The Inhibition of SARS-CoV-2 3CL M\textsuperscript{pro} by Graphene and Its Derivatives from Molecular Dynamics Simulations

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ABSTRACT: At present, the most powerful new drugs for COVID-19 are antibody proteins. In addition, there are some star small molecule drugs. However, there are few studies on nanomaterials. Here, we study the intact graphene (IG), defective graphene (DG), and graphene oxide (GO) interacting with COVID-19 protein. We find that they show progressive inhibition of COVID-19 protein. By using molecular dynamics simulations, we study the interactions between SARS-CoV-2 3CL M\textsuperscript{pro} and graphene-related materials (GRMs): IG, DG, and GO. The results show that M\textsuperscript{pro} can be absorbed onto the surfaces of investigated materials. DG and GO interacted with M\textsuperscript{pro} more intensely, causing the decisive part of M\textsuperscript{pro} to become more flexible. Further analysis shows that compared to IG and GO, DG can inactivate M\textsuperscript{pro} and inhibit its expression effectively by destroying the active pocket of M\textsuperscript{pro}. Our work not only provides detailed and reliable theoretical guidance for the application of GRMs in treating with SARS-CoV-2 but also helps in developing new graphene-based anti-COVID-19 materials.

KEYWORDS: graphene-related materials, SARS-CoV-2, COVID-19, 3CL M\textsuperscript{pro}, interactions, molecular dynamics simulations

1. INTRODUCTION

Recently, a new type of coronavirus named serve acute respiratory coronavirus (SARS-CoV-2), caused a world pandemic, corona virus disease 2019 (COVID-19), which heavily threatens the lives and property of the people around the world.\textsuperscript{1,2} Though its single-stranded RNA genomes are mostly identical to the earlier SARS coronavirus (SARS-CoV) with a ratio of 82\%, SARS-COV-2 seems to be more cunning, that is, higher infectivity and variability.\textsuperscript{3} Its genome encodes more than 20 proteins, and among them, spike protein is the most important structural protein.\textsuperscript{4} This protein binds to cellular surface receptor angiotensin-converting enzyme 2 (ACE2), which is an important step for the virus to enter the host cell.\textsuperscript{5−7} However, previous studies have shown that in the absence of ACE2, spike protein alone can still damage the phospholipid membranes and produce lung injury in vivo.\textsuperscript{8,9} In addition, like other single-stranded RNA viruses, other non-structural proteins necessary for replication of virus are achieved by a protease similar to chymotrypsin (3CL main protease or 3CL M\textsuperscript{pro}).\textsuperscript{10−12}

According to previous research studies, the structure of M\textsuperscript{pro} consists of three domains.\textsuperscript{13,14} Domains I and II contain the active site of the protein, and domain III is composed of five roughly antiparallel helixes, responsible for the stability of its symmetric homodimer. Since M\textsuperscript{pro} plays a key role in mediating replication and transcription of the virus, it has become an attractive drug target for SARS-CoV-2.\textsuperscript{15,16} Until now, many studies have been devoted to searching potential inhibitors of M\textsuperscript{pro} by experiment, computer-aided drug design, high-throughput and virtual screening, molecular docking, molecular dynamic (MD) simulations, and so on to achieve the goal of inhibiting the activity of the virus.\textsuperscript{17−21}

With the rapid development research of nanomaterials, graphene-related materials (GRMs) including intact graphene (IG), graphene oxide (GO), reduced graphene oxide (RGO), and defective graphene (DG) have been widely applied in the biomedical field, such as drug loading and transportation, intelligent targeting, and antiviral treatment, due to their outstanding physical and chemical properties.\textsuperscript{22,23} For example, IG and GO can inhibit the activity of \( \alpha \)-chymotrypsin by experiment and MD simulations.\textsuperscript{24,25} IG is a hydrophobic material composed of \( \text{sp}^2 \)-hybridized carbon atoms.\textsuperscript{26} Meanwhile, GO exhibits hydrophilicity due to the existence of hydroxyl (OH), epoxy (O), and carboxyl groups (COOH) on the surface.\textsuperscript{27} RGO is chemically reduced from GO, which is
highly hydrophilic with low oxygen content.\textsuperscript{28} In addition, previous experiments revealed that various local defects can occur in IG during ion irradiation or chemical treatment, leading to the existence of DG. The “defect” brings new physical and chemical properties to the practical applications of IG.\textsuperscript{29,30}

