NADH Model Reaction

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Detection of a Radical Cation of an NADH Analogue in Two-Electron Reduction of a Protonated *p*-Quinone Derivative by an NADH Analogue**

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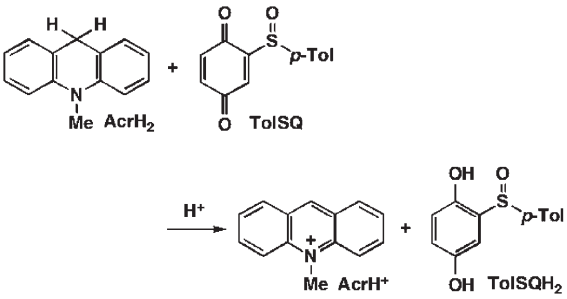
Dihydronicotinamide coenzyme (NADH) plays a vital role as a source of two electrons and a proton (equivalent to a hydride ion) in a number of biological redox processes.[1] On the other hand, quinones (Q) act as biological electron acceptors that can undergo either one- or two-electron reductions coupled with protonation to afford the corresponding semiquinones ($QH^{\bullet}$) and hydroquinones (QH_2), respectively.[2] Two mechanisms are possible in hydride transfer from NADH and analogues to Q: one-step hydride transfer ($NADH + Q \rightarrow NAD^+ + QH^-$) and electron transfer (ET) followed by proton/electron (or hydrogen) transfer ($NADH + Q \rightarrow NADH^{\bullet+} + Q^{\bullet-} \rightarrow NAD^{\bullet} + QH^{\bullet} \rightarrow NAD^+ + QH^-$).[3–6] In contrast to the one-step hydride-transfer pathway, which proceeds without an intermediate, the ET pathway would produce radical cations of NADH and its analogues as reaction intermediates. Such one-step versus multistep pathways of hydride-transfer reaction of NADH and analogues,[7–11] particularly with inclusion of the effect of metal cations[12–14] and acids,[15–18] have been extensively studied because of the essential role of acid catalysis in the enzymatic reduction of carbonyl compounds by NADH.[19] However, the resulting $NADH^{\bullet+}$ or its analogue in the ET pathway has never been detected directly in two-electron reduction of carbonyl compounds by NADH or its analogues.[11–13,15–17,20–22]

We report herein the successful detection of a radical cation of an NADH analogue, namely, 10-methyl-9,10-dihydroacridine ($AcrH_2$), in two-electron reduction of the protonated *p*-quinone derivative 1-(*p*-tolylsulfinyl)-2,5-benzoquinone (TolSQ) by $AcrH_2$. This is the first direct evidence that hydride transfer from an NADH analogue to a hydride acceptor actually proceeds via an ET pathway.[23] $AcrH_2$ and TolSQ were chosen as an acid-stable NADH model compound and a *p*-quinone derivative that can be readily protonated, respectively.[24,25] This study reveals how electron transfer from $AcrH_2$ to $TolSQH^+$ occurs in preference to direct hydride transfer from $AcrH_2$ to $TolSQH^+$.

Efficient reduction of TolSQ by $AcrH_2$ occurs to yield $AcrH^+$ and $TolSQH_2$ in the presence of perchloric acid ($HClO_4$) [Eq. (1)],[26] whereas no reaction occurs between $AcrH_2$ and TolSQ in the absence of $HClO_4$.



(1)

The stoichiometry of Equation (1) is confirmed by spectral titration of TolSQ with $AcrH_2$ in the presence of $HClO_4$ (Figure 1a), in which all TolSQ molecules are consumed by addition of 1 equivalent of $AcrH_2$ to yield 1 equivalent of $AcrH^+$.[27] The promoting effect of $HClO_4$ on the reduction of TolSQ by $AcrH_2$ should result from protonation of TolSQ ($TolSQ + H^+ \rightarrow TolSQH^+$), which is confirmed by UV/Vis spectral changes of TolSQ in the presence of various concentrations of $HClO_4$ (Figure S1 in the Supporting Information). Note that no protonation of unsubstituted *p*-benzoquinone occurs under the same conditions.

