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The Effect of Carbon Nanotube on Improving Catalytic G-quadruplex Sensing Properties

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Abstract

In the use of G-quadruplex as DNAzyme, the background signal corresponding to Hemin is high. Therefore, it limits the application of DNAzyme. If carbon nanotubes are added to the system containing G-quadruplex and Hemin, this problem will be solved. However, the mechanism of the nanotube effect on DNAzyme is not known. In this work, molecular dynamics simulation was used to clarify this mechanism. The interaction between G-quadruplex and Hemin was simulated in the presence and absence of carbon nanotubes. The calculated distance between the center of mass of G-quadruplex and Hemin during the simulation time shows that the nanotube increases the affinity of hemin to G-quadruplex. Also, the calculation of the conformational factor of the G-quadruplex residues shows that Hemin is bonded to the G-quadruplex from the top and side. The obtained results are in good agreement with the available experimental evidence.

Keywords: Biosensor, Signal, Conformational factor, DNA, Enzyme.

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Introduction

Catalysts are important components in industrial processes [1, 2]. In using catalysts, the specificity of the catalyst is very important [3]. Nowadays, due to the high specificity of biocatalysts, their use has become common in various industries [4, 5]. The use of amylases in syrup industries and bread baking industries [6], the use of lipases in food and dairy industries [7], and the use of various types of lyases in detergent industries [8, 9]are among the cases of using biocatalysts in the industry.

However, the use of biocatalysts in industries also has limitations. One of the main limitations of using biocatalysts in the industry is their low stability in vitro [10, 11]. This limitation has made the use of these compounds in the industry expensive. Therefore, various methods are used to increase the stability of biocatalysts. Immobilizing the biocatalyst on a fixed substrate is one of these methods [12]. Complex formation between a biocatalyst and a chemical compound is another method in this field. Using DNA with Hemin is one of the methods of creating biocatalysts with high stability and low price. The special combination of DNA and Hemin, which has a sensor with catalytic properties, is called DNAzyme [13-15]. It has been found that the DNA with a G-quadruplex structure gives a better complex for DNAzyme formation [16, 17].

DNAzyme obtained from G-quadruplex and Hemin is used to catalyze the oxidation of 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) by H_2O_2 to form the respective colored product $ABTS^{\bullet+}$ [18]. In addition, this DNAzyme sensing platform has been used to detect targets from proteins of various sizes and DNA to small molecules and metal ions. Despite the widespread use of G-quadruplex–hemin DNAzyme, there is a significant challenge in its implementation [19, 20]. DNAzyme-sensor property is based on colorimetry[21]. On the other hand, Hemin's background signal is large. Therefore, it makes it difficult to use G-quadruplex-Hemin as a sensor.

One of the solutions used to eliminate this challenge is the use of carbon nanotubes. It has been determined that in the presence of single-walled carbon nanotubes, the Hemin background signal decreases [22-24]. Therefore, the sensor properties of the G-quadruplex–hemin DNAzyme are improved. However, the interaction mechanism of carbon nanotubes with G-quadruplex–hemin DNAzyme has not yet been elucidated. In this work, using molecular dynamics simulation, the interaction of G-quadruplex with hemin has been studied in the presence and absence of single-walled carbon nanotubes.

Experimental

Two simulation boxes were designed. Chiral carbon nanotubes (6, 5) with a diameter of 7.46 Å and 83.1 Å length were designed and placed at the center of one simulation box. In this box, Hemin was randomly placed. In the second box, Hemin was randomly placed along with DNA. To compare the

