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# ESR Spectra of Transient Radical Cations of NADH Analogues

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Electron spin resonance (ESR) spectra of transient radical cations of 9,10-dihydro-10-methylacridine and derivatives, which are NADH analogues, are observed in electron transfer reactions from the NADH analogues to $Fe^{3+}$ or $Cu^{2+}$ in acetonitrile.

The biological importance of dihydronicotinamide adenine dinucleotide (NADH) used as an electron source has attracted considerable interest in electron-transfer reactions from NADH and its analogues to various one-electron oxidants.[1-3] However, no ESR spectra of the radical cations of NADH and analogues have so far been reported because of the instability, although the electronic spectra of NADH and analogues have recently been reported by applying picosecond laser technology.[4,5] This study reports the first observation of ESR spectra of transient radical cations of NADH analogues formed in electron transfer reactions with $Fe^{3+}$ and $Cu^{2+}$ in acetonitrile by applying a rapid-mixing flow technique.

Mixing an acetonitrile (MeCN) solution of $Fe(ClO_4)_3$ with an NADH analogue, 9,10-dihydro-10-methylacridine ($AcrH_2$),[3] by using a rapid-mixing flow apparatus results in the observation of an ESR signal as shown in Fig. 1a. Essentially the same transient ESR signal is obtained when the oxidant $Fe(ClO_4)_3$ is replaced by $Cu(ClO_4)_2$. The ESR signal decayed rapidly when the flow of the reactant solution was stopped (half life is shorter than 0.1 s). The observed ESR signal may be ascribed to the radical cation of $AcrH_2$ ($AcrH_2^{+\cdot}$) formed by electron transfer from $AcrH_2$ to $Fe^{3+}$ (or $Cu^{2+}$), although the resolution of the spectrum is not good enough to confirm this assignment. In such a case, deuterium substitution at appropriate known sites may permit an experimental verification of the assignment of the observed radical species, since a single deuteron gives a triplet (instead of doublet) hyperfine pattern and the deuteron splitting should decrease by the magnetrogyric ratio of proton to deuterium (0.153).[6] In fact, deuterium substitution of two hydrogen atoms at C-9 position and that of three hydrogen atoms at N-$CH_3$ position of $AcrH_2$ result in drastic changes in the

splitting pattern from broad 8 major lines to 17 and 4 lines as shown in Figs. 1b and 1c, where $AcrH_2$ is substituted by [9,9-$^2H_2$]-dihydro-10-methylacridine ($AcrD_2$) and 9,10-dihydro-10-([$^2H_3$]methyl)-acridine ($AcrH_2$-$CD_3$), respectively. The substitution of one hydrogen atom with isopropyl group at C(9) position also causes the change in the splitting pattern as shown in Fig. 1d where $AcrH_2$ is replaced by 9,10-dihydro-9-isopropyl-10-methylacridine $AcrH(Pr^i)$.[7]

The observed ESR spectra in parts a-d in Fig. 1 can be simulated with the parameters listed in Table 1 as shown in parts e-h of Fig. 1, respectively. The assignments of radical cations of $AcrH_2$ and derivatives in Table 1 are ensured by comparing the hyperfine splitting (hfs) values of $a_H$(C-9), $a_D$(C-9), $a_H(CH_3)$, and $a_D(CD_3)$, since the hfs values of 2.34 and 0.74 mT due to C-9 and N-$CH_3$ protons of $AcrH_2^{+\cdot}$ are decreased by the factor of the magnetrogyric ratio of proton to deuterium (0.153) to 0.36 and 0.11 mT due to C-9 and N-$CD_3$ deuterons of $AcrD_2^{+\cdot}$ and $AcrH_2$-$CD_3^{+\cdot}$, respectively, when other hfs values are identical. As shown in Table 1, $a_N$(N-$CH_3$) value (1.01 mT) of $AcrH_2^{+\cdot}$ is larger than the $a_H$(N-$CH_3$) value (0.74 mT), although the reported $a_N$(N-$CH_3$) value (1.6 mT) of $Me_3N^{+\cdot}$ radical cation, which is known to be planar, is smaller than the $a_H$(N-$CH_3$) value (2.82 mT).[8] In the case of planar $Me_3N^{+\cdot}$ radical the SOMO (semi-occupied molecular orbital) is