The dynamics of the reduction of TolSQ by $AcrH_2$ were examined by using a stopped-flow technique. Addition of $AcrH_2$ (6.0×10^{-3} M) to a deaerated solution of TolSQ (4.6×10^{-4} M) in MeCN containing $HClO_4$ (4.9×10^{-2} M) results in instant appearance of a transient absorption band at $\lambda_{max} = 640$ nm (Figure 1b), which is ascribed to formation of $AcrH_2^{\bullet+}$, which was fully characterized including ESR detection.[21a] Formation of $AcrH_2^{\bullet+}$ clearly indicates ET from $AcrH_2$ to $TolSQH^+$ (Scheme 1a). In the absence of $HClO_4$, ET from $AcrH_2$ ($E_{ox} = 0.81$ V vs SCE)[21a] to TolSQ ($E_{red} = -0.26$ V vs SCE)[13a] is highly endergonic because of the highly positive free-energy change of ET ($\Delta G_{et} = 1.07$ eV), and therefore no ET reaction occurs. In the presence of $HClO_4$ (5.0×10^{-2} M), however, the one-electron reduction potential of TolSQ is shifted to 0.69 V vs SCE due to protonation of TolSQ (Figure S2).[28] The free-energy change of ET from $AcrH_2$ to $TolSQH^+$ is still slightly positive ($\Delta G_{et} = 0.12$ eV). This suggests the occurrence of subsequent chemical

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Supporting information for this article (including experimental details) is available on the WWW under http://www.angewandte.org or from the author.

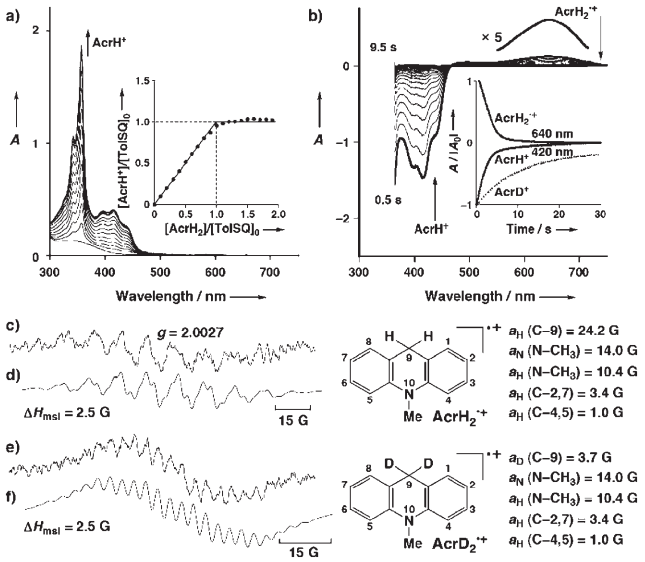
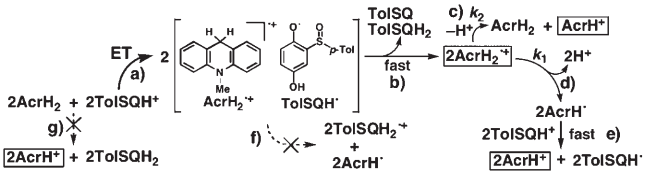


Figure 1. a) Absorption spectral changes observed on addition of $AcrH_2$ (0 to 1.9×10^{-4} M) to a deaerated solution of TolSQ (1.0×10^{-4} M) in MeCN in the presence of $HClO_4$ (1.0×10^{-1} M) at 298 K. b) Differential spectral changes in the reduction of TolSQ (4.6×10^{-4} M) by $AcrH_2$ (6.0×10^{-3} M) in the presence of $HClO_4$ (4.9×10^{-2} M) in deaerated MeCN at 298 K. c) ESR spectrum of $AcrH_2^{\bullet+}$ generated by oxidation of $AcrH_2$ (2.9×10^{-3} M) with TolSQ (2.8×10^{-3} M) in the presence of $HClO_4$ (7.0×10^{-2} M) in deaerated MeCN at 298 K and d) the computer-simulated spectrum with hfc values. e) ESR spectrum of $AcrD_2^{\bullet+}$ generated by oxidation of $AcrD_2$ (2.9×10^{-3} M) with TolSQ (2.8×10^{-3} M) in the presence of $HClO_4$ (7.0×10^{-2} M) in deaerated MeCN at 298 K and f) the computer-simulated spectrum with hfc values. Maximum slope line width $\Delta H_{msl} = 2.5$ G. Insets: a) Plot of $[AcrH^+]/[TolSQ]_0$ vs $[AcrH_2]/[TolSQ]_0$, where $[TolSQ]_0$ is the initial concentration of TolSQ (1.0×10^{-4} M). b) Time course of the absorption change at $\lambda = 640$ and 420 nm for the reduction of TolSQ by $AcrH_2$ (circles) and $AcrD_2$ (triangles), where A_0 is the initial absorbance.



