Direct Observation of NADH Radical Cation Generated in Reactions with One-Electron Oxidants

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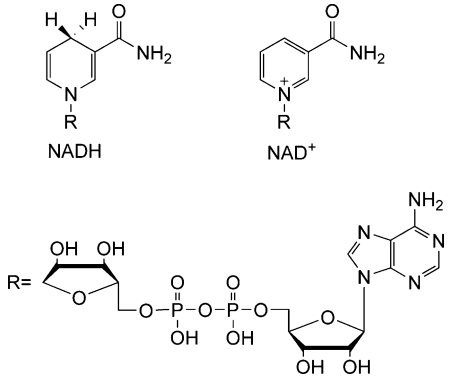
The formation of NADH radical cation in reactions with one-electron oxidants was observed for the first time. Transient products involving two tautomeric (keto and enol) forms of radical cation and neutral radical were spectroscopically characterized by means of pulse radiolysis. The kinetics of the decay of keto radical cation and neutral radical was investigated. The pK_a value of the enol form of NADH radical cation was determined. The simple analogues of NADH and NAD^+, namely, 1-methyl-1,4-dihydronicotinamide and its oxidized form, were studied for comparison.

1. Introduction

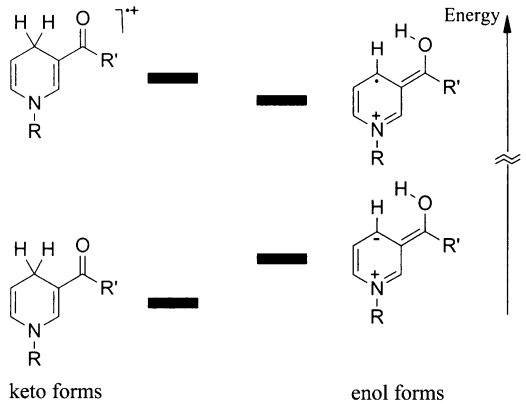
The importance of dihydronicotinamide adenine dinucleotide (NADH) and its oxidized form (NAD^+) in biological systems



is based on the ability of the NADH/NAD^+ couple to act as a source or acceptor of two electrons and a proton. In the overall reaction, a hydride anion is transferred to or from a suitable substrate.[1] Although the mechanism of hydride transfer in NADH ⇄ NAD^+ conversion is still, in many cases, controversial, it is accepted that one-step hydride transfer dominates in enzymatic reactions whereas in many chemical, electrochemical, and photochemical reactions of NADH analogues a multistep hydride transfer occurs.[2−5]

Despite the important role of NADH as an electron source, a dominant number of reports concerning one-electron oxidation of NADH characterize the radical cations formed only from NADH model compounds, mainly 1,4-dihydropyridine derivatives. Two different forms of radical cations generated from NADH analogues can be distinguished: the keto and enol form (see Scheme 1). In the neutral molecule, the enol form is much less stable than the keto tautomer (by over 30 kcal/mol) and is of no importance in the chemistry of this group of compounds. However, as a result of stability inversion, which is one of the unique features of molecular ions, the enol radical cation is more stable than its keto tautomer by over 10 kcal/mol and both forms of radical cation can play a role in the stepwise NADH ↔ NAD^+

SCHEME 1



conversion.[6,7] On oxidation of NADH analogues, the radical cations initially formed in the keto form cannot easily tautomerize to a more stable enol form unless a favorable geometry, that is, a favorable orientation of a carbonyl group for 1,4-hydrogen atom transfer, is provided.[7] Indeed, for a dominant number of NADH analogues, a direct observation of radical cation in a keto form was reported.[3,5−7] The recent successful detection of radical cation of NADH analogue (1-benzyl-1,4-dihydronicotinamide) by electron spin resonance (ESR) spectroscopy clearly confirms previous findings that these radical cations represent the keto form.[8]

On the other hand, the reverse process of reduction of NAD^+ analogues followed by the protonation of an appropriate radical leads exclusively to the enol radical cation. Along with the experimental evidence for spontaneous intramolecular hydrogen atom transfer (tautomerization of keto form to the enol form), it proves the inversion stability order for this group of compounds,[7,9−10] although suggestions that the keto form of the radical cation may be thermodynamically more stable because of solvation appear occasionally.[8]

In contrast to the well-documented direct observations of radical cations of NADH analogues, the identification and spectral characterization of radical cations of NADH itself are very limited.[5] It may be somewhat surprising that the spectral characterization of NADH radical cation comes only from the photoionization experiments and that this species has never been identified in thermal reactions of NADH with one-electron

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oxidants. In this context, we can agree with recent opinion that the direct detection of NADH radical cation and its definite assignment has yet to be attained.[8] This paper fulfills that deficiency because we report the first direct detection of NADH radical cations in reactions with one-electron oxidants and present spectroscopic characterization of both keto and enol forms of NADH radical cation in aqueous solution.

