Acid-catalysed Reduction of *p*-Benzoquinone Derivatives by an NADH Analogue, 9,10-Dihydro-10-methylacridine. The Energetic Comparison of One-electron *vs.* Two-electron Pathways

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The energetic comparison of one-electron *vs.* two-electron pathways has been made for the acid-catalysed reduction of a series of *p*-benzoquinone derivatives (Q) by an NADH analogue, 9,10-dihydro-10-methylacridine ($AcrH_2$), in aqueous solutions (H_2O–EtOH; 5:1 v/v) at 298 K. The pH dependences of the second-order rate constants (log k_{obs}) as well as the primary kinetic isotope effects (k_H/k_D) for the acid-catalysed reduction of Q by $AcrH_2$ have been correlated well with the pH dependences of the one-electron reduction potentials of *p*-benzoquinone derivatives. On the other hand, no direct correlation of log k_{obs} or k_H/k_D with the corresponding two-electron reduction potentials has been observed. Mechanisms of the acid-catalysed hydride transfer reactions from $AcrH_2$ to Q have been discussed based on the energetic comparison of one-electron *vs.* two-electron pathways.

The redox reactions of 1,4-dihydropyridine derivatives have attracted considerable interest in terms of the vital role of the pyridine nucleotide coenzymes, *i.e.*, NADH (dihydronicotinamide adenine dinucleotide) used as electron sources in various enzymatic redox reactions.[1,2] When one-electron oxidants are used as electron acceptors for the two-electron oxidation of NADH and NADH model compounds, it has been well established that the two-electron oxidation proceeds by an electron–proton–electron sequence.[3,4] When two-electron oxidants are used as hydride acceptors, however, it is very difficult to distinguish between an inner-sphere electron-transfer process resulting in the subsequent proton–electron (or hydrogen) transfer in the cage and a one-step hydride-transfer process, since there are no detectable intermediates in either case.[5–7] In such a case, the distinction between one-electron and two-electron pathways may be made by examining the correlations of the activation barriers of the reactions with the energetics of the one-electron and two-electron pathways. However, the energetics of one-electron pathways of homologous series of reactants are usually in parallel with those of the corresponding two-electron pathways.[7] Thus, it is difficult to distinguish between one-electron and two-electron pathways based simply on the energetic comparison of homologous series. In this context, we have recently reported that the rate constants of hydride-transfer reactions of various NADH model compounds including different types to a series of *p*-benzoquinone derivatives are correlated with the energetics of one-electron pathways much better than those of two-electron pathways.[8] However, such mechanistic distinction has so far been limited to non-catalytic or metal-ion catalysed reductions of substrates by NADH model compounds.[5–8] Since acid catalysis is known to play an essential role in the enzyme-catalysed reduction of substrates by NADH,[9] it seems more important to study the mechanisms of acid-catalysed reduction of substrates by NADH model compounds than those of non-catalytic reactions. Thus, it is the purpose of this study to examine the energetic comparison of the one-electron *vs.* the two-electron pathways in acid-catalysed reduction of various substrates by NADH model compounds.

The substrates we have chosen are *p*-benzoquinone derivatives, since the redox and acid–base properties of *p*-benzoquinone derivatives and the reduced forms (one-electron and two-electron) have been well established and they are important thermodynamic parameters in biological redox systems.[10–13] Although the one-electron redox properties of *p*-benzoquinone derivatives are also in parallel with the corresponding two-electron redox properties in the absence of acid,[10] the pH dependences of their one-electron reduction potentials are known to be quite different from those of the corresponding two-electron reduction potentials.[10–12] Thus, we have examined the pH dependences of the rate constants for the reduction of *p*-benzoquinone derivatives by an NADH model compound, which will provide a nice opportunity to distinguish between the one-electron and the two-electron pathways.

Experimental

Materials.—9,10-Dihydro-10-methylacridine ($AcrH_2$) used as an acid-stable NADH model compound was prepared from methylacridinium iodide ($AcrH^+I^-$) by the reduction with $NaBH_4$ in methanol, and purified by recrystallization from ethanol.[14] The dideuteriated compound, 9,9′-[$^2H_2$]-9,10-dihydro-10-methylacridine ($AcrD_2$), was prepared from 10-methyl-(9H)-acridone by reduction with $LiAlD_4$,[15] which was obtained from Aldrich Chemical Co. 9,10-Dihydro-10-methyl-[$^2H_3$]acridine ($AcrH_2$–CD_3) was prepared by $NaBH_4$ reduction of 10-methyl[$^2H_3$]acridinium iodide which was obtained by reaction of acridine with CD_3I in methanol.[16] Various *p*-benzoquinone derivatives and tetracyanoquinodimethan used in this study were obtained commercially and purified according to the literature.[17] Perchloric acid (70%) was obtained from Wako Pure Chemicals.

