Selective Oxidation of Nitric Oxide against Nitrite by Oxo-iron(IV) Porphyrin at an ITO Electrode

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Electrochemical and spectroelectrochemical experiments at an ITO electrode clarified that O=Fe^{IV}(4-TMPyP) was formed via 1e$^-$ + 1H$^+$ oxidation process of Fe^{III}(4-TMPyP) in pH 7.4 PBS and the electrogenerated O=Fe^{IV}(4-TMPyP) selectively reacted with NO to give NO_2^- as the final product through an electrocatalytic cycle.

It is now well known that nitric oxide (NO) plays fundamental roles as a cell-signal molecule, anti-infective agent and an antioxidant in many biological processes.[1] Most of the above phenomena can be explained by the binding of NO to lower valent Fe^{III} or Fe^{II} porphyrins. However, recent researches suggested that high-valent porphyrins are important biological transients in numerous hemoproteins.[2] For example, reduction of ferrylmyoglobin (O=Fe^{IV}Mb) and ferrylhemoglobin (O=Fe^{IV}Hb) by nitric oxide is considered as a protective process against ferryl hemoprotein-induced oxidations in inflammation cells.[3]

Most of oxoiron(IV) porphyrins have been generated by chemical oxidants such as hydrogen peroxide, iodosylbenzene, and hypochlorites.[4] On the other hand, the direct electrochemical oxidation of iron(III) porphyrins to oxoiron(IV) species has also been reported.[5] many oxoiron(IV) porphyrins are known to electrocatalytically oxidize organic substrates[6] and some anions.[7] However, the electrocatalytic oxidation of NO by oxoiron(IV) porphyrin has not yet been studied thoroughly. In the present study, we studied from the standpoint of electrochemistry on the role of high-valent O=Fe^{IV}(4-TMPyP) (4-TMPyP=-*meso*-tetrakis(*N*-methyl-pyridinium-4-yl)porphyrin) in NO oxidation, and found that O=Fe^{IV}(4-TMPyP) showed an effective catalysis towards NO oxidation and an excellent selectivity for NO against nitrite.

Electroconductive and optically-transparent ITO(indium tin oxide)-coated glass was used as the test electrode. The resistance of the ITO was about 10 Ωcm^{-2}. The optically transparent thin layer electrode (OTTLE) cell was prepared by using two ITO glass plates (4 × 1 cm), and the light pass length was about 0.2 mm. NO-saturated solution was prepared by bubbling 1% or 5% NO-Ar mixed gas (Nippon Sanso) into 50 mM phosphate buffer solution (PBS). Saturated concentration of NO has been estimated from Ostwald's[8] solubility coefficient for a given partial pressure of NO.

Electrochemical generation of O=Fe^{IV}(4-TMPyP) has been confirmed by oxidation of Fe^{III}(4-TMPyP) in an OTTLE cell at room temperature. Figure 1 shows the spectral change of Fe^{III}(4-TMPyP) at different potentials. Fe^{III}(4-TMPyP) exhibits λ_{max} at 423, 600, and 630 nm, while the oxidized form exhibits λ_{max} at 426, 517, and 553 nm, also clear isosbestic points were observed at 409, 445, 518, and 572 nm. All these values were in good agreement with those observed for electrochemical generation of O=Fe^{IV}(4-TMPyP) in an alkaline solution.[7] A plot of log[O=Fe^{IV}(4-TMPyP)]/[Fe^{III}(4-TMPyP)] vs $E_{appl.}$ with a slope of 60 mV suggested that the number of electrons transferred, n, is 1. The pH dependence of the formal potential ($E^{o'}$) between 6.0 and 8.8 was found to be −60 mV/pH (inset of Figure 1). These results indicate that O=Fe^{IV}(4-TMPyP) was formed via 1e$^-$ + 1H$^+$ process as shown in Eq 1.