results with available experimental data, DNA was used in G-quadruplex form. The G-quadruplex structure with the entry 1KF1 was obtained from the protein data bank [25]. Two designed simulation boxes were filled with TIP3P model water [26]. Appropriate ions were added to each box to neutralize the system. Molecular dynamics simulation calculations were performed with Gromacs software version 5.1.2 using the Amber force field ff99SB [27]. Since the Gromacs software does not have the force field parameters of the Hemin and carbon nanotube by default, these parameters were obtained from the *Ambertools* package [28, 29]. For this purpose, the Hemin and carbon nanotube structures were optimized using the density functional theory method with the basis set of 6-31G by the software GAMESS [30, 31]. To control the optimization of structures, frequency calculations at the same level were carried out using the density functional theory method, and no imaginary frequency was observed. To eliminate the bad contacts between particles in the simulation boxes, all systems are minimized in terms of energy using the steepest-descents method. In two steps, each for 10 nanoseconds at the time step of 2 fs, the systems were equilibrated in the NVT and NPT ensembles, respectively. In the final step, the molecular dynamics simulation was performed for 300 nanoseconds at a time step of 2 fs. To increase the accuracy of the simulations and to evade any dependency of results on the initial conditions, each simulation was repeated two times under different initial conditions. The control of the system temperature and pressure in all the simulation stages was performed with V-rescale and Berendsen thermostats, respectively. The chemical bond between G-quadruplex and the Hemin was bound by the LINCS algorithm [32, 33] and the chemical bonds of water molecules were bound by the SETTLE algorithm [34]. The PME algorithm was used to calculate the electrostatic interactions.

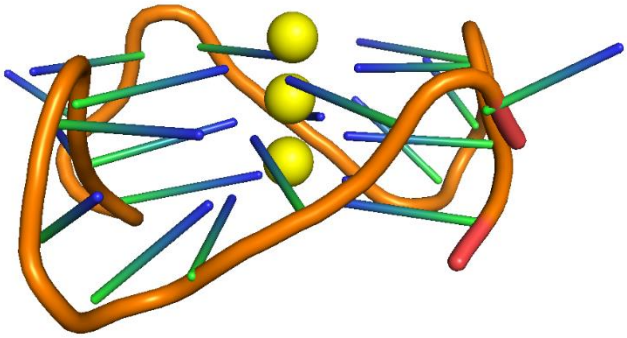
Techniques and software used

Gromacs V. 5.1.2 for molecular dynamics

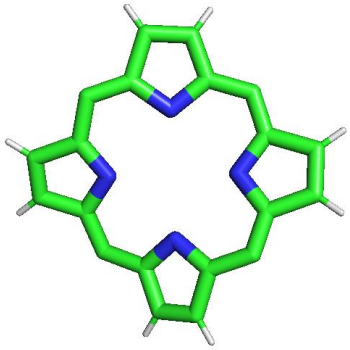
GAMESS for quantum calculations

Results and discussion

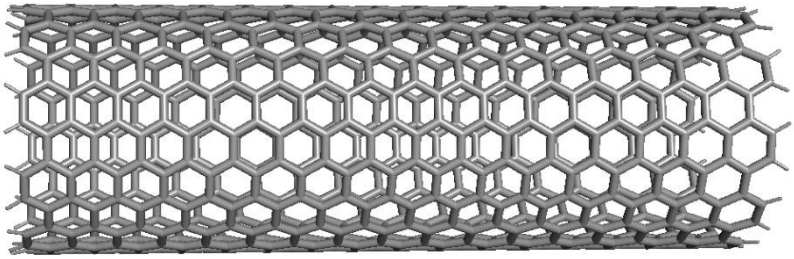
In Figure 1, the structure of G-quadruplex, DNA, and CNT is shown.



G-quadruplex



Hemin



Single-walled carbon nanotube

Figure 1. The structure of compounds studied in this work.

