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RAPID COMMUNICATION

Molecular interactions of graphene with HIV-Vpr, Nef and Gag proteins: A new approach for treating HIV infections

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Abstract−Graphene draws considerable attention among biomedical researchers because of its unique physical, chemical and biological properties. The wide applications of graphene in the biomedical arena such as diagnostics, drug immobilization and drug delivery were well documented in the literature. However the therapeutic potential of the graphene towards retroviruses and the interactions of the graphene with receptors/proteins are still unexplored. Herein we report the antagonistic molecular interactions of graphene with the three key target proteins of HIV infections namely HIV-Vpr, Nef and Gag proteins. The docking investigations were performed to find the binding energy of the graphene ligands to the key target proteins of HIV. The high binding affinity of the graphene to these proteins indicates the antagonistic molecular interaction of graphene to the disease targets. The therapeutic potential of graphene was also studied by changing the size and the number of layers of the graphene. The experimental results confirm the good therapeutic potential of the graphene to combat HIV mediated retroviral infections.

Keywords: Graphene, HIV, Immobilization, Therapeutic Potential, Binding Affinity

INTRODUCTION

Human Immuno Deficiency virus (HIV), the etiologic agent of Acquired Immuno Deficiency Syndrome (AIDS), affects several million human populations throughout the world. The advancements in medical sector accelerate at rapid speed; however, the ideal drug for HIV is still unexplored. 3′-Azido-3′-deoxythymidine (AZT) and dideoxynucleosides have been shown to reduce the mortality of HIV patients. But, AZT drugs are not specific to the pathogens and they also pose toxicity to the host cells. They also affect the cell-mediated immune functions [1]. The continuous use of some antiretroviral drugs for a long duration leads to several complicated ailments including hepatobiliary side effects and metabolic disorders. Retroviral drugs also cause hyperglycemia, hyperlipidemia, lipodystrophy, and bone mineral loss including osteonecrosis [2]. Rapid emergence of drug resistance and drug toxicity hinders the identification of ideal drug for HIV. Hence there is an urgent need to develop new anti-HIV agents for the treatment of AIDS. Nanomaterials are most promising candidates for treatment of several devastating diseases [3]. Carbon materials, including the carbon nanotubes and the carbon dots, were also found to have anti HIV activity [4]. Graphene materials are promising materials for biomedical applications because of their unique physical, chemical and biological properties. Graphene finds its use in several areas of the biomedical sector including therapy and drug delivery [5].

HIV-Viral Protein R (Vpr), Negative Regulatory Factor (Nef) and Group-specific antigen (Gag) proteins are chosen as the key targets to combat HIV mediated infections. HIV-Vpr is a 14-kDa protein with 96 amino acids that plays a most crucial role in regulating the entry of the HIV-1 pre-integration complex into the host cell and replication of virus in non-dividing cells [6]. Nef is small 27-35 kDa myristoylated accessory protein of HIV-1which promotes virus replication and pathogenesis in the infected host [7]. Gag protein is the genetic material that codes for the core structural proteins of a retrovirus that supports the formation of structures required for the correct assembly, budding, and maturation of viruses [8]. The inhibitors of the HIV Vpr, Nef and Gag proteins can serve as good therapeutic molecules to combat the HIV mediated viral infection. Previously, a report was made in the literature on the use of graphene oxide for reducing the Vpr mediated cytotoxicity. Graphene oxide alters the conformation of the Vpr, thereby preventing its pore formation activity [9]. Few investigations were also made on the interactions of graphene with peptides using the techniques such as circular dichorism and scanning electron microscopy [10,11]. Herein, the interaction of graphene with the HIV key target proteins Vpr, Nef and Gag is investigated using computational molecular approaches. Computational approaches are widely used for screening ligands for various biocontrol applications [12]. Recently we have documented our work on the interaction of carbon nanotubes with the key target proteins of HIV [13].

MATERIALS AND METHODS

1. Interaction of Single Layered Graphene with the Disease Targets

Single layered graphene of dimension 9.8 Å×9.8 Å was modeled

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with the Avogadro software. The modeled structures were characterized and were used as the ligand. The modeled graphene ligand is processed by adding hydrogen atom with the MGL tools. The ligand energy is minimized by computing gasteiger charges before storing in the pdbqt format for the docking studies with the AutoDockVina 4.2.

The PDB structures of the HIV-Vpr protein were collected from the PDB id'1CEU from the PDB database. Initially, the native ligands present in the retrived HIV-Vpr protein structure were removed. To check the conformation of the HIV-Vpr protein structure, the RMSD value was calculated between the retrieved original protein structure with the ligand and the ligand deleted protein structure.

