3-31-2015

Interaction pattern for the complex of B-DNAFullerene compounds with a set of known replication proteins using docking study

Sumbul Firdaus

Anupam Dhasmana
The University of Texas Rio Grande Valley

Vandana Srivastava

Tasneem Bano

Afreen Fatima

See next page for additional authors

Recommended Citation

Firdaus, S., Dhasmana, A., Srivastava, V., Bano, T., Fatima, A., Jamal, Q. M., Jahan, R., Wadhwad, G., & Lohani, M. (2015). Interaction pattern for the complex of B-DNA Fullerene compounds with a set of known replication proteins using docking study. Bioinformation, 11(3), 122–126. https://doi.org/10.6026/97320630011122

BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 11(3)

Interaction pattern for the complex of B-DNA-Fullerene compounds with a set of known replication proteins using docking study

Sumbul Firdaus[1], Anupam Dhasmana[2], Vandana Srivastava[2], Tasneem Bano[2], Afreen Fatima[2], Qazi Mohammad Sajid Jamal[3], Roshan Jahan[3], Gulshan Wadhwad[4] & Mohtashim Lohani[3]*

[1]Department of Physics, Integral University, Lucknow, India; [2]Departments of Bioengineering, Integral University, Lucknow, India; [3]Departments of Biosciences, Integral University, Lucknow, India; [4]Department of Biotechnology, Ministry of Science and Technology, CGO complex, Lodhi Road, New Delhi-110003, India; Mohtashim Lohani – Email: mlohani@rediffmail.com; Phone: +91-522- 2890730, 2890812 (O) +91-9161984134 (Personal); Fax: +91-522- 2890809; *Corresponding author

Received January 21, 2015; Accepted February 23, 2015; Published March 31, 2015

Abstract:
Fullerenes have attracted considerable attention due to their unique chemical structure and potential applications which has opened wide venues for possible human exposure to various fullerene types. Therefore, in depth knowledge of how fullerene may interfere with various cellular processes becomes quite imperative. The present study was designed to investigate how the presence of fullerene affect the binding of DNA with different enzymes involved in replication process. Different fullerenes were first docked with DNA and then binding scores of different enzymes was analyzed with fullerene docked DNA. C30, C40 & C50 once docked with DNA, reduced the binding score of primase, whereas no significant change in the binding score was observed with the helicase, ssb protein, dna pol δ, dna pol ε, ligase, DNA clamp, and topoisomerases. On the contrast, the binding score of RPA14 decreases in fluctuating manner while interacting with increasing molecular weight of fullerene bound single-stranded DNA complex. The study revealed the affect of fullerene family interacting with DNA on the binding pattern of enzymes involved in replication process. Study suggests that the presence of most of fullerenes may not affect the activity of these enzymes necessary for replication process whereas C30, C40 & C50 may disrupt the activity of primase, (strating point for DNA polymerase) its docking score decreases from 13820 to 10702.

Keywords: Fullerene, Fullerene family, RPA, replication enzymes.

Background:
Nanotechnology, actually means the exploitation of the substances at their nano-meter size, and is expected to enhance the quality of life and economic development on the global basis. A decade ago, nanoparticles were studied because of their size-dependent physical and chemical properties, but now they have crossed the threshold of commercial exploration period. Understanding of biological processes on the nanoscale level is a strong driving force behind development of nanotechnology **[1].** Out of surplus of size-dependant physical properties of nanomaterials like optical and magnetic effects have been exploited for a number of biological/medical applications, e.g.: their use as fluorescent biological labels for the drug and gene delivery, Probing of DNA structure, for the treatment of cancer, for the separation and purification of biological molecules and cells etc.. These unlimited advantages of nanoparticles lead to thier mass-production, making the exposure of almost enevitable. The human exposure to these nanoparticles raises concern about their potential risk to human health. Nanoparticles could easily enter the body through the

food or water we consume, both accidently or intentionally via nose and lungs just like other aerosols. Some nanoparticles readily travel throughout the body, deposit in target organs, penetrate cell membranes, lodge in mitochondria, and may trigger injurious responses. As related research on smoking's effects on lung tissue has found, foreign particles inhaled into the lungs have the potential to do great damage **[2].** Earlier studies revealed that inhaled nanoparticles not only cause lung damage, but also can move into the bloodstream; potentially causing cardiac damage and other observations indicate that inhaled nanoparticles in humans caused damage both to the point of entry, and to the brain itself. There are some special kinds of nanoparticles made up of carbon are termed as fullerenes which occur naturally in the form of C20, C60, C70, C82, molecules whereas C24, C30, C40 etc can be produced by various industrial processes. Because of small size and easy entry into the human body, they get readily adsorbed to macromolecules affecting the regulatory mechanism of macromolecules, proteins and genetic materials.

