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Interaction of graphene oxide with proteins and applications of their conjugates

Abstract

Graphene oxide (GO) has abundant surface oxygen-containing groups such as epoxide, hydroxyl, and carboxylic groups; it can be prepared through the oxidative intercalation and exfoliation of graphite on a mass scale. Owing to the enriched surface functionalities, the GO is water-soluble and chemically versatile. The surface functional groups can also provide plenty of reaction sites for linking nanoparticles, proteins, enzymes, peptides, bacteria, cells, and nucleic acids through covalent and non-covalent binding. GO has been used as a matrix for protein immobilization in different biotechnological applications such as fluorescence- or electrochemical-based sensors, labeling and imaging, therapy, and targeted delivery. This paper reviews the main strategies for the assembly of proteins onto graphene oxide surface and their applications, especially in the biomedical area.

Keywords: Graphene Oxide, Protein, Interaction, Conjugate

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Introduction

Graphene oxide is a highly oxidized form of chemically modified graphene, produced by oxidation of crystalline graphite followed by sonication or other dispersion methods to produce monolayer material, typically in aqueous suspension. The structure of GO consists of single-atom-thick carbon sheets with phenol, hydroxyl and epoxide groups above and below each plane and carbonyl and carboxyl functional groups attached to the edges of the graphene sheets. Due to functional groups the edges of GO sheets are hydrophilic whereas the basal plane is hydrophobic and the result is an amphiphilic giant sheet-like molecule. In recent years, there has been a surge of interest in exploring graphene oxide for wide array of medical and biological applications due to their in vitro and in vivo biocompatibility, low impact on the environment, structural features, large surface area, low cost, and facile chemical processing. GO based materials have shown the potential applications in drug and gene delivery, near-infrared photothermal treatment for cancer and Alzheimer's disease, biosensing, cellular probing, real-time monitoring based on fluorescence, and as a scaffold for cell culture. The application of materials to the organism for medical purposes leads to exposure of proteins; interaction with these proteins and their nature depends very much on the properties of used materials, such as surface energy, surface charge, and hydrophobicity.

In general, upon the exposure, the surface of GO interacts with proteins of biological fluids like serum or blood plasma which significantly affect the biological characteristics of GO. For example, it has been demonstrated that surface-bound proteins enhance specific cellular uptakes and activate intracellular signalling pathways. It represents the first possibility of the formation of protein-graphene oxide complexes. The second possibility represents purposefully preparing a novel complex between protein and graphene oxide because a significant portion of GO-based bioapplications is based on the molecular interactions between GO and proteins. Immobilization of proteins on solid substrate is an efficient way to improve their performances. The GO-protein conjugates have several advantages over the free ones. They showed unique biological and chemical properties, higher biocompatibility and stability in biological environment, ability of conjugation to target receptors or sites and cell internalization.

Biomedical applications of GO critically depend on the interactions of biomolecules with it; therefore, the understanding the interaction between GO and proteins is fundamentally essential. The surface of GO allows electrostatic, hydrophobic, hydrogen bonding, and π-π stacking interactions which are generally favoured for molecules with poor water solubility. The covalent and non-covalent bindings have been used to attach proteins, enzymes, peptides, bacteria, cells, and nucleic acids to graphene oxide for various applications including fluorescence- or electrochemical-based sensors, labeling and imaging, therapy and targeted delivery, and energy storage. The present review is intended to investigate the interaction between GO and proteins and the potential of these conjugates in bioapplications.

Interaction between graphene oxide and proteins

Non Covalent interaction (Physical adsorption)

Non-covalent protein adsorption onto solid supports represents the most simple and desirable strategy of physical immobilization. Obviously, the mechanisms of proteins adsorption on GO is a kind of non-covalent self-assembly including weak Van der Waals forces, hydrophobic, electrostatic, and π-π stacking interaction. These types of attractions between proteins and graphene oxide involve solution phase incubation, or direct sonication, followed by a washing step to remove the unbound proteins. The non-covalent bonds responsible for the interaction between GO and proteins vary depending on the surface properties of graphene oxide, such as morphology and hydrophobicity.

GO consists of sp2 hybridized honeycomb carbon lattice; therefore, the direct physical adsorption method is predominantly hydrophobic, where the hydrophobic regions of the protein can interact with the carbon lattice.[1] In addition, hydrogen bonds between proteins and side groups of graphene oxide also support surface adhesion. The hydrophobic interaction between GO and proteins strongly depends on electron density and geometry of protein molecule. Lee et al. [2] demonstrated that the lower electron density of the low-molecular weight heparin (LMWH) resulted in the decrement of hydrophobic interaction. With LMWH instead of unfractioned heparin, the conjugation between graphene and LMWH is not effective. Thus,

the hydrophobic interaction between the hydrophobic graphene plates and heparin backbones contributes to the effective conjugation between graphene oxide and heparin. These results suggest that the hydrophobic interaction between the proteins and reduced GO or graphene is stronger than the electrostatic interaction between the proteins and GO .[2]

The interaction between protein molecules and GO could be very complicated because the charge status of the surface functional groups of the protein depends strongly on the environmental conditions, including the pH value, and the ionic strength of the buffer. The protein can be negatively (for instance carboxylate) or positively charged (for instance protonated amino groups), and the surface density of the oxygen-containing groups on the GO also varies with the preparation procedure and storage conditions. Based on this, various proteins could interact with GO through electrostatic interaction with different stability.

