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Research Article

SILENCING DNA FUNCTION BY FORMING TRIPLEX USING LNA-TFO

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ABSTRACT
miRNA play important role in cancer cell in terms of gene regulation, gene expression and other vital activity. It play major role in cancer therapy, while unmodified naked miRNA mimics are quickly degrade and clears in blood circulation. LNA (Locked Nucleic acids) is modified RNA locked in sugar C3'-endo conformation (RNA like), can silence and inhibit the function of miRNA, can inhibit the function of HCV replication in-vivo and in-vitro. The therapeutic approach of nucleic acids triplex in gene silencing, inhibition of replication and homologous recombination etc. can be made more efficient by formation of stable Triplex using LNA Triplex-forming Oligonucleotide (TFO). We design (DNA) 2-LNA triplex and (DNA) 2-RNA Triplex with 15-mer mixed sequence 3'(AGGCCGGACCCGGCG)5' by using Recombinant-type(R-type) of H-bonding of DNA with Gaussian 03 in-silico. The complexes were optimized and a 100ns dynamics run using Particle Ewald Sum Method with AMBER 12 code. The binding energy of LNA-TFO in (DNA)2-LNA triplex and RNA-TFO in (DNA) 2-RNA triplex were calculated. The root-mean-square-deviation of (DNA) 2-LNA triplex and (DNA) 2-RNA triplex was constant and is about 2.5 to 3.5 Angstrom during 100ns of dynamics. The negative binding energy and constant rmsd of LNA-TFO and RNA-TFO shows that the complexes are stable during course of simulation. With properties of cell penetration, higher half-life in-vivo together with moderate affinity in-vitro LNA-TFO may be more applicable in triplex strategies in cancer therapy. These findings suggest LNA-TFO may be used as more efficient anticancer drug rather than miRNA.

Keywords: *LNA, Triplex, TFO, miRNA, R-Type*

INTRODUCTION
The present technique chemotherapy used in treatment of cancer disrupts the cell function in terms of DNA replication, transcription and translation or directly DNA damage (Arora & Scholar 2005). Triplex technology promotes a new way of cancer therapeutics can efficiently target gene in terms of gene silencing and regulation of transcription etc. With property of low toxicity, inhibition of angiogenesis and tumor fibrosis, it may play prominent role in cancer therapy. Moreover, with great response in cancer therapy application of miRNA limits in terms of degradation in biological process, short half-life of oligonucleotide (Raemdonck *et al.,* 2009; Yu *et al.,* 2009). RNA like modified nucleotide Locked Nucleic Acid (LNA) locks sugar with methylene linkage between C2' atom and C4' atom, shows C3'-endo puckering; successfully delivered in Phase II trials in treatment of HCV infection *in-vivo* (Tenvang *et al*., 2012; Elmen *et al.,* 2008). A molecular dynamics study and binding energy calculation with using LNA nucleotide has been done human telomeric G-quadraplexes, enhance the application of LNA in human gene with greater binding affinity using LNA in comparison to natural oligonucleotides (Shchyolkinaa 2016; Chaubey *et al*., 2014; Chaubey *et al*., 2012). A comparative study between LNA, RNA and DNA for duplex has been reported, it was found that LNA is less hydrated compared to DNA and RNA but has well organized structure in water. Stable structure of (DNA)2-LNA triplex has been also proposed with modified Guanine base in LNA strand with Hoogsteen type of Hydrogen bonding with NMR study (Conde *et al*., 2016; Pande & Nilsson, 2008; Eichert *et al*., 2010).
In present study two intermolecular triplex taken with same sequence [3'd(AGGCCGGACCCGGCG)5'-5'd(CGCCGGGTCCGGCCT)3'-5'lna(AGGCCGGACCCGGCG)3'] as (DNA)2-LNA triplex and [3'd(AGGCCGGACCCGGCG)5'-5'd(CGCCGGGTCCGGCCT)3'-5'rna(AGGCCGGACCCGGCG)3'] as (DNA)2-RNA triplex with TFO binds by using R-type of H-bonding. A comparative study presented

here that able to describe the ability of binding of LNA-TFO with R-type of H-bonding (Ojha & Tiwari, 2003; Ojha & Tiwari, 1999; Mukharjee and Vasquez, 2011). A comparative study has been done for 100ns of Dynamics using AMBER 12 code. The result shows LNA-TFO binds in major groove of DNA forms stable triplex and therefore able to interfere in various vital processes.

