



Flow-amperometric biosensor for respiratory toxins using myoglobin-adsorbed carbon-felt, based on an inhibitory effect on bioelectrocatalytic reduction of oxygen

Yasushi Hasebe^{a,*}, Yue Wang^b

^a Department of Life Science and Green Chemistry, Saitama Institute of Technology, 1690, Fusaiji, Fukaya, Saitama 369-0293, Japan

^b School of Chemical Engineering, University of Science and Technology LiaoNing, 185 Qianshan Middle Road, High-tech Zone, Anshan, LiaoNing 114501, China

ARTICLE INFO

Article history:

Received 2 December 2011

Received in revised form 4 March 2012

Accepted 7 March 2012

Available online 17 March 2012

Keywords:

Flow-biosensor
Respiratory toxin
Myoglobin
O₂ reduction
Carbon-felt

ABSTRACT

Novel, simple and highly sensitive reagentless amperometric flow-biosensor for respiratory toxins (i.e. azide and cyanide) has been developed by using myoglobin (Mb)-adsorbed carbon-felt (CF), based on the inhibitory event of azide and cyanide on the Mb-catalyzed O₂ reduction. The Mb-adsorbed CF (Mb-CF) showed a sufficient bioelectrocatalytic activity for O₂ reduction in the potential region from 0 to −0.4 V vs. Ag/AgCl at pH 5.0, due to the direct electron transfer (DET) between Mb-heme and the CF electrode. The heterogeneous electron transfer rate constant (k_s) of the electrochemical redox reaction of Mb-heme-Fe(II)/(III) was estimated to be 15.5 s^{−1}. This Mb-CF-catalyzed O₂ reduction was reversibly inhibited by azide and cyanide, which bind to sixth coordination position of heme-iron center of Mb. When air-saturated 0.1 M phosphate/citrate buffer (pH 5.0) was used as a carrier under the applied potential of −0.2 V vs. Ag/AgCl, the steady-state current due to the Mb-catalyzed O₂ reduction was reversibly inhibited by the injection (200 μl) of azide and cyanide, resulting in peak-shape current responses. The magnitude of the inhibition peak currents linearly increased with increasing concentrations of azide (up to 3 μM) and cyanide (up to 5 μM), and the detection limit of azide and cyanide were found to be 0.12 and 0.23 μM, respectively (S/N = 2). The apparent inhibition constant, K_i' of azide and cyanide were estimated to be 5.71 and 8.95 μM, respectively.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Myoglobin (Mb) is a 16.7-kD heme-protein with a single polypeptide chain with an iron heme as its prosthetic group [1]. Mb transports and stores oxygen in muscle cells in mammals. Although Mb does not work as an electron carrier in the body, Mb would be an ideal model protein for the study of electron transfer of heme proteins and enzymes, because the structure of Mb is well understood and Mb exhibits enzyme-like redox-catalytic activities [2,3].

Electrochemical behaviors of Mb-modified electrodes have been extensively studied [4–20]. Since direct electron transfer (DET) between heme-center of Mb and bare solid electrode is generally difficult, various efforts have been devoted to enhance the electron transfer rate of Mb. For example: employment of electron transfer mediators or promoters [4]; incorporation of Mb into appropriate films (such as hydrogel film [5–7] and sol-gel film [8,9]) that modified on the electrode surface; layer-by-layer film formation of cationic Mb and anionic polymers [10]; the modification of Mb on

self-assembled monolayer-modified electrode [11,20]; employment of various nano-materials (such as carbon nanotube [12,13], gold nanoparticle [14], titania-nanotube [15]); the use of special materials (such as ionic liquid [16,17] and mesoporous silica [18]) as electrode modifiers; and employment of special electrode materials such as boron-doped diamond [19].

Namely, microenvironment of immobilized Mb molecule is one of the important factors to achieve fast DET between Mb and electrode. In these cases, Mb-modified electrodes show a pair of well-defined and nearly reversible cyclic voltammetric peaks for Mb-Fe(III)/Fe(II) redox couple, based on a DET with single-proton transportation. Moreover, the Mb-modified electrodes showed electrocatalytic activities for the reduction of oxygen [5,6,8,10,13,16,19], hydrogen peroxide [7,15,17,18], trichloroacetic acid [5,6,8,10,17], nitric oxide [7,12] and nitrite [6,8,14,18] without any electron transfer mediators.

