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ENERGETICS OF THE ONE-ELECTRON STEPS IN THE $NAD^+/NADH$ REDOX COUPLE

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Summary

The one-electron reduction potential of NAD^+ has been determined using pulse radiolysis to study electron-transfer equilibria between it and a low potential bipyridylium compound. The determined value E^1_7 ($NAD^+/NAD^•$) = −922 ± 8 mV (NHE scale) is used to calculate E^2_7 ($NAD^•/NADH$) = +282 mV. E^1_7 for 1-methylnicotinamide, E^1_7 ($MeN^+/MeN^•$) = −918 ± 7 mV.

Redox couples involving an oxidant S and a reductant SH_2 are of major importance in electron transport sequence such as oxidative phosphorylation. The involvement of intermediate free radicals, e.g. semiquinones, in the reduction of S to SH_2 have long been known [1], but it is only recently that the energetics and kinetics of the reactions of some such intermediates have been characterized in detail [2]. The pulse radiolysis method has been used to generate semiquinones (by one-electron reduction of quinones) and measurements of their electron-transfer equilibria [3] were used to calculate the potential of $O_2/O_2^{\bar{\cdot}}$ couple [4, 5].

The $NAD^+/NADH$ couple is an important component of many biochemical electron transport chains, but although the one-electron intermediate ($NAD^•$) was characterized some years ago [6, 7] its existence in biochemical reactions is unknown. We now report measurements of the energetics of the one-electron reduction of $NAD^+/NAD^•$ which are of importance in assessing the possible role of $NAD^•$.

Abbreviations: NAD^+, nicotinamide adenine dinucleotide; MeN^+, 1-methylnicotinamide; V^{2+}, 1,1′-butano-4,4′-dimethyl-2,2′-bipyridylium; AM-1, 1-(2-hydroxy-3-methoxypropyl)-2-methyl-4-nitroimidazole; NHE, normal hydrogen electrode.

Nicotinamide adenine dinucleotide (NAD^+) 98% and 1-methylnicotinamide chloride were obtained from Sigma Chemical Company and used as supplied. 1,1′-Butano-4,4′-dimethyl-2,2′-bipyridylium dibromide (V^{2+}) was prepared from 4,4′-dimethyl-2,2′-bipyridyl by the method described by Homer and Tomlinson [8].

1-(2-Hydroxy-3-methoxypropyl)-2-methyl-4-nitroimidazole (AM-1) was donated by Dr. A. Michalowski, Instityut Onkologii, Warsaw, Poland and 5-guanidino-1-methyl-4-nitroimidazole (NSC 43805) was donated by Professor G.E. Adams, Institute of Cancer Research, Sutton, Surrey.

The pulse radiolysis method was used to generate $NAD^{\bullet}$ and measure the position of its electron-transfer equilibrium (Reaction 1) with the redox indicator, V^{2+}.

$$NAD^{\bullet} + V^{2+} \rightleftharpoons NAD^{+} + V^{\overset{+}{\bullet}} \qquad (1)$$

Pulse radiolysis was first used to measure the equilibrium constants of free-radical electron-transfer reactions by Dorfman [9] and the general applicability of the method has been recently reviewed [10]. V^{2+} was chosen as other well known viologens such as methylviologen are of too high a reduction potential such that equilibrium (1) was too far to the right for measurement.

Pulse radiolysis of a deaerated aqueous solution containing 0.8 M isopropanol, 2 mM NAD^+ and 2 mM phosphate buffer, pH 7 results in the production of $NAD^{\bullet}$ which is the only radical present ~1 [illegible] after the radiation pulse (0.2 μs duration, 1.5 Gy dose). NAD^+ is reduced by e^-_{aq} to give $NAD^{\bullet}$; $^{\bullet}OH$ and $H^{\bullet}$ are scavenged by the alcohol and the resulting radical also gives rise to $NAD^{\bullet}$ [6, 7].

