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Graphene oxide destabilizes myoglobin and alters its conformation

Zhaohua Zhu [b, 1], Yanqing Wang [a, b, *, 1], Yijun Kang [c], Hongmei Zhang [b, **], Zhengming Zhang [a], Zhenghao Fei [a], Jian Cao [b, ***]

[a] Jiangsu Key Laboratory for Bioresources of Saline Soils, Yancheng Teachers University, Yancheng City, Jiangsu Province, 224002, People's Republic of China
[b] Institute of Applied Chemistry and Environmental Engineering, Yancheng Teachers University, Yancheng City, Jiangsu Province, 224002, People's Republic of China
[c] College of Marine and Bio-engineering, Yancheng Teachers University, Yancheng City, Jiangsu Province, 224002, People's Republic of China

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ABSTRACT

In this work, a series of biophysical assays were performed in order to analyze the effects of a novel two-dimensional carbon nano-material graphene oxide (GO) on the conformational changes of myoglobin (Mb). This study, for the first time, reveals the molecular interactions of GO with Mb. The conformation of the protein is obviously affected due to the binding interaction of GO with Mb. GO has high ability in disturbing the secondary of Mb by forming the Mb-GO conjunction. Multi-noncovalent interactions including hydrophobic, hydrogen bonds, van der Waals interactions and electrostatic forces contribute to the formation of Mb-GO conjunction. Our findings also show that the existence of GO can obviously decrease the thermal stability of protein. In addition, molecular modeling was used to analyze the lowest energy binding mode of GO with Mb. Taken together, this work can provide an insight into the biological interaction GO-heme protein in some biological applications.

1. Introduction

Carbon-based nanomaterials including carbon nanodots, carbon nanotubes (CNTs), graphene, graphene oxide (GO), fullerenes and nanodiamonds have some biological properties and are potential candidates for various applications in medicine [1]. As one of two-dimensional nanomaterials, GO is the most intensively investigated carbon-based nanomaterials to date in the field of nanomedicine [1,2]. It consists of a single atomically thin sheet of hexagonally bound sp 2 carbon atoms and abundant oxygen-containing groups like epoxy, hydroxyl and carboxyl group at the edge [3,4]. The utilization of GO has been extended to biomedicine field, especially as drug and gene delivery system. Numerous studies on the toxicity of GO on cells and peptides, but the mechanism has not been studied [5,6]. Therefore, one critical issue that the biosafety and biocompatibility must be addressed before widespread application of GO. Studies have revealed that the nonspecific interaction of GO with protein may change the conformation and function of certain bioactive proteins [7]. In addition, since graphene based materials have excellent flexibility and electrical conductivity, they can also act as excellent cellular substrates for muscle tissue engineering [8] Shin et al. have discussed the recent applications of graphene-based materials in tissue engineering and regenerative medicine, especially, bone, skeletal muscle, and skin/adipose tissue engineering [9]. Some graphene-based materials become a good kind of candidates for bone and neural tissue engineering [10,11]. Graphene-based materials can also enhance cellular alignment and thus maximize cellular contractile power for skeletal muscle tissues [12]. There are still concerns regarding the potential toxicity and biocompatibility of GO with skeletal muscle tissues. As skeletal muscle tissues have limited regeneration capacities, once they are severely damaged, permanent loss of tissue function can occur [9]. Graphene-based materials can effect some properties including adhesion, proliferation, and myogenesis of skeletal muscle cells [13]. In addition, graphene-based materials can also interact with many biomolecules such as proteins, or peptides for regenerative medicine and tissue engineering [9]. Therefore, a better understanding on the interaction of graphene-based materials with biological molecules of skeletal muscle tissue is a necessary. For the

* Corresponding author. Jiangsu Key Laboratory for Bioresources of Saline Soils, Yancheng Teachers University, Yancheng City, Jiangsu Province, 224002, People's Republic of China.
** Corresponding author.
*** Corresponding author.
E-mail addresses: wyqing76@126.com (Y. Wang), hxzhm@126.com (H. Zhang), yctu_caojian@126.com (J. Cao).
[1] Zhaohua Zhu and Yanqing Wang contributed equally.

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purpose of investigation about the biocompatibility and toxicity of GO, the studies on the conformational and structural behaviors of proteins in the presence of GO are of critical importance for the integration of biology with GO.

In muscle cells, myoglobin buffers mitochondrial oxygen availability, facilitates oxygen diffusion [14]. It is a globular hemoprotein that reversibly binds oxygen in cardiac and skeletal muscle tissue [15], the high concentration of Mb in muscle cells allows mammals to hold their breath for a longer time. Mb was the first protein to have its three dimensional structure rendered with 16.7 kDa molecular weight [16,17]. Mb consists of 153 amino acid residues and an iron porphyrin, like hemoglobin, the iron atom covalently bound to four nitrogen atoms [18]. The iron atom forms other two bonds after bounding with oxygen, one with oxygen atom and the other with nearest histidine residue. It remains a good heme-protein model for studying relationship between protein structure and function in protein chemistry [19–23].

Herein, Mb was used as a protein model in skeletal muscle tissue to explore the conformational and properties changes of protein after binding with GO. In this study, we studied the interaction of GO with Mb via the binding affinity, the thermodynamics property, and the thermal stability of GO-Mb conjunction. Then, the results of our work are expected to provide a better understanding on the interaction of GO with biological molecules of skeletal muscle tissue.

