COMMUNICATION www.rsc.org/chemcomm | ChemComm

# Electroreduction of nitrite on gold electrode modified with Cu-containing nitrite reductase model complex†

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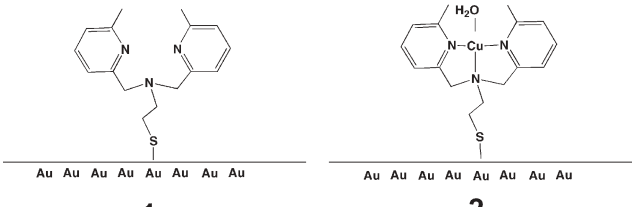
*Received (in Cambridge, UK) 7th June 2005, Accepted 22nd July 2005*
*First published as an Advance Article on the web 9th August 2005*
**DOI: 10.1039/b507932b**

**A gold electrode modified with copper complexes containing a tridentate aromatic amine compound (bis(6-methyl-2-pyridylmethyl)amine ethyl sulfide, which is a model for the nitrite reduction centre of copper-containing nitrite reductase, catalyzed electrochemically the reduction of nitrite to nitrogen monoxide under acidic conditions.**

The nitrogen cycle has received much attention in recent years because of its ecological importance concerning the widespread agricultural use of potentially polluting nitrate and nitrite. Inorganic nitrogen is introduced into the biosphere by biological fixation of dinitrogen and removed from there again by denitrification. Denitrification is the dissimilatory reduction of nitrate ($NO_3^-$) or nitrite ($NO_2^-$) to produce dinitrogen by prokaryotic organisms. It is part of the bioenergetic apparatus of the cell of denitrifying bacteria which occupy a wide range of natural habitats including soil and water. Nitrite reductase, a key enzyme of denitrification, catalyzes the reduction of nitrite to nitrogen monoxide.[1] Copper-containing nitrite reductase (CuNIR) has two kinds of Cu centres per subunit. The type 1 Cu accepts an electron from an external electron donor protein, and the type 2 Cu, which accepts an electron from the reduced type 1 Cu site, is the reduction centre of nitrite. The type 2 Cu site showing a distorted tetrahedral geometry is bound by three His residues and one solvent water. Uptake of nitrite at the type 2 copper appears to involve displacement of water. Subsequently, the reduction of nitrite to NO proceeds with attack of protons on the nitrite bound to Cu site and a concomitant electron transfer reaction from the type 1 copper.[2] Several Cu(II) complexes have been so far reported as models of the CuNIR active site.[3,4] Recently, we have reported that $[\mathrm{Cu(II)Me_2bpa}]^+$ ($\mathrm{Me_2bpa}$: bis(6-methyl-2-pyridylmethyl)-amine) catalyzes the reduction of nitrite with acid to produce NO effectively.[4] The coordination modes of the nitrite ligands in $\mathrm{CuMe_2bpa}$ complexes depend on the oxidation state of copper ion; nitrite is coordinated to Cu(II) through two oxygen atoms (*O*,*O′*-coordination mode) and to Cu(I) through one nitrogen atom (*N*-coordination mode). In this study, we prepared a gold electrode modified with $\mathrm{CuMe_2bpa}$ complex, and investigated the electrochemical reduction of nitrite to NO on the modified electrode.

To prepare the $\mathrm{Me_2bpaEtS}$ monolayer on the gold surface (**1** in Scheme 1) a well-polished and ultrasonically cleaned Au electrode (od 1.6 mm, BAS, USA) was immersed in a 50 mM solution of thiol-derivatized ligand[5] $(\mathrm{Me_2bpaEtS})_2$ in 1 : 3 $H_2O$–methanol for 10 min at room temperature, and then the electrode was sufficiently rinsed with the mixed solvent, distilled water, and methanol to remove the unbound ligand molecules. The insertion of copper ion was performed by soaking the ligand monolayer on the gold surface in 10 mM $\mathrm{Cu(ClO_4)_2}$ aqueous solution for 30 min at room temperature. The surface was thoroughly washed with distilled water to remove excess free Cu ion. In the X-ray photoelectron spectrum (XPS, SHIMADZU ESCA-850) of the Cu complex on Au electrode (**2** in Scheme 1) the signals of the sulfur 2p and copper $2p_{3/2}$ photoelectrons were observed at 163 and 932 eV,[6] respectively.† The results suggest that $\mathrm{CuMe_2bpaEtS}$ is bound to the Au surface by sulfur. The copper : sulfur ratio in **2** of 1:1.0 ± 0.1 indicated that copper ion was quantitatively coordinated to **1**.

