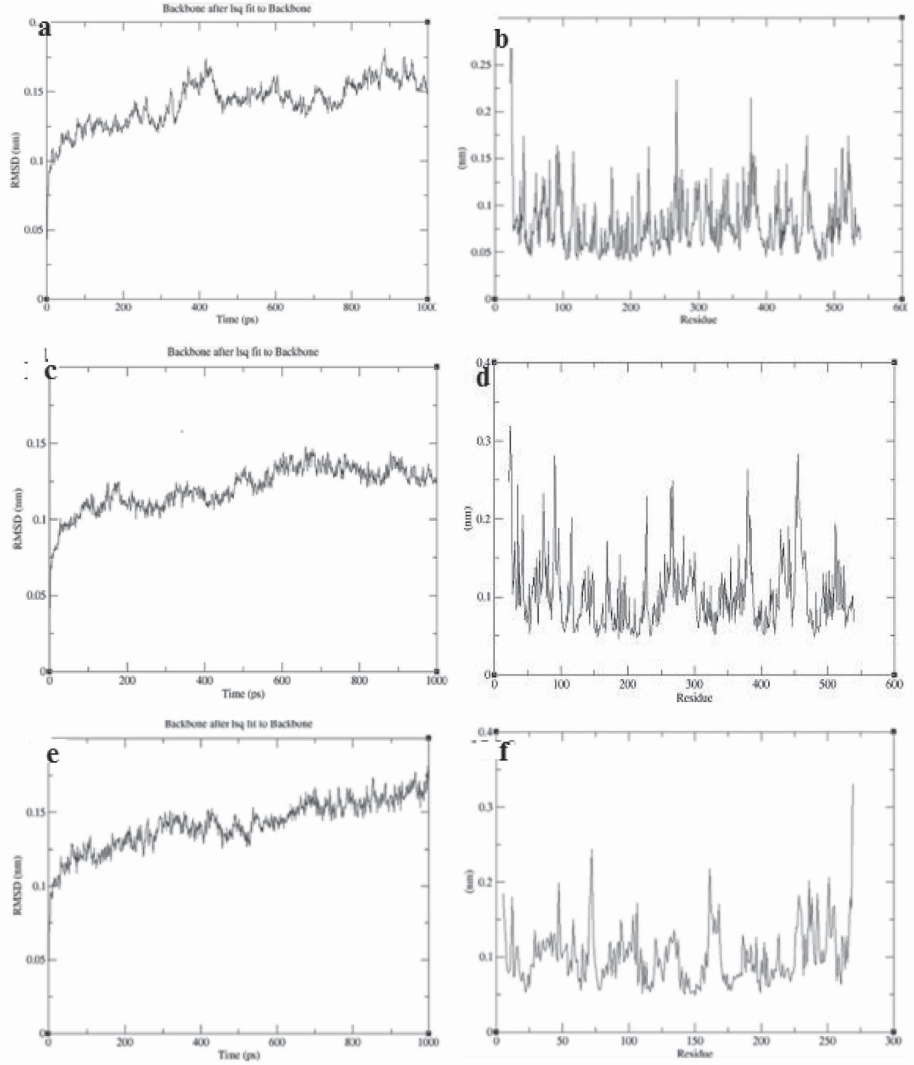

Fig 2: RMSD and RMSF deviations after 1000ps MD simulation. a - RMSD and b- RMSF graph of EstA in aqueous environment, c- RMSD and d-RMSF graph of EstA in acidic environment, e- RMSD and f- RMSF graph of 1TGL in acidic environment.

2c) whereas at neutral pH (aqueous environment) values reach up to 0.175nm and was stabilised after 800ps (Figure 2a). RMSD values at acidic pH stabilised earlier as compared to that of neutral environment. A low RMSD indicates stability and active structure for backbone atoms in EstA. Very small relative fluctuations in RMSD define the system as stable during monitored molecular dynamics (16). A comparative study was also performed on *Rhizomucor mehei* lipase (1TGL), the RMSD found increased to 0.15nm and stabilised after 400ps and remained stable till end of simulation in acidic condition (Figure 2e).

Root mean square fluctuations in atoms were recorded during course of simulation of 1000ps in both aqueous and acidic environment. Large fluctuations were observed at acidic pH, which justify large flexibility (fig. 2d). Moreover, large fluctuations relate to unfolded conformation of protein. Substrate binding, enzymatic process and product release are often associated with conformational changes in structure, and these changes require certain flexibility of protein (17-18).