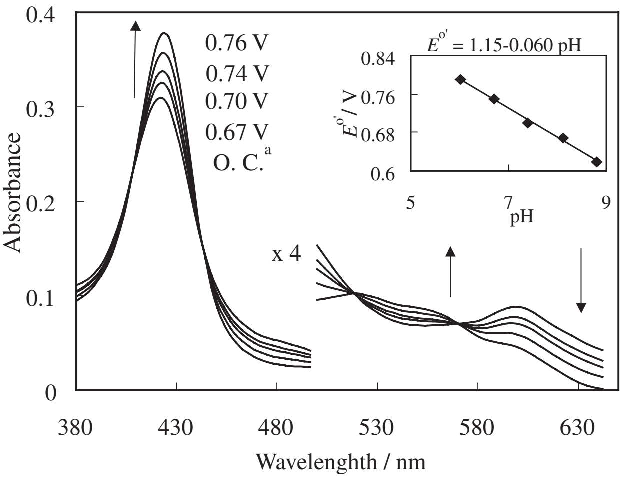


Figure 1. Typical absorption spectra of 0.2 mM Fe^{III}(4-TMPyP) in an OTTLE cell at different applied potentials. The inset shows the Nernst plot of $E^{o'}$ vs pH. ^{a}O.C. means open circuit potential.

$$\text{HO-Fe}^{III}\text{(4-TMPyP)-e}^- \rightarrow \text{O=Fe}^{IV}\text{(4-TMPyP)} + \text{H}^+ \quad (1)$$

Further, $E^{o'}$ at a physiological pH (7.4) was estimated to be 0.71 V vs Ag|AgCl.

Figure 2 shows cyclic voltammograms (CVs) of NO and Fe^{III}(4-TMPyP) in pH 7.4 PBS at an ITO electrode. The direct oxidation currents of NO (Figure 2d) is small because of the large overpotential on hydrophilic ITO electrode, but the presence of Fe^{III}(4-TMPyP) (Figure 2a) causes a significant current increase. The enhanced currents are larger than a simple sum of oxidation currents in Figures 2b and 2d. A comparison of Figure 2a with Figure 2b clarifies that the oxidation of NO proceeds along with the oxidation of Fe^{III}(4-TMPyP). These results suggest the electrocatalytic properties of Fe^{III}(4-TMPyP) towards NO oxidation through an E-C mechanism.

Interestingly, an addition of nitrite into pure Fe^{III}(4-TMPyP) solution causes little change in the cyclic voltammograms (Figures 2b and 2c). This indicates that nitrite can not be oxidized by O=Fe^{IV}(4-TMPyP). Figure 2b showed further

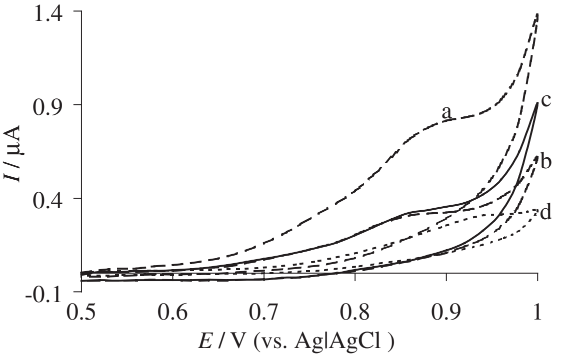


Figure 2. Cyclic voltammograms of 20 μM Fe^{III}(4-TMPyP) at an ITO electrode in 50 mM PBS with pH 7.4, (a) with and (b) without 18.3 μM NO, and (c) with 20 μM NO_2^-. Curve (d) was obtained in pure PBS with 18.3 μM NO. Scan rate, 10 mV s^{-1}.

oxidation of $O{=}Fe^{IV}$(4-TMPyP) above 0.9 V. It was confirmed by the spectroelectrochemistry that the oxidized species at 1.1 V was very unstable and degraded into non-porphyrinic species. Such an oxidized specie is speculated to be $O{=}Fe^{IV}$(4-TMPyP) π-cation radical, corresponding to so-called Compound I in horse radish peroxidase.

