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COMPARATIVE CHAINS DYNAMICS OF TRIOSEPHOSPHATE ISOMERASE INVESTIGATED BY MOLECULAR DYNAMICS SIMULATION

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ABSTRACT

Triosephosphate isomerase is an enzyme which catalyzes the inter-conversion between glyceraldehyde-3-phosphate and dihydroxyacetone phosphate in the fifth step of the glycolytic pathway. In this study, molecular dynamics simulation technique was employed to investigate the dynamics of the two monomer chains of triosephosphate isomerase of *trypanosoma brucei brucei* (TbTIM) using GROMACS 2016.04 software. The MD simulation of the x-ray structure of TbTIM was performed using the GROMOS 9643a1 force field and simple point charge water model under isothermal-isobaric condition with periodic boundary conditions imposed on x, y, z directions. The Root Mean Square Deviation (RMSD) Root Mean Square fluctuation (RMSF), Radius of Gyration (ROG), Solvent accessible surface area (SASA) and hydrogen bonds were computed. The RMSD values indicate that chain-B shows transition between two conformational states with higher RMSD value of 0.45 nm when compared to chain-A with RMSD of 0.27 nm. The RMSF values indicates that residues of loop 6 (residues 166 to 177) have the highest fluctuations in both chains compared to other residues with chain-A having higher fluctuations peak. The ROG values of chain-A varies from 1.675 nm to 1.761 nm while that of chain-B varies from 2.480 nm to 2.590 nm implying that chain-A is more compact during the simulation than chain-B. The SASA values indicates that chain-B has more contact with solvent than chain-A. 160 hydrogen bonds were found which indicates the stability of the protein during the simulation. From the RMSD, ROG, SASA values, it is evident that chain-B of triosephosphate isomerase of *trypanosoma bruceibrucei* displayed greater structural dynamics than chain-A during the MD simulation.

Keywords: Triosephosphate isomerase, force field, hydrogen bonds, isothermal-isobaric, Molecular dynamics simulation

INTRODUCTION

Human African Trypanosomiasis (HAT) is a parasitic disease caused by the protozoan *Trypanosoma brucei brucei*, transmitted by the bite of an infected tse-tse fly [1].The parasite multiplies extracellularly in the mammalian bloodstream, and it has been established that trypanosomes rely mainly on glycolysis as an energy source [2] therefore; blocking this pathway could result in an energy deficit that might impair the survival of the parasite. Thus, the glycolytic enzyme triosephosphate isomerase (TIM) has been proposed by different researchers as a validated drug target against *T. brucei brucei* [3]. Proteins carry out the most difficult tasks in living cells. They do so by interacting specifically with other molecules. This requires that they fold

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to a unique, globular conformation that is only marginally more stable than the large ensemble of unfolded states [4]. The conformational changes in the protein will be examined. Triosephosphate isomerase (TIM) is an enzyme which catalyzes the inter-conversion between glyceraldehyde-3-phosphate and dihydroxyacetone phosphate in the fifth step of the glycolytic pathway [5]. Structurally, TIM is formed by two monomers, and each monomer consists of 250 residues forming eight parallel β-strands surrounded by eight ∝-helices, showing the classical TIM barrel folding [6]. The active site of the enzymes is in the center of the barrel formed by Lys-13, His-95, and Glu-167 with each monomer having an independent catalytic site [7]. Similar to other isoforms, TIM is active only in its dimer form [8]. Minini *et al*., 2015, studied the inhibition mechanism and selectivity of *Trypanosma Cruzi* triosephosphate isomerase (TcTIM) using molecular docking and molecular dynamics simulation. In the study, the binding modes of 1, 2, 4 – thiadiazol, phenazine and 1, 2, 6 – thiadiazine derivatives to TcTIM was investigated. The results show that phenazine and 1, 2, 6 – thiadiazine derivatives, acts as dimer disrupting inhibitors of TcTIM having also allosteric effects in the conformation of the active site. Also, Vazquez-Raygoza *et al*., studied the kinetic and molecular dynamics of species-specific inactivation of Triosephosphate isomerase from trypanosoma *brucei brucei* in the study, three new benzimidazole derivatives were found as Trypanosoma *brucei brucei* Triosephosphate isomerase (T_bTIM) inactivators (compound 1, 2 and 3) with and I_{50} value of 84, 82 and 73 μM respectively[9]. Kinetic analyses indicated that the three molecules were selective when tested against human TIM (hTIM) activity. Additionally, to study their binding mode in (T_bTIM), 100ns molecular of (T_bTIM) inactivator complexes was performed. Simulations shared that the binding of compounds disturbs the structure of the protein, affecting the three new benzimidazole derivatives were found with the capacity to inactivate selectively the (T_bTIM) with respect to the hTIM, and with characteristics to be considered as potential drug candidates.