In fact, the interactions between GRMs and biomolecules (including proteins, lipids, DNA, etc.)\textsuperscript{31–35} demonstrate their great applications in antiviral and antibacterial applications.\textsuperscript{36–39} It was reported that the bilayer lipid of feline coronavirus will adsorb onto the surface of the GO through the hydrogen bond and electrostatic interaction. Subsequently, the binding of GO will destroy the viral membrane and cause the death of feline coronavirus.\textsuperscript{38} Also, Unal et al. demonstrated that GO sheets can interact with SARS-CoV-2 surface components and disrupt infectivity even in the presence of any mutations on the viral spike.\textsuperscript{40} Interestingly, since the outbreak of COVID-19, there have been many reviews summarizing the applications, opportunities, and challenges of GRMs dealing with COVID-19, including protection, sanitization, degradation, filtration, diagnosis, decontamination, detection, etc.\textsuperscript{41–45} However, the detailed molecular interaction mechanisms between GRMs and SARS-CoV-2 have not been systematically studied.

MD simulations can provide detailed molecular interactions and valuable atomic information, so it is widely used to study the interactions between biomolecules and nanomaterials.\textsuperscript{44} Therefore, in this study, we used MD simulations to study the interactions between investigated materials (including IG, DG, and GO) and M\textsuperscript{pro}, aiming to provide details of the interactions between them. Specifically, we discussed the binding process between M\textsuperscript{pro} and investigated materials and analyzed the structural changes of M\textsuperscript{pro}, and we found that investigated materials can be used as an effective interaction platform. Finally, further analysis showed that compared to IG and GO, DG can inactivate M\textsuperscript{pro} and inhibit its expression effectively by destroying the active pocket of M\textsuperscript{pro}. Our work not only provides detailed and reliable theoretical guidance for the application of GRMs in treating with SARS-CoV-2 but also helps in developing new graphene-based anti-COVID-19 materials.

2. SIMULATION METHODS

2.1. System Preparation. The initial structures of M\textsuperscript{pro} and ligand N3 (PDB:6LU7\textsuperscript{13}) were obtained from the Protein Data Bank (http://www.pdb.org/pdb/). The initial structural optimization of M\textsuperscript{pro} includes residual repair, geometric optimization, and prediction protein ionization.\textsuperscript{35} The final structure of M\textsuperscript{pro} and ligand N3 contains 306 amino acid residues and 4779 atoms (Figure 1a).

The model of DG is consistent with that established by Zhou et al.\textsuperscript{19,33,46} The model used to establish GO is $C_{10}(OH)_{1}(O)_{1}(COOH).$\textsuperscript{37–39} IG, DG, and GO were constructed through the atomic simulation environment (ASE), a software package written in the Python programming language.\textsuperscript{20} The dimensions of investigated materials are consistent. Then, the structures of investigated materials were optimized by the Forcite module of Material Studio. The force field is the Dreiding force field.\textsuperscript{31,52} The Gasteiger charges\textsuperscript{53} (the maximum iteration was set to 50,000 and the convergence was set to $5.0 \times 10^{-5}$) and the ultrarfine quality were used for the energy calculation. The final structures of investigated materials were used for MD simulations (Figure S1).

The initial minimum distance between M\textsuperscript{pro} and the surfaces of investigated materials is about 2.0 nm. To observe the change of the active pocket of M\textsuperscript{pro} visually, we chose the direction that the active pocket is facing the surfaces of investigated materials. Then, the M\textsuperscript{pro}-investigated material complexes were embedded into a TIP3P water box.\textsuperscript{54} The water molecules located within 4 Å of the M\textsuperscript{pro}-investigated material complexes were removed. Finally, some counter ions were added to all systems to neutralize the charge (Figure 1b).

