Lifetime of Veratryl Alcohol Radical Cation Electrogenerated in Acetonitrile

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The electrochemical oxidation of veratryl alcohol at a carbon fiber microdisk electrode with relatively high sweep rates in acetonitrile shows a chemically reversible response. Analysis of the cathodic and anodic current ratio allows one to estimate the lifetime of the short-lived radical cation in the solution.

The electrochemical oxidation of benzyl alcohols has received attention to date.[1,2] It was reported that a methoxy group substituted on an aromatic ring allows oxidation at less positive potentials because it concentrates the charge density on the ring and stabilizes the corresponding radical cation.[3,4] A further methoxy substituted compound, veratryl alcohol (3,4-dimethoxybenzyl alcohol, VA) is a secondary metabolite produced by *P. chrysosporium* and it is thought to be a one-electron redox mediator from the enzyme to an insoluble and hydrophobic lignin polymer during biodegradation processes.[5–7] However, the question has been raised why such a short-lived species acts as an efficient mediator. Though initial studies suggested that $VA/VA^{\bullet+}$ works as a diffusible mediator and a later correction indicated that the VA-enzyme complex stabilizes the radical cation,[8] a complete mechanism has not been formally accepted. Thus the estimation of the lifetime of $VA^{\bullet+}$ is an important subject, and enzymatic and radiation chemistry techniques have been employed to evaluate the lifetime in connection with ESR and conductivity measuerments.[9–12] On the other hand, according to the electrochemical measurements on a conventional time scale, VA shows irreversible oxidation properties either in aqueous or aprotic solvents.[13,14] In this study, we attempted to carry out cyclic voltammety of VA in acetonitrile at a relatively fast sweep rate.[15] Aprotic solvent can be used as a hydrophobic model atmosphere of enzymatic reactions. The reverse cathodic peak current proves the existence of the radical cation intermediate and it enables us to approximately estimate the lifetime of $VA^{\bullet+}$.

Veratryl alcohol (Aldrich), anisyl alcohol (4-methxybenzyl alcohol, Wako Pure Chemical), ferrocene (Fc, Tokyo Kasei), sodium perchlorate (Wako Pure Chemical) and acetonitrile (∞ pure grade, Wako Pure Chemical) were used as received. The electrochemical measurements were carried out using the conventional three-electrode cell configuration. The electrode potential was controlled by a potentiostat (Hokuto Denko, HA-501G) and a function generator (Kenwood, FG-281), and the voltammograms were displayed and stored using a digital storage oscilloscope (Tektronics, TDS-1012). A carbon fiber (CF) microdisk electrode (diameter 33 μm, BAS) was used as the working electrode. An Ag/Ag^+ (0.01 M (mol dm^{-3}) $AgNO_3$ in acetonitrile) electrode and a Pt spiral electrode were the reference and auxiliary electrodes, respectively. The potential-current curves at fast sweep rates up to 5 kV s^{-1} were confirmed for measuring the Fc/Fc^+ redox couple in 0.6 M $NaClO_4/CH_3CN$. All experiments were performed at laboratory temperature (22 ± 1 °C).

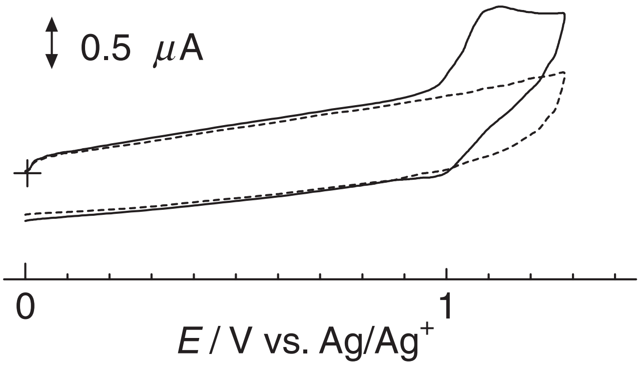

Figure 1. Cyclic voltammogram for 5 mM veratryl alcohol (solid line) in 0.6 M $NaClO_4/CH_3CN$ solution at a CF electrode. Dashed line is the background. The sweep rate was 200 V s^{-1}.

Figure 1 shows the cyclic voltammogram for the VA oxidation at 200 V s^{-1}. Since the current should exhibit the time dependence expected of a planar electrode for the stated conditions of electrode size and time scale and VA undergoes a reversible electron transfer to form the radical cation as the initial stage,[3] the absence of a reverse current response indicates the following chemical reaction. At higher sweep rates (>500 V s^{-1}), a reverse cathodic current appeared. After subtraction of the background current, the ratio of the cathodic and anodic peak currents (i_{pc}/i_{pa}) of the voltammogram at 2 kVs^{-1} approaches unity. Voltammograms obtained at 1 kV s^{-1} are shown in Figure 2. Deaeration with argon gas did not affect the shape of the voltammograms at any sweep rates. For the monomethoxy substituted, anisyl alcohol, the reverse current could not be observed in the time scale we employed. These results suggest that two methoxy groups in VA as electron-donating substituents would stabilize the positive charge and extend the lifetime of the radical cation. In order to explain the formation of the corresponding aldehydes as a result of the benzyl alcohols oxidation, the following mechanism has been proposed: VA undergoes a reversible electron transfer to form the radical cation ($VA^{\bullet+}$) followed by proton elimination, and the resulting neutral radical ($VA^{\bullet}$) should be oxidized to an intermediate (I^+) and complete the reaction via further deprotonation.[3,4,14]

$$VA \rightarrow VA^{\bullet+} + e^- \rightarrow VA^{\bullet} + H^+ \rightarrow I^+ + e^- \rightarrow (\text{aldehyde}) + H^+$$