Spectral and Kinetic Measurements.—Rates of acid-catalysed reduction of *p*-benzoquinone derivatives by $AcrH_2$ were monitored by measuring the rise of the absorbance due to $AcrH^+$ $\lambda_{max.}$ 358 nm (ε 1.8×10^4 dm^3 mol^{-1} cm^{-1}), using a JASCO UVDEC-220B spectrophotometer. Fast reactions of

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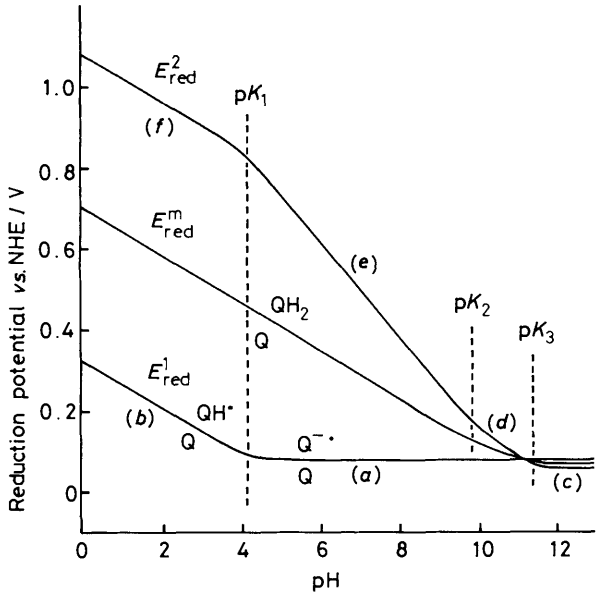


Figure 1. Dependence of the reduction potentials of *p*-benzoquinone (Q) in H_2O.[11] The lower line is the one-electron reduction process of Q. The upper line is the one-electron reduction process of $Q^{-\bullet}$ and QH•. The middle line is the two-electron reduction process of Q [equation (1)].

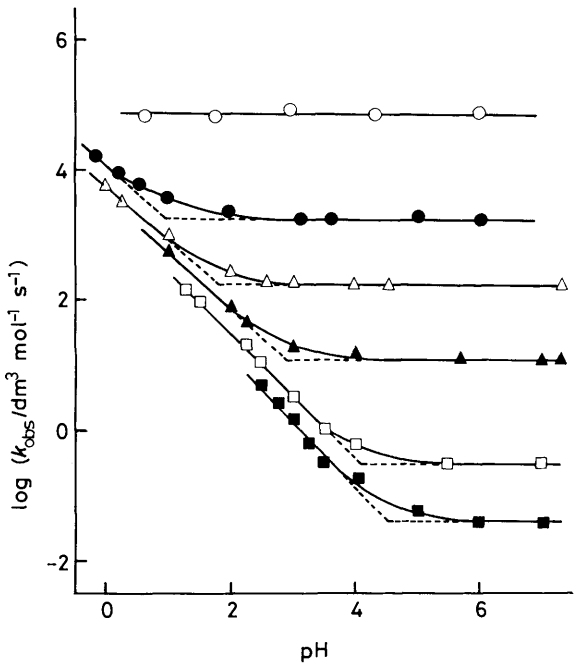


Figure 2. Plots of log k_{obs} *vs.* pH for the reduction of TCNQ (○), *p*-chloranil (●), 2,6-dichloro-*p*-benzoquinone (△), chloro-*p*-benzoquinone (▲), *p*-benzoquinone (□), and methyl-*p*-benzoquinone (■) in H_2O–EtOH (5:1 v/v) at 298 K.

$AcrH_2$ with *p*-benzoquinone derivatives in the presence of $HClO_4$ in aqueous solutions (H_2O–EtOH; 5:1 v/v) at 298 K with half-lives shorter than 10 s were determined by the increase in the absorbance at λ_{max} of $AcrH^+$, using a Union RA-103 stopped-flow spectrophotometer. Kinetic measurements were normally carried out under the conditions in which the concentrations of substrates and $HClO_4$ were maintained at more than ten-fold excess of the concentration of $AcrH_2$. In the pH region where QH• is protonated,[13] the kinetics were complicated by the comproportionation reactions between Q and QH_2. All the rate constants were determined within a pH range within which the rates obeyed pseudo-first-order kinetics, using a microcomputer.

