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Facile Interfacial Electron Transfer of Hemoglobin Mediated by Conjugated Polymers

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Abstract: We herein describe a method of depositing hemoglobin (Hb) and sulfonated polyaniline (SPAN) on GC electrodes that facilitate interfacial protein electron transfer. Well-defined, reproducible, chemically reversible peaks of Hb and SPAN can be obtained in our experiments. We also observed enhanced peroxidase activity of Hb in SPAN films. These results clearly showed that SPAN worked as molecular wires and effectively exchanged electrons between Hb and electrodes.

Keywords: Hemoglobin, sulfonated polyaniline, biosensors, protein electrochemistry, conducting polymers.

1. Introduction

Interfacial protein electron transfer has received wide and significant research interest. In particular scientists are interested in developing novel bioelectronic interfaces that are suitable for facile protein electrochemistry [1-4]. Such study should deepen the understanding for biological protein electron transfer and promote the development of bioelectronic sensors for a variety of applications [5-7].

Hemoglobin (Hb, MW=64 500), a molecular vehicle for oxygen and carbon dioxide in red blood cells, has been regarded as the paradigm of allosteric proteins [8]. The studies on its structure-function relationship have intrigued researchers over the years [9-11]. While Hb does not function physiologically as an electron transfer protein, it does undergo oxidation and reduction at the heme

center in certain cases in vivo [12-14]. Traditionally it was only possible to employ spectroelectrochemical techniques in the presence of mediators to explore the redox reactions of Hb [15]. This might arise due to the fact that the heme ring of hemoglobin is much more buried than that in small redox proteins (e.g. cytochrome c), thus hindering its interaction with the electrode surface [16].

Rusling and coworkers first successfully developed a novel approach, which entrap proteins in lipid films, to achieve direct electrochemistry of Hb and other heme proteins. This has motivated great interest in design new protein films that are suitable for direct protein electrochemistry. For example, Xia and coworkers reported the direct chemistry of Hb on a three-dimentionally ordered macropourous gold film [17]. More importantly, efforts have been taken to develop novel biosensors and bioelectronic devices based on this research. Recently, conducting polymers (CPs) have attracted considerable attention due to their unique properties and potential applications in various fields [18, 19]. CPs serve as an optimal matrix for biomolecular interfaces that might enhance speed, sensitivity and versatility of biosensor systems [20-27]. Yu et al. demonstrated that the use of a kinds of CPs, sulfonated polyaniline (SPAN) effectively wires myoglobin and horseradish peroxidase (HRP) to electrodes. Lisdat and co-workers also obtained the direct electrochemistry of cytochrome c in the SPAN containing polyelectrolyte assembly [28]. The enhanced electron transfer might arise due to the "molecular wire"-like feature of conjugated polymers. Other possibilities include the biocompatibility of SPAN which prevents protein denaturation or tunes orientation of proteins [28-30]. In this work, we demonstrated that SPAN facilitated the electron transfer of a larger protein, Hb. We studied the effect of SPAN on Hb electrochemistry and explored the possibility of developing Hb-based sensors.

2. Experimental

Chemicals

Hemoglobin (pig) was from Sigma. SPAN was from Aldrich. Water was purified with a Millipore MilliQ system to specific resistance of about 18 MΩ•cm.All other chemicals were reagent grade. Several buffers were used in this work: 0.05 M acetate buffer (pH 5.5) for preparation of Hb； The buffer for electrochemistry is 0.05 M acetate,0.05 M NaCl,pH4.5.

Film Deposition

Glasy Carbon working electrodes (GC) (2 mm in diameter, CH Instruments Inc.)were first mechanically cleaned by polishing on microcloth pads (Buehler) with 1.0μm alumina,followed by 0.3 and 0.05μm alumina slurry each for 5 min. These electrodes were then sonicated in ethanol and Milli-Q water for 5 min, respectively. After dried with nitrogen, the GC electrodes were modified by SPAN and Hb at specific ratios and dried air. The electrodes were finally rinsed with Milli-Q water.

Electrochemical Measurements

A CHI 660 electrochemical workstation was used for cyclic voltammetry. A three-electrode cell containing a Ag/AgCl reference electrode, a platinum wire counter electrode and a GC working electrode was employed.

3. Results

3.1. Cyclic voltammetry of SPAN on GC electrodes

As demonstrated in Figure1, SPAN deposited on GC electrodes exhibited two pair of peaks in CV. One peak pair was centered at 0.36 V vs. Ag/AgCl, and the other was centered at 0 V vs. Ag/AgCl. Notably, this two peak pairs were much smaller than those obtained at layer-by-layer assembled polyelectrolyte films. For example, we observed significantly larger peaks by first depositing one layer of poly(diallyldimethylammonium) (PDDA) on GC electrodes and then depositing a layer of SPAN [30]. This suggested that cationic polyelectrolytes had higher affinity to GC electrodes than anionic polyelectrolytes. Nevertheless, we note that the two peak pairs were roughly located at the same positions both in the presence and in the absence of PDDA.

