



Insights into Ferrocene-Mediated Nitric Oxide Sensing – Elucidation of Mechanism and Isolation of Intermediate



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ARTICLE INFO

Article history:

Received 26 March 2016
Received in revised form 19 May 2016
Accepted 21 May 2016
Available online 24 May 2016

Keywords:

Self-assembled monolayer
ferrocene
nitric oxide
ferrocene-hyponitrite
sensing

ABSTRACT

An amperometric nitric oxide (NO) sensor based on a ferrocene methanol functionalized electrode was fabricated. The sensor could work at low over potential with a detection limit of 50 nM. The sensor exhibited excellent analytical performance, including a NO sensitivity of $6 \mu\text{A nM}^{-1} \text{cm}^{-2}$. The surface coverage of ferrocene on the modified electrode was calculated to be $2.06 \times 10^{-10} \text{mol cm}^{-2}$. The heterogeneous rate constant calculated for ferrocene mediated NO reduction was found to be $3.5 \times 10^5 \text{cm}^3 \text{mol}^{-1} \text{s}^{-1}$. Further, the blue colored intermediate (Ferrocene hyponitrite) obtained during chemical and electrochemical reduction of NO mediated by ferrocene was isolated and characterized spectroscopically. Nitrous oxide (N_2O), the final product of electrochemical reduction of NO was characterized using GC-MS. The mechanism of ferrocene-mediated nitric oxide reduction was found to be similar to biological NO reduction exhibited by the enzymes.

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1. Introduction

Nitric oxide (NO), a small diatomic free radical and biologically synthesized molecule plays a significant role in the field of biology, medicine and pharmacy. The biological role of NO is of importance in the cardio-vascular system, neurotransmission, wound healing, antiplatelet aggregation etc., [1–3]. Due to its biological importance, the demand for the analytical techniques for the detection and quantification of NO is receiving considerable interest. Most common techniques of NO detection are spectroscopic and electrochemical methods. Some spectroscopic techniques offer high sensitivity and selectivity for NO, enabling its detection in picomolar range [4]. But the major obstacle in spectroscopic methods for *in vivo* detection of NO is the complex instrumentation which is difficult to miniaturize. The electrochemical techniques offer a good analytical platform with high sensitivity, selectivity, device miniaturization, low-cost fabrication and operation. Electrochemical detection of NO is carried out via two different approaches. The first approach is based on the electrochemical oxidation of NO, on Pt coated with Nafion/cellulose acetate/chlorophrene or metalloporphyrins [5–8]. The second approach is the electrocatalytic reduction of NO with metal complexes such as metal porphyrins (Fe, Mn, Co), Fe-phenanthroline, Fe-pyridine and

EDTA complexes [9,10]. Among the two approaches electrocatalytic reduction is more advantageous compared to electrocatalytic oxidation as the interferences arising from easily oxidisable biological compounds like dopamine, ascorbic acid, uric acid etc., can be overcome completely by this approach.

We have identified ferrocene as a suitable mediator for the reduction of NO at low over potentials in our earlier work and we have demonstrated the analytical utility of the reaction for the detection of NO by electrochemical and spectrophotometric techniques [11–13]. In the present work, we have attempted to covalently tether the mediator ferrocene to the gold electrode surface through a self-assembled monolayer and demonstrated a reagent less detection of NO up to nanomolar levels. Also the mechanistic details of the ferrocene mediated NO reduction has been elucidated and the intermediate and final products of NO reduction are isolated and characterized by techniques such as FT-IR, UV-Vis, EPR, XPS, EDAX, Raman spectroscopy and GC-MS.

2. Experimental

2.1. Materials and methods

Sodium nitrite, Dithiodipropionic acid, absolute ethanol, potassium ferricyanide, sodium chloride (all GR grade) were purchased from Merck. Ferrocene (Fc) was purchased from Alfa aesar. Ferrocene methanol and β -hydroxypropylcyclodextrin were procured from Aldrich Chemicals. All these chemicals were used as

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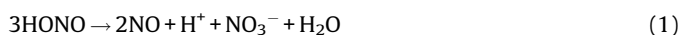
received without any further purification. All solutions were prepared using Millipore water. All the electrochemical studies were performed using MetrohmAutolab (PGSTAT302N, 30 V/2000 mA), GPES 4.9002 version potentiostat/galvanostat. A three electrode cell was used for electrochemical studies. A spiral platinum electrode was used as counter electrode and Hg/Hg₂SO₄ was used as reference electrode. Au or ferrocene modified gold electrode was used as working electrode. All X-ray photoelectron spectroscopic (XPS) measurements were performed using SPECS, phoibos 100 MCD analyzer with a pass energy of 20 eV (AlK α anode, 1486.6 eV) in ultrahigh vacuum (5×10^{-10} millibar). Initially, a full scan from 10 to 1100 eV was performed and later individual scans of 30–50 eV were carried out. The spectra were recorded with pass energy of 50 eV. Agilent technology 7890A GC A.01.08 GC-MS system was used to identify the product obtained during the electrochemical reduction of nitric oxide. Laser Raman spectrum was recorded using Renishaw Raman system 2000 model that employed 514 nm argon laser source. Energy dispersive spectroscopic (EDS) analysis was performed using QUANTEX.

