Electrocatalytic Oxidation of Cysteine on a Nafion-Ruthenium Oxide Pyrochlore Chemically Modified Electrode

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(Received April 19, 1999; CL-990308)

The electrocatalytic oxidation of cysteine (CySH) to cystine (CyS-SCy) was noticed on the Nafion-ruthenium oxide pyrochlore chemically modified electrode. The electrocatalytic oxidation was mediated by the $Ru^{VI/IV}$ redox sites in the oxide pyrochlore network in terms of the Michaelis-Menten kinetics. The obtained heterogeneous catalytic rate constant (k_c) and transfer coefficient (α) are 6.33 s^{-1} and 0.99, respectively.

Cysteine (CySH) is a sulfur containing amino acid that plays crucial roles in biological systems.[1] Electrochemical detection and determination of CySH and its oxidized residue, i.e., cystine (CyS-SCy),[1] are important in the protein characterization. Unfortunately, the oxidation of cysteine at a carbon electrode is sluggish; while, it is very complicated at the Hg, Au, and Pt electrodes.[2a,2b] Different chemically modified electrodes using $Os(bpy)_3^{2+}$,[3a] vitamin B12,[3b] Co-phthalocyanine (CoPc),[3c] and Ni-tetrasulfonated phthalocyanine (NiTsPc)[3d] mediators were thus developed to solve this analytical problem. In contrast, oxide electrodes are hardly used for this purpose, although they possess large surface area and specific redox species. We report here a Nafion-ruthenium oxide pyrochlore chemically modified electrode (NCME) for the electrocatalytic oxidation of CySH. The preparation of the NCME was achieved by ion exchange of Ru^{3+} and Pb^{2+} followed by *in situ*-precipitation method.[4]

The typical cyclic voltammetric behavior of the NCME towards CySH oxidation in pH 7.2 phosphate buffer (I = 0.1 M) is shown in Figure 1. A clear catalytic anodic peak (E_{pa}) at +0.63 V (vs. Ag/AgCl), corresponding to the oxidation of CySH to CyS-SCy, was noticed at the NCME. Further experiments involve the examination of changes in magnitude of the voltammetric peak current with variations in the scan rate. The plot of the peak current (i_{pa}) vs. the square root of the scan rate ($v^{1/2}$) was found to linear up to 200 mV s^{-1}. This observation implies that the oxidation process is diffusion-controlled. For a totally irreversible wave, i_{pa} is proportional to its concentration (C_{CySH}) and $v^{1/2}$, but E_{pa} is a function of scan rate, shifting (for a oxidation) in a positive direction by an amount $1.15RT/\alpha nF$ (or $30/\alpha n$ mV at 25 °C) for each tenfold increase in v.[5] The current function, $i_f = i_{pa}/(v^{1/2}C_{CySH})$, and $\partial E_{pa}/\partial \log(v)$ were calculated to be 54.01 $AV^{-1/2}s^{1/2}mol^{-1}cm^3$ and 30.3 mV/decade, respectively. Since n is equal to 1, the obtained value for α is 0.99 at 25 °C. The corresponding Tafel slope ($2.303RT/\alpha nF$) is therefore equal to 60 mV. The measured values clearly indicate that the NCME provides an efficient electrocatalytic system.[6]

Based on the previous studies on RuO_2,[6,7] the $Ru^{VI/IV}$ redox transition lies in the range of about +0.15 to +0.4 V (vs. Ag/AgCl) in pH 7.2.[7a] Since the starting oxidation potential (E_s) is at around +0.26 V on the NCME, it is thus expected that the $Ru^{VI/IV}$ in the Ru_2O_6 framework of corner-shared octahedral of the oxide pyrochlore[8] may effectively participate in the kinetics of the catalytic oxidation of CySH. The possible mediated oxidation mechanism is proposed as illustrated in Scheme 1. The formation of CyS-SCy from CySH follows the EC type of homogenous-coupled chemical reaction on heterogeneous NCME.[5]

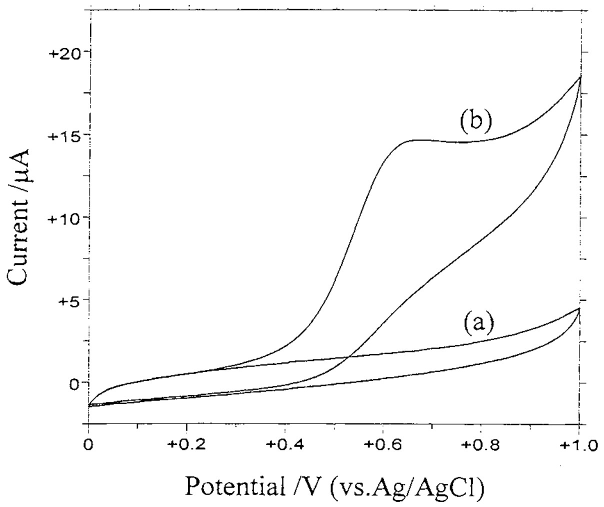


Figure 1. CV behavior of the NCME in the absence (a) and presence (b) of 4.09 mM cysteine in pH 7.2 phosphate buffer at a scan rate of 10 mVs^{-1}.