MATERIALS AND METHODS

Model Building

Two complex using [3'd(AGGCCGGACCCGGCG)5'-5'd(CGCCGGGTCCGGCCT)3'-5'lna(AGGCCGGACCCGGCG)3'] as (DNA)2-LNA triplex; [3'd(AGGCCGGACCCGGCG)5'-5'd(CGCCGGGTCCGGCCT)3'-5'rna(AGGCCGGACCCGGCG)3'] as (DNA)2-RNA triplex and [3'd(AGGCCGGACCCGGCG)5'-5'd(CGCCGGGTCCGGCCT)3'-5'd(AGGCCGGACCCGGCG)3'] as (DNA)2-DNA triplex with TFO binds by forming R-type of H-bonding were modelled using NAB (nucleic acid builder) program and CHIMERA. The complexes were neutralized by Na+ ions, Hydrogen added and solute by using TIP3P water molecule in 10Angtrom range of complex in box shape and coordinate and parameter file were generated by LEaP (Case *et al.*, 2014). Density function theory (DFT) is orthogonal to Hartree-Fock Quantum Mechanical methodology with cheap computational cost (Backe, 1993). The glucose modified and natural nucleotide with Guanine (G-C*G base composition) were modelled by using Arnott fiber structure. The structure were optimized by B3LYP using 6-31G* basis set in Gaussian 09 (Frisch, 2009). The energy of both the nucleotide was calculated by exchange correlation HF QM method.

Minimization and Equilibration

The structure were minimized by using steepest decent method for 500 steps and generalized born method for next 500 steps with decreasing restraints in six steps. Complex was heated till 300 K and equilibrated in 5 steps. The dynamics starts with zero initial velocity, 2.0 ps time step was taken, initial interaction other than hydrogen atom involved.

Production Dynamics

The production dynamics of complexes with water solvent starts with equilibrated structure by the molecular dynamics. These dynamics performed under constant pressure periodic boundary condition with constant pressure of 1 atmosphere with isotropic position of scaling with reference pressure relaxation time of 2.0 ps.

Bond involving hydrogen were neglected. The mdcrd (molecular dynamics coordinate file) will be written after each 2500 steps with dt=0.002 ps in constant temperature of 300.0±3.0 K. In the same manner 100 ns dynamics performed with AMBER 12 code. The Molecular dynamics movie is recorded to see the variation during dynamics for each simulation.

Energy Calculation

The binding free energy of DNA to the DNA duplex, RNA to the DNA duplex and LNA to the DNA duplex was calculated by MMPBSA method of AMBER 12 code (Pandey *et al.*, 2017). Before starting production dynamics for binding free energy calculation, the system was followed by minimization, 50 ps of heating, then density and constant pressure equilibration with shake algorithm of 2fs time step. A weak restraint of 2.0 KCal/mol was kept during heating and density equilibration steps. Further production dynamics run for 20 ns in 4 steps each of 5 ns using Particle Mesh Ewald Sum method. The binding free energy is sum of solvation energy and gas energy.

RESULTS AND DISCUSSION

Energy of Triplets and Hydrogen Bonding Pattern

The total energy of LNA and natural G-C*G triplets were calculated by B3LYP using DFT; it is found that energy of triplet with LNA base is -3081.69 Kcal/mol and natural triplet is -2739.49 Kcal/mol. The third strand base recognizes both the base of double strand by forming R-type of Hydrogen bonds Shown in figure 1. The recognition of both the bases by third base leads to the formation of stable triplex with LNA sugar in mixed sequence.

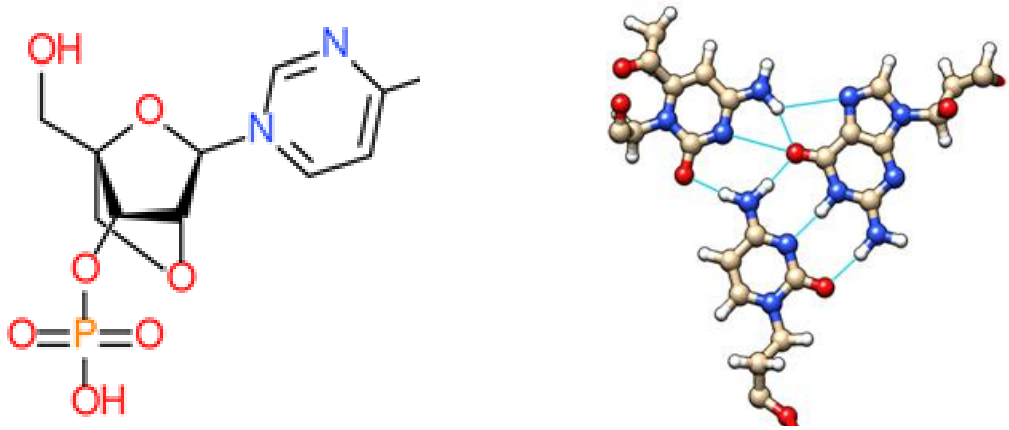

Figure 1: (Left) Nucleotide Structure of LNA with Guanine Base, here C2' Atom and C4' Atom Linked by Methylene Bridge (Nucleotide Locks in C3'-Endo (RNA like) Conformation)
(Right) Hydrogen Bonding Pattern of G-C*G Triplet with R-Type of Bonding Pattern; the Third Base Guanine Binds G-C Base Pair by Forming R-Type of H-Bonds (Image Taken by CHIMERA)

The Root-Mean-Square-Deviation (RMSD)

The rmsd over the course of simulation is used to measure the conformational stability of the complex during simulation. The dynamics study of RNA and LNA triplex show that LNA is less hydrated and show ordered structure during simulation, the intra-strand Phosphate-phosphate distance slightly lesser and inter-strand phosphate-phosphate distance(x-displacement) is larger than natural oligonucleotide. The root-mean-square-deviation plots of these entire complexes are shown in Figure 2. The result shows the stable triplex form by using LNA and RNA-TFOs.

