# First Estimation of C₄−H Bond Dissociation Energies of NADH and Its Radical Cation in Aqueous Solution

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Because the redox coenzyme couple NADH/NAD$^+$ (reaction 1)

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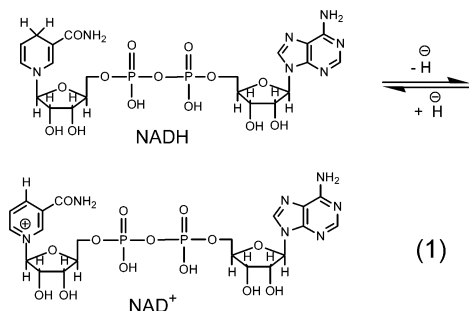
is ubiquitous and controls so much of our oxidation/reduction nature, there has been a long-standing interest in the mechanisms of the redox interconversions. Chemists have given considerable attention to the chemistry of NADH and its models.[1] An interesting controversy is whether the formal hydride transfer from NADH to the surrounding substrates occurs in a one-step or multistep sequence involving electron transfer as the initial step: electron−proton−electron, electron−hydrogen, or hydrogen−electron (Scheme 1). [2]

From Scheme 1, it is conceivable that the heterolytic and homolytic C$_4$−H bond dissociation in NADH and its radical cation (NADH$^{•+}$) could be involved in the course of the biologically re-lated reversible hydride transfer between NADH and NAD$^+$, which indicates that thermodynamic data of the heterolytic and homolytic C$_4$−H bond dissociation energies of NADH and NADH$^{•+}$, espe-cially the thermodynamic data in aqueous phase, should be not only very important but also urgently required for chemists and bio-chemists to thoroughly elucidate the detailed mechanism of the re-versible hydride transfer between NADH and NAD$^+$, since all the reactions mediated by NADH in vivo occur in aqueous phase. Systematic examination of the past publications on this subject shows that although much attention has been paid to the thermo-dynamic analysis on the course of the formal hydride transfer from NADH and its models,[3,4] no efficient method can be used to deter-mine the values of the heterolytic and homolytic C$_4$−H bond dissoc-iation energies of NADH and NADH$^{•+}$ in aqueous phase until now.

In a previous contribution,[5] we reported the determination of heterolytic and homolytic C$_4$−H bond dissociation energies of NADH models in organic phase (dried acetonitrile) according to the reaction of NADH models with *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine radical cation perchlorate (TMPA$^{•+}$ClO$_4^-$) in acetonitrile. In subsequent studies, we found by surprise that TMPA$^{•+}$ClO$_4^-$ is quite stable in water in addition to exhibiting good solubility in water. The test on the stability of TMPA$^{•+}$ClO$_4^-$ in water using UV−vis absorption method showed that for 2 days no significant decrease of the UV−vis absorbance of TMPA$^{•+}$ClO$_4^-$ was found when TMPA$^{•+}$ClO$_4^-$ was dissolved into water, which makes it possible to determine the heterolytic and homolytic C$_4$−H

Scheme 1. Possible Pathways of the Hydride Transfer from NADH
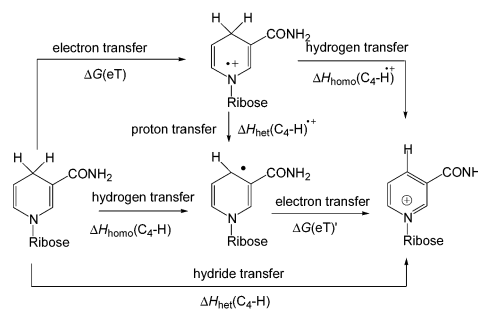
Ribose = adenosine diphosphoribosyl

bond dissociation energies of NADH and NADH$^{•+}$ in aqueous solution using the reaction of NADH with TMPA$^{•+}$ClO$_4^-$ in water. In this communication, we report the first estimation of the heterolytic and homolytic C$_4$−H bond dissociation energies of NADH and NADH$^{•+}$ in aqueous solution.

The reduction of TMPA$^{•+}$ClO$_4^-$ by β-NADH in aqueous solution gives *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPA), TMPAH$^+$, and NAD$^+$ cation. The stoichiometry of the reaction determined from the ^{1}H NMR and ESR suggests that two moles of TMPA$^{•+}$ is required to thoroughly consume one mole of NADH in aqueous solution (reaction 2).

