Spectroscopic and Electrochemical Response to Nitrogen Monoxide of a Cationic Iron Porphyrin Immobilized in Nafion-coated Electrodes or Membranes

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Nafion membranes containing immobilized Fe^{III} tetrakis(*N*-methyl-4-pyridyl)porphyrin show spectroscopic changes in the UV–VIS range after exposure to gaseous NO, indicating the formation of a nitrosyl complex; electroreduction of NO is mediated by this metalloporphyrin when immobilized in Nafion-coated glassy-carbon electrodes and the use of these film electrodes for the amperometric determination of NO in aqueous solutions is demonstrated.

The chemistry of nitrogen monoxide (NO) involves a redox array of species with distinctive properties: NO^+ (nitrosonium), $NO^·$ (nitric oxide) and NO^- (nitroxyl anion). The NO molecule readily forms complexes with transition metal ions, including those found in metalloproteins and haem-containing proteins, such as haemoglobin.[1] The biochemical reduction of nitrite to ammonia that occurs in green plants is a six-electron, seven-proton process catalysed by the nitrite reductase enzymes.[2] The biocatalysis has been mimicked synthetically by using water-soluble iron porphyrins.[3] Moreover, investigations of spectroscopic and electrochemical properties of these systems in the presence of nitrite ions reveal that key intermediates in the metalloporphyrin-mediated reduction of nitrite are nitrosyl complexes.[4] This can be exploited for the development of metalloporphyrin-film electrodes for the detection and quantitative determination of nitrite ions as previously demonstrated[5] and NO as shown in the present study. This latter use is important in view of the implication of the NO molecule and its interrelated redox forms in a number of diverse physiological processes, including muscle relaxation, neurotransmission and immune regulation.[6] A recent publication deals with an NO microsensor in which oxidation of this molecule is mediated by a nickel hydroxo-porphyrin.[7] The present report describes the reduction steps of NO mediated by a cationic iron porphyrin immobilized in a cation-exchange polymer and are compared with those reported in the literature for dissolved porphyrins. Use of these films for the quantitative determination of NO in aqueous solutions is also demonstrated.

Nafion is a negatively charged perfluorinated polysulfonate membrane (Aldrich, 0.17 mm thick) which is capable of attaching to the positively charged Fe^{III} tetrakis(*N*-methyl-4-pyridyl)porphyrin [Fe^{III} TMPy**P**$^{5+}$]. This was achieved by dipping the membrane for 30 min in a 0.5 mol dm^{-3} H_2SO_4 solution containing 2×10^{-4} mol dm^{-3} [Fe^{III} TMPy**P**$^{5+}$]$(Cl^-)_5$, washing the membrane with water and then drying in air. The UV–VIS spectrum obtained for the membrane with immobilized FeTMPy**P** shows two Soret bands at 404 and 427 nm and a peak at 518 nm. Exposure of the membrane to an NO flow (10 cm^3 min^{-1}; 30 min) causes the disappearance of the 404 and 427 nm bands and the appearance of a new band at 439 nm with an approximate 20% increase in molar extinction coefficient (compared with those of the Soret bands before NO exposure). The 518 nm band is shifted to 547 nm after NO exposure.

Nafion-coated glassy carbon (g.c.) electrodes were prepared by spreading 20 μl of a 2% Nafion polymer solution (dilution of the 5% Aldrich solution with ethanol) over the electrodes (A = 0.03 cm^2), followed by drying in air. The porphyrin film electrodes were prepared by dipping the g.c./Nafion film electrodes in the acidic Fe^{III} TMPy**P** solution. Fig. 1(*a*) shows a typical differential pulse voltammogram obtained for a Nafion+FeTMPy**P**/g.c. film electrode in a deaerated Na_2SO_4 + potassium phthalate (KHP) buffer solution at pH 2.5 (full curve). The two waves, at potentials of +0.1 and −0.2 V are suggested to be due to Fe^{III} reduction. Splitting of the Fe^{III}/Fe^{II} wave has been reported for a variety of complexes in Nafion films and it has been suggested that molecules (depending on charge and concentration) are located both in the ionic cluster and interfacial regions of the films.[8] The reduction wave with E_p = −0.66 V and the current increase at potentials more cathodic than −1 V are results that are usually attributed to ligand reduction.

NO additions were accomplished by adding controlled volumes of an NO saturated aqueous solution to the KHP buffer solution (final concentration was calculated using the reported solubility of NO in water, disregarding salt effects). The effect of adding 0.1 mmol dm^{-3} NO in the KHP solution