2.2. Electrode pre-treatment, self-assembled monolayer (SAM) formation and ferrocene methanol functionalization

Prior to SAM modification, gold electrodes were cleaned ultrasonically using acetone and water for 10 min, respectively. This was followed by electrochemical cleaning by potential cycling between -0.4 V to 1.2 V vs Hg/Hg₂SO₄. Then these electrodes were rinsed with millipore water several times and immediately used for SAM formation. Dithiodipropionic acid (DTDPA) monolayer films were formed on the electrode surface by dipping the pre-cleaned gold electrode in 10 mM DTDPA in ethanol solution for overnight followed by washing with excess ethanol and millipore water before further experiments. In order to tether ferrocene methanol with DTDPA SAM, ester coupling method was adopted. To accomplish this, dicyclocarbodiimide along with dimethyl aminopyridine was used as coupling reagent in dichloromethane (DCM). After activating the carboxyl group the electrode was further treated with ferrocene methanol in ethanol, overnight for ferrocene functionalization.

2.3. Preparation of NO solutions for analytical studies

It is well known that sodium nitrite (NaNO₂) would spontaneously undergo disproportionation reaction in acidic conditions (pH < 4) and generate free NO as shown in Eq. (1) [14]. So we selected NaNO₂ as NO precursor whose electrocatalytic reduction was studied by means of ferrocene functionalized gold electrode.



2.4. Preparation of Ferrocene – β -cyclodextrin complex

50 mM of Fc and Hydroxypropyl β -cyclodextrin (HP- β -CD) solutions were prepared in DCM and water respectively. These two solutions were mixed and stirred for 6 h. Ferrocene gets transferred to aqueous phase by forming a complex with HP- β -CD which is water soluble.

2.5. Isolation of Ferrocene-hyponitrite intermediate by chemical reduction of NO

For preparing ferrocene-hyponitrite, ferrocene (20 mg) was first dispersed in water by sonication for 2 hours. Excess undispersed ferrocene was removed by filtration. To this solution Conc. HCl was added drop wise and the pH was adjusted to 3. This solution was

deaerated by purging nitrogen gas. To this solution nitric oxide gas was purged, when the yellow color solution was turned to dark blue due to the intermediate formation. This solution was dried to a powder by using rotary evaporator and vacuum desiccator and was used for further analysis.

2.6. Bulk electrolysis of NO

Bulk electrolysis of nitric oxide was performed using galvanostatic technique in the electrochemical cell containing 10 ml of 0.5 M H₂SO₄ and 0.2 ml of Fc- HP- β -CD (50 mM) complex, which was saturated with nitric oxide gas. Electrolysis was performed by applying a constant current density of $-0.610 \text{ mA cm}^{-2}$ using a glassy carbon plate as working electrode of dimensions $1 \text{ cm} \times 1 \text{ cm}$ along with Pt counter electrode. The gaseous product obtained on electrolysis was collected using a gas-tight syringe. The collected gaseous product was dissolved in 1 ml of doubly distilled ethanol in vacuum for GC-MS analysis.

2.7. Identification of product on NO reduction

The gaseous product obtained during electrolysis of NO was dissolved in ethanol using a gas-tight syringe. The Gas Chromatographic-mass spectroscopic analysis was performed under the conditions with ion source temperature of 200 °C and with a fused silica column of dimensions $30 \text{ m} \times 250 \mu\text{m} \times 0.25 \mu\text{m}$. The temperature was programmed to 50 °C for 5 min holding, 5 °C/min to 120 °C for 10 min then 10 °C/min to 200 °C. Total run time was 37 min in the mass range of 10 to 100 m/z.

3. Results and Discussions

3.1. Electrocatalytic response of NO at ferrocene methanol functionalized electrode

Cyclic voltammograms shown in Fig. 1 correspond to the electrocatalytic reduction of NO by ferrocene tethered SAM modified gold electrode for successive additions of NO. The redox peak is attributed to the ferrocene-ferrocenyl redox couple. From the cyclic voltammograms shown in Fig. 1, it is clear that by increasing the concentration of NO, the electrocatalytic reduction current also increases. Surface coverage (Γ) of ferrocene

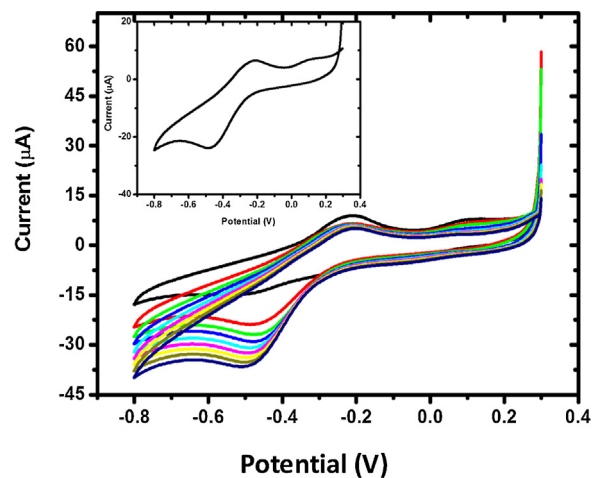


Fig. 1. Electrocatalytic reduction of NO at the ferrocene-functionalized gold electrode in 0.5 M H₂SO₄ as supporting electrolyte at a scan rate of 50 mV/s. Each addition corresponds to 3 μM of NO. The inset shows the cyclic voltammetric response of ferrocene-modified gold electrode in 0.5 M H₂SO₄.

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