Electrochemical Evaluation of Redox Enzyme Reaction Kinetics Based on Mediated Bioelectrocatalysis in Solution

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Approximate and more precise empirical equations have been derived for the steady state limiting currents of bioelectrocatalysis based on homogeneous enzyme and mediator at sufficiently high concentrations of the substrate. These equations allow us to evaluate the catalytic constant and the Michaelis constant of the mediator.

Electron transfer mediators such as quinones and ferrocenes allow to conjugate between redox enzyme reactions and electrode reactions. Such redox compound-mediated and enzyme-catalyzed electrode process is called mediated bioelectrocatalysis.[1] The catalytic current in the bioelectrocatalysis is governed by the enzyme reaction kinetics as well as the electrode kinetics and the mass transport process. Therefore, if the catalytic current is analytically interpreted, one can get several important information concerning the enzyme kinetics. For this purpose, the systems involving homogeneous enzymes and mediators seem to be superior to the immobilized systems to minimize the parameters to be considered. In this work, we focus our attention to derive approximate and empirical equations representing the catalytic current under the conditions that the substrate concentration ([S]) is sufficiently larger than the corresponding Michaelis constant (K_S).

The steady state reaction rate of the redox enzyme reaction (v_E) at [S] >> K_S is expressed by the Michaelis-Menten equation (in terms of the substrate oxidation),

$$v_E = \frac{k_{cat}[E]}{1+K_M/[M_{ox}]} \quad (1)$$

where k_{cat} is the catalytic constant, [E] is the concentration of the soluble enzyme, and K_M and $[M_{ox}]$ are the Michaelis constant and the concentration of the soluble electron acceptor (the oxidized form of the mediator in this case), respectively. In mediated bioelectrocatalysis, the reduced mediator in solution (M_{red}) is oxidized at the electrode surface to generate M_{ox}, which takes part in the enzyme reaction. Under such conditions, the bioelectrocatalysis exhibits the steady state limiting current (I_s) at sufficiently positive potential compared with the redox potential of the mediator. I_s seems to be very convenient to be measured. However, simple analysis of I_s vs. the bulk concentration of M_{red} ($[M_{red}]^*$) plots in terms of the Michaelis-Menten model is incorrect as evidenced in the following.

Digital simulation technique can provide the numerical solution of I_s ($I_{s,sim}$) at any $[M_{red}]^*$.[2] In our case, the explicit point method[3] was applied to get $I_{s,sim}$, in which the concentration change of for example M_{ox} ($\Delta[M_{ox}](j)$) resulting from the enzyme reaction in the j-th volume element at a certain time interval (Δt) is written by $\Delta[M_{ox}](j) = -v_E(j)\Delta t$ and the calculation was continued to reach the steady state.

When $[M_{red}]^* << K_M$, the analytical equation of I_s has been already reported as,[4]

$$I_s = nFA\sqrt{D_M k_{cat}[E]/K_M}[M_{red}]^* \quad (2)$$

where n, F, A, and D_M are the number of electrons, the Faraday constant, the electrode surface area, and the diffusion constant of the mediator, respectively. Here let us consider the other limiting case, that is $[M_{red}]^* >> K_M$. Our assumption under such conditions is that $[M_{ox}]$ is sufficiently larger than K_M within almost whole region of the diffusion layer of the mediator. Taking the assumption, the diffusion coupled with the enzyme reaction is written by $D_M\partial^2[M_{ox}]/\partial x^2 - k_{cat}[E] = 0$ under the steady state conditions. This can be solved to get the analytical equation for I_s:

$$I_s = nFA\sqrt{2D_M k_{cat}[E][M_{red}]^*} \quad (3)$$

by coupling the equation: $I_s/nFA = -D_M(\partial[M_{ox}]/\partial x)_{x=0}$ with the boundary conditions that $(\partial[M_{ox}]/\partial x)_{x=\delta} = 0$ and $[M_{ox}]_{x=\delta} = 0$, δ being the diffusion layer thickness. The values predicted by equation (3) agree well with $I_{s,sim}$ at excess $[M_{red}]^*$, the error being 2.9 % at $[M_{red}]^*/K_M = 80$ and decreasing with an increase of $[M_{red}]^*/K_M$.

