**C₆₀ Binds to and Deforms Nucleotides**

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**ABSTRACT**  Atomistic molecular dynamics simulations are performed for up to 20 ns to monitor the formation and the stability of complexes composed of single- or double-strand DNA molecules and C₆₀ in aqueous solution. Despite the hydrophobic nature of C₆₀, our results show that fullerenes strongly bind to nucleotides. The binding energies are in the range −27 to −42 kcal/mol; by contrast, the binding energy of two fullerenes in aqueous solution is only −7.5 kcal/mol. We observe the displacement of water molecules from the region between the nucleotides and the fullerenes and we attribute the large favorable interaction energies to hydrophobic interactions. The features of the DNA-C₆₀ complexes depend on the nature of the nucleotides: C₆₀ binds to double-strand DNA, either at the hydrophobic ends or at the minor groove of the nucleotide. C₆₀ binds to single-strand DNA and deforms the nucleotides significantly. Unexpectedly, when the double-strand DNA is in the A-form, fullerenes penetrate into the double helix from the end, form stable hybrids, and frustrate the hydrogen bonds between end-group basepairs in the nucleotide. When the DNA molecule is damaged (specifically, a gap was created by removing a piece of the nucleotide from one helix), fullerenes can stably occupy the damaged site. We speculate that this strong association may negatively impact the self-repairing process of the double-strand DNA. Our results clearly indicate that the association between C₆₀ and DNA is stronger and more favorable than that between two C₆₀ molecules in water. Therefore, our simulation results suggest that C₆₀ molecules have potentially negative impact on the structure, stability, and biological functions of DNA molecules.

**INTRODUCTION**

Several recent reports have highlighted the potentially hazardous nature of nanomaterials (1–3). For example, it has been shown that carbon nanotubes, arguably the best known nanomaterials, can accumulate in the lungs of rats, and possibly cause the development of granulomas in rats (4,5). The nanomaterials we consider in this work are buckminsterfullerenes (C₆₀) (6,7), because they show several promising potential applications in biology and pharmacology (8–11) and are regarded as one of the building blocks for nanotechnology applications.

The interest in biological applications of C₆₀, as well as that for possible applications in materials science and in other disciplines, has been tempered by concerns that fullerenes may exhibit adverse environmental and health impacts. Early studies showed low toxicity for the C₆₀ molecule itself (12,13). Pharmacokinetic studies with radio-labeled water-soluble C₆₀ showed that after intravenous injection into mice the compound quickly migrates through the body, accumulates in the liver after a few hours (14), and is excreted, either slowly or rapidly depending on the functionalization of the C₆₀ surface (15). These and other studies suggested that fullerene molecules do not exhibit short-term toxicity (16). However, more recent studies suggest that fullerenes may induce oxidative stress in the brain of juvenile largemouth bass (17), and that certain types of C₆₀ derivatives can be quite toxic in cell membrane (18), thus raising the specter of significant negative health impacts from exposure to fullerenes. It is widely believed that severely negative biological outcomes (e.g., replication errors, self-repair mismatches, abnormal functionalities, etc.) can occur when molecules bind to DNA and impact its shape. Such outcomes have the potential of leading to diseased conditions, including cancer. DNA damage of this kind typically requires long times to become detectable and, to the best of our knowledge, no long-term toxicity study related to DNA damage has been reported for fullerenes.

Fullerenes are known to be essentially insoluble in water. However, their solubility can be increased (up to 2 mM) through appropriate coatings (13) or by being suspended in colloidal solution (19). A colloidal solution of uncoated C₆₀ in water can be very stable (up to nine months) at room temperature (20). Hence, it is useful to consider the exposure of biological molecules, such as DNA, to fullerenes in aqueous solutions. In this work, we are interested in investigating the potential long-term toxic effects of fullerenes by understanding how C₆₀ derivatives approach and interact with nucleic acids. To this end, we performed molecular dynamics simulations of aqueous DNA fragments in the presence of C₆₀.

