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Dynamic Insights into DNA Conformational Changes and the Impact of Magnesium Ions on Topoisomerase IA Enzymes: A Molecular Dynamics Simulation Study

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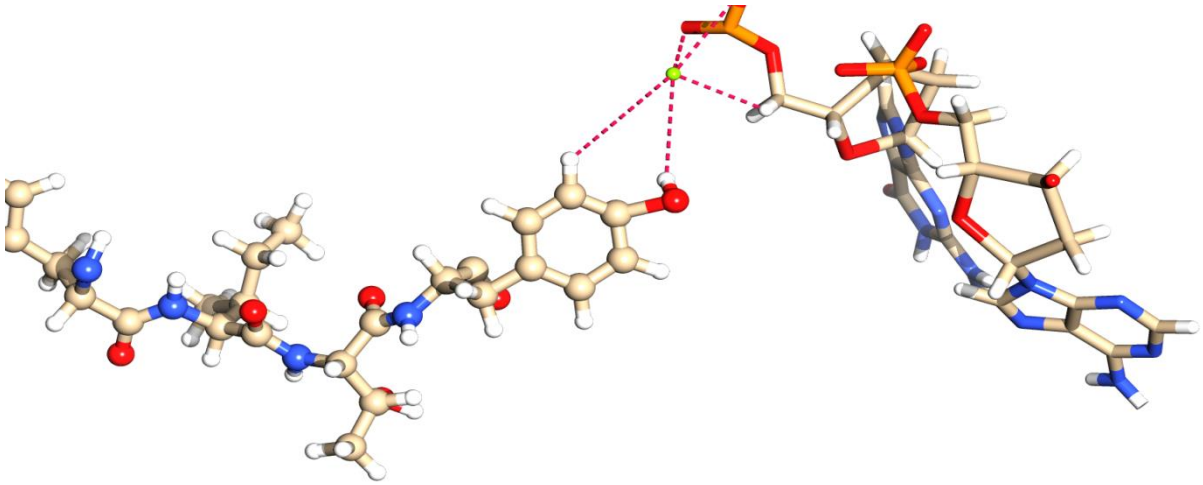
Abstract:
Molecular dynamics simulations were employed to scrutinize the conformational changes in DNA strands and elucidate the influence of magnesium ions on Topoisomerase IA enzymes. Through the computation of Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), hydrogen bond distances, dihedral angles, and Solvated Accessible Surface Area (SASA), we meticulously examined the structural dynamics. The results reveal intricate patterns of DNA strand alterations, showcasing the profound role of magnesium ions in modulating the behavior of Topoisomerase IA enzymes. This study contributes essential insights into the molecular mechanisms governing DNA conformational changes, offering a foundation for further understanding the biochemical intricacies of Topoisomerase IA enzyme function.

Keywords: Topoisomerase IA, MD simulations, DNA cleavage, genomic stability.



1. Introduction: The essential transmission of genetic information across generations hinges on the core concept of genes. At the heart of this genetic relay, the central dogma serves as the guiding principle, directing the intricate processes of DNA transcription and replication [1]. Amidst the diverse enzymes facilitating these chemical reactions, DNA Topoisomerase (topo) enzymes emerge as pivotal players. These remarkable enzymes meticulously control and coordinate changes in DNA topology during the

central dogma, facilitating the transfer of genetic material through generations [1-2].The saga of DNA Topoisomerases traces back to James Wang's groundbreaking discovery in 1971, identifying the first DNA Topoisomerase in bacteria initially named omega (ω) protein and later termed Escherichia coli (E.Coli) Topoisomerase-I (topo-I) [3]. Categorically based on their catalytic activities, DNA topoisomerases fall into two types: type-I and type-II [4]. Topoisomerase-I distinguishes itself by forming a covalent bond solely on one DNA strand through a hydrophilic attack at the active site amino tyrosine, resulting in a break in the DNA strand [5]. This critical enzymatic intervention leads to the creation of an intermediate bond known as the G-strand, while the untouched DNA strand adopts the moniker of the T-strand. Further sub-classification of topo-I enzymes, such as topo-IA, topo-IB, and topo-IC, delineates the nuances of their functionalities [5-6].Specifically, Topoisomerase-IA takes center stage by creating a covalent intermediate bond at C-5^{I} of the DNA strand. Employing a strand passage mechanism, these enzymes rely on divalent metal ions, notably magnesium (Mg2+) [6]. This intricate dance of molecular interactions highlights the complexity and precision orchestrated by DNA topoisomerases, unraveling the genetic tapestry with finesse and ensuring the perpetuation of life across generations.

