The Enthalpy Change for the Reduction of Nicotinamide—Adenine Dinucleotide

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The heat of the reaction $\text{NAD}^+ + \text{propan-2-ol} = \text{NADH} + \text{acetone} + \text{H}^+$ was determined to be $42.5 \pm 0.6 \text{ kJ/mol}$ ($10.17 \pm 0.15 \text{ kcal/mol}$) from equilibrium measurements at $9\text{--}42^\circ\text{C}$ catalysed by yeast alcohol dehydrogenase. With the aid of thermochemical data for acetone and propan-2-ol the values of $\Delta H = -29.2 \text{ kJ/mol}$ (-6.99 kcal/mol) and $\Delta G^\circ = 22.1 \text{ kJ/mol}$ (5.28 kcal/mol) are derived for the reduction of NAD ($\text{NAD}^+ + \text{H}_2 = \text{NADH} + \text{H}^+$). These values are consistent with analogous but less accurate data for the ethanol–acetaldehyde reaction. Thermodynamic data for the reduction of NAD and NADP are summarized.

Although a large body of consistent free-energy data is now available for many biochemical reactions, there is much less information about the enthalpy changes. An accurate determination of the heat of reduction of NAD^+ would be a useful datum for evaluating the heats of NAD- and NADP-linked oxidation–reduction reactions. In this paper we report the determination of the heat of the reaction

Since there are accurate thermochemical data for both acetone and propan-2-ol, a reliable value for the

enthalpy change on the reduction of NAD can be calculated. Free-energy data can also be calculated for other temperatures than 25°C .

Experimental and Results

Equilibrium measurement

Crystalline, salt-free, yeast alcohol dehydrogenase and NAD^+ were obtained from Sigma Chemical Co. Ltd., Kingston-upon-Thames, Surrey KT2 7BH, U.K. The enzyme was dissolved in water at 5 mg/ml . The NAD^+ , A.R. propan-2-ol and A.R. acetone were

Table 1. *Equilibria between propan-2-ol, NAD^+ , acetone and NADH*

$$K_{(1)} = [\text{acetone}][\text{NADH}][\text{H}^+]/[\text{propan-2-ol}][\text{NAD}^+] \text{ at } I = 0.013$$

Where more than one observed value is shown the NAD and NADH values are averages of individual determinations. Values in the last column were calculated from $K_{(1)} = 0.219147e^{-5117.13/T}$, obtained by linear regression of $\ln K_{(1)}$ against $1/T$ where T is the absolute temperature.

Temperature ($^\circ\text{C}$)	Final pH	Final concentrations				$10^9 K_{(1)} \text{ (M)}$	
		Acetone (mm)	Propan-2-ol (mm)	NADH (μM)	NAD^+ (μM)	Observed	Calculated
9.3	7.650	9.9	94.8	148	118	2.87, 2.89, 2.95	2.970
9.4	7.621	10.8	98.6	190	163	3.07	2.988
9.5	7.621	10.8	98.6	192	161	3.14	3.008
12.4	7.653	10.2	98.3	153	101	3.44, 3.68, 3.71	3.615
12.5	7.653	28.4	92.5	127	241	3.60	3.637
12.7	7.653	28.4	92.5	123	239	3.52	3.684
12.95	7.663	37.4	91.3	104	249	3.70, 3.72, 3.74	3.741
25.6	7.581	19.6	89.1	153	106	8.02, 8.07, 9.24	7.98
36.8	7.548	43.8	85.4	167	172	13.8, 14.6	14.82
37.0	7.552	34.2	89.1	163	114	13.8, 15.6, 15.8	14.98
37.2	7.547	43.8	85.4	172	171	14.7	15.13
38.2	7.546	34.2	94.8	168	109	14.9, 16.0, 16.1	15.96
40.6	7.516	37.4	91.2	209	145	17.6, 17.8, 18.7	18.10
41.7	7.565	44.0	80.2	139	107	18.9, 19.7, 19.8	19.16