**SIMULATION METHODOLOGY**

The double-helix DNA molecule used in our simulations consists of 12 basepairs in the sequence of d[AGTCAGT-CAGTC]₂. The length and sequence of the DNA molecule was chosen as a balance between computational efficiency

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and biological realism. In particular, a DNA chain of 12 basepairs shows most of the typical properties of a short DNA segment, including a complete 360° rotation in the double-helix in both the A- and B-forms and the ability to spontaneously transition from its A-form to its B-form. Although the simulation of longer chains is clearly desirable, 12 basepairs is approaching the current limit of feasibility for performing multi-nanosecond, atomistically detailed simulations of a DNA segment in an explicit aqueous solution. We chose the sequence [AGTCAGTCAGTC]₂ because it includes and repeats the whole four types of basepairs. The rationale behind this choice consists in our desire to understand if C₆₀ binds preferentially to one specific basepair.

DNA structures were obtained from the AMBER (21) utility software. The head-to-tail length of the DNA is ~4 nm. Single-strand DNA was obtained by taking one helix from the double-strand B-DNA created. Damaged double-strand DNA was obtained by removing a section of consecutive nucleotides from one helix of the undamaged B-DNA. The section of nucleotide removed contains four bases, together with the backbone connected to them. Resultant gap was of ~1.4 nm. The dangling bonds in the DNA helix resulting from the removal were saturated by hydrogen atoms. DNA and fullerenes were solvated in 4500–6100 (depending on the system size) TIP3P water molecules (22), within a cubic simulation box of initial size 7 nm. In the simulation box, at least 1.2 nm of water buffer separates the solute (DNA and C₆₀) surface and the box boundaries in any simulation cell directions. Periodic boundary conditions were applied in all three directions. Twenty-two Na⁺ (11 Na⁺ for single-strand DNA simulations) countions were added to electrically neutralize the system. The simulations were performed in the NPT (constant pressure, constant temperature) ensemble; hence, the size of the simulation box is adjusted automatically during the course of the simulation to maintain the pressure at 1 bar.

The potential model for DNA was the all-atom AMBER force field, 1999 version (21). The sp² carbon atoms in the C₆₀ molecule were modeled as Lennard-Jones (LJ) particle with ε/k = 43.2 K and σ = 0.34 nm (23). LJ interaction parameters between different atoms were calculated by the standard Lorentz-Berthelot combing rules. Lennard Jones interactions were computed within a 0.9-nm spherical cutoff without long-range corrections. TIP3P water model was chosen based on previous simulation works (24,25). The potential applied for Na⁺ was the one built in AMBER. The particle-mesh Ewald method with a fourth-order interpolation was applied to evaluate electrostatic interactions. Initial configurations were obtained by placing one, two, or three C₆₀ molecules near the DNA molecule. The nearest initial distance between the C₆₀ and DNA surfaces was ~0.7 nm, which is within the LJ cutoff distance. The initial configurations permitted a clear separation between the fullerenes and DNA strands at the beginning of the simulation, while maintaining the simulation box size within a computationally manageable size.

Molecular dynamics simulations were performed within the constant pressure (1 bar) and temperature (300 K) (26) ensemble. The AMBER software package was employed to integrate the equations of motion. Each simulation includes 5000 steps of energy minimization, 40 ps of solvent relaxation, five cycles (each cycle with 1500 steps of energy minimization) of solutes relaxation, 20 ps of equilibration, and 5–20 ns of production. A time step of 2 fs was used and the structural configurations were saved every 2 ps for subsequent analysis.

The binding energy between one DNA and one C₆₀ molecule is defined as

\[ \Delta E = E_{\text{DNA} + C_60} - E_{\text{DNA}} - E_{C_60} - \Delta E_{\text{deform}}, \]

where \( E_{\text{DNA} + C_60} \), \( E_{\text{DNA}} \), and \( E_{C_60}^0 \) represent the potential energy of the bound DNA-C₆₀ pair, the potential energy of DNA in the bound pair, and the potential energy of the C₆₀ in the bound pair. In our calculations, it is necessary to account for \( \Delta E_{\text{deform}} \) because significant deformations were observed for DNA segments and thus \( \Delta E_{\text{deform}} \) is not negligible compared to the total potential energy change. We define \( \Delta E_{\text{deform}} \) as

\[ \Delta E_{\text{deform}} = \left( E_{\text{DNA}} - E_{\text{DNA}}^0 \right) + \left( E_{C_60} - E_{C_60}^0 \right). \]