In recent years, scientists have dedicated their efforts to studying the workings of a minute molecular machine called Topoisomerase-IA. The curiosity revolves around understanding how the components of our genetic material (DNA) undergo shape changes, especially when double-stranded DNA does not precisely fit into enzyme binding grooves. The focus is particularly on comprehending these processes in the presence of magnesium ions (Mg^{2+}). It resembles solving a puzzle to unveil the secrets behind Topoisomerase-IA and its intricate dance with DNA and magnesium ions.

2. Methodology: The calculation of MD simulations was performed in two steps. In the First steps all the basic input and its parametric generation were performed while in the other all the dynamics calculations were completed.

1. Basic Input preparation:

To conduct the molecular dynamics simulation, a series of meticulously crafted steps were undertaken, rendering the process not only methodical but also aesthetically compelling. Commencing this scientific endeavor, the crystal structure of a captivatingly intricate complex featuring the H365R mutant of the 67 kDA N-terminal fragment of E. coli DNA Topoisomerase I, a distinguished subgroup known as topo-IA, was acquired from the esteemed Protein Data Bank. To embellish the structural integrity, a symphony of missing atoms in the enzymes was artfully composed through the virtuoso techniques of the Chimera modeler. Meanwhile, the lacunae within the DNA strands were elegantly addressed and perfected using the masterful Leap program, an integral component of the sophisticated AMBER-18 (8-10) package.The choreography continued as the charge fitting ballet was performed using the exquisite RESP (11-13) method within the enchanting Gaussian 09 package. This ritual was vital in bestowing the modeled molecules with the harmonious resonance needed for the ensuing molecular dynamics performance. For the protagonist, the protein, a spellbinding force field known as ff14.SB was chosen, casting a captivating aura around its dynamic structure. In parallel, the nucleic acid stars, the DNA strands, were adorned with the equally enchanting DNA.bsc1 force field, ensuring a mesmerizing interplay of molecular forces.The grand finale unfolded as the coordinates and parameter files were conjured into existence, akin to the unveiling of a masterpiece. Adding a touch of cosmic elegance, neutralizing ions of Mg^{+2} gracefully descended upon the protein surface, enhancing the celestial balance of the ensemble. The entire spectacle culminated with a poetic immersion, as the system was bathed in the ethereal waters of an octahedral box,

reminiscent of a TIP3P model. This aqueous tapestry extended its enchanting tendrils up to a minimum cutoff of 8Å from the protein boundary, creating a celestial stage for the molecular dynamics ballet to unfold (14).

2. Set-up of MD simulation:

After generating all the required parameter of the system for md simulation completed suitably ,to get the stable structure and to dislodge the bad contiguity minimization was done in two parts having a combination of steepest descent (5000steps) and conjugate gradient (5000 steps) methods(15-17)Firstly the protein and DNA complex was restrained while the water and neutralizing ions were free and in the second step of minimization whole system were minimized without any restrained. Thereafter the system is gradually heated up to 300K under the ensemble NVT for 50picosecond.After this density equilibration for 1ns was done in case of NPT ensemble having target temperature and pressure of 300K and 1 amt pressure respectively, using a Langvin thermostat and Barendsen barostat with the collision frequency of 2ps and pressure relaxation time of 1ps having a weak MD simulation restrained where the system was gradually released to get the constant density after heating dynamics. Finally the production MD for 200ns was done followed by a 3ns equilibration without any restrained(18). The 200ns production was run using the Monte Carlo Barostate for each complex system(19). The SHAKE algorithm and particle mesh Ewald (PME) method was used for the long-range Columbic interaction with the cutoff set as 10 angstrom(20). The whole MD simulation AMBER18 package with GPU version was used.

