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REACTIVITY OF BIOLOGICALLY IMPORTANT REDUCED PYRIDINES V. RELATIVE IMPORTANCE OF ELECTRON VERSUS PROTON LOSS IN FERRICYANIDE-MEDIATED OXIDATION OF DIHYDRONICOTINAMIDES

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Abstract: Kinetics of ferricyanide-mediated oxidation of various 1-(4-substituted phenyl)-1,4-dihydronicotinamides were studied assuming either a rate determining initial electron loss or a rate determining proton loss following a rapid pre-equilibrium step. Values obtained using both methods were similar. In addition, vertical ionization potentials generated using the AM1 molecular orbital approximation were found to be highly correlated with log(oxidation rates) while calculation on the proton loss from the dihydropyridine radical cations showed much less dependence. Measured kinetic deuterium isotope effects were small. These data suggest that the initial electron loss from the dihydronicotinamides is the most important event determining the reaction rates.

Introduction

Various models of electron transfer have been studied in order to gain insight into the biologically important NAD(P)H↔NAD(P)$^+$ interconversion[3,4]. In many instances, system involving dihydronicotinamides have been applied to this purpose[5-7]. Interestingly, the manner in which electrons are shuttled between the redox couples strongly depend upon the reaction conditions. If a good hydride acceptor, such as the 1-methylacridinium ion, serves as the oxidant, the oxidation most likely proceeds via concerted hydride migration, i.e., the simultaneous transfer of a proton and two electrons from the dihydropyridine to the quaternary salt[8,9]. If, on the other hand, a good kinetic and thermodynamic one electron oxidant is present in the reaction media, a sequential electron, proton, electron "hydride-like" transfer occurs[10-14]. Species which are suitable to induce this type of chemical behavior include the ferricyanide anion and the ferricenium cation. Miller has characterized the reactions of ferricenium derivatives with 1-substituted-1,4-dihydronicotinamides as being first order with respect to each species[10-12]. The rates of these reactions were unaffected by pH or by substitution of the 4-hydrogen(s) on the dihydronicotinamide moiety with deuterium atoms. These data are fully consistent with an initial, rate-determining electron transfer. The reaction of the ferricyanide ion with dihydropyridines does not give as unambiguous a picture. Various studies have shown that this redox interaction is first order with respect to each species and that a small to negligible kinetic isotope effect is observed for oxidation of the 4,4-dideuterio analogs[13-15]. These data suggest that a simple

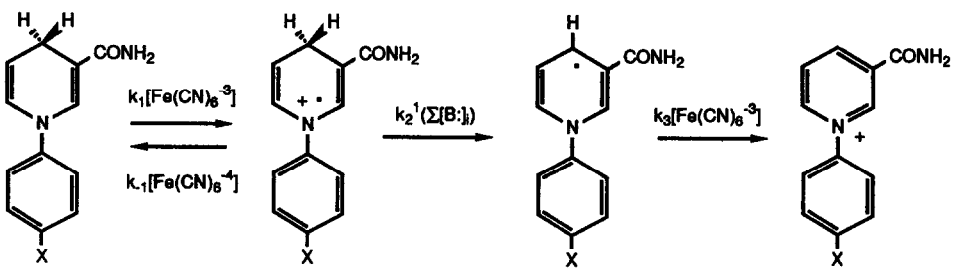
second order reaction adequately describes the oxidative mechanism. In this system the observed rate constant is given by:

$$k_{obs} = k_1[Fe(CN)_6^{-3}] \qquad (1)$$

The fact that added base accelerated the oxidation and that ferrocyanide ion inhibited the reaction rate suggested an alternative mechanism, in which the oxidant and the dihydropyridine species interacted in a rapid pre-equilibrium step to yield the corresponding dihydropyridine radical cation occurred[16]. According to this scheme, the radical cation was then deprotonated in the rate determining step giving rise to the pyridinyl radical which then underwent a non rate-determining electron loss to give the pyridinium salt. This mechanism was shown to operate in the case of ferricyanide-mediated oxidation of 1-methylacridan in that this reaction demonstrated general-base catalysis[17]. The observed rate constant for oxidation of dihydropyridines according to this series of events is given by:

$$k_{obs} = \frac{k_1 k_2'[Fe(CN)_6^{-3}](\Sigma[B:]i)}{k_{-1}[Fe(CN)_6^{-4}]+k_2'(\Sigma[B:]i)} \qquad (2)$$

where $k_2'(\Sigma[B:]i)$ represents the rate of deprotonation of the formed radical cation and $\Sigma[B:]i$ is the concentration of the general-base. This equation is derived from the following reaction sequence:



Clearly, equation (2) collapses to equation (1) if k_{-1} is small. Earlier studies on the ferricyanide-mediated oxidation of a series of 1-(4-substituted phenyl)-1,4-dihydronicotinamides indicated a highly linear relationship existed between log(reaction rate) and Hammett σ values (r=0.9994)[18]. The compounds studied varied in oxidation rate over a range of 30,000-fold and the linearity of the data suggested that a common mechanism operated over the entire reactivity gamut. The present study was designed to examine the relative importance of the initial electron loss and the subsequent proton loss in determining the rate of ferricyanide-mediated oxidations of dihydronicotinamides.

Materials and Methods

All compounds prepared were subjected to microcombustion analysis (Atlantic Microlabs, Atlanta, GA.), melting point determinations (uncorrected, Thomas-Hoover Melting Point Apparatus), nuclear magnetic resonance spectrometry (Varian EM 360 Spectrometer) and infra-red spectrophotometry (Beckman 620 MX

Spectrophotometer). For kinetic studies, a Hewlett Packard 8451A diode array spectrophotometer was used in conjunction with an HP85 microprocessor. High performance liquid chromatography (HPLC) was performed on a system consisting of a Perkin-Elmer Series 4 pump, a Kratos 757 variable wavelength detector, a Perkin-Elmer ISS-100 autosampler and a Perkin-Elmer LC1-100 integrator. All chemicals used in this study were of reagent or spectroscopic grade and were obtained from Aldrich Chemical Company.

1-(4-Substituted phenyl)-1,4-dihydropyridines were prepared by reacting the appropriate aniline with 1-(2,4-dinitrophenyl)nicotinamide chloride[19,20], a so-called Zincke salt. The resulting 1-phenyl derivative was then reduced in aqueous basic sodium dithionite to give the appropriate reduced pyridine derivative as previously described. For p-substituted anilines which contained highly electron withdrawing groups such as nitro or trifluoromethyl, the addition of a base catalyst was required to ensure pyridinium exchange[21].

In generating the 4-deuterio and 4,4-dideuterio derivative of 1-phenyl-1,4-dihydronicotinamide, 1-phenylnicotinamide chloride (19.6 mmol) was dissolved in 170 mL of degassed D_2O[22]. Sodium carbonate (43.4 mmol), which had been dried at 200°C in vacuo for 24 hours, and sodium dithionite (58.2 mmol) were then added to the stirring solution at room temperature under nitrogen. After stirring for 3 hours at 35°C, the yellow precipitate which formed was removed by filtration and washed with cold basic ($NaHCO_3$, 1%) D_2O. The monodeuterio compound was then dried in vacuo overnight. The yield was 71% (2.83 g).

The dideuterio product was obtained by oxidation and reduction of the above product. This was repeated four times to yield the pure 4,4-dideuterio analog. This was accomplished by dissolving 9.2 mmol of the monodeuterio product in 15 mL of dimethyl formamide and 9.6 mmol of chloranil (tetrachloro-1,4-benzoquinone). The reaction mixture was stirred for 5 min and then quenched with 50 mL of 1.0 N HCl. The aqueous phase was washed with ethyl acetate, decolorized with charcoal and evaporated to dryness under reduced pressure. The crude material was recrystallized from methanol to give 1.5 g of product (71% yield). This material was again reduced in aqueous sodium dithionite and the cycle repeated.