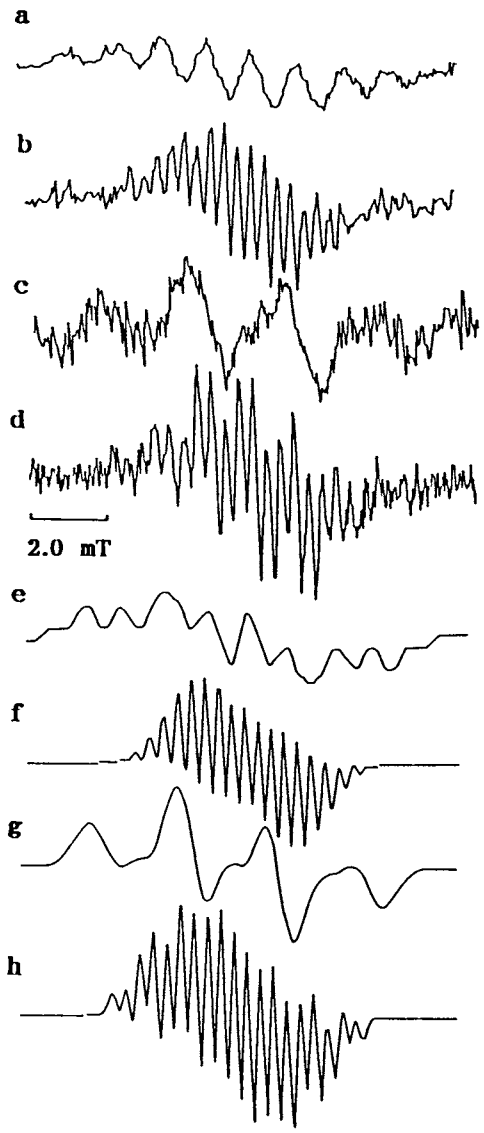
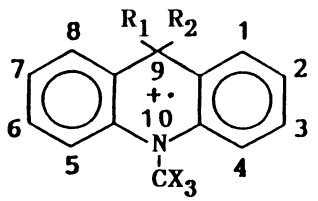



Fig. 1. ESR Spectra of transient radical cations of NADH analogues, observed in electron transfer from (a) $AcrH_2$ ($1.2 \times 10^{-3}$ mol $dm^{-3}$), (b) $AcrD_2$ ($2.0 \times 10^{-3}$ mol $dm^{-3}$), (c) $AcrH_2$-$CD_3$ ($1.2 \times 10^{-3}$ mol $dm^{-3}$), and (d) $AcrH(Pr^i)$ ($1.5 \times 10^{-3}$ mol $dm^{-3}$) to $Fe(ClO_4)_3$ ($1.0 \times 10^{-3}$ mol $dm^{-3}$) in MeCN. (e-h) Computer simulation spectra of $AcrH_2^{+\cdot}$, $AcrD_2^{+\cdot}$, $AcrH_2$-$CD_3^{+\cdot}$, and $AcrH(Pr^i)^{+\cdot}$, using the parameters in Table 1, respectively.

Table 1. Hyperfine Splitting Values of Radical Cations of NADH Analogues




$R_1$ = H, D, $Pr^i$
$R_2$ = H
X = H, D

| Radical Cation | hyperfine splitting value (hfs) / mT[a)] | | | | |
|---|---|---|---|---|---|
| $AcrH_2^{+\cdot}$ | $a_H$(C-9) | $a_N$(N-$CH_3$) | $a_H$(N-$CH_3$) | $a_H$(C-2) | $a_H$(C-7) |
| | 2.34 | 1.01 | 0.74 | 0.34 | 0.34 |
| $AcrD_2^{+\cdot}$ | $a_D$(C-9) | $a_N$(N-$CH_3$) | $a_H$(N-$CH_3$) | $a_H$(C-2) | $a_H$(C-7) |
| | 0.36 | 1.01 | 0.74 | 0.34 | 0.34 |
| $AcrH_2$-$CD_3^{+\cdot}$ | $a_H$(C-9) | $a_N$(N-$CD_3$) | $a_D$(N-$CD_3$) | $a_H$(C-2) | $a_H$(C-7) |
| | 2.34 | 1.01 | 0.11 | 0.34 | 0.34 |
| $AcrH(Pr^i)^{+\cdot}$ | $a_H$(C-9) | $a_N$(N-$CH_3$) | $a_H$(N-$CH_3$) | $a_H$(C-2) | $a_H$(C-7) |
| | 1.42 | 1.01 | 0.74 | 0.34 | 0.34 |

