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ACCERELATION EFFECT OF β-CYCLODEXTRIN ON ELECTROCATALYTIC OXIDATION OF NADH BY FERROCENECARBOXYLIC ACID

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β-Cyclodextrin (β-CD) shows an accelaration effect on the electrocatalytic oxidation of NADH by ferrocenecarboxylic acid (Fca). Quantitative analysis using digital simulation has suggested that the Fca^+-β-CD complex existing in only minute amount acts as an effective oxidant for NADH.

Cyclodextrin (CD) has been revealing its mysterious property which affects the electrochemical response of electroactive materials. The previous studies[1,2] on the effect of cyclodextrin on the electrochemical behaviors of ferrocenes have demonstrated that the formal potential for a ferrocene/ferricinium redox couple in the complexed state with β-CD is far positive from that in the uncomplexed state. This suggests the highly potential ability of the ferricinium-CD complex as an oxidizing reagent. We report herein the acceleration effect of β-CD on the electrocatalytic oxidation of NADH (β-nicotinamide adenine dinucleotide, reduced form) by ferrocenecarboxylic acid (Fca). The present results have clearly suggested that Fca^+-β-CD species acts as an effective oxidant for NADH.

The oxidation of NADH by various ferricinium cations have been investigated spectroscopically in detail by Miller and coworkers[3] in 1-propanol/water (46/54 in w/w) solution. In order to obtain the quantitative feature in an aqueous buffered solution (pH 7.0), we have first carried out the cyclic voltammetric investigation on the electrocatalytic oxidation of NADH by Fca. The electrocatalysis of the present system occurs according to the following scheme:

$$\mathrm{Fca} - e \rightleftharpoons \mathrm{Fca}^+ \qquad E_f^\circ\ ;\ \text{formal potential} \tag{1}$$

$$\mathrm{NADH} + \mathrm{Fca}^+ \xrightarrow{k_{obsd}} \mathrm{NAD}^\bullet + \mathrm{H}^+ + \mathrm{Fca} \tag{2}$$

$$\left(\begin{array}{ll} \mathrm{NADH} + \mathrm{Fca}^+ \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{NADH}^{\dot{+}} + \mathrm{Fca} & (3) \\ \mathrm{NADH}^{\dot{+}} \xrightarrow{k_4} \mathrm{NAD}^\bullet + \mathrm{H}^+ & (4) \end{array}\right)$$

$$\mathrm{NAD}^\bullet + \mathrm{Fca}^+ \xrightarrow{fast} \mathrm{NAD}^+ + \mathrm{Fca} \tag{5}$$

Cyclic voltammetry was carried out with the same instruments and electrode system as those used before.[4)] A phosphate buffer (pH 7.0) was used as an electrolyte solution and the measurement was conducted at 25 °C. β-CD and Fca were obtained commercially and recrystallized from ethanol/water and water, respectively. NADH was obtained from Sigma (Grade III) and used as received.

Cyclic voltammetry with NADH in the solution showed catalytic currents and the quantitative analysis[5)] by using degital simulation[1,6)] afforded k_3=33 $dm^3mol^{-1}s^{-1}$ and k_4=6×10^6 s^{-1}. The formation contstants of Fca-β-CD and Fca^+-β-CD complexes at pH 7.0 were determined to be 2100 and $\lesssim$20 dm^3mol^{-1}, respectively, by the method reported previously.[1,7)] From these results, the formal potentials for the Fca/Fca^+ and NADH/$NADH^{+\bullet}$ couples (E_f° and E_{NADH}°, respectively) are obtained to be 0.281 and 0.75 V vs. SCE, respectively. The exact value of the formal potential (E_c°) for the Fca-β-CD/Fca^+-β-CD couple could not be determined but the present results implies that it resides in the potential range, 0.40-0.75 V vs. SCE. These values were used throughout the present research to obtain theoretical cyclic voltammetric response from the digital simulation.

Table 1 lists the i_p/i_d values, the ratios of the peak current with NADH to

Table 1. Experimental and theoretical i_p/i_d values[a)]

γ [b)]	Scan rate mV s^{-1}		Concentration of β-CD / mmol dm^{-3} 0	3.0	5.0	7.0	10.0
2.0	50	Experimental	1.07	1.13	1.19	1.23	
			(28)	(54)	(78)	(95)	
		Theoretical(III,f=0.3)[c)]	1.07	1.16	1.19	1.22	
		Theoretical(III,f=1)[c)]	1.07	1.35	1.48	1.57	
		Theoretical(II)[d)]	1.07	1.07	1.06	1.05	
	100	Experimental	1.04	1.08	1.11	1.12	1.16
			(29)	(62)	(88)	(97)	(129)
		Theoretical(III,f=0.3)[c)]	1.03	1.08	1.10	1.12	1.13
		Theoretical(III,f=1)[c)]	1.03	1.18	1.25	1.31	1.37
		Theoretical(II)[d)]	1.03	1.03	1.03	1.03	1.03
5.0	100	Experimental	1.08	1.16	1.22		
		Theoretical(III,f=0.3)[c)]	1.08	1.20	1.24		
	200	Experimental	1.04	1.09	1.12		1.16
		Theoretical(III,f=0.3)[c)]	1.04	1.11	1.13		1.16

a) Initial concentration of Fca; 1.0 mmol dm^{-3}. b) Excess factor; the ratio of the concentration of NADH to that of Fca. c) Based on mechanism III. d) Based on mechanism II. Numbers in parentheses are the k_{obsd} values ($dm^3mol^{-1}s^{-1}$) determined from the experimental i_p/i_d ratios.

