Water dispersible graphene noncovalently functionalized with tryptophan and its poly(vinyl alcohol) nanocomposite

Juan Guo, Lulu Ren, Ruiyu Wang, Chao Zhang, Yang Yang, Tianxi Liu *

Key Laboratory of Molecular Engineering of Polymers of Ministry of Education, Department of Macromolecular Science, Fudan University, Shanghai 200433, China

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Graphene sheets functionalized noncovalently with aromatic amino acid, tryptophan (Tryp), were prepared by reducing graphene oxide through hydrazine hydrate. Tryp-functionalized graphene is water dispersible and can be stabilized for several months. Atomic force microscopy (AFM), X-ray diffraction (XRD), UV–vis absorption and Raman spectroscopy were used to investigate the nanostructures and the properties of graphene. Application of the graphene dispersion to poly(vinyl alcohol) (PVA) with the help of tryptophan to prepare nanocomposite was also carried out. And the PVA/graphene nanocomposite was characterized by thermogravimetric analysis (TGA) and tensile testing. A 23% improvement in tensile strength and moderate increases in Young’s modulus and thermal stability for PVA were achieved by adding only 0.2 wt% graphene sheets.

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1. Introduction

Graphene, a single layer of graphite, is a two-dimension (2D) honeycomb nanostructure, which has attracted great interest in the field of composite materials in the past several years because of its preeminent thermal, electrical and mechanical properties [1–4]. It has been demonstrated that the incorporation of well-dispersed graphene sheets into polymers can remarkably improve the properties of the polymer host materials [5,6]. For example, Zhang et al. [5] reported that a 150% improvement of tensile strength and a nearly 10 times increase of Young’s modulus were achieved at a graphene loading of 1.8 vol.%. Recently, Yang et al. [6] demonstrated that the modulus and tensile stress of PVA/3.5 wt% graphene nanocomposites were 16% and 32% higher than those of neat PVA, and the glass transition temperature and the thermal stability were improved to some extent.

However, there are no functional groups on the surface of graphene, which makes graphene hydrophobic, and the high specific surface area facilitates graphene forming irreversible aggregation or even restacking to graphite through strong π–π stacking and van der Waals interactions, which greatly limits the applications of graphene in numerous areas. Thus like many other nanomaterials, the prevention of aggregation is of significant importance in the preparation and processing of graphene, which will ensure the unique and outstanding properties of an individual graphene sheet. In recent years, some methods have been used for obtaining graphene dispersion in aqueous media by functionalizing graphene surfaces through either covalent [7] or noncovalent approaches [8–10]. For covalent functionalization, the intrinsic electrical and thermal conductivity properties of graphene are usually severely demolished, while in this regard the noncovalent functionalization is fascinating because it is considered to have less impact or destruction on the structure and the electronic network of graphene, and subsequently the intrinsic properties of graphene can be preserved. In the noncovalent interaction, dispersing graphene in water, based on van der Waals forces or π–π interactions using small molecules or polymers containing aromatic structure, such as pyrenebutyric acid [9] and sulfonated polyaniline [11], has been proved feasible.

Recently, the biological applications of graphene have also been concerned. For example, Shan et al. reported a convenient method to prepare water dispersible graphene sheets functionalized by biocompatible poly-L-lysine as a linker through covalent amide groups [12]. However, as mentioned above, the covalent functionalization does destroy the intrinsic properties of graphene. Herein, we report a noncovalent strategy for stabilizing aqueous dispersion of graphene by adding tryptophan (Tryp) molecules which may make graphene have potential biological applications. And then, the noncovalently functionalized graphene was used as reinforcing nanofiller to fabricate poly(vinyl alcohol) (PVA) nanocomposite with the help of tryptophan.

2. Experimental

2.1. Materials

Natural graphite power (325 mesh) was obtained from Alfa Aesar and used without further purification. L-Tryptophan was...
purchased from Sinopharm Chemical Reagent Co. Ltd. PVA (Mw = 85,000–124,000, 98–99% hydrolyzed) was commercially received from Sigma–Aldrich. 98% H2SO4, 50% hydrate hydrazine, 30% H2O2, KMnO4, NaNO3, NaCl and HCl were supplied by China Medicine Co. and used as received. Deionized (DI) water was used as the solvent in all the experiments.

