Molecular Dynamics Simulation of siRNA Encapsulated in Carbon Nanotube

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Abstract

We investigated the encapsulation of small interfering RNA (siRNA) in carbon nanotube (CNT) using molecular dynamics simulation. siRNAs can be used to silence specific genes effectively if they remain intact while they are delivered to their target cells. Along with the various drug delivery systems designed for this purpose, CNTs are a promising one. Based on their shape, siRNA can encapsulate inside CNTs and protect them from degradation. However, several factors can affect siRNA encapsulation inside CNTs including temperature and CNT diameter. Herein, we conducted a simulation study to evaluate the impact of these factors in the placement of siRNA. Our results can be considered in designing further experimental siRNA delivery systems using carbon nanotubes.

Introduction

Carbon nanotubes (CNTs) are one of the most widely used nanomaterials in biomedical fields. Over the past decades, CNTs have gained lots of attention in the development of different biosensors, imaging, cancer therapy, tissue engineering, and designing drug delivery systems (1). Carbon nanotubes are fabricated by rolling up a graphene layer. Additionally, they can be classified as single-walled carbon nanotubes (SWNTs) or multi-walled CNT (MWCNT) (2). Considering the needle structure of CNTs, they can penetrate the cytoplasmic membrane of cells without resulting in cell death, therefore, it is possible to use them as a carrier in drug delivery systems (3).

Recently, carbon nanotubes have attracted researchers in small interfering RNA (siRNA) delivery due to their shape which can protect siRNA from degradation. Expression of a specific gene will reduce by the action of a double-stranded RNA (dsRNA); a cellular process known as RNA interference (RNAi). The protein expression will stop when siRNA binds to its complementary site on mRNA. Following siRNA hybridization with mRNA, RNA-induced silencing complex (RISC) will be activated (4). An important challenge in using siRNA-based therapies is designing an efficient delivery system. The use of siRNA in gene silencing is effective when siRNA can be inserted into the cell without degradation. Several delivery systems have been used in recent years to accomplish this goal including polymer-mediated, peptide-based, and lipid-based delivery systems (5).

If a CNT-based delivery system is used, siRNA dissociates from carbon nanotube following transfection into the target cells. siRNA can bind or adsorb to the surface of carbon nanotubes; however, encapsulation of siRNA inside CNTs can protect them from environmental damages such as enzymatic degradation. Moreover, two important factors can significantly affect siRNA delivery using CNTs including temperature and CNTs diameter. In this study, we used molecular dynamics simulation to study the encapsulation of siRNA inside carbon nanotubes.

Methods

Utilizing NAMD (6) package, molecular dynamics (MD) simulations were conducted. To evaluate the effect of temperature on the encapsulation of siRNA in CNT, we simulated the system at four different temperatures including, 300, 310, 320 and 330 K. Selected temperatures are around the body temperature, and this range of temperature is achievable through therapeutic methods. In the next step, we studied the effect of CNT diameter in the encapsulation of siRNA. For this purpose, we employed three nanotubes with the chirality of (20,25), (20,20) and (20,15). The diameter of the mentioned nanotubes is 3.06, 2.71 and 2.38 nm, respectively. The length of the different nanotubes was set to 10 nm. According to the chirality numbers, selected CNTs are in armchair and chiral forms. Figure 2a to 2c compare the diameter of employed carbon nanotubes. It is noteworthy that in all the simulation we used a same siRNA chain. The sequence of the siRNA chain is presented in Table 1.

As Figure 2d indicates, the encapsulated siRNA is located in aqueous environment. To neutralize the system, the positive sodium ions were added to simulation box. The TIP3P model (7) was applied to the water molecules, and the CHARMM36 force field (8) was utilized to model the interaction of siRNA and CNT. Nonbonded interactions were modeled by 6-12 Lennard-Jones (9) (LJ) and electrostatic potentials. The cutoff radius was adjusted to 12 Å for all the nonbonded interactions. Electrostatic interactions were calculated using the particle-mesh Ewald (PME) method (10). The SETTLE algorithm (11) is used to keep hydrogen bonds rigid. The simulation system was minimized for 1000 steps with conjugate gradient method (12) and then relaxed for 100 ps. The simulations ran for 1 ns with the time-step of 2 fs. We applied NPT ensemble to the simulation system using the Langevin (13) and Nosé-Hoover (14, 15) dynamics to maintain the temperature and the pressure. The pressure of the simulation systems was set to 1.01 bar.