Scheme 1. Mechanism of reduction of $TolSQH^+$ by $AcrH_2$.

processes. In such a case, efficient ET from $AcrH_2$ to $TolSQH^+$ may be followed by rapid disproportionation of $TolSQH^\bullet$ (Scheme 1b), which makes the ET reduction of $TolSQH^+$ go to completion.

The decay of the absorption at 640 nm due to $AcrH_2^{\bullet+}$ is accompanied by a rise in absorption at 420 nm due to $AcrH^+$, as shown in Figure 1b.[29] The decay dynamics of $AcrH_2^{\bullet+}$ (and rise dynamics of $AcrH^+$) consist of both first- and second-order processes (circles in Figure 1b, inset), which correspond to deprotonation and disproportionation of $AcrH_2^{\bullet+}$ (Scheme 1d and c, respectively).[30] Both the first- and second-order processes exhibit large primary kinetic isotope effects ($k_H/k_D = 3.2$ and 10, respectively) when $AcrH_2$ is replaced by the dideuterated compound ($AcrD_2$, triangles in Figure 1b inset).[31] $AcrH^\bullet$ produced by deprotonation of $AcrH_2^{\bullet+}$ is a much stronger reductant than $AcrH_2$, and rapid ET from $AcrH^\bullet$ ($E_{ox} = -0.46$ V vs SCE)[11] to $TolSQH^+$ thus occurs to yield $AcrH^+$ and $TolSQH^\bullet$ (Scheme 1e). As a consequence, 1 equivalent of $TolSQH^+$ is reduced by 1 equivalent of $AcrH_2$ to yield 1 equivalent of $AcrH^+$ and $TolSQH_2$.

We also detected $AcrH_2^{\bullet+}$ by applying a rapid-mixing ESR technique in the thermal oxidation of $AcrH_2$ (2.9×10^{-3} M) with TolSQ (2.8×10^{-3} M) in the presence of $HClO_4$ (7.0×10^{-2} M). The resulting ESR spectrum (Figure 1c) reasonably agrees with the computer simulation spectrum (Figure 1d) produced using values of the hyperfine coupling constants (hfc) (a_H(C-9) = 24.2, $a_N(NCH_3) = 14.0$, $a_H(NCH_3) = 10.4$, a_H(C-2,7) = 3.4, and a_H(C-4,5) = 1.0 G) of $AcrH_2^{\bullet+}$ that were previously reported.[21a,32] The hfc assignment in Figure 1d was further confirmed by deuterium substitution of two hydrogen atoms at the C-9 position of $AcrH_2$. The observed ESR spectrum (Figure 1e) agrees well with the computer simulation (Figure 1f) with the same hfc values except for that of deuterium ($I = 1$; a_D(C-9) = 3.7), which is reduced by the magnetogyric ratio of proton to deuteron (0.153).[32] Complete assignment of the ESR spectrum due to $AcrH_2^{\bullet+}$ observed in the thermal oxidation of $AcrH_2$ with $TolSQH^+$ strongly supports the formation of $AcrH_2^{\bullet+}$ in the two-electron reduction of $TolSQH^+$ by $AcrH_2$ (Scheme 1). On the other hand, the absence of an ESR signal due to $TolSQH^\bullet$ in ET oxidation of $AcrH_2$ by $TolSQH^+$ (Figure 1c) suggests rapid disproportionation of $TolSQH^\bullet$ (Scheme 1b). This is the reason why we successfully detected only $AcrH_2^{\bullet+}$ in the two-electron reduction of $TolSQH^+$ by $AcrH_2$.

