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Revealing graphene oxide toxicity mechanisms: A reactive molecular dynamics study

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ABSTRACT

We report the first atomistic-scale study on graphene oxide (GO) toxicity, revealing potentially harmful chemical mechanisms of GO during its interactions with biomolecules. ReaxFF-based reactive molecular dynamics study was utilized to assess the impact of different functional groups on GO biocompatibility. Our study predicts different chemical reactions between the GO sheet and peptides that lead to reactive oxidative species (ROS), acidic or basic pHs and cell surface adhesions. We observe that cell-surface adhesion is a result of strong H-bonding and stable π–π stacking interaction. This stacking can also lead to the disruption of the polypeptide secondary structure.

1. Introduction

Geim and Novoselov referred to graphene as "a rapidly rising star on the horizon of materials science" since every day we are confronted by its new applications in science and industry [1]. Amongst such unique applications, medical applications of graphene have recently been in the center of attention [2–6]. Fullerene, nanodiamond, carbon nanotubes (CNTs), and graphene oxide (GO) all are accounted as a family of carbon based materials and are used widely in places where living tissues are involved [7]. Given considerable applications of carbon based materials in medicine and biotechnology such as biosensors, gene delivery and drug delivery [8–10], a thorough investigation on the possible hazards that it can pose on living organisms seems vital [7,11,12]. Despite various studies on toxicity of different carbon based materials, there are still controversies regarding the amounts of toxicity and the underlying mechanisms [13–15].

Among different graphene based materials, graphene oxide has its own particular applications in medicine because of its hydrophilicity. Graphene oxide has similar structure to graphene but the presence of distinct functional groups enhances GO hydrophilicity. Although today a significant number of reports regarding the number and the range of these functional groups are on hand, one may refer to epoxide (–O–), hydroxyl (–OH) and carboxyl (–COOH) as the main underlying functional groups that form GO family. The density and the distributions of these functional groups vary based on the manufacturing methodologies [16]. While Hofmann and Holst's suggest a model which only consists of epoxide [17], Ruess considers a more complex model by adding hydroxyl groups [18]. Nakajima and Matsuo, on the other hand, changed the hypothetical sheet geometry of GO while keeping the functional groups of Ruess model and thereby, they suggested a lattice structure analogous to $(C_2F)_n$ [19,20]. Other functionalizations are also reported by adding amines [21], polyethylene glycol [22] and polyethylenimine [23].

Given its increasing applications, a systematic analysis of its potential hazards to human health seems necessary. Generally carbon based materials are either biocompatible or have insignificant toxic effect. However, functionalized graphene materials namely GO have been reported to show cytotoxic effects on bacteria [24]. To explore such possibilities, different studies have been performed at cellular level [14,25–32]. Not only are these studies controversial, but they also do not represent the precise mechanisms that bring about the described cytotoxicity. For instance, several studies show that graphene based materials that are functionalized with chitosan, peroxide or PEG have excellent biocompatibility; however, other studies have reported toxic properties for GO because of the production of ROS and different metallic compounds [33]. However, because of the complex interactions between GO and cellular membrane, the final conclusion has often been postponed to

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the future investigations. Moreover, to our knowledge, there is no report available to illustrate the underlying chemical reactions that may cause cytotoxicity at atomic level.

Here, we report the role of different functional groups on GO-peptide interactions at atomic level. We performed a series of ReaxFF-based reactive molecular dynamics simulations to see the explicit interactions between a GO sheet and two peptides in water. First, different functional groups are analyzed separately and their reactivity is studied. Afterwards, a system consisting of all of the previous groups is built and investigated. Our results show epoxide and carboxyl groups have catalytic effects on thiol bond (–CSH) deprotonation reaction as well as aldehyde to carboxyl transition in the presence of ROS. Hydroxyl groups are primarily responsible for secondary structure denaturation. In addition, by forming strong hydrogen bonds with hydrophilic side chains of the peptides, hydroxyl groups can augment cell-surface adhesion as reported by Lee et al. [34]. Moreover, our model describes how π–π stacking interaction between the aromatic rings of different amino acid residues (e.g. Tryptophan) plays a vital role in the experimentally observed peripheral cell-surface adhesion [35]. Our procedure to question GO toxicity can also be employed for further studies on other functional groups on graphene as well as evaluation of toxicity ranking for different metal oxide nanoparticles [49].

2. Methods

ReaxFF based reactive molecular dynamics is utilized to assess the cytotoxicity of graphene oxide based materials. First, different systems consisting of distinct functional groups are created separately and their interactions with a peptide are studied. Afterwards, a unified model is built that contains all the previously analyzed groups and their combined effects are investigated. Also the possible pathways that peptide-graphene adhesion may arise from are studied.