\( E_{\text{DNA}}^0 \) and \( E_{C_60}^0 \) represent the potential energy of DNA and C₆₀ in their native conformations. We use the term “native conformation” to refer to the state that a free DNA or C₆₀ takes when it is in the same solvent and ion environment but with no association interaction force on it. We performed very short simulations (200 ps for C₆₀, B-DNA, ssDNA, and damaged DNA, 10 ps for A-DNA because A-DNA tends to convert into its B-form under the simulation solvent conditions) of free DNA and C₆₀ in water with counterions to calculate \( E_{\text{DNA}}^0 \) and \( E_{C_60}^0 \). We should point out that the binding energy reported in this work is not free energy. Free-energy calculations are preferable, but the tools available to us for the systems of interest can be highly approximate (such as the Poisson-Boltzmann/surface-area free-energy estimation techniques in MMTSB, The Scripps Research Institute) and we did not attempt to carry out such calculation.

The potential energies were computed from the simulation structures collected, using the software MMTSB. MMTSB operates on the equilibrated structures of DNA and C₆₀ collected during molecular dynamics and invokes an instantaneous potential energy calculation, employing the AMBER package and its utilities. It reports the potential energies of the molecules of interest, or of the associated hybrids.

RESULTS AND DISCUSSION

Our results indicate that C₆₀ forms stable complexes with aqueous DNA (Fig. 1). In our initial simulation configurations
molecule and that of one DNA end (defined as the center of mass of the DNA end basepairs C12–G13) as a function of simulation time (b). The same quantity for the C60 molecule and the minor groove site versus simulation time is shown in d. In both cases, it can be seen that after ~2 ns the fullerene is able to find a docking site in the nucleotide. Once the C60 molecule reaches the docking site, the hybrid remains stable for the remainder of the simulation.

C60 and DNA are separated, with solvent molecules in between. The first contact between the C60 molecule and the DNA typically occurs after 1–2 ns. Once the fullerenes diffuse and bind with the nucleotides, C60-DNA complexes are formed and remain stable for up to 20 ns of the simulations. Initially it appeared that the C60 molecules are prone to bind only to the free ends of the double-strand DNA. However, extensive simulations indicate that association of C60 can also occur at the minor groove sites. No association has been observed at the major groove sites in our simulations. These observations are different from a recent experimental observation, according to which a water-soluble (i.e., functionalized) C60 derivative can interact strongly with DNA, the major groove of the double-helix and phosphate backbone acting as the binding sites (27). However, we observe that the C60 considered here is hydrophobic and not hydrophilic.

We note that the free ends of the double-strand DNA fragment are the explicit hydrophobic sites exposed in solution, and this may favor the diffusion of hydrophobic fullerenes toward their docking sites. The two free ends of our double-strand DNA segment are constituted by either A-T or G-C basepairs. We performed simulations with C60 in proximity of both ends and did not notice appreciable differences in the binding features. Careful calculation of the binding energy ΔE reveals that the hybrid C60-DNA complexes are energetically favorable compared to the unpaired molecules (the binding energy goes from ΔE = −35.9 kcal/mol, for C60 docked in the minor groove, to −30.4 kcal/mol, for C60 docked at the DNA ends). For comparison, the thermal fluctuations under the simulation condition are in the magnitude of 0.6 kcal/mol (300 K). We also found that the deformation energy of B-DNA is ~6.5 kcal/mol, which indicates that the C60-DNA association causes an energetically unfavorable deformation in the DNA molecule. However, the deformation energy of C60 observed in simulations is <0.5 kcal/mol and so is negligible.

We also simulated the self-association of C60 molecules in the presence of DNA molecules in the same aqueous solution. The result is summarized in Fig. 2. In this simulation, we placed two C60 molecules and one B-DNA in the simulation box. Self-association between C60 molecules was observed at early stages during the simulation, with a binding energy of ΔE = −7.5 kcal/mol. However, the stable configuration obtained after 5 ns of simulation indicates that the self-association between C60 molecules did not prevent one of the C60 molecules from binding to one end of the DNA. Both C60 molecules migrated to the end of the DNA, with one C60 docked to the DNA end; the other C60 remains associated with the DNA-docked C60.