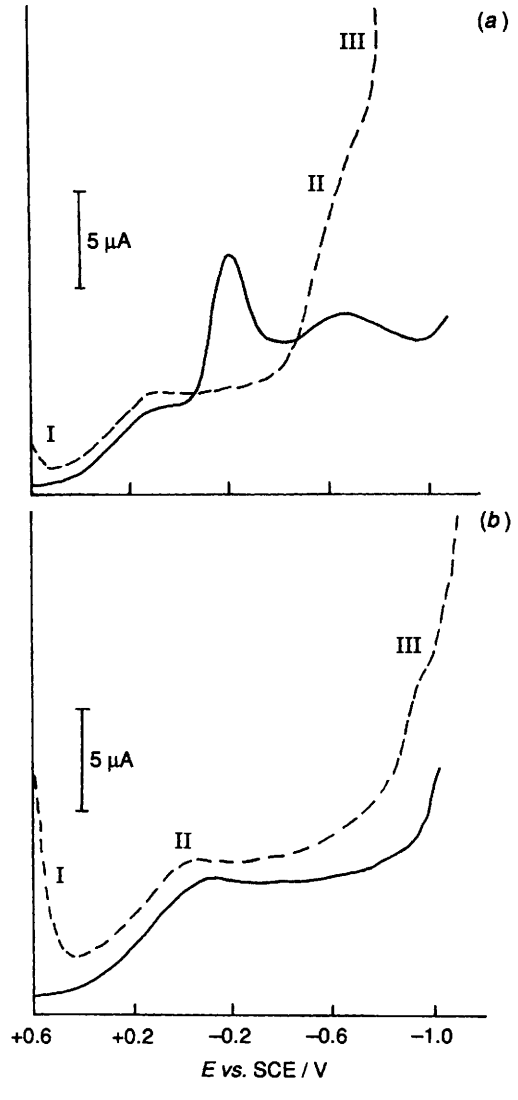

Fig. 1 Differential pulse voltammograms (scan rate: 10 mV s^{-1}; modulation amplitude: 25 mV) for a Nafion + FeTMPy**P**/g.c. film electrode in a deaerated 0.1 mol dm^{-3} Na_2SO_4 + 5×10^{-2} mol dm^{-3} KHP solution at pH 2.5 (*a*) and pH 4 (*b*), before and after introducing 0.1 mmol dm^{-3} NO (full and dashed curves, respectively)

at pH 2.5 is also shown in Fig. 1(*a*) (dashed curve). Three new reduction regions are observed: at $E > +0.5$ V, $E_{1/2} = -0.6$ V and $E < -0.8$ V (I, II and III, respectively). The Fe^{III} reduction wave at -0.2 V almost completely disappears after adding NO, suggesting the formation of a nitrosyl complex, $Fe^{II}(NO^+)$TMPyP, as also supported by the UV–VIS changes when exposing the Nafion membrane to NO. As also suggested for dissolved iron porphyrins,[3] electroreduction of the $Fe^{II}(NO^+)$, $Fe^{II}(NO^{\cdot})$ and $Fe(NO^-)$ complexes occurs in potential regions I, II and III, respectively. The smaller Fe^{III}/Fe^{II} wave at +0.1 V remains almost unchanged after NO addition, for a reason which is still unclear.

At pH 4, the differential pulse voltammogram for a Nafion + FeTMPyP/g.c. film electrode in the absence of NO [full curve, Fig. 1(*b*)] shows one Fe^{III}/Fe^{II} wave with $E_p = -0.1$ V, indicating that splitting of this wave occurs only at low pH. Ligand reduction occurs at $E < -1$ V. Electroreduction of the iron-NO complexes occurs at $E > +0.5$ V, $E_p = -0.04$ V and

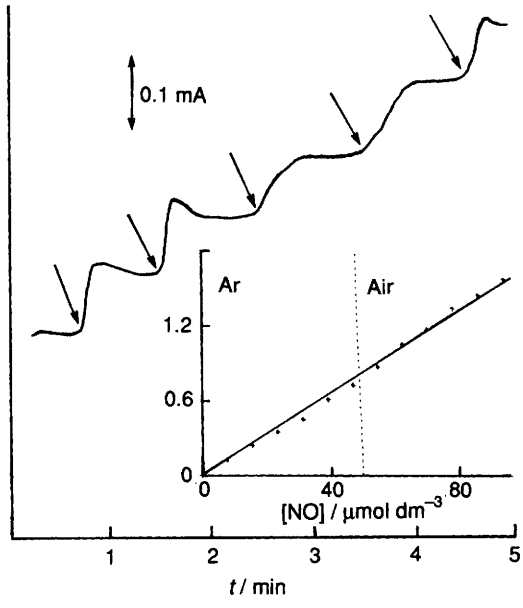

Fig. 2 Typical amperometric responses obtained at +0.7 V for a Nafion + FeTMPyP/g.c. film electrode in a deaerated 0.1 mol dm^{-3} Na_2SO_4 + 5 × 10^{-2} mol dm^{-3} KHP solution at pH 4 to successive NO additions of 10 μmol dm^{-3} NO. Inset: current at +0.7 V *vs.* NO concentration in deaerated as well as air-saturated solutions.

at the -1 V region [I, II and III, respectively in Fig. 1(*b*), dashed curve]. This seems to indicate a similar mechanism to that suggested for pH 2.5. However, since the current of the Fe^{III}/Fe^{II} wave at pH 4 remains almost unchanged after NO addition (although a positive 60 mV shift of E_p is observed), this seems to indicate that electroreduction of the nitrosyl complex (at region I) forms an $Fe^{III}(NO^-)$ complex, rather than $Fe^{II}(NO^{\cdot})$ as suggested at low pH. These two possible formulations have been suggested for nitrite reduction mediated by dissolved FeTMPyP.[3]

Fig. 2 shows the Nafion + FeTMPyP/g.c. electrode response to successive additions of NO in a KHP solution at pH 4 and at a constant potential of +0.7 V. This relative positive potential, corresponding to nitrosyl electroreduction, was chosen in order to prevent the interference of dioxygen reduction catalysed by Fe^{II} porphyrin. The response time is less than 3 s for [NO] < 20 μmol dm^{-3} and increases to about 10 s at higher concentrations. The current is linearly proportional to NO concentrations as low as a few μmol dm^{-3} and as high of a few tenths of mmol dm^{-3}. Moreover, it can be seen that the presence of dioxygen (introduced after the addition of 50 μmol dm^{-3} NO in a deaerated solution) has no effect on the linear response of the electrode (inset of Fig. 2). Although the characteristics of the electrodes, including response time at low NO concentrations (the biological halflife of NO is generally assumed to be a few seconds) and linearity in the absence and presence of dioxygen make them good candidates as electrochemical sensors in tissue and cell culture studies.

Received, 17th September 1993; Com. 3/05628G

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