Catalytic selectivity of metallophthalocyanines for electrochemical nitric oxide sensing

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A B S T R A C T

The catalytic properties of metallophthalocyanine (MPc) complexes have long been applied to electrochemical sensing of nitric oxide (NO) to amplify sensitivity and reduce the substantial overpotential required for NO oxidation. The latter point has significant ramifications for in situ amperometric detection, as large working potentials oxidize biological interferents (e.g., nitrite, L-ascorbate, and carbon monoxide). Herein, we sought to isolate and quantify, for the first time, the selectivity benefits of MPc modification of glassy carbon electrodes. A series of the most catalytically active MPc complexes towards NO, including Fe(II)Pc, Co(II)Pc, Ni(II)Pc, and Zn(II)Pc, was selected and probed for NO sensing ability under both differential pulse voltammetry (DPV) and constant potential amperometry (CPA). Data from DPV measurements provided information with respect to MPc signal sensitivity amplification ($\sim 1.5\times$) and peak shifting (100–200 mV). Iron-Pc exerted the most specific catalytic activity towards NO over nitrite. Catalyst-enabled reduction of the working potential under CPA was found to improve selectivity for NO over high potential interferents, regardless of MPc. However, impaired selectivity against low potential interferents was also noted.

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1. Introduction

Nitric oxide (NO) is an endogenous free radical implicated in a number of essential physiological processes, including inflammation [1], wound healing [2], vasodilation [3], and neurotransmission [4]. Despite a growing understanding of NO's roles, the ways in which endogenous levels of NO naturally fluctuate and the concentration-dependence of its activity are generally still not agreed upon. Unfortunately, dynamic detection of NO is constrained by NO's reactive nature and the presence of scavengers, greatly reducing its half-life in biological media [5–7]. Electrochemical sensors enable direct, real-time measurements of NO with fast response, high temporal resolution, low cost, and freedom from exogenous reagents [8,9].

Selectivity remains perhaps the greatest challenge to accurate electrochemical detection of NO in situ [5,10,11]. While electrochemical oxidation of NO is thermodynamically favorable, formation of the initial nitrosyl cation (NO^+) is kinetically slow, requiring large overpotentials (Fig. 1, adapted from reference 8) that are also capable of oxidizing interferent species present in biological milieu (e.g., nitrite, L-ascorbate, and carbon monoxide) [12]. In this manner, sensor accuracy is diminished greatly. Working electrodes must therefore be chemically modified to differentially enhance the NO signal and/or impede interferent species' access to the electroactive surface [11]. Selective NO detection is traditionally achieved by the use of either selectively permeable (i.e., *permselective*) polymer membranes or electrocatalysts [13]. Permselective barriers confer NO selectivity through well-understood size-exclusion, hydrophobic interaction, or charge-repulsion sieving mechanisms—exploiting NO's small size, lipophilicity, and neutral charge, respectively [14]. In contrast, electrocatalysts do not physically obstruct interferents; rather they enhance the NO signal and/or reduce the overpotential required for oxidation by facilitating electron transfer kinetics.

Metallophthalocyanines (MPc) represent the most common catalysts used in the fabrication of electrochemical NO sensors (Figure S1) [13,15]. Belonging to the same class of transition metal-coordinated macrocycles as metalloporphyrins (MP), MPcs have extended π-systems, which allow them to undergo fast redox processes and thereby facilitate electron transfer to a variety of molecules [16]. Moreover, MPc catalysts are more chemically and thermally stable than their MP counterparts [17]. Coordination of

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$$\cdot NO \rightarrow NO^+ + e^-$$
$$NO^+ + OH^- \rightarrow HNO_2$$
$$HNO_2 + H_2O \rightarrow NO_3^- + 3H^+ + 2e^-$$
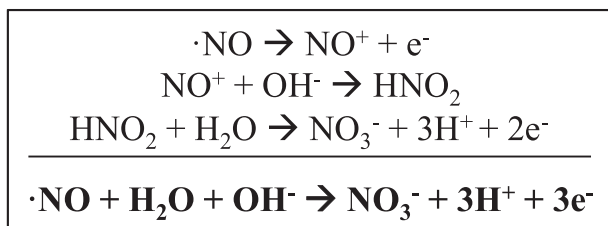$$\overline{\cdot NO + H_2O + OH^- \rightarrow NO_3^- + 3H^+ + 3e^-}$$

Fig. 1. Three-step electrochemical oxidation of nitric oxide.

NO with an electrode surface-confined MPc complex (chemisorptive or physisorptive) facilitates initial electron transfer from NO to the MPc, which then relays that electron to the electrode charge sink, generating the current response (Figure S2). The presence of certain MPc complexes is also believed to stabilize the transient NO^+ cation before further oxidation to nitrate [16,18]. It is through these stabilizing effects that the NO signal is amplified and voltammetric features are shifted to lower potentials.

The identity of the transition metal (M) has been reported to influence the site of NO coordination [19], bond orientation [20], electronic structure [21], and oxidation potential [12]. While separate studies have come to different conclusions as to the most catalytically active MPc complex towards NO [12,18,22], no study has yet evaluated the concomitant effects on sensor selectivity. For instance, an electrocatalyst that enhances both the NO and interferent signals will not bear any improvement on sensor selectivity. Signal enhancement from such a catalyst is more likely the result of increased electroactive surface area rather than targeted ligation of NO. This hypothetical catalyst is significant in light of the fact that several MPc complexes do not specifically bind NO at the metal core [19]. For example, nickel-Pc is the most routinely used MPc for NO sensor fabrication due to its substantial NO signal sensitivity amplification, yet several studies have reiterated that the oxidation is not "metal-based" (i.e., mediated through the metal core) [13,18,22,23]. First-principles density functional theory (DFT) calculations demonstrate that nickel- and zinc-Pc only weakly physisorb NO at the metal core, preferring instead to hybridize with the C atoms on the macrocycle's periphery (Figure S1) [19,20]. Clearly, it is important to determine if these non-metal-based interactions actually furnish selectivity benefits to the detection of NO.

