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Energetics of Multistep versus One-step Hydride Transfer Reactions of Reduced Nicotinamide Adenine Dinucleotide (NADH) Models with Organic Cations and *p*-Quinones

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Free energy changes of each elementary step involved in the formal hydride transfer ($H^-{}_T$) reactions (including the so-called "one-step" $H^-{}_T$ and "multistep" $H^-{}_T$ mechanisms) of the reduced nicotinamide adenine dinucleotide (NADH) models with various cations and quinones (**17**) were investigated either by direct thermodynamic measurements or by calculations from thermochemical cycles. Based on the energetic data thus derived, combined with kinetic observations (particularly kinetic isotope effects), the mechanistic characteristics of the NADH-mediated reductions were systematically analyzed. Practical guidelines that resulted from these analyses suggest that a mutistep mechanism (e^--$H^\bullet$ or e^--H^+-e^-) would be followed if the energy gap of the initial electron transfer [$\Delta G(e^-{}_T)$] between the NADH model compound and the reducing substrate is considerably smaller than the empirical critical limit of 1.0 V for an endothermic $e^-{}_T$. In contrast, if $\Delta G(e^-{}_T)$ is much greater than 1.0 V, a concerted one-step $H^-{}_T$ may take place. The guideline also suggests that a "hybrid" mechanism is possible if the $\Delta G(e^-{}_T)$ is in an intermdiate situation.

Introduction

The mechanisms of reduced nicotinamide adenine dinucleotide (NADH) model-mediated reductions have long been a subject of extensive investigations in the overlapping areas of chemistry and biology because of their relevant importance in understanding the vital functions of the NADH coenzyme in living bodies. Although there have been many discussions in the literature regarding this issue in recent years,[1−3] a working solution to clarifying the long-debated superficial hydride transfer mechanisms [i.e., (i) the one-step hydride transfer[2] and (ii) the multistep hydride transfer[1b−e,3]] has not yet been reached. Investigations showed that when a one-electron oxidant was used in the reaction, it was often observed that the reductions followed the electron transfer-initiated multistep sequence (e^--H^+-e^- or e^--$H^\bullet$).[4,5] On the other hand, it was also shown that a good electron acceptor (which may also be a good hydride acceptor[6]) may initiate a one-step hydride transfer reaction as well.[1a,b,2e−g] Cases also exist where distinctions between a true one-step hydride process and a multistep process are quite difficult to make if only conventional techniques such as electron spin resonance (ESR), chemically reduced dynamic nuclear polariation (CIDNP), ultraviolet−visible (UV−vis), isotope labeling, and product analysis are relied upon.[1−5,7−11] In these cases, knowledge about thermodynamic driving forces of every mechanistically feasible elementary step for each proposed mechanism may play a valuable or sometimes even decisive role in mechanism distinction, especially when combined with kinetic evidence from other sources.[3c] However, thermodynamic analysis of this kind is very sparse in the literature largely because of the transient nature of the intermediate species involved in NADH-mediated reductions (Scheme 1). In this paper, we report our recent evaluations of the detailed energetics for each elementary step shown in Scheme 1 to facilitate mechanism differentiation. The approaches used in this work to derive these otherwise hard-to-get energetic data are primarily various thermochemical cycles (eqs 1−5, see Results), which are similar to the general strategy applied frequently in recent years in evaluating important quantities such as bond dissociation energy (BDE),[6,12] pK($HA^{+\bullet}$),[13] hydride affinities,[14] bond energies of radical ions,[15] and so on.

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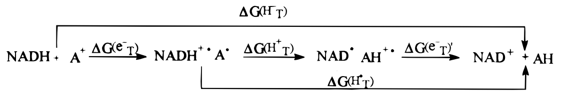
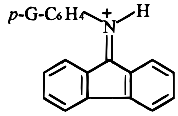
Scheme 1



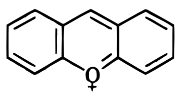
A^+=9-*p*-$GC_6H_4NHFL^+$(**1**), Xn^+(**2**), 9-$PhXn^+$(**3**), Ph_3C^+(**4**), *p*-BQ(**5**), *p*-BQH^+(**6**), $AcrH^+$(**7**);

NADH = BNAH or $AcrH_2$

The substrates chosen in this work are protonated *N*-arylfluorenimines (**1**, 9-$GC_6H_4NHFL^+$), xanthylium ion (**2**, Xn^+), 9-phenylxanthylium ion (**3**, 9-$PhXn^+$), trityl cation (**4**, Ph_3C^+), *p*-benzoquinone (**5**, *p*-BQ) and its derivatives, protonated *p*-benzoquinone (**6**, *p*-BQH^+), and *N*-methylacridinium ion (**7**, $AcrH^+$). Thermodynamic driving forces (i.e., free energy changes) for each step involved in the one-step or multistep pathways (Scheme 1) for reductions of substrates **1**−**7** by the chosen NADH models (i.e., 1-benzyl-1,4-dihydronicotinamide, BNAH, or 10-methylacridan, $AcrH_2$) were estimated. Based on these parameters, combined with the experimental evidence from other sources, (e.g. isotope labeling and kinetic measurements), it becomes more likely that a clarification of possible mechanisms for an apparent hydride transfer reaction can be better reached.