Results and Discussion

Acid-catalysed Reduction of p-*Benzoquinone Derivatives by an* NADH *Model Compound.*—Figure 1 shows the pH dependences of both the one-electron and two-electron reduction potentials of *p*-benzoquinone (Q).[10–12] The variations of the reduction potentials with pH are governed by the acid– base properties of the reduced species. As such, the one-electron reduction potential of Q (E^1_{red}) is determined by the standard one-electron reduction potential of Q, $E^0(Q/Q^{-\bullet})$, and the acid dissociation constant of QH• (pK_1). At high pH values ($pH > pK_1$), $Q^{-\bullet}$ predominates as the reduced species, and thereby E^1_{red} [*vs.* NHE (normal hydrogen electrode)] is independent of pH, equal to $E^0(Q/Q^{-\bullet})$ as shown by line (*a*) in Figure 1. In the region $pH < pK_1$, QH• predominates as the reduced species when the E^1_{red} value is shifted to the positive direction with a slope of $2.3RT/F$ (F is the Faraday constant) which corresponds to 0.0592 at 298 K [line (*b*), Figure 1]. The one-electron reduction potential of the reduced species ($Q^{-\bullet}$ and QH•), E^2_{red} (*vs.* NHE), also varies depending on the acid dissociation constants of QH^- (pK_2) and QH_2 (pK_3) as shown by lines (*c*)–(*f*) in Figure 1. The two-electron reduction potential of Q (E^m_{red} *vs.* NHE) is obtained from equation (1). The pH

$$E^m_{red} = \tfrac{1}{2}(E^1_{red} + E^2_{red}) \quad (1)$$

dependence of E^m_{red} is shown by the middle line in Figure 1, where the E^m_{red} value is shifted to the positive direction in the region $pH < pK_3$. Thus, the pH dependence of the energetics of the two-electron reduction of Q (E^m_{red}) is quite different from that of the one-electron reduction (E^1_{red}). If the two-electron reduction of Q proceeds by a one-step process, the pH dependence of the rate would be correlated directly with the pH dependence of E^m_{red}, and thereby the rate would increase in the region $pH < pK_3$. On the other hand, if the two-electron reduction of Q proceeds *via* the one-electron reduction, the pH dependence of the rate would be correlated with the pH dependence of E^1_{red}, and thereby the rate would increase in the region $pH < pK_1$. Thus, examination of the pH dependence of the rate of the two-electron reduction of Q by an NADH model compound will provide an unequivocal basis to determine whether the activation barrier is determined by the one-electron or two-electron process.

Since NADH and ordinary NADH model compounds are knownt to be subjected to the acid-catalysed hydration,[18] 9,10-dihydro-10-methylacridine ($AcrH_2$), which is stable in the presence of $HClO_4$,[19] is used as an NADH model compound. The rates of the two-electron reductions of various *p*-benzoquinone derivatives (Q) and 7,7,8,8-tetracyanoquinodimethan (TCNQ) by $AcrH_2$ in aqueous solutions (H_2O–

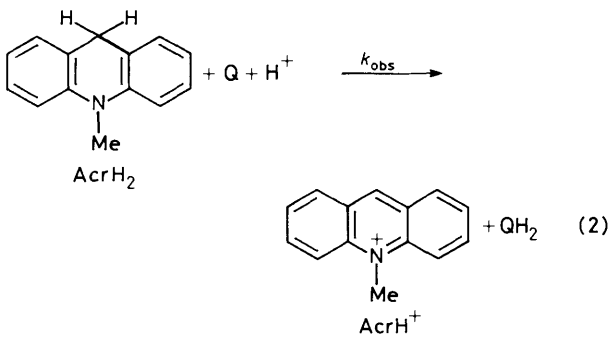


Table 1. Comparison of the observed acid dissociation constants pK_{obs} of reduced *p*-benzoquinone derivatives (Q) with pK_1 of $QH^{\bullet}$, pK_2 of QH_2, and pK_3 of QH^-.

p-Benzoquinone derivative	pK_{obs} [a]	pK_1 [b]	pK_2 [b]	pK_3 [b]
p-Chloranil	0.9	0.9 [c]	5.6	8.2
2,6-Dichloro-*p*-benzoquinone	1.8	2.1	7.9	10.0
Chloro-*p*-benzoquinone	2.9	3.1 [c]	8.9	10.7 [d]
p-Benzoquinone	4.1	4.1	9.9	11.4
Methyl-*p*-benzoquinone	4.5	4.45	10.1	11.5

[a] Determined from the results in Figure 2. [b] Taken from ref. 10 unless otherwise noted. [c] Taken from ref. 13. [d] Assumed to be the average of 2,6-dichloro-*p*-benzoquinone and *p*-benzoquinone.