each dissolved or diluted in 0.65M-NaH₂PO₄-4.35M-Na₂HPO₄. To minimize pipetting errors, a single stock mixture of propan-2-ol, buffer and NAD⁺ was prepared for each series of measurements. Each mixture contained 250 µg of enzyme in a total volume of 1.3–1.5 ml with a final concentration of 4.8 mM-phosphate, $I = 0.013$. The initial pH was 7.69 at 25°C as measured by a glass electrode and calomel half cell by using a standard of pH 7.413 (KH₂PO₄, 1.179 g/litre; Na₂HPO₄, 4.30 g/litre). Semi-micro cells were used in a temperature-controlled compartment of a Hilger-Gilford spectrophotometer. The enzyme was added last in some reaction mixtures, but in others the acetone was added several minutes after the enzyme so that the final equilibrium was reached from the side of NADH excess (Burton & Wilson, 1953). Equilibrium was reached 10–60 min after the last addition. The temperature was measured at equilibrium in each cell by a thermistor probe thermometer (Light Laboratories, Brighton, Sussex, U.K.), and the concentrations were determined as described by Burton & Wilson (1953).

The principal results are shown in Table 1. The final pH values are calculated for the known effects of temperature on the pK of phosphate (Bates & Acree, 1935), and for the pH change owing to the liberation of H⁺ ions by the reduction of NAD⁺. Since the effect of ionic strength on the pH of a buffer depends on the Debye-Hückel constant A , which is a function of temperature, a further small correction was applied. For this purpose it was considered sufficient to use the equation $pK' = pK_a - 0.3A$ at $I = 0.013$. These procedures were adopted because it was considered that calculation would introduce smaller errors than direct measurement of the final pH. The interpolated value at 25°C is $K_{(1)} = 7.7 \times 10^{-9}$ M, in excellent agreement with 7.19×10^{-9} M

which was originally reported by Burton & Wilson (1953).

The ethanol-NAD⁺-acetaldehyde equilibrium was also measured in a similar way but by using only 50 µg of enzyme and with no addition of acetaldehyde. The equilibrium constants were calculated assuming that the concentrations of acetaldehyde and NADH were identical with each other. The interpolated value of $K_{(2)}$ at 25°C is 6.92×10^{-12} M, corresponding to $\Delta G_{(2)}^0 = 63.7$ kJ/mol (15.23 kcal/mol).

Discussion

The data in Table 1 were analysed by a least-squares fit of $\ln K$ and of $1/T$ to a straight line, T being the absolute temperature. ΔH was then calculated from the slope, $[\Delta H = -R \ln K/d(1/T)]$, for which the s.d. was $\pm 1\%$. R was taken as $8.31433 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$. Hence, $\Delta H_{(1)} = 42.5 \pm 0.6$ kJ/mol (10.17 ± 0.15 kcal/mol) at 25°C including an allowance for other errors. Also, $\Delta G_{(1)}^0 = 46.5$ kJ/mol (11.11 kcal/mol; Burton & Wilson, 1953) again at 25°C.

Likewise, from the data of Table 2, $\Delta H_{(2)} = 46.9 \pm 1$ kJ/mol (11.20 ± 0.25 kcal/mol) and $\Delta G_{(2)}^0 = 63.7$ kJ/mol (15.23 kcal/mol) for the reaction:

[The subscripts (1), (2) etc. in $\Delta G_{(1)}^0$, etc. indicate that the thermodynamic property applies to the reaction of the same number.]

Relevant thermochemical data are summarized in Table 3. They were derived with the same values for atomic weights of C, H and O and for the enthalpies of formation (ΔH_f^0) of CO₂ and water as used in earlier compilations of such data (e.g. Rossini *et al.*, 1952; Burton & Krebs, 1953; Green, 1961; Stull *et al.*, 1969; Cox & Pilcher, 1970). The primary data

Table 2. *Equilibria between ethanol, NAD⁺, acetaldehyde and NADH*

$K_{(2)} = [\text{acetaldehyde}][\text{NADH}][\text{H}^+]/[\text{ethanol}][\text{NAD}^+]$ at $I = 0.013$. The calculated values are from $K_{(2)} = 0.00112438e^{-5637.22/T}$.