1



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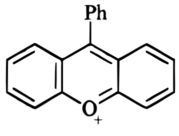
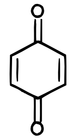


3

G = MeO(a), H(b), Cl(c)

Ph_3C^+

4



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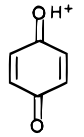

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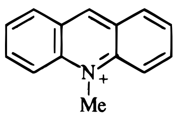
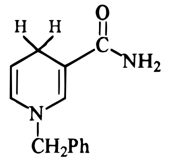
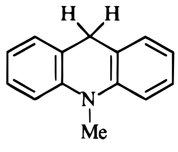


7



BNAH



$AcrH_2$

Results

All the thermodynamic data required in this study were obtained at 25 °C in dimethyl sulfoxide (DMSO). Free energy changes (Δ*G*) for each elementary step of the e^--H^+-e^- mechanism in Scheme 1 were either directly measured (i.e., for electron transfers) or calculated using eqs 1−3 (here NADH represents its models BNAH or $AcrH_2$, NAD^+ represents their oxidized forms), where $E(NADH^{+\bullet}/NADH)$ and $E(AH^{+\bullet}/AH)$ are the oxidation potentials (E_{ox}s) of BNAH, $AcrH_2$, and substrates (AH), respectively; and $E(NAD^+/NAD^\bullet)$ and $E(A^+/A^\bullet)$ are the reduction potentials (E_{rd}s) of BNA^+, $AcrH^+$ and substrate cations (A^+), respectively.

Table 1. Redox Potentials of the Relevant Species Involved in Evaluation of the Energetic Data Using Thermodynamic Cycles for NADH Model Reactions[a]

compound(AH)	$E_{ox}(A^-)$	$E_{ox}(AH)$	$E_{rd}(A^+)$
9-(p-GC_6H_4NH)FLH			
$G = CH_3O$	−1.817	0.214	−1.445
$G = H$	−1.768	0.438	−1.362
$G = Cl$	−1.705	0.506	−1.352
XnH	−1.685	1.135	−0.293[c]
9-PhXnH	−1.531	1.215	−0.366[c]
Ph_3CH	−1.486	1.415	−0.257[c]
p-BQH^-	−1.746[b]	−0.784	0.856[b]
p-BQH_2	−1.784	0.715	−0.04[d]
BNAH		0.182	−1.528
$AcrH_2$		0.497	−0.876

[a] E^p values in volt measured in the present work (except otherwise specified) in DMSO at 25 °C versus ferrocenium/ferrocene (Fc^+/Fc) redox couple under the conditions as described in Experimental Section. [b] From ref 20. [c] From ref 14. [d] From ref 2 g and 21.

$$\Delta G(e^-{}_T) = -F\Delta E[(A^+/A^\bullet) - (NADH^{+\bullet}/NADH)] \quad (1)$$

$$\Delta G(H^+{}_T) = 2.303RT[pK(NADH^{+\bullet}) - pK(AH^{+\bullet})] \quad (2)$$

$$\Delta G(e^-{}_T)' = -F\Delta E[(AH^{+\bullet}/AH) - (NAD^+/NAD^\bullet)] \quad (3)$$

Reduction potentials of 9-$GC_6H_4NHFL^+$ (**1**) were obtained from the second oxidation wave of the 9-arylaminofluorenide ions (the first wave was reversible). Acid dissociation constants of radical cations of the substrates (AH = **1**H−**6**H), or the $pK(AH^{+\bullet})$, were obtained from the combination of p*K*(AH) with oxidation potentials of the parent molecule (E_{ox}(AH)) and its conjugated base ($E_{ox}(A^-)$) using eq 4.[13]

$$pK(AH^{+\bullet}) = pK(AH) + F\Delta E[(A^\bullet/A^-) - (AH^{+\bullet}/AH)]/2.303RT \quad (4)$$

The redox potential data and the acidity and hydride affinity values of the relevant species that were necessary for calculating these energetic quantities are listed in Tables 1 and 2, respectively.