2.2. Preparation of graphite oxide

Graphite oxide (GO) was synthesized from natural graphite flake by a modified Hummers method [13]. One gram of graphite powder was gradually added to a 500 mL beaker containing 0.5 g of sodium nitrate, 23 mL of 98% H2SO4 and then stirred for 30 min in an ice-salt bath at 0 °C. 3 g of KMnO4 was slowly added into the solution with a temperature under 20 °C. After being stirred for 2 h, the solution temperature was raised gradually to 35 ± 3 °C in a water bath and maintained for more than 30 min. After that, 46 mL of DI water was added to the solution, and then increased to 98 °C and maintained there for 15 min. After additional 140 mL of DI water was used to dilute the solution, 30% H2O2 was added into the mixture until the color of the suspension changed to brilliant yellow. The products were filtered and washed by 5% HCl in order to eliminate SO2−4 followed by 300 mL of DI water to remove the acid. The resulting solid was dried in a vacuum oven at 50 °C for 24 h to obtain graphite oxide.

2.3. Preparation of the Tryp-functionalized graphene dispersion

Ten milligrams of GO powder was added to 100 mL of DI water and then sonicated for 30 min to obtain homogeneous yellow–brown dispersion (Fig. 1a)1. Then, 20 mg of tryptophan was added to 20 mL of GO dispersion (0.1 mg/mL), and then the mixture was reduced with 100 μL of 50% hydrazine hydrate at 90 °C for 24 h. After reduction, a homogeneous black dispersion (i.e. Tryp-functionalized graphene dispersion, labeled as Tryp-G dispersion) was obtained (Fig. 1c), which can be stabilized for more than 3 months without precipitation. In comparison, however, the reduction of GO dispersion without Tryp stabilizer produced black reduced graphene oxide (r-GO), precipitating to the bottom of the solution (Fig. 1b).

2.4. Preparation of PVA/graphene nanocomposite

In a 100 mL flask, 1 g of poly(vinyl alcohol) (PVA) powder was dissolved in 30 mL of DI water at 90 °C and 20 mL of the as-synthetic Tryp-G dispersion (0.1 mg/mL) was gradually added to the PVA solution. After being stirred for 1 h, the mixture was poured into a culture dish and evaporated slowly at 50 °C until the weight reached an equilibrium value to obtain the composite film containing 0.2 wt% graphene nanosheets. And a parallel sample of neat PVA with the same amount of Tryp was prepared. The thickness of the resulting films was about 50 μm.

2.5. Characterization

Atomic force microscopy (AFM) images were taken in a tapping mode with a Veeco Scanning Probe Microscope from Digital Instruments with a Nanoscope IV controller. Samples for AFM observations were prepared by depositing a dispersed GO or Tryp-G solution onto a freshly cleaved mica surface and making them dry in air at room temperature. The UV–vis absorption spectra were recorded on a Lambda 35 (Perkin–Elmer) spectrometer.

Raman spectra were measured on LabRam-1B French Dilor Com (λex = 632.8 nm). X-ray diffraction (XRD) measurements were carried out using a PANalytical (X’Pert PRO) X-ray diffractometer with Cu Kα radiation (λ = 0.1548 nm). Transmission electron microscopy (TEM) observation was performed with a Philips CM 300 FEG TEM under an acceleration voltage of 200 kV. Thin section (with a thickness below 100 nm) for TEM observation was cut from the as-prepared PVA/Tryp-G nanocomposite film under cryogenic conditions using a Leica ultramicrotome with a diamond knife. Thermogravimetric analysis (Pyris 1 TGA) was performed under nitrogen flow from 50 to 800 °C at a heating rate of 10 °C/min. Scanning electron microscope (SEM, Tescan) was used to observe the failure surfaces of PVA/Tryp-G nanocomposite after tensile tests. The failure surfaces were sputtered with gold before SEM observations. The mechanical properties of neat PVA and its graphene nanocomposite were measured by a universal tensile testing machine. The extension rate was 5 mm/min and the samples were cut into strips with a width of 10 mm using a razor blade. The thickness of each strip was measured utilizing a digital micrometer. In all cases, more than five samples were tested.