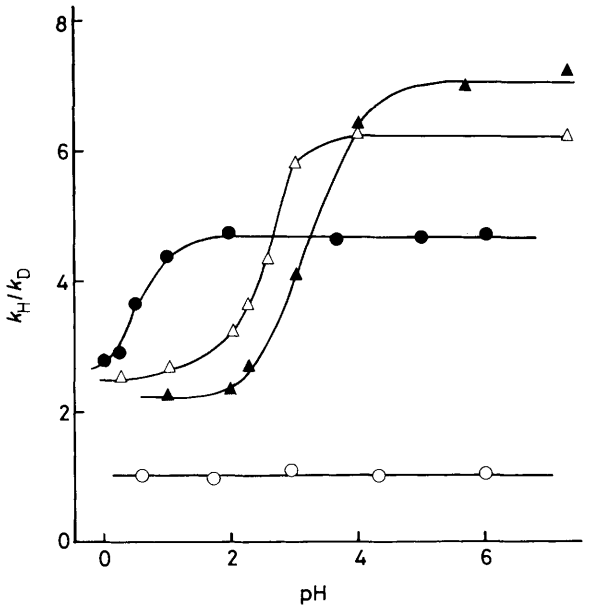


Figure 3. Plots of the primary kinetic isotope effects k_H/k_D *vs.* pH for the reduction of TCNQ (○), *p*-chloranil (●), 2,6-dichloro-*p*-benzoquinone (△), and chloro-*p*-benzoquinone (▲) by $AcrH_2$ and $AcrD_2$ in H_2O–EtOH (5:1 v/v) at 298 K.

EtOH; 5:1 v/v) at 298 K were determined by monitoring the formation of $AcrH^+$ (λ_{max} 358 nm), equation (2). The rates in the presence of greater than ten-fold excess of Q or TCNQ obeyed the pseudo-first-order kinetics at the pH region examined (see Experimental), and the pseudo-first-order rate constants were proportional to the substrate concentrations. The pH dependences of the observed second-order rate constants (log k_{obs}) are shown in Figure 2. The log k_{obs} value of each Q in Figure 2 exhibits variation with pH in agreement with the pH dependence of the one-electron reduction potential of Q (E^1_{red}) in Figure 1. The log k_{obs} value of each Q is independent of pH in the region pH > pK_{obs}, but increases with decreasing pH in the region pH < pK_{obs}. The pK_{obs} values, thus determined for five different *p*-benzoquinone derivatives, agree well with the corresponding pK_1 values of $QH^{\bullet}$, which are clearly different from the pK_2 and pK_3 values as shown in Table 1. In the case of TCNQ, the log k_{obs} value is independent of pH. The $TCNQ^{-\bullet}$ (λ_{max} 842 nm) [20] was stable in the pH region examined in Figure 2, thus, no protonation of $TCNQ^{-\bullet}$ has occurred in this pH region.

The primary kinetic isotope effects (k_H/k_D) were also examined by replacing $AcrH_2$ with the 9,9′-dideuteriated analogue ($AcrD_2$). The k_H/k_D values were determined from the ratios of the rate constants of $AcrH_2$ to those of $AcrD_2$ when the

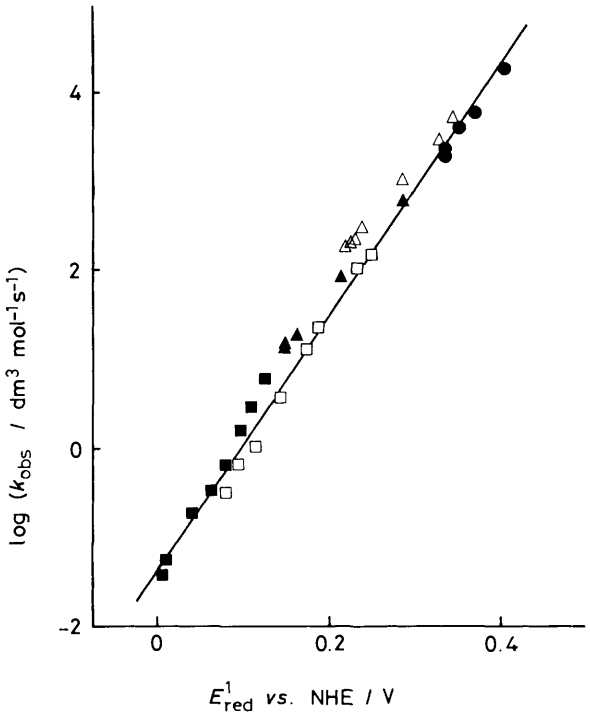


Figure 4. Plots of log k_{obs} *vs.* the one-electron reduction potentials of Q (E^1_{red}) for the reduction of *p*-benzoquinone derivatives by $AcrH_2$ in H_2O–EtOH (5:1 v/v) at 298 K. The symbols are the same as those used in Figure 2.