2.2. MD Simulations. All MD simulations were performed with NAMD simulation package\textsuperscript{55} using the CHARMM27 force field\textsuperscript{56} in the NPT ensemble at 1 atm and 310 K. Before performing the ceremonial MD simulations, we performed (1) 5000 steps of energy minimization, (2) a 1 ns equilibration of water molecules by fixing the heavy atoms of M\textsuperscript{pro} and investigated materials, and (3) a further 4 ns equilibration in the absence of positional restraints of M\textsuperscript{pro}. In addition, the Dreiding force field was used to describe the interactions between M\textsuperscript{pro} and investigated materials.\textsuperscript{51,52} Periodic boundary conditions were applied for all systems. The particle mesh Ewald (PME)\textsuperscript{57} summation was applied to model the full system electrostatic interaction, where a cutoff of 12 Å was used for the separation of the direct and reciprocal space summation. The SWITCH algorithm, with a cutoff distance of 12 Å, was also applied to calculate van der Waals interactions.\textsuperscript{58} For each system, we performed three independent 100 ns MD simulations to ensure the accuracy of the results. In addition, in the absence of ligand N3, we also performed the same MD simulations for each system. Of course, in the absence of investigated materials, we also performed 100 ns MD simulations to study M\textsuperscript{pro} and ligand N3. Finally, all trajectories were generated for VMD analysis.\textsuperscript{59} The detailed MD simulation information is shown in Table S1.

3. RESULTS AND DISCUSSION

3.1. Adsorption of M\textsuperscript{pro} onto Investigated Materials. From the trajectories, M\textsuperscript{pro} that adsorbed onto the surfaces of investigated materials undergo the same process: M\textsuperscript{pro} adjusted its spatial conformation first, then contacted the surfaces quickly, and finally adjusted its spatial conformation again and achieved the relatively stable state. As shown in Figure 2a, M\textsuperscript{pro}
began to contact the surface of IG at 12 ns, and about 35 amino acid residues were adsorbed on the surface at 100 ns. Due to the high hydrophobicity of IG, the hydrophobic interaction occupies the main driving force for adsorption. However, when Mpro were adsorbed onto the surfaces of DG and GO, there were about 50 amino acid residues that contacted the surfaces (Figure 2b,c). The existence of hydrophilic functional groups on the surfaces of DG and GO caused hydrophilic amino acid residues to be adsorbed.

To understand the adsorption mode between Mpro and investigated materials profoundly, we calculated the root-mean-square deviation (RMSD) of all backbone carbon atoms of Mpro, the interaction energy, and the contact area between them.

As shown in Figure 3a, for the "Mpro-IG" system, the RMSD of Mpro is 3.3 Å approximately; for the "Mpro-DG" and "Mpro-GO" systems, the RMSD of Mpro are 4.1 and 3.6 Å, respectively. So, we believed that the structural change of Mpro adsorbed onto the surface of DG was more serious followed by GO and IG. In addition, the evolution of RMSD also reflected that 100 ns was sufficient to study the interactions between Mpro and investigated materials.

Generally, when the interaction energy and contact area increase rapidly, it means that the adsorption process proceeds quickly. As shown in Figure 3b,c, the trend of interaction energy and contact area with the evolution of time is consistent. However, their adsorption affinities are different. Specifically, for the "Mpro-IG" system, the interaction energy and contact area maintain stability at about −260 kcal/mol and 1550 Å² finally. When Mpro adsorbed onto the surfaces of DG and GO, the interaction energy and contact area increased rapidly at about 10 ns and remained stable at about −330 and −320 kcal/mol and 2090 and 2050 Å² finally, respectively. The results indicated that Mpro are preferentially attached on DG and GO rather than IG. This is consistent with the adsorption process mentioned above. In short, Mpro can adsorb onto investigated materials, while DG and GO interact with Mpro more intensely. This shows their potential application in detecting and diagnosing the SARS-CoV-2.