The ESR detection of $TolSQH^\bullet$ was then performed in photoinduced ET from dimeric 1-benzyl-1,4-dihydronicotinamide ($(BNA)_2$)[33] to $TolSQH^+$ in propionitrile (EtCN) at 193 K. The ESR spectrum obtained by steady-state photoirradiation of an EtCN solution of TolSQ (2.1×10^{-2} M) and $(BNA)_2$ (1.6×10^{-2} M) in the presence of 6.0×10^{-1} M $HClO_4$ (Figure 2a) is well reproduced by the computer-simulated spectrum with hfc values of a(3H) = 4.90, 2.18, and 0.55 G (Figure 2b).[34] When $HClO_4$ is replaced by $DClO_4$, the drastic change in the ESR spectrum (Figure 2c) provide experimental verification of the assignment of the observed radical species, because the deuteron splitting should decrease by the magnetogyric ratio of proton to deuteron (0.153; vide supra). The complete agreement of the observed ESR spectra (Figure 2a and c) with the computer-simulated spectra (Figure 2b and d) clearly indicates formation of $TolSQH^\bullet$ ($TolSQD^\bullet$).

The ESR signal due to $TolSQH^\bullet$ disappears immediately when the light is cut off, and therefore steady-state photoirradiation is required to generate $TolSQH^\bullet$ for detection by ESR (vide supra). This is also consistent with the fast disproportionation of $TolSQH^\bullet$ in Scheme 1b. The hfc values, calculated on the optimized structure by DFT at the BLYP/6-31G** (in parentheses in Figure 2), agree well with the observed hfc values within the errors due to the large line width resulting from self-exchange ET with $TolSQH^+$.[35] Such agreement indicates that the proton from $HClO_4$ is bound to the C=O oxygen atom on the opposite side to the S=O group (see the optimized structures of $TolSQH^\bullet$ in Figure 2 and

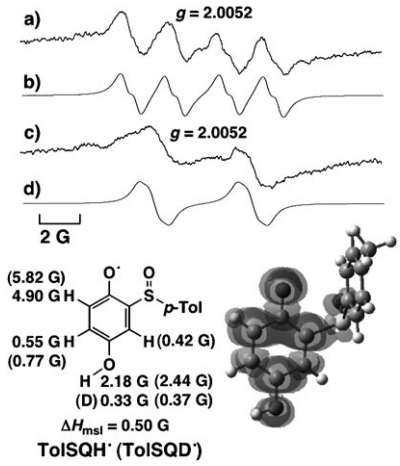


Figure 2. ESR spectra of a deaerated solution of TolSQ (2.1×10^{-2} M) and $(BNA)_2$ (1.6×10^{-2} M) in EtCN in the presence of a) $HClO_4$ (6.0×10^{-1} M) and c) $DClO_4$ (6.0×10^{-1} M) under photoirradiation at 193 K. The computer-simulated spectra are shown in b) and d). Maximum slope line width $\Delta H_{msl} = 0.50$ G. The calculated hfc values in parentheses and spin-density plot of TolSQH· were obtained by DFT at the BLYP/6-31G** level.

Figure S4). There is no ESR signal due to the corresponding hydroquinone radical cation ($TolSQH_2^{\bullet+}$) even in the presence of an extremely high concentration of $HClO_4$ (6.0×10^{-1} M, Figure 2a). This indicates that proton transfer from $AcrH_2^{\bullet+}$ to TolSQH· (Scheme 1f) is unlikely to occur.

Protonation of TolSQ is expected to result in enhanced electrophilicity of TolSQ and thus to accelerate direct hydride transfer from $AcrH_2$ to $TolSQH^+$ (Scheme 1g), but no one-step hydride transfer occurs. The electrostatic potential map for $TolSQH^+$ indicates that the positive charges (blue) due to protonation of TolSQ are fully delocalized over the entire ring systems (Figure 3b) as compared to TolSQ (Figure 3a).[36] In such a case, delocalization of the positive charge (due to H^+) in $TolSQH^+$ results in a decrease in the electrophilicity of $TolSQH^+$, which leads to deceleration of the direct hydride-transfer pathway. On the other hand, the ET pathway is promoted by protonation, as indicated by the significant positive shift of the E_{red} value. This may be the reason why ET from $AcrH_2$ to $TolSQH^+$ occurs instead of direct hydride transfer.

