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MD Simulation Studies of Fumarase Reveal Thermo Dynamical Stability

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Abstract

Fumarase enzyme is known to catalyse the stereo specific inter conversion of fumarate to L-malate which is a part of the Krebs cycle. Despite the biological significance and importance of this enzyme, the reaction mechanism of fumarase is not completely understood or known. In this context an experiment on molecular dynamics simulation was carried out for at least 10 nanoseconds molecular dynamics simulation run using Nano Scale Molecular Dynamics program implemented in Discovery Studio 4.0. The trajectory analysis of various energy parameters revealed the thermo dynamical stability of the enzyme. The present findings may aid in understanding the biological significance of this enzyme.

Keywords: Fumarase; Molecular dynamics; Krebs cycle

Background

Fumarase enzyme catalyses the stereo specific inter conversion of fumarate to L-malate as part of the metabolic citric acid or Krebs cycle [1]. The recent three-dimensional structure of the fumarase C of *Escherichia coli* has identified a binding site for anions which is generated by side chains from three of the four subunits within the tetramer [2]. These same side chains are found in the three most highly conserved regions [3]. This enzyme belongs to the family of lyases, specifically the hydro-lyases, which cleave carbon-oxygen bonds [4]. Despite its biological significance, the reaction mechanism of fumarase is not completely understood [5]. The reaction itself can be monitored in either direction; however, it is the formation of fumarate from S-malate in particular that is less understood due to the high pKa value [5]. There are also reports of the reaction that suggest the formation of fumarate from S-malate involved dehydration of malate to a carbocationic intermediate, which then loses the alpha proton to form fumarate [6]. Another, e recent trials have provided evidence that the mechanism actually takes place through an acid-base catalysed elimination by means of a carbanionic intermediate E1CB elimination [7]. In an attempt to understand the actual mechanism of the enzyme, a molecular dynamics simulation experiment was designed which may provide the insights of the thermo dynamical behaviour. During this investigation, the molecular dynamics simulation (MD) was performed to understand the stability of the enzyme in various energy related behaviors to understand its thermodynamic behaviours which might contribute in the stability of the enzyme [8]. Initially, the three dimensional crystal structure of fumarase having a resolution of 1.98 A was retrieved from the Protein Data Bank. And only Chain A was considered for the molecular dynamics simulation studies. The 3D structure was prepared using the protein preparation cascade in implemented in Discovery Studio 4.0. The system was initially solvated in a cubic box of TIP3 waters. It was further minimized using steepest descent energy minimization and equilibrated for at least 100 ps with NVT (Canonical) and NPT (Isothermal–isobaric). MD simulation was run for 10 ns production using NAMD implemented in the licensed version of Discovery 4.0. The results of the various energy calculation is described in Figure 1, the overall heat map of fumarase enzyme (PDB ID: 1FUO) for various energy calculation (angle energy, torsion energy, electrostatic energy, Van der Waals energy, kinetic energy, temperature, potential energy, bond energy and total energy) is shown. In Figure 1, the red colour indicated high energy and the blue colour indicates negative or stable energy which is revealed that the kinetic energy of the enzyme is high while the rest of the energy parameters are stable to some extent.

Figure 2 describes the MD simulation profile of electrostatic energy on fumarase enzyme run for 10 ns. As evident from Figure 2, the trajectory profile tends to attend stability just after 2 ns till 10 ns without any further unusual shift in the trajectory profile, which claims the electrostatic stability of the enzyme.

Further, the torsion energy (dihedral angle) and bond angle energy profile of fumarase enzyme is depicted in Figure 3a and 3b respectively. In Figure 3a the energy shifts range from ~1450 to ~1500 which reveals the stability profile of the torsion angle energy of the enzyme during the 10 ns MD simulation run. Also, from Figure 3b the angle energy shifts of the enzyme ranges from ~3300 to ~3550 which is very stable without any further deviation and confirms the stability of all the bond angles of the enzyme.

Figure 4 a and b depicts the potential energy and total energy profile, where in Figure 4a revealed the stability of the enzyme with an energy shifting from -17200 kJ/mol to -16800 kJ/mol which is very stable in the dynamic equilibrium. In case of Figure 4b, the total energy value shifts from -12000 kJ/mol to -11600 kJ/mol with a mean difference of ~400 kJ/mol which indicates the system is stable enough the during the 10ns MD simulation run.

Also, the secondary structure of the fumarase enzyme (PDB ID: 1FUO) is shown in Figure 5. Lastly, the RMSD graph which was plotted from the result of trajectory analysis is shown in Figure 6 which indicates the conformational stability of the enzyme during the 10 ns MD simulation run.

