Journal of Colloid and Interface Science 433 (2014) 49–57

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Structural characterization and electrocatalytic application of hemoglobin immobilized in layered double hydroxides modified with hydroxyl functionalized ionic liquid

Tianrong Zhan [a,*], Qi Yang [a], Yumei Zhang [a], Xinjun Wang [a], Jie Xu [a], Wanguo Hou [b,*]

[a] State Key Laboratory Base of Eco-chemical Engineering, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China
[b] Key Laboratory of Colloid & Interface Chemistry (Ministry of Education), Shandong University, Jinan 250100, China

ARTICLE INFO

Article history:
Received 26 May 2014
Accepted 8 July 2014
Available online 23 July 2014

Keywords:
Layered double hydroxides
Hydroxyl functionalized ionic liquid
Hemoglobin
Immobilization
Electrocatalytic application

ABSTRACT

Hemoglobin (Hb) was immobilized in Zn_2Al-Layered Double Hydroxides (LDH) modified with Hydroxyl Functionalized Ionic Liquid (HFIL) through adsorption and coprecipitation method, respectively. Adsorption experiments showed that the presence of HFIL could enhance the maximum protein loading. However, the Hb loading through coprecipitation technique was far higher than that for adsorption. The role of HFIL on the interaction between Hb and LDH was investigated by XRD, FTIR, UV–vis and fluorescence spectroscopies. Although the quaternary structure of Hb entrapped in HFIL–LDH through coprecipitation technique (denoted as HFIL–LDH–Hb_{cop}) might be altered slightly more than that in LDH (LDH–Hb_{cop}), its secondary structure and redox-active heme groups kept intact. Morphologies of LDH–Hb_{cop} and HFIL–LDH–Hb_{cop} biohybrids were analyzed through SEM and TEM images. The direct electrochemistry of the immobilized Hb indicated that the coprecipitation bioelectrode performed better than that of the corresponding adsorption one. Regardless of adsorption and coprecipitation, the introduction of HFIL could distinctly promote the electron transport. Among all bioelectrodes, HFIL–LDH–Hb_{cop}/GCE displayed the best electrocatalytic activity for H_2O_2 determination with a larger electroactive Hb percentage (6.76%), higher sensitivity (40.63 A/M cm^2) and lower detection limit (0.0054 μmol/L). So HFIL–LDH could effectively immobilize enzymes via coprecipitation technique, which had potential applications in the fabrication of electrochemical biosensors.

1. Introduction

Bioinorganic interface chemistry of protein and solid inorganic particle has attracted enormous interests in both basic and applied scientific fields because of their extensive potential applications ranging from biocatalysts to biomedical diagnosis as well as biosensor [1–5]. The bioinorganic interfacial assembly involves van der Waals forces, hydrophobic affinity, electrostatic interaction and hydrogen bonds. Although binding of proteins on solid supports can improve their properties [6,7], the absorbed proteins may be deactivated or denatured under some interfacial conditions. In fact, it is of importance to keep the bioactivity of the immobilized protein as intact as possible for all researches. Therefore, in order to retain the native bio-function, a biocompatible and favorable microenvironment is indispensable for the immobilized protein.

Layered Double Hydroxides (LDH) are a class of ionic lamellar solid made up of positively charged brucite-like layers with an interlayer containing charge balancing anions. A generic formula for LDH may be written as $[M_{1-x}^{2+}M_x^{3+}(OH)_2]_x^+[(A^{n-})_{x/n}\cdot mH_2O]$. Owing to the open 2D structure, unique ion-exchange properties as well as good biocompatibility with positive charges, LDH can often be used to immobilize negatively charged biomolecules with wide isoelectric point (*pI*) range through various soft chemical methods including adsorption, delamination/restacking, coprecipitation and electrodeposition [8–10]. Recently, the immobilization of heme proteins in LDH matrices has attracted great attention. Hemoglobin (Hb) was adsorbed on ZnAl–SDS–LDH and on delaminated LDH-lactate sheets [11,12], while myoglobin (Mb) and horseradish peroxidase (HRP) were incorporated in NiAl-Br and NiAl-NO_3-LDH, respectively [2,13]. The bi-protein Hb/HRP was assembled with LDH sheets via layer-by-layer deposition method [14]. The heme proteins (Hb, Mb and HRP) were entrapped into Fe-based LDH [15]. As far as above literatures, immobilization of protein was achieved through the classic adsorption or coprecipitation methods. These results also showed that the interactions between protein and LDH depended on the particle sizes, the nature of interlayer species and LDH composition. For instance, the