Reduction current of I^+ to $VA^{\bullet}$ was not observed, which indicates that deprotonation of I^+ should be facile. The plot of the anodic peak currents normalized by the square root of the sweep rate (v) vs $\log v$ in Figure 3 guarantees that the redox potential of $VA^{\bullet}$ is less positive to that of VA. These results allow us to assume an ECE mechanism.[16] A first-order rate constant of $(5.5 \pm 2) \times 10^4$ s^{-1} is estimated from the working curve of i_{pc}/i_{pa} vs $\log\lambda$, where $\lambda = (RT/Fv)k$ is the dimensionless pa-

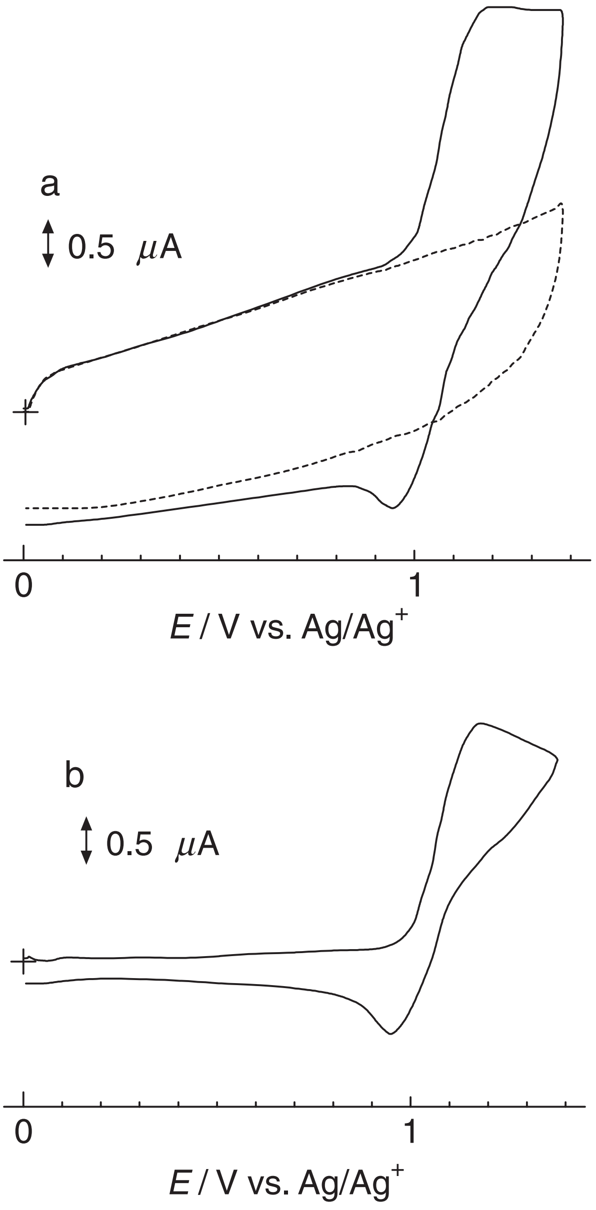

Figure 2. (a) Cyclic voltammogram for 5 mM veratryl alcohol (solid line) in 0.6 M $NaClO_4/CH_3CN$ solution at a CF electrode. Dashed line is the background. The sweep rate was $1\,kV\,s^{-1}$. (b) Cyclic voltammogram obtained by background subtraction from (a).

rameter of interest and k is a first-order rate constant.[17,18] This rate constant corresponds to a half-life of approximately 13 µs. It was reported that the rate of decay of $VA^{\bullet +}$ generated with the enzyme is $k = 1.85\,s^{-1}$ ($t_{1/2} = 375\,ms$),[19] and $k = 1.2 \times 10^3\,s^{-1}$ ($t_{1/2} = 0.58\,ms$) when generated with Ce(IV) both in acidic aqueous solutions.[11] In another case, $k = 17.1\,s^{-1}$ ($t_{1/2} = 40.5\,ms$) in acidic aqueous acetonitrile.[9] Acidic condition is necessary for peroxidase activity. The reason why the lifetime estimated in this study is much shorter than those would probably be due to the basicity of acetonitrile as the solvent compared to that of acidic aqueous solutions.

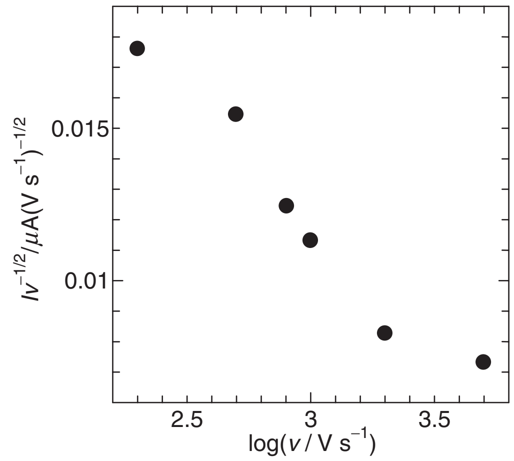

Figure 3. Variation in normalized peak current for the oxidation of 5 mM veratryl alcohol at a CF electrode with sweep rate.

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