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Effect of Temperature on the Polarographic Catalytic Current Produced by Proteins in the Presence of Cobalt Salts

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The effect of temperature on the polarographic catalytic hydrogen evolution current (Brdička current) produced by ribonuclease-A in an ammoniacal buffer containing cobalt salt has been studied using the theoretical equation of Brdička current. With increasing temperature, the Brdička current at −1.3 V increased between 10 and 30 °C then fell off above 30 °C, whereas the currents at −1.4 and −1.5 V decreased continuously. Analysis showed that the initial increase of the Brdička current at −1.3 V was only apparent and that the Brdička current constant, k_B, which represents the catalytic activity of the protein, always decreased with increasing temperature in the potential range of −1.3 to −1.5 V. Further analysis has revealed that the intrinsic catalytic activity of the protein–cobalt(0) complex, which is transiently formed on the electrode surface and which catalyzes the hydrogen evolution, increases with increasing temperature whereas the life time of the complex decreases and the latter decrease cancels the former increase, resulting in the decrease of the k_B value with increasing temperature.

The Brdička current, or polarographic catalytic hydrogen evolution current produced by proteins containing so-called Brdička-active groups, such as SH- and/or SS-groups[1] and others,[2,3] in the presence of cobalt salt, has found a variety of applications in structure analysis as well as trace analysis of proteins (see Ref. 4—6 for review). The effect of temperature on the Brdička current has been studied by many investigators,[5—11] but these studies have remained qualitative or at most semiquantitative. This is probably due to the lack of a suitable theoretical equation to predict the Brdička current in a quantitative way. In previous papers,[6,12] we have presented a theoretical equation for the Brdička current, in which it is expressed as a function of the surface concentration of protein, the bulk concentration of cobalt ion and two parameters representing the properties of the protein–cobalt(0) complex which catalyzes the hydrogen evolution. In this study, the effect of temperature on the Brdička current has been analyzed by the theoretical equation presented in the previous papers.

Experimental

Bovine pancreas ribonuclease-A (Type-A, Sigma Chemical Co., lot No. 58C-0116) was obtained commercially and used as received. All other chemicals were of reagent grade quality. The apparatus for the electrochemical measurements has been described in a previous paper.[12] Potentials were measured against a saturated calomel electrode (SCE). All measurements were made in a thermostat which was controlled within ±0.5 °C.

Results and Discussion

Figure 1 shows d.c. polarograms of ribonuclease-A (RNase) at 10, 25, and 40 °C in a base solution (0.1 mol dm^{-3} ammonia, 0.1 mol dm^{-3} ammonium chloride, and 0.1 mol dm^{-3} potassium chloride, pH 9.5 at 25 °C) containing 2×10^{-4} mol dm^{-3} hexaamine cobalt(III) chloride, Co(III). The Brdička current of RNase showed a typtical double-step wave with two maxima. With increasing temperature, the height of the first wave at −1.35 V (so-called Brdička first wave) increased, whereas that of the second wave at −1.55 V (so-called Brdička second wave) decreased, and the second maximum disappeared at increased temperature. The reduction waves of cobalt(II) to cobalt(0) shifted to less negative potential with increasing temperature both in the presence and in the absence of RNase. The limiting diffusion current of cobalt ion also increased with increasing temperature.

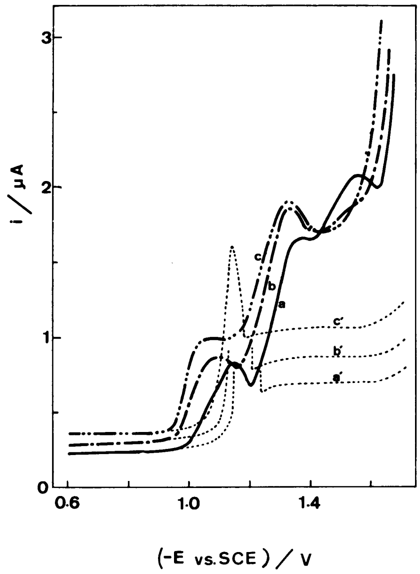

Fig. 1. D.c. polarograms of 4 μg cm^{-3} RNase at a) 10, b) 25, and c) 40 °C in the base solution containing 2×10^{-4} mol dm^{-3} Co(III). The broken lines represent d.c. polarograms in the absence of RNase at a′) 10, b′) 25, and c′) 40 °C.