Evaluation of the free energy changes for the follow-up proton transfer (i.e., $\Delta G(H^+{}_T)$) upon formation of the NADH model radical cation requires a knowledge of their equilibrium acidity constants [i.e., $pK(BNAH^{+\bullet})$ and $pK(AcrH_2{}^{+\bullet})$]. Because these $pK(AH^{+\bullet})$ values in DMSO

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Table 2. Acid Dissociation Constants of Neutral Substrates [p*K*(AH)] and Radical Cations p*K*($AH^{+\bullet}$) and Hydride Affinities of the Corresponding Cations [$\Delta G_{H^-}(A^+)$] in DMSO at 25 °C

compound(AH)	p*K*(AH)[a]	p*K*($AH^{+\bullet}$)[d]	$-\Delta G_{H^-}(A^+)$[g]
9-(*p*-GC_6H_4NH)FLH			
G = CH_3O	19.9	−14.3	46.8
G = H	19.3	−17.8	49.1
G = Cl	19.0	−18.2	50.3
XnH	30.0[b]	−18[e]	90.3[h]
9-PhXnH	27.9[b]	−18[e]	89.3[h]
Ph_3CH	30.6[b]	−18[e]	96.5[h]
p-BQH^-	26.2[c]	10	70.7[i]
p-BQH_2	19.76[b]	−5.5	102.9[i]
BNAH		−3.3[f]	59.0[j]
$AcrH_2$		−6.9[f]	76.5[k]

[a] Determined in the present work (except the ones specified) by the double standard acid (SA) method (see Experimental Section): SA1 = dibenzyl sulfone [p*K*(SA1) = 23.9]; SA2 = *m*-cyanobenzylphenyl sulfone [p*K*(SA2) = 20.6] or SA2 = *p*-cyanobenzylphenyl sulfone [p*K*(SA2) = 18.52]. [b] From ref 12c. [c] From ref 20. [d] Calculated from eq 4. [e] From Cheng, J.-P., Ph.D. Dissertation, Northwestern University, Evanston, IL, 1987. [f] Derived using eq 5 (see text for details). [g] In kcal/mol, calculated from eq 10 or 11. [h] Quoted from ref 14a. [i] From refs 15 and 16. [j] Calculated from eq 11, where ΔG_{BDE} for BNAH, $E(BNA^+/BNA^\bullet)$, and $E(H^\bullet/H^-)$ were taken as 69.2 kcal/mol (see text), −0.991 V (value from Table 1 adjusted to versus NHE by adding 0.537 V), and −0.55 V (vs NHE),[14a] respectively. [k] From ref 16.

Scheme 2

$$\begin{array}{ll} NADH \rightarrow NAD^\bullet + H^\bullet & \Delta G_{BDE} \\ H^\bullet \rightarrow H^+ + e^- & FE(H^+/H^\bullet) \\ NADH^{+\bullet} + e^- \rightarrow NADH & -FE(NADH^{+\bullet}/NADH) \\ \hline NADH^{+\bullet} \rightarrow NAD^\bullet + H^+ & 2.303RTpK(NADH^{+\bullet}) \end{array}$$

Scheme 3

$$\begin{array}{ll} BNAH + AcrH^+ \rightarrow BNA^+ + AcrH_2 & \Delta G(H^-{}_T) \\ AcrH^\bullet + BNA^+ \rightarrow AcrH^+ + BNA^\bullet & F\Delta E[(AcrH^+/AcrH^\bullet) - (BNA^+/BNA^\bullet)] \\ \hline AcrH^\bullet + BNAH \rightarrow AcrH_2 + BNA^\bullet & \Delta\Delta G_{BDE}\,[(BNAH) - (AcrH_2)] \end{array}$$

are not yet reported, eqs 5 and 6, which are based respectively on the thermodynamic cycles shown in Schemes 2 and 3,[6] were used jointly to derive these data.

$$pK(NADH^{+\bullet}) = [\Delta G_{BDE} - FE(NADH^{+\bullet}/NADH) + FE(H^+/H^\bullet)]/2.303RT \quad (5)$$

$$\Delta\Delta G_{BDE}[(BNAH) - (AcrH_2)] = \Delta G(H^-{}_T) + F\Delta E[(AcrH^+/AcrH^\bullet) - (BNA^+/BNA^\bullet)] \quad (6)$$

Bond dissociation free energy of the 9-C−H bond in $AcrH_2$ was evaluated previously by Parker and coworkers as 71.6 kcal/mol[16,17] by modeling with the structurally similar xanthene. Combination of this value with $E(H^+/H^\bullet)$ of −2.48 V[18] and $E(AcrH_2{}^+/AcrH_2{}^\bullet)$ of 1.034 V [from this work, adjusted to versus normal hydrogen electrode (NHE)] gave a p*K* value of −6.9 for the 10-methylacridan radical cation ($AcrH_2{}^{+\bullet}$). Precision of this value is assured on the part of the redox potential measurement of this work (reproducible to 10 mV) if confidence in the accurary of the two literature values used in the evaluation is assumed. Unfortunately, the p*K*($BNAH^{+\bullet}$) cannot be directly calculated in the same simple manner because of the lack of the ΔG_{BDE} value of the 4-C−H bond in BNAH. The missing quantity can be estimated from eq 6, however. For this purpose, a calorimetric measurement of the heat of hydride transfer between BNAH and $AcrH^+$ in DMSO was carried out (see Experimental Section) and a value of −17.4 ± 0.3 kcal/mol was obtained. This value is assumed to be equal to the corresponding free energy because the entropy contribution to this quite symmetrical reaction in acetonitrile was shown to be negligible as judged by the close match of the heat of hydride transfer in MeCN measured in this work ($\Delta H(H^-{}_T) = -12.3$ kcal/mol) with the free energy of hydride transfer ($\Delta G(H^-{}_T) = -11.2$ kcal/mol) determined from an equilibrium study.[6,19] The p*K*($BNAH^{+\bullet}$) evaluated from eq 5 using a ΔG_{BDE} thus estimated from eq 6 is −3.3. Accumulation of all the experimental errors from this rather lengthy evaluation gave an uncertainty of ~1.3 p*K* units, which is acceptable for the purpose of this study and is comparable to or better than the uncertainty of 2 p*K* units generally found for the pK_a quantities of radical cations in the literature.[13]