To evaluate the siRNA release, the steered molecular dynamics (SMD) simulation has been conducted. The siRNA chain was pulled out of carbon nanotube as a rigid fragment. The carbon nanotube was considered rigid during the extraction, as well. The siRNA has been pulled out of the CNT with the constant velocity of 10 Å/ps. The stiffness of the SMD spring has been adjusted to 10 kcal/mol.Å². The potential energy corresponding to the nonbonded interaction between CNT and siRNA was recorded during the extraction process. The parameters required in the calculation of the interaction energy was obtained from CHARMM36 force field (8). It should be mentioned that, the effect of the water molecules was neglected in the calculation of siRNA nonbonded potential energy. As a result, the variation of obtained potential energy is related to the effect of the CNT.

We obtained siRNA sequence from VIRsiRNAdb (16) which is a curated collection of viral siRNA/shRNA that has been validated experimentally. We selected a siRNA sequence that is employed to silence full length hepatitis C virus particles experimentally (17). Discovery Studio was used to generate three-dimensional structure of siRNA duplex (Figure 1).

Table 1. The sequence of the siRNA utilized in all the simulation systems.

Sense sequence	GGCUGAUAACACCAACGGCA
Antisense sequence	UGCCGUUGGUGUUGAUCAGCC

Results And Discussion

Effects of temperature

Figure 3 demonstrates the root mean square deviation (RMSD) values corresponding to the motion of siRNA at temperatures of 300 to 330 K. To obtain the RMSD, all of the siRNA atoms were included. According to the growth rate of RMSD, the interactions of the siRNA atoms with CNT and water molecules are stabilized with in the 300 ps at the temperatures of 300 to 320 K, while the RMSD of siRNA increases with the time at the temperature of 330 K. As the temperature of the siRNA system increases, we observe the growth of average RMSD. As it shown in Fig. 3, siRNA finds similar RMSD at 300 and 310 K, and the average RMSD is 7.3 Å and 7.6 Å at these temperatures, respectively. By increasing the temperature 320 K, siRNA experiences the average RMSD of 9.6 Å, and the value increases to 14.0 Å at the temperature of 330 K. The growth of average RMSD with temperature indicates the change of siRNA structure at higher temperatures. The change of siRNA structure is reasonable due to the increase of thermal energy at higher temperatures. The variation of RMSD at different temperatures reveals that, siRNA molecule is more stable in nanotube at lower temperatures. Once we increase the temperature of the system, the siRNA experiences higher mobility in carbon nanotube. Due to the growth rate of RMSD it is concluded that, siRNA molecule finds higher diffusion coefficient at higher temperature. This property can be employed to control the encapsulation of siRNA inside carbon nanotube.

The potential energy of the siRNA has been computed to evaluate the stability of molecule at different temperatures. The potential energy of the siRNA is related to the van der Waals (vdW) interaction of the molecule with CNT and solvent atoms. Figure 4 illustrates the potential energy of siRNA at the temperatures of 300 to 330 K. The potential energy of the siRNA molecule is almost constant at the end of the simulation time, which shows the stability of the molecules at different temperatures. The potential energy of siRNA increases within the first 400 ps of the simulation time at the temperature of 330 K and it finds a constant value after 400 ps. The change of the potential energy in the initial time is likely related the structure change of the siRNA molecule. The structure of siRNA is more stable after the first 400 ps, due to the constant potential energy in this period of the time. According to the Fig. 4, the change of the temperature has a considerable effect on the potential energy of the siRNA molecule. The average potential energies of siRNA at the temperatures of 300, 310, 320 and 330 K are - 758, -750, -741 and - 720 kcal/mol, respectively. As a result, the average potential energy of siRNA increases with the temperature. The increase of the potential energy at higher temperatures reveals that, siRNA is more stable at lower temperatures. By increasing the temperature from 300 to 330 K, the average potential energy of the molecules increases by 38 kcal/mol. Consequently, siRNA finds the most stable state at the temperature of 300 K and it has the strongest vdW interaction with CNT and water molecules at this temperature.