Many previous authors[7—11] have measured the maximum heights of the first and second waves of Brdička current to study the effect of temperature on the Brdička current. However, as the temperature and other experimental conditions change, the maxima are not always

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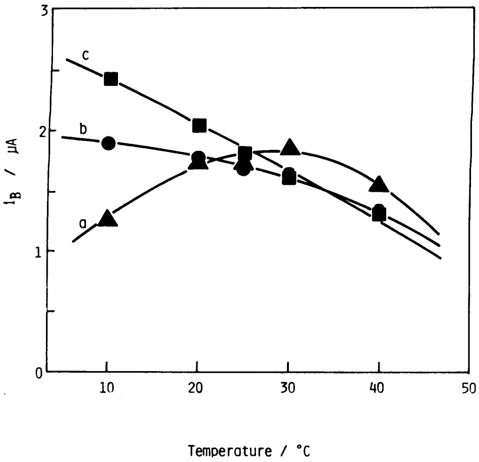

Fig. 2. Temperature dependence of the Brdička currents of 4 μg cm^{-3} RNase at a) −1.3, b) −1.4, and c) −1.5 V in the base solution containing 2×10^{-4} mol dm^{-3} Co(III).

well-defined and the potential of the maxima also shifts as shown in Fig. 1. Accordingly we have measured the heights of the Brdička current at three fixed potentials, *i.e.* −1.3, −1.4, and −1.5 V, as a function of temperature. In measuring the current with a dropping mercury elecrode (DME), the current intensity at the maximum growth of each mercury drop was taken and the net Brdička current, i_B, was obtained by correcting for the diffusion current of cobalt ion, i_{Co}. Figure 2 shows i_B at −1.3, −1.4, and −1.5 V plotted against temperature. With increasing temperature, i_B at −1.3 V increased initially from 10 °C to 30 °C, then decreased, whereas at −1.4 and −1.5 V, i_B decreased continuously.

It has been shown[6,12] that the Brdička current observed in the presence of Co(III) at low concentration, usually less than 2×10^{-4} mol dm^{-3}, can be expressed by

$$i_B = FAk_B\Gamma f_{Co}, \qquad (1)$$

where F is Faraday, A the surface area of mercury electrode, k_B the Brdička current constant representing the catalytic activity of the protein, Γ the surface concentration of the protein adsorbed on the electrode surface, and f_{Co} the flux of cobalt ion at the electrode surface. In Eq. 1, f_{Co} and Γ as well as k_B depend on temperature. f_{Co} at a given temperature can be estimated from i_{Co} (broken lines in Fig. 1) according to the Ilkovič equation; $f_{Co}=i_{Co}/nFA=(7D_{Co}/3\pi t)^{1/2}C_{Co}$ at DME, where n is the number of electrons involved (here n=3), D_{Co} the diffusion coefficient of the cobalt ion, C_{Co} the bulk concentration of the cobalt ion and t the drop time. It has been shown[6,12] that Γ is given by the Koryta equation,[13] $\Gamma=(12D_pt/7\pi)^{1/2}C_p$ at DME in good approximation, for the Brdička current of RNase at the potential $E\geqq-1.5$ V and $C_p\leqq4$ μg ml^{-1} under the usual DME conditions, where D_p and C_p are the diffusion coefficient and the bulk concentration of the protein, respectively. The values of D_p at different temperatures were estimated by D_p=(const.)T/η,[14] where $D_p=13.1\times10^{-7}$ cm^2 s^{-1} at 20 °C,[15] T; the absolute temperature, and η; the viscosity of water at each temperature,[16] respectively. Thus f_{Co} and Γ for a given temperature were calculated and k_B's at three different potentials were determined by Eq. 1. The results are plotted against temperature in Fig. 3. Note that k_B decreased continuously with increasing temperature at all potentials.

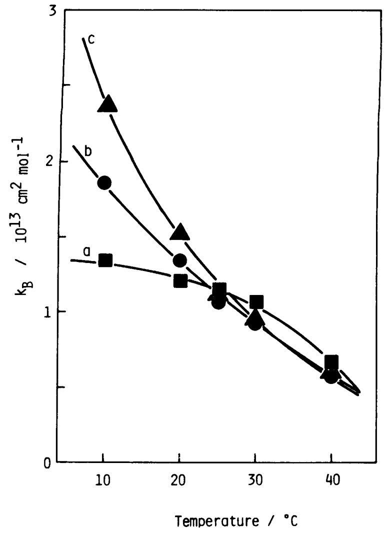

Fig. 3. Temperature dependence of the k_B values of RNase at a) −1.3, b) −1.4, and c) −1.5 V in the base solution.

The negative temperature coefficient of the Brdička current has been pointed out by many previous authors,[7−11] who have interpreted this phenomenon by the decreased adsorption of protein on the electrode surface at increased temperature. The present result clearly indicates that the negative temperature coefficient of the Brdička current is due to the negative *temperature coefficient of k_B, and is not attributable to* the decreased adsorption of protein. Also it is clear that the initial increase of i_B at −1.3 V (the Brdička first wave, curve a in Fig. 2) is due to the increase in Γ and f_{Co}, and does not indicate any increase in the catalytic activity itself of the protein with increasing temperature, as Březina and Gultajaj[7] have suggested.