2. Results and Discussion

2.1. NADH Radical Cation—Keto Form. A direct detection of the one-electron oxidized species derived from NADH is a challenging task, especially in aqueous solution, because of the instability of NADH radical cation ($NADH^{\bullet+}$). Unquestionably, there are numerous examples of NADH reactions that indirectly confirm an involvement of $NADH^{\bullet+}$ as the transient species.[11−13] However, no reaction has ever been reported leading to a direct experimental evidence for the participation of NADH radical cations. The transient absorption spectrum of $NADH^{\bullet+}$ has been only detected upon laser excitation of NADH in aqueous solution, exhibiting absorption bands at 370 and 550 nm.[5] However, the electron-transfer reaction from NADH excited state cannot be recognized as a typical chemical reaction of NADH. Moreover, Fukuzumi et al. have recently suggested that radical cations of NADH and of its analogues generated in highly exergonic photochemical electron-transfer processes may be formed directly in the enol form.[8]

Unfortunately, the matrix isolation methods are not suitable for investigation of transient species generated from large organic molecules of biological importance, although a use of ionic liquids as novel transparent matrixes may soon challenge that view.[14] Therefore, at present only the time-resolved techniques of generation and detection of radical ions can be applied for that purpose, for example, the pulse radiolysis technique. First, this time-resolved method can be equipped with a sensitive UV−vis detection. Second, it allows a generation of strong one-electron oxidants[15] capable of effective NADH oxidation in diffusion-controlled reactions, faster than a decay of $NADH^{\bullet+}$.

In a series of experiments, we have tested a group of the well-known one-electron oxidants generated radiolytically. The use of some of them for oxidation of NADH as, for example, $SO_4^{\bullet-}$ radical anion ($E°(SO_4^{\bullet-}/SO_4^{2-}) = 2.43$ V)[15] turned out to be unsuccessful because its precursor, $S_2O_8^{2-}$ anion, reacted with NADH. On the other hand, the much weaker $N_3^{\bullet}$ radical ($E°(N_3^{\bullet}/N_3^{-}) = 1.33$ V)[15] reacts effectively with NADH, but we were not able to observe the formation of transient radical cation of NADH most likely because that reaction was too slow compared with the rate of $NADH^{\bullet+}$ decay.

Finally, we have found that dibromide radical anion ($Br_2^{\bullet-}$, $E°(Br_2^{\bullet-}/2Br^{-}) = 1.63$ V),[15] which is a stronger oxidant than $N_3^{\bullet}$, is very suitable for that purpose. It has already been demonstrated that NADH is oxidized by the $Br_2^{\bullet-}$ radical anion, although because of the short lifetime of $NADH^{\bullet+}$ no evidence for its presence was found.[11,12] As it is shown in the Experimental Section $Br_2^{\bullet-}$ radical anions can be effectively generated by pulse radiolysis of aqueous solutions containing KBr. Unfortunately, $Br_2^{\bullet-}$ absorbs ($\lambda_{max} = 360$ nm) in the region of short wavelength absorption of $NADH^{\bullet+}$ shown in photoionization experiments,[5] but it should not interfere with the second, weaker, but more characteristic band of $NADH^{\bullet+}$. This band was well-characterized for radical cations generated from the simple NADH analogues.

The spectrum obtained by pulse radiolysis of NADH in aqueous solution containing KBr is presented in Figure 1. It is

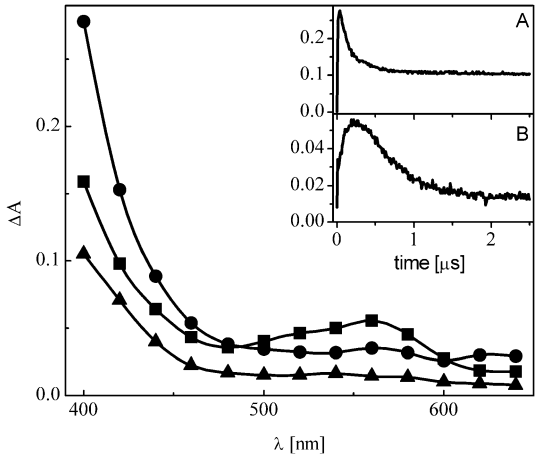


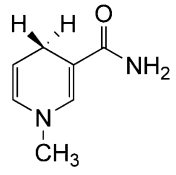
Figure 1. Transient absorption spectra obtained by pulse radiolysis of NADH (0.004 M) in N_2O-saturated aqueous solution containing 1 M KBr. The spectra were collected (●) 100 ns, (■) 240 ns, and (▲) 2 μs after the electron pulse. The inset shows the changes of absorbance at (A) 400 and (B) 560 nm. The sample was 1 cm thick and received a radiation dose of 80 Gy.