2. Materials and methods

2.1. Reagents

Myoglobin (Horse) was purchased from Sigma (St., Louis, MO, USA) and used without further purification. Graphene oxide solution (1 wt%) was obtained from Aladdin Industrial Corporation (Shanghai, China). The stock solution of Mb (5.0×10^{-6} mol/L) was dissolved in a 0.05 mol/L pH 7.40 potassium phosphate buffer by gentle stirring at room temperature and were stored at 4 °C. Ultrapure water was used throughout.

2.2. Methods

2.2.1. Circular dichroism (CD) spectral measurements

A Chirascan spectrometer (Applied Photophysics Ltd., Leatherhead, Surrey, UK) equipped with temperature control quantum to control temperature was used to determine the conformation of Mb in the absence and presence of GO. The CD spectra were recorded from 200 to 280 nm using 0.1 and 1 cm quartz cells, respectively. A scan speed was set 50 nm/min, and a bandwidth was set 1 nm. The concentration of Mb was kept constant 5.0×10^{-6} mol/L. The circular dichroism neural networks software (CDNN) was used to analyze the changes in the contents of different secondary structures of Mb in the absence and presence of GO [24]. Furthermore, the thermal denaturation experiments of Mb in the absence and presence of GO were carried out with a wavelength range from 200 to 280 nm in 0.1 cm path length cells. The temperature during the thermal denaturation of Mb was varied from 20 to 90 °C in 5 °C step with 240 s increments. The melting temperature (T_m) and the enthalpy changes (ΔH_m) at the melting temperature were obtained by using Global Analysis Software equipped with the spectrometer.

2.2.2. Fluorescence spectral measurements

A LS–50B Spectrofluorimeter (Waltham, Massachusetts, USA) equipped with 1.0 cm optical path quartz cell and a thermostat bath was used for fluorescence spectral measurements. Mb concentration was kept constant at 5.0×10^{-6} mol/L and the GO concentrations have been varied from 0 to 20 mg/L. The fluorescence intensities of GO-Mb system with different concentration of GO were measured at λ_{ex} 280 nm with scan range from 300 to 500 nm. The synchronous spectra were recorded at $\Delta\lambda = 20$ nm or $\Delta\lambda = 60$ nm to analyze the effects of GO on tyrosine (Tyr) or tryptophan (Trp) amino residues in Mb. In addition, for three-dimensional fluorescence spectra, the emission wavelengths range and the excitation wavelengths were selected from 270 to 500 nm and from 200 to 340 nm, respectively. For all fluorescence measurements, the excitation and emission slit widths were set at 10.0 and 8.0 nm respectively with the scan speed of 300 nm/min. In addition, the inner filter effect of GO absorbance on the fluorescence intensity of Mb was corrected by using Eq. (1) due to GO solution absorbance at excitation wavelength and the maximum emission wavelength for Mb-GO system [25],

$$F_{cor} = F_{obs} \times e^{(A_{ex}+A_{em})/2} \quad (1)$$

where F_{cor} and F_{obs} are the fluorescence intensity corrected and observed, respectively; A_{ex} and A_{em} are the absorbance of system at excitation and emission wavelength, respectively.

2.2.3. UV–vis absorption spectral measurements

The UV–vis absorption measurements were carried out at room temperature using a SPECORD S600 spectrophotometer (Jena, Germany). In the absorption spectra experiment, the concentration of Mb was 5.0×10^{-6} mol/L. A fixed concentration of Mb (5.0×10^{-6} mol/L, 2.5 mL) with GO solution was added to the 1 cm optical path quartz cell and was mixed to obtain the different

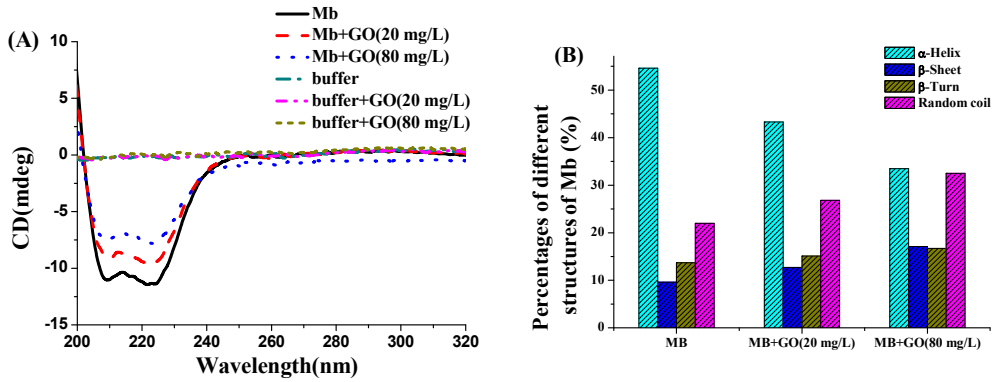

Fig. 1. (A) Effects of GO on the CD spectra of Mb. (B) The percentages of the different structures of Mb in the absence and presence of GO. c (Mb) = 5.0×10^{-6} mol/L, pH = 7.4, T = 298 K. (A colour version of this figure can be viewed online.)