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Direct Determination for the pK_a Values of Radical Cations of NADH Analogues

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The pK_a values of radical cations of NADH analogues, 10-methyl-9,10-dihydroacridine ($AcrH_2$) and 9,10-dimethyldihydroacridine (AcrHMe), have been determined directly by measuring the decay of the transient absorption spectra of the radical cations, formed by the electron transfer oxidation with Fe^{3+}, through the deprotonation in the presence of various concentrations of perchloric acid in acetonitrile at 298 K.

The biological importance of dihydronicotinamide adenine dinucleotide (NADH) used as an electron source has attracted considerable interest in the electron transfer oxidation of NADH and its analogues, in which the radical cations of NADH and analogues should be formed.[1-3] We have previously evaluated the pK_a values of radical cations of NADH analogues by analyzing the kinetic data for electron transfer from NADH analogues to one-electron oxidants such as $[Fe(bpy)_3]^{3+}$ (bpy = 2,2'-bipyridine) in the presence of various bases in MeCN.[4] On the other hand, Savéant et al. have recently reported the pK_a value of the radical cation of 10-methyl-9,10-dihydroacridine ($AcrH_2$) used as an NADH analogue based on the thermochemical analysis.[5] The pK_a value is, however, 8 pH unit lower than the pK_a value derived from the kinetic analysis.[4,5] The origin of such large discrepancy for the pK_a values has remained to be solved, since the pK_a is one of the most essential properties in understanding mechanisms of the oxidation of NADH analogues. In this study we report the direct determination for the pK_a values of $AcrH_2^{+\bullet}$ and 9-methyl substituted analogue ($AcrHMe^{+\bullet}$) by measuring the decay of the transient absorption spectra of the radical cations through the deprotonation to clarify the origin of the discrepancy for the pK_a values.

Mixing an acetonitrile (MeCN) solution of $Fe(ClO_4)_3$ with $AcrH_2$ and AcrHMe in a stopped-flow spectrometer results in an instant appearance of a new absorption band at lmax = 640 nm and 660 nm followed by its decay, respectively.[6,7] Essentially the same transient spectra are obtained when the oxidant $Fe(ClO_4)_3$ is replaced by $[Fe(phen)_3]^{3+}$ (phen = 1,10-phenanthroline) or $Cu(ClO_4)_2$. Thus, appearance of a new transient absorption band may be ascribed to formation of radical cation $AcrHR^{+\bullet}$ (R = H, Me). In fact, the ESR spectra are observed in the electron transfer oxidation of AcrHR with Fe^{3+} in deaerated MeCN by applying a rapid-mixing ESR technique.[7] A typical example of the decay of $AcrHR^{+\bullet}$ (R = Me) is shown in Fig. 1. At the initial stage of the decay obeys the second-order kinetics as shown in the linear plot of $[AcrHMe^{+\bullet}]^{-1}$ *vs.* time. On the other hand, the decay at the longer time obeys the first-order kinetics as shown in the plot of $\ln(A_0 - A)$ *vs.* time, where A_0 is the initial absorbance at lmax = 660 nm due to $AcrHMe^{+\bullet}$ and A is the absorbance at the time t. When the deviation from the second-order plot starts, the first-order decay kinetics begins to hold (Fig. 1). Thus, the decay kinetics of $AcrHR^{+\bullet}$ is given by Eq. 1, where k_1 and k_2 are the first-order and the

$$-d[AcrHR^{+\bullet}]/dt = k_1[AcrHR^{+\bullet}] + k_2[AcrHR^{+\bullet}]^2 \quad (1)$$

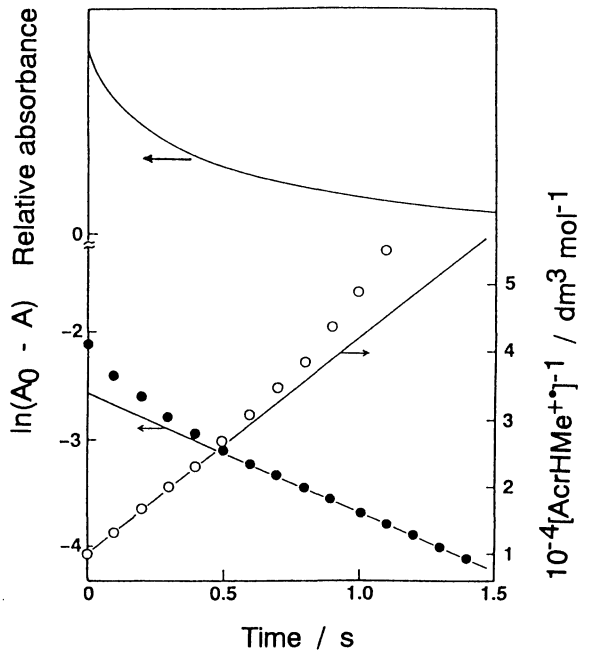


Fig. 1. Decay of the absorbance due to $AcrHMe^{+\bullet}$ formed in the electron transfer oxidation of AcrHMe (1.0×10^{-4} mol dm^{-3}) by $[Fe(phen)_3](PF_6)_3$ (5.0×10^{-4} mol dm^{-3}) in deaerated MeCN at 298 K; the first-order plot (●) and the second-order plot (○).

