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# Direct Electrochemistry of Copper-Containing Nitrite Reductase from *Achromobacter xylosoxidans* NCIB 11015

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Nitrite reductase containing type I and type II coppers from *Achromobacter xylosoxidans* NCIB 11015 shows the direct electrochemical response corresponding to the type I copper site at a di-(4-pyridyl) disulfide (4-pyds) modified gold electrode.

Direct electrochemistry of several redox enzymes has been studied in recent years.[1] Direct electrochemistry of redox enzymes provides static aspects, such as redox potentials and the very important information to understand the mechanism of redox catalysis because the promoter induced electrode surface is resemble to the protein surface composed of functional groups, polar and non-polar amino-acid residues.[2] Very recently, Azab et al. reported the direct electrochemistry and the reactivities of "non-blue" copper-containing mammalian superoxide dismutase.[3]

Nitrite reductase from *Achromobacter xylosoxidans* NCIB 11015 reduces nitrite ion to nitrogen oxide as a key enzyme of the anaerobic nitrate respiration chain.[4] Figure 1 and 2 shows the electronic absorption and EPR spectrum of the reductase, respectively. Nitrite reductase (monomer; 35 kDa) contains two types of copper, type I (blue; $g_1$ = 2.20) and type II (non-blue; $g_2$ = 2.34),[5] and the type I site shows an intense absorption band at 593 nm ($\varepsilon$ 3700 $M^{-1}$ $cm^{-1}$), which from investigations on Co(II)-substituted protein is assigned to be a cystein $S^- \rightarrow$ copper(II) charge transfer.[6] Godden et al. reported the three-dimensional structure of *A. cycloclastes* nitrite reductase containing type I and type II coppers, which exits as a trimer of the three identical subunits.[7]

We here report the observation of direct electrochemical response of type I copper in nitrite reductase

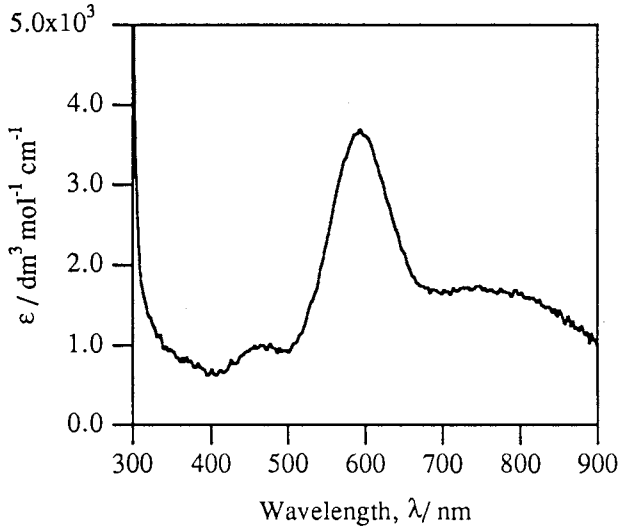


Fig. 1. Electronic absorption spectrum of nitrite reductase at pH 7.0 (0.1 mol $dm^{-3}$ phosphate).

from *Achromobacter xylosoxidans* NCIB 11015 at a 4-pyds modified gold electrode.

Nitrite reductase from *Achromobacter xylosoxidans* NCIB 11015 was purified to $A_{280}/A_{593}$ ratio of 12.6 by previous method.[8] Protein homogeneity was checked by polyacrylamide gel electrophoresis, and the specific enzymatic activity was evaluated to be 230 μmol / min mg as the consumption of nitrite by one mg of the enzyme per one minute.[9]