The cyclic voltammograms of **2** as working electrode in 100 mM $\mathrm{NaClO_4}$ aqueous solution ($E_{1/2}$: +294 mV *vs.* Ag/AgCl) are presented in Fig. 1. The surface coverage of **2** on the gold surface was determined to be $9.0 \times 10^{-11}$ mol cm$^{-2}$ by integrating the charge under the redox wave at $E_{1/2}$. This is consistent with the theoretical value expected from close-packed monolayers, $1.2 \times 10^{-10}$ mol cm$^{-2}$, assuming a molecular area of *ca.* 132 Å$^2$ based on X-ray crystallographic data of $[\mathrm{Cu(II)Me_2bpa(ClO_4)}]^+$.[7] After the addition of $\mathrm{NaNO_2}$ to the $\mathrm{NaClO_4}$ solution, the half-peak potential was shifted to +140 mV (**b** in Fig. 1). The result implies that the fourth ligand of the Cu complex bound on the gold electrode was replaced by nitrite. As the redox potentials of $\mathrm{CuMe_2bpaEt}$ in the same condition are +41 and −35 mV in the absence and presence of nitrite, respectively, the gold matrix was found to affect the redox potentials of the Cu complexes. By the further addition of acid ($\mathrm{HClO_4}$) to the $\mathrm{NaClO_4}$ solution containing nitrite (final pH 5.3), the shape of the voltammogram is dramatically changed and an enhanced sigmoidal cathodic current–potential curve (catalytic current) was observed (**c** in



Scheme 1

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† Electronic supplementary information (ESI) available: XPS core level spectra for $\mathrm{CuME_2bpaEt}$ and **2** on Au surface. See http://dx.doi.org/10.1039/b507932b

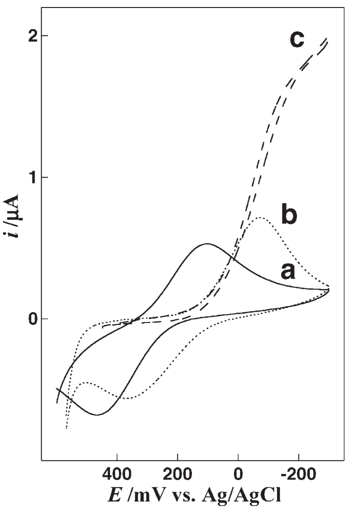

**Fig. 1** Voltammetric behaviour of **2** as working electrode (**a**) in 100 mM $NaClO_4$ aqueous solution at 25.0 °C and scan rate of 10 mV $s^{-1}$ (counter electrode: Au wire, reference electrode: Ag/AgCl), (**b**) after addition of 500 mM $NaNO_2$ to the electrolyte solution **a**, and (**c**) after addition of $HClO_4$ to **b** for adjustment of pH to 5.3.

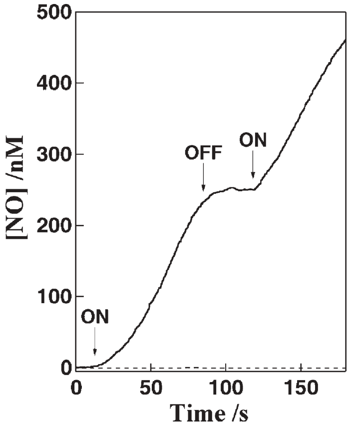

**Fig. 2** NO generated upon electron supply on the modified electrode **2** at −200 mV in the solution (2 ml) of 100 mM $NaClO_4$ and 500 mM $NaNO_2$ at pH 5.3 and 25.0 °C. Solid line: electron supply, broken line: no electron supply.