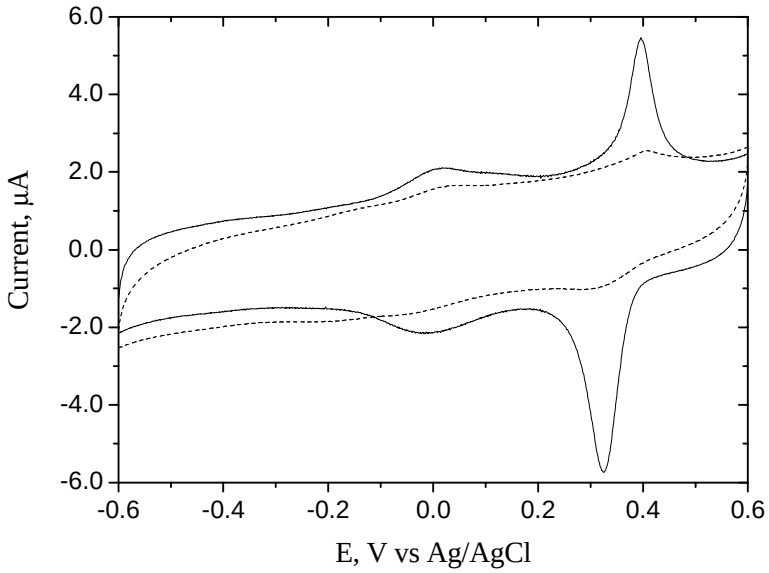


Figure 1. Cyclic voltammetry of GC electrodes coated with PDDA/SPAN (solid line) or SPAN (dashed line), in pH 4.5 buffer.

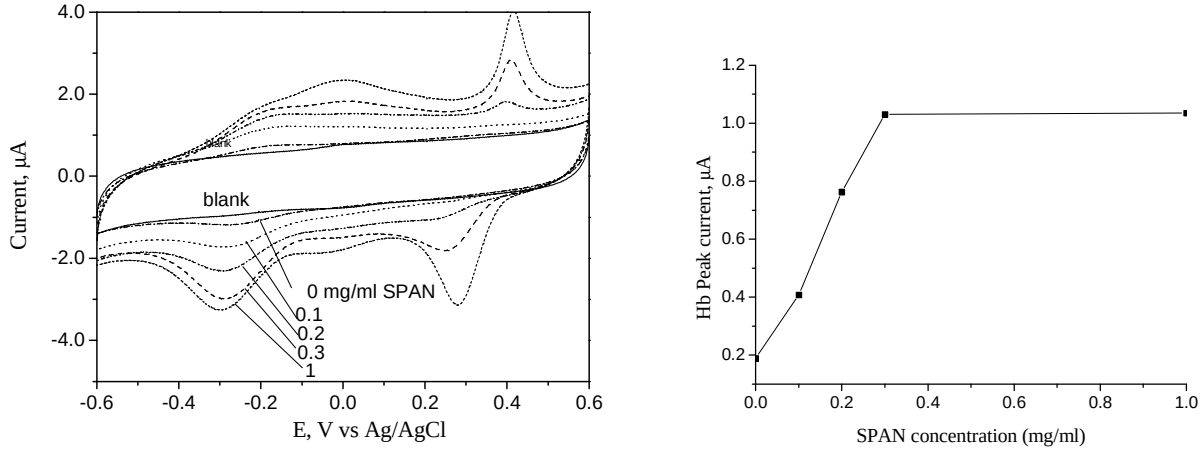
3.2. CV of Hb entrapped in SPAN films

As demonstrated in Figure 2, one additional peak pair appeared when Hb was entrapped in the SPAN film. This should arise due to the reduction and oxidation of Hb since the peak potential was close to previously reported redox potential of Hb [6]. The peak currents are linearly proportional to scan rates (100 mV/s~1 V/s), indicating that this is a surface-confined process. Interestingly, we observed that the peaks of Hb increased along with the ratio of SPAN/Hb. We observed that the peak

currents of Hb increased rapidly at low concentrations of SPAN, while they reached a plateau when the SPAN concentration reached 0.3 mg/ml. Note that we only observed a pair of very small Hb peaks in the absence of SPAN. This suggested that SPAN should facilitate the redox reaction of Hb. As is well known, hemes are deeply buried in Hb molecules [31], which makes them difficult to exchange electrons with electrodes. Since SPAN is conductive, they could work as "molecular-wires" to relay electron transfer (Figure 3). These molecular wires wrap Hb molecules and facilitate the heme-electrode electron transfer. This also explained the observation of the plateau, which arises due to the saturation of SPAN.