that without NADH, together with k_{obsd} values. The k_{obsd} values increase with increasing concentrations of β-CD in solution, demonstrating the acceleration effect of β-CD. The observed rate constants in the presence of β-CD are fairly large as compared with those using typical electron-transfer mediators; 1,4-benzoquinone,[8] 5 $dm^3mol^{-1}s^{-1}$; $Fe(CN)_6^{3-}$,[3] 3 $dm^3mol^{-1}s^{-1}$. This acceleration effect might be discussed by the following three possible mechanisms: (I) oxidation of the included NADH by Fca^+, (II) suppression of the relative contribution of the backward process of reaction (3) by forming the inclusion complex of Fca with β-CD, and (III) oxidation of NADH by Fca^+ included in β-CD.

Mechanism I is least likely since the induced circular-dichroism measurements have shown that NADH is hardly included in β-CD (formation constant: ca. 50 dm^3 mol^{-1}). In addition, the formal potential of the NADH-β-CD/$NADH^{+\bullet}$-β-CD couple would be far positive from that for the uncomlexed couple deduced from the phenomena obtained previously.[1,4] Thus, the electron-transfer from NADH-β-CD to Fca^+ would be energetically very difficult even though the following reaction is rapid.

The relative importance of mechanism II was examined by digital simulation. As shown in Table 1, the thoretical i_p/i_d values are almost independent of the concentration of β-CD, indicating negligibly small influence of mechanism II. The present measurement was conducted at the low initial concentration of Fca. This might reduce the backward reaction.

Next we have investigated the possibility of mechanism III. The equilibrium constant for the following electron-transfer reaction (6) is larger than that for reaction (3), since E_c° is more positive than E_f°:[1]

$$\text{NADH} + \text{Fca}^+\text{-}\beta\text{-CD} \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} \text{NADH}^{+\bullet} + \text{Fca-}\beta\text{-CD} \qquad (6)$$

This means that Fca^+-β-CD is a thermodynamically stronger oxidant compared with Fca^+, but the concentration of the former species is much lower than that of the latter. The digital simulation for this mechanism was also carried out. In this case, the program contained an efficient factor, f,[9] for the electron-transfer reaction (6). For ordinary electron-transfer reactions, f=1 and $0 \leq f < 1$ correspond to the diffusion-controlled and activation-controlled situations, respectively. If E_c° is more positive than E_{NADH}°, $f \approx 0$. This situation is practically equivalent to that in mechanism II. The theoretical i_p/i_d values under various concentrations of β-CD for f=1 and 0.3 in this mechanism are listed in Table 1. This mechanism clearly demonstrates the acceleration effect of β-CD on the electrocatalysis. However, the ratios obtained by f=1 are always larger than those observed experimentally. Excellent agreement between the experimental and simulated values are observed when f=0.3. This means that k_6 is three-tenth of the value expected from the fully diffusion-controlled process (f=1.0). The calculated k_6 ($\gtrsim 1\times10^3$ $dm^3mol^{-1}s^{-1}$) is, nevertheless, sufficiently larger than the k_3 value (33 dm^3 $mol^{-1}s^{-1}$). The observed smaller value (f=0.3) than unity could be a consequence of the surrounding effect of the CD wall which acts as a barrier to the electron-transfer reaction.[1)]

In conclusion, the acceleration effect of β-CD on the electrocatalytic oxidation of NADH by Fca is reasonably accounted for by the effective oxidation of NADH by the small amount of Fca^+ included in β-CD. Since the formal potential for the Fca-β-CD/Fca^+-β-CD couple is much positive than that for the Fca/Fca^+ couple, Fca^+-β-CD acts as an effective oxidant for NADH. The increase in the concentration of β-CD results in an increase in that for Fca^+-β-CD in solution. Therefore, i_p/i_d ratio for the electrocatalytic oxidation of NADH increases with the increasing concentration of β-CD.

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9) The meaning of the efficient factor, f, is expressed by $f=k_6K^+/(k_3K)$ where K and K^+ are the formation constants of the Fca-β-CD and Fca^+-β-CD complexes, respectively. In the present simulation, the following steady-state assumption was used in every time increment on the bases of the above relationship:

$$\frac{d[NAD\cdot]}{dt} = \frac{(fK[CD]+1)k_3[Fca^+][NADH]}{1+\frac{k_{-3}}{k_4}[Fca]}$$

Note that this equation does not contain K^+. In the present case, a definite value for K^+ is not necessary as long as K^+ is sufficiently small.

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