Interaction of graphene oxide with other molecules can be mediated by van der Waals interactions. This non-covalent bonding enables the use of graphene oxide flakes as carriers for water-insoluble drugs or creates nanocomposites between graphene and polymers that can be further bound to proteins .[3] However, the electrostatic interactions are more pronounced on GO, whereas both van der Waals and electrostatic interactions play a major role in the adsorption of proteins on reduced GO. The increase in the van der Waals interaction on reduced graphene oxide is attributed to the increase in unfunctionalized regions on the surface .[4]

The basal plane of the GO enriched with π electrons (each carbon atom of GO bonded with three adjacent carbon atoms with sp2 hybridized orbitals forming robust σ bonds, and the rest of the electrons in the p atomic orbitals are delocalized all over the basal plane of the GO sheet forming a superb π bond) making it possible for the GO to interact with proteins through π-π stacking interaction. The softness of GO could help the protein binding by adapting its own shape to fit better with the aromatic residues of the protein, forming stronger π-π stacking. This phenomenon was observed experimentally by Alwarappan et al.[5] who demonstrated that a strong π-π interaction existed between the individual hexagonal cells of the GO basal planes and the glucose oxidase .[5]

Apparently, the enzyme immobilization onto GO might be a result of the synergic effect of different interactions. De et al. [6] studied the interaction between GO and chymotrypsin and found that GO could strongly inhibit the activity of chymotrypsin, which might be due to the coexistence of anionic, hydrophobic, and π-π stacking interactions .[6] The interaction between protein and surface of graphene oxide substrate should be a comprehensive result of several attractive and repulsive interactions and the exact interaction, or "driving force", for it should be different for various classes of proteins. Zang et al. [7] found that HRP and lysozyme were immobilized on GO sheets through electrostatic interactions if the pH level was below the isoelectric point; if the pH level was above the isoelectric point they suggested that hydrogen bonds interaction prevailed.

Covalent interaction

The enriched reactive oxygen functional groups of GO should render it a good solid substrate for protein immobilization through covalent binding. Covalent binding of a protein with GO is most commonly based on chemical reactions between the side groups of amino acid residues located on the protein surface and functionalized group available on GO surface. Often, GO can be activated before binding to proteins, usually by directly introducing electrophilic functionalities on the surface. Various functional groups can be easily incorporated on the surface of graphene oxide by chemical modification such as chitosan, folic acids, and polyethylene glycol; some of which are non-covalent while the rest are covalent functionalization.

The covalent immobilization generally ensures the highest binding strength between the GO and the protein while minimizing leakage issues and increasing operational stability towards heat, pH, organic solvents, and storage. On the other hand, covalent immobilization may result in steric modifications of the protein, leading to a decrease of protein functionality or enzymatic activity. However, the use of appropriate cross-linking molecules between protein and graphene oxide can often prevent these changes. The cross-linking molecule binds to graphene oxide through non-covalent functionalization such as hydrophobic and π-π interactions, and covalently binds the protein through, for example, an amide bond. Shen J et al. [8] have reported the two-step method of GO functionalization with bovine serum albumin (BSA) through diimide-activated amidation.

However, the most of the covalent immobilization methods are nonspecific. In contrast, the click chemistry is an excellent platform for biomedical applications especially for the effective bio-conjugation of nanomaterials. The click chemistry strategy provides a facile and general method for functionalization of graphene oxide with biomolecules. The azide-alkyne click chemistry has been demonstrated to be quite efficient for the functionalization of GO. Kou et al. [9] employed azide-alkyne click chemistry to functionalize GO with linear polymers, various amino acids, aliphatic chains; they investigated the effect factors of reaction conditions, as well.