evident that one-electron oxidation of NADH by $Br_2^{\bullet-}$ radical anion leads to the formation of the corresponding radical cation, which has the absorption band at 560 nm (Figure 1, reaction 1). Because of the high concentration of NADH in this

$$NADH + Br_2^{\bullet-} \rightarrow NADH^{\bullet+} + 2Br^{-} \qquad (1)$$

experiment absorbing effectively at 340 nm and also high absorption of $Br_2^{\bullet-}$ itself, the short wavelength band of $NADH^{\bullet+}$ cannot be observed. The assignment of the absorption band at 560 nm to the NADH radical cation is not only based on the similarity with the results of Czochralska et al.[5] and with the spectra of radical cations generated from the simple NADH analogues[7] but also based on the kinetic analysis. The rate of disappearance of the absorbance of one-electron oxidant $Br_2^{\bullet-}$ ($k = 1.1 \times 10^9$ M^{-1} s^{-1}) is very close to the rate of appearance of radical cation band ($k = 1.5 \times 10^9$ M^{-1} s^{-1}) (see inset to Figure 1). To our knowledge, this is the first direct observation of $NADH^{\bullet+}$ identified in the chemical reaction. The earlier observation of NADH radical cation comes from the flash photolysis experiments.[5]

Similarity of the spectrum of $NADH^{\bullet+}$ (band location and its shape) with the spectra of previously characterized radical cations generated from the model analogues[3−7] (mainly 1,4-dihydropyridine derivatives) indicates that unpaired spin and positive charge of radical cation are mainly located in the dihydropyridine moiety. The spectrum of radical cation generated from the simple analogue of NADH, namely, a 1-methyl-1,4-dihydronicotinamide (MNAH), is presented in Figure 2.



MNAH

Because of the lower oxidation potential or the higher diffusion coefficient of MNAH than of NADH or both, the rate constant of the reaction with $Br_2^{\bullet-}$ is higher: $k = 4.5 \times 10^9$ M^{-1} s^{-1} determined from the dibromide radical anion decay or $k = 4.2 \times 10^9$ M^{-1} s^{-1} determined from the growth of MNAH

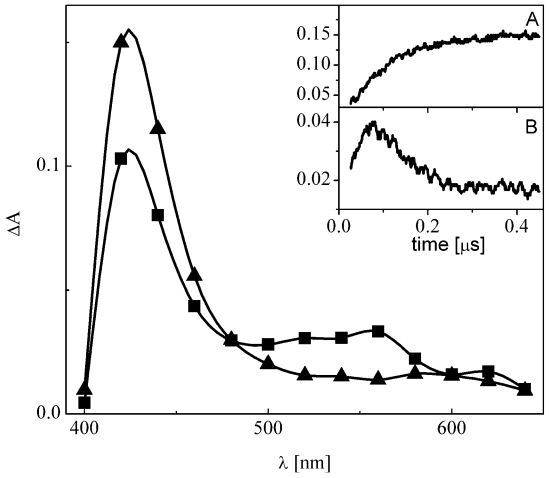


Figure 2. Transient absorption spectra obtained by pulse radiolysis of MNAH (0.005 M) in N_2O-saturated aqueous solution containing NaN_3 (0.05 M). The spectra were collected (■) 100 and (▲) 500 ns after the electron pulse. The inset shows the changes of absorbance at (A) 420 and (B) 560 nm. The sample was 1 cm thick and received a radiation dose of 65 Gy.