For the purpose of this work, the structural activities of the monomer chains in triosephosphate isomerase of *trypanosoma brucei brucei* during simulation will be examined to understand the dynamical interplay between the two monomer chains in their transitions between the open and closed conformations of the enzyme which could help in further understanding of the inactivation and inhibition mechanisms of the glycolytic pathway of *trypanosoma brucei brucei* by triosephosphate isomerase.

MATERIALS AND METHODS

The Triosephosphate isomerase to be used for the simulation studies was taken from the Research Collaborator for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) [10] with PDB code 1IIH[11]and the starting configurations was appropriately edited to mimic the environment of

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the protein in the body system the starting structure of protein was solvated explicitly in cubic box of water (figure 1). All simulation was carried out using the Groningen machine for chemical simulations (GROMAC 2016.5) software [12]. The GROMOS 9643a1 force field and simple point charge water model was used. Two rounds of energy minimization were carried out (the steepest descents and conjugate gradient methods).

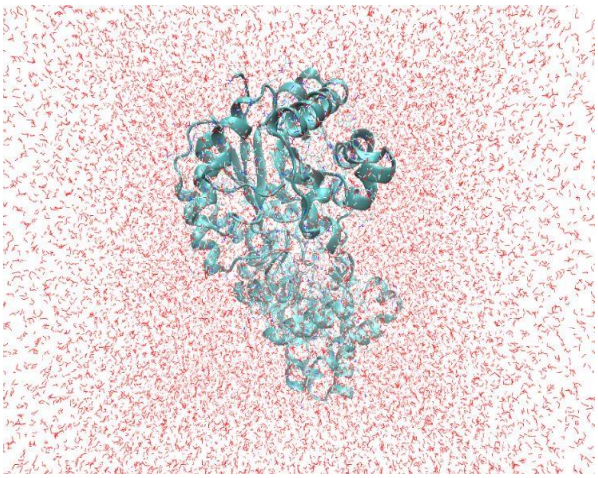

Figure 1: Solvated triosephosphate isomerase in a cubic box

Two rounds of equilibration were done, the first equilibration was done after the first energy minimization. After the second round of energy minimization, the entire system was then subjected to 500 ps equilibration at constant pressure of 1atm under an isotropic condition, by gradually heating up system from 0 – 300 K at 20 K interval. The temperature and the pressure were controlled by coupling the system with Nose-Hoover thermostat [13] andisotropic Parrinello-Rahman barostat [14].

The production was carried out at constant temperature and pressure (NPT) of 300 K and 1 atm for one microsecond with time-step of 2 fs.

RESULTS AND DISCUSSION

Root Mean Square Deviation

The Root Mean Square Deviation (RMSD) is a measure of the average variation in the displacement of a selection of atoms for a specific frame with respect to a reference frame [15]. It is calculated for all frames in the trajectory. RMSD is defined by the equation below:

$$\text{RMSD:} \sqrt{\frac{1}{N}\sum_{i=1} N\,\delta_i^2} \qquad (1)$$

Where δ_i is the distance between atom i and either a reference structure or the mean position of the N equivalent atoms [15]. This is mostly calculated for the backbone heavy atoms C, N, O and C_α or sometimes just the C_α atoms. TbTIM C_α of the monomers RMSD values (compared to the energy minimized starting structure) along the simulation is shown in the Figure 2.

From figure 2, it can be deduced that chain-A was relatively stable during the MD simulation initially maintaining a RMSD value of 0.20 nm at the start of simulation till around 450 ns after which there is a slight change in the RMSD value to 0.27 nm indicating a change in conformation as the simulation progresses till 1000 ns. Similarly chain-B also showed two conformational states but with higher RMSD values of 0.3 nm from 0 to 440 ns and 0.45 nm

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from 570 ns and maintained stability till 1000 ns. The structural activity of TIM during MD simulation resulting in two conformational states agrees with previously reported findings that all the dimeric TIM crystal structures have two conformations with one of the chains in open conformation and the other chain in closed conformation due to crystal packing effects [16].