Effects of CNT diameter

In this step, the encapsulation of siRNA was examined in three carbon nanotubes with different diameters. The chirality indices of the employed CNTs are (20,25), (20,20) and (20,15), which are different in diameter size. The simulations of different CNTs were carried out at the temperature of 300 K. Figure 5 illustrates the RMSD of siRNA in different carbon nanotubes. Comparing the RMSD of Fig. 5 with the values of Fig. 3 it is concluded that, the change of temperature impacts the RMSD more. As a result, the increase of temperature is a better option to control the stability of siRNA in CNT compared to the change of CNT diameter. However, the change of CNT diameter affects the RMSD of encapsulated siRNA. As Fig. 5 indicates, the lowest RMSD value is attributed the motion of siRNA inside a (20,15) CNT. The structure of siRNA is more stable in this nanotube. The RMSD of siRNA shows higher values with the increase of the CNT diameter. As a result, the siRNA is more flexible in (20,25) CNT and it is allowed to move more in this nanotube. The decrease of siRNA RMSD in smaller nanotubes is likely attributed the attraction term of the vdW interaction. Since the siRNA and CNT atoms have smaller distance in (20,15) CNT case, siRNA molecule experiences more attraction from the nanotube and it does let molecule to move a lot.

Here, we evaluated the potential energy of siRNA molecules during the simulation time. Figure 6 demonstrates the vdW potential energy of the siRNAs encapsulated in different CNTs. The variation of potential energy reveals that, (20,15) CNT has the strongest vdW interaction with siRNA. The smaller diameter of this CNT causes the siRNA to be located at closer distance to the nanotube. Thus, the siRNA atoms are more attracted by (20,15) carbon nanotube atoms. The stronger attraction of (20,15) CNT decrease the mobility of the molecule, which is consistent with the results of RMSD analysis. On the other hand, the potential energy of siRNA is higher in (20,25) CNT. The higher potential energy of siRNA indicates that the molecules is less stable in this nanotube. Since the vdW interaction of siRNA and (20,25) CNT is weaker, the molecule is able to have higher mobility inside the nanotube. This perception is confirmed by the RMSD of molecule located in different CNTs (i.e. Figure 5). It is noteworthy that, the temperature has higher effect on the potential energy of siRNA. Comparing Fig. 4 and Fig. 6 one can realize that the potential energy of the siRNA increases more with temperature than CNT diameter.

Ultimately, we evaluated the potential energy of siRNA during its extraction from (20,20) carbon nanotube. The potential energy of an encapsulated siRNA has been indicated in Fig. 7 as function CNT displacement. The displacement of siRNA is related to the displacement of the center of mass of molecule. Since the siRNA atoms are located in larger distance to the CNT during the extraction process, we observe the decrease of vdW interaction in Fig. 7. As we pull out the siRNA, the potential energy of the CNT-siRNA interaction decreases by almost 800.16 kcal/mol. Since the siRNA and CNT atoms are located in large distance to each other at high displacement range, the vdW interaction between the molecules finds a value close to zero; which means that siRNA is came out of the nanotube completely at the end of process. The decrease of CNT-siRNA interaction energy is almost linearly. The linear decrease of potential energy is probably related to the extraction of siRNA as a rigid fragment from the CNT.

Conclusion

Using siRNA as a gene therapy strategy requires a reliable drug delivery system to prevent siRNA degradation in the extracellular environment. Carbon nanotubes are promising candidates to deliver the desired siRNA into target cells. siRNAs can bind to CNTs in different ways, however, encapsulation of siRNA inside CNTs can be more effective to prevent enzymatic degradation. In our study, we evaluated siRNA encapsulation inside CNTs using molecular dynamics simulation. We investigated the effectiveness of this process by focusing on two important factors including temperature and CNT diameter. Based on our findings, the growth rate of RMSD related to an increase in temperature can be considered as an important factor to control the encapsulation of siRNA inside a carbon nanotube. siRNA is more stable at a lower temperature according to the average potential energies of siRNA, which is consistent with the results of the RMSD.

Next, we evaluated the effect of CNT diameter on the encapsulation of siRNA using three nanotubes with the diameters of 3.06, 2.71 and 2.38 nm. The RMSD of the siRNA indicated a higher mobility for siRNA in larger nanotube. The variation of potential energy revealed that siRNA is more stable in smaller carbon nanotube, which is in agreement with the results of RMSD analysis. Based on our findings, by changing the temperature we are able to control the stability of siRNA in CNT more precisely compared to the CNT diameter changes. However, further experimental evaluation is needed to confirm our findings.