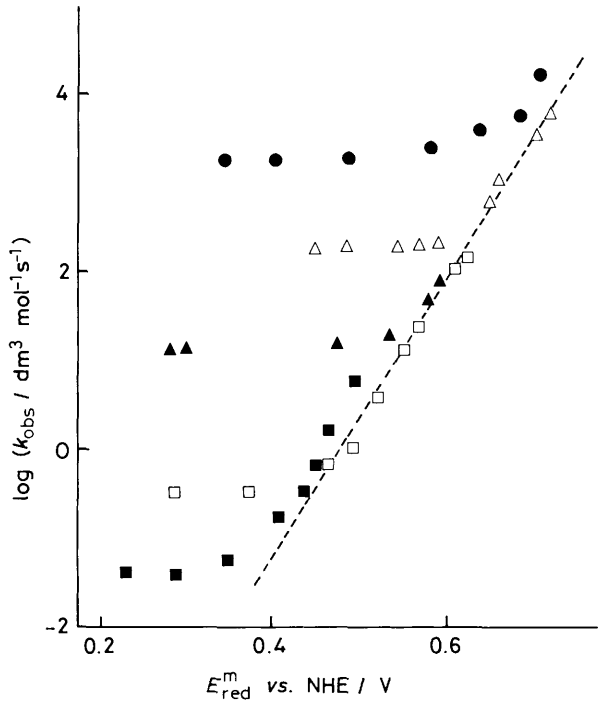


Figure 5. Plots of log k_{obs} *vs.* the two-electron reduction potentials of Q (E^m_{red}) for the reduction of *p*-benzoquinone derivatives by $AcrH_2$ in H_2O–EtOH (5:1 v/v) at 298 K.

secondary kinetic isotope effects are assumed to be unity. The pH dependences of k_H/k_D are shown in Figure 3. It has been confirmed that no primary kinetic isotope effects are observed when $AcrH_2$ is replaced by 10-methyl-[$^2H_3$]acridine ($AcrH_2$–CD_3). In the case of TCNQ, the k_H/k_D value is close to unity,

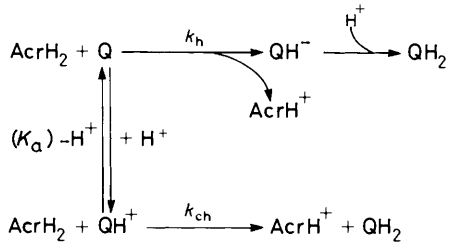
independent of pH, when the k_{obs} value is also independent of pH (compare Figure 3 with Figure 2). The k_H/k_D value of each Q is constant in the region pH > pK_1, where the k_{obs} value is independent of pH, but decreases to a much smaller value in the region pH > pK_1 where the k_{obs} value increases with decreasing pH (compare Figure 3 with Figure 2).

Thus, the pH dependences of both k_{obs} and k_H/k_D strongly indicate that the activation barrier of the two-electron reduction of Q by $AcrH_2$ is correlated with the energetics of the one-electron pathway but not with the two-electron pathway. This can be shown more clearly by the plots of the log k_{obs} values of various *p*-benzoquinone derivatives (Q) *vs.* the one-electron reduction potentials of Q (E^1_{red}) and the two-electron reduction potentials (E^m_{red}) as shown in Figures 4 and 5, respectively. All the values of k_{obs} in Figure 2 are unified as a single correlation between log k_{obs} and E^1_{red} (Figure 4) as given by equation (3). On

$$\log k_{obs} = 14.4\ E^1_{red} - 1.3 \qquad (3)$$

the contrary, the plot in Figure 5 shows no single correlation between log k_{obs} and E^m_{red}. Closer examination of the plots in Figure 5 reveals that a correlation between k_{obs} and E^m_{red} exists only in the region pH > pK_1 where the E^1_{red} values are in parallel with the E^m_{red} values (Figure 1).

Mechanisms of Acid-catalysed Reduction of Q *by* $AcrH_2$.—As demonstrated above, the acid-catalysed two-electron reduction of Q by $AcrH_2$ may proceed by a one-electron pathway. However, one might argue that a direct transfer of a hydride ion is still possible when the reaction occurs by the combination of acid-independent hydride transfer (k_h) and acid-catalysed hydride transfer (k_{ch}) as shown in Scheme 1, where K_a is the



Scheme 1.

acid-dissociation constant of a protonated quinone QH^+. According to Scheme 1, the observed rate constant (k_{obs}) may be given by equation (4). Then, at pH < log ($k_{ch}K_a^{-1}k_h^{-1}$), k_{obs}