Kinetic analysis for the ferricyanide-mediated oxidation of the 1-(4-substituted phenyl)-1,4-dihydronicotinamides was performed according to two previously published methods. In the first,[13,14] the rate of disappearance of the 360 nm absorbance of the dihydropyridine was determined in buffered 20% aqueous acetonitrile solutions[0.1 mM $K_4Fe(CN)_6$, 60 mM KCl and 1.0 mM K_2CO_3] containing various concentrations of $K_3Fe(CN)_6$ (1-50 mM). All determinations were made at 37°C at constant ionic strength. The dihydropyridine in acetonitrile was added to the test solution using a Hamilton syringe. The solutions were contained in anerobic screw-top cuvettes (Spectrocell, Inc.) fitted with a teflon septa. For a given ferricyanide concentration, the pseudofirst order rate constant was determined and then this value was correlated with the ferricyanide ion concentration to yield a slope from which the second order rate constant could be derived. In the second method[16], ferricyanide ion concentration was maintained while ferrocyanide ion concentration was varied. Specifically, the pseudofirst order rates of oxidation for the dihydronicotinamides were determined in an 20% aqueous acetonitrile buffer [4 mM $K_3Fe(CN)_6$, 60 mM KCl and 1.0 mM K_2CO_3] containing various concentrations of $K_4Fe(CN)_6$ (10-100 mM). The reciprocal of the k_{obs} values obtained were then plotted as a function of the reciprocal of ferrocyanide ion concentration. The intercept (see equation 2) then gave the second-order rate constant.

For kinetic runs which were characterized by long half-lives, HPLC was used to monitor the reaction. In the case of the p-nitro and p-trifluoromethyl derivatives, the reaction mixtures were injected at various times post-dihydronicotinamide addition onto a Spherisorb C-18 Alltech Associates/Applied Science

4.6 mm i.d. x 25 cm reversed phase analytical column. The mobile phase consisted of 70:30 acetonitrile: H_2O, the flow rate was I mL/min and the column operated at ambient temperatures. The 1-(4-nitrophenyl)-1,4-dihydronicotinamide eluted at 7.8 min while 1-(4-trifluoromethylphenyl)-1,4-dihydronicotinamide eluted at 7.0 min. All components were detected at 360 nm. The change in the log(peak height) with time generated the pseudo-first order rate constants which were then manipulated accordingly.

For AM1 semi-empirical calculations[23, 24], an IBM 3084 Model K dual processor computer operating at 15 MIPS was used. The structural input was generated using the SYBYL/MOPAC interface and geometries found by minimizing the total molecular energy with respect to all structural descriptors using the standard Davidon-Fletcher-Powell optimization procedure. Vertical ionization potentials were estimated using Koopman's theorem.

Results and Discussion

In the present study, the rate constant (k_1) for ferricyanide-mediated oxidation of dihydronicotinamides was calculated according to the assumptions made in either equation (1) or (2). Experimentally, in assuming that the initial electron loss was rate determining, ferrocyanide concentrations were maintained in the reaction media and ferricyanide levels altered. Second order rate constants were obtained from a plot of ferricyanide ion concentrations and pseudo-first order rate constants for dihydronicotinamide oxidation. If the reaction is assumed to proceed as described by equation (2), ferricyanide levels were maintained while ferrocyanide ion concentrations were varied. In the ferrocyanide inhibition studies, second order rate constants were derived from the y-intercept of a plot of the reciprocal of the ferrocyanide ion concentrations and the reciprocal of the observed rate constants.