Despite the large deformation energies discussed earlier, visual observation of the simulation results suggests that the overall shape of the double-strand DNA molecule is not appreciably affected by the association of C60, in agreement with our previous studies.
with experimental observations of DNA/fullerene derivative hybrid materials (28). However, the impact on the DNA structure is more pronounced when C$_{60}$ interacts with a double-strand DNA fragment in the A-form (Fig. 3). DNA fragments in their A-form differ from their B-form counterparts in that more hydrophobic contacting surfaces are exposed at the end because of the open channel along the axis. The nucleotide segment modeled in this work is expected to transform into the narrower B-form under the solution conditions considered here (24,25). The simulation started from initial conditions similar to those for C$_{60}$/B-DNA simulations (see Fig. 1) except the initial DNA structure was replaced by its A-form counterpart. Our results (Fig. 3 a) indicate that a C$_{60}$ molecule is able to penetrate into one free end of an A-form DNA fragment. A closer look at the basepairs being penetrated indicates that the hydrogen bonds between them are disconnected. The C$_{60}$ molecule docked in the DNA end is able to come in contact with the face of the basepairs second from the end. The binding energy that corresponds to this docking amounts to $-41.9$ kcal/mol. The observed deformation energy of the A-DNA is $\sim -4.5$ kcal/mol. We observe that the deformation energy is negative, while it was positive in our C$_{60}$/B-DNA simulations. The favorable deformation energy observed for A-DNA is due to the transition of the nucleic acid to its more stable B-form. Although it is known that the hydrogen bonds between the end basepairs of a double-strand DNA may in some cases break during the course of molecular simulation (24,25), such bond breakage is temporary and recovered through thermal fluctuations as the simulation proceeds. In contrast, in our study, we find that the hydrogen-bond breakage encountered when a C$_{60}$ molecule docks into the end of the A-form DNA.

FIGURE 3 Deformation of an A-form DNA when it interacts with C$_{60}$ molecules. (a) Two C$_{60}$ are associated with the DNA moleucle. One penetrates into the A1–T24 end of DNA, the other docked by the minor groove. The observed deformation energy of the A-DNA is $\sim -4.5$ kcal/mol. The binding energy between the two C$_{60}$ molecules is $-7.5$ kcal/mol. It can be seen from the final configuration that the self-association of the two C$_{60}$ molecules did not inhibit the binding between one C$_{60}$ and one hydrophobic end of the DNA.

FIGURE 2 Self-association of C$_{60}$ molecules in the presence of a DNA molecule. (a) The starting configuration of two C$_{60}$ molecules and a B-DNA molecule. (b) The stabilized hybrid of C$_{60}$-DNA after 5-ns simulation. One C$_{60}$ binds with an end of the DNA, while the other C$_{60}$ associates with it. The binding energy between the two C$_{60}$ molecules is $-7.5$ kcal/mol. It can be seen from the final configuration that the self-association of the two C$_{60}$ molecules did not inhibit the binding between one C$_{60}$ and one hydrophobic end of the DNA.
DNA is not restored during the course of the simulation, and appears to be permanent. The docked C₆₀–DNA complex is very stable and the broken hydrogen bonds are not reconnected even after 10 ns of simulation (see Fig. 3d).

In addition, we observe an appreciable deformation on the basepair stacking angles of the A-form double-strand DNA when the C₆₀ docks on its minor groove sites (Fig. 3b). Although the A-form DNA under our simulation condition is expected to transform into its B-form with the stacking planes between consecutive basepairs parallel to each other (25), the docking of one C₆₀ into the minor groove induces an angle of 20° between the basepair planes that bite the C₆₀. The deformation of the nucleotide structure results in a slightly greater binding energy (−36.9 kcal/mol) than that of C₆₀ binding to the minor groove of a regular B DNA (−35.7 kcal/mol). Examining the structural details of C₆₀/B-DNA and C₆₀/A-DNA hybrids more carefully, we see that the binding of C₆₀ on the minor groove of A-DNA is somewhat prohibited from fully relaxing into its B-form, as indicated by the nonparallel basepairs in the region near to where C₆₀ docks. This allows the C₆₀ molecule to contact deeper, and with a larger contact area into the A-DNA groove than it does with a B-DNA (see Fig. 4).