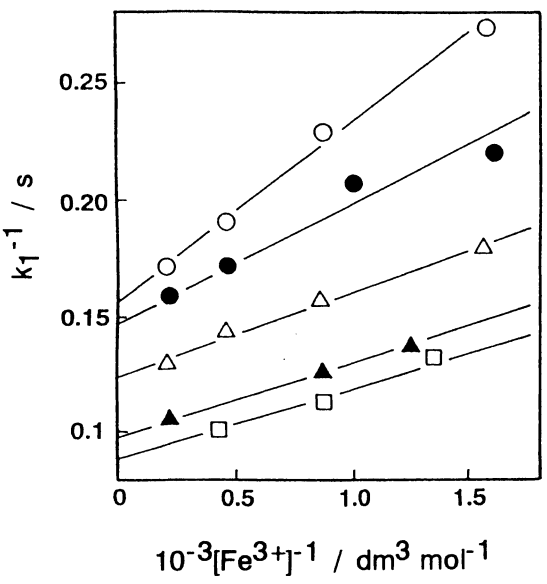


Fig. 2. Plots of k_1^{-1} *vs.* $[Fe^{3+}]^{-1}$ for the decay of $AcrH_2^{+\bullet}$ in the presence of $HClO_4$ in deaerated MeCN at 298 K; $[HClO_4]$ (70 %) = 1.2×10^{-2} mol dm^{-3} (○), 2.3×10^{-3} mol dm^{-3} (●), 5.8×10^{-2} mol dm^{-3} (△), 8.6×10^{-2} mol dm^{-3} (▲), and 1.2×10^{-1} mol dm^{-3} (□).

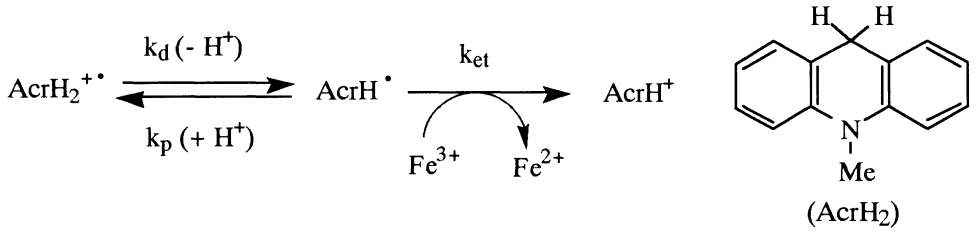
second-order decay rate constants, respectively. The k_1 and k_2 values of $AcrHR^{+\bullet}$ are obtained from the slopes of the first-order and second-order plots, respectively. When $AcrH_2$ is replaced by the 9,9'-dideuterated compound ($AcrD_2$), the large primary kinetic isotope effects are observed for both the first-order and second-order decay (k_H/k_D = 9.0 and 8.0, respectively), indicating that both processes involve the transfer of hydrogen nucleus. The second-order decay of $AcrHR^{+\bullet}$ may be ascribed to the disproportionation of $AcrHR^{+\bullet}$ to yield $AcrR^+$ and $AcrHRH^+$ (Eq. 2).

$$2AcrHR^{+\bullet} \longrightarrow AcrR^+ + AcrHR + H^+ \qquad (2)$$

The first-order decay (k_1) is ascribed to the deprotonation of $AcrH_2^{+\bullet}$, which may be in equilibrium with the protonation of $AcrH^\bullet$ (Eq. 3). In the oxidation of $AcrH_2$ with Fe^{3+}, the facile electron transfer from $AcrH^\bullet$ to Fe^{3+} (k_{et}) may also occur to yield $AcrH^+$, judging from the low oxidation potential of $AcrH^\bullet$ (E^0_{ox} *vs.* = -0.43 V) as compared with that of $AcrH_2$ (E^0_{ox} *vs.* SCE = 0.80 V).[4] By applying the steady state approximation to the reactive intermediate radical ($AcrH^\bullet$), the observed first-order decay rate constant k_1 is given by Eq. 4. The plots of k_1^{-1} *vs.* $[Fe^{3+}]^{-1}$ for the first-order decay of $AcrH_2^{+\bullet}$ in the presence of various concentrations of $HClO_4$ give straight lines as expected from Eq. 4, as shown in Fig. 2. Similar linear plots are