Free energy changes of the hydrogen atom transfer step in the e^--$H^\bullet$ mechanism [$\Delta G(H^\bullet{}_T)$] and of the direct hydride transfer step in the one-step mechanism [$\Delta G(H^-{}_T)$] were evaluated from eqs 7 and 8, respectively, based on the thermodynamic cycles shown in Scheme 1.

$$\Delta G(H^\bullet{}_T) = \Delta G(H^+{}_T) + \Delta G(e^-{}_T)' \quad (7)$$

$$\Delta G(H^-{}_T) = \Delta G(e^-{}_T) + \Delta G(H^+{}_T) + \Delta G(e^-{}_T)' \quad (8)$$

Alternatively, $\Delta G(H^-{}_T)$ may also be evaluated from the difference between the hydride affinities of substrate [$-\Delta G_{H^-}(A^+)$] and of the oxidized form of model compound [$-\Delta G_{H^-}(NAD^+)$] (eq 9) according to Scheme 1. Calculation of hydride affinities for the individual cationic species can be easily made using eq 10[14] or eq 11.[16] The $\Delta G(H^-{}_T)$ values of the overall reactions thus evaluated are in fact identical to those from eq 9.

$$\Delta G(H^-{}_T) = -\Delta G_{H^-}(NAD^+) - [-\Delta G_{H^-}(A^+)] \quad (9)$$

$$-\Delta G_{H^-}(A^+) = 2.303RTpK(AH) + F\Delta E[(A^\bullet/A^-) + (A^+/A^\bullet)] - F\Delta E[(H^\bullet/H^-) + (H^+/H^\bullet)] \quad (10)$$

$$-\Delta G_{H^-}(A^+) = \Delta G_{BDE} + FE(A^+/A^\bullet) - FE(H^\bullet/H^-) \quad (11)$$

The energetic data derived in this work for each processes of interest (Scheme 1) are summarized in Table 3.

Discussion

It is well recognized that thermodynamic driving forces (i.e., energetics of a reaction) can usually serve as a good guide (though may not always be in an exclusive sense)

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(17) This value should be considered the upper limit of this bond scission because *N*-methylanilino is a stronger radical stabilizing group than phenoxy, which implies that the p*K*($AH^{+\bullet}$) derived using this number would also be a maximum value.

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Table 3. Free Energy Changes (kcal/mol) of Each Mechanistic Step for Various Hydride Transfer Mechanisms Shown in Scheme 1[a]

	mechanisms					
	e^- - $H^\bullet$		e^- -H^+ -e^-			H^-
model/subst	$\Delta G(e^-_T)$	$\Delta G(H^\bullet_T)$	$\Delta G(e^-_T)$	$\Delta G(H^+_T)$	$\Delta G(e^-_T)'$	$\Delta G(H^-_T)$
Group I						
BNAH/Xn^+	11.0	−41.3	11.0	20.1	−61.4	−30.3
BNAH/9-$PhXn^+$	12.6	−43.1	12.6	20.1	−63.2	−30.5
BNAH/Ph_3C^+	10.1	−47.8	10.1	20.1	−67.9	−37.7
$AcrH_2$/p-BQH^+	12.4	−38.6	12.4	−1.9	−36.7	−26.2
Group II						
BNAH/9-$GC_6H_4NHFL^+$						
G=CH_3O	37.5	−25.1	37.5	15.1	−40.2	12.4
G=H	35.6	−25.4	35.6	19.9	−45.3	10.2
G=Cl	35.4	−26.5	35.4	20.4	−46.9	8.9
$AcrH_2$/p-BQ	31.2	−25.2	31.2	−23.1	−2.1	6.0
Group III						
BNAH/p-BQ	23.9	−35.4	23.9	−18.2	−17.2	−11.5
BNAH/$AcrH^+$	24.4	−41.8	24.4	4.9	−46.7	−17.4
$AcrH_2$/*p*-chloronil[b]	19.9	−26.4	19.9		−6.5	

[a] Values measured in DMSO at 25 °C or derived using eqs 1−3 and 7−9. [b] Data evaluated similarly, using reported values from ref 16.

for analyzing various possibilities of reaction mechanisms. Because general agreement on assigning reaction mechanism from the ones proposed previously for NADH-mediated reductions has not been reached, the detailed thermodynamic parameters derived in this work may be of value in providing hints for differentiating the true paths for at least certain types of NADH model reactions. The reactions chosen for this purpose are those listed in Table 3 and are grouped into three categories (Groups I−III) according to the similarities of their energetic behaviors.