$$k_{obs} = k_h + k_{ch}K_a^{-1}[H^+] \qquad (4)$$

($=k_{ch}K_a^{-1}[H^+]$ increases with decreasing pH, while at pH > log ($k_{ch}K_a^{-1}k_h^{-1}$), k_{obs} ($=k_h$) becomes independent of pH. In this case, however, pK_{obs} in Figure 2 corresponds to $-\log(k_{ch}K_a^{-1}k_h^{-1})$, and the agreements of the $k_{ch}K_a^{-1}k_h^{-1}$ values with the K_1 values in Table 1 should be considered as merely fortuitous. Moreover, the k_{ch} values would exceed the diffusion rate constant in aqueous solution (3×10^9 dm^3 mol^{-1} s^{-1}).[21] For example, the k_{ch} value of *p*-benzoquinone at pH = 2.25 ($k_{ch} = k_{obs}K_a[H^+]^{-1}$) can be evaluated from the k_{obs} value at pH = 2.25 (2.2×10 dm^3 mol^{-1} s^{-1}) and the pK_a value of *p*-benzoquinone (−7),[22] as 4×10^{10} dm^3 mol^{-1} s^{-1}, which is much larger than the diffusion rate constant. Although the pK_a values of other *p*-benzoquinone derivatives are not known, the pK_a value of *p*-chloranil may be much more negative than that of *p*-benzoquinone, judging from the larger electron affinity of *p*-chloranil than *p*-benzoquinone.[23] Then, the lowest estimate of the k_{ch} value of *p*-chloranil is obtained as $>1.1 \times 10^{11}$ dm^3 mol^{-1} s^{-1}, which also exceeds the diffusion limit. As such, Scheme 1 cannot account for the pH dependence of k_{obs} in Figure 2. Even if all the k_{ch} values are assumed to be diffusion limited, the k_H/k_D values in such a case would be unity for all the *p*-benzoquinone derivatives when catalysed by acid. This is certainly not the case as shown in Figure 3.

As discussed above, a protonated quinone QH^+ may not be involved in the reduction of Q. Thus, the most plausible mechanism which can account for all the experimental results (Figures 2–5) may be given as shown in Schemes 2 and 3. In the

$$AcrH_2 + Q \underset{}{\overset{k_{et}}{\rightleftharpoons}} (AcrH_2^{+\bullet}\ Q^{-\bullet}) \xrightarrow{k_{H^+}} (AcrH^\bullet\ QH^\bullet) \xrightarrow{fast} AcrH^+ + QH^-$$

Scheme 2.

$$AcrH_2 + Q + H^+ \underset{k_{cb}}{\overset{k_{cet}[H^+]}{\rightleftharpoons}} (AcrH_2^{+\bullet}\ QH^\bullet) \xrightarrow{k_H} AcrH^+ + QH_2$$

Scheme 3.

region pH > pK_1 (Scheme 2), the one-electron reduction of Q gives $Q^{-\bullet}$ (Figure 1). The free-energy change of electron transfer (ΔG^0_{et}) from $AcrH_2$ to Q is highly endothermic judging from the one-electron oxidation potential of $AcrH_2$ (E^1_{ox} 1.04 V *vs.* NHE, which corresponds to 0.80 V *vs.* SCE)[8] and the one-electron reduction potentials of Q ($E^1_{red} \ll 1.04$ V *vs.* NHE)[10] in H_2O; $\Delta G^0_{et} = F(E^1_{ox} - E^1_{red})$. In such a case, the endothermic electron transfer from $AcrH_2$ to Q (k_{et}) to give a radical ion pair ($AcrH_2^{+\bullet}\ Q^{-\bullet}$), may be followed by the proton transfer from $AcrH_2^{+\bullet}$ to $Q^{-\bullet}$ (k_{H^+}) to give a radical pair ($AcrH^\bullet\ QH^\bullet$) in competition with the back electron transfer (k_b). The free-energy change of electron transfer from $AcrH^\bullet$ to $QH^\bullet$ is highly exothermic judging from the one-electron oxidation potential of $AcrH^\bullet$ (E^2_{ox} −0.19 V *vs.* NHE)[8] and the one-electron reduction potentials of $QH^\bullet$ in H_2O ($E^2_{red} \gg -0.19$ V *vs.* NHE, *e.g.*, see Figure 1).[10] Thus, the facile electron transfer from $AcrH^\bullet$ to $QH^\bullet$ may occur to yield $AcrH^+$ and QH^-. At pH < pK_2, the QH^- is protonated to yield QH_2. We have previously delineated this mechanism (Scheme 2) for uncatalysed hydride-transfer reactions from various NADH model compounds to Q in detail.[8]