3. Results and discussion

3.1. Exfoliation of graphene

As we know, tryptophan is an essential amino acid for human being, which has an indole conjugate structure and can theoretically stabilize the aqueous dispersion of graphene through π–π interactions. Actually, a theoretical study on the interaction of aromatic amino acids with graphene has been investigated by Rajesh et al. using density functional theory and the Møller–Plesset second-order perturbation theory within linear combination of atomic orbitals–molecular orbital approach [14]. In this study, they believe that the aromatic rings of the amino acids prefer to orient in parallel with the benzene rings’ plane of graphene, which is attributed to the π–π interactions. And Rajesh et al. have already proved that the binding strength or interaction energy between the aromatic ring of tryptophan and graphene is the highest among all the amino acids by theoretical calculation. After reduction, the yellow–brown graphene oxide suspension (Fig. 1a) changed into stable black graphene dispersion (Fig. 1c) with the help of tryptophan, suggesting that deoxygenation of the graphene oxide effectively happened.

Fig. 2 shows the AFM images of GO and Tryp-G nanosheets. The AFM image revealed that most GO sheets (Fig. 2a) had an average thickness of about 0.80 nm, which is characteristic of a fully exfoliated graphene oxide sheet [8,15–17]. And, the mean thickness of...
single Tryp-G was determined to be about 4 nm (Fig. 2b). If we assume that both sides of graphene sheet are covered with aromatic rings of Tryp molecules oriented parallel to the graphene plane through \( \pi-\pi^* \) interactions, the average interplanar distance between Tryp and graphene sheet is about 0.35 nm [14]. Considering the thickness of chemically converted graphene sheet is generally about 1 nm [9], there may be multilayer adsorption of Tryp molecules onto the graphene surface, we can expect that these sheets are 2–3 graphene layers.

As can be seen from Fig. 3, UV–vis spectra of the samples also confirm the transition of graphene oxide into graphene. The curve of graphene oxide dispersion has a maximal absorption at 228 nm and a tiny shoulder at about 300 nm, which is ascribed to \( \pi-\pi^* \) transitions of aromatic \( \text{C} = \text{C} \) bonds and \( n-\pi^* \) transitions of \( \text{C} = \text{O} \) bonds [5]. Theoretically speaking, the absorption peak at 228 nm for the graphene dispersion will be red-shifted to \( \sim 270 \) nm. Since Tryp molecule has an absorption peak at \( \sim 279 \) nm, as a comparison, Tryp solution with the same concentration was also measured. The inset (enlarged figure) illustrates the absorption spectra of Tryp and Tryp-G dispersion from 240 nm to 400 nm. Compared with Tryp solution, Tryp-G dispersion has an obvious absorption peak at 271 nm, which suggests that the electronic conjugation within the carbon framework of the graphene nanosheets has been restored upon hydrazine reduction, being in agreement with the previous reports [10,16,18].

Raman spectra were recorded from 875 to 2000 \( \text{cm}^{-1} \) to evaluate the transformation from pristine graphite to graphite oxide and then to graphene. As shown in Fig. 4, pristine graphite displays a very weak band at 1328 \( \text{cm}^{-1} \) and a strong band at 1570 \( \text{cm}^{-1} \), corresponding to the well-documented D and G bands. The D and G bands are ascribed to structure defects and the first-order scattering of the \( E_{2g} \) vibrational mode in graphite sheets, respectively [19]. The G and D bands are also observed for graphite oxide (at 1337 and 1590 \( \text{cm}^{-1} \), respectively) and graphene (at 1328 and 1587 \( \text{cm}^{-1} \), respectively), as shown in Fig. 4. The D band becomes prominent and the ratio of D to G-band intensity \( (I_D/I_G) \) increases from 0.06 for pristine graphite to 1.25 and 1.44 for graphite oxide and graphene, respectively. According to the previous work in which \( I_D/I_G \) can be used to qualitatively characterize the change of defects in the carbon framework, \( I_D/I_G \) should decrease in the process of reduction. In our work, however, \( I_D/I_G \) of graphene is
higher than that of graphite oxide. Stankovich et al. [16] has interpreted such a phenomenon by assuming that the reduced state increases the number of aromatic domains of smaller size in graphene, leading to an increase of the $I_D/I_G$ ratio. Therefore, Raman results also clearly indicate that GO has been well deoxygenated after reduction by hydrazine hydrate.

The XRD pattern of graphite oxide has a peak centered at $2\theta = 11.1^\circ$ (Fig. 5), corresponding to the layer-to-layer distance of $\sim 0.80$ nm, which is significantly larger than that of pristine graphite ($2\theta = 26.6^\circ$, corresponding to a d-spacing of 0.336 nm), due to the intercalation of oxide functional groups. Whereas the XRD pattern of graphene is obviously different from those of pristine graphite and graphite oxide. The absence of the characteristic peaks indicated that graphene was exfoliated into monolayer or few-layers.