Thermodynamics of the Multistep Hydride Transfer Mechanism. As already mentioned, there are two distinct cases for the so-called multistep mechanism: (i) an electron transfer followed by a proton transfer and then a second electron transfer (i.e., e^--H^+-e^- pathway); and (ii) an electron transfer followed by a hydrogen atom transfer (i.e., e^--$H^\bullet$ pathway). Thus, in either case, transfer of an electron is required to initiate the multistep reaction. According to what has been extensively studied and suggested in the literature,[22] for the best result of an electron-transfer-initiated process, the energy gap between the donor and acceptor should not exceed the 1.0 V (i.e., 23.1 kcal/mol) limit. This empirical criterion for a conventional electron transfer to occur would require that the NADH model used in the reduction not be too poor an electron donor and that the substrate not be too poor an electron acceptor. Previous studies have shown that reductions of some good electron acceptors, such as substituted ferrocenium series,[2e,4] ferricynide ion,[5] and some radical cations,[4b] by certain NADH models did proceed via the multistep mechanism (i.e., initiated by an electron transfer). As shown by the data in Table 1, the reduction potential of the protonated quinone (**6**, E_{rd} of −0.04 V) is very close to that of ferrocenium (0.00 V, serving as the internal reference) and those of **2**−**4** are not too much more negative. Therefore, the situation for the Group I reductions are expected to be similar (i.e., via electron transfer). In fact, the very low e^-_T barriers found for this group of reactions[23] suggest that this should be the case.

One may have noted from Table 1 that although $AcrH_2$ is actually not as good an electron donor as BNAH (E_{ox}s are 0.182 and 0.497 V, respectively), the electron transfer energetics (i.e., $\Delta G(e^-_T)$) of the $AcrH_2$/*p*-BQH^+ donor−acceptor pair, and of the other three combinations of Group I in Table 3 (11.0, 12.6, 10.1, and 12.4 kcal/mol, respectively), however, all fall into the favorable range (<23.1 kcal/mol) proposed for an electron transfer reaction, indicating that the initial electron transfer is nevertheless an energetically feasible path for these reactions to choose. Furthermore, it is conceivable that the small energy required for initiating an electron transfer process can certainly be fully compensated because both the driving forces of the overall reaction (−30.3, −30.5, −37.7, and −26.2 kcal/mol, respectively) and of the immediate follow-up hydrogen atom transfer (−41.3, −43.1, −47.8, and −38.6 kcal/mol, respectively) are highly exothermic. The experimentally observed negligible kinetic isotope effect ($k^H/k^D = 1.4$) and the small enthalpy of activation for reaction of Xn^+(**2**) with BNAH ($\Delta H^\ddagger = 7.6$ kcal/mol) described in our earlier report[23] provide further evidence for ruling out the involvement of a direct C−H bond cleavage of BNAH in the rate-limiting step, though the overall reaction does at first sight look like a one-step hydride process. The fact that the BNAH/Ph_3C^+(**4**) reaction was indeed observed to follow the multistep pathway[24] also supports this driving force-based argument.

The kinetic isotope effect of the BNAH/DDQ reaction (DDQ: 2,3-dichloro-5,6-dicyanoquinone) was reported in the literature to be 1.5 in MeCN,[3a] again indicative of the absence of a C−H bond cleaving process in the rate-limiting step. The electron transfer energetics are easily evaluated in this work from their respective redox potentials[16] to be 2.2 kcal/mol, a value well below the 1.0 V criterion. It is therefore reasonable to conclude that this reaction also proceeded by an electron-transfer-initiated multistep mechanism, even though the free

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energy of the one-step hydride transfer is much more negative [$\Delta G(\text{H}^-{}_\text{T})$ of -42.6 kcal/mol is calculated from eq 9 using the data in ref 16].

It is of course desirable to be able to predict which one of the two follow-up paths (i.e., $\text{H}^+{}_\text{T}$ or $\text{H}^\bullet{}_\text{T}$) is more likely to occur for the Group I reactions. From the thermodynamic point of view, one would immediately note from the corresponding data in Table 3 that the $\text{H}^+{}_\text{T}$ step in e^--H^+-e^- mechanism is once again energy requiring (except for case 4, $\text{AcrH}_2/\text{QH}^+$, which is slightly energy releasing), which is in a sharp contrast to the huge driving force provided by the $\text{H}^\bullet{}_\text{T}$ step (-38.6 to -47.8 kcal/mol) in the e^--$\text{H}^\bullet$ mechanism. Thus it is reasonable to propose that the energetically less favorable e^--H^+-e^- mechanism would not likely to be the one for this group of reactions to choose. Fukuzumi and co-workers studied the $\text{AcrH}_2/p\text{-BQH}^+$ case (which is in fact the least favorable one in this group for the assigned mechanism) and reached a similar conclusion on the basis of the observed kinetic isotope effect ($k^\text{H}/k^\text{D} = 1.1$ in MeCN and 2.5 in 5:1 $\text{H}_2\text{O}-\text{EtOH}$)[3e] and the estimated relative pK_a (1.7) of $\text{AcrH}_2{}^{+\bullet}$ and $p\text{-BQH}_2{}^{+\bullet}$.[3c,25]