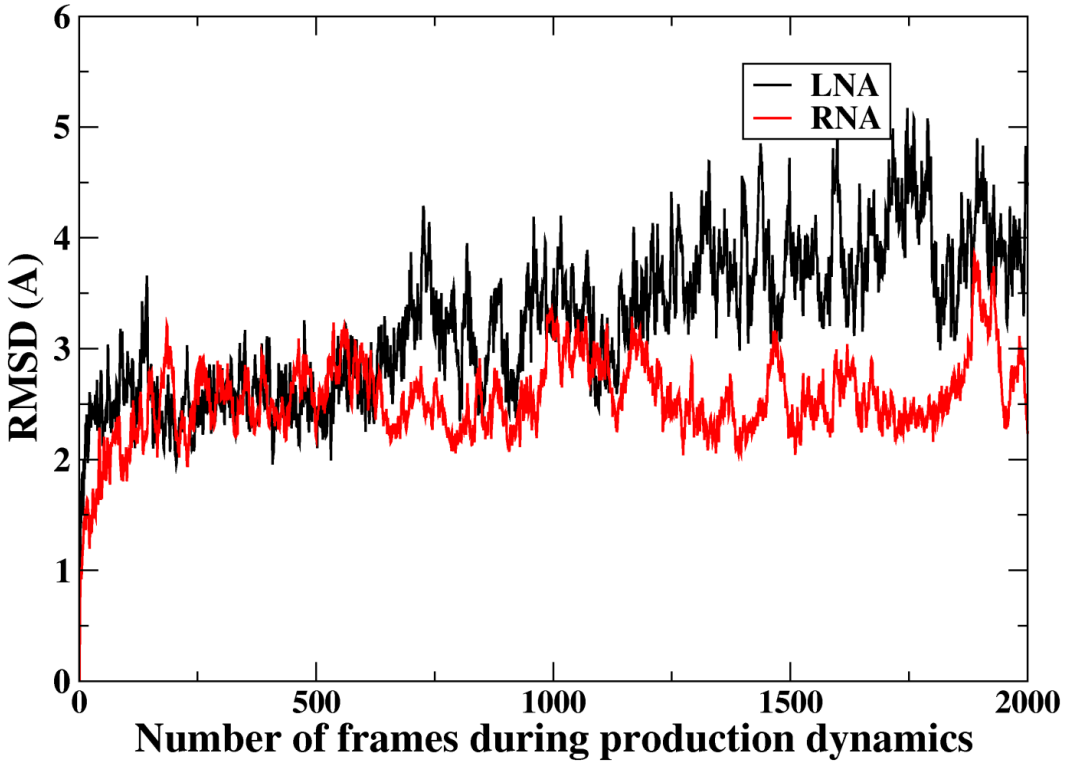

Figure 2: The Root-Mean-Square-Deviation of Triplex with Third Strand with RNA in Red Color and Triplex with Third Strand with LNA in Black Color

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Binding Free Energy Calculation

The binding free energy of both the complex is negative and shown in Table 1. The negative binding free energy is the measure of stable structure during course of simulation. The binding free energy is of RNA strand to DNA duplex (-95.54 Kcal/mol) is even greater than LNA strand to DNA duplex (-49.22 Kcal/mol) but the binding free energy of DNA strand to DNA duplex (+76.53Kcal/mol) is positive.

Table 1: Binding Free Energy of Triplexes with DNA, RNA and LNA Complexes (in Kcal/mol)

Energy Component	(DNA)2-DNA Triplex	(DNA)2-RNA Triplex	(DNA)2-LNA Triplex
van der Waals	-103.11	-115.56	-94.87
Electrical	6198.73	6316.40	-94.87
Poisson Boltzmann	-6100.89	-6283.95	-6241.20
Surface Energy	82.19	-12.43	-10.88
Delta G gas	6095.62	6200.84	6202.85
Delta G solvation	-6019.08	-6296.39	-6252.08
Delta G total	**+76.53**	**-95.54**	**-49.22**

Discussion

The free energy calculations show that LNA can form stable complex in the mixed sequence of DNA. Root-mean-square-deviation shows that structure has stable conformation during dynamics and forming R-type of hydrogen bindings in the base triplets. Since LNA-TFO has cell penetration properties as tested in HCV drug therapy *in-vivo*, moderate binding affinity as RNA-TFO with R-type H-bonding, higher half-life *in-vivo*. These properties of LNA-TFO suggest that LNA-TFO may be more efficient than RNA-TFO in triplex technology of cancer therapy.

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