Calculation: The investigation sated forth in this article refers to the trajectory of production (200ns) from the fashion of root mean square deviations(RMSF) of the enzyme-DNA complex(E-D complex) provide that the system was good stable after

Results:

RMSD & RMSF Calculation:

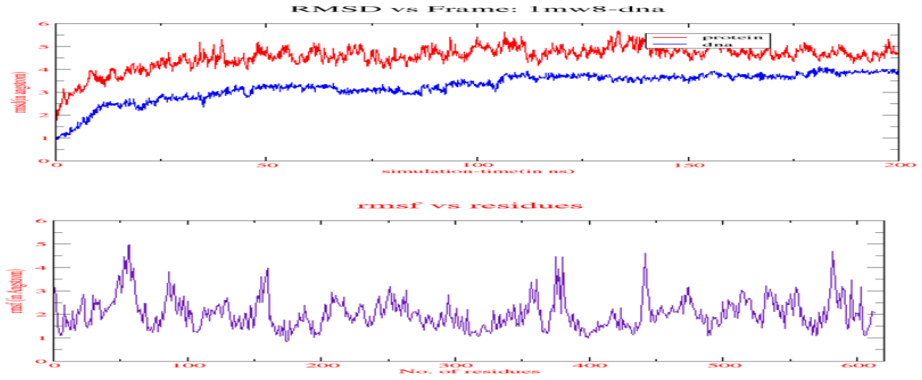


Fig1.a

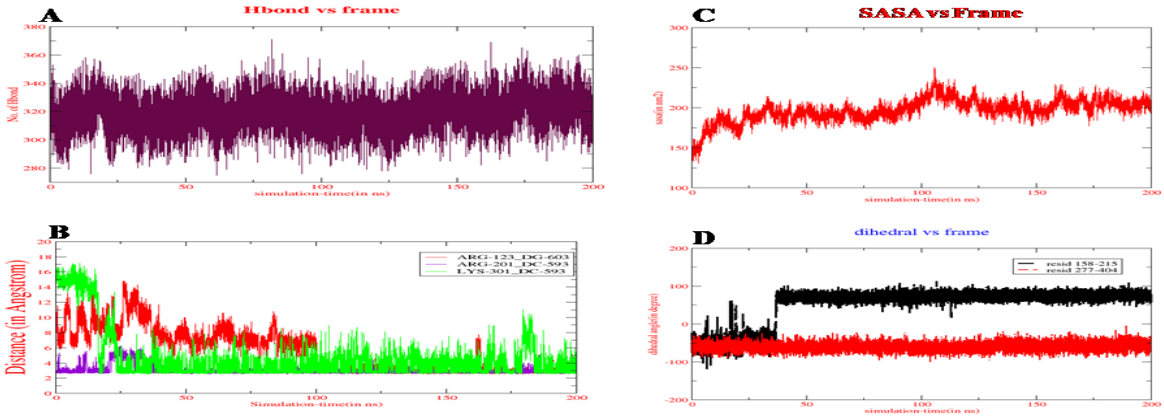
Fig1.b Fig.1a represents the root mean square fluctuation Vs simulation time. The fig1.b represents the complex molecule before and after MD simulation upto 200ns which shows the conformational changes occurs during the molecular dynamics simulation.The domain D1:1-60,81-157 are in cyan ;D2:216-278,405-472 are in green;D3(active domain):279-404 are in red while domain D4:61-80,158-215,473-591 are in red color and the DNA strands in production are in magenta color .