In the presence of low concentrations of the viologen redox indicator $[NAD^+]/[V^{2+}] = 100$—4000) there will be negligible initial reduction of V^{2+} to $V^{\overset{+}{\bullet}}$ by the radiation-produced free radicals during the first few microseconds, but if Reaction 1 occurs there will be a subsequent grow-in of the absorption spectrum of $V^{\overset{+}{\bullet}}$. The equilibrium yield of this absorption will depend on the concentration of NAD^+ and V^{2+} and upon the equilibrium constant K_1.

Oscillogram 1a shows the radical $V^{\overset{+}{\bullet}}$ obtained by pulse radiolysis of a deaerated solution containing only isopropanol and the viologen. This represents the absorption if all the radiation-produced radicals were converted to $V^{\overset{+}{\bullet}}$. Observations at several wavelengths between 300 nm and 900 nm gave the absorption spectrum characteristic of such a radical [11]. Oscillogram 1b shows the build-up of absorption after the radiation pulse of a solution containing 0.8 M isopropanol, 5.2 mM NAD^+ and 13.7 μM V^{2+}. An equilibrium absorption is observed ~50 μs after the pulse. In the presence of a lower concentration of V^{2+} (e.g. 4.6 μM) the approach to equilibrium is slower (oscillogram 1c) and the equilibrium absorption is lower as expected.

The difference in one-electron reduction potential ΔE between two redox compounds is given by

$$\Delta E = (RT/nF)\ln(K_1 \cdot f_r) = 59\,(\log K_1 + \log f_r) \text{ at } T = 295 \pm 2\text{ K} \qquad (2)$$

where K_1 is the equilibrium constant and f_r is the activity coefficient ratio.

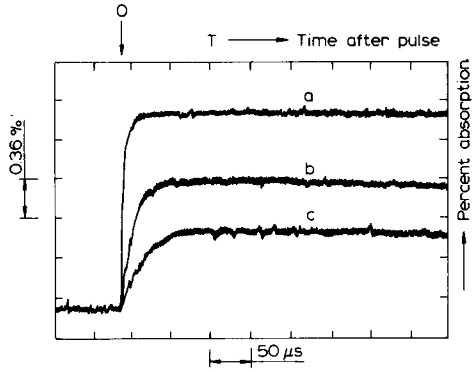

Fig.1. Oscillograms showing the build-up of absorption at 550 nm following pulse radiolysis of deaerated solutions containing 0.8 M isopropanol and (a) 0.25 mM V^{2+}; (b) 13.7 μM V^{2+} and 5.2 mM NAD^+; (c) 4.6 μM V^{2+} and 5.2 mM NAD^+.

The activity of individual ions cannot be determined, but the mean activity coefficient, $f^{\pm}$ of ions of charge z_1 and z_2 in a fully-dissociated aqueous electrolyte can be estimated using the Debye-Hückel expression [12]

$$\log f^{\pm} = -0.507|z_1z_2|I^{1/2}(1 + 3.28\, a_i I^{1/2})^{-1} \tag{3}$$

where I is the ionic strength and a_i is the ion size parameter (taken as 0.5 nm).

Following a suggestion by Drs. Land and Swallow to use the kinetic salt effect to determine the apparent charge on the $NAD^{\cdot}$ radical to use in calculating ionic strength effects on K_1, we measured the rate of formation of the $V^{\dot{+}}$ absorption in solutions containing 1 mM NAD^+, 25 μM V^{2+}, 0.2 M isopropanol and 0—0.113 M sodium perchlorate (7 solutions, $I = 0.003$ to 0.115). The rate decreased 2-fold as I increased over this range; a plot of $\log_{10}k_1$ vs. $[I^{1/2}/(1 + I^{1/2}) - 0.2I]$ gave an initial slope of −2. This indicates an apparent charge of −1 on the $NAD^{\cdot}$ species should be used for ionic strength correction using Equations 2 and 3 in this system.

For $I \leqslant 0.13$ we estimate from 9 solutions of $[NAD^+]/[V^{2+}]$ ranging from 96 to 3846 $K_1 = 848 \pm 123$ and $\log f_r \leqslant 0.297$ (Table I). Hence ΔE $(E^1_7(V^{2+}/V^{\dot{+}}) - E^1_7(NAD^+/NAD^{\cdot})) = 187 \pm 4$ mV. Since $E^1_7(V^{2+}/V^{\dot{+}}) = -735$ ± 5 mV (see below) $E^1_7(NAD^+/NAD^{\cdot}) = -922 \pm 8$ mV.

