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Thermodynamic Analysis of Short Single-Stranded DNA (ssDNA) for Advancing DNA-Based Biosensor-biocatalyst Development

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Abstract

This study aimed to enhance our understanding of short single-stranded DNA (ssDNA) to facilitate the development of novel DNA-based biosensors-biocatalyst. A 10-base ssDNA model was constructed based on the 130-145 codon sequence of the p53 gene, a key tumor suppressor gene. By employing molecular dynamics (MD) simulations, we delved into the thermodynamic properties and equilibrium states of the ssDNA system, unveiling crucial insights into its behavior. Various macroscopic observables were investigated during the MD simulations, including temperature, energy distributions, and the root mean square deviation (RMSD) of the ssDNA's nucleic acid backbone. The structural model of the ssDNA was meticulously constructed using the AMBER program, ensuring accuracy and reliability. Subsequently, atomistic MD simulations were conducted in three different ensembles utilizing the Gromacs program. The microcanonical, canonical, and isobaric-isothermal ensembles were employed to compare and contrast the equilibrium characteristics of the ssDNA in aqueous solutions. The choice of ensemble played a decisive role in capturing the dynamic equilibrium and conformational behavior of the ssDNA system. The distribution of energy, encompassing both kinetic and potential energy, provided valuable insights into the establishment of thermodynamic equilibrium. Fluctuations in temperature and total energy underscored the finite nature of the system, while the average kinetic energy confirmed the attainment of physiological temperature. Furthermore, the RMSD analysis shed light on the conformational stability of the ssDNA, with both NVT and NPT ensembles exhibiting stable conformational states under their respective thermodynamic conditions. These findings emphasize the intricate interplay between thermodynamic conditions and the conformational flexibility of ssDNA.

Keywords: Catalyst, Biosensor, Molecular Dynamics, Nanodevice

1. Introduction

In recent years, the widespread application of nucleic acid hybridization in various fields, such as nanoelectronics, nanomechanics, and biosensing devices, has led to the development of novel nanostructures [1]. The sensitivity and selectivity of DNA-based biosensors greatly depend on the specific molecular recognition property and hybridization phenomenon of DNA. DNA, with its polyanionic backbone consisting of alternating sugar and phosphate groups, and four distinct bases (Adenine, Guanine, Cytosine, and Thymine), exhibits specific molecular recognition through selective base pairing: A binds to T and C binds to G. Additionally, the polyanionic backbone imparts physiochemical characteristics to DNA, including flexibility, electrostatic properties, and binding capacity to cationic nanoparticulates. DNA-based biosensors heavily rely on the hybridization process, where a single-stranded (ss) DNA selectively binds to its complementary strand under ambient conditions. Compared to the structurally rigid double-stranded (ds) DNA, ssDNA exhibits notable differences in electrostatic properties and flexibility due to its exposed bases and lack of a stable helical structure. Consequently, ssDNA plays a crucial role in both the hybridization process and the proper functioning of nanostructure devices. This study focuses on the 130-145 codon (15 codon) sequence of the p53 gene, which is associated with tumor suppression, with mutations in this codon sequence being linked to specific cancers like lung cancer [2-4].

The specific objective of this research is to gain theoretical insights into the conformational characteristics of short single strands of nucleic acids under various thermodynamic conditions. By understanding the behavior of these short DNA strands, we can enhance the hybridization process and improve the performance of biosensing devices. Molecular dynamics (MD) simulations were conducted to investigate the behavior of ssDNA in this study.

2. Method

Molecular dynamics (MD) simulations are valuable tools for investigating the atomistic details of DNA systems, as they calculate atomic trajectories by solving equations of motion using empirical force fields that accurately describe atomic forces in biomolecular systems. In this study, the Gromacs molecular dynamics program was employed to simulate the DNA system. The force field used was the AMBER force field. The MD simulations were conducted with explicit solvent molecules and Na+ counter ions using a periodic boundary condition. The ssDNA system was solvated with a TIP3P water model. The structural parameters and coordinates for the ssDNA system were obtained from a helix of double-stranded B-DNA generated by the AMBER program, based on the specified p53 sequence (130-140 codon sequence). A single DNA strand consisting of 12 bases was prepared for the molecular dynamics study. The simulation process began with energy minimization of the ssDNA structure followed by equilibration under specific thermodynamic conditions appropriate for the target ensemble. Energy minimization involved using the conjugate gradient method in the Gromacs program, while equilibration was performed in the microcanonical (NVE), canonical (NVT), and isobaric-isothermal (NPT) ensembles. The temperature was maintained at 300 K for all ensembles, and pressure was set at 1 bar in the NPT ensemble. Temperature consistency was achieved through Langevin Dynamics, and constant pressure control was implemented using the Langevin piston Nose-Hoover method available in the Gromacs program. Visualization of system structure was accomplished using the PyMol molecular viewing program. The achievement of dynamic equilibrium was assessed by examining the distribution of energy, pressure, and temperature, which are fundamental thermodynamic properties of the system, over a specified period of time [5-7].

