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Protein structure preservation by MWCNTs/RTIL nano-composite

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ABSTRACT

The structure changes of hemoglobin (Hb) and glucose oxidase (GOx), as two typical proteins, upon interaction with MWCNTs and MWCNTs-[BMIM]BF_4^- was studied. The ability of MWCNTs and MWCNTs-([BMIM]BF_4^-) for the achievement of direct electrochemistry of GOx and Hb and enhancing the electron transfer rate between proteins and electrode surface was compared. Also the data obtained by various techniques including UV–vis, fluorescence and circular dichroism spectroscopy revealed that the structure of Hb or GOx conjugated with supramolecular network of the nano-composite could be preserved much better than those absorbed on MWCNTs. It seems that ionic liquid provides a favorable microenvironment to maintain the protein native structures.

1. Introduction

The compounds developed for protein structure preservation are important specially for retaining the stability of proteins. Carbon nanotubes (CNTs) and ionic liquids (ILs) seems to be very effective on structure stability of proteins from different points of view. Feng et al. [1] have reported that hydrophobicity of CNTs greatly changes the conformation of glucose oxidase (GOx). Also Vaitheeswaran et al. [2] showed that the folding/unfolding equilibrium of the adsorbed protein on nano-materials depends on the strength of its connection to the surface of nano-materials. Several groups have claimed that the activity and stability of enzymes were in agreement with the hydrophobicity of ILs [3–7]. It has been suggested that the hydrophobic ILs have a tendency to make strong interaction (such as hydrogen bonding) with enzymes [8–11]. On the other hand, ILs can form the organized nano-structures, so-called hydrogen-bonded polymeric supramolecules containing polar and non-polar regions in either solid or liquid states [12,13]. For instance, in case of 1-Butyl-3-methyl imidazolium hexa fluoroborate ([BMIM]PF_6^-) each imidazolium cation bound to three anions and each PF_6^- anion is enclosed by three cations [14]. Dupont [13] proposed that free enzymes in aqueous solution might be embedded in IL network, which could keep the essential water of proteins, and also the solvophobic interactions are critical for preserving the native structure of enzymes. Therefore, it could be possible to assume that enzymes stability in ILs is primarily determined by direct enzyme-ion interactions [15], while protective water on the enzymes surface might still play a significant secondary role.

In the present work, a nano-composite was developed by combining 1-Butyl-3-methyl imidazolium tetra fluoroborate ([BMIM]BF_4^-) as a typical IL and MWCNTs as unique carriers for proteins. The nano-composite was examined for the achievement of direct electrochemistry of two redox proteins including hemoglobin (Hb) and GOx and also enhancing the electron transfer rate between the redox proteins and electrode surface. In addition, Hb and GOx were entrapped in supramolecular network of the nano-composite; then, the ability of nano-composite in preserving the protein native conformation and/or bioactivity was studied.

2. Experimental

2.1. Reagents and apparatus

Glucose Oxidase (GOx, EC 1.1.3.4) Type X-S from Aspergillus Niger and human hemoglobin (Hb) were purchased from Sigma. Multi-wall carbon nanotubes (MWCNTs), prepared by chemical vapor deposition, were supplied by Timesnano Co. (China). ([BMIM]BF_4), as a typical room temperature ionic-liquid (RTIL) was purchased from Kimia Exir (Tehran, Iran). Potassium dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were obtained from Merck (Germany).

Electrochemical experiments were carried out using a Potentiostat/Galvanostat (model 263-A, EG&G, USA) controlled by a

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PowerSuite software package and a GPIB interface. All experiments were carried out using a three-electrode cell consisting of a glassy carbon (GC) electrode (2 mm in diameter) as working electrode (from Azar Electrode, Uromia, Iran), a silver/silver chloride (Ag/AgCl, saturated KCl) reference electrode (from Metrohm) and a platinum rode as a counter electrode. Circular dichroism (CD) spectroscopy was done with Aviv, model 215 spectropolarimeter (Lakewood, NJ, USA). Fluorescence spectroscopy was carried out using Hitachi spectrofluorimeter (MPF-4 model, Japan). UV–vis spectroscopy was carried out using Cary spectrophotometer (100 Bio-model, USA).

Unless otherwise stated, phosphate buffer solution (PBS, 0.1 M, pH 7.4) was used as a supporting electrolyte.

2.2. Nano-composite preparation

For preparing MWCNTs/RTIL nano-composite, 2 mg of MWCNTs were dispersed in 1 mL of [BMIM]BF_4^- by grinding them in an agate mortar for about 1 h. The positively charged imidazolium ion would be wrapped on the surface of MWCNTs during the course of grinding. Afterward, the resultant nano-composite of MWCNTs/[BMIM]BF_4^- was centrifuged at 18,000 rpm for 30 min. Then, the transparent liquid phase (upper phase) was separated from the black gel phase of MWCNTs/[BMIM]BF_4^-. The nano-composite was finally collected by removing the supernatant with a pipette. The prepared nano-composite was dispersed in 1 mL of double-distilled water and preserved for usage in next experiments.