A number of in vitro as well as in vivo studies have proven that these nanomaterials are also capable of inducing DNA damages **[3, 4 & 5].** C60 and its derivatives were reported to inhibit the replication of simian immunodeficiency virus (SIV) in vitro and the activity of Moloney murine leukaemia virus (M-MuLV) reverse transcriptase **[6].** Large numbers of researches are being done on DNA replication and causes of mutations, checkpoint control and also on the enzymes involved in replication process **[7, 8 & 9].** Simulation studies conducted earlier revealed that C60 strongly binds to nucleotides in aqueous solution at the hydrophobic ends or at the minor groove of the nucleotide **[10].** This C60-ssDNA binding can significantly deform the nucleotides. Some studies revealed CNP -DNA binding leading to DNA aggregation in vivo and *in vitro* **[11]** .The binding mechanism of water-soluble C60 derivative-ss-DNA was found to be similar to native C60–DNA, while forming more stable C60–DNA complex. Molecular dynamics study reveals the distortion of DNA/RNA by the fullerene **[12].** It has been already reported that C60 can binds to DNA via hydrophobic interactions *in silico*. Some *in vitro* studies are also done to investigate the toxicity mechanism of C60 in biological system show that C60 molecules may interfere with the biological functions performed by DNA, resulting in disruptions to DNA replication, transcription and repair processes **[13].** More studies are needed to establish the interactions of fullerenes with the molecular machineries and processes and how these interactions may affect various biological functions. In depth studies are required to investigate the interference of fullerenes in DNA replication machinery, how fullerene bound DNA is interacts with the enzymes involved in replication process etc. Here, we propose the application of *in-silico* approach to investigate the interaction of enzymes involved in DNA replication process with fullerene (C20 to C180) bound DNA.

Methodology:

In one set of investigation, the docking scores of DNA with eight different enzymes involved in replication process were determined. Further, the docking score of eight enzymes were determined with the fullerene (C20 to C180) bound DNA complexes. The two sets were compared to determine the effect of fullerene on the binding of enzymes with DNA.

Generation and procurement of macromolecules

Double stranded & single stranded DNA structures were constructed using Discovery Studio Visualizer (Version 2.5.5). And the structures of enzymes involved in the DNA Replication process were obtained from RCSB Protein Data Bank. Published structures were edited to remove HETATM using Discovery Studio Visualizer (Version 2.5.5). Chimera was used for energy minimization, removal of steric collision with the steepest descent steps 1000, steepest descent size 0.02 Å, Conjugated gradient steps 1000 and the conjugate gradient step size 0.02 Å for the conjugate gradient minimization **[14, 15].**

Procurement of fullerene family

Nanotube Modeller is a program for generating XYZ co-ordinates of nano geometries (nanocone, nanotube, fullerenes, viruses etc.). Fullerenes of various molecular sizes were obtained through fullerene library of Nanotube Modeller. Generated geometries of C20, C30, C40, C50, C60, C70, C80, C90, and C100 & C180 were viewed using the integrated viewer.

Molecular Docking Studies

All the *in silico* docking analyses were performed using PatchDock (Schneidman *et al*, 2005) **[16].** The fullerenes were docked with the DNA. The resultant pdb file obtained after fullerenes and DNA docking was used as fullerene-DNA complex, and was docked with different enzymes along with some replication factors involved in the replication process of DNA by uploading them as a receptor and ligand molecules in PatchDock Server, an automatic server for molecular docking. Clustering RMSD was chosen as 4.0Å.