### 3.2. PVA/graphene nanocomposite

As mentioned above, Tryp was first added into graphene oxide solution, and then the mixture was reduced by hydrazine hydrate, and finally, the stabilized Tryp-G complex was incorporated into PVA matrix by solution blending to fabricate the PVA nanocomposite. Fig. 6 shows a TEM image of PVA/Tryp-G nanocomposite with 0.2 wt% graphene. The image demonstrates that the graphene sheets are fully exfoliated and clearly well-dispersed in the PVA matrix.

TGA was performed to investigate the effect of graphene sheets on the thermal stability of the polymer matrix. TGA and the corresponding differential thermogravimetric (DTG) curves for PVA/Tryp and PVA/Tryp-G nanocomposite with 0.2 wt% graphene are shown in Fig. 7. Both PVA/Tryp and its nanocomposite showed a two-step weight loss process. The TGA curve of the nanocomposite (Fig. 7A) was shifted toward a higher temperature, compared with that of PVA/Tryp. The peak temperature of the DTG curve represents the temperature at which the maximum weight loss rate was reached. The peak degradation temperatures of PVA/Tryp-G/0.2 wt% composite were observed at about 267°C and 457°C (Fig. 7B) for the two-step weight loss process, thus increased by 5°C and 17°C, respectively, compared with those of PVA/Tryp without graphene. All of these indicate that addition of graphene at such an extremely low concentration can improve the thermal stability of the PVA nanocomposite.

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**Fig. 4.** Raman spectra of (a) pristine graphite, (b) graphite oxide and (c) graphene.

**Fig. 5.** XRD patterns of (a) Tryp, (b) pristine graphite, (c) graphite oxide and (d) graphene.

**Fig. 6.** TEM image of PVA/Tryp-G nanocomposite with 0.2 wt% graphene.

**Fig. 7.** (A) TGA and (B) DTG curves of (A) PVA/Tryp and (B) PVA/Tryp-G nanocomposite with 0.2 wt% graphene.
It is expected that the mechanical properties of the nanocomposite should be improved by adding graphene sheets, due to the molecular-level dispersion of the graphene sheets in PVA matrix and the strong interfacial interaction (mainly hydrogen bonding) between graphene and PVA matrix [5,6,20]. Fig. 8 shows the typical stress–strain curves for the films of PVA/Tryp and PVA/Tryp-G/0.2 wt% nanocomposite. Compared with the PVA/Tryp film, the addition of only 0.2 wt% graphene results in an increase of the tensile stress by 23% from 38.4 (±3.9) to 47.2 (±1.5) MPa, and a moderate increase of the Young’s modulus from 1.48 (±0.11) to 1.56 (±0.07) GPa. The elongation at break of the nanocomposite is comparable with that of PVA/Tryp film. The increase to some extent of the tensile stress and modulus of the PVA nanocomposite film is probably ascribed to homogeneous dispersion of the graphene sheets in the matrix and strong interfacial interaction between them.

The fracture surfaces of PVA films were further investigated by SEM after tensile testing. As shown in Fig. 9a, the failure surface of PVA/Tryp film exhibits a smooth surface, whereas in contrast, the fracture surface of the nanocomposite film shows a laminar loose structure (Fig. 9b). Just as reported previously [6], they believed that the paper-like graphene could exhibit preferential orientation within the nanocomposite film and tend to align parallel to the film surface, thus resulting in the formation of a layered-like structure.

### 4. Conclusions

In conclusion, graphene sheets prepared by reduction of graphene oxide utilizing hydrazine hydrate can be noncovalently functionalized with tryptophan (Tryp), which has been theoretically reported to have the strongest interactions among all the aromatic amino acids with graphene planes through π–π stacking. The resulting Tryp-G complex can be stably dispersed in water for several months. PVA/graphene nanocomposite was prepared with the help of tryptophan. Enhancement of the mechanical properties of PVA/Tryp-G nanocomposite was obtained at extremely low concentration of graphene sheets. By adding only 0.2 wt% graphene sheets, a 23% improvement in tensile strength and a mild increase of Young’s modulus were achieved, indicating that the graphene sheets were homogeneously dispersed in the PVA matrix. Also, the addition of graphene sheets at low concentration improved the thermal stability of the nanocomposite. The simple method experimentally developed here, graphene sheets stabilized by aromatic amino acid (i.e., tryptophan), can be extended to synthesize various biocompatible polymer/graphene nanocomposite.

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