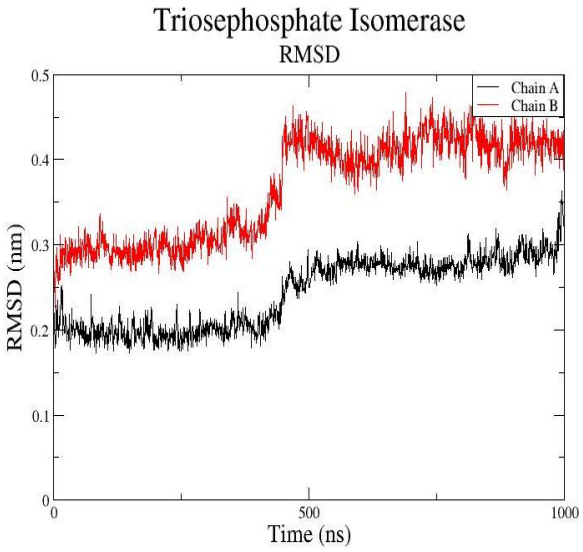

Figure 2: RMSD plot of monomer chains of Triosephosphate Isomerase during the simulation

From Figure 2, it is evident that chain-A slightly changes it conformation from its starting open conformation to a closed conformation while chain-B which shows a significant change in RMSD value changes it conformation from the starting closed conformation to a more active open conformation as the simulation progresses, thus, chain-B can be said to be the most active of the two monomers during the MD simulation.

Root Mean Square Flunctuation

Root Mean Square Flunctuation is the degree of the displacement of a particular atom or group of atoms that is correlated to the reference structure averaged over the number of atoms [17]. Trajectories variability can be investigated by plotting the root mean square fluctuation against number of atoms or residues as plotted in Figure 3.

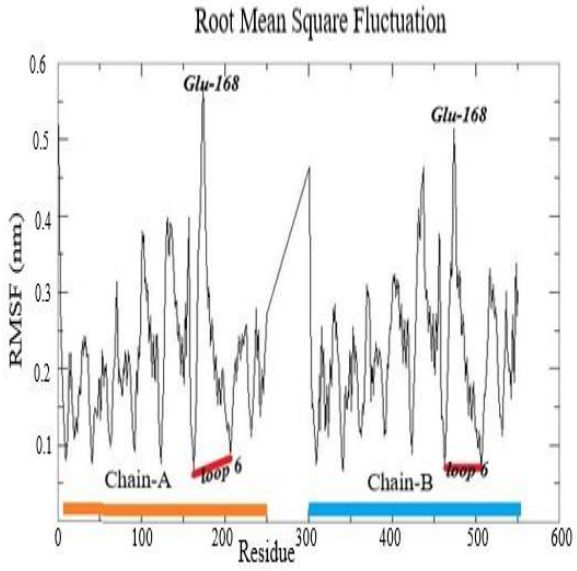

Figure 3: RMS fluctuation plot of triosephosphate isomerase in (nm) against the number of residues

Figure 3 reveals that there is significant mobility difference between the residues in both chains. However, higher fluctuation was observed between residue 166 to 179 (loop 6) of both chains and residue 168 has the highest fluctuations with RMSF of 0.56 nm and 0.51 nm for chain-A and chain-B respectively. It has been reported that optimal function of TIM relies on the ability of an active site loop (loop 6, residues 166 to 177) to move between open and close conformation [18] . Thus, it is evident from

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Figure 3 that the highest fluctuation peak in both chains corresponds to the residues of the catalytic loop 6 (residues 166 to 179) and confirms the fact that the catalytic loop 6 residues are free and enhance amino acid transitions and high solvent accessibility.

Radius of Gyration (ROG)

Radius of gyration (ROG) is the distribution of protein atoms around its axis. It provides the length that signifies the distance between the point when it is rotating and the point where the transfer of energy has the maximum effect [19].

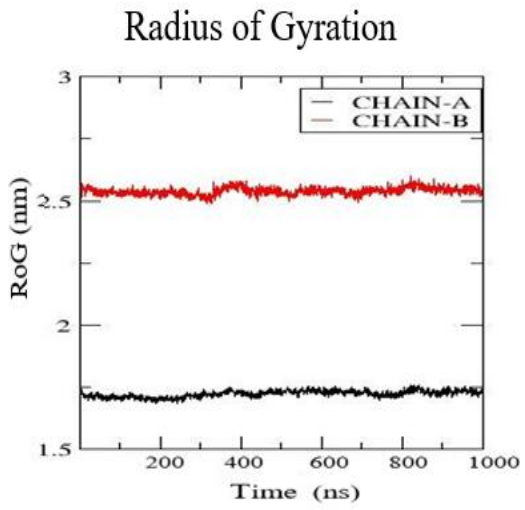

Figure 4: Radius of gyration plot of triosephosphate isomerase during the simulation

The calculation of ROG is a significant indicator that is used in predicting the structural activity of a macromolecule and its compactness. Figure 4 shows that the radius of gyration of TIM chain-A values vary from 1.675 nm to 1.761 nm with an average value of 1.725 nm while that of chain-B vary from 2.480 nm to 2.590 nm with an average value of 2.50 nm. This implies that chain-A is more compact than chain-B during the simulation.

Solvent Accessible Surface Area (SASA)

Solvent Accessible Surface Area (SASA) of proteins has always been considered as a significant factor in protein folding and stability studies. It is defined as a factor used to measure the area of a protein that is in contact with the solvent [20]. Figure5 shows the SASA plot of TIM during the MD simulation. It is evident from figure 5 that chain-A has lower solvent accessible surface area value than chain-B.

