

## Cyclic Voltammetric Responses of Nitrate Reductase on Chemical Modified Electrodes

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**Abstract:** Electrochemistry of nitrate reductases (NR) incorporated into 2-aminoethanethiol self-assembled on the gold electrode and polyacrylamide cast on the pyrolytic graphite electrode was examined. NR on chemical modified electrode showed electrochemical cyclic voltammetric responses in phosphate buffers.

**Keywords:** NR, cyclic voltammetric response, chemical modified electrode.

Nitrate reductase catalyzes the reduction of nitrate to nitrite in the pathway of inorganic nitrogen assimilation, which is considered as a rate-limiting and regulated step, so NR plays a potentially important role in the solution of the nitrate pollution and more efficient use of N-fertilizers. NR is a high-molecular-weight redox enzyme, and contains three internally electroactive sites<sup>1</sup> (FAD, Fe, and Mo). In order to limit or prevent the strong adsorption of NR at the bare electrode, chemically modified electrodes, which may provide a unique microenvironment for electrode reaction and improve the electrochemical properties of NR, were prepared.

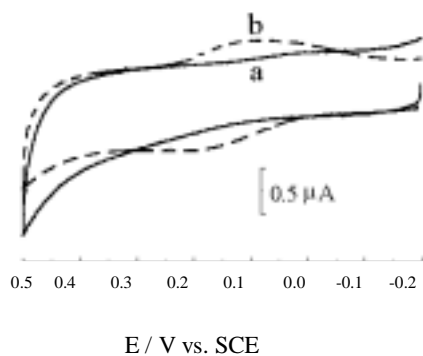
NR and 2-aminoethanethiol (AET) were from Sigma Chem. Co.. Pyrolytic graphite electrode (PGE) and polyacrylamide (PAA) were from Beijing Normal University. Cyclic voltammetry (CV) was taken by CHI830 electrochemical system. Modified chemical electrode acted as the working electrode, a platinum flag as the counter electrode and SCE electrode as the reference electrode.

A bare gold electrode was immersed in 0.01 mol/L AET aqueous solution for 24 h at room temperature. The freshly prepared AET / Au-modified electrode was thoroughly rinsed with water and incubated in phosphate buffer of NR<sup>2</sup>. The CVs of the AET / NR / Au system in a 0.1 mol/L phosphate buffer (pH 7.0) at scan rate 0.1 V s<sup>-1</sup> was shown in **Figure 1**. A pair of pre-reversible peaks appeared at around 0.174 V, and irregularly grew with soaking time in blank buffer. The cathodic peak current had linear relationship with scan rate between 0.02 and 0.5 V s<sup>-1</sup>. The rate of electron transfer of the AET / NR / Au system was slow because the difference between the cathodic and anodic peak potentials ( $\Delta E_p$ ) became larger with the scan rate increase and  $\Delta E_p$  was about 0.224 V at 0.1 V s<sup>-1</sup>. The direct electron transfer of the AET / NR / Au system was strongly affected by the solution environment including pH, the component and the concentration of the buffer.

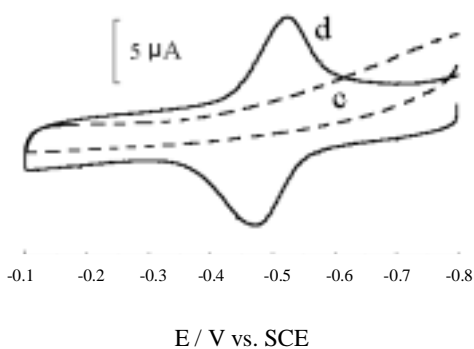
Compared with in pH 7 buffers, in pH 6 buffers the cathodic potential of NR shifted negatively, while there was no clear redox peak in pH 8 buffers. When the concentration of phosphate solution was increased from 0.005 mol/L to 0.2 mol/L, the formal potential changed from 0.165 V to 0.128 V, respectively. The introduction of  $\text{SO}_4^{2-}$  or  $\text{Cl}^-$  enhanced the rate of electron transfer, while the  $\Delta E_p$  became larger with the addition of  $\text{NO}_3^-$  or  $\text{ClO}_4^-$ .

PAA films were prepared by casting 10  $\mu\text{L}$  of 1 mg/mL PAA aqueous solution onto a PGE. After water was evaporated, 10  $\mu\text{L}$  phosphate buffer in the presence of NR was cast onto PAA / PG-modified electrode<sup>3</sup>. In pH 7.0 buffers, the PAA / NR / PGE system showed a pair of well-defined and nearly reversible peaks at about  $-0.473$  V (**Figure 2**). There was a good linearity in peak current with scan rate between 0.02 and  $0.5 \text{ V s}^{-1}$ , suggesting the characteristic for thin layer electrochemical behavior. The formal potential had a linear relationship between pH 3-12 with a slope of  $-56.3 \text{ mV / pH}$ . It suggested that one proton was coupled with one electron in the electrochemical reaction. However, the influence of the component and the concentration of the buffer on the direct electron transfer of the PAA / NR / PGE were much less than the AET / NR / Au.

**Figure 1** CVs of the AET / Au (a) and AET / NR / Au (b) at scan rate  $0.1 \text{ V s}^{-1}$  in a 0.1 mol/L phosphate buffer (pH 7.0)



**Figure 2** CVs of the PAA / PGE (c) and PAA / NR / PGE (d) at scan rate  $0.1 \text{ V s}^{-1}$  in a 0.1 mol/L phosphate buffer (pH 7.0)



### Acknowledgments

We are grateful to the NNSFC (29973026), BNSF (2992007) and Foundation for University Key Teacher by the Ministry of Education for the provision of financial support.

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Received 11 October, 2001