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Enhanced electron transfer for hemoglobin entrapped in a cationic gemini surfactant films on electrode and the fabrication of nitric oxide biosensor

Fang Wang [a,b], Xiaoxia Chen [a], Yanxia Xu [a], Shengshui Hu [a,b,*], Zhinong Gao [a]

[a] *Department of Chemistry, Wuhan University, Wuhan 430072, PR China*
[b] *State Key Laboratory of Transducer Technology, Chinese Academy of Sciences, Beijing 100080, PR China*

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Abstract

The direct electrical communication between hemoglobin (Hb) and GCE surface was achieved based on the immobilization of Hb in a cationic gemini surfactant film and characterized by electrochemical techniques. The cyclic voltammograms showed that direct electron transfer between Hb and electrode surface was obviously promoted and then a novel unmediated nitric oxide (NO) biosensor was constructed in view of this protein-based electrode. This modified electrode showed an enzyme-like activity towards the reduction of NO and its amperometric response to NO was well-behaved with a rapid response time and displaying Michaelis–Menten kinetics with a calculated K_m^{app} value of 84.37 μmol L^{-1}. The detection limit was estimated to be 2.00×10^{-8} mol L^{-1}. This biosensor was behaving as expected that it had a good stability and reproducibility, a higher sensitivity and selectivity and should has a potential application in monitoring NO released from biologic samples.
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1. Introduction

Research on NO, its physiological and pathological roles or clinical potential is currently one of the most exciting sources of knowledge in biology (Cals-Grierson and Ormerod, 2004; Valverde et al., 2000; Maeda et al., 2004; Prast and Philippu, 2001). The discovery of biological functions of NO in the 1980s came as a complete surprise and caused quite a stir, in particular after its identification as the endothelium-derived relaxing factor (EDRF) which is responsible for promoting blood vessel relaxation and regulating vascular tone (Ignarro et al., 1987; Palmer et al., 1987). In 1992, NO was named “Molecule of the Year” by the journal Science (Koshland, 1992) and now it has been demonstrated to play a role in a variety of biological processes as an important signalling molecule (Edelman and Gally, 1992; O’Dell et al., 1991). The detection and quantification of NO is very difficult because it is a free radical with half-life of a few seconds (Snyder and Bredt, 1992). As a result, a variety of approaches have been developed for NO measurements including chemiluminescence (Kikuchi et al., 1993), UV–vis spectroscopy (Miranda et al., 2001), fluorescence (Kojima et al., 2001), electron paramagnetic/spin resonance spectroscopy (EPR/ESR) (Korth et al., 1994), electrochemical method (Malinski and Taha, 1992; Maskus et al., 1996) and so on (Kiechle and Malinski, 1996). However, electrochemical techniques are especially well used for NO analysis because of their high sensitivity and stability as well as easy construction with growing interesting in the direct and in vivo NO detection nowadays (Isik et al., 2004; Lee et al., 2004; De Groot et al., 2005). The most widely familiar detection method is amperometry. Early amperometric sensor for NO was based on direct oxidation of NO at bare basic electrodes including platinum (Shibuki and Okada, 1991) and gold electrodes (Bedioui et al., 1994). In 1992, a NO sensor based on polymeric nickel porphyrin/Nafion covered carbon fiber electrode was reported (Malinski and Taha, 1992). Since then the NO sensors based on chemically modified electrode have been widely researched by using all

* Corresponding author at: Department of Chemistry, Wuhan University, Wuhan 430072, PR China. Tel.: +86 27 8721 8904; fax: +86 27 6786 4573.
E-mail address: sshu@whu.edu.cn (S. Hu).

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kinds of modifier such as metal porphyrin (Malinski and Taha, 1992), metal phthalocyanine (Bedioui et al., 1997), metal protein (Casero et al., 2000), non-conducting polymer (Park et al., 1998), carbon nanotubes (Wang et al., 2005; Zhang et al., 2005).

Gemini surfactants consist of two or more pairs of hydrophilic and hydrophobic groups connected to each other with a spacer. This kind of surfactant has a number of unique aggregation properties in comparison to conventional surfactants, such as much lower critical micelle concentration (CMC) and strong hydrophobic microdomain (Li et al., 2006). In fact, Rusling has reported that the electron transfer rate of protein in surfactant film with bad water-solubility could be remarkably enhanced (Nassar et al., 1995; Rusling and Nassar, 1993). Considering the interaction of NO with proteins (Ye et al., 2006; Kim and Lim, 2005; Lisdat et al., 2000) heme protein-based NO biosensors have been presented by other groups (Haruyama et al., 1998; Fan et al., 2000; Pang et al., 2003). However, a NO biosensor based on hemoglobin embedded in a gemini surfactant with good water-solubility has not been reported. Here, a cationic gemini surfactant (Mathias et al., 2001) was used to construct a novel NO biosensor via immobilizing Hb on GCE surface. The direct electron transfer between Hb and electrode surface was achieved and this NO biosensor showed an enzyme-like activity towards the reduction of NO with a K_m^{app} value of 84.37 μmol L^{-1}. Because this biosensor had a good stability and reproducibility, a higher sensitivity and selectivity, it could be applied in monitoring NO released from real sample rat liver.