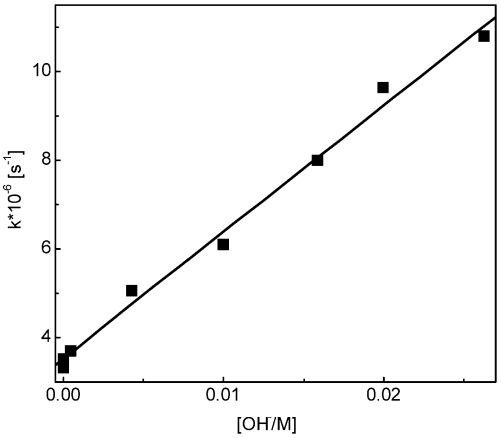


Figure 3. The dependence of the pseudo-first-order rate constant of deprotonation of *keto*-$NADH^{\bullet+}$ radical cation on pH of the solution.

radical cation absorption. In this case also, $N_3^{\bullet}$-mediated oxidation of MNAH was fast enough to allow the observation of $MNAH^{\bullet+}$ (see Figure 2).

On the microsecond time scale the 560 nm absorption band of radical cation disappears and only the product with absorption band at 400 nm is seen. For NADH, that band is obscured by the strong absorptions of $Br_2^{\bullet-}$ and $NADH^{\bullet+}$ radical cation. However, in the case of oxidation of MNAH by $N_3^{\bullet}$ radical, which has no absorption above 300 nm, it was possible to observe the simultaneous disappearance of radical cation absorption band and formation of the absorption band at 420 nm. The bands located around 400 nm can be assigned to the neutral radical, the product of radical cation deprotonation (Figure 2).[11,12]

This assignment remains in agreement with our previous spectral characterization of radicals of NADH analogues supported by quantum mechanical calculations.[9] But it is also supported by the kinetic analysis of the radical cation decay and formation of the product. The rate of the radical cation decay is pH-dependent (Figure 3), and most likely, two processes contribute to $NADH^{\bullet+}$ decay under our experimental conditions (reactions 2 and 3).

$$NADH^{\bullet+} + OH^- \rightarrow NAD^{\bullet} + H_2O \qquad (2)$$

$$NADH^{\bullet+} + H_2O \rightarrow NAD^{\bullet} + H_3O^+ \qquad (3)$$

The rate constants for radical cation deprotonation onto OH^- or water molecules are $k_2 = 2.9 \times 10^8\ M^{-1}\ s^{-1}$ and $k_3 = 3.5 \times 10^6\ s^{-1}$, respectively. Similar values were found for the decay of $MNAH^{\bullet+}$ ($4.7 \times 10^8\ M^{-1}\ s^{-1}$ and $2.7 \times 10^6\ s^{-1}$ for deprotonation onto OH^- or water molecules, respectively). The decay rates of $NADH^{\bullet+}$ and $MNAH^{\bullet+}$ do not depend on their concentration, as well as on the concentration of parent compounds. This may indicate that the disproportionation reaction or proton transfer from radical cation to parent molecule are less important in the aqueous solutions. According to these results, the radical cations of NADH and MNAH do not tautomerize to the more stable enol forms but instead lose protons to give neutral radicals.

Neutral radicals $NAD^{\bullet}$ and $MNA^{\bullet}$ can be formed independently by one-electron reduction of the corresponding pyridinium cations. Also this method leads to the product, which absorbs at 400 nm (reduction of NAD^+) and at 420 nm (reduction of MNA^+). In neutral deoxygenated solution, these radicals undergo dimerization.[16,17]

$$2NAD^{\bullet} \rightarrow (NAD)_2 \qquad (4)$$

The rate constants for dimerization of $NAD^{\bullet}$ and $MNA^{\bullet}$ radicals are 7.4×10^7 and $7.3 \times 10^8\ M^{-1}\ s^{-1}$, respectively. The rate constants of the decay of radicals were determined independently in the experiments using different methods of radical generation, that is, oxidation of NADH (MNAH) followed by the deprotonation of radical cation or reduction of NAD^+ (MNA^+). The agreement between these two methods additionally supports an assignment of the products of these reactions to the appropriate radicals. Similarity of the spectra of radicals and their dimerization process suggests that $NADH^{\bullet+}$ deprotonates from the same 4-position of the 1,4-dihydropyridine ring as its simple $MNAH^{\bullet+}$ analogue.

In the presence of oxygen, the radicals decay mainly by the electron transfer to oxygen molecule.[18]

$$NAD^{\bullet} + O_2 \rightarrow NAD^+ + O_2^{\bullet-} \qquad (5)$$

Assuming the concentration of O_2 in aerated and oxygenated solution as, respectively, 2.5×10^{-4} and 1.25×10^{-3} M, the rate constant for the reaction of $NAD^{\bullet}$ and $MNA^{\bullet}$ radicals with the oxygen are 1.6×10^9 and $3.5 \times 10^9\ M^{-1}\ s^{-1}$, respectively.