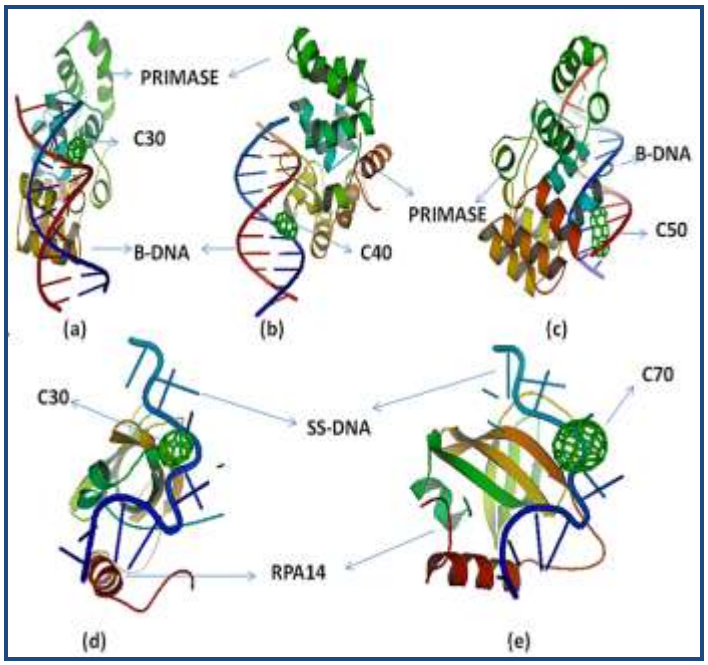

Figure 1: Presence of some fullerene molecules affects the binding of **Primase** and **RPA14 1(a):** shows the interaction of primase with c30 bound DNA, **1(b):** shows the interaction of primase with c40 bound DNA, **1(c):** shows the interaction of primase with c50 bound DNA, **1(d):** shows the interaction of RPA14 with c30 bound ss-DNA, **1(e):** shows the interaction of RPA14 with c70 bound ss-DNA.

Results:
Fullerenes of various molecular weights (c20, c30, c40, c50, c60, c70, c80, c90, c100, c180) were separately docked with DNA to form fullerene-DNA complex. Docking score ranged from 2062(C20) to 3888(C180). Further, fullerene-DNA complexes were used as a receptor and enzymes involved in replication process as a ligand to show the effects of fullerenes on the DNA replication process. Each of the eight enzymes was docked with the different forms of fullerene-DNA complex. Then, each of these enzymes involved in replication process were separately docked with DNA alone i.e. in the absence of fullerene. And their docking scores were compared and analyzed to investigate the effect of presence of fullerene. Analysis of all the docking results revealed that c30, c40 and c50 may affects the activity of Primase as the docking score of primase with B-DNA is 13820, but the docking score of primase with B-DNA-C30, BDNA-C40, BDNA-C50 complexes are 10702, 11734 & 11270 respectively **(Figure1).**

Apart from the enzymes, replication proteins A (RPA14, RPA32 & RPA70) were docked with single-stranded DNA alone then with the fullerene bound ssDNA in order to analyse the affect of of fullerene. **Figure 2** Graph 1 showed the docking result which explains that the presence of fullerene caused considerable reduction in the docking score of RPA14, whereas RPA32 & RPA70 binding score increased in the presence of fullerene. Docking score of RPA14 with ssDNA is11170 whereas the docking score decreases when RPA14 interact with fullerene bound ssDNA, ranged from 10116(ssDNA-C20) to 10284(ssDNA-C180). All these scores are shown in **Table 1 (see supplementary material).**

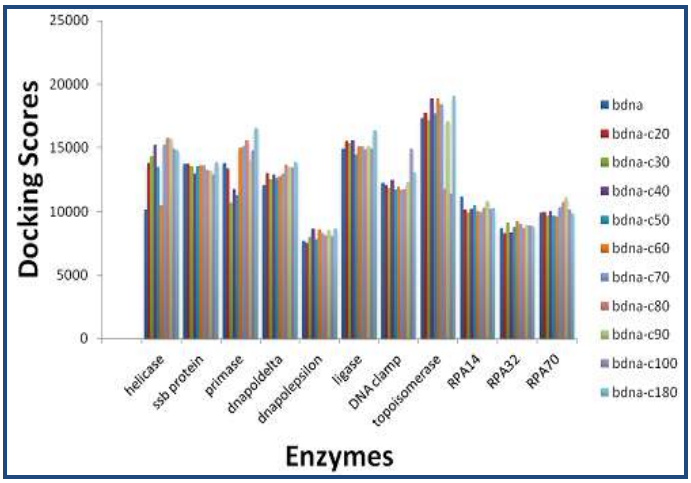

Figure 2: Graph shows functional loss analysis in expressions of docking score of B-DNA in the presence of fullerene with enzymes and factors involved in replication machinery.