The sampling of the suitable sample from molecular trajectory for analysis has always been one of the issues which are interested in molecular dynamics simulation. The sampling of the averaging of the end several time steps in the simulation or sampling of the averaging of the several nanoseconds in equilibrium condition is among these methods. In these methods, variations of a quantity such as root-mean-square deviation or radius of gyration are used. But there is no physical concept in the average of atomic coordinates. So, for the sampling of the simulations, the free energy landscape (FEL) analysis method is used. Calculations of the root-mean-square-deviation (RMSD) and the G-quadruplex's radius of gyration (Rg), obtaining the possibility of the presence of G-quadruplex configuration in every corresponding value of RMSD and Rg and calculation of the free energy of configurations based on the probability values of presence are the three main stages of FEL analysis[35]. RMSD values (nm) and Rg value of G-quadruplex for the studied system during simulation time (ns) are shown in Figure 2. The Root Mean Square Deviation (RMSD) is obtained from the following equation:

$$\text{RMSD}\ (t_1, t_2) = \left[\frac{1}{M}\sum_{i=1}^{N} m_i ||r_i(t_1) - r_i(t_2)||\right] \qquad (1)$$

Where r_i is the atom position at time t and $M = \sum_{i=1}^{N} m_i$.

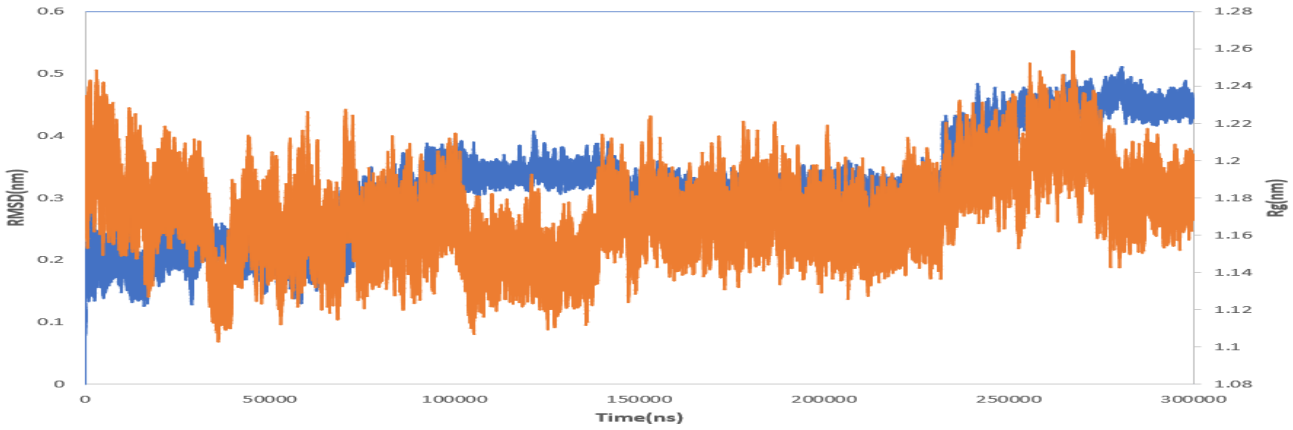
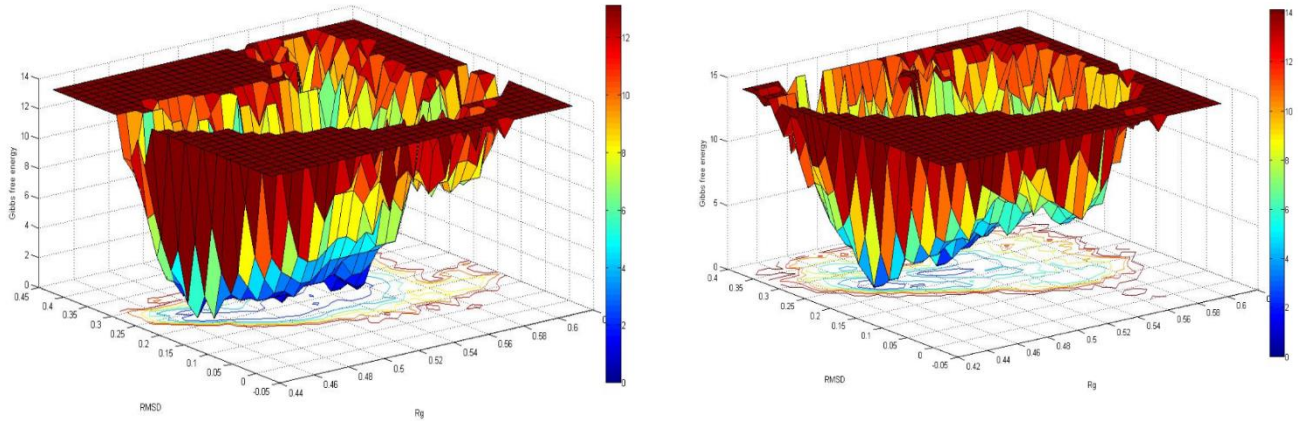