Sodium azide is a toxic chemical widely used in hospitals and laboratories as a chemical preservative, in agriculture for pest control, detonators, and other explosives. In addition, sodium azide is the starting materials of a variety of medicines. Moreover, sodium azide is used daily in the air-bags of vehicles with large tonnage. The azide poisoning depends on amount, rout, and length of time

* Corresponding author. Tel.: +81 48 585 6840; fax: +81 48 585 6840.
E-mail address: hasebe@sit.ac.jp (Y. Hasebe).

of exposure, as well as the age and pre-existing medical conditions of the person exposed. It prevents the cells of the body from using oxygen, and is thus very harmful to organs, especially the heart and the brain, because the heart and the brain use a lot of oxygen.

On the other hand, cyanide is commonly used in industrial applications such as metal-plating, metal mining, plastic manufacture, and extracting gold and silver from ores. In addition, cyanide is used in agricultural fields (e.g. fumigating fruit trees). Similar to azide, cyanide is also very toxic to humans, in particular its role in the suppression of oxygen transport and respiration system. For example, uptake of cyanide results in the cellular hypoxia, by inhibiting enzymes in the terminal respiratory chain.

Therefore, the quantitative determination of azide and cyanide is quite important. Several conventional methods such as titrimetric, colorimetric, and chromatographic methods have been established for the determination of these respiratory toxins [21–23]. However, these methods usually need relatively expensive equipments, complicated sample treatments and time-consuming analytical procedures. In contrast, electrochemical biosensors are attractive tools for simple and highly sensitive determination of these toxins.

Azide and cyanide are known to bind (coordinate) the active-metal center of redox enzymes, and inhibit their catalytic activities. Therefore, various types of inhibition-based enzyme electrodes have been fabricated by using heme-proteins (e.g. peroxidase [24–30]; catalase [31,32]; and cytochrome oxidase [33]) and copper-proteins (e.g. tyrosinase [34–36] and laccase [37–40]) as bio-recognition catalytic elements for the electrochemical determination of azide and cyanide. However, the application of Mb-modified electrode for the electrochemical sensing of azide and cyanide has not been reported until now, to our best knowledge.

Most of the enzyme-inhibition-based biosensors for azide and cyanide are batch-type sensors [24–32,34–37,40]. From more practical view point, as compared with biosensors with batch-type mode, a biosensor with flow-injection mode (FIA-based biosensor) has a number of advantages: (1) potential applicability for on-line analysis (automated system); (2) possible high sample throughput; (3) negligible effect of sample dilution as compared with batch-type sensor; and (4) the detectable sample concentration range and sensitivity can be altered by changing the sample injection volume and carrier flow rate.

In general, porous three dimensional macro-electrode is useful as a working electrode unit of electrochemical flow-through detector. The low diffusion barrier of porous matrix facilitates mass transport, and high surface area permits higher coulometric efficiency, which enables highly sensitive determination even at higher flow rate. Carbon-felt (CF) is a micro-electrode ensemble of micro-carbon fiber (ca. 7 μm diameter) and possesses a random three-dimensional structure [41]. The CF has high conductivity and a large effective surface area (estimated to be 0.1–20 $\text{m}^2 \text{g}^{-1}$), which allows large measurable current density and high electrolytic efficiency. In addition, high porosity of the CF (>90%) permits low diffusion barrier of solution-flow. On the basis of these features, the CF has been extensively used in wide fields: e.g. redox flow battery [42]; electrochemical synthesis [43]; rapid coulometry [44]; and electrochemical waste-water purification [45].

As compared with other porous electrode materials (e.g. platinum mesh electrode, gold mesh electrode, and reticulated vitreous carbon electrode), the CF has the following advantages: (1) inexpensive; (2) physically and mechanically stable; (3) easily handled; and (4) easily be manufactured to be arbitrary shapes. Therefore, the establishment of convenient and efficient immobilization biomolecules onto the CF would promise the development of highly functional electrochemical flow-biosensors. From this context, in our laboratory, we are engaging in developing the CF-based electrochemical flow-detectors [46,47] and flow-biosensors [30,48–56].