It is worth pointing out that even though the e^--$\text{H}^\bullet$ mechanism could be assigned for the aforementioned NADH-mediated reactions, it may still not guarantee a trap of the radical intermediate. The failure to observe a deuterated product in the reaction of **3** or **4** with BNAH in the presence of deuterated trifluoroacetic acid (see Experimental Section) and the absence of a radical inhibitor effect for reaction of **2** with BNAH[23] suggest that in these cases the incipient radical pair generated upon electron transfer must have been kept within the solvent cage during the entire course of hydrogen atom transfer, or alternatively, the $\text{BNAH}^{+\bullet}/\text{Xn}^\bullet$ (or /9-$\text{PhXn}^\bullet$ or /$\text{Ph}_3\text{C}^\bullet$) radical pair may look more like a transition state in a continuous spectrum rather than an isolated intermediate, so there should be no chance for the deuterium or radical inhibitor to catch them. This result can be further understood by examining the free energies of hydrogen atom transfer for this category of reductions. As already mentioned, the 38.6 to 47.8 kcal/mol driving forces for the hydrogen atom transfer steps [i.e., $\Delta G(\text{H}^\bullet{}_\text{T})$] are certainly qualified to drive the $\text{H}^\bullet{}_\text{T}$ reaction to go extremely fast, so it is almost impossible in a practical sense for radicals to escape out of the solvent cage.

Thermodynamics of the One-Step Hydride Transfer Mechanism. Reductions of imines and iminium ions by the NADH model compounds have been intensively investigated in the literature.[2a,b,26] It is generally suggested that the reactions followed the one-step hydride transfer mechanism.[2a,b,27] Thermodynamic quantities have, however, not been touched so far for this type of reactions primarily because of the lack of means of manipulating the transient species involved in direct measurement. Therefore, a thermodynamic study for reductions of **1** by BNAH (i.e., the Group II reactions) using a strategy similar to that just applied was performed in this work. In a sharp contrast to the energetic pattern found for the Group I reactions, electron transfers that are necessary for initiating the multistep mechanism were all observed to be extremely endothermic for this group, as judged by their respective $\Delta G(\text{e}^-{}_\text{T})$ values in Table 3 (from 37.5 kcal/mol for BNAH/**1a** to 31.2 kcal/mol for AcrH_2/**5**). In addition, it is noted that neither the follow-up proton transfer in the e^--H^+-e^- mechanism nor the hydrogen atom transfer in the e^--H mechanism could provide enough energy to drive the reduction to completion. On the other hand, the energy required for the direct one-step hydride transfer is nevertheless small (ranging from 6.0 to 12.4 kcal/mol, Table 2), and thus is relatively easy to overcome.[28,29] Thus, this path is realized to be the only energetically plausible one left for the reduction to proceed to avoid the huge energy barrier of electron transfer. The high k^H/k^D ratio of 6.3 observed for the $\text{AcrH}_2/p\text{-BQ}$ reaction in 5:1 $\text{H}_2\text{O}-\text{EtOH}$,[3e] the failure of incorporating deuterium into the products of the BNAH/**1** reaction found in this work, together with the thermodynamic argument presented here, indeed seem to be much in favor of the one-step $\text{H}^-{}_\text{T}$ mechanism for the Group II reactions. This conclusion is surely in good accordance with those proposed earlier,[2a,b,27] but is based instead on the detailed energetic analysis (i.e., in terms of driving forces).

If the thermodynamic features just described for the Group I and Group II reactions are considered as the two energetic extremes, one may then notice that the Group III reactions (Table 3) are characteristic of an intermediate situation. The three reactions in Group III are in fact among those cases where much debate about their mechanism has arisen.[1b,2e,f,30,31] On inspection of the data in the last three entries of Table 3, it is obvious that the energy required for initiating an electron transfer reaction (23.9, 24.4, and 19.9 kcal/mol, respectively) is very close to the previously mentioned 1.0 V (i.e., 23.1 kcal/mol) criterion[22] and therefore it is not very likely that an exclusive electron-transfer-initiated multistep reaction will occur. On the other hand, the exothermicity of the direct hydride transfer path (-11.5, -17.4, and -6.5 kcal/mol) suggests that the one-step mechanism may be plausible for this group of reactions. The k^H/k^D ratio of 6.2 observed experimentally by Fukuzumi et al.[3a] for reaction of p-BQ with BNAH seems to agree well with the one-step $\text{H}^-{}_\text{T}$ mechanism.[2f]