Applications of GO-proteins conjugates

GO-proteins conjugates, considering the low toxicity, cost-effectivity, optical properties, high surface area, and possibility of functionalization, are crucial for their application in imaging, sensing, and biomedical applications. Guo et al.[10] developed a complex of GO and herceptin by simply incubating the graphene oxide and herceptin under an alkaline condition. It was found that the prepared conjugate exhibited a constant fluorescent intensity. Additionally, the stability of GO-herceptin complex under high ionic conditions, near-infrared excitation characteristics, and non-photobleaching properties render it an excellent bio-probe for live cell imaging. Li et al.[11] created a novel class of hydrophilic, bio-inspired and biodegradable composite materials with well-organized layered structures of alternating graphene and amyloid layers. This new composite material is inexpensive, highly conductive and can be degraded by enzymes. Furthermore, it can reversibly change shape in response to variations in humidity, and can be used in the design of biosensors for quantifying the enzymatic activities.[11]

The complexes of GO with proteins exhibit an exceptional set of material properties which can be used to provide targeted (cellular or tissue) delivery of small drug molecules, improve bioavailability, sustain release of drugs or solubilize drugs for systemic delivery. This process can be adapted to protect therapeutic agents against enzymatic degradation (i.e., nucleases and proteases). Xing et al.[12] reported a co-assembly strategy for the fabrication of complex of GO and bovine serum albumin as a biologically-derived degradable component for accelerating the intracellular release. Prepared complex has been demonstrated as a smart photosensitizer delivery system which prevents to the inactivation of photosensitizer before it has reached the targeted tissues. The transferrin GO conjugates were used as therapeutic agents for photothermal therapy, causing cell damage under high power laser irradiation, as well as optical probes for the targeted malignant cells using their two photon photoluminescence.[13]

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The results indicate that GO is effective against Gram-positive and Gram-negative bacteria. The results suggest that antimicrobial actions are contributed to by membrane and oxidation stress; and a three-step antimicrobial mechanism, previously used for carbon nanotubes is applicable to graphene-based materials as well. Mahmoudi et al.[14] showed that protein covered graphene oxide delays the fibrillation of proteins via adsorption of amyloid monomers by decreasing the kinetic reaction and Li et al.[15] locally heated and dissociated amyloid aggregation using thioflavin-modified GO with NIR laser irradiation.

GO can be used as a template for further inorganic nanoparticle assembly that affords the as prepared nanocomposites with more feasible structures and unique properties over the composites of bare GO or inorganic nanoparticles. It was demonstrated that the incorporation of protein successfully turns GO into general platforms toward the efficient assembly of nanoparticles with varying sizes, shapes, compositions, and properties. GO functionalized by BSA was used as an extremely versatile and highly efficient self-assembly platform for a preparation of series of metal nanoparticle.[16] To achieve this presynthesized Au, Ag, Pt, Pd, and latex nanoparticles were allowed to interact with the GO-BSA complex. The adsorption of noble metal particles on graphene oxide sheets functionalized by BSA is enabled by a specific chemical bonding between bovine serum albumin and metal nanoparticles due to the presence of thiol, amine, and imidazole groups in BSA (i.e., cysteine, lysine and histidine residues) where the hydrophobic and electrostatic interactions play an important role. The incorporation of large inert particles such as latex into GO-BSA materials was demonstrated as well. The surface of pristine polystyrene latex is hydrophobic; thus hydrophobic patches on BSA may be responsible for the strong interactions between latex and BSA. The proteins are known to be strongly adsorptive to a wide range of particles; therefore, the graphene oxide can be a truly universal "adhesive" for the attaching of numerous nanomaterials.

The interaction of GO with proteins has also been applied in protein crystallization. Controlling the crystal nucleation is a crucial step in obtaining high quality protein crystals for structure determination by X-ray crystallography which is the most successful and prolific method for high-resolution structural determination of three-dimensional structures of proteins. Graphene oxide has been shown to lead improvement in crystalline output and nucleation. Stacks of graphene sheets present a large surface heterogeneity with a number of differently sized, inter-particle 'pockets' in which protein crystals may be able to nucleate. As nucleating agents, GO showed the universal applicability for many proteins under different conditions.[17,18]

With a two-dimensional sheet-like structure, graphene represents an interesting geometrical support for molecular catalysts with a large open surface area that is readily accessible to substrates/products with a small diffusion barrier which is distinct from conventional high surface area porous materials. Moreover, graphene oxide also possesses a rich surface chemistry and has the potential to further promote the catalytic activity and stability of the supported molecular systems such as hemin and other porphyrin species through cation-π interactions or π-π stacking.[19] Hunag et al.[20] prepared a GO-haemoglobin composite supramolecular hydrogel by directly mixing the dispersions of both components and explored its application as a catalyst for a peroxidatic reaction in organic solvents. The activity and stability of the enzyme-containing hydrogel were tested to be much higher than those of haemoglobin itself in organic solvents.

Conclusion

As novel 2D materials, the atomic flat surface, abundant surface functional groups, and ultra-large aromatic structure render graphene oxide as an ideal platform for elucidating the immobilization mechanisms of proteins. GO-protein conjugates represent a promising platform for the drug and gene delivery, cancer therapy, biosensing, cellular probing and tissue engineering. The understanding of the interaction between GO and proteins is crucial because the applications of GO in biosystems depends on the interactions between them. The surface of GO allows weak van der Waals, electrostatic, hydrophobic, hydrogen bonding, π-π stacking bonds and covalent interactions as well. However, the results indicate that the protein binding onto GO might be a result of the synergic effect of the different interactions.

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Conflicts of interest

None.

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