Declarations

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Conflicts of interest/Competing interests

The authors declare that there is no conflict of interests.

Availability of data and material

All related data are deposited in the manuscript.

Authors' contributions

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References

1. Simon J, Flahaut E, Golzio M. Overview of Carbon Nanotubes for Biomedical Applications. *Materials (Basel)*. 2019;12(4).
2. Eatemadi A, Daraee H, Karimkhanloo H, Kouhi M, Zarghami N, Akbarzadeh A, et al. Carbon nanotubes: properties, synthesis, purification, and medical applications. *Nanoscale Research Letters*. 2014;9(1):393.
3. Bianco A, Kostarelos K, Prato M. Applications of carbon nanotubes in drug delivery. *Current Opinion in Chemical Biology*. 2005;9(6):674-9.
4. Agrawal N, Dasaradhi PV, Mohmmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK. RNA interference: biology, mechanism, and applications. *Microbiol Mol Biol Rev*. 2003;67(4):657-85.
5. Dana H, Chalbatani GM, Mahmoodzadeh H, Karimloo R, Rezaiean O, Moradzadeh A, et al. Molecular Mechanisms and Biological Functions of siRNA. *Int J Biomed Sci*. 2017;13(2):48-57.
6. Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, et al. Scalable molecular dynamics with NAMD. *Journal of computational chemistry*. 2005;26(16):1781-802.
7. Mark P, Nilsson L. Structure and dynamics of the TIP3P, SPC, and SPC/E water models at 298 K. *The Journal of Physical Chemistry A*. 2001;105(43):9954-60.
8. Huang J, MacKerell Jr AD. CHARMM36 all-atom additive protein force field: Validation based on comparison to NMR data. *Journal of computational chemistry*. 2013;34(25):2135-45.
9. Honeycutt JD, Andersen HC. Molecular dynamics study of melting and freezing of small Lennard-Jones clusters. *Journal of Physical Chemistry*. 1987;91(19):4950-63.
10. Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG. A smooth particle mesh Ewald method. *The Journal of chemical physics*. 1995;103(19):8577-93.
11. Miyamoto S, Kollman PA. Settle: An analytical version of the SHAKE and RATTLE algorithm for rigid water models. *Journal of computational chemistry*. 1992;13(8):952-62.
12. Navon I, Legler DM. Conjugate-gradient methods for large-scale minimization in meteorology. *Monthly Weather Review*. 1987;115(8):1479-502.
13. Ceriotti M, Bussi G, Parrinello M. Langevin equation with colored noise for constant-temperature molecular dynamics simulations. *Physical review letters*. 2009;102(2):020601.
14. Nosé S. A unified formulation of the constant temperature molecular dynamics methods. *The Journal of chemical physics*. 1984;81(1):511-9.

15. Hoover WG. Canonical dynamics: Equilibrium phase-space distributions. *Physical review A*. 1985;31(3):1695.
16. Thakur N, Qureshi A, Kumar M. VIRsiRNAdb: a curated database of experimentally validated viral siRNA/shRNA. *Nucleic Acids Res*. 2012;40(Database issue):D230-6.
17. Ansar M, Ashfaq UA, Shahid I, Sarwar MT, Javed T, Rehman S, et al. Inhibition of full length hepatitis C virus particles of 1a genotype through small interference RNA. *Viol J*. 2011;8:203.

Figures

Figure 1

3D structure of duplex siRNA.

a)

b)

c)

d)

Figure 2

(a-c) Top view representation of simulation systems. The diameter of CNT varies from (a) 2.38 to (c) 3.06 nm. (d) Side view representation of simulation system. The encapsulated siRNA was placed in aqueous environment and the sodium ions (red spheres) were added for neutralization.

Figure 3

The root mean square deviation of encapsulated siRNA at the temperatures of 300 to 330 K.

Figure 4

Potential energy of siRNA as function of time at different temperatures. The obtained potential energy is originated from the vdW interaction of the molecule with CNT and water molecules.

Figure 5

The RMSD of siRNA molecule as function of simulation time. The siRNA has been encapsulated in CNTs with different chirality indices.

Figure 6

Potential energy of siRNA inside different carbon nanotubes with different diameters.

Figure 7

The vdW interaction of CNT and siRNA during the extraction of siRNA.