After the protein conformation studies, the heteroatoms including water molecules were removed from the HIV-Vpr protein structure. Polar hydrogens were added to HIV-Vpr proteins and Kollman's partial atomic charges were applied to minimize the energy. The processed protein structure was saved in PDBQT file format that contains a protein structure with hydrogens in all polar residues. Q-SiteFinder is the most simple energy-based method for the prediction of active sites of the protein [14]. The active sites of the HIV-Vpr protein were found with the Q site finder. The active binding region of HIV-Vpr protein for docking was found such that the entire ligand binding region of the protein was covered within the GRID. The Autodock tool was used to select the active region based on the amino acid sequence obtained with the Q site finder. The dimensions of the Grid were 40 along all the three directions. AutoDock Vina is a docking tool to improve the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading [15]. Docking study is performed with the AutoDock Vina with AMBER force field and Monte Carlo simulated annealing. The processed HIV-Vpr protein is kept as rigid and the ligand molecules are kept flexible throughout the docking process. For searching the phase space of the ligand and the protein a novel hybrid global-local evolutionary algorithm was used. Similar procedures were followed for accessing the molecular interaction of the modeled graphene ligands to the Nef and Gag proteins. The virtual analysis of the docked site of the protein with the ligand was analyzed with the PyMOL viewer.

2. Effect of the Size of Graphene on the Interaction with the Disease Targets

Single layered graphene sheets with the dimension of 17 Å×17 Å and 22 Å×22 Å are modeled with the Avogadro software. The interaction of these two modeled structures with the Vpr, Nef and Gag proteins was done by docking studies as described in the previous section.

3. Effect of the Number of Layers of Graphene on the Interaction with the Disease Targets

Further studies were made of the therapeutic effect of the layer number of graphene. The graphene structures with 1, 2, 3, 4 and 5 layers with a standard dimension of 9.8 Å×9.8 Å were modeled and were processed as described previously. Then the molecular interactions of the modeled graphene structures with the Vpr, Nef and Gag proteins were analyzed and their binding affinities were identified.

RESULTS AND DISCUSSION

Single layered graphene sheets of dimension 9.8 Å×9.8 Å were modeled using the Avogadro software (Fig. 1A). They had the molecular weight of 652.948 (g/mol). The structure of the target proteins Vpr, Nef and Gag is shown in the Fig. 1B, 1C and 1D, respectively.

1. Interaction of Single Layered Graphene with the Disease Targets

The binding affinity of modeled single layered graphene of dimen-

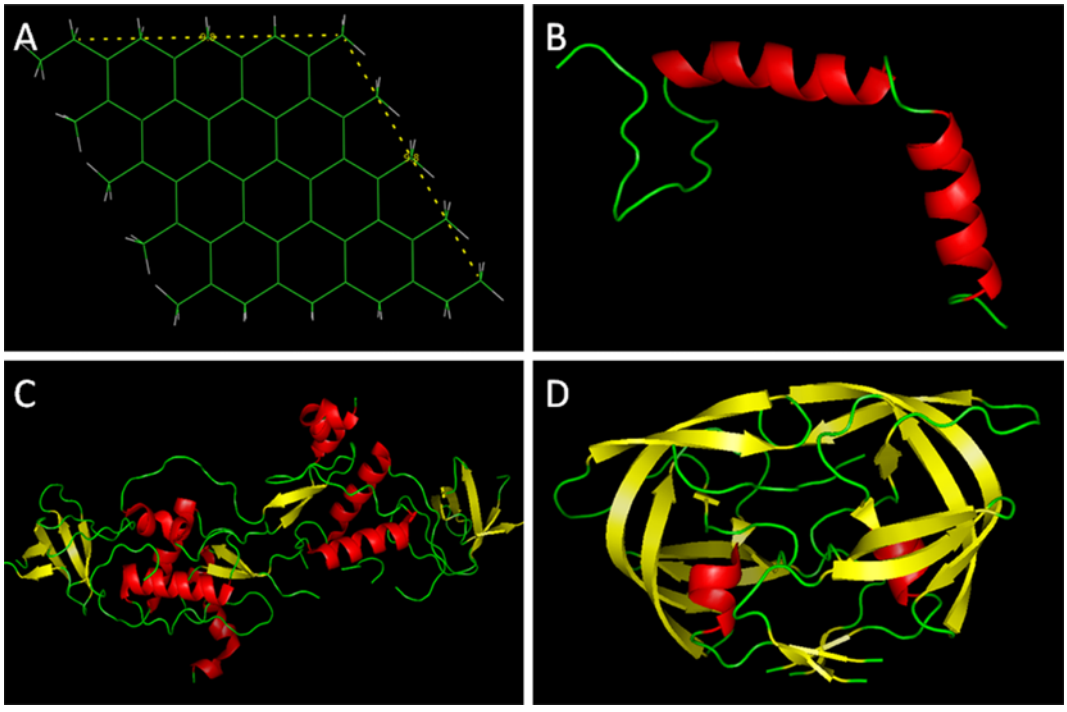

Fig. 1. (A) Modeled graphene of dimension 9.8×9.8 Å, (B) structure of HIV-Vpr protein (C) Nef (D) Gag.