Fig. 1). The appearance of the catalytic current indicates the regeneration of the oxidized Cu complex on the gold surface by the reduction of nitrite ligand.[4]

The generated NO was measured directly in real time with an NO electrode (World Precision Instruments, USA). Fig. 2 shows that NO was not generated in $NaClO_4$ solution at pH 5.3 with 500 mM $NaNO_2$ as indicated by the line with zero slope prior supply of electron. On supply at −200 mV *vs.* Ag/AgCl on the gold electrode modified with $CuM_2bpaEtS$ (**2**), linear generation of NO was observed, which stopped when the electron supply was turned off. The subsequent electron supply on the electrode at −200 mV triggered further NO generation until the electron supply was turned off. The rate of NO generation was 4.2 nM $s^{-1}$. The current efficiency and the turnover number of Cu complex on the gold electrode for the reduction of nitrite to NO were estimated to be *ca.* 94% and $2.3 \times 10^3$ $s^{-1}$, respectively.[8] A continuous supply of electrons, however, causes a decrease in current efficiency to 50% for 6 min. When the bare and ligand only (**1**) modified gold electrodes were used as working electrodes, no NO generation was detected at all.

In conclusion, the electroreduction of nitrite to NO on the gold electrode modified with Cu complex, which is a model for the nitrite reduction centre of CuNIR, was observed under acidic conditions. The results suggest that the modification of an electrode by metal complexes would be useful for the catalytic redox reaction of substrate, such as nitrite. Furthermore, the electrode might be applicable to detection of nitrite in some environments and electrochemically controlled generation of NO as a vasodilator, an anticancer agent, and a neurotransmitter in living bodies. The substrate selectivity is being investigated and the improvements of the modified electrode are in progress.

We thank Dr Keita Kobayashi and Dr Hiroshi Nakao (Sakai Chemical Industry Co., LTD.) for XPS measurement. This work was supported by Mitsubishi Chemical Corporation Fund, The Association for the Progress of New Chemistry, and Grant-in Aid for Scientific Research on Priority Areas (No. 17036037 "Chemistry of Coordination Space" to K.Y.) from Ministry of Education, Science, Sports and Culture, Japan.

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8 When nitrite was reduced electrochemically at −200 mV on the gold electrode modified with $CuM_2bpaEtS$ (**2**) in 2 ml solution containing 100 mM $NaClO_4$ and 500 mM $NaNO_2$ at pH 5.3 and 25.0 °C for 187 s, cathodic charge flow and generated NO concentration were observed to be $1.61 \times 10^{-4}$ C and $7.82 \times 10^{-7}$ M, respectively. Accordingly, the amounts of supplied electron and generated NO were calculated to be $1.66 \times 10^{-9}$ mol ($=1.61 \times 10^{-4}$ C/96 485 mol $C^{-1}$) and $1.56 \times 10^{-9}$ mol ($=7.82 \times 10^{-7}$ M $\times$ 0.002 L), respectively. The current efficiency (*ca.* 94%) was estimated from the calculation ($1.56 \times 10^{-9}$ mol/ $1.66 \times 10^{-9}$ mol). Meanwhile, as the surface coverage of **2** on the gold surface (od1.6 mm) was $9.0 \times 10^{-11}$ mol $cm^{-2}$, the amount of Cu complex was estimated to be $1.8 \times 10^{-12}$ mol. Therefore, turnover number of Cu complex on the gold electrode for the reduction of nitrite to NO was calculated to be $2.3 \times 10^3$ $s^{-1}$ ($=4.2$ nM $s^{-1}/1.8 \times 10^{-12}$ mol).