Bioelectrochemistry 91 (2013) 8–14

Contents lists available at SciVerse ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Electrochemistry of heme proteins entrapped in DNA films in two imidazolium-based room temperature ionic liquids

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A R T I C L E I N F O

Article history:
Received 20 April 2012
Received in revised form 20 September 2012
Accepted 25 November 2012
Available online 8 December 2012

Keywords:
[bmim][PF_6]
[bmim][BF_4]
Heme protein
Electrochemical properties

A B S T R A C T

Two imidazolium-based ionic liquids (ILs), hydrophilic 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]) and hydrophobic 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF_6]), have been chosen as solvents for investigating bioelectroanalysis of four heme proteins. Heme proteins including hemoglobin, myoglobin, catalase and horseradish peroxidase immobilized in DNA can form stable and electrochemically active films on glassy carbon electrode. The morphology studies of films were demonstrated by atomic force microscopy. The direct electrochemistry of heme proteins were performed in ILs, and a pair of well-defined and nearly reversible redox peaks were observed. The electron transfer rate and reversibility of heme proteins in [bmim][BF_4]/water were better than those in [bmim][PF_6]. Through comparing several electrochemical parameters such as formal potentials and electron transfer rate constant of proteins in ILs, this paper tried to explain the differences of electrochemical properties of proteins as a function of viscosity, solubility characteristics, etc. of ILs. The possibility to specifically vary the properties of ILs by the selection of suitable cations and anions make them ideal candidates for wide applications in cell biological processes.

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1. Introduction

Deoxyribonucleic acid (DNA) is a well-known natural macromolecular consisting of three parts: a phosphate acid group, a basic group, and a sugar unit. A well-defined linear double helix and repetitive structure is found in double strand DNA. Experiments by Barton et al. [1] confirmed that efficient charge migration within the DNA duplex might be possible over long distance. It seems that DNA has an inherent potential for transport of electrons between protein and electrode. Immobilized DNA on electrode can act as a DNA sensor, and example applications are the sensitive determination of riboflavin at ds-DNA-modified pencil graphite electrode [2], and cytochrome C on DNA-modified glassy carbon electrode (DNA-GCE) [3].

Room temperature ionic liquids (ILs), which are composed entirely of ions and are liquid at ambient or even far below ambient temperature, have attracted much attention as novel environmentally benign solvents due to their negligible vapor pressure, wide potential window, good solubility and electrolytic conductivity. Moreover, the physicochemical properties of ILs, such as the hydrophobicity and viscosity, can be tuned by simply selecting different combinations of cations and anions as well as attaching substituents to customize ILs for many specific demands [4]. It is important that one is allowed to design an IL for specific reaction conditions. Such as to increase the substrate solubility, modify the enzyme selectivity, or tailor the reaction rate by manipulating the solvent properties [5]. As designer solvents, ILs have been used for extraction, separation, enzymatic reactions and so on.

ILs have potential applications in electrochemical study and much work has been carried out [6–8]. As we know, one primary application of ILs in electrochemical aspect was to design ILs-modified electrodes [9–11]. In recently years, their application as reaction media for enzyme reactions has gained increasing attention. Good activity and improved stability of enzymes have been obtained in the ILs [12–14]. An excellent review published by Naushad has highlighted the solvent properties of ILs, and their effects on enzyme performances such as activity, stability and selectivity [5]. Since both the thermodynamics and kinetics of reactions carried out in ILs are different from those in conventional molecular solvents, there are many good reasons to study ILs as alternative solvents in bio-electrochemistry as well. Most of the research works have been concentrated on investigating the electrochemical properties of proteins in one or more ILs [15–20]. Our group had carried out a great deal of work in this area [16,17]. DiCarlo et al. [18] have demonstrated that the characteristic Fe(II)/Fe(III) redox signal of Cytochrome c immobilized on alkylthiol self-assembled monolayers was lost when exposed to ionic liquids composed of a butylimidazolium cation combined with either hexafluorophosphate or bis(trifluoromethylsulfonyl) imide anion. Lu et al. have investigated [19] the effect of the structure of imidazolium cations in $[BF_4]^-$-type ionic liquids on direct electrochemistry and electrocatalysis of horseradish peroxidase in Nafion films. In this paper, two kinds of imidazolium-based ILs, hydrophobic 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF_6]) and hydrophilic 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim]

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http://dx.doi.org/10.1016/j.bioelechem.2012.11.002

[BF_4]), have been chosen for investigating the influence of the solvent properties of ILs on the electrochemical properties of heme proteins.

In this work, ILs with the same cations but different anions showed diverse physical and chemical properties. They were used as electrolytes for electrochemical reactions of the heme protein-DNA films modified electrodes. Then the effects of their solvent properties on the electrochemical response of heme proteins were investigated. This paper tried to explain the differences of electrochemical properties of heme proteins in the two ILs as a function of viscosity, solvent strength, and conductivity. The study could help us to choose and design ILs with specific properties for wide applications in biological process.

2. Experimental

2.1. Materials

Horse heart myoglobin (Mb) and cattle hemoglobin (Hb) were purchased from Fluka. Horseradish peroxidase (HRP) was from Dongfeng Biotechnology Co. Ltd. (Shanghai, China). Catalase (Cat) and DNA were obtained from Sigma. All heme proteins and DNA were used as received. All other chemicals were of reagent grade. Deionized double-distilled water was used in all experiments.

[bmim][PF_6] and [bmim][BF_4] were prepared by our laboratory as described in the literature [14,21]. The purity of the ILs was checked by elemental analysis, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The residual water content was analyzed by standard Karl-Fischer titration to be below 0.13% (w/w).