2.3. Electrode preparation

The GC electrode was first polished with 10 and then with 0.3 μm alumina slurry and rinsed with double-distilled water thoroughly. Next, 2.5 μL of MWCNTs/[BMIM]BF_4^- nano-composite was dropped on the cleaned GC electrode and dried at room temperature. Then, the modified GC electrode was immersed in 5 mg mL^{-1} of either Hb or GOx solution in PBS (0.1 M, pH 7.4) and kept at 4 °C for 10 h. Finally, immobilized Hb or GOx was denoted as either Hb/MWCNTs/[BMIM]BF_4^-/GC or GOx/MWCNTs/[BMIM]BF_4^-/GC electrode. The modified electrodes were stored at 4 °C when not in use.

2.4. Protein sample preparation

In all spectroscopic studies (UV–vis, CD and fluorescence), the native protein (Hb or GOx) solutions were prepared in the concentration of 0.2 mg mL^{-1} in PBS (0.1 M, pH 7.4) and the bioconjugate systems were prepared according to the following procedure: The protein (Hb or GOx, 5 mg mL^{-1}) was mixed with either MWCNTs or MWCNTs/[BMIM]BF_4^-, with a V/V ratio of 5:1, respectively. Then, the mixture was incubated at 4 °C for at least 10 h. Thereafter, the mixture was centrifuged at 4000 rpm for 30 minutes. In order to remove the suspended nano-material from supernatant and obtain a clear bioconjugate, based on the experiences reported in the literature [16], the centrifugation process was repeated three times. Finally, the concentration of protein in the supernatant was adjusted to 0.2 mg mL^{-1} by diluting in PBS (0.1 M, pH 7.4). The protein concentration was controlled by UV–vis absorption peak at 280 nm and applying the extinction coefficients of 13.1936×10^4 and 16.7×10^4 cm^{-1} M^{-1} for Hb and GOx, respectively.

3. Results and discussion

3.1. Electrochemical behavior of protein on MWCNTs/[BMIM]BF_4/GC electrode

The electrochemical behavior of Hb and GOx immobilized on MWCNTs/[BMIM]BF_4^-/GC electrode was investigated, using cyclic voltammetry in N_2-saturated PBS (0.1 M, pH 7.4,) at potential scan rate of 50 mV s^{-1}. Fig. 1 shows the cyclic voltammograms (CVs) of different modified electrodes.

As seen, when either Hb or GOx was immobilized on bare GC electrode, no electrochemical response was observed (Fig. 1A and B, curves a). This may be due to the fact that the redox center of GOx (flavine adenine dinucleotide, FAD) and the heme groups of Hb is deeply buried in protective protein shells (hydrophobic pocket) [17,18]. After immobilization of Hb or GOx on MWCNTs/GC electrode, in both cases, a pair of well-defined oxidation-reduction peaks was observed (Fig. 1A and B, curves b). The formal potentials ($E^{o'}$, the average of cathodic and anodic peak potentials) were obtained as −0.33 and −0.42 V, respectively. The value of $E^{o'}$ for Hb was close to the value obtained previously for the heme Fe^{III}/Fe^{II} redox couple [19]. Also, the value of $E^{o'}$ for GOx was close to that obtained previously for FAD/$FADH_2$ redox center [20,21]. It has been reported that [16,22–24] nano-structures, such as carbon nanotubes, nano-particles and CdTe quantum dots can denature the protein, probably through destroying their quaternary, tertiary, and/or secondary structures. These changes in the structure can be occurred due to the transition of the heme or FAD group from the hydrophobic pocket to the solvent. The exposure of these redox groups lead to enhance in electron transfer rate [16,24].

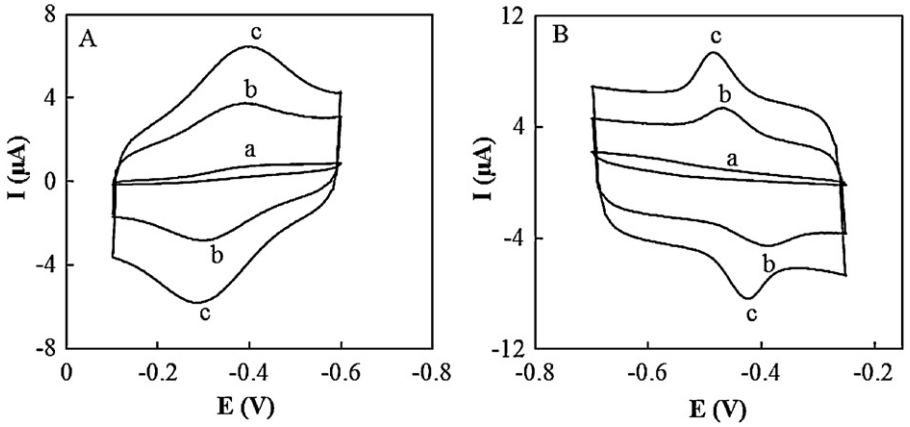

Fig. 1. (A) CVs of (a) Hb/GC, (b) Hb/MWCNTs/GC and (c) Hb/MWCNTs/[BMIM]BF_4/GC electrodes. (B) CVs of (a) GOx/GC, (b) GOx/MWCNTs/GC and (c) GOx/MWCNTs/[BMIM]BF_4/GC electrodes. The electrochemical experiments were carried out in PBS (0.1 M, pH 7.4, under N_2 saturated) at scan rate of 50 mV s^{-1}.