Figure 1b illustrates the structural alignment of the crystal structures of the two states of Topoisomerase I before and after MD simulation(21). The multi-colored representation in Figure 1b captures a moment at 120ns during the production run. The overall root mean square deviation (RMSD) for the protein is 5.5Å, whereas for the DNA, it is 3.5Å, as depicted in Figure 1a(22).At the specific simulation time of 120ns, a noteworthy observation emerges, wherein both the protein and DNA closely approach each other, leading to interactive dynamics between the DNA and Topoisomerase enzymes. This interaction is evident from the root mean square deviation at this time point(23).The root mean square fluctuation (RMSF) analysis, as illustrated in Figure 1b, highlights distinct regions, with notable variations concentrated in domain IV(22-24). This fluctuation provides compelling evidence of a substantial conformational transition associated with domain III (residues 279-404). Supporting this observation, Changela et al. (18) reported that the helix at the interface of domain IV undergoes a slight shift upon binding of dsDNA to Topoisomerase-I. This shift in the helix may, in turn, prompt domain II to tilt forward, initiating the motion of domain III, thereby facilitating the entry of a DNA strand into the central hole(25-26).

The significance of hydrogen bonds and their distance calculation in molecular dynamics simulations cannot be overstated. Achieving an accurate representation requires the initial structure to undergo a meticulous process of minimization. This step holds paramount importance, aligning with the fundamental principle that the structure employed for MD simulation should be situated at the energy surface's minimum (19).Following a methodical removal of constraints, the foundational structure undergoes a transformation. This refined structure is then meticulously compared with the 200ns production structure, a critical step showcased in Figure 1c. This comparative analysis serves as a vital checkpoint, ensuring that the dynamic evolution of the molecular system aligns with the underlying principles of energy minimization (27).

Hydrogen Bond Calculation: In the course of MD simulations, Figure 2a illustrates that the hydrogen bonds maintained an average value of 340%. Notably, the simulation recorded the lowest percentage of hydrogen bonding, dropping below 280% at 60 ns. The analysis of hydrogen bonds provided insights into intricate interactions among different residues.Initially, ARG^{123} was observed in close proximity to DC3 ($cytocine^{603}$), establishing hydrogen bonds. This interaction was influenced by the proximity of ARG^{201}

and DC^{593}, as depicted in Figure 2(C). Subsequently, LYS^{301} formed interactions with DC^{593}, leading to its proximity to ARG^{201}. Interestingly, as ARG^{123} interacted with $DC3^{603}$ and approached LYS^{301}, ARG^{201} shifted away by 5Å.Another noteworthy interaction involved LYS^{301}, which, upon interacting with DC^{593}, previously distant, moved closer and interacted with ARG^{201}. Consequently, ARG^{201} shifted to engage with LYS^{301}. These interactions remained stable until 65 ns. However, beyond this point, ARG^{201} and showed increased interaction with LYS^{301}.In summary, the dynamic interplay of ARG^{123}, ARG201, and LYS^{301} during MD simulations highlights the intricate nature of hydrogen bonding events. Distinct shifts and interactions occurred over time, as evidenced by the observed structural changes in Figure 2(C).



Eternal Hydration: The Enduring Ballet of Water Molecules at the Active Site: The analysis of our simulation focused exclusively on H_2O (water) molecules, extending beyond 160 ns, revealing a sustained choreography of water molecules within the protein-DNA system. Notably, twenty of these molecules congregated to form a water cloud in close proximity to the active domain residues, suggesting an aqueous affinity in this specific region. Within this aqueous enclave, a distinctive pattern emerged: two water molecules established hydrogen bonds with DT^{605}, four engaged with DC^{607}, and a pair interacted with GLY^{314}(28). The hydrogen bonding configuration of these water molecules also involved a significant interaction with the phosphate group of DG^{599}, depicted in Fig. 3(a).Remarkably, the last water molecule in this arrangement positioned itself optimally to accept a proton from the active TYR^{318} residue. This observation lends support to the hypothesis that a water molecule in close proximity to the active domain serves a catalytic role(29). The intricate dance of water molecules, particularly their strategic hydrogen bonding, emphasizes the dynamic interplay within the aqueous environment of the protein-DNA system(30-32)

