Biochimica et Biophysica Acta, 590 (1980) 273—276

BBA Report

BBA 41325

THE ONE-ELECTRON REDUCTION POTENTIALS OF NAD

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(Received October 12th, 1979)

Key words: Bipyridylium; Reduction potential; One-electron reduction potential; NAD; 1-Methylisonicotinamide; Pulse radiolysis

Summary

The one-electron reduction potential (E_7^1) of NAD^+ has been determined by pulse radiolysis to be −0.94 V. E_7^2 (E_7^1 for the free radical, $NAD^\cdot$) is +0.30 V. E_7^1 for 1-methylisonicotinamide is −0.77 V.

Full understanding of the oxidation-reduction reactions of NAD requires knowledge of the potentials for the two one-electron steps, but although there are values in the literature [1, 2] these are subject to uncertainties. Pulse radiolysis can be used to make unambiguous measurements of relative one-electron reduction potentials [3] and so by using suitable reference compounds can give absolute values. E_7^1 and hence E_7^2 for NAD^+ have now been determined by this method.

1,1′-Trimethylene-2,2′-bipyridylium (TRIQ) and 1,1′-tetramethylene-2,2′-bipyridylium (TETQ) [4] as dibromide were dried at 100° C for 2 h before use. 1-Methylisonicotinamide (MeN) as perchlorate had been kindly provided by Professor E.M. Kosower. NAD^+ was Sigma Grade III. Sodium formate was from BDH.

The pulse radiolysis equipment and method of use were essentially as before [5]. Solutions were deoxygenated by bubbling with oxygen-free nitrogen (from British Oxygen Company). Pulse doses were such as to produce approx. 10^{-6}M free radicals, and were measured at the beginning and end of each run using 10^{-4}M paraquat dichloride (methyl viologen) in 10^{-1} M sodium formate, assuming an extinction coefficient for the radical of $1.35 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ at 606 nm.

Abbreviations: MeN, 1-methylisonicotinamide; NHE, normal hydrogen electrode.

To measure the difference in reduction potentials, ΔE, between a pair of compounds, an approximate value was first estimated from literature values for related compounds. Solutions were then prepared containing the more-difficult-to-reduce compound at about $10^{\Delta E/0.059}$ times the concentration of the other compound. Sodium formate was present to replace the H and OH radicals produced by the pulse by CO_2^-, which like e^-_{aq}, reduces all the compounds. The concentration of formate was 10^{-1}M except in the experiments with NAD itself, where the high NAD^+ concentration demanded 1 M formate to ensure that H and OH would not attack the NAD^+.

Pulses were delivered to the solutions and the maximum change in absorbance recorded at wavelengths where, from the measurements on solutions containing the single pyridinium compounds (10^{-4}M) the extinction coefficients of the two radicals differed the most. This maximum change was attained in two parts, a rapid change due to reaction of e^-_{aq} and CO_2^- with the compound in excess, and a slower change (half-life $\sim$20 μs when the minor component was 10^{-5}M) due to equilibration between the free radical forms of the two compounds. In solutions containing NAD there was a very slow change following equilibration due to combination or disproportionation of the radicals present. A typical trace is shown in Fig.1. The equilibrium concentrations R_1 and R_2 of the two radicals were calculated from oscilloscope traces obtained at different wavelengths, effects of combination or disproportionation being eliminated by extrapolating the very slow changes to time zero. The difference between the observed absorbance, A_{obs}, for the mixture and that calculated from the measured extinction coefficients of the two radicals separately is $A_{obs} - (R_1\epsilon_1 + R_2\epsilon_2)$, where R_1 and R_2 are the concentrations of the two radicals and ϵ_1 and ϵ_2 their respective extinction coefficients. It was assumed that $R_1 + R_2$ was constant and equal to the total radical concentration, R_0, produced by the pulse, as determined by dosimetry. This expression for the difference therefore becomes $A_{obs} - (R_1\epsilon_1 + (R_0 - R_1)\epsilon_2)$. R_1 was calculated by setting to zero the differential with respect to R_1 of the sum of the squares of these differences taken over all the wavelengths used.

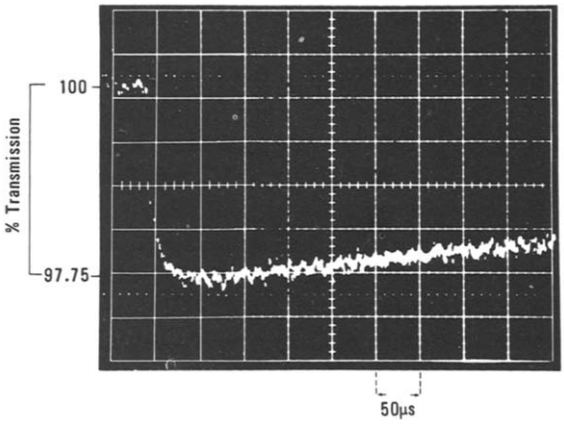

Fig.1. Oscilloscope trace showing formation of $NAD^\cdot$ followed by equilibration $NAD^\cdot + MeN^+ \rightleftharpoons NAD^+ + MeN^\cdot$, and very slow dimerisation. Wavelength 390 nm. The solution contained 10^{-2} M NAD, $2 \cdot 10^{-5}$ M MeN and 10^{-1} M HCOONa. Pulse dose 1 Gy, path length 2.5 cm.