Figure 1 gives the Hammett relationships for log k_1 derived from either equation (1) ($k_1(1)$) or equation (2) ($k_1(2)$). Both correlations are highly linear ($r>0.999$). The reaction constants (ρ) are, however, different. The rate constant for $k_1(2)$ was found to be steeper ($\rho=-3.63$) than that derived from $k_1(1)$ ($\rho=-2.77$). The greater dependence of the rate on substitution for $k_1(2)$ is consistent with the fact that this constant represents only the pure electron loss component of the rate constant while $k_1(1)$ is a composite constant which includes other processes. At sigma values close to zero, however, the values of $k_1(1)$ and $k_1(2)$ are similar. Deviations occur at low values of sigma where $k_1(1)$ underestimates $k_1(2)$ and at large sigma values where the circumstance is reversed. It is important to note, however, that $k_1(1)$ approximates $k_1(2)$ relatively well. The Hammett data are consistent with an initial electron loss in that the relatively large negative reaction constant indicates that significant positive charge is accumulating in the transition state[18, 25]. Electron withdrawal slows the rate of reaction by decreasing the electron availability. To more closely evaluate the importance of the electron removal and the reaction rate, several theoretical molecular orbital (MO) studies were performed. The energy required to remove an electron from the highest occupied molecular orbital (HOMO) of various 1-(4-substituted phenyl)-1,4-dihydronicotinamides was estimated using the AM1 semi-empirical all valence electron MO approximation[23, 24]. Correlation of the resulting vertical ionization potentials with either $k_1(1)$ or $k_1(2)$ is given in Figure 2. As shown, a linear segment is present in both plots for substituents ranging from p-nitro to p-methoxy ($r=0.981$ and 0.991, respectively). This significant correlation suggests that the initial electron loss is important in determining the reaction rate. The measured vertical ionization potentials for the dimethylamino substituent were larger than expected in both the $k_1(1)$ and $k_1(2)$ cases. This observation may relate to the basicity of the dimethylamino group in that

partial protonation of this molety would prevent electron donation to the π-system. This would, in essence, decrease the reactivity of the substituent. This was not accounted for by the AM1 method. Nonetheless, the highly significant correlation between nonionizable substituents and vertical ionization potentials strongly argues that the initial electron removal is primarily responsible for determining the reaction rate.

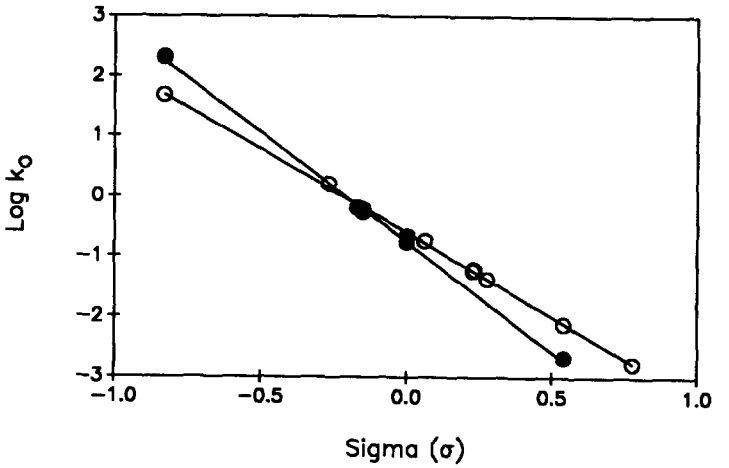


Figure 1. Hammett plots for the rates of ferricyanide-mediated oxidation of 1-(4-substituted phenyl)-1,4-dihydronicotinamides assuming either an initial rate determining electron loss ($k_1(1)(\bullet)$) or a rapid pre-equilibrium step followed by a rate determining proton loss ($k_1(2)(o)$).

