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Investigation on the conformational structure of hemoglobin on graphene oxide

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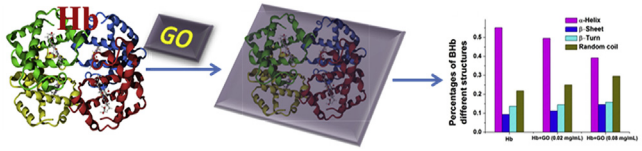
HIGHLIGHTS

- Multi-noncovalent interactions exist between BHb and GO.
- GO has high ability of disturbing the secondary of BHb.
- GO acts as a structure destabilizer during the thermal denaturation process of BHb.
- GO can obviously prevent the non-enzymatic glycosylation of BHb.

GRAPHICAL ABSTRACT



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ABSTRACT

The binding interaction between bovine hemoglobin (BHb) and a novel two-dimensional carbon nanomaterial, graphene oxide (GO), has been investigated in this work. It is found that GO strongly disturbs the secondary structure of BHb by forming BHb-GO aggregates. The binding affinity between the GO and BHb is shown to be mainly from non-covalent interactions including hydrophobic, hydrogen bonding, van der Waals and electrostatic interactions. In addition, two possible binding modes are proposed, insert binding mode and surface binding mode, as shown by molecular modeling. Our findings also show that the existence of GO can significantly prevent the non-enzymatic glycosylation of BHb and decrease the thermal stability of the protein. This work elucidates the effects of the binding interactions of GO with BHb on some biological properties and functions of heme protein.

1. Introduction

Nowadays, carbon nano-materials have been widely used in biological applications due to their unique and outstanding properties [1–3]. A novel two-dimensional (2D) carbon nanomaterial, graphene oxide (GO), has shown potential biotechnological applications in drug and gene delivery systems [4–8]. Therefore, the understanding GO's influence on structure and activities of biomolecules are essential for before any biological applications of GO, especially for disease- and drug-related proteins [9,10], cellular bioimaging [11,12], protein adsorption [13,14] etc. Many studies showed that GO strongly interacted with amino acids, peptides, and serum proteins [9]. The adsorption of serum proteins on GO occurred spontaneously and rapidly, leading to significant changes in size, zeta potential, and morphology [15]. However, so far very few studies on the biocompatibility of GO with heme proteins, or on the toxic effects of GO on heme proteins at the molecular level have

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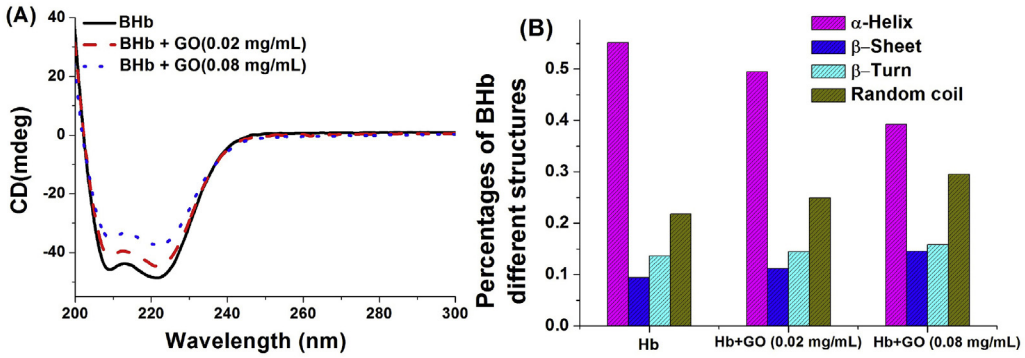

Fig. 1. (A) Circular dichroism (CD) spectra and (B) the percentages of the different structures of free BHb and its GO-BHb complex, c(BHb) = 5 × 10^{-6} mol/L, pH = 7.4, T = 298 K.

been conducted [16,17].

Hemoglobin (Hb) is one of heme proteins that makes up about 92% of the Red blood cells' dry content and plays various roles including in the transport of oxygen, in the dispersion of hydrogen peroxide, and in the affection of electron transfer in human body parts and organs in living beings [18,19]. Hb is oxygen carriers in red blood cells [18]. The amount of Hb in blood is associated with many clinical diseases such as leukemia, anemia, heart diseases, etc [20]. When GO penetrates plasma membrane, the interactions of them with Hb maybe affect human health. In addition, Hb can successfully combine with carboxyl grapheme through condensation reaction to form the bio-nanocomposites that exhibits good ability of oxygen-carrying [17]. For the purpose of investigation about the biocompatibility, bioavailability, and toxicity of GO, the fundamental understandings of the binding behaviors of Hb are of critical importance for the integration of biology with GO.