* Corresponding authors. Fax: +86 532 84023927.
E-mail addresses: trzhan@sdu.edu.cn (T. Zhan), wghou@sdu.edu.cn (W. Hou).

http://dx.doi.org/10.1016/j.jcis.2014.07.012
0021-9797/

maximum absorption amount of Hb on exfoliated LDH sheets [12] is much higher than that for MgAl–LDH [16]. Iron-enriched LDH can promote the direct electron transfer of Hb and enhance the electrocatalytic activity of the bioelectrode towards H_2O_2 with a low detection limit [15]. They also found that Hb captured in SDS–LDH can realize the direct electron transfer, whereas no reduction peak for LDH treated with poly(ethylene oxide) (PEO) or cetyltrimethyl-ammonium (CTA) [11]. Obviously, it is still a challenge to acquire LDH with an appropriate interface to retain the bioactivity of the immobilized protein.

Functionalized ionic liquids (FIL) contain different functional groups in cation and/or anion part. They can offer some novel merits in several processes except for the general properties such as high chemical and thermal stability, high ionic conductivity, negligible vapor pressure and unique solubility for classic ionic liquid (IL) [17]. Recent studies revealed that hydroxyl FIL (HFIL) could fill the polarity gap between water and general organic solvents and replace traditional alcohols in certain application [18]. Some HFILs were employed to stabilize enzymatic catalysts in organo-active systems by providing a more water-like microenvironment [19]. They were also applied to prepare some solid nano-catalyst with better performance [20]. Additionally, HFIL could enhance the catalytic efficiency of iron (III) porphyrin toward the reaction of phenol with H_2O_2 [21], and was also used as binder to prepare carbon ionic liquid electrode [22]. Therefore, we think that the modification of LDH with HFIL will increase active sites of the support surface and produce a more biocompatible interfacial microenvironment for protein binding. Furthermore, HFIL–LDH nanohybrid can not only prevent the secondary structure of protein from damaging, both of them could also cooperatively accelerate the electron transfer of protein.

Thus in this work, HFIL, 1-(3-hydroxypropyl)-3-methylimidazolium tetrafluoroborate ($HPMMBF_4$, Scheme S1) was chosen as an interfacial binder for the assembly of Hb with LDH. The immobilization of Hb in HFIL–LDH host matrices was accomplished by the adsorption and coprecipitation methods, respectively. The role of HFIL on the specific interaction between Hb and LDH was investigated. The morphologies of the biohybrids were characterized. And the structure and electrocatalytic activity of the immobilized Hb were also comparatively analyzed.

2. Materials and methods

2.1. Materials

The bovine hemoglobin (Hb, from Tianjin Chuanye Biochemical Co. Ltd., China) was used without further purification. HFIL, $HPMMBF_4$, was obtained from Lanzhou Greenchem ILS, LICP, CAS. Other chemicals were analytical reagent grade and used as received.

2.2. Physical characterization

Powder X-ray diffraction (PXRD) patterns were collected on a Rigaku D/MAX-2500/PC diffractometer using a Cu Kα irradiation source ($\lambda = 0.15405$ nm). FTIR spectra were recorded on a Nicolet iS10 spectrometer. A TU-1901 UV–vis spectrophotometer (PUXI) was used for Hb quantification by absorbance at 406 nm. Florescence spectra of aqueous suspension samples were recorded on a F-2700 fluorescence spectrophotometer (Hitachi) at an excitation wavelength of 270 nm. The surface morphologies of samples were determined by scanning electron micrographs (SEM, JSM-6700F). Their transmission electron microscope (TEM) images were recorded on a JEM-2000EX microscope by placing the sample suspension on a carbon grid and drying at room temperature. Hydrodynamic nanohybrid particle sizes were measured by photo-correlation spectroscopy (Zetasizer Nano-ZS90 Malvren). Cyclic voltammetry (CV) experiments was performed by a CHI 660D electrochemical work station with three-electrode electrochemical cell, including a Ag/AgCl reference electrode, a platinum auxiliary electrode, and a glassy carbon electrode (GCE) modified by LDH films as working electrode.