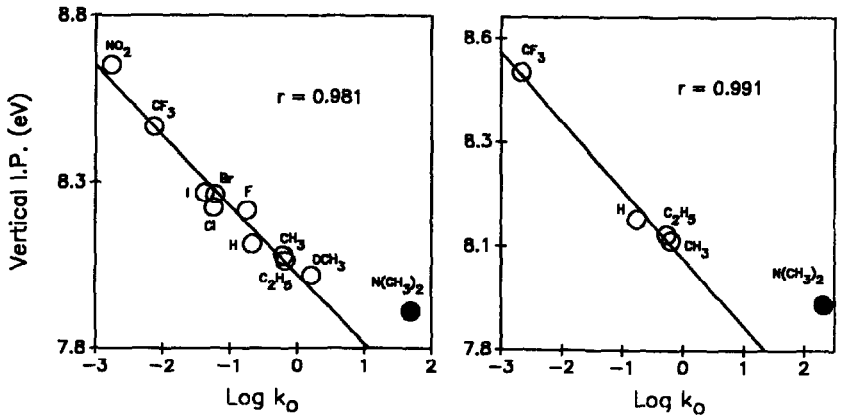
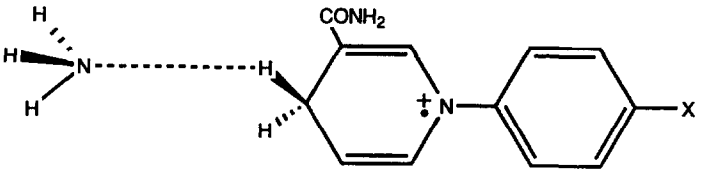


Figure 2. Correlation between vertical ionization potentials and $k_1(1)$ (left panel) or $k_1(2)$ (right panel). Vertical ionization potentials were estimated using the AM1 molecular orbital approximation.

The next step to be considered in the reaction is the removal of a proton from the 1-phenyl-1,4-dihydronicotinamide radical cation. In studying this process, a model system was constructed in which

ammonia was used as the proton acceptor. In the system, the ammonia molecule was placed at some distance from the C-4 sp^3 carbon bearing hydrogen with the appropriate geometry to remove the pro-H_R hydrogen. The starting intermolecular separation (10 Å) was slowly decreased until a separation (N-H) of 1.0 Å was achieved (see below).



At each distance increment (0.1 Å) the heat of formation (ΔH_f) of supermolecule was optimized with respect to all geometric variables. Figure 3 gives the effect of the ammoniacal N--pro-H_R hydrogen bond length on the energy of the supermolecule as well as on the C(4)-H_R bond length. As ammonia approaches the C(4)-H_R atom, there is little change in either the system energy (ΔH_f) or the C(4)-H_R bond length. At 3.4-3.3 Å separation, the energy of the system rapidly drops and the C(4)-H_R bond length concomitantly lengthens indicating the reaction is substantially complete. No energy barrier was associated with this process. When different substituents (NH_2, F, Cl, Br and I) were attached to the 4-position of the pendant phenyl group, similar energy and bond length profile were generated. Comparison of the energies of isolated ammonia/radical cation species (at 10 Å) and the complex at its lowest energy are collected in Table I. As expected, there is only a slight effect of substitution on the $\Delta\Delta H_f$'s.

Table I. AM1-generated heats of formation (ΔH_f, kcal/mol) for various 1-(4-substituted phenyl)-1,4-dihydronicotinamide radical cation-ammonia complex at 10 Å and at 3.3 Å separation and the corresponding differences in ΔH_f ($\Delta\Delta H_f$).

	H_3N-HC (sp^3) separation		
Substituent	10 Å	3.3 Å	$\Delta\Delta H_f$
NH_2	185.1	160.5	24.6
H	188.7	164.5	24.2
F	146.1	121.1	24.9
Cl	184.4	159.1	25.3
Br	197.0	171.4	25.6
I	208.3	182.7	25.6

The kinetic isotope effects were also measured for 1-phenyl-1,4-dihydronicotinamide using either $k_1(1)$ or $k_1(2)$ paradigms and using both the 4-deuterio and 4,4-dideuterio analogs. As shown in Table II, there is a small isotope effect in each case. In all cases the secondary isotope effects are close to one. The primary isotope

effect (k_H/k_D) is slightly greater in the case of $k_1(1)$ (1.83) compared to $k_1(2)$ (1.27) consistent with the more defined function of the latter constant. The primary isotope effects obtained, however, are modest and lower than those reported for reactions involving hydrogen transfer[28].