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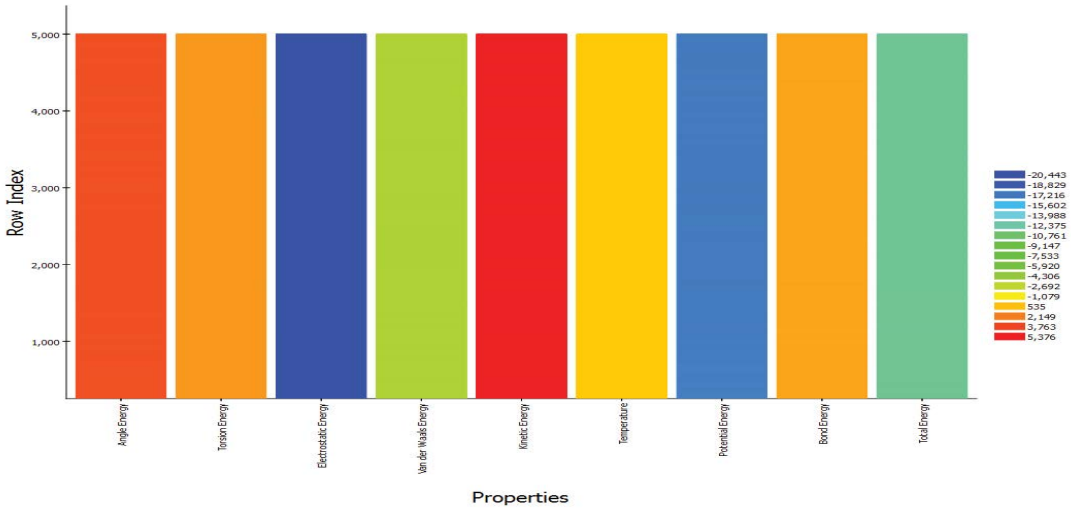

Figure 1: Heat map of fumarase enzyme revealing the stability of various energy parameters.

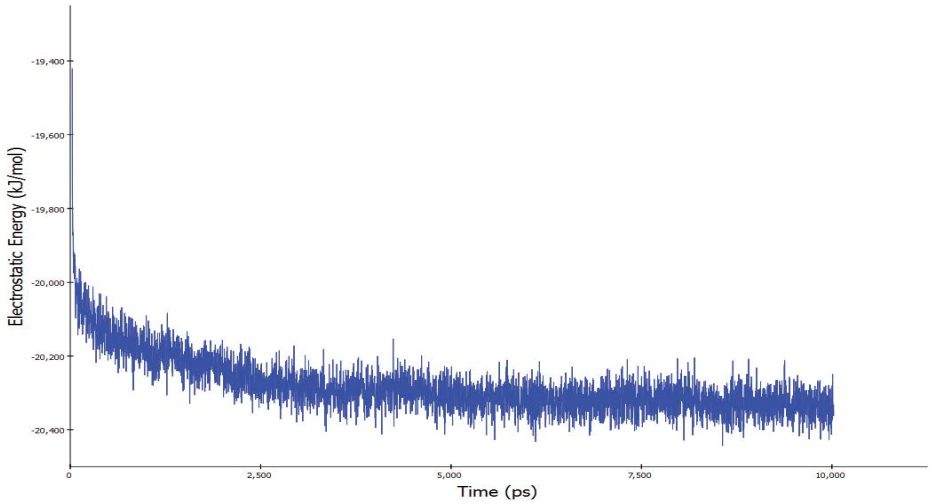

Figure 2: Trajectory profile of electrostatic energy of fumarase during 10 ns MD simulation run.

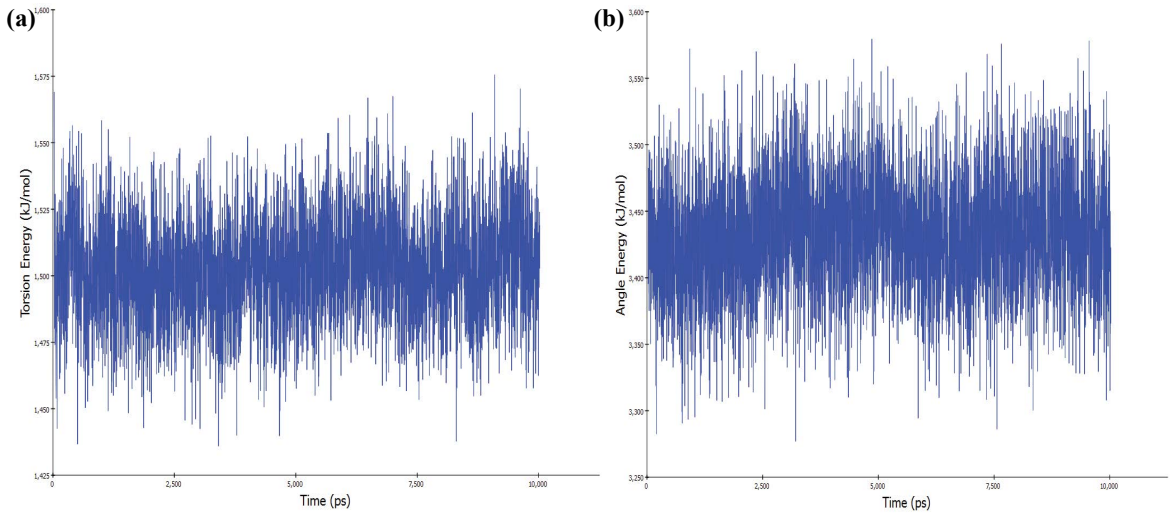

Figure 3: (a) Torsion energy and (b) angle energy profile of fumarase enzyme during 10 ns MD simulation run.