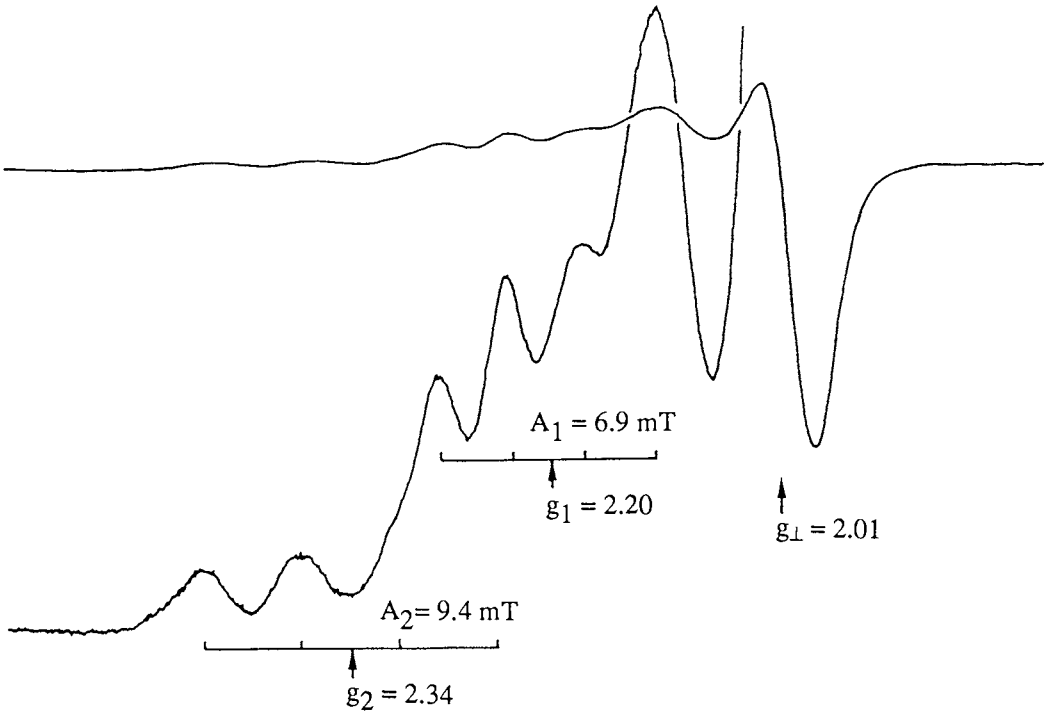


Fig. 2. EPR spectrum of nitrite reductase at 77 K.

Direct electrochemical response of nitrite reductase at pH 7.0 was not observed at a bare gold electrode in the potential range of +0.6 V to 0 V vs. NHE, however, well-defined response was obtained at a 4-pyds modified gold electrode as well as electron-transfer proteins.[1) 10)] The voltammetric behavior of nitrite reductase indicated a slow electron transfer process at pH 7.0 (0.1 M phosphate buffer), and observed half-wave

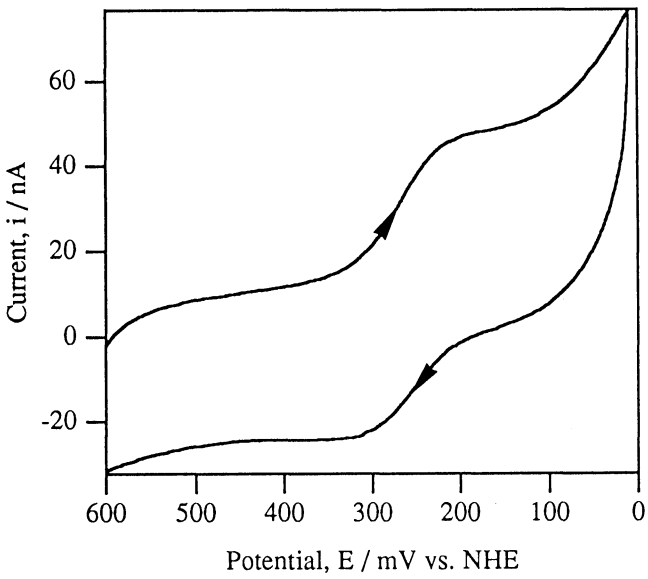


Fig. 3. Cyclic voltammogram of nitrite reductase (100 μmol $dm^{-3}$ in 0.1 mol $dm^{-3}$ phosphate buffer, pH 7.0) at a 4-pyds modified gold electrode at a scan rate of 20 mV $s^{-1}$.

peak potential was determined to be 0.26 V vs. NHE from the midpoint between the reduction and subsequent re-oxidation peak potentials (Fig. 3). The redox potential was good consistent with the previous value of type I copper site in type II copper-depleted nitrite reductase from the same bacterium, $E_{1/2}$ = 260 mV vs. NHE by potentiometric titration.[8] The peak current, $i_{pc}$ was found to increase linearly with the square root of the scan rate, $v^{1/2}$ up to a potential scan rate 100 mV $s^{-1}$ as expected for a diffusion-controlled process. Diffusion coefficient of nitrite reductase (0.1 M phosphate buffer, pH 7.0) was estimated to be 4 ± 1 x $10^{-8}$ $cm^2$ $s^{-1}$, and the heterogeneous electron-transfer rate constant of the enzyme was calculated to be 4 x $10^{-5}$ cm $s^{-1}$ from the cyclic voltammetric peak-to-peak separation.[11] The diffusion coefficient is reasonable to that of large protein molecule and supports that the voltammetric response is due to the redox of nitrite reductase. The estimated redox potential of the reductase is very resemble to those of other blue copper electron-transfer proteins,[12] and hence the observed redox couple is assumed to be the response of the type I copper site. Godden et al. reported that the type II copper site of *A. cycloclastes* nitrite recutase is located 12 Å deep in a solvent channel formed by two monomers.[7] Synchrotron radiation small-angle X-ray scattering indicated that the three-dimentionl structure of the reductase is very similar to that of *A cycloclastes*.[14] It might be impossible to observe the direct electron-transfer of type II copper site, because the type II copper site is far from the protein surface which interets with electrode surface. Nitrite reductase from *A. xylosoxidans* has positively charged protein surface which may interact with pyridine moiety of the 4-pyds modified gold electrode like other positively charged electron transfer proteins,[1, 2] and the interfacial electron-transfer of the reductase will arise through the hydrogen-bonding between the pyridine moiety and the positively charged protein surface. For reasons mentioned above, the type I copper site seems to be located near the positively charged patch of the enzyme.

Further studies on spectroscopic and redox properties of the enzyme are under way.

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