The most movable/fluctuating residues in acidic environment were found in the following regions: 75-100 (part of â turn), 220-300 (part of helix, loops and strands), 375-395 (part of helix and loops) and 430-475 (part of helix and a small part of loop) in EstA. During simulation in acidic environment these fragments were found displaced from their original position and hence provide flexibility to the protein to perform catalytic activity. The MD Simulations carried out at neutral pH environment revealed that these were found highly compact, closed and in folded conformation.

The radius of gyration was predicted for all simulated models in acidic and aqueous environments. It gives information about the compactness of protein structure. The higher the value of gyration the less is the compactness (19). At acidic environment the radius of gyration reached to a maximum value of 2.25nm which shows that EstA is less compact hence, found in open conformation after MD simulation for 1000ps (fig. 3c). Whereas, in neutral environment radius of gyration reached a maximum value of 2.20nm which means the simulated structure is more compact and ultimately in closed conformation as compared to acidic environment MD simulation (fig. 3a). For the comparative study the radius of gyration of 1TGL was also predicted after MD simulation for 1000ps in acidic pH which was found to be 1.65nm, hence it signifies less compactness and stability of structure (fig. 3e).

Superimposed structures were obtained to show the fluctuations/ shifts in the residues after molecular dynamics simulation with respect to original pdb structure. These conformational changes in the structure have role in the activity of protein EstA. Superimposition of simulated structure after MD simulation in acidic environment for 1000ps on original pdb showed interesting results. The structure showed movements/shifts in three regions (A, B and C) after simulation in acidic environment as compared to aqueous environment. Region A represents nucleophilic elbow G-X_1-S-X_2-G (20-23). It is required for nucleophilic attack by serine of catalytic triad. It was identified by signature motif of the áâ hydrolase family as Gly208, Val209, Ser210, Ala211, and Gly212. A low barrier hydrogen bond is formed by Histidine and Glutamate of active site residue that facilitates nucleophilic attack by serine (24).