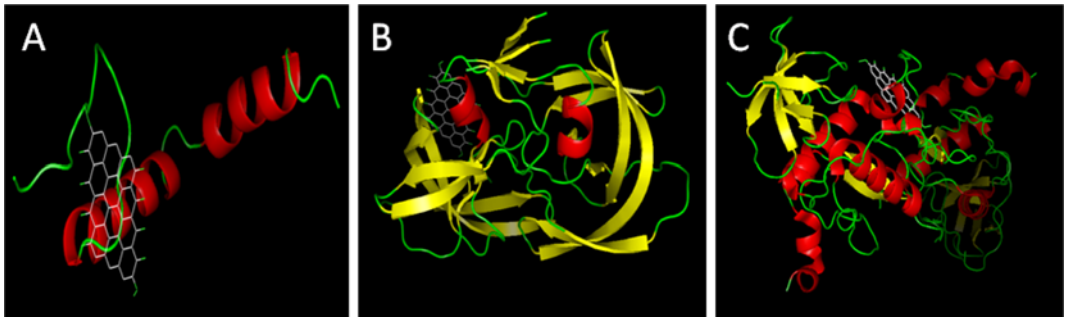

Fig. 2. Molecular interaction of the graphene with the (A) Vpr, (B) Gag and (C) Nef proteins.

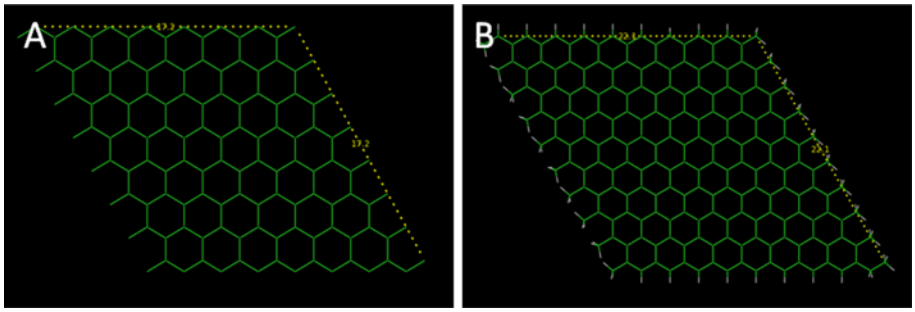

Fig. 3. Modeled graphene of dimension (A) 17×17 Å (B) 22×22 Å.

Table 1. The binding affinity of the graphene with the disease target proteins Vpr, Gag and Nef

Dimension of graphene (Å)	Layers of graphene	Binding affinity with Vpr (Kcal/mol)	Binding affinity with Gag (Kcal/mol)	Binding affinity with Nef (Kcal/mol)
9.8×9.8	1	−8.5	−9.6	−11.3
9.8×9.8	2	−8.5	−9.6	−10.6
9.8×9.8	3	−8.9	−10.1	−11.4
9.8×9.8	4	−8.9	−10.1	−11.8
9.8×9.8	5	−8.9	−10.1	−11.4
17×17	1	−11.9	−13.5	−16.7
22×22	1	−13.3	−15.7	−17.6

sion 9.8 Å×9.8 Å with the Vpr, Gag and Nef proteins was found to be –8.5, –9.6 and –11.3 Kcal/mol, respectively. Such a strong binding interaction of graphene with three different target proteins of HIV indicates the good antagonistic activity of the graphene molecules to the key target proteins. The molecular interaction of the graphene with the Vpr, Gag and Nef proteins is shown in the Fig. 2A, 2B and 2C, respectively.

2. Effect of the Size of Graphene on the Interaction with the Disease Targets

17 Å×17 Å and 22 Å×22 Å were modeled and are shown in the Figs. 3A and 3B, respectively. The molecular weight of the modeled graphene sheets of dimension 17 Å×17 Å and 22 Å×22 Å was found to be 1618.005 daltons and 2508.982 daltons, respectively. The dipole moment was found to be 0.003. The modeled graphene of dimension 20 Å×20 Å had a dipole moment of 0.677. The docking studies showed good binding affinities of the graphene with the HIV Vpr, Gag and Nef. A substantial increase in binding interactions on increasing the ligand size from 9.8 Å×9.8 Å, 17 Å×17 Å and 22 Å×22 Å was observed. The increase in binding affinities with size is due to the increase in contact area. High throughput screening approaches for anti-HIV drugs were reported based on the antagonistic activity of the compounds to the Nef proteins [16].

Modeled graphene with 1, 2, 3, 4 and 5 layers of dimension 9.8 Å ×9.8 Å had the molecular weight of 652.948 daltons, 1305.890 daltons, 2181.338 daltons, 2798.423 daltons and 3635.563 daltons, respectively. The dipole moment was found to be 0.004D, 0.244D, 1.622D, 1.802D and 2.700D, respectively. The binding affinities of graphene do not significantly increase on increasing the number of layers from 1 to 5. It confirms that the number of layers has no effect on the therapeutic potential of the graphene. Also, the increase in the dipole moment will substantially increase the hydrophobicity. Highly lipophilic ligands bind effectively to the protein and this decreases the period of action of the ligand.

CONCLUSION

The investigations on the molecular interactions of graphene with the disease targets of HIV show the therapeutic efficacy of graphene

to combat the HIV mediated infections. The modeled graphene structures had the strong binding affinity to all the three disease target proteins of HIV. Further studies on dynamics will aid in understanding the stability of graphene-target protein complex.

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