Comparison of Adsorption of Proteins at Different Sizes on Pristine Graphene and Graphene Oxide

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Using all-atom molecular dynamics (MD) simulations, we have investigated the adsorption stability and conformation change of different proteins on the surface of pristine graphene (PG) and graphene oxide (GO). We find that: (i) with the cooperation of the electrostatic interactions between proteins and oxygen-containing groups, GO shows better adsorption stability than PG; (ii) the peptide loses its secondary structure on both PG and GO surface, and the $\alpha$-helix structure of the protein fragment is partially broken on PG surface, but is well preserved on GO surface, while the secondary structure of globular protein has no distinct change on both PG and GO surface. In general, GO presents better biocompatibility than PG. Our results are of significant importance to understand the interactions between proteins and PG/GO and the applications of PG/GO in biotechnology and biomedicine.

Key words: Graphene, Graphene oxide, Protein, Adsorption, Molecular dynamics simulation

I. INTRODUCTION

Because of their excellent physical and chemical properties, graphene and its derivatives have exhibited growing potential in various biomedical applications\cite{1-3}, such as drug delivery and biosensors\cite{4-7}. Pristine graphene (PG) is hydrophobic, therefore, graphene oxide (GO) has been adopted in biological experiments to achieve better water-solubility. GO possesses strong physisorption ability and acts as an ideal substrate for adsorbing biomolecules\cite{8-11}, due to its large specific surface area and abundant functional groups. One such biomolecule is protein, and the adsorption of protein on graphene has triggered great concern both experimentally and theoretically\cite{12, 13}, since the interactions between proteins and graphene may result in proteins’ conformation change. For example, recent experiments have reported that the fragment of viral protein R (Vpr) can assemble and change its conformation from \$\alpha$-helix to \$\beta$-sheet after adsorbed on GO and then reduce its cytotoxicity\cite{14}.

However, different groups reported inconsistent results. Dravid \textit{et al.} found that the secondary structure of the protein chymotrypsin on GO surface was well conserved through fluorescence spectroscopy and circular dichroism studies, indicating that GO was highly biocompatible\cite{15}. It can be seen that proteins with different sizes present different results after adsorbed on GO, which need further explanation. Molecular dynamics (MD) simulation can provide detailed information at atomic level, and has been widely used to explore the interactions of proteins with PG/GO\cite{16, 17}. For example, MD simulations performed by Ou \textit{et al.} and Zuo \textit{et al.} showed that peptides or proteins can be adsorbed on the PG surface, and unfolded or lost most of their native secondary structures\cite{18, 19}. Our previous simulations revealed that the secondary structure of GA53 were well preserved on GO surface, while GA53 will partially lost its secondary structure after adsorbed on PG\cite{20}.

In all the aforementioned studies, a single protein was chosen to study the effect of PG/GO on proteins’ structure, which lacks of systematic comparison. In this work, we will use MD simulations to investigate the adsorption of proteins at different sizes on PG/GO, including the adsorption stability, conformation change, and the protein-PG/GO interactions. The results may provide molecular mechanisms of GO’s good biocompatibility.

II. COMPUTATIONAL METHODS

To study the adsorption stability and biocompatibility of graphene and its oxide, we chose the proteins with the increasing number of amino acid residues, namely, peptide, protein fragment and globular protein, which consisted of 26, 53, and 130 residues. They were frequently used to explore the mechanisms of protein unfolding and the interactions between proteins...
and carbon nanoparticles [21]. Their native structures were downloaded from Protein Data Bank (PDB ID1VTP1PRB and 1AKI) and modeled by the AMBER03 force field [22].

The basal planes of GO and PG were 5 nm x 6 nm, and were composed of 1258 carbon atoms. GO were constructed by a molecular formula of C_{10}O_{1}(OH)_{1}-(COOH)_{0.5} (i.e., 2 epoxy, 2 hydroxyl on both sides of graphene basal plane, and 1 carboxyl group on the edges of graphene, per 20 carbon atoms), which was widely employed in MD simulations [16, 23, 24]. The hydroxyl and epoxy groups were randomly on the basal plane, and the carboxyl groups were only attached to the edge randomly. The sp^2 carbon atoms in GO were treated as uncharged Lennard-Jones (LJ) spheres with a cross section of \(\sigma_{cc}=0.34\) nm and a depth of the potential well of \(\varepsilon_{cc}=0.36\) kJ/mol. The bonded parameters of carbon atoms in the basal plane of PG and GO were obtained from Patra et al. [25], while those of hydroxyl, carboxyl and epoxy groups were taken from the AMBER03 force field. Water molecules were realized by the standard SPC model [26].