2.2. NADH Radical Cation—Enol Form. In strongly acidic solution, the absorption bands of neutral radicals undergo red shift as the result of their protonation (Scheme 2).[9−10,16] The radical cation formed is not identical with the radical cation formed by the one-electron oxidation of dihydro forms; instead of an absorption band at 560 nm, the absorption band at 430−440 nm is observed (Figure 4). Because the spectra of NAD^+ and MNA^+ reduction products under acidic conditions are very similar, one can assume that protonation took place on the nicotinamide moiety. Because the protonation of radical leads to the radical cation and because the radical cation in the keto form, which is obtained by protonation at C-4, is less stable than the corresponding enol form, which arises upon protonation of the carbonyl function, it is reasonable to assume that the latter is being formed predominantly.[6−7,9−10,16] On the basis of our results obtained for NADH model compounds supported by the quantum mechanical calculations, this absorption band can be assigned to the enol form of radical cation.[7] The estimated pK_a

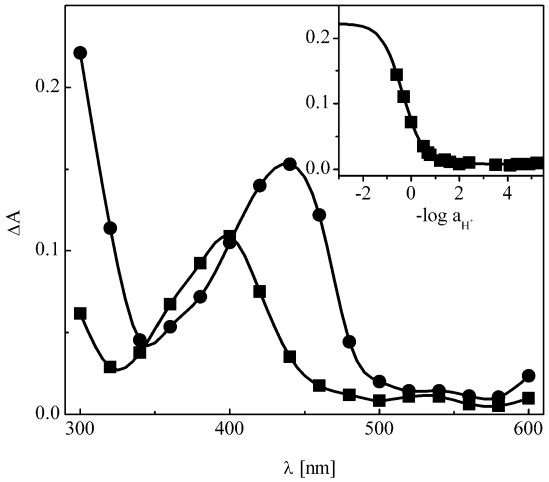


Figure 4. Transient absorption spectra of the acid−base forms of $NAD^{\bullet}$ radical obtained by pulse radiolysis of NAD^+ (0.003 M) in N_2O-saturated aqueous solution containing *iso*-PrOH (1 M): (■) spectrum obtained in neutral aqueous solution; (●) spectrum observed in 1.5 M $HClO_4$ aqueous solution. The spectra were collected 1.5 μs after the electron pulse. The inset shows the titration curve determined at 460 nm. For pH < 0.5, the H_0 scale for perchloric acid has been used. The sample was 1 cm thick and received a radiation dose of 60 Gy.

SCHEME 2

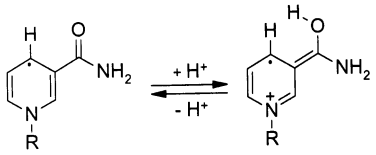


TABLE 1: Spectroscopic Features of the Transient Species Identified in This Study

	NADH		MNAH	
transient species	λ_{max} [nm]	$\epsilon \times 10^{-3}$ [M^{-1} cm^{-1}]	λ_{max} [nm]	$\epsilon \times 10^{-3}$ [M^{-1} cm^{-1}]
keto radical cation	560	1.6	560	1.4
enol radical cation	430	5.2	440	5.2
neutral radical	400	2.5	420	3.5

values are −0.3 and 1.3 for *enol*-$NADH^{\bullet+}$ and *enol*-$MNAH^{\bullet+}$ radical cations, respectively.

Scheme 3 summarizes the reactions of NADH oxidation or NAD^+ reduction and transient species identified in this study. Spectroscopic features of these species are presented in Table 1. Direct spontaneous tautomerization of NADH radical cation to its enol form is not observed, most likely because of a short lifetime of the keto form (fast deprotonation dominates in aqueous solutions) and rather high barrier for intramolecular hydrogen atom transfer. It is possible that in an environment of low proton affinity the tautomerization of radical cation can be observed. We have shown recently the formation of the enol form of MNAH radical cation upon oxidation of MNAH in ionic liquid glass at 150 K.[14] This radical cation can be formed by intramolecular hydrogen atom transfer in *keto*-$MNAH^{\bullet+}$ although intermolecular protonation of neutral radicals can be a more important route to the enol radical cation. It is also possible that some changes in the geometry of the molecule are required as, for example, those taking place in the binding sites of enzymes,[19] to facilitate intramolecular hydrogen transfer in radical cations of NADH.

