行政院國家科學委員會補助專題研究計畫成果報告

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※ 微過氧化酵素的電化學及電催化反應 ※
※ Electrochemistry and Electrocatalysis of Microperoxidases ※
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計畫主持人：蘇玉龍　教授　台灣大學化學系

共同主持人：

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執行單位：台灣大學化學系

中　華　民　國　　　年　　　月　　　日

Electrocatalytic Reactions of Nitric Oxide on Prussian Blue Film Modified Electrodes
普魯士藍修飾電極對一氧化氮電催化反應

計畫編號：NSC 89-2113-M-002-039-
執行期間：八十八年入月一日至入十九年七月三十一日

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中文摘要

研究使用普魯士藍薄膜修飾電極，在酸性的緩衝溶液中電氧化及還原催化一氧化氮。從循環伏安法及光譜電化學法的結果，我們發現普魯士藍的還原態"普魯士白"，對於一氧化氮有電還原催化的效應。一氧化氮的還原催化電流從負 0.4 伏特開始產生，而且會隨著電位的增負而變大。用普魯士藍薄膜修飾電極在負 0.7 伏特大量電解亞硝酸鹽水溶液，可以得氨及羥胺二種產物。普魯士藍的氧化態"柏林綠"，對於一氧化氮有氧化催化的活性，在正 1.3 伏特下可以使一氧化氮生成硝酸根。在文章中，普魯士藍的反應性被探討並呈現反應的圖表。

關鍵鍵字：普魯士藍修飾電極、一氧化氮、電催化反應、電氧化、電還原。

Abstract

Electrocatalytic reduction and oxidation of nitric oxide on electrodes modified with Prussian blue films was studied in acidic aqueous buffer solutions. The results from the cyclic voltammetry and spectroelectrochemistry show that Prussian white, the reduced form of Prussian blue, acted as an electrocatalyst toward NO reduction. The reduction current started at –0.4 V and became higher at more negative potential. Bulk electrolysis of nitrite solution at PB modified electrodes at –0.7 V led to the formation of NH_3 and NH_2OH. Berlin green, the oxidized form of Prussian blue, has the activity to catalyze NO oxidation to yield nitrate at +1.3V. The reactivity of Prussian blue was discussed and the reaction schemes were presented.

Keywords: Prussian blue-modified electrode, Nitric oxide, Electrocatalytic reaction, Electroreduction, Electrooxidaton.

1. Introduction

Nitric oxide (NO) has been of interest to chemists because of its important role in environmental problems [1]. It is one of the most studied molecules in the biological science recently due to its multiple roles. It has a role as a signaling molecule in the brain [2,3], in the regulation of blood pressure [4] and in the cardiovascular systems [5]. Also, it is identified as an important neurotransmitter in central and peripheral nervous systems [6]. Moreover, it is related to some tissue damage and fatal effect [7].

Prussian blue (PB-Fe(III)Fe(II)), known as a mixed-valence compound [8], can be deposited onto electrode surfaces [9-12]. The modified electrodes have a wide range of application in electrocatalysis [9], electrochromic displays [10], ion-selective electrodes [11] and biosensors [12]. Prussian blue exists in the solid state in two forms. Both forms, insoluble PB ($Fe_4[Fe(CN)_6]_3$) and soluble PB ($KFe[Fe(CN)_6]$), are electroactive. PB may be oxidized and reduced associated with color changes. Prussian white

(PW-Fe(II)Fe(II)) is the completely reduced form, which was reported to reduce oxygen [13], hydrogen peroxide [13] and CO_2 [9]. Berlin green (also known as Prussian yellow, PY-Fe(III)Fe(III)) is the completely oxidized form, which was able to oxidize hydrogen peroxide [14], thiosulfate [15] and hydrazine [16].

