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Electrostatic and redox potential effects on the rate of electron-transfer reaction of nicotinamide adenine dinucleotides with 1-substituted 5-ethylphenazines

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The effects of redox potential and electric charge on the rate of electron-transfer reaction by a two-electron process were investigated. For electron donors, β-NADH, β-NADPH and α-NADH were used; they have similar structures but different charges and different redox potentials. For electron acceptors, the following 5-ethylphenazine derivatives were used: 1-(3-carboxypropyloxy)-5-ethylphenazine, 1-(3-ethoxycarbonylpropyloxy)-5-ethylphenazine, and 1-[*N*-(2-aminoethyl)carbamoylpropyloxy]-5-ethylphenazine. They have similar structures and different charges. Using these donors and acceptors, the potential and the charge effects were estimated separately. In the potential effect, a linear free energy relationship was observed for the change in the redox potential of the donor with a Brønsted slope of about unity. On the other hand, the slope for the change in the potential of the acceptor was about 0.5. These results show that the potential effect due to electron donors is different from that due to electron acceptors. A linear relationship was also observed between activation free energy and electrostatic force (or potential). The redox potential effect and the electrostatic effect are independent and additive. New theory for the mechanism of electron-transfer reactions is needed to explain these results.

Introduction

NAD and NADP are coenzymes for dehydrogenases, and are electron carriers in biological systems. The reaction mechanisms of dehydrogenases have been extensively investigated, but little is known about the step of electron transfer. One way to understand the electron-transfer step is to study nonenzymic reactions for NAD(H) and NADP(H), and to find how the reaction rates depend on factors such as redox-potential differences, steric interactions and electrostatic effects. The effects of these factors have been investigated for electron-transfer reactions among organic and inorganic molecules [1–3] and also biological redox proteins [4–9], and the results have been analyzed using Marcus' theory [10,11]. However, these reactions follow the one-electron process, and for the two-electron process, which the reactions of NAD(H) and NADP(H) follow, such studies have to our knowledge not been reported.

Recently, we have prepared 1-substituted 5-ethylphenazine derivatives, and showed that these derivatives are stable and can accept electrons nonenzymatically from NAD(P)H [12]. Using these derivatives we can design a series of experiments which provide clear results showing the relationships between the reaction rates and the difference in redox potential or electric charge. The study reported here used the following 5-ethylphenazine derivatives as electron acceptors: 1-(3-carboxypropyloxy)-5-ethylphenazine (**I**), 1-(3-ethoxycarbonylpropyloxy)-5-ethylphenaizine (**II**), and 1-[*N*-(2-aminoethyl)carbamoylpropyloxy]-5-ethylphenazine (**III**). The structural differences between them are the size and the terminal charge of the substituent at position 1 (see Fig. 1). For electron donors, β-NADH, β-NADPH and

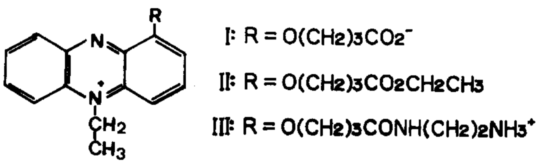

Fig. 1. Phenazine derivatives.

Abbreviation: MTT, (3-(4′,5′-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide.

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α-NADH were used, and the values of their standard oxidation-reduction potential ($E^{\circ\prime}$) are −320, −324 and −341 mV, respectively [13]. Their structural differences are that β-NADPH has an extra phosphate group at the 2′ position of the adenosyl moiety of β-NADH, and that α-NADH is an epimer of β-NADH. Assuming that the structural differences between the nicotinamide adenine dinucleotides do not have steric effects on the electron-transfer rates, we can quantitatively estimate the effects of redox potential and charge on the electron-transfer rate. These results provide us basic information for understanding the electron-transfer step in dehydrogenase reactions and for designing artificial oxidoreductases and coenzymes.