2. Experimental

2.1. Reagents and apparatus

Pig hemoglobin (MW 68,000) was purchased from the Sigma Chemical Company and used as received by dissolving in water to form 16 mg mL^{-1} solutions. Gemini surfactant $(C_{12}H_{25})[N^+(CH_3)_2]CH_2CHOHCH_2[N^+(CH_3)_2](C_{12}H_{25})\cdot 2Cl^-$ designed as C_{12}-C_3(OH)-C_{12} was offered by Wuhan University and dissolved in water to form 2 mg mL^{-1} (about 3.6 mmol L^{-1}) stock solution. NO standard solution was freshly prepared by dropping H_2SO_4 (2 mol L^{-1}) into deoxygenated $NaNO_2$-saturated solution slowly. The concentration of NO in saturated stock solution is 1.8 mmol L^{-1} at 20 °C (Butler and Williams, 1993). Male rat (about 4 weeks) was used for preparation of rat liver samples. The final biological sample was clear solution in red color. All solutions were prepared with doubly distilled water.

All electrochemical measurements were performed on a CHI 830 electrochemical analyzer (Shanghai Chenhua Co., China) in a three-electrode arrangement, equipped with a platinum wire counter electrode, a saturated calomel electrode (SCE) reference electrode and GC working electrode. For the deoxygenated experiments, the electrolyte was bubbled with highly-purity (99.999%) nitrogen for 15 min and maintained nitrogen condition during the experiments. UV–vis absorption spectra were recorded on a UV–vis spectrophotometer Tu-1901 (Purkinje General Instrument Co. Ltd., Beijing, China). The samples were, respectively, prepared by dropping hemoglobin and hemoglobin–surfactant solution on a glass slide, then air dried overnight.

2.2. Preparation of modified electrode

Firstly, 16 mg mL^{-1} hemoglobin solution and 2 mg mL^{-1} surfactant solution were mixed together according to the ratio of 1:1 (v/v). Prior to preparation of modified electrode, a GCE of 3.0 mm in diameter were mechanically polished to a mirror finish with wet microcloth containing 0.05 μm alumina powder, and then carefully cleaned in 1:1 HNO_3–H_2O (v/v), ethanol (C_2H_5OH) and water by ultra-sonication bath, each for 2 min. At last 3 μL of mixed protein–surfactant solution was cast on the GCE surface and air dried for several hours. In the same way, surfactant or protein modified GCE was also obtained.

2.3. Analysis procedures

A solution of 5 mL of 0.1 mol L^{-1} phosphate buffer solution (PBS) was used as supporting electrolyte and the direct electron transfer of hemoglobin at the electrode surface was mainly investigated by cyclic scans between −0.8 and +0.2 V. As far as NO measurement was concerned, a conventional amperometric method was introduced at 0.92 V under a stirred condition. The NO-saturated solution was added into the PBS after steady electrochemical responses were obtained. As to NO measurement in real biological sample, 500 μL sample solution was firstly added into 2.0 mL of 0.1 mol L^{-1} deoxygenated PBS (pH 7.4) bubbled with nitrogen for 10 min, then 0.8 mmol L^{-1} L-Arg was added in electrolyte under stirring condition.

3. Results and discussion

3.1. Characterization of the modified electrode

Curve a in Fig. 1 illustrates typical cyclic voltammograms (CVs) of Hb-C_{12}-C_3(OH)-C_{12} electrode in 0.1 mol L^{-1} phosphate buffer solution (PBS, pH 7.4). A couple of well-defined and reversible redox peaks could be observed at about −0.34 and −0.30 V attributing to the Fe(III)/Fe(II) couple of hemoglobin (King et al., 1992). The potential difference (ΔE_p) between cathodic peak potential (E_{pc}) and anodic one (E_{pa}) was 40 mV. The formal potential ($E^{0'}$) estimated from the mean of E_{pc} and E_{pa} was −0.32 V. The ratio of cathodic peak current over the anodic one was close to 1. Based on these data, the direct electrochemistry of Hb in this gemini surfactant film was considered to proceed by a quasi-reversible process. Correspondingly, no redox peaks were observed at C_{12}-C_3(OH)-C_{12} modified GCE and bare GCE under the same conditions (curves c and d). Only one poor reduction peak at Hb modified GCE could be found (curve b), indicating that hemoglobin can exchange electron directly and quickly with GCE when it was well-immobilized in this surfactant film. There are two weak interaction effects between Hb molecules and C_{12}-C_3(OH)-C_{12} film. One of

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