Self-deposition of the PB occurred when electrodes such as Pt, Au and glassy carbon were immersed directly in solutions of $K_3Fe(CN)_6$ and $FeCl_3$. Trace ferric ion was reduced to ferrous ion from the reaction of ferric ion and water molecules. Solid PB was formed on the electrode surface owing to the following equation,

$$K^+ + Fe^{2+} + Fe(CN)_6^{3-} = KFeFe(CN)_6 \qquad (1)$$

The PB film formed without potential control was unstable and redissolved into the solution when the modified electrode was used in the follow-up experiments.

Deposition of PB on electrode can be conducted by electrochemical methods and the cycled film may be composed of a mixture of the soluble PB and insoluble PB [17]. The electrochemistry of the PB film has been well studied in the literatures. In the reduction, K^+ ions are incorporated in the PB lattice to maintain film electroneutrality, which is proved from the electrochemical quartz crystal microbalance (EQCM) experiments [17-19]. The transport of K^+ ion causes the increase and decrease of the frequency [20,21]. The reduction reaction results from the redox reactions of high spin $Fe^{+3/+2}$ [22]. In the anodic scan, electrooxidation of PB to Berlin green occurs in chloride medium [23]. The oxidation reaction corresponds to electrochemical reactions of low spin $Fe(CN)_6^{4-/3-}$ [22].

Oxidation and reduction can be described according to Itaya et al [18]

$$Fe_4^{III}[Fe^{II}(CN)_6]_3 + 4K^{=} + 4e^- = K_4Fe_4^{II}[Fe^{II}(CN)_6]_3 \qquad (2)$$
(PB) (PW)

$$Fe_4^{III}[Fe^{II}(CN)_6]_3 + 3Cl^- = Fe_4^{III}[Fe^{III}(CN)_6]Cl_3 + 3e^- \qquad (3)$$
(PB) (PY)

The lattice structure is converted to the 'soluble' form [24] when the films are cycled several times in potassium medium. The reactions can be rewritten as [25,26]

$$KFe^{III}Fe^{II}(CN)_6 + K^+ + e^- = K_2Fe^{II}[Fe^{II}(CN)_6] \qquad (4)$$
(PB) (ES)

$$KFe^{III}Fe^{II}(CN)_6 + Cl^- = KFe^{III}[Fe^{III}(CN)_6]Cl + e^- \qquad (5)$$
(PB) (PY)

It is well known that electrochemical technique is very promising for NO measurement [27,28]. Also, PB was found to be a good catalyst of some biological molecules electrochemically [29]. However, to our knowledge, there is no report concerning its application in NO determination. In this work, we develop the PB modified electrodes to investigate its electrocatalytic activity on nitric oxide. The lattice of the PB film is expected to allow the transferring of cation and neutral species. Both electroreduction and electrooxidation of nitric oxide at the PB modified electrodes are under studied. Ruthenium purple (RP) was also used for comparison. Such information may be useful in the application of PB as a NO detector.

2. Experimental

Reagents – The solution used for PB deposition was prepared from equal volumes of freshly prepared $FeCl_3 \cdot 6H_2O$ (0.01 M) and $K_3Fe(CN)_6$ (0.01M). The solution used for the analogue RP (ruthenium purple) deposition was the same except that $K_4Ru(CN)_6$ was instead. The electrolyte was 1.0 M KCl and the solution was buffered to pH 2.0. Double-distilled water, additionally purified in a Milli-Q ultrapure water system, was used in all experiments.

Electrodes – Indium-tin oxide (ITO, 5cm × 0.8cm) were used as working electrodes. It was cleaned by soaking in concentrated ammonia solution followed by rinsing in deionized water. The electrode was then immersed in acetone and ultrasonicated for 3 minutes. The PB film was deposited in potentiostatic conditions (+0.6 V) for 15 to 120 seconds. For the purpose of stabilizing the Prussian blue film, the modified electrode was cycled 15 times between +0.6 and –0.2 V in pH 2.0 buffer solution [24]. A home-

made Ag/AgCl electrode was used as a reference electrode. All potentials were reported with respect to this reference electrode. Platinum wire was used as a auxiliary electrode. Spectroelectrochemistry was conducted in a 1 cm path length cuvette, consisting the same three-electrodes system.

