

Electrochemical study on selective detection of nitric oxide mediated by iron-porphyrin in solution and in DNA film

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学位授与の題目	Electrochemical Study on Selective Detection of Nitric Oxide Mediated by Iron-Porphyrin in Solution and in DNA Film (溶液中および DNA 膜中の鉄-ポルフィリンの触媒作用による一酸化窒素の選択的検出に関する電気化学的研究)
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学 位 論 文 要 旨

Abstract:

A stepwise catalytic oxidation of nitric oxide and nitrite by water-soluble iron(III) meso-tetrakis(*N*-methylpyridinium-4-yl)porphyrin ($\text{Fe}^{\text{III}}(4\text{-TMPyP})$) was first revealed by using an indium-tin oxide (ITO) electrode. Electrochemical and spectroelectrochemical studies characterized the formation of oxoiron(IV) porphyrin and oxoiron(IV) porphyrin π -cation radical in the oxidation of $\text{Fe}^{\text{III}}(4\text{-TMPyP})$. A mechanism of typical chemical catalysis was proposed for the catalytic oxidations of NO and NO_2^- by oxoiron(IV) porphyrin complexes.

On the other hand, a stable $\text{Fe}(4\text{-TMPyP})\text{-DNA-PADDA}$ (FePyDP) film was prepared on pyrolytic graphite electrode (PGE) or ITO electrode through the supramolecular interaction between water-soluble $\text{Fe}(4\text{-TMPyP})$ and DNA matrix, where PADDA (poly(acrylamide-*co*-diallyldimethylammonium chloride)) is employed as a co-immobilizing polymer. This assembly film was characterized by quartz crystal microgravimetry (QCM), UV-vis spectroscopy and electrochemistry. The catalytic activity for NO reduction and oxidation were investigated at FePyDP film modified electrode, respectively.

Introduction

It is now well established that nitric oxide (NO) plays fundamental roles in biochemical processes such as blood pressure control, neurotransmission, and immune response, and subsequent reports have identified a number of disease states involving NO imbalances [1,2]. Thus, in order to clarify the function and control the concentration of NO in biological systems, it is significant challenge to develop highly selective and sensitive detection of NO.

Nitric oxide electrochemical sensors have been mainly developed on three different approaches. The first one is based on the direct oxidation of NO on metal anodes coated with different membranes such as poly[4,4'-dihydroxybenzophenone], electrodeposition Pb and Ir oxide in PVP film, $\text{Co}(\text{phen})_2$ and Nafion film and so on. The second amperometric approach for nitric oxide detection is based on the catalysis of NO by metalloproteins such

as cytochrome c, hemoglobin and myoglobin. Since Malinski and Taha reported a carbon fiber modified by electropolymerization of nickel(II) tetrakis(3-methoxy-4-hydroxyphenyl)-porphyrin (NiTMHPP), metalloporphyrin film modified electrode became very significant in the detection of NO due to high sensitivity, selectivity, and in vivo capability [3,4].

In the present thesis, the author reported the electrochemical study on selective detection of nitric oxide mediated by iron-porphyrin in solution and in DNA film.

Experimental

Chemicals

Fe^{III}(4-TMPyP) was prepared according to the methods of Pasternack et al and Bedioui et al. Salmon testis double strand (ST-ds) DNA (Sigma) was used as received. DNA concentration was estimated in nucleotide phosphate, which was determined using spectrophotometer with ϵ of $1.31 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ at 260 nm.

Electrochemical and Spectroelectrochemical measurements

Electrochemical experiments were performed on a digital universal processing unit (Fuso, HECS 326) connected to a head box (Fuso, HECS 326-1) in one three-electrode cell. The working areas of an optically transparent ITO electrode and a PG electrode were 18.2 mm² and 7.1 mm², respectively. The Ag | AgCl | 3 M NaCl electrode (BAS, RE-5) and a platinum coil were utilized as reference and counter electrodes, respectively.

Preparation of DNA-PADDA composite and cast films

The mixture of 0.3 ml aqueous solution of 15 mg ml⁻¹ ST-dsDNA and 0.2 ml aqueous solution of 15 mg ml⁻¹ PADDA was set at a room temperature for 30 min until a white precipitate of DNA-PADDA was formed. After being set at ambient temperature overnight, the solution was centrifuged, and the sediment was washed with pure water and then dried at a room temperature. Such dried DNA-PADDA composite powder was dispersed in water by ultrasonication for 30 min to obtain a DNA-PADDA cloudy suspension of 3 mg ml⁻¹. 10 μ l 3 mg ml⁻¹ DNA-PADDA suspension was cast on the cleaned electrode surface and dried in air overnight. After the electrode was soaked in water for at least 4 h and rinsed with water to remove any unadsorbed composite, one DNA-PADDA film modified PGE was obtained. The electrode was then immersed in 2×10^{-4} M Fe(4-TMPyP) solution for 60 min to prepare Fe(4-TMPyP)-DNA-PADDA (FePyDP) film.