$$NADH + 2TMPA^{•+} \rightarrow NAD^+ + TMPA + TMPAH^+ \quad (2)$$

From reaction 2, four thermodynamic cycles were constructed as shown in Schemes S1 and S2 in Supporting Information, from which four formulas (eqs 3−6) to evaluate the heterolytic and homolytic C$_4$−H bond dissociation energies of NADH and NADH$^{•+}$ were derived, respectively.[6]

$$\Delta H_{het}(C_4-H)^{•+} = F[E_{1/2}(TMPA^{•+}) - E_{1/2}(NADH)] - F[E_{1/2}(NAD^+) - E_{1/2}(TMPA^{•+})] + \Delta H_a(TMPAH^+) + \Delta H_{rxn} \quad (3)$$

$$\Delta H_{homo}(C_4-H)^{•+} = F[E_{1/2}(TMPA^{•+}) - E_{1/2}(NADH)] - F[E_{1/2}(H^{+/0}) - E_{1/2}(TMPA^{•+})] + \Delta H_a(TMPAH^+) + \Delta H_{rxn} \quad (4)$$

$$\Delta H_{het}(C_4-H) = \Delta H_{homo}(C_4-H)^{•+} - F[E_{1/2}(H^{0/-}) - E_{1/2}(NADH)] \quad (5)$$

$$\Delta H_{homo}(C_4-H) = \Delta H_{het}(C_4-H)^{•+} - F[E_{1/2}(H^{+/0}) - E_{1/2}(NADH)] \quad (6)$$

In eqs 3−6, $\Delta H_{het}(C_4-H)$, $\Delta H_{homo}(C_4-H)$, $\Delta H_{het}(C_4-H)^{•+}$, and $\Delta H_{homo}(C_4-H)^{•+}$ are heterolytic and homolytic C$_4$−H bond dissociation energies of NADH and NADH$^{•+}$, respectively; $E_{1/2}(TMPA^{•+})$, $E_{1/2}(NADH)$, $E_{1/2}(NAD^+)$, $E_{1/2}(H^{+/0})$, $E_{1/2}(H^{0/-})$ are redox potentials of TMPA$^{•+}$, NADH, NAD$^+$, H$^+$, and H$^•$, respec-

Table 1. Enthalpy Changes of Reaction 2 and the Deprotonation of TMPAH$^+$ in Aqueous Solution (kcal/mol), Heterolytic and Homolytic C$_4$–H Bond Disassociation Energies of NADH and NADH$^{\bullet+}$ (kcal/mol) along with Redox Potentials of the Relative Species (V vs NHE)

$\Delta H_{rxn}{}^a$	−24.8	$\Delta H_{het}(C_4{-}H)^{\bullet+\,g}$	5.1
$\Delta H_a(TMPAH^+)^b$	5.71	$\Delta H_{homo}(C_4{-}H)^{\bullet+\,g}$	36.3
$E_{1/2}(TMPA^{\bullet+})^c$	0.520	$\Delta H_{het}(C_4{-}H)^g$	53.6
$E°(NAD^+)^d$	−0.94	$\Delta H_{homo}(C_4{-}H)^g$	79.3
$E°(NADH)^e$	0.93		
$E°(H^{+/0})^f$	−2.29		
$E°(H^{0/-})^f$	0.18		

^{a} ΔH_{rxn} obtained from the reaction heat of reaction 2 by switching the sign of the heat value, the latter was measured by titration calorimetry in phosphate buffered (pH 7.0) aqueous solution at 25 °C under an atmosphere of argon. The data in kcal/mol given were average values of at least three independent runs, each of which was again an average value of 10 consecutive titrations except the first. The reproducibility is 0.5 ± 0.5 kcal/mol. ^{b} $\Delta H_a(TMPAH^+)$ is the enthalpy change of the deprotonation of TMPAH$^+$ equal to the reaction heat of TMPA with HClO$_4$ in buffer aqueous solution, the latter was measured by titration calorimetry at 25 °C under an atmosphere of argon. Uncertainty is smaller than 0.6 kcal/mol. ^{c} Measured by cyclic voltammetry in aqueous solution at 25 °C, under an atmosphere of argon, and reproducibile to 5 mV. ^{d,e,f} From refs 7, 8, and 9, respectively. ^{g} Calculated from reactions 3–6, respectively. Estimated uncertainties: 2.3 kcal/mol.

tively; ΔH_{rxn} and $\Delta H_a(TMPAH^+)$ are the enthalpy changes of the reaction 2 and the deprotonation of TMPAH$^+$, respectively. It is not difficult that from eqs 3–6 the heterolytic and homolytic C$_4$–H bond dissociation energies of NADH and NADH$^{\bullet+}$ in aqueous solution can be obtained only if ΔH_{rxn}, $\Delta H_a(TMPAH^+)$, and $E_{1/2}(TMPA^{\bullet+})$, $E_{1/2}(NADH)$, $E_{1/2}(NAD^+)$, $E_{1/2}(H^{+/0})$, $E_{1/2}(H^{0/-})$ are available. Evidently, the enthalpy changes can be obtained from the corresponding heats of reactions, which can directly determined by titration calorimetry. The redox potentials can be obtained from the literature or from measurements by cyclic voltammetry. The detailed experimental results are summarized in Table 1.

