Molecular simulations of conformation change and aggregation of HIV-1 Vpr13-33 on graphene oxide

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Recent experiments have reported that the fragment of viral protein R (Vpr), Vpr13-33, can assemble and change its conformation after adsorbed on graphene oxide (GO) and then reduce its cytotoxicity. This discovery is of great importance, since the mutation of Vpr13-33 can decrease the viral replication, viral load and delay the disease progression. However, the interactions between Vpr13-33 and GO at atomic level are still unclear. In this study, we performed molecular dynamics simulation to investigate the dynamic process of the adsorption of Vpr13-33 onto GO and the conformation change after aggregating on GO surface. We found that Vpr13-33 was adsorbed on GO surface very quickly and lost its secondary structure. The conformation of peptides-GO complex was highly stable because of π-π stacking and electrostatic interactions. When two peptides aggregated on GO, they did not dimerize, since the interactions between the two peptides were much weaker than those between each peptide and GO.

Graphene oxide (GO) is a versatile derivative of graphene, functionalized with oxygen-contained groups1–3. Because of its water solubility, large specific surface area and functional groups, GO possesses strong physiosorption ability and serves as an ideal substrate for adsorbing biomolecules without any surface modification4–6. GO presents growing potential in biomedical applications, such as enzyme immobilization7–10, drug delivery11–13 and biosensors14–19. For example, graphene-peptide complex could monitor the protein-peptide interactions20. Due to the π-π stacking and hydrophobic interactions, the pyrene-labeled peptide was strongly adsorbed on GO surface. The preferential adsorption of single-stranded DNA over double-stranded DNA on GO was also observed via π-π stacking and electrostatic interactions18,19,21,22.

Therefore, understanding the interactions between biomolecules and GO is fundamentally essential, especially for drug- and disease-related peptides or proteins. One such peptide or protein is virus protein R (Vpr), which is a small nuclear accessory protein of HIV-123. The segment of Vpr, Vpr13-33, plays an important role in regulating nuclear importing of HIV through ion channel24. Recent scanning tunneling microscopy and circular dichroism studies have shown that Vpr13-33s aggregate on GO accompanied by conformation change from α-helix to β-sheet25. However, the atomic level information about the peptides-GO complex is largely unknown. Molecular dynamics (MD) simulations can thus be used to provide detailed information on the interactions between peptides or proteins and carbon nanoparticles. For example, Sun et al. have successfully explained the mechanism of the activity of α-chymotrypsin inhibited by GO using MD simulations26.

In this paper, we used all-atom MD simulations to investigate the conformation change and aggregation of Vpr13-33 on GO. Vpr13-33 was adsorbed on GO surface very quickly and then bent into U-shape. Both π-π stacking and electrostatic interactions contributed to the binding of Vpr13-33 on GO. When multi-peptides interact with GO, we observed that the peptides could assemble on GO surface with lower root-mean-square deviation (RMSD) because of steric effect.

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by the binding energy (ΔGbind1). As shown in Fig. 3, the binding energy was increasingly higher when the peptide...
was far away from the GO surface. When the COM distance was more than 25 Å, their interactions were negligible. The value of $\Delta G_{\text{bind1}}$ was close to $-50 \text{ kcal/mol}$. Therefore, once the COM distance was less than 25 Å, the peptide could continue to approach GO until tightly adsorbed on its surface.

To better understand the interactions between Vpr13-33 and GO, we found that there were three hydrophobic $\pi-\pi$ structures formed between Tyr15, Trp18 and His33 of the peptide and GO, and the aromatic or heterocyclic rings were in the flat mode (see Fig. 2A, $t = 150 \text{ ns}$). We then calculated the distances between the COM of the above three residues and GO (see Fig. 2B). Till $t = 4.4 \text{ ns}$, the distances between Tyr15, His33 (which are located on or near the termini of Vpr13-33) and GO reached about 4 Å successively, and stayed at this value until the end of simulation, indicating that the two $\pi-\pi$ structures were the main forces to keep the peptide U-shape.

Since there are a large number of oxygen-contained groups on GO surface, electrostatic interaction is another important force contributing to the binding affinity of Vpr13-33 on GO. We then analyzed the number of hydrogen bond formed between the peptide and GO, as illustrated in Fig. 4A. The hydrogen bond was sensitive to the position of each atom, therefore, the number fluctuated dramatically because of thermal motion. As average, there were about 5 hydrogen bonds between the peptide and GO after equilibrium. For example, there were 5 hydrogen bonds in Fig. 4B, with 3 between Tyr15 and GO, and 2 between Arg32 and GO, respectively.

The previous studies have revealed that the unfolding of $\alpha$-helical peptides after adsorbed on graphene is induced by the strong vDW and hydrophobic interactions, while electrostatic interaction and steric effect prevent the peptide from further unfolding. On the contrary, electrostatic interaction enhances the stability of the binding of proteins on GO. Therefore, Vpr13-33 had no essential conformation change and the RMSD as well as the $\alpha$-helical residues only fluctuated slightly after adsorbed on GO, as shown in Fig. 2C.