Similar experiments were carried out using 1-methylnicotinamide. For $I \leqslant 0.018$ we estimate from 4 solutions of $[MeN^+]/[V^{2+}]$ ranging from 188 to 528 $K_1 = 1007 \pm 136$ and $\log f_r \leqslant 0.112$ (Table I). Hence $\Delta E(E^1_7(V^{2+}/V^{\dot{+}})$ · $E^1_7(MeN^+/MeN^{\cdot}) = 183 \pm 4$ mV and $E^1_7(MeN^+/MeN^{\cdot}) = -918 \pm 7$ mV.

The value may be compared with a reported polarographic half-wave potential in phosphate buffer for the one-electron reduction of NAD^+ of −0.69V when corrected to the NHE scale [13]. The very rapid (diffusion-controlled) bimolecular disappearance of $NAD^{\cdot}$ in aqueous solution presents a major problem in the achievement of thermodynamic reversibility under polarography conditions, but does not interfere with the present measurements because the position of equilibrium is determined before this natural radical decay can occur.

TABLE I

$[V^{2+}]$ (μmol·l^{-1})	$[NAD^+]$ (mmol·l^{-1})	$[MeN^+]$ (mmol·l^{-1})	K_1*	ΔE**
4.6	5.2		826	186
9.1	5.2		772	184
9.1	10.3		1004	194
13.7	5.2		904	188
26.0	5.0		664	180
26.0	10.0		686	184
46.8	9.0		798	188
52.0	5.0		971	190
52.0	6.0		1003	192
26.5		7.5	1062	184
26.5		14.0	1204	189
53.0		10.0	892	180
53.0		15.0	869	180

*observed equilibrium constant, when corrected for depletion of solute; all solutions contained 1 mmol·l^{-1} phosphate (pH 7) and 0.8 mol·l^{-1} isopropanol.

**ΔE is corrected for ionic strength effects.

The reduction potential of V^{2+} was determined by similar measurement of electron-transfer equilibria between V^{2+} and the two 4-nitroimidazole compounds. The potentials of NSC 43805, −602 ± 5 mV (O'Neill, P., personal communication) and AM-1, −583 ± 4 mV were both determined using 1,1′-propano-2,2′-bipyridylium dibromide, (−548 ± 3 mV [14]), as the reference compound. Establishing these reference potentials in this step-wise manner was necessary because of the exceptionally low potential of the $NAD^+/NAD^{\bullet}$ couple.

The two-electron potential E'_0 $(NAD^+/NADH) = -320$ mV [15] and using the relationship $E'_0 = (E^1_7 + E^2_7)/2$ we find $E^2_7(NAD^{\bullet}/NADH) = +282$ mV.

NADH can only act as a one-electron donor in reducing substrate S if the free energy change derived from the $E^2_7(NAD^{\bullet}/NADH)$ couple is less positive than that from the $E^1_7(S/S^{\bar{\bullet}}(SH^{\bullet}))$ couple.

We now see that $E^2_7(NAD^{\bullet}/NADH) >> E^1_7(O_2/O_2^-)$ (−155 mV, 1 mol O_2 [4, 5]) in contrast to flavins where $E^2_7(FH^{\bullet}/FH_2)$ (−150 mV [11, 16]) $\sim E^1_7$ (O_2/O_2^-). If the reaction of reduced flavins or NADH with molecular oxygen proceeds via a rate-determining step of the form

$$FH_2\,(NADH) + O_2 \rightarrow FH^{\bullet}(NAD^{\bullet}) + O_2^-$$

then the rate of NADH oxidation would be markedly slower than that of FH_2, in agreement with the known stabilities of NADH and FH_2 in oxygenated solutions.

NADH may still reduce substrates of potentials $E^1_7(S/S^{\bar{\bullet}}(SH)) > E^2_7$ $(NAD^{\bullet}/NADH)$ by a two-electron process (H^- transfer) to give SH_2 if $E'_0(S/SH_2) > E'_0$ (NAD/NADH).

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