On the other hand, the one-electron reduction gives $QH^\bullet$ in the region pH < pK_1 (Figure 1), when acid-catalysed electron transfer from $AcrH_2$ to Q becomes energetically more favourable as pH decreases (Figure 1). In such a case, the acid-catalysed electron transfer from $AcrH_2$ to Q (k_{cet}) results in the formation of a radical pair ($AcrH_2^{+\bullet}\ QH^\bullet$) as shown in Scheme 3. Without a subsequent exothermic reaction, no net reaction would occur, since the acid-catalysed electron transfer may still be endothermic judging from the one-electron reduction potentials of various *p*-benzoquinone derivatives in the presence of acid (*e.g.*, Figure 1) which are still more negative than the one-electron oxidation potential of $AcrH_2$ (E^0_{ox} 1.04 V *vs.* NHE).[8] The proton affinity of $QH^\bullet$ is known to be much less than $Q^{-\bullet}$.[12,13] For example, the pK_a value of $AcrH_2^{+\bullet}$ (pK_a = 2.0)[8] is larger than the pK_a value of $QH_2^{+\bullet}$ (pK_a = 0.3),[13] and thereby the proton transfer from $AcrH_2^{+\bullet}$ to $QH^\bullet$ is endothermic. On the other hand, the pK_a value of $AcrH_2^{+\bullet}$ is smaller than the pK_1 value of $QH^\bullet$ (pK_i = 4.1; see Table 1),[10] and thereby the proton transfer from $AcrH_2^{+\bullet}$ to $Q^{-\bullet}$ is exothermic. Then, the proton transfer from $AcrH_2^{+\bullet}$ to $QH^\bullet$ may be endothermic, in contrast to the case of the proton transfer from $AcrH_2^{+\bullet}$ to $Q^{-\bullet}$. Thus, the subsequent endothermic reaction may proceed by the hydrogen transfer from $AcrH_2^{+\bullet}$ to $QH^\bullet$ (k_H) to yield $AcrH^+$ and QH_2 in competition with the back electron transfer (k_{cb}) as shown in Scheme 3.

According to Schemes 2 and 3, the observed rate constants (k_{obs}) may be given by equation (5), where K_{et} and $K_{cet}[H^+]$ are

$$k_{obs} = \frac{K_{et}}{(k_{H^+}^{-1} + k_b^{-1})} + \frac{K_{cet}[H^+]}{(k_H^{-1} + k_{cb}^{-1})} \quad (5)$$

the equilibrium constants of electron transfer, k_{et}/k_b and $k_{cet}[H^+]/k_{cb}$, respectively. The equilibrium constant of electron transfer, log K_{et} (pH > pK_1) or log $K_{cet}[H^+]$ (pH < pK_1) is given as a function of E^1_{red}, equation (6), where C_1 =

$$\log (K_{et} \text{ or } K_{cet}[H^+]) = FE^1_{red}/(2.3RT) + C_1 \quad (6)$$

$-(FE^1_{ox} + w_p)/(2.3RT)$; E^1_{ox} is the one-electron oxidation potential of $AcrH_2$ and w_p is the work term used to bring the products ($AcrH_2^{+\cdot}$ and $Q^{-\cdot}$ or QH$^\cdot$) to the mean separation of the complex.[8] From equations (5) and (6), log k_{obs} can then be correlated with E^1_{red} by equation (7), where $C_2 = -(FE^1_{ox} +$

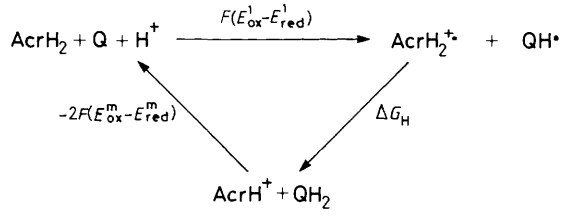
$$\log k_{obs} = FE^1_{red}/(2.3RT) + C_2 \quad (7)$$

$w_p)/(2.3RT) - \log C_3$; $C_3 = (k_{H^+}^{-1} + k_b^{-1})$ at pH > pK_1, $C_3 = (k_H^{-1} + k_{cb}^{-1})$ at pH < pK_1. Assuming that the C_2 values are rather constant with the variation of pH and substrates, log k_{obs} may be correlated linearly with E^1_{red} in agreement with the experimental plot in Figure 4. Such an assumption may be reasonable, since the proton transfer (k_{H^+}), hydrogen transfer (k_H), and the back electron transfer (k_b, k_{cb}) are exothermic and thus, these rate constants may be large enough to be rather constant with the change of pH and substrates. Moreover, the slope in Figure 4 [14.4, equation (3)] shows a reasonable agreement with the slope in equation (7) ($F/2.3RT$) at 298 K, which corresponds to 16.9. The small deviation of the observed slope in equation (3) from the calculated slope in equation (7) may be ascribed to the small variation of the C_2 in equation (7). According to equation (3) and the relation between E^1_{red} and E^m_{red} in Figure 1, only the log k_{obs} values at pH < pK_1 are expected to be correlated linearly with the two-electron reduction potentials E^m_{red}. In fact, such a limited correlation is observed in Figure 5.