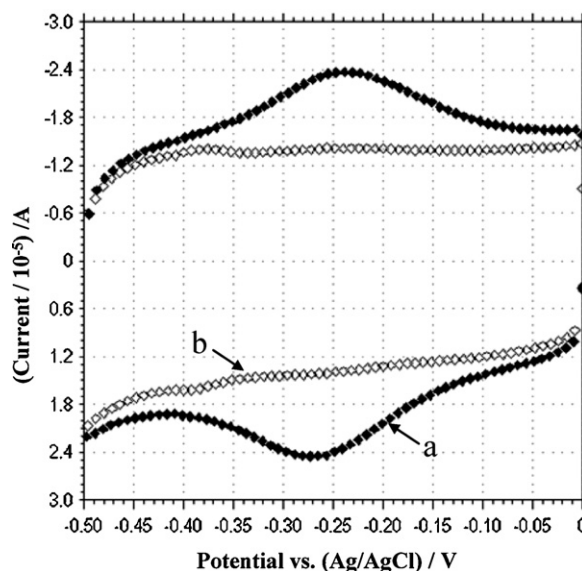


Fig. 1. CVs of Mb-adsorbed CF (curve a: solid circle) and bare-CF (curve b: open circle) in deoxygenized 0.1 M phosphate/citrate buffer (pH 5.0). Potential scan rate is 5 mV/s. Starting potential is 0.05 V vs. Ag/AgCl.

Various enzymes were immobilized onto the CF surface by physical adsorption [30,48–51] or covalent-modification [52–56], and the resulting enzyme-modified-CFs have been successfully used as flow-biotectors [30,48–50,52–55] and column-type bioreactors [51,56].

In this study, Mb was physically adsorbed on the CF surface, and the resulting Mb-adsorbed CF (Mb-CF) was successfully used for bio-recognition catalytic element for the flow-determination of respiratory toxins. The adsorbed Mb on the CF surface exhibited excellent bioelectrocatalytic activity for the reduction of oxygen, based on the DET between Mb and CF without any special materials (and mediators). Furthermore, this bioelectrocatalytic activity was reversibly inhibited by the respiratory toxins such as azide and cyanide. When air-saturated 0.1 M phosphate/citrate buffer (pH 5.0) was used as a carrier, the steady-state background current, which is due to bioelectrocatalytic O_2 reduction, was reversibly changed by the injection of azide and cyanide, leading to peak-shape current responses. This Mb-CF-based flow-biosensor enables direct (without mediator), simple (reagentless), and relatively highly sensitive (sub-micro molar) flow-determination of respiratory toxins. This would be a first report describing amperometric flow-determination of respiratory toxins based on the monitoring of inhibitory effect of respiratory toxins on the Mb-catalyzed electrochemical O_2 reduction.

2. Experimental

2.1. Reagents and materials

Myoglobin (Mb, from horse heart) was purchased from Sigma Co. Before use, the Mb was purified by dialysis against deionized water and isolated by lyophilization. Sodium cyanide (NaCN) and sodium azide (NaN_3) were purchased from Wako Pure Chemicals (Osaka, Japan) and used as received. All of the other chemicals were of the highest grade available, and were used without further purifications. Millipore Milli-Q water (resistivity > 18 $\text{M}\Omega \text{cm}$, TOC, 3 ppb) was used for preparation of all solutions throughout the experiments. 0.1 M phosphate/citrate buffer solution (pH 5.0) was made up from K_2HPO_4 and citric acid monohydrate, and used as electrolyte and carrier solutions. A carbon-felt (CF) sheet was obtained from Nippon Carbon Ltd. (Japan). Effective surface

Download English Version:

<https://daneshyari.com/en/article/6618237>

Download Persian Version:

<https://daneshyari.com/article/6618237>

[Daneshyari.com](https://daneshyari.com)