Each difference was weighted inversely as the amount of noise in the traces used to compute it. The observed equilibrium constant, K_{obs}, is equal to $R_1(C_2-R_2)/R_2(C_1-R_1)$ where C_1 and C_2 are the initial concentrations of the two compounds. Values of K_{obs} are shown in Table I. It can be seen that for a given mixture K_{obs} is, within the experimental error, independent of the concentrations used, the error being largest for TRIQ/TETQ, where the radical spectra do not differ greatly.

For equilibria where the forward and reverse reactions are symmetrical with respect to the charges on the reacting species, the equilibrium constant at zero ionic strength, K_{corr}, is equal to K_{obs}. The equilibrium constant involving TETQ and MeN was corrected by using the Brønsted-Debye equation [6] taking the closest-approach parameter to be 2. It is less easy to correct the equilibrium constant involving MeN and NAD because of the high ionic strength of the solution and uncertainty in the appropriate charge for the NAD radical. An estimate was therefore made by calculating the value that K_{corr} would have if the Brønsted-Debye equation still applied at 1 M, the effective charge on the NAD radical being −1 (see accompanying paper by Dr. R.F. Anderson [13]). Values of K_{corr} together with ΔE, equal to $0.059 \log_{10} K_{corr}$ are given in Table I.

The value for ΔE for TRIQ/TETQ (Table I) agrees with published data [4, 7, 8]. The value of ΔE for MeN/NAD is consistent with movement of the weakly electron withdrawing substituent $CONH_2$ from the 4- to the 3-position since differences of 0.11 and 0.16 V have been measured between the half-wave potentials of 3- and 4-isomers of cyano- and ethoxycarbonyl-substituted 1-methylpyridinium salts respectively [9]. Taking a mean value of −0.64 V for TETQ, the values of E_7^1 for MeN^+ and NAD^+ become −0.77 and −0.94 V respectively (vs. NHE). If no Brønsted-Debye correction for MeN/NAD were attempted, E_7^1 for NAD^+ would be −0.92 V, not greatly different from −0.94 V.

Previous measurements of the one electron reduction potential of NAD^+ [1, 2] have been made by electrochemical methods. Values in the range of −0.67 to −0.88 V (vs. NHE) have been obtained but interpretation is complicated by dimerisation of $NAD^\cdot$. Using measured rate constants for this dimerisation, E_7^1 has been estimated as −0.82 V [10]. The present value is significantly different from this, but is more reliable since both the redox equilibrium and the absence of significant dimerisation of $NAD^\cdot$ can be verified. Moreover, there are no complications due to electron transfer at a solid surface where stereochemical fit, adsorption and hydrogen evolution may cause difficulties. Using the accepted value of $E_7^m = -0.32$ V [11] for the

TABLE I

Mixture	Concentrations (M)	Wavelengths (nm)	K_{obs}	K_{corr}	ΔE (V)
TRIQ/TETQ	$4\cdot10^{-5}/6\cdot10^{-4}$	370, 380, 570	47.7	47.7	−0.099
	$2\cdot10^{-5}/8\cdot10^{-4}$	370, 380, 570	67.8	67.8	−0.108
TETQ/MeN	$2\cdot10^{-5}/1\cdot10^{-3}$	360, 370, 410	104	164	−0.131
	$1\cdot10^{-5}/1\cdot10^{-3}$	360, 370, 410	82.2	130	−0.125
MeN/NAD	$1\cdot10^{-5}/1\cdot10^{-2}$	390, 400, 410	362	793	−0.171
	$2\cdot10^{-5}/1\cdot10^{-2}$	390, 400, 410	407	891	−0.174

two-electron reduction potential of NAD^+ at pH 7, the calculated value for E_7^2 becomes +0.30 V.

If NADH is to act as a one-electron donor [12] in reducing a substrate S, the free energy change will only be favourable if E for $NAD^\cdot/NADH$ is less positive than for E for $S/S^{\cdot-}$. NADH cannot therefore reduce S by a one-electron process if the one-electron reduction potential of S is appreciably less than +0.30 V. This rules out, in aqueous solution, one-electron reductions by NADH of flavins, quinones (except those with strongly electron withdrawing substituents), bipyridylium salts and many metal co-ordination compounds. If NADH reduces these compounds directly it must be by a two-electron process (e.g. H^- transfer) which is allowed on thermodynamic grounds if E_7^m of S is not more negative than −0.32 V. Even for compounds whose E_7^1 is more positive than +0.30 V, two-electron reduction is still possible if E_7^m is not more negative than −0.32 V.

In enzyme reactions involving NAD, other factors must affect the relative importance of one- and two-electron reductions. First the local pH may be very different from 7. While this would not affect the reduction potentials of NAD^+ itself, raising the pH will cause E^2 to become more negative, so increasing the chance of one-electron reduction. Second the polarity of the molecular environment of the NAD may be very different from that of water. A less polar environment for the pyridine ring might destabilise both NADH and $NAD^\cdot$ less than NAD^+, so making E^1 less negative and E^m more positive. This will enhance both one- and two-electron oxidations by NAD^+. The effect on E^2 will be small. Thus the likelihood of one-electron reductions will be increased in comparison with two-electron reduction. Third the steric requirements for molecular interaction to allow electron transfer will be less demanding than those for hydride ion transfer. Thus, even where one-electron reactions are unlikely in free aqueous solution, it is still not possible to rule out one-electron transfer as a mechanism for the action of NAD in enzyme systems.

This work was supported in part by grants from the Cancer Research Campaign and the Medical Research Council.

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