Results and discussion

A stepwise catalytic behavior of oxoiron(IV) 4-TMPyP complexes towards NO and NO₂⁻ oxidation

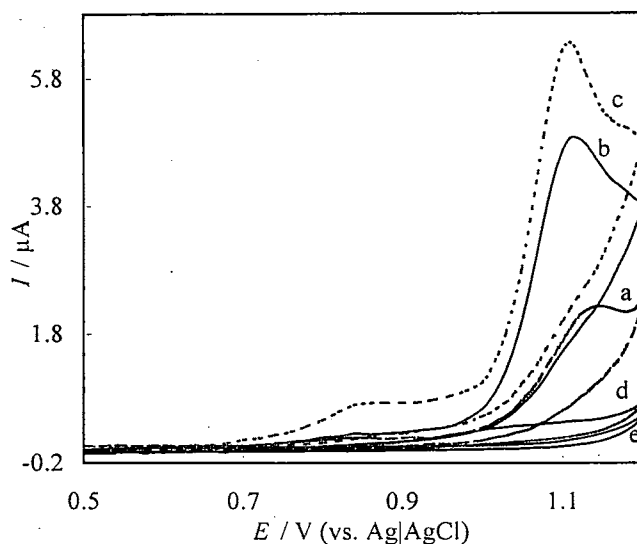


Fig. 1. Cyclic voltammograms of 20 μM $\text{Fe}^{\text{III}}(4\text{-TMPyP})$ at an ITO electrode in 50 mM pH 7.4 PBS (a) without and (b) with 100 μM NO_2^- , and (c) with 100 μM NO_2^- and 18.3 μM NO. Curves (d) and (e) are obtained in pure PBS with and without 18.3 μM NO and 100 μM NO_2^- , respectively. Scan rate, 10 mV s^{-1} .

Fig. 1a shows the cyclic voltammogram (CV) of $\text{Fe}^{\text{III}}(4\text{-TMPyP})$ in pH 7.4 PBS at an ITO electrode. At pH 7.4 the hydroxy-aqua iron porphyrin, $\text{HO-Fe}^{\text{III}}(\text{H}_2\text{O})(4\text{-TMPyP})$, predominates over diaqua or dihydroxy iron porphyrin, since proton equilibria exist with the pK_a values of 5.5 and 11.8. Two separate oxidation peaks are observed for $\text{HO-Fe}^{\text{III}}(4\text{-TMPyP})$ (ligand H_2O is omitted for simplicity) around +0.85 V and +1.15 V, corresponding to the oxidation of the central iron and porphyrin ring, respectively. The CV of pure NO and NO_2^- in pH 7.4 PBS at an ITO electrode was shown in Fig. 1d. The oxidation peak potential of NO was observed around +1.0 V compared with +0.65 V at a GC electrode. On the other hand, the peak potential of NO_2^- oxidation was not clear and estimated by subtracting residual currents to be roughly +1.2 ~+1.3 V (+0.8 V at a GC electrode), but the peak currents were small owing to the large overpotentials for the direct oxidation on ITO electrode. However, a large peak current was observed at the CV of NO_2^- in the presence of $\text{Fe}^{\text{III}}(4\text{-TMPyP})$ (Fig. 1b). By comparing Fig. 1b with Figs. 1a and 1d, it could be concluded that the enhanced current proceeded from the catalytic oxidation of NO_2^- by $\text{Fe}^{\text{III}}(4\text{-TMPyP})$. Fig. 1c shows the CV of NO and NO_2^- mixture in the presence of $\text{Fe}^{\text{III}}(4\text{-TMPyP})$. Simultaneously, two enhanced peak currents were observed around the first and second oxidation potentials of $\text{Fe}^{\text{III}}(4\text{-TMPyP})$, which are shown in inset of Fig. 1. These results clearly suggested a stepwise catalytic oxidation behavior of NO and NO_2^- oxidation by high-valent iron porphyrins.