3. Conclusions

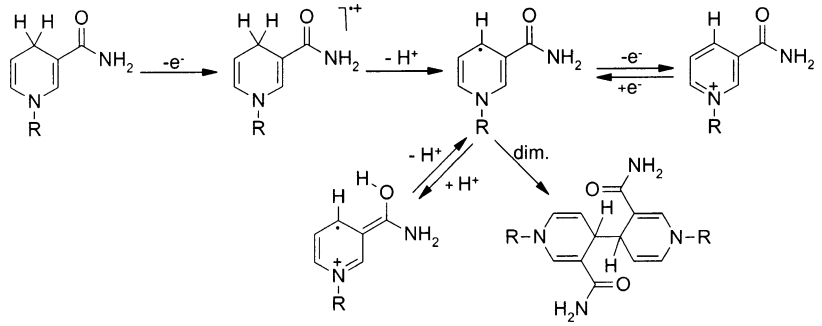
We have presented for the first time an evidence for the direct formation of the radical cation of NADH in its chemical reaction with a strong one-electron oxidant. The transient species formed on one-electron oxidation of NADH and reduction of NAD^+, and their simple analogues MNAH and MNA^+, have been spectroscopically and kinetically characterized. Removal of an electron from NADH produces the radical cation in the keto form, while the reduction of NAD^+ under acidic conditions leads to the enol form of radical cation. The formation of enol form of $NADH^{\bullet+}$ indicates its higher stability than the keto form, although the intramolecular tautomerization in the latter was not observed. However, in the stepwise electron−proton−electron reduction of NAD^+, one should take into consideration the formation of the enol form of radical cation as the product of neutral radical protonation. Because protonation occurs only in strongly acidic solution, the process is unlikely in bulk aqueous solution. But in the contact pair between NAD^+ and the reductant or in the environment of low proton affinity, the multistep NAD^+ reduction may take place with the participation of enol form of NADH radical cation.

4. Experimental Section

4.1. Compounds. *1-Methylnicotinamide, Chloride Salt (MNA^+Cl^-).* The iodide (MNA^+I^-) was prepared via reaction of nicotinamide with methyl iodide in methanol at room temperature and then converted to the chloride salt by shaking its aqueous solution with freshly precipitated silver chloride. Mp 243 °C.

1-Methyl-1,4-dihydronicotinamide (MNAH). To a stirred, degassed solution of sodium dithionite (6 g) and sodium carbonate (2 g) in 50 mL of water, 3 g (17.4 mmol) of MNA^+Cl^- was added during a 20-min period. The solution was extracted with chloroform (3 × 100 mL), the extract was dried with anhydrous $MgSO_4$, and the solvent was removed on a rotary evaporator. The residual oil was pumped out and dried over P_2O_5 to give a yellow solid (60% yield). ^1H NMR (Bruker 250 MHz, $CDCl_3$) δ ppm: 2.94 (s, 3H, CH_3), 3.05 (m, 2H, CH_2), 4.73 (m, 1H), 5.68 (m, 1H), 7.01 (s, 1H).

SCHEME 3



Other Reagents. β-NADH, NAD^+, and KSCN were obtained from Sigma-Aldrich. KBr, NaN_3, $(NH_4)_2S_2O_8$, 2-propanol, perchloric acid, and sodium hydroxide were from POCH (Poland).

4.2. Pulse Radiolysis. Pulse radiolysis experiments were carried out with a high-energy (6 MeV) 17 ns electron pulse generated from ELU-6 linear electron accelerator. The dose absorbed per pulse was determined with N_2O saturated aqueous solution of KSCN (0.01 M), assuming $G((SCN)_2^{\bullet-}) = 6.0$ and $\epsilon((SCN)_2^{\bullet-}) = 7600\ M^{-1}\ cm^{-1}$ (G represents yield of radicals per 100 eV of energy absorbed, and ϵ is molar extinction coefficient at 475 nm).[20] The dose delivered per pulse was within the range 5−80 Gy. Details of the pulse radiolysis system are given elsewhere.[21]

The pulse radiolysis of neutral water produces three highly reactive species, e_{aq} (2.6), $^{\bullet}OH$ (2.7), and $H^{\bullet}$ (0.6), in addition to the formation of less-reactive products, H_2O_2 (0.7), H_2 (0.45), and H_3O^+ (2.6) (numbers in parentheses are the G values 100 ns after electron pulse).[22]