The mechanism of the Brdička current is not yet fully understood, but it is now generally accepted[5,6,17] that zero-valent cobalt, Co(0), which is liganded to a Brdička active group of protein to form a protein-Co(0) complex on the electrode surface, may catalyze the hydrogen evolution before the complex is decomposed into Co(0)-amalgam. The proposed mechanism has led to a theoretical equation for the Brdička current,[6,12]

$$i_B = H_1C_{Co}/[H_2+C_{Co}], \qquad (2)$$

with

$$H_1 = FAn_ck_c\Gamma, \qquad (3)$$

$$H_2 = (k_d/k_f)C_{Co}/f_{Co}, \qquad (4)$$

where k_c is the constant representing the intrinsic catalytic activity of the protein-Co(0) complex, n_c the number of sites where the protein-Co(0) complex can be formed per protein molecule, and k_f and k_d the rate constants of formation and decomposition of the protein-Co(0) complex, *i.e.* k_f/k_d represents the stability or life time of the complex. Equation 2 also predicts that $n_c k_c(k_f/k_d)=k_B$ in Eq. 1, which is valid for $f_{Co}(k_f/k_d)\ll 1$ or at sufficiently low value of C_{Co}, usually[12] less than 2×10^{-4} mol dm^{-3}. Thus, the dependence of i_B on C_{Co} was analyzed using Eqs. 2—4 in the C_{Co} range between 5×10^{-5} and 2×10^{-3} mol dm^{-3} at different temperatures to give the $n_c k_c$ and k_f/k_d values as functions of temperature. The results are shown in Figs. 4 and 5.

As seen in Fig. 4, $n_c k_c$ increases with increasing temperature at all potentials, except at -1.5 V where $n_c k_c$ tends to decrease slightly. The decreasing tendency of $n_c k_c$ at -1.5 V is probably due to overestimation of Γ at such a negative potential. The adsorption of protein is usually weaker,[18] so that the Koryta equation is not valid, but gives an overestimated value of Γ, *i.e.* $\Gamma < (12D_p t/7\pi)^{1/2}C_p$, and the weaker the adsorption, the lower is Γ. On the other hand, k_f/k_d always decreases with temperature (Fig. 5). Thus the decrease in the stability or life time of the protein-Co(0) complex cancels the increase in the intrinsic catalytic activity of the complex, resulting in the decrease in the over-all catalytic activity of the protein, *i.e.* the k_B value, with increasing temperature. In the above discussion, the change of the pH with temperature is not taken into account; this is calculated to be from 9.95 to 9.05 for the temperature change from 10 to 40 °C in ammoniacal buffer.[19] It has been shown[20] that both $n_c k_c$ and k_f/k_d decrease with decreasing pH. This implies that the "true" temperature coefficient should be larger for $n_c k_c$ and smaller for k_f/k_d. However this correction for pH change can be shown to be not sufficiently significant to modify the above conclusion.

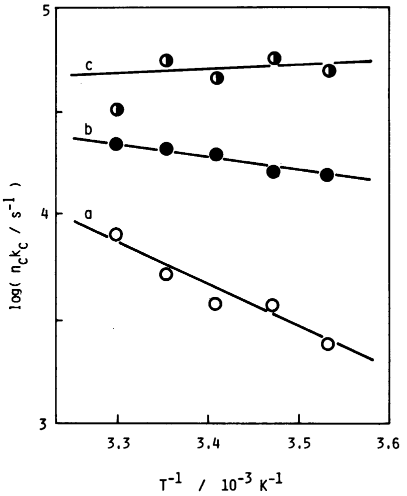

Fig. 4. Temperature dependence of the $n_c k_c$ values of RNase at a) -1.3, b) -1.4, and c) -1.5 V in the base solution.

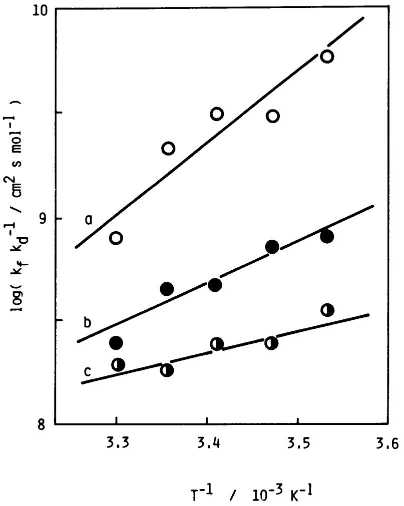

Fig. 5. Temperature dependence of the $k_f k_d$ values of RNase at a) -1.3, b) -1.4, and c) -1.5 V in the base solution.

The slopes of the linear plots in Fig. 4 give the apparent activation energy of the catalytic hydrogen evolution on the electrode surface, which was calculated to be 37 kJ mol^{-1} at -1.3 V, 17 kJ mol^{-1} at -1.4 V (and -1 kJ mol^{-1} at -1.5 V). Also, the plots in Fig. 5 give the difference between the apparent activation energies of the formation and the decomposition of the protein-Co(0) complex, which was calculated to be -64 kJ mol^{-1} at -1.3 V, -36 kJ mol^{-1} at -1.4 V and -20 kJ mol^{-1} at -1.5 V. The potential dependence of these energy values may be associated with the conformational change of the protein absorbed on the electrode surface.

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