Discussion:
Buckminsterfullerene (C (60)) has received great research interest due to its extraordinary properties and increasing applications in manufacturing industry and biomedical technology. AN H *et al.* **[17]** recently reported C (60) could enter bacterial cells and bind to DNA molecules and determine how the DNA-C60 binding affected the thermal stability and enzymatic digestion of DNA molecules, and DNA mutations. Some in vitro studies have also been done to investigate the toxicity mechanism of C60 in biological system show that C60 molecules may interfere with the biological functions performed by DNA, resulting in disruptions to DNA replication, transcription, and repair processes **[18].** More studies are needed to reveal the interactions of various species of fullerenes with enzymes involved in DNA processes, and how this interaction may affect the biological functions. However to the best of our knowledge no such study has been done involving fullerene and fullerene family interaction with all the enzymes of eukaryotic DNA replication process. Therefore, the present study was designed to reveal the effect of molecular weight and size of fullerene on its capacity to interact with different enzymes of DNA replication.

We have previously shown the applicability of PatchDock to determine interaction between nanoparticles and biomolecules **[19].** In the present study, we performed molecular docking between various enzymes & factors involved in replication with the DNA bound with fullerene molecule of different sizes (C20 to C180) separately in order to evaluate the effect of fullerene on the binding receptor of enzymes with DNA. Docking score of DNA-enzyme complexes were compared with the fullerene bound DNA- enzyme complexes, which reveals that most of the enzymes activity were not affected by the presence of fullerene. Docking score of only Primase and RPA14 decreased when they interacts with the fullerene-DNA complex in comparison to the score when they were interacted directly with the DNA. So this decrease in the score may be considered because of the presence of fullerene and in-vitro and in vivo studies are needed to conclude that the presence of fullerene may hamper the activity of enzymes during replication process of DNA. All these enzymes are having their specific function; involvement of fullerene may affect its functionality. Previous studies have shown that the nanoparticle was found to bind with the minor grooves of double-stranded DNA and trigger unwinding and disrupting of the DNA helix, which indicates C60 can potentially inhibit the DNA replication and induce potential side effects and it has been proved that pristine fullerene nanoparticles are capable of adsorbing polymerase and significantly inhibiting its biologically important replication activity; however, the inhibition can be partially mitigated by abundant proteins through competitive binding **[20].** Zhao *et.al* work theoretically to show how C60 binds to and deforms a DNA fragment suggesting the potential for C60 molecules to disrupt the replication and repair of DNA **[10].** The DNA-C60 complexes depend on the nature of the nucleotide. Yong Liang et.al, shows that that C60 can disrupt DNA replication in vitro by binding to DNA and changing the conformation of DNA templates.

Our finding suggests that activity of primase might be affected by C30, C40 & C50 fullerene-DNA complex. Interaction shows significant decrease in their docking score such as c30, 40, 50-DNA complexes with Primase **(Figure 1).** Primase provides a starting point of RNA (or DNA) for DNA polymerase to begin synthesis of the new DNA strand but the presence of fullerene may hamper the replication at the starting point itself. Whereas interaction of fullerene with other enzymes showed, no significant change in the docking scores. But the interaction of replication factor RPA14 with ssDNA-fullerene complex shows significant decrease in the docking score from 11170 to10284 shown in **Table 1 (see supplementary material).** As the Replication protein A (RPA) binds with high affinity to ssDNA

that is formed transiently during DNA replication, recombination and repair, protecting it from nucleases, and destabilizing unwanted secondary structures (e.g., hairpins and G-quadruplexes) but the decrease in binding score is due to fullerene presence which may affect the proper functioning of these particular enzymes, necessary for replication process; suggesting that defect in DNA replication may be the source of this damage.

Conclusion:

Primase provides a starting point of RNA (or DNA) for DNA polymerase to begin synthesis of the new DNA strand but this activity of primase might be impaired by fullerene **(**C30, C40 & C50). While the interaction of fullerenes with other enzymes (helicases, ssb protein, dna pol δ, dna pol ε, ligase, DNA clamp, and topoisomerases) do not show significant reduction in their docking score. Apart from enzymes replication factor RPA14 when docked with ssDNA-fullerene complex shows significant decrease in the docking score, which means RPA14 would not able to destabilize unwanted secondary structures of ssDNA and may leads to poor replication.