2.3. Synthesis of materials based on LDH

Zn_2Al-CO_3-LDH (denoted as LDH) was prepared by a modified coprecipitation technique as described elsewhere [23]. Briefly, a mixed solution (0.5 mol/L) were prepared by dissolving $Zn(NO_3)_2{\cdot}6H_2O$ and $Al(NO_3)_3{\cdot}9H_2O$ (Zn^{2+}:Al^{3+} = 2 molar ratio) in deionized water. The second solution containing NaOH (2.0 mol/L) and Na_2CO_3 [Na_2CO_3:$Zn(NO_3)_2$ = 1:1 molar ratio] in deionized water was prepared. The solutions were simultaneously added into a reactor, maintaining pH at 9.0. The suspension was kept vigorous stirring at room temperature for 24 h. The final product was washed several times with deionized water after centrifugation. The samples were dried in air at room temperature for physical characterization.

HFIL–LDH hybrid was prepared by the similar method as that of LDH except that the mixed solution was $Zn(NO_3)_2$ and $Al(NO_3)_3$ and HFIL with a molar ratio of 2:1:0.5.

LDH–Hb_{cop} (Hb immobilized in LDH through coprecipitation technique) samples with different Hb/LDH (w/w) ratio (Q = 0.05, 0.25, 0.5, 1 and 2) were prepared by the same coprecipitation method in enzyme solution [24,25]. Typically, a mixed aqueous solution (0.5 mol/L) dissolving $Zn(NO_3)_2{\cdot}6H_2O$ and $Al(NO_3)_3{\cdot}9H_2O$ (Zn^{2+}:Al^{3+} = 2 molar ratio) was carefully introduced into a reactor containing 10, 50, 100, 200 and 400 mg Hb in 20 mL H_2O. Simultaneously, a prepared 2.0 mol/L NaOH and Na_2CO_3 [Na_2CO_3:$Zn(NO_3)_2$ = 1:1 molar ratio] mixed solution were added to maintain pH at 9.0. The suspension was stirred at room temperature for 12 h. The products were centrifuged and washed several times with deionized water, and finally dried in air at room temperature for physical characterization or kept as a fresh aqueous gel (20 wt%) for preparing modified electrodes.

HFIL–LDH–Hb_{cop} (Hb immobilized in HFIL–LDH through coprecipitation technique) phases with Hb/HFIL–LDH (w/w) ratio (Q = 0.05, 0.25, 0.5, 1 and 2) were prepared by the same method as for LDH–Hb_{cop} except that the molar ratio of $Zn(NO_3)_2$ and $Al(NO_3)_3$ and HFIL was 2:1:0.5.

2.4. Adsorption experiments

2.5 mg of LDH or HFIL–LDH dry powder was added to 10 mL phosphate buffer solution (PBS 7.0, 0.05 mol/L) of Hb with different concentrations ($2.5 \times 10^{-3} \sim 3.0 \times 10^{-2}$ mg/mL). After stirring at 160 rmp for 3 h, the mixture was centrifuged for 30 min at 10,000 rmp. The protein contents in the supernatant were quantified directly by the UV–vis absorbance. The amount of Hb bound to the supports was calculated by the mass balance equation $C_s = [(C_i - C_e)V]/m$ where C_i and C_e are the concentrations of the initial Hb solution and supernatant, respectively (mg/mL); V is the volume of the suspensions (mL); and m is the weight of the adsorbent (mg). The adsorption isotherms were obtained by plotting the amount of adsorbed Hb (C_s w/w) vs. the Hb equilibrium concentration in the solution (C_e mg/mL). The resulting composites were designated as LDH/Hb_{ads} and HFIL–LDH/Hb_{ads}.

2.5. Electrochemical experiments

The GCE (3 mm) was firstly polished with alumina slurry (0.05 μm) and then sonicated in water and ethanol and finally rinsed with water. For the preparation of LDH/Hb_{ads}/GCE, HFIL–LDH/Hb_{ads}/GCE, 7 μL LDH/Hb or HFIL–LDH/Hb (both Q = 5)