The objective of this study is to provide a detailed analysis of the chemical interactions between graphene oxide functional groups and different amino acids at atomic scale to clarify the amount of cytotoxicity of GO and the underlying mechanisms of such interactions. Computational limitations hinder larger scale investigations such as full atomistic study on GO-protein system to further analyze tertiary structure interactions, allosteric effects, etc., which would be more descriptive of GO toxicity. However, our results can be generalized for larger systems consisting of significant number of amino acids.

In practice, it is hardly possible to purify GO in order to obtain specific functional groups on GO. Hence, using simulation is an obvious way to perform such studies. Additionally, by employing molecular dynamics techniques, a time dependent picture can be obtained that allows one to see atom-by-atom interactions, which can ultimately describe the toxicity of the material at the femtosecond level. In contrast to experimental approaches, our molecular dynamics method is able to probe chemical reactions in a time dependent manner for each of functional groups that facilitates drawing conclusion about the relevance of each of these groups.

It is essential to mimic the biological environment in our simulations so that the obtained results are trustable. As a first step towards that goal, we consider a 12-residues alpha helix to represent the biomaterial. This peptide is designed to cover most of the significant activities of amino acid side chains such as ionic interactions, H-bonds, hydrophobic interactions and π–π stacking interaction. In order to show that the presented results are not sequence specific, a mutated version of this peptide sequence is also studied (AHAGADACAMWA → ARAGADACAMGA). Note that the peptides are capped by aldehyde to prevent fast denaturation [48].

Graphene with periodic boundary conditions (29.55882 by 34.15774 Å) is built for all studies unless otherwise mentioned. Subsequently, different functional groups are added to graphene. Chemical formula of $C_{96}O_7$, $C_{96}(OH)_7$ and $C_{96}(COOH)_7$ are used for epoxide, hydroxyl and carboxyl, respectively. Fig. 1 shows these different systems schematically. Obviously, such high density of functional groups usually is not used for practical applications; however, we study the extreme case in our model to magnify any possible phenomena. The GO sheet and the peptides are then placed in the vicinity (with an initial 3 Å gap) and then the simulation box

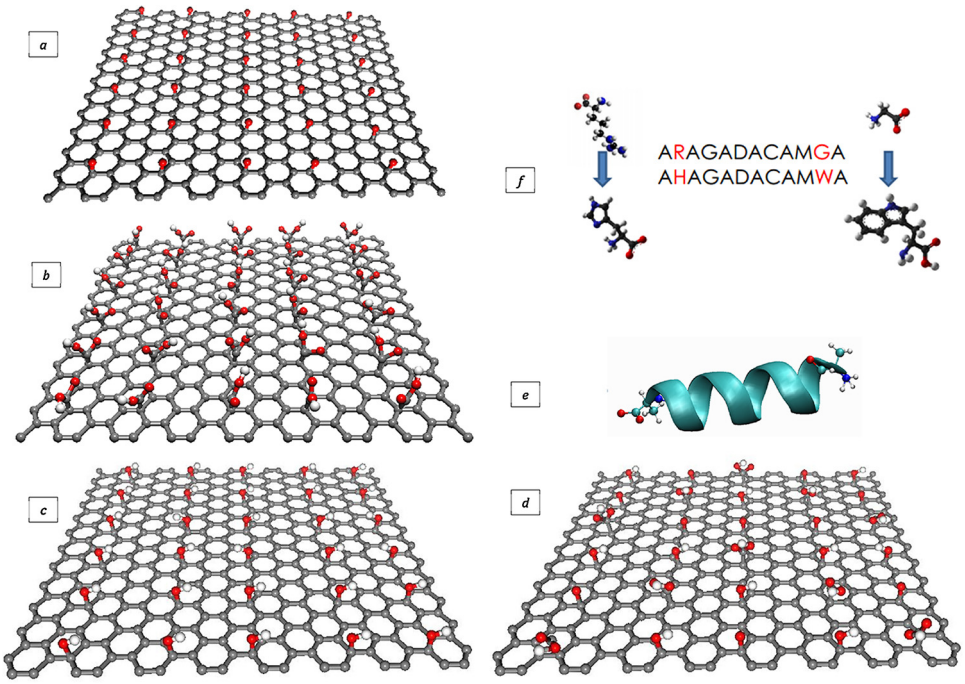

Fig. 1. Graphene oxide in the presence of (a) epoxide, (b) carboxyl, (c) hydroxyl, and (d) combination of all functional groups. (e) polypeptide; (f) R→H and G→W mutation.