It should be warned, however, that the situation for the Group III reactions may sometimes become sensitive to variation of the actual experimental conditions applied because they are energetically so close to the borderline. For instance, the kinetic isotope effect for reaction of AcrH^+ with BNAH was 4.6[31] in acetonitrile, which may

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(25) It should be noted, however, that the relative p$K(\text{AH}^{+\bullet})$ in DMSO evaluated in this work is a small negative number (-1.4) whereas in MeCN, it is a small positive number (1.7[3c]). This difference implies that the e^--H^+-e^- sequence should also be a feasible choice for the case 4 reaction ($\text{AcrH}_2/\text{QH}^+$) in DMSO, even though in MeCN it was suggested to follow the e^--$\text{H}^\bullet$ pathway.[3c]

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(28) This reduction is expected to be relatively slow, however, because of the energy barrier. Experimentally, the reduction yield was observed low in this work due possibly to this reason and also to the instability of BNAH in acid media.[29]

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be indicative of a "hybrid" of the one-step and the multistep mechanisms. A midranged k^H/k^D ratio of 4.8 found for the $AcrH_2$/*p*-chloronil reaction in 5:1 H_2O−EtOH[3e] seems also to support the concept of the hybrid description.

The so-called $H^\bullet$-e^- mechanism, as alternatively suggested by Carlson and Miller for the BNAH/**5** reaction,[2e] may also be taken as a potential candidate for the reaction mechanism. The free energy changes of the initial $H^\bullet_T$ and the following e^-_T steps of this mechanism were evaluated in this work to be 5.7 and −17.2 kcal/mol, respectively. It is therefore reasonable to conceive that the $H^\bullet$-e^- mechanism is in fact an equivalent of the one-step mechanism because the e^-_T step is even more exogonic and would proceed so fast that a separation from its preceding step is practically not feasible in this case.

The reported observation of ESR signals (proposed for *p*-$BQ^{-\bullet}$) during the BNAH/**5** reaction[3a] seems at first glance conflicting with the one-step mechanism already suggested. However, a simple calculation indicates that only 1.7 kcal/mol energy is required to transfer an electron from *p*-BQH^- (the reduced product upon hydride transfer) to the starting *p*-BQ to form its radical anion (*p*-$BQ^{-\bullet}$). This process could also be driven by the energy released from the overall reaction [$\Delta G(H^-_T) = -11.5$ kcal/mol]. The failure of observing *p*-$BQ^{-\bullet}$ under slightly different conditions where an equal amount of *p*-BQ and BNAH was used[1f] (which eliminated the chance for the aforementioned process) does agree with this inference. Thus, it is realized that observation of ESR signals may not always ensure the occurrence of an electron transfer initiated process.

Conclusion

A systematic thermodynamic investigation of each mechanistically distinctive elementary steps involved in the three types of hydride transfer mechanisms of NADH model-mediated reductions are presented. Based on the energetic data collected in this work, jointly with the kinetic observations reported in the literature, practical guidelines for differentiating or predicting the mechanistic details for certain types of NADH reactions are proposed. It is suggested that if the energy required for an initial electron transfer is well below the empirical energy limit (1.0 V), a multistep reaction (e^--$H^\bullet$ or e^--H^+-e^-) will possibly take place. If on the other hand the barrier for an initial electron transfer is well above the line, the one-step process would then most likely be the one for the reaction to follow, provided that it is energetically feasible. Cases between may proceed by a merged ("hybrid") mechanism.

Experimental Section

Materials. 1-Benzylnicotinamide bromide (BNA^+Br^-) and 1-benzyl-1,4-dihydronicotinamide (BNAH) were prepared according to literature procedure.[2a] *N*-Methylacridinium ion ($AcrH^+I^-$, **7**) and 10-methylacridan ($AcrH_2$) were obtained by the method described in ref 32. *N*-Arylfluorenimines were synthesized by condensation of 9-fluorenone with substituted anilines according to the method of Rajgopal Srinivasan.[33] *N*-Arylaminofluorenes were prepared from reactions of 9-bromofluorene with corresponding amines in acetonitrile.[34] 9-Phenylxanthylium and trityl ions were prepared by dehydroxylation of the corresponding carbinols (Aldrich) with 48% HBF_4 in propionic anhydride.[35] The compounds synthesized in this work were characterized by verifying their melting points, ^1H NMR spectra, and/or elemental analysis. Purification of DMSO solvent and preparation of dimsyl base ($CH_3SOCH_2^-K^+$) were carried out according to the standard procedure in the literature.[36] Acetonitrile (spectroscopic grade) was refluxed over $KMnO_4$ and K_2CO_3 for several hours and was doubly redistilled over P_2O_5 under argon before use. The commercial tetrabutylammonium hexafluorophosphate (Bu_4NPF_6, Aldrich) was recrystallized from CH_2Cl_2 and was vacuum dried at 110 °C overnight before preparation of supporting electrolyte solution.