Experimental procedures

Materials

β-NADH and β-NADPH were purchased from Oriental Yeast (Tokyo); α-NADH was from Sigma (St. Louis, MO); MTT was from Dojindo Laboratories (Kumamoto). 5-Ethylphenazine derivatives, **I**, **II** and **III**, were prepared as described in our previous paper [12].

Electron-transfer reaction

The reactions were measured at 30°C in 0.1 M sodium phosphate buffer (pH 7.5) as described in Ref. 12. The reaction mixture contained 1.5 mM MTT, one of the following phenazine derivatives: 0.626 μM of **I**, 0.611 μM of **II** and 0.516 μM of **III**, and one of the following reduced nicotinamide adenine dinucleotides: α-NADH, β-NADH or β-NADPH; the concentrations of the nucleotides were varied from 0 to 0.2 mM. The reactions were recorded as the increase in absorbance at 570 nm (formazan concentration), and the rates were calculated from the initial linear parts. The assays were made at least in triplicate with a reproducibility of less than 5% error. The concentration of the formazan produced from the reduction of MTT was calculated using a millimolar absorption coefficient of 13 $\text{mM}^{-1}\cdot\text{cm}^{-1}$ at 570 nm [14]. The concentrations of β-NADH (and also α-NADH and NADPH) and **I** (and also **II** and **III**) were measured spectrophotometrically using millimolar absorption coefficients of 6.3 $\text{mM}^{-1}\cdot\text{cm}^{-1}$ at 340 nm and 17 $\text{mM}^{-1}\cdot\text{cm}^{-1}$ at 386 nm [12]. The second-order rate constants were obtained from the data as described in our previous paper [12].

Results

Potential effects

The apparent second-order rate constants, $k_{\alpha N}$ and $k_{\beta N}$, for the electron-transfer reactions of α-NADH and β-NADH, respectively, with phenazine derivatives are listed in Table I. The difference between $k_{\alpha N}$ and $k_{\beta N}$ for each phenazine derivative is due to the difference between α-NADH and β-NADH. Actually the ratio of $k_{\alpha N}/k_{\beta N}$ is almost the same for these derivatives, and the values are 5.0, 5.0 and 4.7 for **I**, **II** and **III**, respectively. These differences are due mostly to the difference in $E^{\circ\prime}$ between α- and β-NADH because the steric effect due to their structural difference seems to be small.

TABLE I

Rate constants of electron-transfer reactions and Bronsted slope for redox potential effect

Phenazine derivative	Z_A	$k_{\alpha N}$ ($\text{mM}^{-1}\cdot\text{s}^{-1}$)	$k_{\beta N}$ ($\text{mM}^{-1}\cdot\text{s}^{-1}$)	k_{NP} ($\text{mM}^{-1}\cdot\text{s}^{-1}$)	a
I	0	7.05	1.42	1.83	1.00
II	+1	8.50	1.68	2.63	1.01
III	+2	9.47	2.03	3.72	0.96

The simplest linear relationship between the free energy of activation (ΔG^*) and the standard free-energy change (ΔG°) is expressed as:

$$\Delta G^* = a\cdot\Delta G^\circ + b \tag{1}$$

where a and b are constants. The differences in ΔG^* and in ΔG° between the reactions of α-NADH and β-NADH are expressed as:

$$\Delta G^*_{\alpha N} - \Delta G^*_{\beta N} = -RT\ln(k_{\alpha N}/k_{\beta N}) \tag{2}$$

$$\Delta G^\circ_{\alpha N} - \Delta G^\circ_{\beta N} = n\mathscr{F}(E^{\circ\prime}_{\beta N} - E^{\circ\prime}_{\beta N}) = -4.05\ \text{kJ} \tag{3}$$

where the subscripts, αN and βN stand for α-NADH and β-NADH, respectively. Then the values of the slope, a, in Eqn. 1 for **I**, **II** and **III** are calculated and listed in Table I; the average value is 0.99.