The findings above motivated us to systematically evaluate the selectivity benefits of MPc complexes in isolation (i.e., in the absence of a co-deposited permselective membrane). Based on independent studies carried out by Caro et al. and Vilakazi et al., iron-phthalocyanine (FePc), cobalt-phthalocyanine (CoPc), nickel-phthalocyanine (NiPc), and zinc-phthalocyanine (ZnPc) were identified as the most catalytically active MPc complexes [18,22]. These catalysts were drop-cast on glassy carbon (GC) electrode surfaces and evaluated for selectivity against: nitrite (NO_2^-), an oxidative product of NO oxidized at similarly high potentials; L-ascorbate (AA), a biologically ubiquitous, redox-active molecule; and carbon monoxide (CO), a molecule of similar size and structure that has been shown to exert similar physiological effects to NO through heme-coordination (e.g., with hemoglobin) [24]. These properties are summarized in Table 1.

Table 1
Interferents to electrochemical detection of nitric oxide.

Interferent	E_a (mV)	Concentration (nM)	Media	Reference
NO_2^-	800–1200	>20,000	Blood	[25]
AA	100–300	43,000	Serum	[26]
CO	200–500	500–1500	Kidney tissue	[27]

In addition to varying the interferent and MPc metal, selectivity was also monitored as a function of electrochemical technique. One of the ways MPcs purportedly improve selectivity is by lowering the required potential for NO oxidation, though actual selectivity benefits have never been rigorously quantified [13]. Herein, NO selectivity was measured using both differential pulse voltammetry (DPV) and constant potential amperometry (CPA). DPV was used to determine shifts, if any, in the NO oxidation potential ($E_{a,NO}$) relative to the bare glassy carbon electrode. Measured $E_{a,NO}$ values of the catalyst-modified electrode were then used as the working potentials for CPA measurements. Comparisons in selectivity when the working potential was set at either the $E_{a,NO}$ of the bare or MPc-modified electrode allowed for the determination of whether catalyst-enabled reductions of the working potential improved selectivity.

2. Experimental

2.1. Materials and reagents

Iron (II) phthalocyanine (FePc), cobalt (II) phthalocyanine (CoPc), nickel (II) phthalocyanine (NiPc), zinc (II) phthalocyanine (ZnPc), sodium nitrite ($NaNO_2$), and L-ascorbic acid (AA) were purchased from Sigma (St. Louis, MO). Pyridine was obtained from Fischer Scientific (Hampton, NH). Nitric oxide (99.5%), carbon monoxide (99.3%), nitrogen (N_2), and argon (Ar) gases were purchased from National Welders Supply (Raleigh, NC). Chemicals and solvents were analytical-reagent grade and used as received without further purification.

Distilled water was purified to a final resistivity of 18.2 MΩ cm and a total organic content of ≤6 ppb using a Millipore Milli-Q UV Gradient A10 System (Bedford, MA). Saturated solutions of gaseous NO (1.9 mM) and CO (0.9 mM) were prepared by purging ~20 mL of phosphate buffered saline (PBS; 10 mM, pH 7.4) on ice with Ar for 30 min to remove oxygen, followed by purging with NO or CO gas for 30 min [28]. Saturated solutions were prepared on the same day as use and stored at 4 °C between calibrations. All electrochemical experiments were carried out in 20 mL of deoxygenated PBS at room temperature (23 °C) using a CH Instruments 1030 8-channel Electrochemical Analyzer (Austin, TX). The electrode configuration consisted of glassy carbon (GC; 3 mm dia.) inlaid disc working electrodes sealed in Kel-F (6 mm total dia.; CH Instruments), a silver-silver chloride (Ag|AgCl) reference electrode (3.0 M KCl; CH Instruments), and a coiled Pt wire counter electrode. All potentials are reported versus the Ag|AgCl reference electrode.

2.2. Preparation of catalyst-modified glassy carbon electrodes

Glassy carbon disc working electrodes were sequentially polished with 1.0, 0.3, and 0.05 μm alumina slurries to achieve a mirror finish. To avoid surface cracking and loss of electrical contact, glassy carbon electrodes were wiped lightly with tissue and rinsed copiously with DI water (in place of sonication) to remove imbedded alumina. After drying under a N_2 stream, catalysts were drop-cast onto the GC electrode surface using 30 μL of 1.5 mM MPc solution dissolved in pyridine. After evaporation of the pyridine in ambient (~20 min), excess catalyst not physi- or chemisorbed to the surface was removed by rinsing with ethanol and then pyridine. Catalyst-modified electrodes were dried under a N_2 stream before immediate use. Adsorption of the catalyst was confirmed by X-ray electron spectroscopy (XPS). Briefly, a Kratos Axis Ultra DLD X-ray photoelectron spectrometer with a monochromatic Al Kα X-ray source (base pressure $= 6 \times 10^{-9}$ torr) was used to collect both survey (80 eV pass energy) and high-resolution scans (20 eV pass energy) of the signature peaks of the transition metal centers. All