Reduction of Protonated *N*−Arylfluorenimines (1a−c) by BNAH. To a mixture of *N*-arylfluorenimine (1 mmol) and BNAH (2 mmol) was added 5 mL of CF_3CO_2D−DMSO (1:4). After stirring the mixture at room temperature overnight, water was added to quench the reaction. Extraction of the organic layer by benzene, followed by preparative thin-layer chromatography (TLC) gave a white powder which was identified as *N*-arylaminofluorene upon comparison with authentic samples. Analysis by ^1H NMR showed that there is no deuterium in the product.

Reduction of 9-Phenylxanthylium and Trityl Cations by BNAH. To a mixture of 9-phenylxanthylium ion (0.5 mmol) and BNAH (0.5 mmol) was added 3 mL deaerated acetonitrile containing 0.5 mL CF_3COOD under argon. Ten minutes later, water was added to quench the reaction. The product mixture was extracted with benzene and was isolated by preparative TLC to give 9-phenylxanthene as the sole product, mp, 142.0−143.0 °C (CRC, 145.0−146.0 °C). ^1H NMR ($\delta CDCl_3$): 7.01−7.52 (m, 13H), 5.32−5.41 (s, 1H). Triphenylmethane was similarly obtained from reaction of trityl cation with BNAH; mp 91.0−93.0 °C. ^1H NMR ($\delta CDCl_3$): 7.09−7.53 (m, 15H), 5.58−5.65 (s,1H).

***pK*(AH) Measurement.** The p*K*(AH) values were measured in DMSO at 25 °C by the "Overlapping Indicator Method"[12,36] on a Beckman DU-8B UV−vis spectrophotometer. Because the UV absorptions of the 9-arylaminofluorenide ions and the indicator anions were seriously overlapped, the so-called "double standard acid method"[37] (an analogue of the indicator method) was used to derive the data. The general procedure for deriving pK_as in this way is as follows.

A certain amount of a chosen standard acid whose acidity is between the estimated values of the N−H bond and C−H bond in a substrate is first added to an optical cuvette containing 2 mL of diluted dimsyl base ($CH_3SOCH_2^-K^+$) solution. The anion generated upon a complete deprotonation of the standard acid is then titrated with the substrate of interest in small aliquots to give a linear Beer's Law plot from which the extinction coefficient (ϵ) can be calculated. Then, another standard acid whose pK_a is in a close vicinity of the estimated p*K* value of the particular bond is added to the solution and the UV absorption is recorded. The UV data thus derived are manipulated by a standard computer program for the standard acid method to give the p*K*(AH) value.

The p*K*(AH) measured in this work are the average values of at least two independent runs and are expected to be accurate to within 0.15 p*K* units.

Electrochemical Measurement.[12,13] Cyclic voltammograms were recorded on a BAS-100B electrochemical analyzer (Purdue Research Park, West Lafayette, IN) equipped with a three-electrode assembly. The working electrode was a 1-mm diameter platinum disk embedded in a cobalt glass seal and was polished consecutively with polishing alumina and dia-

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mond suspensions (supplied by BAS as a kit) between runs. The potentials were scanned at a sweep rate of 0.1 V/s versus the 0.1 M $AgNO_3$/Ag (in 0.1 M Bu_4NPF_6−DMSO) reference electrode whose potential was periodically checked against the ferrocenium/ferrocene (Fc^+/Fc) internal standard. The auxiliary electrode was a platinum wire. The anions were generated in situ under argon by titrating a stock dimsyl solution (enough to produce 1 mM anion concentration) into a 0.1 M supporting electrolyte solution containing the substrate. Immediately after preparation of the anion, a cyclic voltammetry (CV) spectrum was recorded. The potentials for neutral or cationic compounds were similarly measured under argon. The redox values thus measured were generally reproducible to 5 mV for anions and 10 mV for cationic and neutral species.

Titration Calorimetry. Heats of reaction of BNAH with $AcrH^+$ in DMSO and acetonitrile were measured at 25 °C with a Tronac 458 titration calorimeter whose performance was periodically checked by measuring the standard heat of neutralization with standard aqueous sodium hydroxide and hydrogen chloride solutions.

The BNAH solutions (0.017−0.02 M) were prepared inside the argon-filled drybox. Before each calorimetric run, the calibrated motor-driven buret (filled with BNAH solution) and the reaction vessel containing ~35 mL of $AcrH^+$ cation solution (0.02 M) were connected to the calorimeter insert assembly. A dry argon blanket was maintained at the top of the reaction vessel to protect the $AcrH^+$ solution from exposure to air. The operation of the calorimeter itself was followed according to the procedure described by Arnett et al.[38] The heat obtained is an average value of at least two enthalpy measurements with the same stock of BNAH. Reproducibility of the data was verified by comparing with the heat data obtained on a different day with another samples of BNAH and $AcrH^+$ prepared differently and was 0.3 kcal/mol.

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