To study the reaction of NADH with $Br_2^{\bullet-}$, the pulse radiolysis was carried out in the solution of KBr so that the $^{\bullet}OH$ radicals react with bromide anions to form dibromide radical anions.[15]

$$Br^- + {}^{\bullet}OH \rightarrow {}^{\bullet}Br + OH^- \quad (6)$$

$$^{\bullet}Br + Br^- \rightarrow Br_2^{\bullet-} \quad (7)$$

The solution was saturated with N_2O to convert e_{aq} into hydroxyl radicals.[15]

$$e_{aq} + N_2O \rightarrow {}^{\bullet}OH + OH^- + N_2 \quad (8)$$

Pulse radiolysis of aqueous solution of NaN_3 leads to the formation of $N_3^{\bullet}$ radicals in reaction of $^{\bullet}OH$ radicals with N_3^-.

$$N_3^- + {}^{\bullet}OH \rightarrow N_3^{\bullet} + OH^- \quad (9)$$

The radical $NAD^{\bullet}$ was produced by the reaction of NAD^+ with 2-propanol ketyl radical formed via reactions 10 and 11.

$$^{\bullet}OH + (CH_3)_2CHOH \rightarrow (CH_3)_2{}^{\bullet}COH + H_2O \quad (10)$$

$$^{\bullet}H + (CH_3)_2CHOH \rightarrow (CH_3)_2{}^{\bullet}COH + H_2 \quad (11)$$

The reduction was carried out in aqueous solutions containing 1 M 2-propanol saturated with N_2O to convert e_{aq} into $^{\bullet}OH$ radicals. α-Hydroxy alkyl radicals formed in reactions 10 and 11 with 85% yield (the remaining 15% are unreactive β-hydroxy radicals) are strongly reducing species that react with many compounds via one-electron transfer ($E^\circ((CH_3)_2CO,H^+/(CH_3)_2{}^{\bullet}COH) = -1.39$ V).[15] This reduction method was also used in the determination of the pK_a of enol forms of radical cations, assuming that yield of generated radicals does not change within the pH range used.

To study the reactivity of $NAD^{\bullet}$ radical toward oxygen, the radiolysis was carried out in the aerated or oxygenated solutions of NAD^+ containing HCOONa. The reducing agents in this case are e_{aq} ($E^\circ(aq/e_{aq}) = -2.87$ V) and $CO_2^{\bullet-}$ ($E^\circ(CO_2/CO_2^{\bullet-}) = -1.9$ V).[15]

$$HCOO^- + {}^{\bullet}OH \rightarrow CO_2^{\bullet-} + H_2O \quad (12)$$

The pH of the solutions was adjusted with perchloric acid or sodium hydroxide and measured with ORION 420A pH meter (Orion Research, Inc.).

Kinetic analysis was done with the Levenberg−Marquardt algorithm. The first-order rate constant values (k_{obs}) were evaluated from the plot of ΔA vs time. The bimolecular rate constants were determined from the slope of the linear plot of k_{obs} vs solute concentration.

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References and Notes

(1) Stryer, L. *Biochemistry*; Freeman: New York, 1995.

(2) (a) Abeles, R. H.; Hutton, R. F.; Westheimer, F. H. *J. Am. Chem. Soc.* **1957**, *79*, 712. (b) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 1014. (c) Carlson, B. W.; Miller, L. L. *J. Am. Chem. Soc.* **1985**, *107*, 479. (d) Kreevoy, M. M.; Lee, I.-S. H. *J. Am. Chem. Soc.* **1984**, *106*, 2550. (e) Ostovič, D. H.; Lee, I.-S. H.; Roberts, R. M. G.; Kreevoy, M. M. *J. Org. Chem.* **1985**, *50*, 4206. (f) Kreevoy, M. M.; Ostovič, D.; Lee I.-S. H.; Binder, D. A.; King, G. W. *J. Am. Chem. Soc.* **1988**, *110*, 524. (g) Lee, I.-S. H.; Jeoung, E. H.; Kreevoy, M. M. *J. Am. Chem. Soc.* **1997**, *119*, 2722. (h) Bunting, J. *Bioorg. Chem.* **1991**, *19*, 456. (i) Yasui, S.; Ohno, A. *Bioorg. Chem.* **1986**, *14*, 70.

(3) (a) Carlson, B. W.; Miller, L. L.; Neta, P.; Grodkowski, J. *J. Am. Chem. Soc.* **1984**, *106*, 7233. (b) Moiroux, J.; Elving, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 6533. (c) Anne, A.; Hapiot, P.; Moiroux, J.; Neta, P.; Savéant, J.-M. *J. Phys. Chem.* **1991**, *95*, 2370. (d) Anne, A.; Hapiot, P.; Moiroux, J.; Neta, P.; Savéant, J. -M *J. Am. Chem. Soc.* **1992**, *114*, 4694. (e) Miller, L. L.; Valentine, J. R. *J. Am. Chem. Soc.* **1988**, *110*, 3982. (f) Powell, M. F.; Wu, J. C.; Bruice, T. C. *J. Am. Chem. Soc.* **1984**, *106*, 3850. (g) Almarsson, Ö.; Sinha, A.; Gopinath, E.; Bruice, T. C. *J. Am. Chem. Soc.* **1993**, *115*, 7093.