Product analysis – The reduction products were reacted with *o*-phthaldehyde and mercaptoethanol, and then quantitated by a fluorescence methods [30].

Instruments – Electrochemical experiments was performed using EG&G Princeton Applied Research Model 174A Polarographic Analyzer and PAR Model 175 Universal Programmer. Cyclic voltammetry (CV) curves were recorded using a BAS (Bioanalytical Systems, West Lafayette, IN, USA) X-Y recorder. The absorption spectra were obtained with a Hewlett-Packard Model 8453 spectrophotometer. The fluorescence spectra were obtained with a HITACHI F-4500 spectrophotometer.

3. Results and Discussion

3.1. Nitric oxide reduction at the PB modified electrodes

Figure 1A shows the cyclic voltammograms for the electron transfer of PB/PW under a nitrogen atmosphere. These ITO modified electrodes are prepared by various deposition time. The amount of PB film on the electrodes can be measured from the voltammograms. It can be seen that the cathodic peak area between +0.4 to –0.2 V increases as the deposition time increases, which indicates that the PB film becomes thicker at more deposition time. When these modified electrodes are transferred into a KNO_2 solution buffered to pH 2.0, the cyclic voltammograms are shown in Figure 1B. The reduction current starts at –0.4 V and a fairly large increase in cathodic current is found, while bare ITO electrode has no catalytic ability for the reduction of a KNO_2 solution. The electroreduction current at –0.6 V is also larger when the deposition time increases, which means that the more of the PB molecule deposition, the more efficiently it act as a reduction catalyst. That is to say, the electroreduction reactions occur within the PB lattice.

Nitrite ion was disproportionated according to the following equation [31]

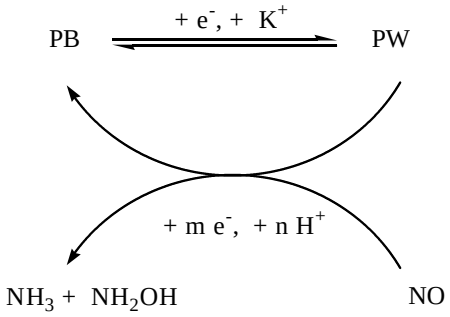
$$2NO_{(aq)}+NO_3^-+2OH^- = 3NO_2^-+H_2O \quad K=1.1\times10^{20}M^{-2} \quad (6)$$

In acidic medium, nitric oxide is generated thermodynamically. PW, the reduced form of PB, was reported to have the catalytic activity for the reduction of molecular oxygen to water via four-electron-transfer reactions [14]. Owing to the similar molecular size with oxygen, nitric oxide can be expected to transport through the lattice of PB during reduction. NO is supposed to be electrocatalytically reduced by PB. Both nitrite and nitrate anions were expelled by the negatively charged PB film. Thus, the reduction current is resulted from the reduction of NO by PB.

The reduction of NO by PB modified electrode using spectroelectrochemistry methods is shown in Figure 2. At open circuit (trace A), absorption near 350 nm (multiples) and 700 nm (broad) are attributed to the nitrite and PB, respectively. At –0.7 V (traces B ~ D), the broad band at 700 nm is completely disappeared, which indicates that PW is generated electrochemically. At the same time, the absorbance of nitrite decreases gradually. At bare ITO electrode, the absorbance decrease of nitrite is not significant. The electrocatalytic reduction of NO at the PB film apparently causes the chemical equilibrium shifting to the consumption of NO_2^-.

3.2. Product analyses

The reduction products obtained by prolonged potentiostatic electrolysis with the prepared electrodes are shown in Table 1. The products are NH_3 and NH_2OH of nearly equal quantity using PB modified electrodes. When a ruthenium purple (RP) electrode was used, the higher yield product was NH_2OH but the total quantity were less than the PB case. The electrocatalytic reduction scheme can be shown as



It was reported that NO binds to Fe^{II} ion in the PB lattice [32], which make the reduction possible. Accordingly, reduction products were obtained via electron transfer accompanied by proton transfer.