3.3. Catalytic Reduction of Hydrogen Peroxide

Hb is potentially a peroxidase. H_2O_2 converts the iron heme cofactors of Hb to oxyferryl radicals that can be reduced backed to the Fe(III) form. When Hb is coupled to an electrode, a complex catalytic cycle for the reduction of H_2O_2 is set up that can be detected via a catalytic reduction current [32-34]. However in the absence of SPAN, the peroxidase activity of Hb was very low. We observed that this effect was greatly enhanced in the presence of SPAN. Addition of micromolar amounts of H_2O_2 to buffer solutions bathing SPAN/Hb film gave increases in CV reduction current at the Fe(III) peak, accompanied by a disappearance of the Fe(II) oxidation peak. Figure 4 showed influence of concentration of H_2O_2 on the catalytic CV peak of SPAN/Hb film at pH 4.5. We observed that catalytic peak currents increased along with H_2O_2 concentration. This clearly showed the nice catalytic effect of Hb entrapped in SPAN films.



（a） （b）

Figure 2. (a) Influence of concentration of SPAN on CV response of Hb (the concentration of Hb was 1 mg/mL). The inner blank curve stands for CV in the absence of Hb. (b) The peak current of Hb increased with increasing SPAN concentration.

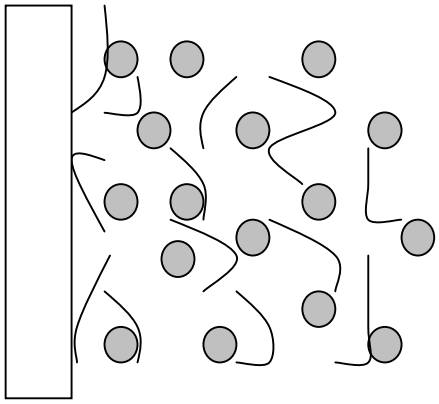


Figure 3. Schematic depiction of the multiplayer electrode. Circles=Hb, lines=SPAN, rectangle=GC electrode.

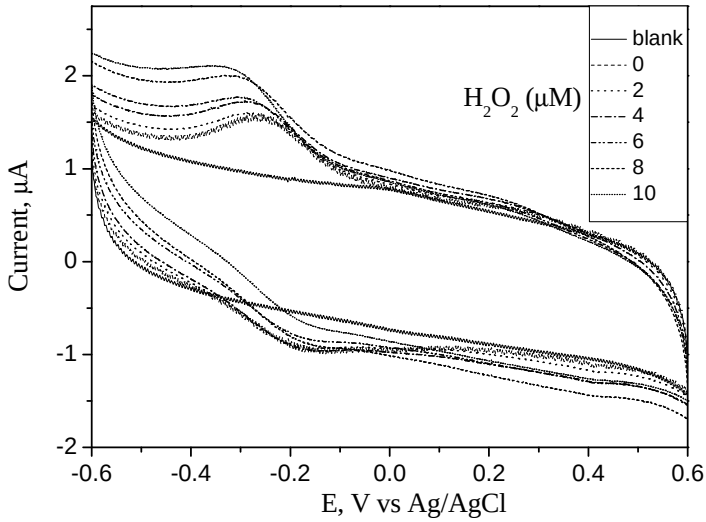


Figure 4. Influence of concentration of H_2O_2 on the catalytic CV peak at 0.3V/S for SPAN/HB=1:1 at pH 4.5

4. Discussion

Electrochemistry of hemoglobin in organic films has been extensively studied [7, 35]. However, most previous systems involve the use of organic solvents as co-immobilizing reagents [7, 35-37]. We note that organic solvents by itself can facilitate the electron transfer of proteins [36], thus it is difficult to separate the effect of organic films from the solvent effect. In addition, organic solvents are well known to induce structure alteration of proteins, thus complicating the explored system. In contrast, since SPAN is essentially water-soluble, it offers a protein-encapsulating medium free of organic solvents. Compared with other organic solvent-free systems [38], SPAN is a conjugated polymer, therefore it acts as a molecular wire to effectively communicate electrons with substrate electrodes [30]. Moreover, SPAN is electroactive by itself, therefore we can conveniently monitor the layer-by-layer assembly process of SPAN and hemoglobin from the peaks corresponding to the redox reaction

of SPAN. Given these advantages, we propose that the present system might provide an ideal system for studying the electrochemistry of heme proteins.

5. Conclusion

In this work we report the direct electrochemistry of hemoglobin in the presence of a molecular wire-like polymer, SPAN. This water-soluble conjugated polymer not only facilitates the electron transfer reaction of hemoglobin, but also can be facilely assemble on electrodes via a layer-by-layer approach. Also important, hemoglobin exihibits nice catalytic activity toward reduction of hydrogen peroxide in the SPAN film. We thus expect that this described system might offer a new approach for investigation of protein electrochemistry as well as design of novel bioelectronic devices.

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