References:

[1] Whitesides GM, *Nat Biotechnol*. 2003 **10:** 1161 [PMID: 14520400]
[2] Ryu JH *et al. Eur Respir J*. 2001 **17**: 122 [PMID: 11307741]
[3] Rahman Q *et al. Environ Health Perspect*. 2002 **110:** 797 [PMID: 12153761]
[4] Srivastava RK *et al. Nanotoxicology*. 2011 **5:** 195 [PMID: 20804439]
[5] Srivastava RK *et al. Hum Exp Toxicol*. 2013 **32**: 153 [PMID: 23111874]
[6] Nacsa J *et al. Fullerenes, Nanotubes and Carbon Nanostructures.* 1997 969–976**.**
[7] Pray L, *Nature Education*. 2008 **1**: 214
[8] Das-Bradoo S & Bielinsky A, *Nature Education*. 2010 **3:** 50
[9] Pursell ZF & Kunkel TA, *Prog Nucleic Acid Res Mol Biol*. 2008 **82:** 101 [PMID: 18929140]
[10] Zhao X *et al. Biophys J.* 2005 **89:** 3856 [PMID:16183879]
[11] An H *et al. Biochem Biophys Res Commun*. 2010 **393:** 571 [PMID: 20156419]
[12] Xu X *et al. Nucleic Acids Res.* 2012 **40:** 7622 [PMID: 22661584]
[13] Connie A *et al. Journal of Experimental Microbiology and Immunology*. 2006 **9:** 68
[14] Wang J *et al. J Comput Chem*. 2004 **25:** 1157 [PMID: 15116359]
[15] Wang J *et al. J Mol Graph Model*. 2006 **25:** 247 [PMID: 16458552]
[16] Schneidman-Duhovny D *et al. Nucleic Acids Res.* 2005 **33:** W363
[17] An H & Jin B, *Environ Sci Technol*. 2011 **45:** 6608 [PMID: 21718073]
[18] Xu X *et al. Nucleic Acids Res.* 2012 **40:** 7622 [PMID: 22661584]
[19] Dhasmana A *et al. PLoS One*. 2014 **9:** e107068 [PMID: 25215666]
[20] Maoyong S *et al. Chem Commun (Camb)*. 2010 **46:** 1404 [PMID: 20162129]

Edited by P Kangueane
Citation: **Firdaus *et al.*** Bioinformation 11(3): 122-126 (2015)

BIOINFORMATION

open access

Supplementary material:

Table 1: Functional loss analysis of activity of enzymes and factors involved in replication machinery when docking score/ binding values of BDNA with enzymes and factors were compared in the presence and absence of fullerene molecule of different molecular sizes.

S.NO.	Enzymes	Bdna	bdna-c20	bdna-c30	bdna-c40	bdna-c50	bdna-c60	bdna-c70	bdna-c80	bdna-c90	bdna-c100	bdna-c180
1.	Helicase	10170	13822	14368	15252	13478	10496	15254	15794	15720	14932	14794
2.	ssb protein	13764	13768	13580	12989	13550	13610	13632	13236	13178	12896	13876
3.	Primase	13820	13386	10702	11734	11270	14990	15102	15588	14050	14804	16570
4.	Dnapoldelta	12038	13038	12572	12876	12578	12764	12958	13678	13498	13470	13880
5.	dnapolepsilon	7674	7482	8010	8662	7804	8592	8302	8104	8504	8074	8676
6.	Ligase	14966	15526	15338	15610	14450	15108	15140	14874	15120	14916	16394
7.	DNA clamp	12218	12084	11832	12512	11706	11916	11688	11740	12328	14916	13094
8.	topoisomerase	17310	17730	17168	18924	17692	18884	18402	11740	17078	11428	19100
9.	RPA14*	11170	10116	9928	10182	10528	10020	9984	10278	10834	10186	10284
10.	RPA32*	8714	8268	9106	8330	8782	9274	9034	8726	8970	8874	8814
11.	RPA70*	9892	9990	9656	10048	9680	9582	10304	10724	11082	10156	9820

NOTE: "*" sign denotes replication factors interacting with single–strand DNA, scores in red color denote major functional loss of enzymes activity.