3.3. Nitric oxide oxidation at the PB modified electrodes

Figure 3 shows the cyclic voltammograms for the PY/PB redox couple under nitrogen. The voltammogram charge (the cathodic peak charge between +0.6 to +1.2 V) increases linearly with the deposition time, indicating again that the PB film is thicker at more deposition time. When these PB modified electrodes were used to detect NO, the oxidation current starts at +0.9 V and is enhanced greatly at the PB modified electrodes under NO atmosphere (Fig 3B) compared to that under nitrogen (Fig 3A). The oxidation current at +1.3 V is larger at the thicker PB film electrodes, which means the electrooxidation reactions occur within the PB lattice. Bare ITO shows no catalytic current to NO oxidation, which means that PB is also a catalyst toward NO oxidation.

The oxidation of NO by PB modified electrodes using spectroelectrochemistry methods is shown in Figure 4. The electrode is set at the right angle of the path length, so that the solution composition during the oxidation reactions can be monitored. The multiple peaks in the range of 330 ~ 400 nm are characteristic of potassium nitrite in pH 2.0 solution. At +1.3 V, the multiples absorbance decreases gradually and a band characteristic of nitrate grows at 300 nm. After 110 min, the absorbance of nitrite is 80 % lower than the original. In the absence of PB, the occurrence of the reaction (6) may contribute to the decrease of NO_2^- and the increase of NO_3^- in absorbance, however, the contribution is less significant. Chen [33] has reported the absorption spectral change of nitrite in a buffer solution (pH 1.5) in the presence of oxygen. It took 2610 min to lower nitrite absorbance of the same quantity. Evidently, PB can act as a catalyst for NO oxidation.

The oxidation product of NO by PB modified electrodes is proposed to be nitrate anion as shown in Figure 4. The half-reactions for some nitrogen species in aqueous solution [34] are shown below:

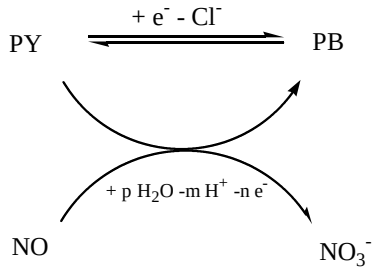
$$HNO_2 + H^+ + e^- = NO + H_2O \qquad E^0 = +0.983\ V \quad (7)$$

$$NO^{3-} + 4H^+ + 3e^- = NO + 2H_2O \qquad E^0 = +0.957\ V \quad (8)$$

$$N_2O_4 + 4H^+ + 4e^- = 2NO + 2H_2O \qquad E^0 = +1.035\ V \quad (9)$$

$$2NO_3^- + 4H^+ + 2e^- = N_2O_4 + 2H_2O \qquad E^0 = +0.803\ V \quad (10)$$

Thermodynamically, nitrate anion may be formed via (7)—>(8) or (7)—>(9)—>(10) pathway. The electrocatalytic NO oxidation scheme at PB is thus shown as



The study for detailed reaction mechanism is presently in progress.

Table 1. Reduction products of 20mM $NaNO_2$ by modified electrodes for 3 hr. at –0.7 V. Solution conditions : 1.0 M KCl and buffered to pH 2.0.

Product	NH_3 (M)	NH_2OH (M)
PB/C[a]	8.3×10^{-4}	8.1×10^{-4}
RP/C[a]	1.6×10^{-4}	5.6×10^{-4}
Bare C[a]	1.0×10^{-5}	6.6×10^{-6}

a. vitreous carbon electrode

4. 計畫成果自評

本報告乃一系列電催化還原一氧化氮研究的延伸，之前我們用各種金屬紫質作為催化劑，其中以鐵紫質的效果最好，我們亦嘗試以鐵的無機高分子，即普魯士藍，作為其比較研究。整個實驗計畫已經有一個階段性的完整度，且實驗的內容為一新發現，在學術上有其研究之價值，近期應可以在國際性的學術期刊上發表。整體來說，研究計畫的這個部份尚稱圓滿。