All simulations were carried out for 300 ns in an NVT ensemble using Gromacs package 4.5.6 [27, 28]. We applied periodic boundary conditions in all directions. The temperature was kept stable at 300 K using the V-rescale thermostat [29]. The particle-mesh Ewald method (PME) was used to calculate the long-range electrostatic interactions [30, 31], whereas the vDW interactions were treated with smooth cutoff at 1 nm. Bond lengths within water and graphene/proteins were constrained by the SETTLE and the LINCS algorithms [32, 33], respectively. The secondary structure of the peptide was determined by DSSP program and the snapshots in the work were depicted by PYMOL software.

III. RESULTS AND DISCUSSION

A. Comparison of the peptide on PG and GO surface

First, we simulated the adsorption of an \(\alpha\)-helical peptide on PG and GO surface, and the vertical distance between their COMs was initially set to 25 Å, as illustrated in FIG. 1 (a, c). It was found that the peptide had been quickly adsorbed on PG surface. Correspondingly, the vertical COM distance declined from 25 Å to 8 Å in the first 8 ns (FIG. 1(e)). Then, the peptide unfolded gradually and spread on PG, and the vertical COM distance dropped continually to 5 Å. Finally, the vertical COM distance fluctuated at this value till the end of the simulation. Dierent from the adsorption of the peptide on PG, the vertical COM distance between the peptide and GO was 2 Å higher than that of peptide-PG system at equilibrium. Two main factors were responsible for it, namely, the steric effect and the unfolding of peptide. Because of the oxygen-containing groups on GO surface, they prevented the peptide further close to the basal plane of GO. On the other hand, the extent of the unfolding of peptide on PG was much
greater than that on GO surface, which can be testified by the RMSD of the peptide and the number of residues in the α-helix structure, as shown in FIG. 2.

After adsorbed on PG and GO surface, we observed that the α-helix structure of peptide was damaged (FIG. 1 (b, d)). The RMSD on PG surface fluctuated greatly near 6 Å, while that on GO surface was kept stable at 5 Å. And there were averaged about 10 residues in the α-helix structure on GO surface, but only 3 residues on PG surface, which best illustrated that the influence of PG on peptide’s structure was much more serious than GO and the adsorption stability of peptide on GO surface is stronger than that of PG. This was because the PG surface was much smoother, and when the α-helix structure was destroyed, the peptide became easier to sway on the slippery PG surface. On the contrary, the oxygen-containing groups made GO much rougher and the electrostatic interactions between them and the peptide enhanced the adsorption stability. The detailed interactions between peptide and PG/GO would be discussed as follows.

Except for the conformation change, the adsorption stability of peptide on PG and GO surface can be mainly characterized by the horizontal COM distance between peptide and PG or GO (FIG. 1(f)) and the interactions between the peptide and PG/GO (FIG. 3). The horizontal COM distance between peptide and PG shook dramatically during the whole simulation, while that between peptide and GO remained steady at 12.5 Å, which was in good agreement with the conformation change.

Since the carbon atoms of PG are uncharged, vdW interaction was the only non-bonded interaction and represented by the Lennard-Jones potential (Eq.(1)) in the AMBER03 force field,

\[
V_{ij}(r_{ij}) = 4\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^6\right]
\]

where \(V_{ij}\) was the interaction energy between atoms \(i\) and \(j\). Eq.(2) and Eq.(3) were standard combining rules in the AMBER03 force field. The results were given in FIG. 3. As we can see from FIG. 3(a), the vdW energy between peptide and PG was about −700 kJ/mol, about 200 kJ/mol more than that between peptide and GO, due to the lower vertical COM distance. Since the oxygen-containing groups increase the distances between residues of peptide and the basal plane of GO, their vdW interactions were weakened. However, it could not elucidate that the adsorption stability of PG was stronger than GO. On the contrary, the adsorption of peptide on GO was much firmer than PG as mentioned before. It was clear that hydrogen bond and

![FIG. 2](image1.png)

**FIG. 2** (a) RMSD of the backbone of peptide on PG and GO compared with its native structure, (b) the number of residues in the α-helix structure.