Herein, Hb is used as a good protein model for conformational change studies before and after the assembly on GO surface. The binding affinity, the thermodynamics property, the energetic of the interaction, and the thermal stability of GO-Hb association, the effect of GO on the non-enzymatic glycosylation of BHb was carried out by experimental and calculation studies. According to the study, we expect it not only provides the detailed conformation behavior of Hb molecules on GO but also creates a framework for analyzing the biosafety of GO in terms of the biological behavior of biomacromolecules. In addition, these findings also have shed light respective the design of bio-nanocomposites by comprehensive reconsideration of their interaction with Hb.

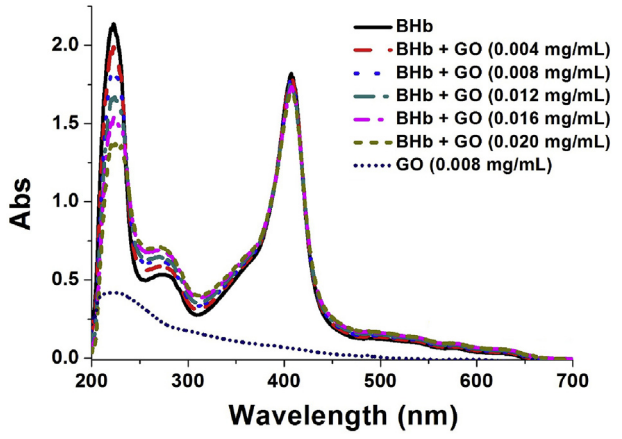

Fig. 2. UV–vis absorption spectra of BHb, GO, and BHb-GO system, c(BHb) = 5 × 10^{-6} mol/L, pH = 7.4, T = 298 K.

2. Material and methods

2.1. Reagents

Bovine hemoglobin (BHb) was purchased from Sigma (St, Louis, MO, USA) and used without further purification. GO solution (5%) was purchased from Aladdin Industrial Corporation (Shanghai, China). The stock solutions of BHb (5.0 × 10^{-6} mol/L) was dissolved in a 0.05 mol/L potassium phosphate buffer with pH 7.4 by gentle stirring at room temperature and stored at 4 °C. All other chemicals were of analytical reagent grade. Ultrapure water was used throughout.

2.2. Methods

2.2.1. UV–vis absorption measurements

The difference UV–vis absorption measurement of BHb (5.0 × 10^{-6} mol/L) in the absence and presence of GO were measured on A SPECORD S600 spectrophotometer equipped with 1 cm quartz cells. A fixed concentration of BHb with different concentration of GO was added to the 1 cm sample cell, Then an equal concentration of GO was added to the reference cell simultaneously [21,22]. The scan speed was set at 200 nm/min and the spectra range was from 200 to 600 nm.

2.2.2. Fluorescence spectra measurements

A LS–50B Spectrofluorimeter equipped with 1.0 cm quartz cells and a thermostat bath was used for fluorescence spectra measurements of BHb in the absence and presence of GO. The fluorescence spectra of BHb-GO reaction solutions were recorded in the range of 300–500 nm with the excitation wavelength 280 nm after 1 h equilibrate. Synchronous fluorescence spectra of BHb were recorded at 20 nm or 60 nm. In addition, for three-dimensional fluorescence spectra, the emission wavelengths range was selected from 270 to 500 nm, the initial excitation wavelength was set from 200 to 340 nm with increments of 10 nm.

2.2.3. Circular dichroism spectra measurements

The CD spectra of BHb (5.0 × 10^{-6} mol/L) in the absence and presence of GO were acquired on an Applied Photophysics Ltd. Chirascan spectrometer. For each CD spectrum, the program CD spectra deconvolution program (CDNN) was used to obtain the percentage of α-Helix, β-Sheet, β-turn, random coil of BHb in the absence and presence of GO in order to analyze the effect of GO on the secondary structure of protein [http://bioinformatik.biochemtech.uni-halle.de/cdnn] [23]. The temperature of thermal denaturations of BHb in the absence and presence of GO were varied from 20 to 90 °C in 5 °C steps, with 250 s increments.