Table 1 shows that the heterolytic C$_4$–H bond dissociation energy of NADH is 53.6 kcal/mol in aqueous solution. This value is much smaller than that of the hydride affinities for some transition-metal hydrides in acetonitrile (e.g., the hydride affinities are 66.2 and 60.4 kcal/mol for [HNi(depp)$_2$]$^+$ and [HNi(dmpp)$_2$]$^+$, respectively);[10] this suggests that NADH with 1,4-dihydropyridine structure as the redox center would be a good hydride donor in aqueous phase, which is also well in line with the relatively negative standard two-electron reduction potential of redox pair NADH/NAD$^+$ [$E°(NADH/NAD^+) = -0.32$ V vs NHE].[7] The reason could be that the pyridinium salt formed by the heterolytic C$_4$–H bond dissociation is quite stable in aqueous solution. Comparing the redox potentials of the redox pairs NADH/NADH$^{\bullet+}$ and NADH/NAD$^+$ shows that redox potential of the redox pair NADH/NADH$^{\bullet+}$ (0.93 V vs NHE) is much more positive than that of NADH/NAD$^+$ (−0.32 V vs NHE) in aqueous solution, which suggests that in living organisms transfer of a pair of electrons (i.e., hydride transfer) from NADH in one step should be much easier than initial single-electron transfer from NADH to an electron acceptor (such as β-hydroxy-β-methylglutaryl-CoA), except that the electron acceptor is a single-electron oxidant (such as Fe^{3+} in hemachrome). In Table 1, the value of the homolytic C$_4$–H bond dissociation energy of NADH is 79.3 kcal/mol in aqueous solution, which is not only larger than the corresponding heterolytic C$_4$–H bond dissociation energy but also quite larger than M–H homolytic bond dissociation energies of some metal hydrides in acetonitrile [such as 61.5 kcal/mol for (η^5-C$_5$H$_5$)Cr(CO)$_3$H, 69.2 kcal/mol for (η^5-C$_5$H$_5$)Mo(CO)$_3$H, 72.3 kcal/mol for (η^5-C$_5$H$_5$)W(CO)$_3$H, 57.1 kcal/mol for (η^5-C$_5$H$_5$)Fe(CO)$_2$H, 64.9 kcal/mol for (η^5-C$_5$H$_5$)Ru(CO)$_2$H, etc.],[11] and even close to the C–H homolytic bond dissociation energies of some familiar hydrocarbon C–H acids in dimethyl sulfoxide (such as

82.1 kcal/mol for Fl–H, 84.1 kcal/mol for Ar–CH$_2$–H, 83.9 kcal/mol for Cp–H, 81.5 kcal/mol for In–H, etc.).[12] It is reasonable to suggest that NADH is not a good hydrogen atom donor in aqueous phase, which indicates that in living organisms hydrogen atom transfer should be few and far between NADH and the surrounding substrates, except when the surrounding substrate is an active radical such as superoxide anion radical O$_2^{\bullet-}$, NO$^\bullet$, α-tocopheroxyl radical, etc.

From Table 1, we also find that the heterolytic and homolytic (C$_4$–H)$^{\bullet+}$ bond dissociation energies of NADH$^{\bullet+}$ in aqueous solution are 5.1 and 36.3 kcal/mol, respectively. Clearly, a simple comparison of the two bond cleavage energies reveals that the heterolytic (C$_4$–H)$^{\bullet+}$ bond cleavage energy is quite smaller than the corresponding homolytic (C$_4$–H)$^{\bullet+}$ bond cleavage energy by 31.2 kcal/mol. This indicates that the proton transfer from NADH$^{\bullet+}$ should be much easier than the hydrogen atom transfer from NADH$^{\bullet+}$. From the very low heterolytic (C$_4$–H)$^{\bullet+}$ bond dissociation energy of NADH$^{\bullet+}$, it is conceivable that the proton transfer should be extremely fast and possibly is diffusion-controlled in aqueous solution. Here it is concluded that the e$^-$–H$^+$–e$^-$ sequence hydride transfer should be most likely among all the possible multistep mechanisms of hydride transfer initiated by single-electron transfer in living organisms.

In conclusion, the heterolytic and homolytic C$_4$–H bond dissociation energies of NADH and NADH$^{\bullet+}$ in aqueous solution were estimated, which, to our knowledge is first reported. This energetic information disclosed in the present work should be believed to furnish hints to the understanding of the mechanisms for the redox interconversions of coenzyme couple NADH/NAD$^+$ in vivo.

Supporting Information Available: Four thermodynamic cycles, titration calorimetry, and measurement of redox potentials (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) In eqs 3–6, free energy changes were used to replace the enthalpy changes for the electron-transfer processes. This treatment is reasonable, since the entropy changes associated with electron transfer have been verified to be small (see *J. Am. Chem. Soc.* **1990**, *112*, 344).

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