Preparation and characterization of FePyDP film modified electrode

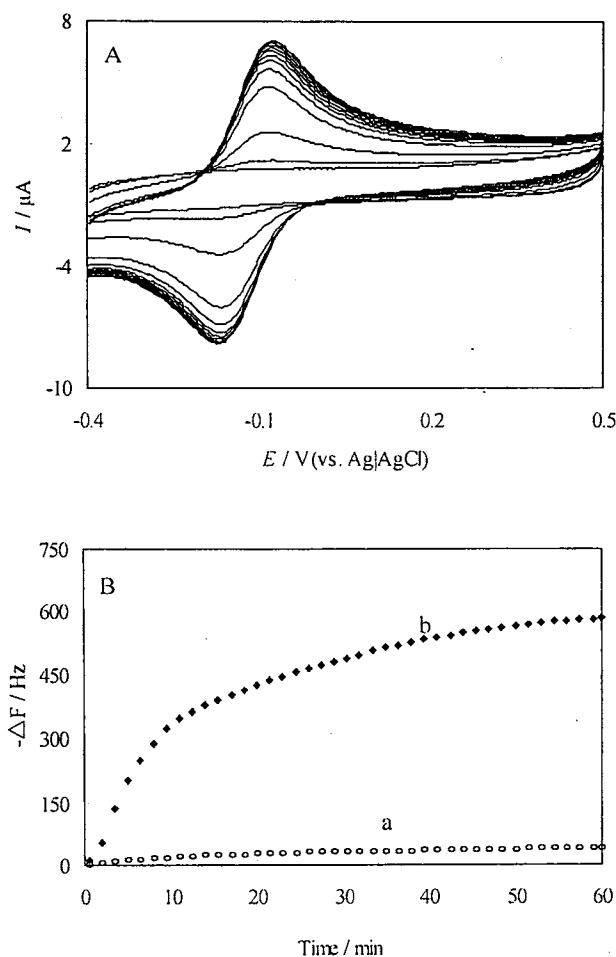


Fig. 2. (A) Cycle voltammograms of DNA-PADDA film in a 0.05 M PBS (pH 7.4) containing 2×10^{-4} M Fe(4-TMPyP) obtained at different soaking times (each 5 minutes) from inner to outside. Scan rate, 200 mV s^{-1} . (B) *In-situ* monitoring of the frequency decreases ($-\Delta F$) with the time for the immobilization of 2×10^{-4} M Fe(4-TMPyP) in pH 7.4 PBS onto (a) a bare Au/quartz crystal, and (b) a DNA-PADDA modified Au/quartz crystal electrode.

Figure 2A shows the electrochemical response of DNA-PADDA film on the PGE in 2×10^{-4} M Fe(4-TMPyP) PBS (pH 7.4), performed by consecutive cyclic voltammetry between potentials of -0.4 and $+0.5$ V. As can be seen, the growth of the cyclic voltammogram current in Fig. 7 shows $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of Fe(4-TMPyP) with formal potential, E^0 , of -0.13 V vs. Ag|AgCl, which was slightly higher than that determined in the same pH solution on bare PGE.

This electrochemical result is consistent with the QCM data (Fig. 2B). On a bare Au/quartz crystal electrode, no significant frequency change was observed on the QCM electrode. However, the DNA-PADDA modified Au/crystal electrode showed an obvious frequency decrease. The change was attributed to the entrapping of Fe(4-TMPyP) in DNA-PADDA film. In the first stage a sharp decrease of the frequency was observed. The first-order rate constant was obtained by curve fitting as a characteristic time of immobilization, τ , of 15 min. The immobilization rate constant was 34 ng min^{-1} . The total change in the frequency corresponded to an accumulation of $0.65 \text{ } \mu\text{g}$ in the film after interaction with Fe(4-TMPyP) for 60 min toward a saturation value.

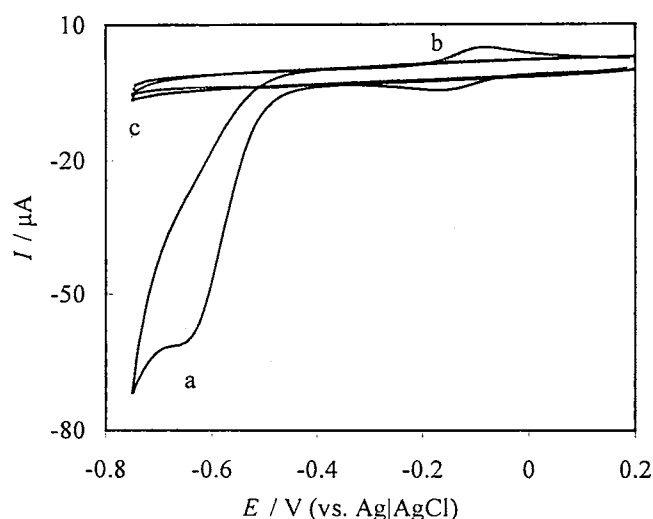


Fig. 3. Cyclic voltammograms of FePyDP film modified PG electrode in 50 mM PBS (pH 7.4) (a) with 1.8 mM NO and (b) with 100 μM NO_2^- . Curve (c) is obtained in pure PBS at DNA-PADDA film with 1.8 mM NO. Scan rate, 200 mV s^{-1} .