Equations (2) and (3) may be utilized to evaluate k_{cat}/K_M and k_{cat}, respectively. However, it seems to be important to express I_s generally at a given $[M_{red}]^*$ for more precise evaluation of k_{cat} and K_M. We have proposed an approximate equation for I_s ($I_{s,app}$) as allowed to satisfy equations (2) and (3) under the limiting conditions.

$$I_{s,app} = nFA\sqrt{\frac{2D_M k_{cat}[E]}{2K_M+[M_{red}]^*}}[M_{red}]^* \quad (4)$$

The $I_{s,app}$ values can predict the $I_{s,sim}$ values well with only about 5 % of the maximum error around $[M_{red}]^*/K_M = 4 - 5$, as evidenced in Figure 1. Thus we can conclude that equation (4) is practically acceptable. We have further found that the $I_{s,app}/I_{s,sim}$ value is empirically expressed as a function of $[M_{red}]^*/K_M$ by:

$$\frac{I_{s,app}}{I_{s,sim}} = 1+\frac{[M_{red}]^*/K_M}{0.85([M_{red}]^*/K_M)^2+11.4[M_{red}]^*/K_M+17.9} \quad (5)$$

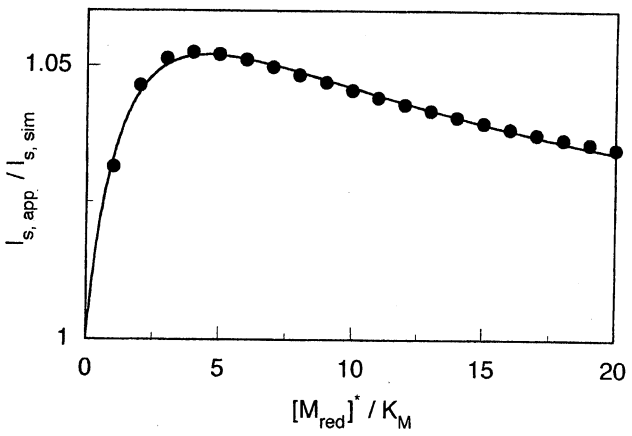

Figure 1. Values of $I_{s,app}/I_{s,sim}$ as a function of $[M_{red}]^*/K_M$. The solid curve represents the function expressed by equation (5).

By substituting equation (4) into equation (5), the $I_{s,sim}$ value can be analytically expressed in very high precision as is illustrated by the solid curve in Figure 1.

The approximate equation (4) and/or the empirical equation (5) can be utilized to analyze $[M_{red}]^*$ dependence of experimental I_s values. Equation (4) is rewritten as the Michaelis-Menten-type expression.

$$\frac{I_{s,app}^{\ 2}}{[M_{red}]^*} = \frac{2(nFA)^2 D_M k_{cat}[E]}{1+2K_M/[M_{red}]^*} \quad (6)$$

Therefore, several linear regression analyses such as Lineweaver-Burk-type plots ($[M_{red}]^*/I_s^2$ vs. $1/[M_{red}]^*$) and Hanes-Woolf-type plots ($([M_{red}]^*/I_s)^2$ vs. $[M_{red}]^*$) are useful in obtaining k_{cat} and K_M values. More precisely, experimental I_s vs. $[M_{red}]^*$ relations may be directly analyzed by a nonlinear regression method based on equation (5) coupled with equation (4).