Figure 2. root-mean-square deviation (blue) and radius of gyration (orange) of G-quadruplex versus simulation time

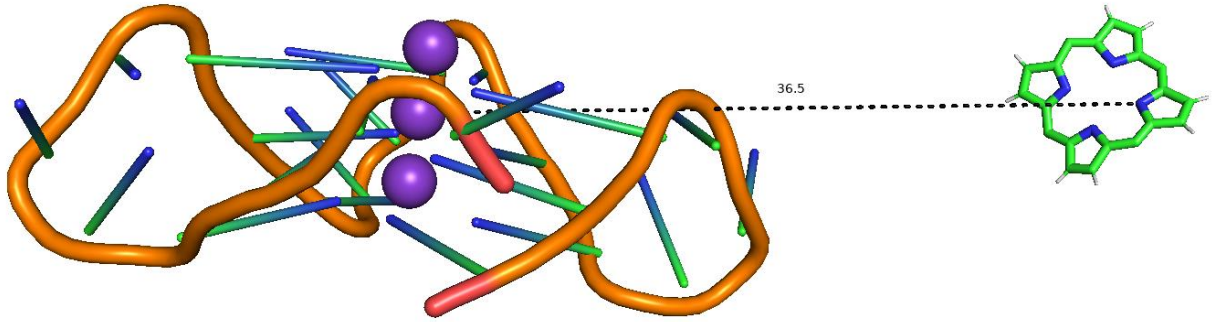
According to Figure 2, RMSD changes are in the range of 0.1 to 0.5 nm. This range of variation is not large considering the size of the G-quadruplex. Therefore, it shows that the simulation time is appropriate. The changes in Rg, which is a measure of the size of the G-quadruplex, show that the G-quadruplex has not changed much. Based on these quantities, the free energy diagram of the studied systems was obtained. The results are shown in Figure 3.



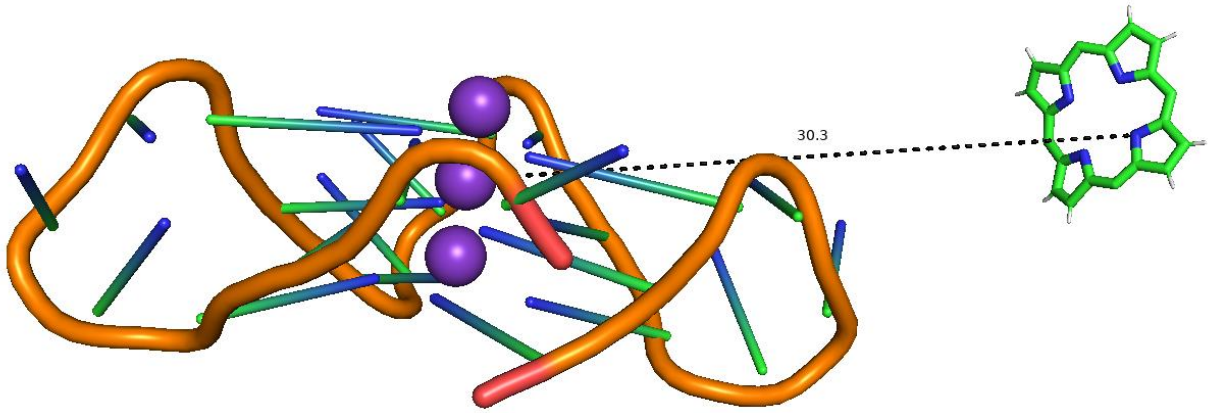
G-guadruplex-Hemin-CNT G-qudruplex-Hemin

Figure 3. Free Energy Landscape of the simulated system.