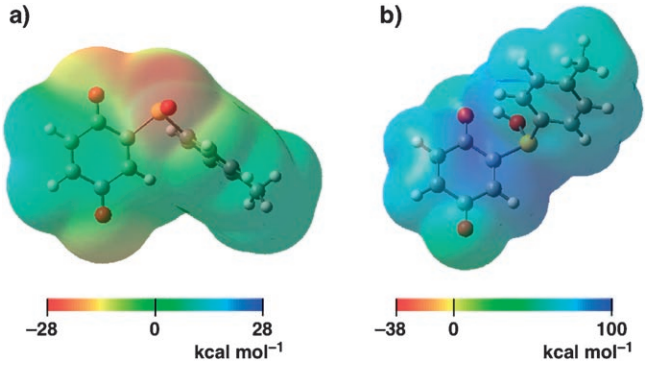


Figure 3. Electrostatic potential maps for a) TolSQ and b) $TolSQH^+$ calculated by DFT at the BLYP/6-31G** level.

In conclusion, we have successfully detected a radical cation of an NADH analogue ($AcrH_2$) in thermal two-electron reduction of a protonated *p*-quinone derivative ($TolSQH^+$) by $AcrH_2$. This is the first direct evidence for an ET pathway in the two-electron reduction of substrates by an NADH analogue. This finding provides valuable insight into how acids promote hydride-transfer reactions of NADH analogues via the ET pathway in preference to the direct hydride-transfer pathway when the substrates act as strong electron acceptors.

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[20] The subsequent proton (or hydrogen) transfer from $NADH^{\bullet+}$ and its analogues to singly reduced species ($Q^{\bullet-}$) acting as strong bases is generally too fast to detect the radical cation.[11–13,15–17]
[21] For direct observation of $NADH^{\bullet+}$ analogues by transient ESR spectroscopy in the oxidation of NADH analogues by one-electron oxidants, see: a) S. Fukuzumi, Y. Tokuda, T. Kitano, T. Okamoto, J. Otera, *J. Am. Chem. Soc.* **1993**, *115*, 8960–8968; b) S. Fukuzumi, O. Inada, T. Suenobu, *J. Am. Chem. Soc.* **2003**, *125*, 4808–4816.
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[23] We recently reported the promoting effect of scandium ion (Sc^{3+}) acting as a strong Lewis acid on the $AcrH_2$/TolSQ system, in which hydride transfer from $AcrH_2$ to TolSQ proceeds via the one-step hydride-transfer pathway at 298 K, with transition to the ET pathway at low temperatures. In this case, however, no $AcrH_2^{\bullet+}$ is observed in hydride transfer of $AcrH_2$ because of subsequent rapid proton transfer from $AcrH_2^{\bullet+}$ to the Sc^{3+} complexes of $TolSQ^{\bullet-}$ [$TolSQ^{\bullet-}$–$(Sc^{3+})_n$ ($n = 1, 2$)].[13a]
[24] The one-electron oxidation potential of NADH ($E_{ox} = 0.76$ V vs SCE)[6,25] in aqueous solution is close to that of $AcrH_2$ ($E_{ox} = 0.81$ V vs SCE)[6,25] in acetonitrile. Thus, $AcrH_2$ is a suitable analogue for the ET oxidation of NADH.
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[26] For safety reasons, $HClO_4$ (70%) containing 30% water was used.
[27] Virtually no protonation of $AcrH_2$ occurs in the presence of $HClO_4$ (4.9×10^{-2} M) containing 30% water.
[28] The E_{red} value of TolSQ in the presence of $HClO_4$ was determined by second-harmonic alternating-current voltammetry (SHACV) because of the instability of $TolSQH^{\bullet}$ (Figure S2).
[29] The differential absorption spectra were recorded by subtracting the final absorption spectrum from the observed spectra during the reduction of $TolSQH^+$ by $AcrH_2$, as shown in Figure 1b. Thus, formation of $AcrH^+$ is represented by the disappearance of the negative absorption band due to $AcrH^+$.
[30] Virtually the same first- and second-order processes were observed in the decay dynamics of $AcrH_2^{\bullet+}$ produced by ET oxidation of $AcrH_2$ by one-electron oxidants.[21a]
[31] The first-order decay rate constant k_1 and second-order decay rate constant k_2 of $AcrH_2^{\bullet+}$ were determined as 1.1×10^{-1} s^{-1} and 6.6×10^3 $M^{-1}s^{-1}$, respectively, separately from the first-order and second-order plots (Figure S3).
[32] Note that water in $HClO_4$ (70%) significantly reduces the sensitivity of ESR spectroscopy.
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[36] In contrast to $TolSQH^{\bullet}$, H^+ may be bound to the S=O oxygen atom, since the larger negative charge (red) is located on the S=O oxygen atom as opposed to the C=O oxygen atoms (Figure 3a).