Charge effects

The apparent second-order rate constants, k_{NP}, for the reaction of NADPH with the phenazine derivatives are also listed in Table I. The difference between k_{NP} and $k_{\alpha N}$ for each phenazine derivative is due to the difference in charge and $E^{\circ\prime}$. As the $\text{E}^{\circ\prime}$ values for these nicotinamide adenine dinucleotides are known, the charge effects are clearly shown by the ratios of $k_{NP}/k_{\alpha N}$ and $k_{NP}/k_{\beta N}$. These ratios for each phenazine derivatives may be written as:

$$\ln(k_{NP}/k_{\alpha N}) = p_\alpha(Z_{NP} - Z_{\alpha N})Z_A - a_\alpha(n\mathscr{F}/RT)(E^{\circ\prime}_{NP} - E^{\circ\prime}_{\alpha N}) \tag{4}$$

$$\ln(k_{NP}/k_{\beta N}) = p_\beta(Z_{NP} - Z_{\beta N})Z_A - a_\beta(n\mathscr{F}/RT)(E^{\circ\prime}_{NP} - E^{\circ\prime}_{\beta N}) \tag{5}$$

where p_α and p_β are constants, Z is the net charge, a_α and a_β are the same constants as the slope, a, in Eqn.

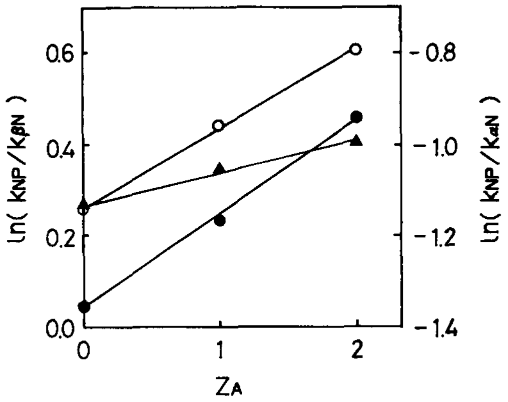

Fig. 2. Plot for Eqns. 4 (●) and 5 (○, ▲). The experimental conditions for the points, ● and ○, are described under Experimental procedures. The values for the points, ▲, were obtained from the reaction rates measured at 30 ° C in the presence of 0.5 M KCl; the reaction mixture contained 0.1 M sodium phosphate buffer (pH 7.5), 0.5 M KCl, 1.5 mM MTT, one of the following phenazine derivatives: 0.517 μM of I, 0.539 μM of II and 0.515 μM of III, and one of the following reduced nicotinamide adenine dinucleotides: 0.158 mM β-NADH and 0.172 mM NADPH.

1, and the subscripts A, αN, βN, and NP stand for the acceptor (phenazine derivatives), α-NADH, β-NADH, and NADPH, respectively.

Fig. 2 shows a linear relationship between Z_A and the logarithm of the ratio of the rate constants. Fig. 2 also shows that the two lines have similar slopes: 0.20 for Eqn. 4 and 0.18 for Eqn. 5. As the 2′-phosphate group of NADP has two net negative charges at pH 7.5,

$$Z_{NP} - Z_{\alpha N} = Z_{NP} - Z_{\beta N} = -2 \quad (6)$$

Then the values of p_α and p_β are -0.10 and -0.09, respectively. The values of a_α and a_β are also calculated from the straight lines to be 1.04 and 0.85, respectively, and are similar to those obtained in Table I.

The values of p_β was also measured in the presence of 0.5 M KCl (see Fig. 2), and is -0.03. This means that the charge effect depends on ionic strength, and a larger effect must be observed in the absence of solvent ions.

These results show that there is a linear relationship between ΔG^* and electrostatic force (or potential), and that this charge effect is independent of the redox potential effect. Therefore, in our reaction system, the linear relationship between ΔG^*, ΔG° and electrostatic force can generally be written as:

$$\Delta G^* = a \cdot \Delta G^\circ - p \cdot RT \cdot Z_D \cdot Z_A + c \quad (7)$$

where c is a constant, Z_D and Z_A are the net charges of the donor and the acceptor, respectively, and the values of a and p are about 1 and -0.1, respectively.