3. Results and discussion

Thermodynamic properties such as pressure, temperature, and volume are crucial in selecting the appropriate ensemble for the simulation study. The distribution of energy, encompassing kinetic and potential energy, indicates the establishment of dynamic thermodynamic equilibrium. In MD simulations, the force field assumes that bonded interactions (bonds, angles, and dihedrals) behave like harmonic oscillators. Consequently, fluctuations in kinetic energy, and thus the temperature distribution, can be attributed to the equilibrium state of the ssDNA system. The simulation was conducted in the microcanonical ensemble, where the number of atoms (N), volume (V), and energy (E) are conserved constants. The system dynamics were observed for 100 ps following an initial equilibration period of 50 ps. The initial equilibration process was performed at a constant temperature of 300 K. During the dynamics study, the temperature fluctuations followed a Gaussian distribution with a mean temperature of 303.99 K (Figure 1). These fluctuations in temperature reflect the finite nature of the system.

As the system reaches dynamic equilibrium, its total energy stabilizes at a constant value. The fluctuations in the total energy depend on the number of atoms or particles in the system. The average kinetic energy determines the temperature of the solvated ssDNA system. The distribution of kinetic energy confirms that the system has reached physiological temperature. To compute the kinetic energy distribution, the dynamics were run for 100 ps in the canonical (NVT) ensemble. The distribution followed the Maxwell-Boltzmann distribution with a standard deviation of 0.602 Kcal/mole, corresponding to a temperature of approximately 300 K (Figure 2). This demonstrates that the desired equilibrium state was achieved through proper sampling of the ssDNA configurations

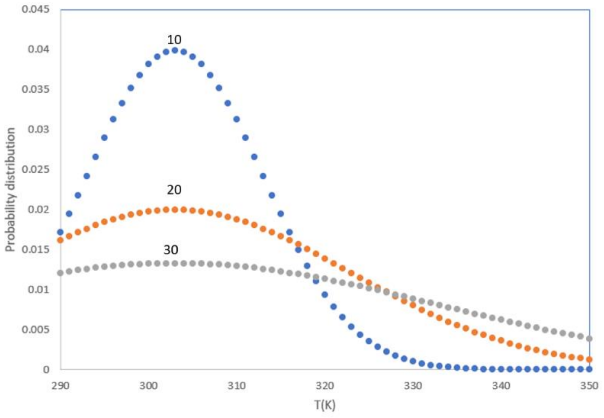

Fig.1 The observed temperature fluctuation in the NVE ensemble of ssDNA at different standard deviation. The numbers corresponding to standard deviation are shown on the curves.

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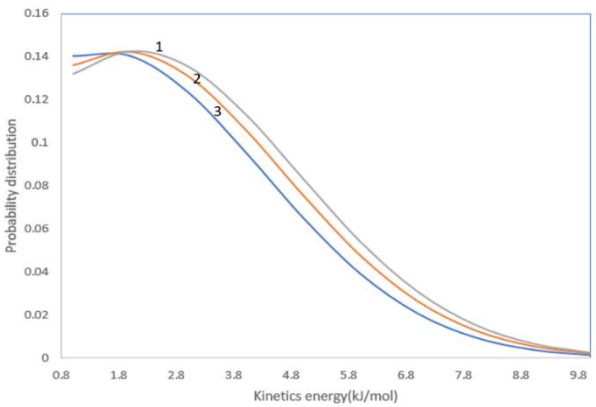

Fig.2 The observed kinetics energy fluctuation in the NVE ensemble of ssDNA at different standard deviation. . The numbers corresponding to standard deviation are shown on the curves.