a) The $\Delta H_{msl}$ values of $AcrH_2^{+\cdot}$, $AcrD_2^{+\cdot}$, $AcrH_2$-$CD_3^{+\cdot}$, and $AcrH(Pr^i)^{+\cdot}$ are 0.21, 0.14, 0.40, and 0.14 mT, respectively.

strongly confined to the $2p_z$ orbital on nitrogen, when the $a_N$(N-$CH_3$) and $a_H$(N-$CH_3$) values are determined by the small 2s population which appears solely by spin polarization of the $\sigma$-bonding electrons and the $2p_z$ population on nitrogen, respectively. Thus, the larger $a_N$(N-$CH_3$) value of $AcrH_2^{+\cdot}$ than $a_H$(N-$CH_3$) value suggests the non-planarity of $AcrH_2^{+\cdot}$ at nitrogen, since the deviation from planarity may result in the increase in the 2s population on nitrogen and thereby the $a_N$(N-$CH_3$) value may increase as compared to the $a_H$(N-$CH_3$) value. Our preliminary MNDO calculations[9)] support this prediction as well as the assignment in Table 1 as follows. The folding angle between the planes of the two benzene rings of 9,10-dihydro-9-t-butylacridine is reported to be 150.2°,[10)] which is increased to 164° in the best-fit calculation for $AcrH_2^{+\cdot}$ where the degree of non-planarity of $AcrH_2^{+\cdot}$ is estimated to be approximately inbetween the reduced form $AcrH_2$ being a boat conformation and the oxidized form $AcrH^+$ being planar. In such a case the hfs value of axial C-9 proton ($a_{Hax}$ = 2.39 mT) is calculated to be larger than that of equatorial C-9 proton ($a_{Heq}$ = 1.98 mT). In the ESR spectrum of $AcrH_2^{+\cdot}$, however, the two C-9 protons are equivalent, $a_H$(C-9) = 2.34 mT, which agrees with the average value (2.19 mT) of $a_{Hax}$ and $a_{Heq}$. Thus, the inversion of the boat structure may occur rapidly in solution in the ESR time scale. The other predicted hfs values

of $AcrH_2^{+\cdot}$ being $a_N(N\text{-}CH_3)$ = 0.99, $a_H(N\text{-}CH_3)$ = 0.67, $a_H(C\text{-}2) = a_H(C\text{-}7)$ = 0.23 mT agree well with the observed hfs values in Table 1.[11] In the case of $AcrH(Pr^i)$ the isopropyl group may be fixed in a boat-axial conformation with respect to the central acridine ring as observed for the t-butyl group of 9,10-dihydro-9-t-butylacridine.[10] This may be the reason why the $a_H(C\text{-}9)$ value of $AcrH(Pr^i)^{+\cdot}$ (1.43 mT), which may be ascribed to the equatorial proton, is smaller than the averaged $a_H(C\text{-}9)$ value of $AcrH_2^{+\cdot}$ (2.34 mT).[12] Although application of the present method to observe the ESR spectra of radical cations of NADH and other analogues has been unsuccessful because of the instability of the radical cations, the present study demonstrates for the first time the presence of large hfs values of two active methylene protons [$a_H(C\text{-}9)$] of NADH analogues.

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11) The hfs values of C-1(8), C-3(6), and C-4(5) protons are estimated as 0.002, 0.052, and 0.12 mT, respectively.
12) The difference in the folding angle between $AcrH_2^{+\cdot}$ and $AcrH(Pr^i)^{+\cdot}$ may also affect the hfs value.

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