At DNA-PADDA film modified electrode the voltammogram of NO in pH 7.4 PBS showed a small current at -0.90 V (Fig. 3c). A large reductive current was observed in the presence of NO at FePyDP film modified electrode, while the redox peaks of Fe(4-TMPyP) disappeared (Fig. 3a). The large reductive current resulted from the catalytic reduction of NO by the immobilized Fe(4-TMPyP), and peak current ratio was ca. 15 for the peak current vs. the noncatalytic $\text{Fe}^{\text{III/II}}$ reduction peak at the saturation concentration of 100 % NO (1.8 mM). The above results indicated a high catalytic activity of iron porphyrin for NO reduction at FePyDP film modified electrode. More interestingly, an addition of nitrite into pure Fe(4-TMPyP) solution caused little change in the cyclic voltammogram. This indicated that nitrite could not be reduced at FePyDP film modified electrode in this potential range. As a conclusion, it was clarified that FePyDP film exhibited selective catalytic reduction of NO against NO_2^- .

Conclusions

A stepwise catalytic oxidation of nitric oxide and nitrite by water-soluble Fe^{III} (4-TMPyP) was first revealed by using an ITO electrode. $\text{O}=\text{Fe}^{\text{IV}}$ (4-TMPyP) showed an effective catalysis towards NO oxidation and an excellent selectivity for NO against nitrite. The electrogenerated $\text{O}=\text{Fe}^{\text{IV}}$ (4-TMPyP) π -cation radical effectively catalyzed the oxidation of NO_2^- by a two-step process to give nitrate as a final product.

On the other hand, we prepared and characterized a novel porphyrin film, Fe(4-TMPyP)-DNA-PADDA (FePyDP) film, through the supramolecular assembly of cationic porphyrins into DNA matrix and provide new insights on the water-soluble porphyrin modified electrode as NO biosensor. The modified electrode displayed an excellent catalytic activity for NO reduction at -0.61 V vs. Ag|AgCl via a CEC electrocatalytic mechanism and NO oxidation at $+0.89$ vs. Ag|AgCl mediated by oxoiron(IV) porphyrin complexes via a typical chemical catalysis, respectively.

References

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学位論文審査結果の要旨

本学位論文に関して、平成 16 年 7 月 23 日に第 1 回の学位論文審査会を開催し、提出された論文および関連資料について検討した。ついで、平成 16 年 7 月 28 日の口頭発表後、第 2 回審査委員会を開催し、審議の結果、以下のとおり判定した。

本論文は、電気化学的手法により生体中の微量一酸化窒素を選択的に検出することを目的とし、水溶性鉄-ポルフィリン (iron (III) meso-tetrakis (N-methylpyridinium-4-yl) porphyrin, Fe (4-TMPyP)) を用いて得た種々の成果をまとめたものである。すなわち、Fe (4-TMPyP) は電位によって、異なる高原子価錯体を生成し、オキソ鉄 (IV) 錯体は一酸化窒素のみの酸化を、オキソ鉄 (IV) π -カチオンラジカルは亜硝酸イオンの酸化を触媒する機構を、ITO (Indium-Tin Oxide) 電極を用いて始めて明らかにした。これにより、亜硝酸イオンが共存していても電位をコントロールすることにより一酸化窒素の選択的検出が可能となることを指摘した。また、この正に荷電した鉄-ポルフィリンを DNA 膜中に固定した電極を工夫し、DNA 膜中でこの鉄-ポルフィリンの酸化還元反応が溶液中と同じように進行し、DNA の塩基対を経由する電子トンネリングの可能性を明らかにするとともに、鉄 (II) 錯体を利用した一酸化窒素の還元的検出およびオキソ鉄 (IV) による一酸化窒素の酸化的検出が高選択的に行えることを実証した。

以上のように本論文は関連分野の発展に大きく貢献する成果を挙げていることから、博士 (理学) の学位を与えるに十分値するものと考え、審査員一致で合格と判定した。