Temperature (°C)	Final pH	[NADH] = [acetaldehyde] (µM)	[NAD ⁺] (µM)	[ethanol] (mM)	10 ¹² K ₍₂₎ (M)	
					Observed	Calculated
10.6	7.691	82.0	239.5	194.9	2.93	2.648
10.6	7.680	96.5	225.1	324.2	2.66	2.648
10.6	7.673	107.7	213.8	454.1	2.54	2.648
17.35	7.658	91.6	229.9	194.9	4.12	4.202
17.35	7.646	109.3	212.2	324.1	3.93	4.202
20.25	7.635	116.6	196.9	324.2	4.93	5.089
34.6	7.605	119.0	202.6	129.0	13.4	12.47
34.6	7.584	147.9	173.6	258.8	12.7	12.47
34.6	7.571	162.2	154.3	389.0	12.5	12.47
42.8	7.568	133.4	188.1	128.9	20.3	20.05
42.8	7.545	165.6	155.9	258.6	19.3	20.05
42.8	7.528	186.5	135.0	388.5	19.7	20.05

Table 3. *Relevant thermochemical data for 298.15°K (25°C)*

To remain consistent with the earlier tabulations, all values in this Table are given in kcal/mol (1 cal = 4.1840 J). The data in aqueous solution follow the convention that they apply to the equilibrium mixture of solvated and non-solvated forms. The ΔH_f° and ΔG_f° values for the ideal gas and the liquid states are from Stull *et al.* (1969). Other details: (1) by interpolation from Henry's Law data of Kurz (1967); (2) Ragaini & Cavenaghi (1969); (3) Beare *et al.* (1930), Hartley (1931), Arnett *et al.* (1972); (4) Rossini *et al.* (1952), Aveyard & Lawrence (1964), Arnett *et al.* (1972); (5) Butler *et al.* (1935); (6) Dimmling & Lange (1951).

Compound	$-\Delta H_f^\circ$	$-\Delta G_f^\circ$	Ancillary data
Acetaldehyde (g)	39.76	31.86	$g \rightarrow aq., \Delta G^\circ = -1.45$ (1)
(l)	45.90		$l \rightarrow aq., \Delta H^\circ = -4.28$ (2)
(aq.)	50.18	33.31	
Acetone (g)	52.00	36.58	
(l)	59.30	37.14	$l \rightarrow aq., \Delta H^\circ = -2.37$
(aq.)	61.67	38.47	$\Delta G^\circ = -1.33$ (3)
Ethanol (g)	56.12	40.22	
(l)	66.20	41.62	$l \rightarrow aq., \Delta H^\circ = -2.49$ (4)
(aq.)	68.69	43.24	$\Delta G^\circ = -1.62$ (5)
Propan-2-ol (g)	65.15	41.49	
(l)	76.00	43.12	$l \rightarrow aq., \Delta H^\circ = -2.83$ (6)
(aq.)	78.83	44.30	$\Delta G^\circ = -1.18$ (5)

are taken from the tabulation of Stull *et al.* (1969), whose ΔH_f° values for acetone, acetaldehyde and propan-2-ol are identical with or very close to those selected by Cox & Pilcher (1970). For ethanol, however, Cox & Pilcher (1970) selected a ΔH_f° value based on the data of Rossini (1932), whereas Stull *et al.* (1969) preferred the value of Chao & Rossini (1965) partly because they considered that the earlier data might have been influenced by water impurity. From Table 3 we obtain $\Delta H_{(3)}^\circ = 71.8$ kJ/mol (17.16 kcal/mol) and $\Delta G_{(3)}^\circ = 24.4$ kJ/mol (5.83 kcal/mol) for the reaction:

Likewise, $\Delta H_{(4)}^\circ = 77.4$ kJ/mol (18.51 kcal/mol) and $\Delta G_{(4)}^\circ = 41.5$ kJ/mol (9.93 kcal/mol) for the reaction:

From eqns. (1) and (3) we obtain $\Delta H_{(5)} = -29.2$ kJ/mol (-6.99 kcal/mol) and $\Delta G_{(5)}^\circ = 22.1$ kJ/mol (5.28 kcal/mol). It is difficult to give reliable assessments of the probable errors, but it seems that the $\Delta H_{(5)}$ value should be at least accurate to ± 1.5 kJ/mol (0.4 kcal/mol) and that the ΔG° value is somewhat more reliable.