3.2. Structural Changes of Mpro. Organized enzymes usually contain multiple structural domains, which have an extensive and close connection, and perform specific physiological functions together. €62,63 Take Mpro as an example, the interface between domain I and domain II constitute the active pocket, while domain II and domain III are connected by a loop (F185-I200), and domain III is responsible for the stability of symmetric homodimer. So, we calculated the root-mean-square fluctuation (RMSF) for each amino acid residue of Mpro to understand the part and overall structural changes. As shown in Figure 4, for all systems, the RMSF fluctuated similarly. The RMSF of the amino acid residues located in the N-terminal and C-terminal of Mpro is the highest owing to their high flexibility in general. Other peaks of RMSF reflect in some specific amino acid residues. Interestingly, these specific amino acid residues with higher RMSF are usually located at the crucial parts of Mpro, such as the active pocket (T24-T26, T45-Y54, F140-C145, and H163-T169), the loop link domain II and domain III (F185-I200), and the five α-helices of domain III. These highly flexible amino acid residues mean that they can adjust their conformation to adapt different substrates. In addition, our results are similar to the previous studies, the active pocket of Mpro has malleable property, thereby promoting the combination of clinical antiviral drugs. €20,62 Moreover, the RMSF of Mpro adsorbed onto the surfaces of DG and GO are higher than that of IG significantly. It means that the combination of DG and GO with Mpro endues Mpro more flexibility than IG. In other words, DG and GO may be more suitable as a variety of effective protease inhibitor carriers to interact with Mpro, showing their great applications in anti-SARS-CoV-2. In addition, the reason why the RMSF of the loop and the five α-helices in domain III also increased may be due to their need to adjust the conformation of Mpro more flexibly to maintain overall structural stability.

3.3. Conformational Changes of the Active Pocket of Mpro. Generally speaking, the specificity and activity of
organized enzymes are mainly related to the conformation of the active pocket. When the structure of the active pocket is destroyed, the detectability of enzyme activity drops sharply, that is, inactivation.25,64 Therefore, we focused on the conformational changes of the active pocket of Mpro in this section. As shown in Figure 5a, for the “Mpro-IG” system, the RMSD of Mpro is 2.6 Å; for the “Mpro-DG” and “Mpro-GO” systems, the RMSD of Mpro are 4.1 and 3 Å, respectively. This indicates that the active pocket of Mpro adsorbed onto the surface of DG experienced the most dramatic structural changes.

As shown in Figure 5b, one terminal of the active pocket was attached to the surface of IG, while the other terminal was far away from it, causing the whole active pocket to assume a “standing posture” binding mode. However, the whole active pocket adsorbed onto the surfaces of DG and GO, resulting in a relatively “parallel posture” binding mode (Figure 5c,d). Then, we counted the number of amino acid residues of the active pocket, which adsorbed onto investigated materials (Figure 6a). Obviously, DG adsorbed more amino acid residues of the active pocket followed by GO and IG. Generally, any changes in the spatial structure of the active pocket of Mpro will reduce its activity. In fact, the stability of the spatial structure of proteins is mainly maintained by secondary bonds such as hydrogen bonds (H-bonds).65 The destruction of H-bonds in the active pocket usually leads to its structural instability, which affects the expression of protein activity. Therefore, we analyzed the average number of H-bonds formed by the active pocket of Mpro (Figure 6b). Here, H-bonds are defined as those formed when the distance between the donor and acceptor is 0.35 nm and the angle of donor–H-acceptor is less than 30°.66 When Mpro adsorbed onto IG, DG, and GO, the average numbers of H-bonds formed by the active pocket were 9.98, 8.15, and 8.55, respectively. It indicates that DG has a more serious destructive effect on the active pocket, and the destruction of the H-bonds may result in the loss of activity of Mpro.

The active pocket consists of 4 subsites: S1, S1’, S2, and S4. The structure of the initial active pocket of Mpro is three-dimensional conformation, with complete and compact residues of the active pocket followed by GO and IG. Generally, any changes in the spatial structure of the active pocket of Mpro will reduce its activity. In fact, the stability of the spatial structure of proteins is mainly maintained by secondary bonds such as hydrogen bonds (H-bonds).65 The destruction of H-bonds in the active pocket usually leads to its structural instability, which affects the expression of protein activity. Therefore, we analyzed the average number of H-bonds formed by the active pocket of Mpro (Figure 6b). Here, H-bonds are defined as those formed when the distance between the donor and acceptor is 0.35 nm and the angle of donor–H-acceptor is less than 30°.66 When Mpro adsorbed onto IG, DG, and GO, the average numbers of H-bonds formed by the active pocket were 9.98, 8.15, and 8.55, respectively. It indicates that DG has a more serious destructive effect on the active pocket, and the destruction of the H-bonds may result in the loss of activity of Mpro.