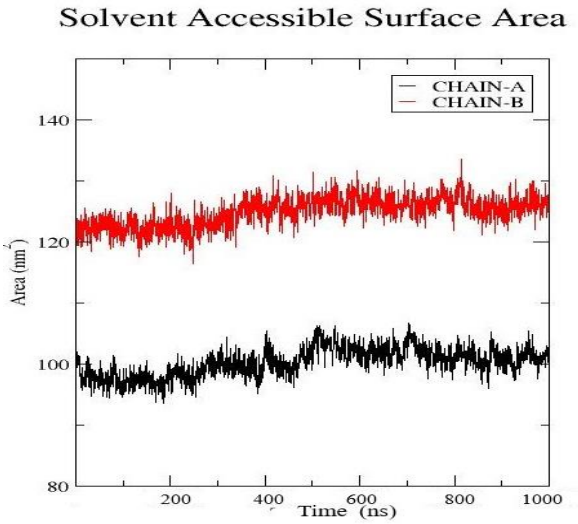

Figure 5: Plot of Solvent Accessible Surface Area (SASA) of triosephosphate isomerase

The SASA range of 93 nm^2 to 106 nm^2 for chain-A implies that more residues of chain-A are buried in the protein as compared to chain-B which has a SASA range of 116 nm^2 to 133 nm^2 indicating that more of chain-B is sticking out into water to have more contact with the solvent than chain-A. This also agrees with the earlier findings that chain-A is structurally more stable.

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Hydrogen Bonds

Hydrogen bond is an interaction involving a hydrogen atom located between a pair of other atoms having affinity for electrons. Hydrogen bonds can occur between molecules inter-molecularly or within same molecule intra-molecularly. Before a Hydrogen bond can be formed there must be a donor and acceptor. The distance between the donor and the acceptor should be less than that of the cut-off distance with 3.0 Å by default [21].

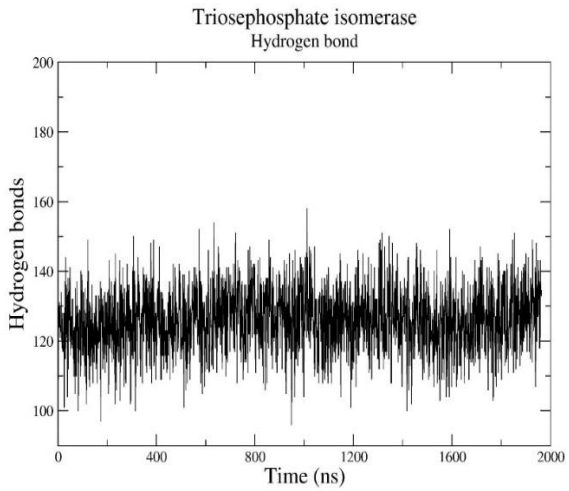

Figure 6: Plot of the hydrogen bonds present in Triosephosphate isomerase during the simulation.

From figure 6, there are 160 hydrogen bonds which are formed because of hydrogen bonds between donor and acceptor atom of protein which also enhance the stability of the protein. A hydrogen bond is significant if occupancy is greater than 38% [21]. For this study, occupancies of 50% to 75% were reported in table 1.

Table 1: Hydrogen bond Occupancy

Donor	Acceptor	Occupancy
THR145-Side	VAL141-Main	59.06%
ARG191-Main	HIS87-Main	54.53%
ALA219-Main	ASN215-Main	58.86%
THR75-Side	GLU397-Side	72.96%
ALA451-Main	ILE447-Main	51.43%
THR31-Side	LEU27-Main	62.22%
SER30-Side	ASP26-Main	50.56%
ARG399-Main	CYS426-Main	57.74%
THR351-Side	HIS347-Main	59.88%
GLU435-Main	LEU431-Main	55.09%
ARG138-Side	GLU133-Side	64.61%
ALA219-Main	ASN215-Main	58.86%
SER396-Side	GLU467-Side	65.17%
THR75-Side	GLU397-Side	72.96%
GLU467-Main	ILE427-Main	51.88%

CONCLUSION

Molecular dynamics simulation of triosephosphate isomerase of trypanosome *brucei brucei* was carried out in an all-atoms explicit solvent simulation study. The dynamics revealed the transitions between the open and closed conformational states of the molecule. From the structural analysis of the conformation changes of TIM using the RMSF, RMSD, SASA and Hydrogen bonds of the monomer chains in triosephosphate isomerase of *trypanosoma brucei brucei,* it can be concluded that chain-B is more active during the conformational transition while chain-A tends to be more stable.

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