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Integrated gold-disk microelectrode modified with iron(II)-phthalocyanine for nitric oxide detection in macrophages

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Abstract

An integrated gold-disk microelectrode (IGME) was fabricated and modified with Fe(II)-phthalocyanine (Fe(II)-PC) for NO detection in biological media. Microanalysis of NO using square wave anodic stripping voltammetry (SWASV) in 0.01 M $HClO_4$ was optimal at the initial potential of 0.1 V, frequency of 100 Hz, pulse amplitude of 25 mV, and a scan rate of 200 mV/s. When the electrode was modified with Fe(II)-phthalocyanines, the anodic peak current and sensitivity of NO were remarkably increased due to the catalytic oxidation of NO. The calibration curve had good linearity in the range from 3.6×10^{-5} to 7.2×10^{-7} M, and the detection limit was $(5.7\pm1.2)\times10^{-7}$ M. Fe(II)-phthalocyanine modified gold-disk microelectrode coated with Nafion was applied to determination of NO released from macrophage cell.
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Keywords: Modified electrode; NO determination; Fe(II)-phthalocyanine; Square wave anodic stripping voltammetry; Macrophage

1. Introduction

Nitric oxide(NO), the unassuming molecule that suddenly grabbed spotlight a little over a decade ago as an ubiquitous biological signaler in mammals, plays a mystic role in various phenomena from blood vessel dilation to smooth muscle relaxation. It is also very important in neural transmittance related to cognition and control of sexual function and other biochemical processes [1–4].

NO was known to be capable of inactivating biologically important enzymes such as ribonucleotide reductase through its reaction with the metal center to form *S*-nitrosylation or sulfhydryl oxidation. NO was previously shown to have inhibition on the glucose oxidase (GOX) activity, although no literature has yet reported about the mechanism of the inhibition of NO on GOX. The inhibition of NO on xanthine oxidase (XOD) activity was reported to be due to the NO reaction with an essential sulfur of the reduced molybdenum center of XOD to produce desulfo-type inactive enzyme [5–8]. Several physiologic phenomena in which NO is involved, such as the biologic mechanisms underlying symptoms of senility and antitumoral activities, would be better understood if NO could be directly detected in biological media.

For measurement and elucidation of NO effects in biological system, various detection methods including spectrometry [9,10], ESR spectroscopy [11,12], laser absorption spectroscopy [13], chemiluminescence detection [14–19], fiber optic sensor [20], and gas chromatography mass spectrometer [21–24] have been reported. Recently, the electrochemical techniques appeared to be an attractive alternative for monitoring NO concentration in situ as well as ex vivo [25–36]. There are currently two ways of amperometric methods available for measuring NO from intact tissues and single cells.

The first amperometric approach was based on the direct electrooxidation of NO on a platinum electrode coated with different membranes such as chloroprene, nitrocellulose, silicone or Nafion, and cellulose acetate [5,37–44]. Shibuki

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[45] recently reported the measurement of NO release in the cerebellum using a microelectrode. A miniature O_2 electrode was modified by sealing its fire-polished 150- to 250-μm tip with a thin chloroprene rubber seal so that only low-molecular weight gases could enter. The reproducibility of this electrode was affected by numerous parameters that were difficult to control, and especially, by variations in the membrane thickness [46].