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especially, the active pocket of MPro that adsorbed onto investigated materials was all destroyed. The conformational change of the active pocket adsorbed onto DG was more significant than that of IG and GO, accompanied by obvious conformational incompleteness. This is consistent with the changing trend of the H-bonds we mentioned above. For the "MPro-IG" system, because the active pocket was not adsorbed onto the surface completely, the active pocket was damaged slightly and still relatively compact and complete (Figure 7b). For the "MPro-DG" system, the active pocket adsorbed onto the surface completely, and the two terminals of the active pocket were torn in opposite directions, causing intense damage (Figure 7c). For the "MPro-GO" system, the active pocket also adsorbed onto the surface completely and underwent deformation, especially the S1′ subsite had a large offset, making the shape of the active pocket similar to a "plane triangle" (Figure 7d). In conclusion, the results in this section show that all investigated materials can adsorb the active pocket of MPro onto their surfaces, but compared to IG, DG and GO adsorbed more amino acids of the active pocket, which changed the posture and conformation of the active pocket and induced the inactivation for MPro. DG and GO may have more advantages in degradation and filtration than IG for the COVID-19.

3.4. Changes in Binding between the Active Pocket of MPro and Ligand N3. Previous studies have shown that the active pocket of MPro in all coronaviruses is conservative extremely, and this active pocket can be used as a drug target for the design of broad-spectrum inhibitors. In addition, according to other literature, ligand N3 (a Michael acceptor inhibitor) can covalently bind with four subsites of the active pocket and specifically inhibit MPro of a variety of coronaviruses, including SARS-CoV and MERS-CoV. Therefore, to analyze the changes in the binding capacity between the active pocket and ligand N3, we calculated their interaction energy. According to Figure 8, the interaction energy of the "control system" is about −60.77 kcal/mol. For DG, the interaction energy is the lowest (−38.61 kcal/mol) followed by GO (−41.39 kcal/mol) and IG (−51.04 kcal/mol). All investigated materials weaken the interactions between the active pocket and ligand N3, but the degree of weakening is different. In general, the deformation of the active pocket and the interactions between investigated materials and ligand N3 are the two reasons for the decline in the interactions between the active pocket and ligand N3. In fact, the initial structure of MPro does not contain ligand N3, so when researching the interactions between MPro and three-dimensional ligand N3, with the tearing of the active pocket, the structure of ligand N3 also changed significantly (from three-dimensional to the two-dimensional plane). Ligand N3 gradually moved away from the S1 and S1′ subsites to approach the S4 and S2 subsites, which weakened the interactions between the S1 and S1′ subsites and ligand N3. In short, through the observation of the active pocket and the analysis of the interaction diagram with the active pocket and ligand N3, we believed that the decrease in the interactions between the active pocket and ligand N3 is related to the conformational change of the active pocket. Of course, we considered that compared to GO and IG, DG had the most intense damage to the active pocket of MPro, which may lead to the inactivation of MPro, showing its great potential for physical protection and filtration for the COVID-19.

3.5. MD Simulation Results about the Interactions between Investigated Materials and MPro without Ligand N3. To profoundly comprehend the interactions between the MPro without ligand N3 and investigated materials and verify our previous analysis, we also performed another 100 ns MD simulations for each system without ligand N3. Our results show that MPro were adsorbed onto the surfaces of investigated materials and reached a relatively stable state (Figure 10). Moreover, for the amino acid residues that make up the active pocket of MPro, DG adsorbed more of these amino acid residues than GO and IG (Figure S4). As shown in
Figure 11a, when Mpro were adsorbed onto DG and GO, the RMSD is 6 Å, which is significantly higher than the value onto the IG, indicating that Mpro underwent the greatest structural change. The interaction energy between Mpro and investigated materials are about $-280$, $-330$, and $-400$ kcal/mol (Figure 11b). This indicates that the interaction strength between Mpro and DG is stronger than that of GO and IG. The trend of the contact area between them is consistent with the interaction energy (Figure 11c). In short, the adsorption strength between DG and Mpro is greater than that of GO and IG.