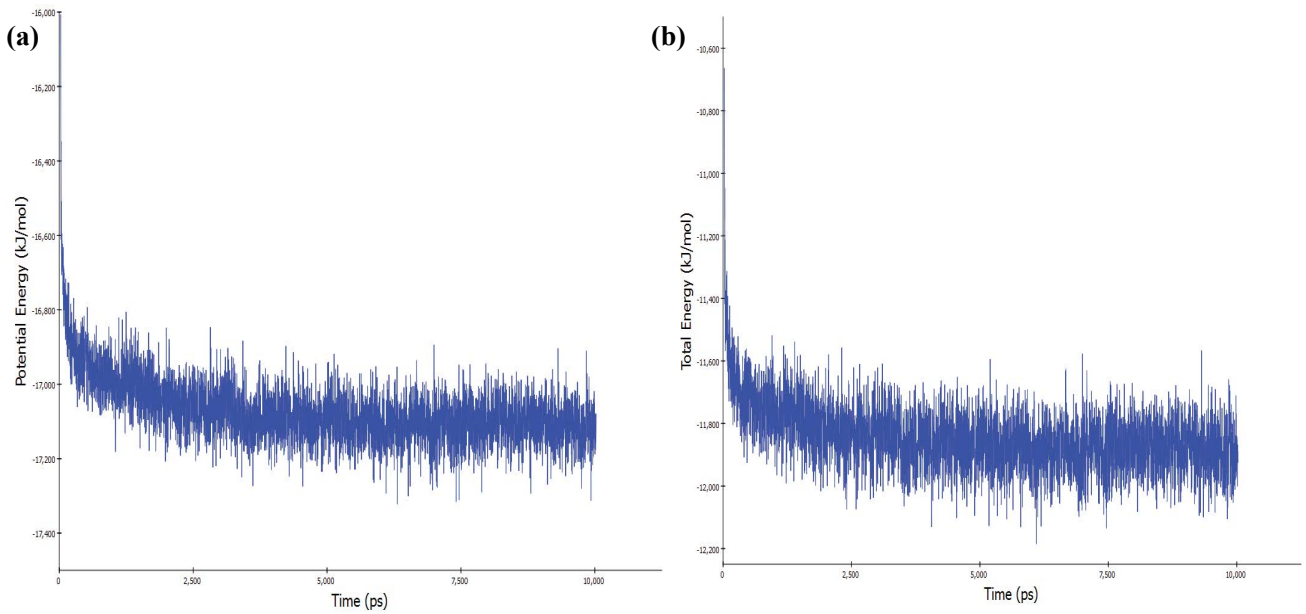

Figure 4: (a) Potential energy and (b) Total energy profile of fumarase enzyme during 10 ns MD simulation run.

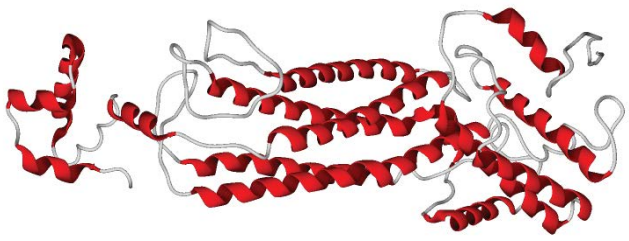

Figure 5: Secondary structure of fumarase enzyme (PDB ID: 1FUO).

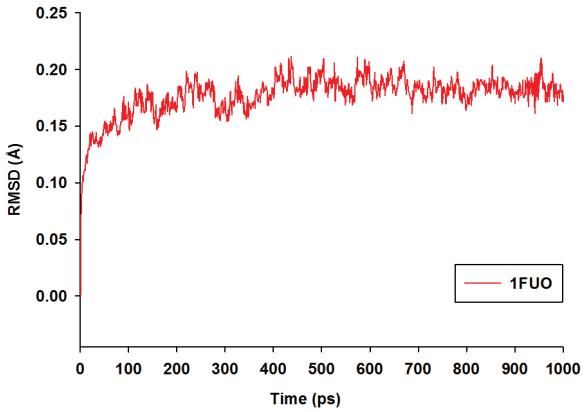

Figure 6: RMSD backbone of fumarase enzyme (PDB ID: 1FUO) during 10 ns MD simulation run.

Conclusion

The MD simulation study of fumarase enzyme was carried out for 10 ns MD production run and the trajectory of the various energy profiles was generated and analysed. As evident from the various energy profiles depicted in the Figures 1-6, the fumarase enzyme is stable enough in its dynamic behaviour.

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