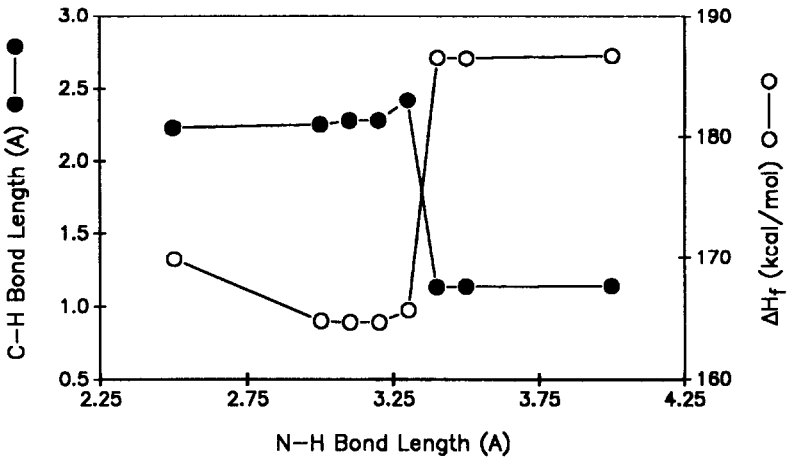


Figure 3. Supermolecular energies and C-H bond lengths as a function of the proximity (in angstroms) of the ammoniacal nitrogen to the pro-H_R hydrogen. System energies and geometries were estimated using the AM1 molecular orbital approach.

Table II. Ferricyanide-mediated oxidation of 1-phenyl-1,4-dihydronicotinamide (4,4-H,H), 1-phenyl-1,4-dihydro-4-deuteronicotinamide (4,4-H,D) and 1-phenyl-1,4-dihydro-4,4-dideuteronicotinamide (4,4-D,D) assuming an initial rate-determining electron loss ($k_1(1)$) or a rapid pre-equilibrium followed by a rate-determining proton loss ($k_1(2)$). All determinations were made at 37°C.

	Rate constant($M^{-1}s^{-1}$)	
Compound	$k_1(1)$	$k_1(2)$
4,4-H,H	0.22	0.176
4,4-H,D	0.157	0.156
4,4-D,D	0.120	0.139
4,4-H,H / 4,4-D,D	1.83	1.27
4,4-H,H / 4,4-H,D	1.40	1.13

The data collected here and elsewhere point to the importance of the initial electron loss in determining the rate of ferricyanide-mediated oxidation of dihydropyridines. These data include: 1) the strong correlation between log (reaction rate) and sigma values which suggest a single mechanism is operating over the entire

range of reactivities, 2) the magnitude of the negative slope obtained by Hammett analysis of the data which is consistent with the development of considerable positive charge in the transition state, 3) the highly significant correlation between log (reaction rate) and vertical ionization potentials, 4) the less important dependence of the reaction rate on the energetics of dihydropyridine radical cation deprotonation, 5) the small deuterium isotope effect and 6) the correlation observed between the rates of ferricyanide-mediated and hydride-mediated oxidation of dihydropyridines and related compounds[26]. These observations do not favor, in an absolute sense, either the reaction described by equation (1) or (2). In fact, the more complicated reaction equation (2) best explains the accumulated experimental data. The events described by equation (2) allow for the initial electron loss to influence the rate by controlling the steady state concentration of the radical cation. In addition, a strong base such as carbonate should make deprotonation rapid. The influence of the electron ionization is apparently strong and is the preeminent factor effecting the reaction rate.

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