Discussion

We have quantitatively demonstrated the effects of redox potential and electric charge on the electron-transfer reactions between the reduced nicotinamide adenine dinucleotides and 5-ethylphenazine derivatives. In that we have assumed that the structural differences between the nucleotides do not have steric effects on the reaction. The assumption seems to be correct because the values of the slope, a, listed in Table I are similar to that of a_α in Eqn. 4 and also to that of a_β in Eqn. 5. The linearity of the data in Fig. 2 and the similarity of the slopes of the two lines also show that the steric effect due to the structural differences is small. Our experimental ranges of potential differences and charge difference are not large, but our data are accurate enough to estimate the pure potential and charge effects. These results provide fundamental information not only for the two-electrontransfer process in dehydrogenase reactions but also for general electron transfer reactions.

The potential effect described here reflects the difference in $E^{\circ\prime}$ between the electron donors, and the Brønsted slope, a, in Eqn. 7 is between 0.9 and 1.0. This means that the difference in ΔG° due to the difference in $E^{\circ\prime}$ of the donors is matched by the difference in ΔG^*, and the molecular structure of the activation complex is common for the donors. A Brønsted slope of unity is unusual, but one example has been reported by Brondwell and Clemens [15,16] for single electron transfer from carbanions.

In our previous paper [12], second-order rate constants for the electron-transfer reactions between β-NADH and 5-methylphenazine and between β-NADH and 5-ethylphenazine are given as 1.83 $mM^{-1} \cdot s^{-1}$ and 0.75 $mM^{-1} \cdot s^{-1}$, respectively. As the values of $E^{\circ\prime}$ for 5-methylphenazine and 5-ethylphenazine are 80 mV and 55 mV, respectively [13], the value of the slope, a, in Eqn. 1 is calculated to be 0.47, which is about half of that obtained in this work. Here it is assumed that the steric effect due to the structural difference between 5-methylphenazine and 5-ethylphenazine is negligible, but it should be noted that if a significant steric effect is present, the true potential effect (i.e., the value of the slope) must be much smaller, because the ethyl group is bulkier than the methyl group. Therefore, we conclude that the degree of the potential effect due to electron donors is larger than that due to electron acceptors in our system; more generally, the rate constant of an electron-transfer reaction is differently related to the donor potential and to the acceptor potential. This difference has not yet been considered probably because of the lack of clear data. The structure of the activation complex must be known for understanding the true relationship between the rate constant and ΔG°.

The presence and the importance of the effect of electric charge on the rate of electron-transfer reactions have been demonstrated [4–11], but the pure charge effect has not been estimated quantitatively. Our experimental system is well designed for measuring the charge effect: the value can be obtained directly by comparing k_{NP} and $k_{\alpha\mathrm{N}}$ or k_{NP} and $k_{\beta\mathrm{N}}$ for each phenazine derivatives having a different electric charge. Our results suggest the presence of a simple relationship shown as Eqn. 7. This means that electrostatic force (or potential) between a donor and an acceptor is linearly related to ΔG^*, and that the two effects due to redox potential and electric charge are independent and additive. It should be noted that the charge effect is observed in 0.1 M sodium phospate buffer solution (pH 7.5, ionic strength of 0.23 M). The observed charge effect seems to be related to the electron-transfer step, and may include the term of ΔG^*_{es} reported by Jordi and Erman [4]; electrostatic force between a donor and an acceptor could contribute to stabilize an activation complex or to shorten the distance of the electron jump.

These potential and charge effects have not been reported, and there are no theories that can explain them. Our results will provide new insight into the mechanism of electron-transfer reactions as well as basic information for designing artificial oxidoreductases and coenzymes.

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