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Figure captions

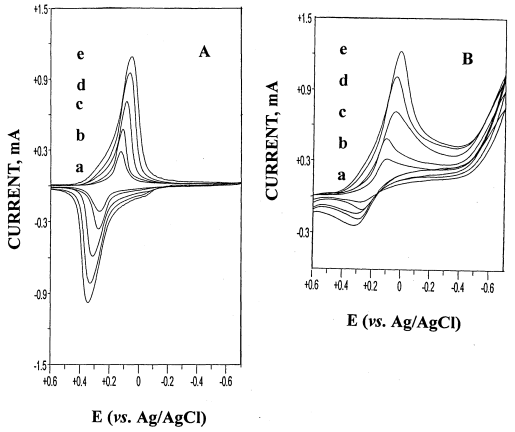

Fig.1 Cyclic voltammograms of PB/PW in pH 2.0 buffer solutions containing 1.0 M KCl and (A)0.0 (B)10 mM KNO_2. ITO electrodes were electrodeposited for (a)15 (b)30 (c)45 (d)90 (e)120 seconds.

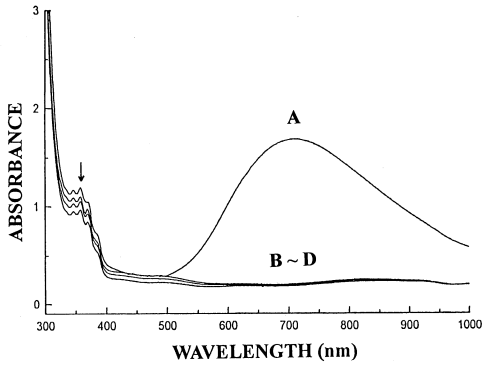

Fig.2 Time-resolved absorption spectral change of PB in pH 2.0 buffer solution containing 20 mM KNO_2 and 0.5 M KCl. Time interval = 10 min. (A)under open circuit (B) ~ (D)at – 0.7 V.

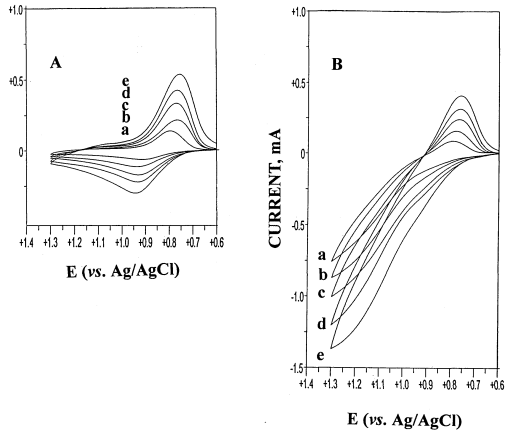

Fig.3 Cyclic voltammograms of PY/PB in pH 2.0 buffer solutions containing 1.0 M KCl and (A)0.0 (B)10 mM KNO_2. ITO electrodes were electrodeposited for (a)15 (b)30 (c)50 (d)70 (e)120 seconds.

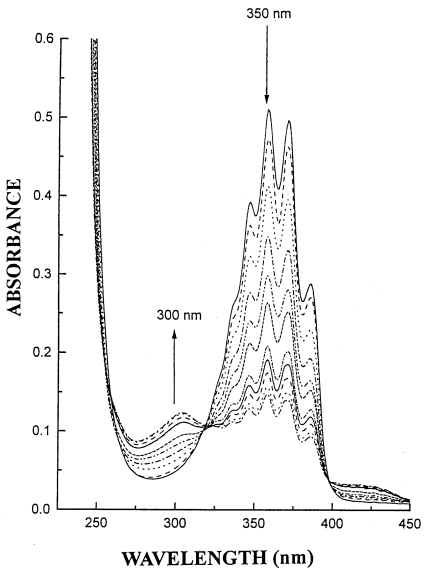

Fig.4 Time-resolved absorption spectral change of PB in pH 2.0 buffer solution containing 10 mM KNO_2 and 1.0 M KCl. $E_{applied}$ = +1.3 V. Time interval = 10 min.