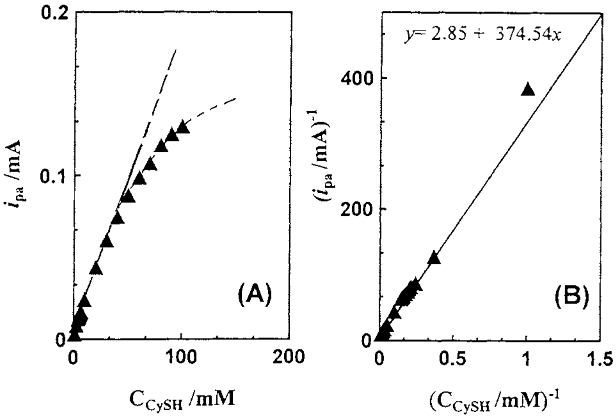
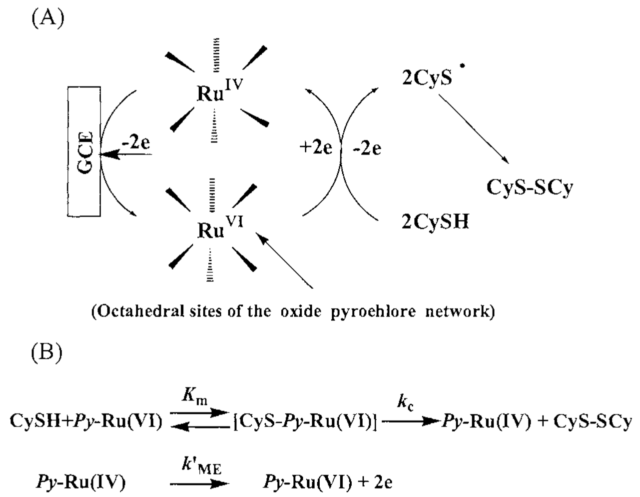


Figure 2. (A) A plot of i_{pa} vs. C_{CySH} for cysteine oxidation on the NCME obtained by CV at 10 mV/s in pH 7.2 phosphate buffer. (B) Typical Lineweaver-Burke (LB) plot for the data in (A).

Figure 2A is a plot of i_{pa} against C_{CySH} obtained at a scan rate of 10 mV s^{-1} (i.e., a quasi steady-state condition). It is linear



Scheme 1. Electrocatalytic mediated mechanism for the CySH oxidation on the NCME. (A) Illustration of the participation of the $Ru^{VI/IV}$ redox sites in the mediated mechanism, (B) Reaction pathway based on the Michaelis-Menten kinetics. *Py* represents the basic network of the ruthenium oxide pyrochlore.

Table 1. Mechanistic features of the cysteine oxidation at the NCME

Parameter	Value	
	NCME	ST-NiTsPc*
pH	7.2	2.0
Starting oxidation potential (E_s, mV)	260	~250
Half-wave potential ($E_{1/2}$, mV)	504	~425
Anodic peak potential (E_{pa}, mV)	630	~475
Current function (i_f, $AV^{-1/2}s^{1/2}mol^{-1}cm^3$)	54.01	36.7
Transfer coefficient (α)	0.99	-
Tafel slope (b, mV/decade)	60	-
Michaelis-Menten constant (K_m, mmol dm^{-3})	131.42	-
Electrochemical rate constant (k'_{ME}, $\times 10^{-4}$ cm s^{-1})	3.91	-
Catalytic rate constant (k_c, s^{-1})	6.33	-
The detection range (mM)	0.5–40	1–7

* Data measured in 0.5 M acetate buffer at $v = 20$ mV/s from Ref. 3d.

up to 40 mM CySH with a regression coefficient of 0.997 ($i_{pa} = 0.0038 + 0.0018C_{CySH}$) and beyond that i_{pa} reaches a plateau. This trend generally follows the Michaelis-Menten type kinetics observed in biological systems.[1,9a] Figure 2B is a typical plot of i_{pa}^{-1} vs. C_{CySH}^{-1} for the expression of Lineweaver-Burke (LB) analysis.[9] The equations used for LB analysis are $1/i_{pa} = K_m/nFAk_c\Gamma_t C_{CySH} + 1/nFAk_c\Gamma_t$ and $q = nFA\Gamma_t$, where Γ_t is the total surface coverage of the $Ru^{VI/IV}$ species[10] and K_m, k'_{ME} ($= k_c\Gamma_t/K_m$), and k_c are Michaelis-Menten kinetic parameters, other symbols have its usual significance. The calculated kinetic parameters values based on the LB plot are listed in Table 1. Note that the obtained catalytic rate constant (k_c) value from the Michaelis-Menten kinetics is very much comparable to that of earlier result (2.3 s^{-1}) for the catechol oxidation reaction on a RuO_2-Nafion composite electrode mediated bv $Ru^{VI/IV}$ species.[9a] Meanwhile, the summarized table evidenced the superior activity of the NCME towards the oxidation of CySH than a similar study on another system of ST-NiTsPC,[3d] where ST is silica gel modified TiO_2 as a immobilizing matrix for NiTsPc. Note that, since the E_{pa} is pH-dependent, the overpotential is actually about 230 mV lower for the NCME than that of the ST-NiTsPC. Further work is in progress for sensitive detection of cysteine using square-wave stripping voltammetric technique, in the presence of glutathione, and in other sulfhydryl compounds using HPLC coupled with electrochemical detection.

The authors gratefully acknowledge financial support from the National Science Council of the Republic of China under the grant NSC 88-2113-M-005-019.

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10 Since the CV peak is poor, the Γ_t value was estimated from subtracting charges ($q_t - q_{dl} = q$) at which the catalytic current occurs in base electrolyte. The charge of q_t was obtained by cycling between the hydrogen and oxygen evolution potential region at a very slow scan rate of 5 mV s^{-1}; while, the value of q_{dl} is measured by the small amplitude CV technique[6,7b] (i.e., measured at a very narrow potential range (150 – 400 mV) in the catalytic region at the same scan rate. The calculated surface coverage (Γ_t) is 8.129×10^{-9} mol cm^{-2}.