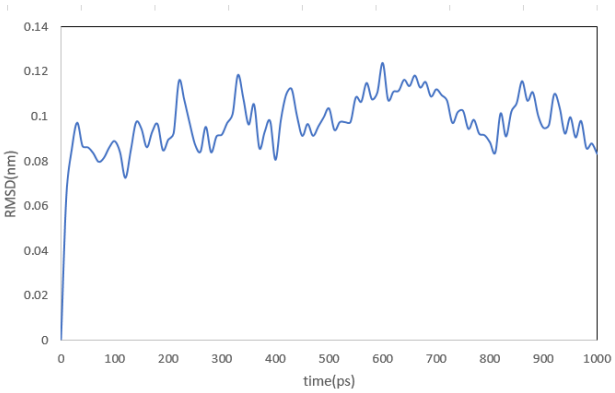

Fig. 3 RMSD of ssDNA backbone in NVT ensemble

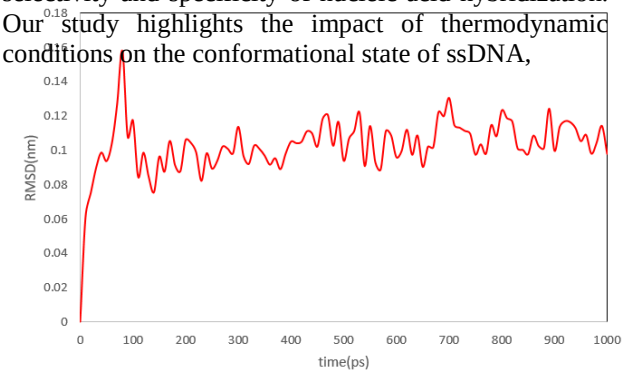

Fig. 4 RMSD of ssDNA backbone in NPT ensemble

The Root Mean Square Deviation (RMSD) was computed for the ssDNA backbone in both NVT and NPT ensembles, as shown in Figures 3 and 4. RMSD provides a numerical measure of the difference between two structural states of the ssDNA, indicating its conformational stability. The RMSD of the nucleic acid backbone reflects the conformational state of the ssDNA throughout the dynamics. By observing changes in the RMSD over time, we can assess the stability of the ssDNA at a specific equilibrium state. In the NVT ensemble study, the ssDNA system was initially equilibrated at 300 K for 100 ps. The achievement of dynamic equilibrium was evaluated by examining the consistency in total energy, temperature, and volume. Subsequently, the system dynamics were studied for an additional 100 ps. During this period, the RMSD of the nucleic acid backbone was calculated to analyze the conformational changes of the ssDNA from its apparent equilibrium structure. The RMSD of the ssDNA exhibited a relatively flat curve (Figure 3) with a deviation ranging from approximately 1.1 Å to 1.5 Å. This result indicates a stable conformational state of the ssDNA under the prevailing thermodynamic conditions. The RMSD of the nucleic acid backbone was computed using a similar method in the NPT ensemble. The simulation was conducted at a temperature of 300 K and a pressure of 1 atm. Initially, the ssDNA system was equilibrated for 100 ps, followed by a 900 ps dynamics study. The RMSD exhibited a change of approximately 1.4 Å over time (Figure 4) during the dynamics study. These results suggest that the ssDNA may have undergone structural changes from its previous conformation over time, despite the system maintaining consistency in the equilibrium thermodynamic conditions. The NPT ensemble, which closely resembles biological conditions, offers insights into the conformational flexibility of ssDNA under ambient conditions. The appropriate thermodynamic conditions and conformational states of ssDNA play a crucial role in regulating the rate of hybridization. The conformational stability and flexibility of ssDNA greatly influence the selectivity and specificity of nucleic acid hybridization. Our study highlights the impact of thermodynamic conditions on the conformational state of ssDNA, potentially influencing the qualitative aspects of DNA-based biosensing devices [7-12].

4. Conclusion

In conclusion, the simulation study focused on the thermodynamic properties and equilibrium states of the ssDNA system. The selection of the appropriate ensemble, such as NVT and NPT, was crucial in capturing the dynamic equilibrium and conformational stability of ssDNA. The distribution of energy, including kinetic and potential energy, indicated the establishment of thermodynamic equilibrium. The fluctuations in temperature and total energy reflected the finite nature of the system, while the average kinetic energy confirmed the attainment of physiological temperature. The Root Mean Square Deviation (RMSD) analysis provided insights into the conformational stability of ssDNA, with both NVT and NPT ensembles showing stable conformational states under their respective thermodynamic conditions. Furthermore, the study emphasized the importance of thermodynamic conditions and conformational flexibility in the hybridization process of nucleic acids. The selectivity and specificity of hybridization depend significantly on the conformational stability and flexibility of ssDNA. The findings suggest that the thermodynamic conditions can modulate the qualitative features of DNA-based biosensing devices. Overall, this study contributes to our understanding of the

thermodynamic properties and conformational dynamics of ssDNA, providing valuable insights for future research in the field of nucleic acid studies and biosensing applications.

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