(4) (a) Fukuzumi, S.; Tanaka, T. In *Photoinduced Electron Transfer*; Fox, M. A., Chanon, M., Eds.; Elsevier: Amsterdam, 1988; Part C, p 578. (b) Fukuzumi, S. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 1.

(5) (a) Czochralska, B.; Lindqvist, L. *Chem. Phys. Lett.* **1983**, *101*, 297. (b) Czochralska, B.; Bojarska, E.; Pawlicki, K.; Shugar, D. *Photochem. Photobiol.* **1990**, *51*, 401. (c) Noguchi, N.; Tachikawa, M.; Takahashi, H. In *Spectroscopy of Biological Molecules: Modern Trends*; Carmona, P., Navarro, R., Hernanz, A., Eds.; Kluwer: Netherlands, 1997; p 161.

(6) Gębicki, J.; Marcinek, A.; Adamus, J.; Paneth, P.; Rogowski, J. *J. Am. Chem. Soc.* **1996**, *118*, 691.

(7) Marcinek, A.; Adamus, J.; Huben, K.; Gębicki, J.; Bartczak, T.; Bednarek, P.; Bally, T. *J. Am. Chem. Soc.* **2000**, *122*, 437.

(8) (a) Fukuzumi, S.; Inada, O.; Suenobu, T. *J. Am. Chem. Soc.* **2002**, *124*, 14539. (b) Fukuzumi, S.; Inada, O.; Suenobu, T. *J. Am. Chem. Soc.* **2003**, *125*, 4808.

(9) Marcinek, A.; Adamus, J.; Rogowski, J.; Gębicki, J.; Bednarek, P.; Bally, T. *J. Phys. Chem. A* **2000**, *104*, 718.

(10) Marcinek, A.; Adamus, J.; Gębicki, J.; Platz, M. S.; Bednarek, P. *J. Phys. Chem. A* **2000**, *104*, 724.

(11) (a) Land, E. J.; Swallow, A. J. *Biochim. Biophys. Acta* **1971**, *234*, 34. (b) Land, E. J.; Swallow, A. J. *Biochim. Biophys. Acta* **1968**, *162*, 327.

(12) Grodkowski, J.; Neta, P.; Carlson, B. W.; Miller, L. L. *J. Phys. Chem.* **1983**, *87*, 3135.

(13) Hore, P. J.; Volbeda, A.; Dijkstra, K.; Kaptein, R. *J. Am. Chem. Soc.* **1982**, *104*, 6262.

(14) Marcinek, A.; Zielonka, J.; Gębicki, J.; Gordon, C. M.; Dunkin, I. R. *J. Phys. Chem. A* **2001**, *105*, 9305.

(15) (a) Neta, P.; Huie, R. E.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 1027. (b) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637.

(16) (a) Brühlmann, U.; Hayon, E. *J. Am. Chem. Soc.* **1974**, *96*, 6169. (b) Neta, P.; Patterson, L. K. *J. Phys. Chem.* **1974**, *78*, 2211. (c) Kosower, E. M.; Teuerstein, A.; Burrows, H. D.; Swallow, A. J. *J. Am. Chem. Soc.* **1978**, *100*, 5185.

(17) Bielski, B. H. J.; Chan, P. C. *J. Am. Chem. Soc.* **1980**, *102*, 1713.

(18) Willson, R. L. *Chem. Commun.* **1970**, 1005.

(19) (a) Almarsson, Ö.; Bruice, T. C. *J. Am. Chem. Soc.* **1993**, *115*, 2125. (b) Olson, L. P.; Bruice, T. C. *Biochemistry* **1995**, *34*, 7335.

(20) Schuler, R. H.; Patterson, L. K.; Janata, E. *J. Phys. Chem.* **1980**, *84*, 2088.

(21) (a) Karolczak, S.; Hodyr, K.; Łubis, R.; Kroh, J. *J. Radioanal. Nucl. Chem.* **1986**, *101*, 177. (b) Karolczak, S.; Hodyr, K.; Połowiński, M. *Radiat. Phys. Chem.* **1992**, *39*, 1.

(22) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513.