![FIG. 3](image2.png)

**FIG. 3** (a) The van der Waals energies between peptide and PG/GO, (b) the number of hydrogen bond between peptide and GO.
electrostatic interaction played an important role in the adsorption of peptide onto GO surface. There were averaged 4 hydrogen bonds between the peptide and GO (FIG. 3 (b)), which made them occlude with each other and strengthened the adsorption stability.

In addition, we observed that π-π stacking structures were established in both systems, which originated from the third and tenth tyrosine residues, as shown in the insets in FIG. 4. The vertical COM distance (denoted as \(d\)) and the relative angle (\(\alpha\)) between the benzene ring on the tyrosine residue and the basal plane of PG/GO were employed to monitor such π-π stacking interactions. The values of \(\cos \alpha\), 1 and 0 mean the two planes are parallel and vertical, respectively. In both systems, the values of \(\cos \alpha\) were close to 1, implying that the two planes were almost parallel. The difference of the π-π structures between the two systems was that the values of \(\cos \alpha\) in PG system varied more obviously than those in GO system. The π-π structures in GO system maintained much stable, benefited from the cooperation with the electrostatic interactions between the tyrosine residues and the surrounding oxygen-containing groups.

B. Comparison of protein fragment on PG and GO

Then, we increased the length of the peptide chain, selected a protein fragment (marked as GA53, consisted of three α-helix segments), and continued to simulate the adsorption of GA53 on PG or GO. The results were given in FIG. 5. Similar to the adsorption process of
peptide, GA53 went to the PG and GO surface quickly, and could slide freely on PG surface, while firmly adsorbed on GO surface (FIG. 5(h)). The vertical COM distance between GA53 and GO was about 2.5 Å farther than that between GA53 and PG (FIG. 5(g)). Different from the adsorption of peptide, the secondary structure of GA53 was well preserved on GO surface (FIG. 5(d)), while GA53 partially lost its secondary structure on PG surface (FIG. 5(b)). As shown in FIG. 6(a, b), the RMSD of GA53 on PG was generally larger than that on GO, since the two α-helix segments on PG surface were completely destroyed. Correspondingly, the number of residues in the α-helix structure on GO surface fluctuated slightly at 33, whereas the number declined to about 15 on PG surface from 220 ns.

The interactions between GA53 and PG/GO were also similar to those between peptide and PG/GO. Interestingly, it was also found that π-π structures were formed between the fifth aromatic residue tryptophan and the unoxidized domain on the basal plane of GO (FIG. 5(e)) or PG (FIG. 5(f)). We then counted the number of hydrogen bond through the whole simulation, as depicted in FIG. 6(c). There were averaged 5 hydrogen bonds existing from 100 ns to 300 ns. On the other hand, these oxygen containing groups increased the distance between GA53 and the basal plane of GO, which weakened their vdW interactions. After equilibrium, the vdW energy between GA53 and GO was −593.4 kJ/mol, which was about 360 kJ/mol bigger than that between GA53 and PG.

C. Comparison of globular protein on PG and GO

Eventually, we chose a globular protein lysozyme to explore its adsorption on PG and GO surface, as shown in FIG. 7. The secondary structure of lysozyme had no significant change and the RMSDs were kept stable near 1.5 Å in both systems (FIG. 7(c)). As a result, PG and GO had little or no effect on global protein’s structure. In addition, the horizontal COM distance between lysozyme and GO remained steady after 50 ns, while that on PG surface fluctuated fiercely, as illustrated in FIG. 7(d). GO still showed better adsorption stability than PG.

IV. CONCLUSION

In summary, MD simulations have been conducted systematically to explore the adsorption of proteins at different scales on PG and GO. The simulation results show that the secondary structures of proteins on graphene surface become more and more stable, with
FIG. 7 The adsorption of globular protein lysozyme on PG and GO. (a, b) The final structures of lysozyme on PG and GO, (c) RMSD of the backbone of lysozyme on PG and GO, (d) the horizontal COM distance between lysozyme and PG/GO.

the increasing peptide chain, especially on GO surface. GO possesses better biocompatibility.

Through analyzing the detailed interactions between proteins and PG/GO, we find that the distance between protein and PG is closer than that between protein and GO, due to the oxygen-containing groups on GO surface, which result in the steric effect and prevent proteins from further approaching the basal plane of graphene and weaken their vdW interactions. However, hydrogen bonds and electrostatic interactions strengthen the binding of proteins on GO. In contrast with PG, GO owns better adsorption stability.

V. ACKNOWLEDGMENTS

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