Figure 11d shows the RMSF of each amino acid residue to reflect their flexibility for all systems. Compared with the “control system”, the amino acid residues of Mpro showed some prominent peaks, especially the Mpro-DG/GO systems. These peaks are consistent with the conclusions we mentioned above. Similarly, these important amino acid residues of Mpro adsorbed onto GO and DG have higher structural flexibility, which indicates that they can adjust their position and structure flexibly to adapt to different ligands. Finally, we analyzed the RMSD (Figure 11e) and H-bond evolution (Figure 11f) of the active pocket during 100 ns MD simulations: the RMSD of Mpro is 6 Å, and the average number of H-bonds of the active pocket of Mpro adsorbed onto the surface of DG is 5.72, which is less than that of GO and IG. Obviously, DG has the greatest impact on the conformational change for the active pocket of Mpro. In short, DG has the greatest damage on the active pocket of Mpro, and the results are identical to our previous MD simulation results.

We found that GRMs can progressively inhibit the activity of Mpro, which is consistent with the latest research work by Donskyi et al. They systematically researched the inhibitory ability of the graphene platform with precise disulfate/alkyl functional groups against SARS-CoV-2, and their results proved that the graphene platform has strong antiviral activity against natural SARS-CoV-2 but no obvious toxicity to human cells. Our research proved that GRMs have infinite potential in the field of resistance to SARS-COV-2. Nevertheless, there are several issues that still need to be further studied and discussed. First, there are many factors that may affect the interaction between GRMs and Mpro, such as temperature, humidity, dust, and various air pollutants. For example, Sharma and Deep explored the effect of pH on Mpro.72 Our work only discusses basic realistic conditions, but the real-life conditions are complicated and changeable, so various real-life conditions still need further research and discussion. Second, the main outer component of SARS-COV-2 is spike protein instead of...
Mpro, although our results show that GRMs can quickly capture Mpro quickly, GRMs also need to couple with other specific DNA chains, small molecule inhibitors, targeted molecules, etc. to achieve precise capture of Mpro. Furthermore, although humans have developed many SARS-COV-2 vaccines, these vaccines seem to be unable to withstand the spread of SARS-COV-2. This may be attributed to the strong variability of SARS-COV-2. Therefore, the interaction between GRMs and other SARS-COV-2 also needs further research and discussion.

4. CONCLUSIONS

In this study, we investigated the interactions between the key target protein (Mpro) of SARS-CoV-2 and IG, DG, and GO by employing MD simulations. Our results show that Mpro can be adsorbed onto the surfaces of investigated materials, and the interaction energy and contact area show that the interaction strength between Mpro and DG and GO are stronger than IG. Next, we explored the influence of investigated materials on the part and overall structure of Mpro. We found that some key parts of Mpro (active pocket, loop, α-helixes, etc.) usually have high flexibility, while investigated materials can enhance the flexibility of these key parts. Compared to IG, DG and GO have a stronger influence on these parts, which indicates that DG and IG enable the active pocket to accommodate ligands in different positions and directions, which is conducive to exploring more inhibitors. Then, we focused on the change of the active pocket and the change of the interactions between it and ligand N3. We found that compared to IG and GO, when Mpro adsorbed onto the surface of DG, the active pocket was damaged significantly, and the interaction between it and ligand N3 decreased obviously. So, we believe that DG can inactivate Mpro and inhibit its expression effectively. Finally, we studied the interactions between Mpro and investigated materials in the absence of ligand N3 by MD simulations and obtained identical conclusions.

Our findings not only provide more details on the interactions between Mpro and GRMs to develop new graphene-based anti-COVID-19 materials but also provide strong support for the application for combating the SARS-CoV-2, including detection, diagnosis, protection, degradation, filtration, etc.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.1c18104.

(Figure S1) Atomic structure of investigated materials (IG, DG, and GO), (Table S1) MD simulation models for all systems, (Figure S2) other representative trajectory snapshots for systems, (Figure S3) two-dimensional interaction diagram between the initial structure of the active pocket and ligand N3, (Figure S4) number of amino acid residues of the active pocket adsorbed onto investigated materials (PDF)

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Notes

The authors declare no competing financial interest.

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