As might be expected on the basis of the double-strand DNA results, the docking of a C₆₀ into a flexible single-strand DNA molecule causes significant deformations in the DNA (Fig. 5a). We started additional simulations by placing three C₆₀ and one single-strand DNA in the simulation box. After the system reaches equilibrium, we observe that all the C₆₀ molecules in the system are essentially wrapped by nucleotides. One end of the single-strand DNA bends severely because of the two fullerenes docked inside. The other end maintains a relatively native conformation because no C₆₀ molecule binds to it. The C₆₀ docked in the central region of the single-strand DNA induces significant structural deformations on the two basepairs it contacts. The stacking of the base rings is completely deformed and the bases contact the C₆₀ in a face-on pattern. We speculate that a sufficiently long single-strand DNA would completely wrap a C₆₀. However, we did not test this hypothesis, because of computing time limitations. The single-strand DNA and C₆₀ hybrids observed in this work are energetically favorable, with binding energies in the range −27.7 to −39.3 kcal/mol, depending on atomistic docking details. The deformation energy computed for one single-strand DNA (Fig. 5) is ~5.1 kcal/mol. We noted that a free single-strand DNA is very flexible and therefore can experience large deformation. That helps to explain the relatively small deformation energy of single-strand DNA, even though significant deformation is seen in the ssDNA-C₆₀ hybrids.

We attribute the binding between the C₆₀ and DNA molecules to the hydrophobic interaction between the C₆₀ and hydrophobic sites on the DNA. As indicated previously, the binding energy being measured is not free energy, but includes contributions from both free energy ΔG and entropic term TΔS. For nonpolar molecules in an aqueous environment, the hydrophobic interaction is dominated by entropy change. This is due to the high entropy decrease...
during water structural rearrangement for the nonpolar solutes to disperse into the solvent (29). This is supported by our structural analysis of water molecules surrounding the binding sites in the nucleotide compared with a hydrophilic site (Fig. 5 b). Water molecules are completely displaced from the binding site after the docking process stabilizes. Our contention that hydrophobic forces dominate agrees with earlier observations according to which electrostatics are not the driving force for the association between fullerences and nucleotides (30). Ideally, one could estimate the entropic contribution to the binding process by analyzing the system dynamics using techniques such as those of Smith and co-workers (31,32). However, such analysis is nontrivial and we did not attempt to estimate the entropic interactions.

The above described tendency of \( C_{60} \) to bind and form stable hybrids with the DNA segments led us to investigate the behavior of \( C_{60} \) in proximity of a damaged nucleotide. In this simulation, we removed a piece of nucleotide with the length of four bases from the central part of one DNA strand. The resulting double-strand DNA shows one defect gap in its structure (Fig. 6). The width of the defect gap is \( \sim 1.4 \) nm. \( C_{60} \) has a diameter of \( \sim 1 \) nm. We find that when a \( C_{60} \) diffuses near this structure it is rapidly attracted within the defect and forms a stable hybrid with a binding energy of \(-48.3 \) kcal/mol. The interaction between the DNA and \( C_{60} \) deformed the stacking of the binding bases for \( C_{60} \) to have a face-on contact with them, as found in \( C_{60} \) and single-strand DNA simulations. The significant deformation of the bases contacting the \( C_{60} \) molecule results in a deformation energy of \( 11.0 \) kcal/mol. The hybrid remains very stable during the entire course of simulation. The results suggest that \( C_{60} \) fills in the defect gap, and so thereby may impact the self-repairing process of the damaged nucleotide by blocking the repair sites.

**CONCLUSIONS**

In summary, we have shown, based on molecular dynamics simulations, that \( C_{60} \) can bind to nucleotides and form energetically stable hybrids in aqueous solution. The favorable binding sites in double-strand DNA are the free ends and minor grooves. The binding between \( C_{60} \) and B-form double-strand DNA does not affect the overall shape of the DNA. In contrast, \( C_{60} \) can penetrate into an A-form DNA from the free end and permanently break the hydrogen bonds between the end basepairs. Furthermore, the \( C_{60} \) docked on the minor groove of A-DNA deforms the stacking angles of bases contacting it. \( C_{60} \) binds strongly with single-strand DNA and results in a significant deformation of the nucleotides. In addition, we find that a \( C_{60} \) molecule can readily occupy a defect site in a double-strand DNA and form a stable complex. The set of simulation results presented here suggest the possibility that \( C_{60} \) molecules may interfere with the biological functions performed by DNA, and therefore cause long-term negative side effects in living organisms. However, additional studies are required to determine whether the binding events observed in these simulations have in vivo relevance.

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