Table 1. Deprotonation Rate Constants (k_d) and pK_a of $AcrH_2^{+\bullet}$ and $AcrHMe^{+\bullet}$ in the Presence of $HClO_4$ in Deaerated MeCN at 298 K

$[HClO_4]$ / mol dm^{-3}	$k_d(AcrH_2^{+\bullet})$ / s^{-1}	$pK_a(AcrH_2^{+\bullet})$	$k_d(AcrHMe^{+\bullet})$ / s^{-1}	$pK_a(AcrHMe^{+\bullet})$
1.2×10^{-2}	6.4	8.1	7.0×10^{-1}	8.5
2.3×10^{-2}	6.8	7.6	8.2×10^{-1}	8.1
5.8×10^{-2}	8.2	7.1	9.5×10^{-1}	7.6
8.6×10^{-2}	10.3	6.9		
1.2×10^{-1}	11.5	6.8		



(3)

$$k_1^{-1} = k_d^{-1} + (k_p[H^+]/k_d k_{et})[Fe^{3+}]^{-1} \quad (4)$$

obtained for the decay of $AcrHMe^{+\bullet}$. From the intercepts are obtained the deprotonation rate constants k_d. Since the electron transfer from $AcrH^\bullet$ to Fe^{3+} may well be assumed to be diffusion-limited, i.e., $k_{et} = 2.0 \times 10^{10}$ dm^3 mol^{-1} s^{-1}, the pK_a [= -log(k_d/k_p)] values are obtained from the intercepts and slopes in Fig. 2. The k_d and pK_a values of $AcrH_2^{+\bullet}$ and $AcrHMe^{+\bullet}$ are summarized in Table 1. The k_d values increase with an increase in the $HClO_4$ concentration, when the pK_a values decrease. Such changes in the k_d and pK_a values may be ascribed to the different concentrations of H_2O contained in the MeCN solution, since $HClO_4$ (70 %) was used for a safety reason. In fact, the pK_a values of $AcrH_2^{+\bullet}$ and $AcrHMe^{+\bullet}$ decrease with an increase in the H_2O concentration ($[H_2O]/[HClO_4]$ = 2.4) contained in the MeCN solution as shown in Fig. 3, where the pK_a value of $AcrH_2^{+\bullet}$ in the presence of added

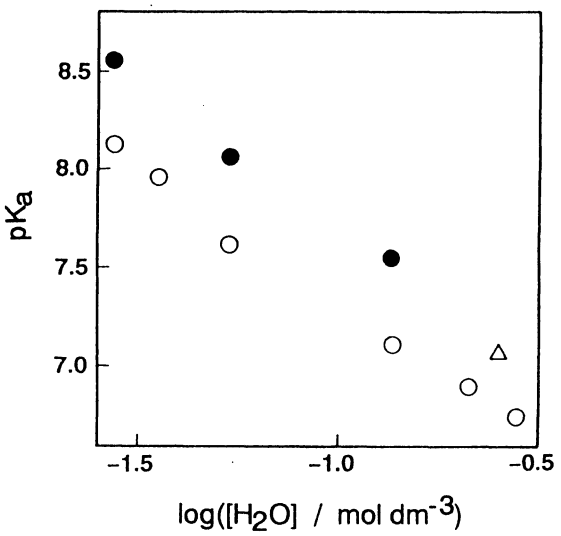


Fig. 3. Dependence of pK_a of $AcrH_2^{+\bullet}$ (○) and $AcrHMe^{+\bullet}$ (●) on the H_2O concentration ($\log[H_2O]$) in MeCN containing $HClO_4$ at 298 K. The ratio of $[H_2O]/[HClO_4]$, 2.4 (○, ●) and 16.7 (△), is changed by addition of H_2O to the reaction system.

H_2O (0.21 M, $[H_2O]/[HClO_4]$ = 16.7) is included. Thus, the deprotonation of $AcrH_2^{+\bullet}$ is accelerated by the presence of H_2O, while the protonation of $AcrH^{\bullet}$ is retarded, resulting in the significant decrease in the pK_a values with an increase in the H_2O concentration in MeCN. Such variation of the protonation and deprotonation rates may be attributed to the strong solvation of H_2O to H^+ as compared to that of MeCN.[8]

The Brönsted plot of the deprotonation rate constant *vs.* the pK_a of bases in H_2O gave the pK_a value of $AcrH_2^{+\bullet}$ as 2.0,[4] which agrees well with the pK_a values obtained in this study (pK_a = 6.8 - 8.1), when the difference in the pK_a values in MeCN and H_2O is taken into account (e.g., the pK_a of pyridine is 12.3 in MeCN, but 5.3 in H_2O).[5] The source of the discrepancy concerning the pK_a values of radical cations of NADH analogues[5] appears to be the neglect of the significant effects of H_2O on the pK_a values in aprotic solvents as clearly demonstrated in Fig. 3.

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