As an example, I_s values in glucose oxidase (GOD)-catalyzed and *p*-benzoquinone (BQ)-mediated system were measured by means of cyclic voltammetry or constant potential amperometry (at 1.0 V vs. Ag/AgCl) with a glassy carbon electrode in the presence of 1 mol dm^{-3} (= M) glucose (glc) (>> K_S = 110 mM, which was spectrophotometrically determined) at pH 5.7 and 30 °C under nitrogen atmosphere. Experimental I_s values increased on successive addition of BQ (Figure 2, ●). In the experiments, the added BQ was immediately reduced by GOD consuming negligible amount of glc compared with the initial [glc]. The Hanes-Woolf-type plots based on equation (6) showed a linear relation between $([BQ]/I_s)^2$ vs. [BQ] (Figure 3, ●). The linear regression analysis (Figure 2, solid line) yielded enzymatic parameters: $k_{cat} = 1.2 \times 10^3$ s^{-1} and K_M = 0.87 mM, using a value of $nFA\sqrt{D_M}$ and [E], which were evaluated by potential step chronoamperometry of BQ in the absence of GOD and spectrophotometry (ε = 18240 at 450 nm), respectively. The I_s values predicted by equation (5) with these parameters reproduce well the experimental ones (Figure 2, broken curve). On the other hand, the direct nonlinear regression analysis of the I_s vs. [BQ] plots using equation (5) resulted in the solid curve in Figure 2,

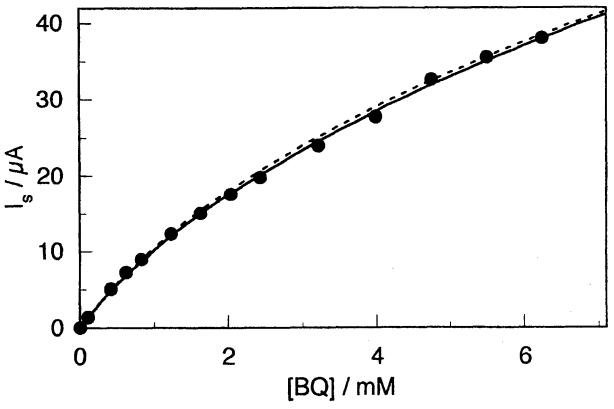

Figure 2. Dependence of I_s on [BQ] in GOD (0.18 μM)-catalyzed and BQ-mediated electrochemical oxidation of glc (1 M). Solid curve: the nonlinear regression result. Broken curve: calculated values with the parameters obtained by the linear regression analysis in Figure 3. See text for details.

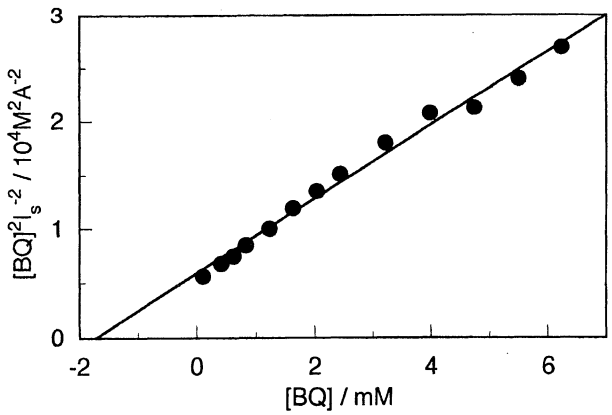

Figure 3. Hanes-Woolf-type linear regression analysis of the data given in Figure 2.

giving parameters as $k_{cat} = 1.5 \times 10^3$ s^{-1} and K_M = 1.1 mM. The two sets of the parameters are in good agreement with each other and also with spectrophotometric data obtained under identical conditions ($k_{cat} = 1.1 \times 10^3$ s^{-1} and K_M = 1.3 mM). These results support the usefulness of the present analytical methods. The linear regression method is satisfactorily acceptable and would be more convenient in practice than the nonlinear one.

In conclusion, we have proposed the novel equations (equation (4) (or (6)) and equation (5)) for the mediated bioelectrocatalysis under the conditions of [S] >> K_S. These equations provide a means to evaluate k_{cat} and K_M separately by electrochemical methods. Compared with the conventional spectrophotometric method, our proposed method(s) is very convenient and the amount of enzyme required is much smaller. In principle, this method can be applied to oxidase reaction kinetic measurements, because O_2/H_2O_2 can work as a mediator at certain electrodes. For evaluation of K_S, we will report a new method elsewhere in near future.

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