A second, more sensitive technique is based on the catalytic oxidation of NO ($NO - e^{-} \rightarrow NO^{+}$) by metalloporphyrins. Malinski and Taha [47] and Malinski et al. [48] coated carbon fibers (chosen as a convenient, strong microprobe material) with a thin polymeric porphyrin layer and measured NO at 0.65 V. To minimize detection of NO_2^-, they coated the carbon fiber with Nafion, a negatively charged material that was highly impermeable to anions. Nafion-coated fibers displayed no change in current although 20-fold excess of NO_2^- was added to the sample [49,50]. This technique allowed detection of NO production from cultured endothelial and smooth muscle cells. There are several reports of porphyrinic sensor for in situ detection of NO production in various biological systems [51–55]. Metallophthalocyanine complexes (M-PCS, Fig. 1) have a similar structure to the metalloporphyrins and have used to mimic the properties of the naturally occurring metalloporphyrin complexes. M-PCS have an added advantage in that they are more stable to degradation than metalloporphyrin complexes. The reactive centers for both phthalocyanines and porphyrins can be identified and so can be varied or optimized. Redox properties are expected at the central metal in Mn-PC, Fe-PC, and Co-PC whilst ring based processes occur in Zn-PC, Ni-PC, and Cu-PC. M-PCS with metal-based oxidation process are expected to show better electrocatalytic activity than ring-based M-PCS [56–64].

In this article, we report an integrated gold-disk microelectrode (IGME) system modified with Fe(II)-phthalocyanine (Fe(II)-PC). We optimized analytical parameters for direct measurement of NO by square wave anodic stripping voltammetry (SWASV) using Fe(II)-PC-modified electrode and used for the analysis of NO generated from sodium nitroprusside (SNP), macrophage (RAW 264.7), and taxol-treated murine peritoneal macrophage.

2. Experimental section

2.1. Reagents

NO-saturated solution was obtained by bubbling NO gas (98.5%, Aldrich) through deoxygenated distilled 0.01 M $HClO_4$ solution for 40 min, giving the value of 1.9 mmol/L when saturated [36,65]. A series of standard NO solutions was prepared by diluting aliquots of NO saturated solution. The NO solutions were kept in a glass flask with a rubber septum, and stored in the dark to ensure stability for 3 h.

Fe(II)-PC and sodium nitroprusside (SNP) were purchased from Aldrich. Deionized water was obtained by filtering through a Millipore Milli-Q filter. About 0.1 M of phosphate-buffered saline (PBS, pH 7.4 at 25 °C) and 0.1 M saline (NaCl) solution were prepared immediately prior to use. Measurement of electrode responses to NO was made under anaerobic conditions by purging electrolyte solutions with nitrogen. All other chemicals used were obtained from common manufacturers in reagent grade.

2.2. Macrophage cell line and culture

The murine macrophage cell line (RAW 264.7) was obtained from the American Tissue Culture Collection (ATCC, Rockville, MD). The cells were maintained in complete Rosewell Park Memorial Institute medium (RPMI-1640) supplemented with 10% fetal bovine serum (FBS) and antibiotics at 37 °C.

2.3. Instruments

Bundles of integrated gold-disk microelectrodes were connected to a PARC model 303 A electrode system, and voltammograms were obtained with a PARC model RE0093

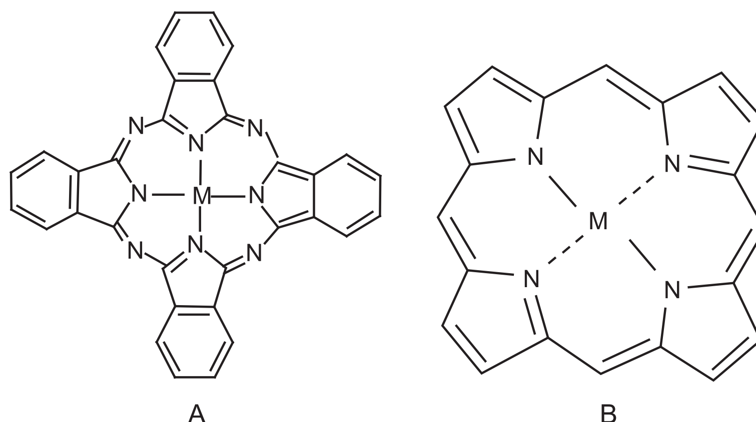

Fig. 1. Typical structure of metallophthalocyanine (A) and metalloporphyrin (B) molecules.

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