According to this figure, the appropriate structure was sampled from the performed simulations. To investigate the effect of carbon nanotube on the affinity of Hemin interaction with the G-quadruplex, the distance between the center of mass of these two molecules was calculated in the presence and absence of the nanotube. The result was shown in Figure 4.



G-quadruplex-Hemin system



G-quadruplex-Hemin-CNT system

Figure 4. The distance between the center of mass of G-quadruplex and Hemin in the simulated systems in angstroms.

The figure shows that the nanotube has caused the distance between G-quadruplex and Hemin to decrease. It means that the nanotube has increased the affinity of binding of Hemin to G-quadruplex. Increasing Hemin to G-quadruplex affinity can reduce the background signal and improve complex sensory performance. To investigate the mechanism of increasing binding affinity more closely, the conformational factor denoted as P_i, was calculated. For this purpose, the model developed by Housainidokht et al., in which P_i is considered to be the affinity, was used[36]. P_i is the contacts mean with a certain residue over the simulation time, which can be obtained from the equation $P = \frac{n_I}{<N>}$. <N> is obtained from equation (2).

$$< N >= \sum_{i=1}^{N} \frac{n_i}{N} \qquad (2)$$

Where, n_i is the number of contacts with the residue i, and N is the number of residues in the G-quadruplex structure. The P_i conformation factor, when greater than 1, represents high amounts of affinity for the ligand, and it can be seen that the residues with $P_i > 1$ are used as binding sites. The calculated values of the conformational factor are listed in Table 1.

Table 1. conformational factor of G-quadruplex residue

Residue	Absence of CNT	Presence of CNT
1	0.03	2.03
2	0.48	1.99
3	0.65	0.88
4	0.78	0.99
5	0.05	4.48
6	1.66	2.66
7	1.09	1.98
8	0.99	5.58
9	0.00	0.66
10	0.00	1.11
11	0.00	1.89
12	0.00	8.84
13	0.00	6.67
14	0.35	3.36
15	0.01	0.58
16	0.00	0.36
17	0.00	0.39
18	0.11	0.65
19	0.05	0.99
20	0.00	0.84
21	0.00	0.69
22	0.11	0.48

According to the table, it can be seen that the nanotube has significantly affected the conformational factor. So, in the presence of nanotubes, the conformational factor of G-quadruplex residues has increased. This increase in conformational factor is observed mostly in the middle and initial residues of the G-quadruplex. In other words, Hemin has interacted with the quadruplex from the top and side. These results are consistent with the experimental results that show the improvement of DNAzyme sensor properties in the presence of nanotubes [37-39].

Conclusion

Since biological sensors are far more efficient than chemical sensors, many efforts are being made to develop these sensors. However, there is an important limitation in the application of biosensors in vitro, which is related to the instability of the biosensor in these conditions. The system including G-quadruplex, Hemin and carbon nanotube is one of the proposed solutions for this problem. Many industries such as food contamination detection, pathogen detection and metal ion detection are industries that use this system as a sensor. However, the stabilization mechanism of the biosensor in the presence of carbon nanotubes is not well understood. DNAzyme-sensor property is based on colorimetry and hemin's background signal is large. Therefore, it makes it difficult to use G-quadruplex-Hemin as a sensor. In this work, molecular dynamics simulation method is used to understand the role of carbon nanotube in this stability. The calculation of the interaction parameters showed that the carbon nanotube reduce the hemin's background signal and stabilizes the sensor system by increasing the binding affinity of Hemin to the G-quadruplex.

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