Figure 3 shows the reactivity of electrogenerated $O{=}Fe^{IV}$(4-TMPyP) for NO and NO_2^-. $O{=}Fe^{IV}$(4-TMPyP) was quickly reduced back to Fe^{III}(4-TMPyP) by the addition of NO (Figure 3a). However, the addition of nitrite (Figure 3c) results in a slow decay rate of $O{=}Fe^{IV}$(4-TMPyP). The initial slope in the decay curve corresponds to the reduction rate of $O{=}Fe^{IV}$(4-TMPyP). The ratio of the slopes between NO and NO_2^-, which were corrected for the natural decay (Figure 3b), was roughly 120. This value is almost the same as estimated ratio of free NO concentration, 128, in the solutions of NO and NO_2^-, because 1 mol% nitrite even in pH 7.4 can be disproportinated into nitrate and NO (0.64 mol%).[9] In this sense, $O{=}Fe^{IV}$(4-TMPyP) can not oxidize nitrite. Further, product analysis[10] showed that the concentration of nitrite was directly proportional to the amount of NO added into $O{=}Fe^{IV}$(4-TMPyP) solution. This indicated that nitrite is formed as the final product of NO oxidation by $O{=}Fe^{IV}$(4-TMPyP).

Catalytic oxidation cycle of NO is shown in Scheme 1. In our experiments, $O{=}Fe^{IV}$(4-TMPyP) selectively oxidizes NO radical against NO_2^- anion. This suggests that NO radical attacks the negatively charged oxygen interacting with iron site, and an electron on the oxygen atom transfers to Fe^{IV} site by a synchronous process. On the other hand, it was observed that the catalytic current for NO oxidation was increased with increasing pH and the presence of NO_2^- gave no change in the spectrum of Fe^{III}(4-TMPyP). These results indicate that the intermediate ONO-Fe^{III}(4-TMPyP) in Scheme 1 is not so stable and is easily replaced by OH^- to regenerate original HO-Fe^{III}(4-TMPyP). Although present $O{=}Fe^{IV}$(4-TMPyP) could not oxidize NO_2^-, the reaction between oxoiron(IV) TMPS and NO_2 radical has been reported to be quite fast.[11] These results suggest that $O{=}Fe^{IV}$(4-TMPyP) has excellent selectivity for NO radical oxidation. $O{=}Fe^{IV}$(4-TMPyP) π-cation radical is speculated to participate in the nitrite oxidation, because the redox potential of $O{=}Fe^{IV}$(4-TMPyP) π-cation radical is higher than that of $O{=}Fe^{IV}$(4-TMPyP). Further work is in process.

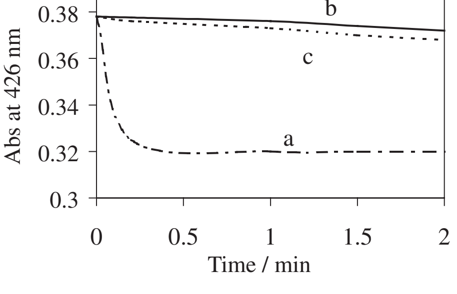
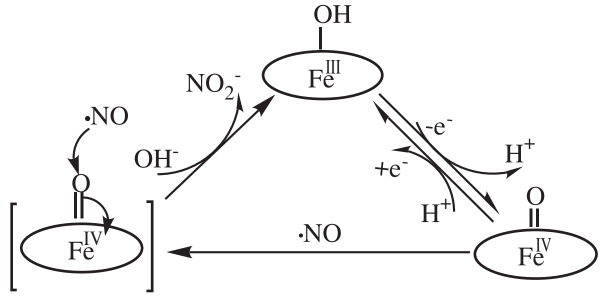


Figure 3. UV–vis spectral change at 426 nm monitored for the decay of electrogenerated $O{=}Fe^{IV}$(4-TMPyP) to Fe^{III}(4-TMPyP) in 50 mM PBS (a) with and (b) without 82.2 μM NO, and (c) with 100 μM NO_2^-.



Scheme 1. Proposed reaction mechanism for the catalytic oxidation cycle of NO by oxoiron(IV)porphyrin.

In conclusion, the present study clearly demonstrates the catalytic activity and selectivity of $O{=}Fe^{IV}$(4-TMPyP) for nitric oxide oxidation under physiological conditions. These observations well lead to possible development of highly selective NO biosensor and further explore the significance of NO upon Fe^{IV}/Fe^{III} redox couple in biological system.

References and Notes

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