2.2. Preparation of heme protein–DNA films

The concentrations of heme protein stock solutions, prepared by dissolving proteins in 0.05 M phosphate buffer solution (PBS, pH 7.0), were 10, 7, 18, 30 mg mL^{-1} for Hb, Mb, HRP, Cat, respectively. DNA stock solution was prepared using PBS (0.05 M, pH 7.0), with a final concentration of 0.5 mg mL^{-1}. Protein solution was mixed with the DNA stock solution with a ratio of 1:1 (v/v). Then 30 μL of the mixture was pipetted onto the surface of the pretreated GCE and spread gently over the entire surface. Finally, the electrode was left in a fuming cupboard to dry for 8 h at room temperature. The construction of the DNA film modified electrode was in the same way but the heme protein stocks were replaced with equivalent volume of PBS. The electrochemical area of the GCE was determined by cyclic voltammetry (CV) using the Randles-Sevcik equation for the redox couple $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$.

2.3. Apparatus and procedures

CV and square wave voltammetry (SWV) were carried out with a CHI 660 electrochemical workstation. CV parameters were as follows: scan rate, 0.1 V/s; initial potential, −0.9 V; final potential, −0.9 V; switching potential, 0.3 V; number of segments, 2; and current sensing range, 1×10^{-6}A. CV data were recorded after the peak currents kept steady state. The experimental conditions for SWV were as followers: scan rate, 0.1 V/s; initial potential, 0 or 0.1 V; final potential, −0.6 V; number of segments, 1; pulse height 75 mV, step height 4 mV. SWV frequencies were listed in figure legends. All electrochemical experiments employed a conventional three-electrode system with a film modified GCE as working electrode, a platinum wire as auxiliary electrode and an Ag/AgCl (3 M NaCl) as reference electrode. ILs were purged with high purity nitrogen for at least 20 min prior to every experiment and the nitrogen atmosphere was maintained during the experiments. All the experiments were performed at room temperature. All potentials given below are referenced to Ag/AgCl.

FTIR spectra were recorded using a Perking–Elmer Spectrum One FTIR spectrometer (U.S.A). All the spectra were obtained with an average of 100 scans and 4 cm^{-1} resolution. UV–Vis spectra recorded with a Perking–Elmer Lambda 17 UV–Visible spectrophotometer (U.S.A) with wavelength range of 190–900 nm. Atomic force microscopy (AFM) images were obtained on a PicoScan system (Molecular Imaging Inc.) operated in contact mode with commercially ultrasharpened Si_3N_4 tips (MAClever II, Molecular Imaging Inc.). In each measurement, the set point was adjusted to minimize the force between the tip and the sample immobilized on mica disks. The AFM measurement parameters were as follows: force constant, 0.12 N/m; number of scans, 8 times. Before the deposition of the films, the mica disks were freshly cleaved. The surface of the mica disks is reasonably flat as viewed under the AFM (data not shown).

3. Results and discussion

3.1. Morphology studies by atomic force microscopy

Fig. 1 shows a set of representative AFM topographs of protein and DNA films immobilized on mica disks. The surface roughnesses of the films were assessed by vertical depth analysis of the data. The image of Mb film (Fig. 1A) had numerous protrusions assignable to the depositing of large aggregates of Mb molecules. The large roughness of the membrane indicated that the deposited enzymes formed a loose and uneven surface. Many small but evenly dispersed particles could be observed in DNA membrane (Fig. 1B). It is known that DNA has a random coil conformation in solution. Thus, DNA could be displayed as small particles on the AFM images. After the addition of Mb, the surface morphology changed dramatically, showing much rougher surface with a lot of big particles (Fig. 1C). The large particles in the image should be induced by the cross-linked Mb molecules. The crater and valley topographic features also existed, but the roughness of this membrane was smaller than Mb alone indicating that the deposited enzymes formed a more uniform layer.

3.2. Electrochemistry behaviors of heme proteins entrapped in DNA in [bmim][PF_6] and [bmim][BF_4]

CVs of heme proteins entrapped in DNA films in [bmim][PF_6] (Fig. 2A) and [bmim][BF_4] (Fig. 2B) were obtained. No peaks were observed on DNA film (curve a) or Mb film (curve b) in [bmim][PF_6] or [bmim][BF_4]. Mb-DNA film in the deoxygenated ILs gave pairs of well-define, quasi-reversible oxidation–reduction peaks (curve c). Obviously, heme proteins entrapped in DNA films retain their electrochemical activity in ILs. Moreover, FTIR spectra [22] and UV–Vis absorption spectra [23] (Fig. S1), which were useful conformational probes for heme proteins, exhibited no shift in the absorbed band from that of Hb film alone, verifying that enzymes entrapped in DNA films could retain the essential features of their original structures that were essential for proteins to keep their bioactivity. But the change in intensity of the Soret band suggested that the interaction of Hb and DNA was obvious.

The larger peak currents and the small peak potential differences suggested that DNA expedited the direct electron transfer between heme proteins and electrode in ILs. As we know, DNA polymer in its double-helical form can be viewed as an extended array of stacked, electronically coupled, aromatic heterocycles within a polyanionic sugar–phosphate backbone [1]. Moreover, DNA film at the surface of GCE is believed to exhibit an ordered liquid crystal construction with the high concentration of DNA in the film [24]. The base pairs stack acts as a conduit for chemistry at a distance and the extended π systems facilitate charge transport [25]. It was deduced that DNA in the thin film might behave as a bridge for electron transfer between the electroactive center of heme proteins and GCE.

It was worth noting that no redox peaks could be obtained if the scans were immediately carried out after the heme protein–DNA films were immersed in both [bmim][PF_6] and [bmim][BF_4]. Referring to [bmim][PF_6] (water content at saturation: 1.8%) [26], it was found

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