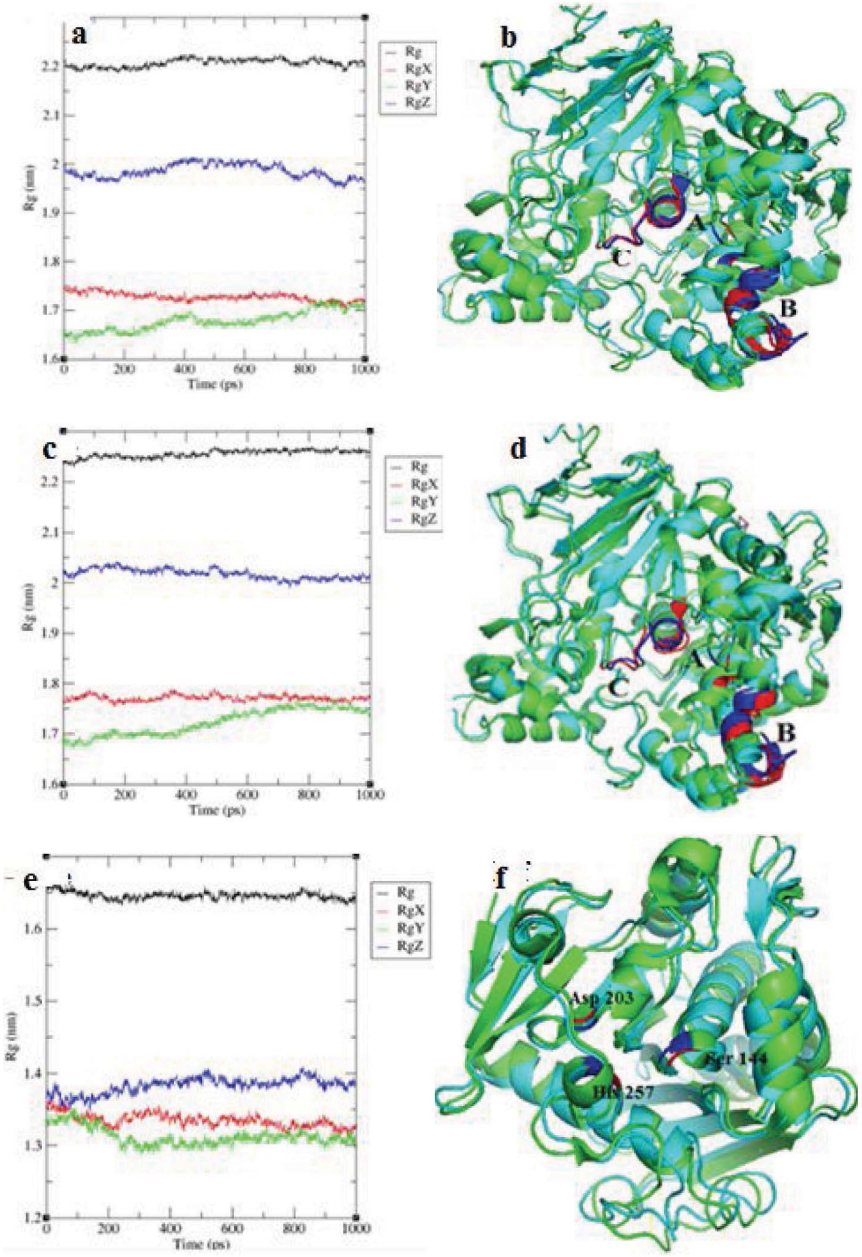

Fig 3: Change in radius of gyration and superimpoed structure in EstA during simulation. a- Change in radius of gyration in aqueous condition, b- Superimposed structure of EstA green (simulated) on original pdb structure. c- Change in radius of gyration in acidic condition, d- Superimposed structure of EstA green (simulated) on original pdb, e- Change in radius of gyration of ITGL in acidic condition, f- Superimposed structure of ITGL green (simulated) on original. [*A: Nucleophilc elbow and active site, B: loop 22, 25, Helix 12, 14. C: Oxyanion hole, Blue (Original pdb* ***structure), (Red simulated structure)***

Regulatory domain of carboxylesterase comprises of á helix and disordered loops that are closed partially over active site. Position of active site residues Histidine and Glutamate are affected by disordered loops that can change catalytic activity of enzyme**.** Region B (Figures 3b and d) forms the part of regulatory domain (helix 12, 14 and loops 22, 25). Simulation in acidic environment showed the displacement in loops 22 and 25 from closed to open form is responsible for correct orientation of active site residues (Figure 3d). Whereas in aqueous environment there was no shift in these regions thus preventing the proper conformation of active site (Figure 3b). Region C (Figures 3b and d) represents the oxyanion hole during simulation in aqueous environment and in acidic environment respectively. The hole is responsible for the stabilisation of transition state formed in reaction it has a glycine rich conserved sequence of G-G-G-X-X as Gly126, Gly127, Gly128, Thr129, Ala130. In esterase the oxyanion hole is preformed (25).

The changes in the conformation of active site residues of EstA after simulation in acidic and aqueous environment were shown in Figure 3. It was observed that the residues aligned properly when simulated in acidic environment than in aqueous environment, because of the displacement of helix of regulatory domain (helix 12, 14) which enables them to carry out the reaction.

To compare the structural changes in lipase/esterase MD simulations were also performed on lipase from *Rhizomucor mehei* (1TGL), which has 63% similarity with EstA. RMSF graph showed fluctuations in residues 70-100 and 200-260 (Figure 13d). The active site residues (Asp20 and His257), lid region residues (Ile77 -Tyr99) and a loop region residues (Ala90 - Phe99) were present in fluctuating regions might have potential role in catalytic activity of 1TGL. Certain specific residues in the lid region (Ala90-Phe99, Ile85, Ile89, and Leu92) of 1TGL form loop and hydrophobic side chain that play an important in lid movement and interfacial activation (26).

CONCLUSION

EstA is a novel class of enzyme superfamily recently found in *Aspergillus niger,* and in certain other fungal strains. Maximum fluctuations in EstA were recorded at in acidic environment under molecular dynamic simulation for 1000ps compared to aqueous environment. Four different regions were found to have maximum mobility than other regions in acidic environment. Many important regions residues like nucleophilic elbow, oxyanion hole, (responsible for nucleophilic attack and stabilisation of transition state respectively), binding pocket and active site residues were found in fluctuating regions. At acidic pH catalytic triad which forms a pocket was found in proper orientation. EstA was found less compact and more flexible in acidic environment which is one of the essential requirements of protein for a reaction, compared to aqueous environment. The study thus concludes that EstA from *Aspergillus niger* favours acidic environment for its maximum activity. At acidic pH it shows high stability of protein structure, high fluctuations in residues and less change in radius of gyration during molecular dynamics simulation in acidic environment.

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