The primary kinetic isotope effects in Figure 3 are also consistent with Schemes 2 and 3 as follows. At pH > pK_1 (Scheme 2), the observed primary kinetic isotope effects may be attributed to those for the proton transfer from $AcrH_2^{+\cdot}$ to $Q^{-\cdot}$. The magnitude of k_H/k_D of the proton transfer process is known to vary with the free-energy change of the proton transfer ($\Delta G^0_{H^+}$).[8,24] The maximum value (k_H/k_D = 7) is known to be obtained at $\Delta G^0_{H^+}$ = 0, when the proton is symmetrically bonded to the atoms between which it is being transferred.[8,24] In fact, the large k_H/k_D values (6.2 and 7.1 at pH > pK_1) are obtained for 2,6-dichloro-*p*-benzoquinone and chloro-*p*-benzoquinone, respectively (Figure 3), when the $\Delta G^0_{H^+}$ values are close to zero, judging from the ΔG^0_H value obtained by the relation, $\Delta G^0_{H^+} = 2.3RT(pK_a - pK_1)$ in which the pK_1 values of the semiquinone radicals (2.9 and 1.8 for chloro-*p*-benzoquinone and 2,6-dichloro-*p*-benzoquinone, respectively)[13] are close to the pK_a value of $AcrH_2^{+\cdot}$ (2.0).[8] The k_H/k_D value of *p*-chloranil (4.7 at pH > pK_1) becomes significantly smaller as is expected when the increase in the $\Delta G^0_{H^+}$ values is considered.[8,24] In the case of a strong oxidant, *i.e.*, TCNQ, the electron transfer from $AcrH_2$ to TCNQ becomes energetically more favourable relative to *p*-benzoquinone derivatives, and no protonation of $TCNQ^{-\cdot}$ occurs. In such a case, the back electron transfer (k_b) may become much smaller than the proton transfer (k_{H^+}), when equation (5) is reduced to equation (8). Thus, no primary kinetic isotope effect is observed as shown in Figure 3.

$$k_{obs} = k_{et} \quad (8)$$

At pH < pK_1, the species which can accept hydrogen may be changed from $Q^{-\cdot}$ to QH$^\cdot$ (Scheme 3). In such a case, the observed primary kinetic isotope effects may be attributed to those for the hydrogen transfer from $AcrH_2^+$ to QH$^\cdot$. The magnitude of k_H/k_D of the hydrogen transfer is also known to vary with the free-energy change of the hydrogen transfer (ΔG^0_H) and is a maximum at ΔG^0_H = 0.[25] When the hydrogen transfer is highly exothermic, the k_H/k_D value becomes significantly smaller.[25] For example, it has been reported that the k_H/k_D value of the hydrogen transfer from 2-methylpropane-2-thiol to benzyl radical is 6.35 when ΔH = 3 kcal mol^{-1},* but the value of the corresponding hydrogen transfer to phenyl radical becomes much smaller, k_H/k_D = 1.88 when ΔH = −24 kcal mol^{-1}.[25] The hydrogen transfer from $AcrH_2^{+\cdot}$ to QH$^\cdot$ may also be highly exothermic as follows. The free-energy change of the hydrogen transfer from $AcrH_2^+$ to QH$^\cdot$ (ΔG_H) may be evaluated using the thermochemical cycle (Scheme 4) and given by the relation,



Scheme 4.

$\Delta G_H = 2F(E^m_{ox} - E^m_{red}) - F(E^1_{ox} - E^1_{red})$, where E^m_{ox} is the two-electron oxidation potential of $AcrH_2$. The E^m_{ox} value at pH 2 is obtained as 0.43 V *vs.* NHE using the relation, $E^m_{ox} = (E^1_{ox} + E^2_{ox})/2$, where E^1_{ox} = 1.04 V *vs.* NHE E^2_{ox} = −0.19 V *vs.* NHE.[8] Then, the ΔG_H value at pH = 2 in the case of *p*-benzoquinone (E^m_{red} and E^1_{red} at pH = 2 are 0.58 and 0.205 V *vs.* NHE, see Figure 1) is obtained as −26 kcal mol^{-1} [$= 2F(0.42 - 0.58) - F(1.04 - 0.205)$]. Thus, the change of the species from $Q^{-\cdot}$ which accepts a proton from $AcrH_2^{+\cdot}$ at pH > pK_1 to QH$^\cdot$ which accepts a hydrogen from $AcrH_2^{+\cdot}$ at pH < pK_1 may cause the observed drastic decrease in the k_H/k_D value as shown in Figure 3.

Under the conditions that $k_H \gg k_{cb}$ at pH < pK_1, equation (5) may be reduced to $k_{obs} = k_{cet}[H^+]$, when the forward acid-catalysed electron transfer becomes the sole rate-determining step, and thereby no primary kinetic isotope effect would be observed. Thus, the increase in forward acid-catalysed electron-transfer rate may be accompanied by the decrease in the back electron transfer rate (k_{cb}), which may also contribute to the decrease in the observed primary kinetic isotope effect with a decrease in pH (Figure 3).

The present results add significant weight to our previous conclusion[8] that the rate constants as well as the primary kinetic isotope effects for two-electron reductions of *p*-benzoquinone derivatives by NADH model compounds are correlated much better with the energetics of the one-electron pathways rather than the two-electron pathways.

* 1 cal = 4.184 J.

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