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**Figure 2.** A representative trajectory of the adsorption of Vpr13-33 onto GO. (A) Side view of snapshots at critical time points. Vpr13-33 was depicted as a cartoon in cyan, but Tyr15, Trp18 and His33 specifically in red. (B) COM Distance between Vpr13-33, Tyr15, Trp18 and His33 and GO along the direction vertical to GO. Subfigure specified the distance in the first 10 ns. (C) The RMSD of Vpr13-33 from its native structure and the number of $\alpha$-helical residues.

**Figure 3.** PMF profiles for the binding energy of Vpr13-33 on GO surface (black) and double peptides in the pure water (red).
Similarly, we first simulated the aggregation of two peptides in water. Here, we employed a new parameter, contacting surface area (CSA), to characterize the dimerization, as shown in Fig. 5C. The CSA was defined by the following formula:

\[ \text{CSA} = \frac{\text{SASA}_1 + \text{SASA}_2 - \text{SASA}_{1+2}}{2} \]

where SASA represents the solvent accessible surface area. Initially, the CSA was zero, since the two peptides were far away and the COM distance between them was set as 25 Å (see Fig. 5A, \( t = 0 \) ns). Then, the CSA rose fast to about 600 Å\(^2\) at \( t = 18 \) ns, indicating that the two peptides had dimerized. Just like single peptide in the water, the two termini of the peptides unfolded and the middle helices were maintained well. Correspondingly, the RMSDs of the two peptides fluctuated at 4 Å.

To investigate the aggregation of Vpr13-33 on GO, we put two peptides in the simulation box and enlarged the size of GO with 55 × 68 Å\(^2\). The COM distances between the two peptides and GO and between the two peptides themselves were the same 25 Å (see Fig. 6A, \( t = 0 \) ns). The two peptides went to GO surface separately and...
were finally adsorbed on GO, since the binding energy of Vpr13-33 on GO ($\Delta G_{\text{bind1}} \sim -50 \text{ kcal/mol}$) was much stronger than that of double Vpr13-33 in the water ($\Delta G_{\text{bind2}} \sim -30 \text{ kcal/mol}$, see Fig. 3). Figure 6 showed one typical trajectory. Since GO possessed large surface area to adsorb biomolecules, the two peptides had enough space to extend after adsorbed on GO surface. Therefore, the COM distances between peptides and GO were close to that of single peptide-GO system, about 6 Å.

The CSAs between each peptide and GO were alike with each other (Fig. 6C), about 700 Å² after equilibrium. However, the CSA between the two peptides was only 150 Å², meaning that the two chains were not dimeric and the interactions between peptides were much weaker than that between each peptide and GO. The two peptides could dimerize in the pure water (see Fig. 5A) or on pristine graphene (PG) (see Figure S1 in the Supplementary Information). Because of the smooth PG surface, peptides could slide on it and interpeptide hydrophobic interactions compel the peptides to form a dimer. In GO system, electrostatic interaction and steric effect that originate from oxygen-contained groups on GO surface enhanced the stability of the adsorption and hindered the peptides from sliding freely. Therefore, the two peptides exhibited no obvious dimerization. However, interpeptide hydrophobic interactions could still make the two peptides approach further. As shown in Fig. 6C, the CSA between the two peptides had a slight climb near $t = 400 \text{ ns}$. This climb happened between Leu22 in P1 and Leu20 in P2 (see Fig. 6A, $t = 425 \text{ ns}$), which are hydrophobic residues.

Both peptides lost their partial secondary structures after adsorbed on GO surface. The RMSDs of the two peptides were close to 4 Å (see Fig. 7A), close to those in pure water, but 2 Å less than that of single peptide on GO surface, because the main chains of the two peptides did not bend. Comparing the adsorption of peptides, protein fragments and globular proteins on GO, we could speculate that the effect of GO on conformation change of peptides or proteins would be more and more weak with the increasing of chain length because of steric effect26,40.
Conclusions

In summary, molecular dynamic simulations have been performed systematically to explore the adsorption of Vpr13–33 on GO. The simulation results confirm that GO can induce conformation change and aggregation of Vpr13–33. The conformation of Vpr13–33 on GO surface is highly stable via π–π stacking and electrostatic interactions, while electrostatic interactions and steric effect prevent Vpr13–33 further unfolding. Compared with the adsorption of peptides on pristine graphene, where two peptides are dimeric, the peptides are separately located on GO surface, since the interactions between each peptide and GO are much stronger than interpeptide hydrophobic interactions.

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Author Contributions
S.Z. wrote the paper. G.Z. and J.G. performed the simulations. F.Z. and J.C. analyzed the results and prepared all figures. All authors discussed the results and reviewed the manuscript.

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