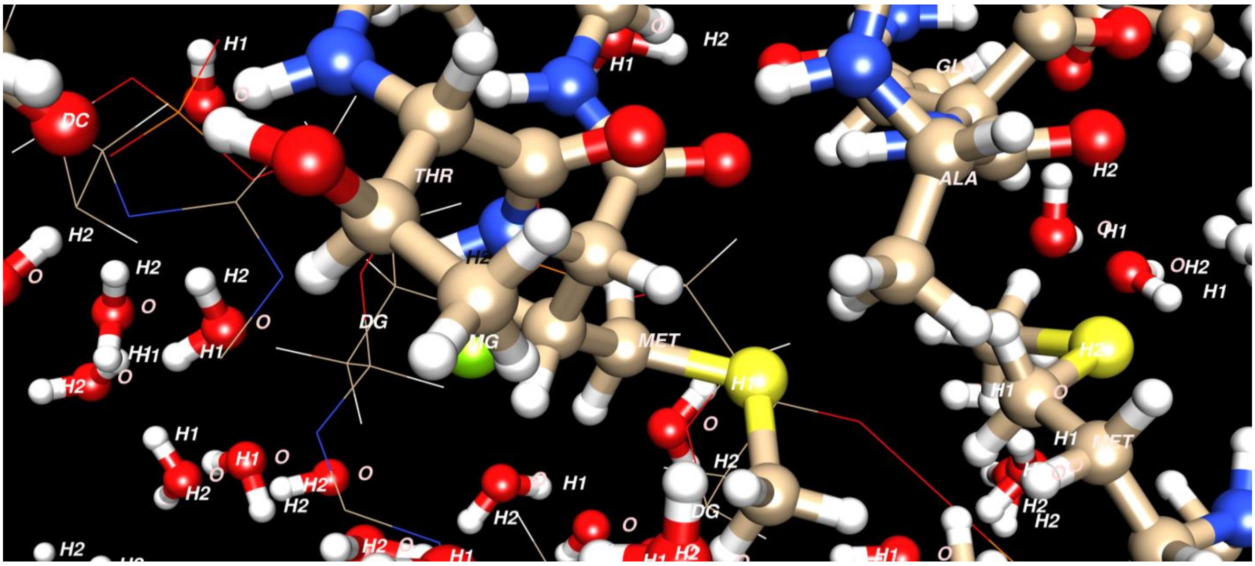


Fig.3a

Master role of Mg^{++} ion in DNA-topoisomeric activity: Throughout the MD simulation and its trajectory analysis, we have observed a significant contribution of Mg^{++} (magnesium ion) in demonstrating the catalytic activity of DNA-Topo-IA(33). Up to the 50ns, the magnesium ion predominantly forms hydrogen bonds with the G-strands of DNA, as illustrated in Fig. 4 (iii & iv). Subsequently, beyond 50ns, the magnesium ion shifts its hydrogen bonding pattern to engage with residues located in the active domain amino acids, as indicated in Fig. 4 (i & ii).As the simulation progresses, the formation of hydrogen bonds between magnesium ion (Mg^{++}) and the active tyrosine (TYR^{318}) lends support to the hypothesis that Mg^{++} ions nearness to the active domain play a catalytic role(34-35). This intricate dance of Mg^{++} ions, particularly their strategic hydrogen bonding, underscores the dynamic interplay within the aqueous environment of the protein-DNA system.

Conclusion:

This study utilizes molecular dynamics simulation (MD simulation) to expand the analysis of the DNA-topoisomerase complex interactions obtained from experimental descriptions. The investigation takes into account the hydration pattern around the DNA-topoisomerase interface. Both water and magnesium ions are acknowledged for their crucial role in mediating protein-DNA interactions, and MD simulation proves effective in exploring the catalytic role in the presence of magnesium ions.This research reveals numerous magnesium-mediated hydrogen bonds, comparable to direct bonds observed in many high-resolution structures. This suggests that magnesium plays a pivotal role in mediating affinity and specificity. The recognition of magnesium-mediated interactions enables the interpretation of experimental data, particularly in cases of site-directed mutagenesis, which may report reduced protein-DNA affinity or the emergence of drug-resistant enzymes.

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