Less certain estimates can also be obtained from reactions (2) and (4). The above values give $\Delta H_{(5)} = -30.6$ kJ/mol (-7.31 kcal/mol) and $\Delta G_{(5)}^\circ = 22.2$ kJ/mol (5.30 kcal/mol), but if the ΔH_f° values for acetaldehyde and ethanol selected by Cox & Pilcher (1970) are used $\Delta H_{(5)}$ and $\Delta G_{(5)}^\circ$ become -31.6 and 21.2 kJ/mol respectively. In these calculations, $\Delta G_{(2)}^\circ$ differs slightly from that used by Burton &

Wilson (1953), based on the value of $K_{(2)} = 1.15 \times 10^{-11}$ M (Racker, 1950). A lower value is indicated by the data of Bäcklin (1958) and other data discussed by him as well as by the measurements in Table 2.

The $\Delta G_{(5)}^\circ$ value of 22.1 kJ/mol (5.28 kcal/mol) is only a minor revision of that derived by Burton & Wilson (1953) (5.22 ± 0.2 kcal/mol). The difference between the two values is still within the known limits of error, but the newer value benefits from the additional thermochemical data that are now available.

The value of -29.2 kJ/mol (-6.99 kcal/mol) for $\Delta H_{(5)}$ agrees well with unpublished calorimetric results of M. Schott & J. M. Sturtevant ($\Delta H_{(5)} = -6.56 \pm 0.6$ kcal/mol, i.e. -27.4 ± 2.5 kJ/mol) as well as with earlier calorimetric data which indicate $\Delta H_{(5)} = -6.7 \pm 1.3$ kcal/mol (Poe *et al.*, 1967). The value of -3.56 kcal/mol based on oxidation-reduction potential measurements (Rodkey, 1959) is evidently wrong and earlier values of about 7 kcal/mol for $\Delta H_{(2)}$ (Gierer, 1955; Bäcklin, 1958) must also be wrong, presumably because of errors in the correction for buffer ionization or of other systematic errors in measuring the variation of K with temperature. Insufficient experimental details were given in these papers to assess the possible sources of error.

NADP

Engel & Dalziel (1967) have measured the equilibria of the glutamate dehydrogenase reactions at different temperatures with NAD⁺ and with NADP⁺ as the nucleotide. As expected, the ΔG° and ΔH values for the reduction of NADP⁺ are close to those for the reduction of NAD⁺. The difference between the two ΔH values (0.6 kcal) is comparable with probable errors in the comparison. It is, however, in good

Table 4. *Thermodynamic data for the reduction of NAD⁺ and NADP⁺*

ΔG^0 values at temperatures above or below 25°C are calculated assuming that ΔH and ΔS^0 are constant in the temperature range.

	Temp. (°C)	NAD ⁺ (Reaction 5)		NADP ⁺ (Reaction 7)	
		(kJ/mol)	(kcal/mol)	(kJ/mol)	(kcal/mol)
ΔH	25	-29.2	-6.99	-25.3	-6.04
ΔG^0	25	+22.1	+5.28	25.1	5.99
$T \cdot \Delta S^0$	25	-51.3	-12.27	-50.3	-12.03
ΔG^0	10	19.5	4.66	22.5	5.38
ΔG^0	15	20.4	4.87	23.4	5.59
ΔG^0	20	21.2	5.07	24.2	5.79
ΔG^0	30	23.0	5.49	25.9	6.19
ΔG^0	35	23.8	5.69	26.7	6.39
ΔG^0	37	24.1	5.77	27.1	6.47
ΔG^0	40	24.7	5.90	27.6	6.60

agreement with unpublished calorimetric work of M. Schott & J. M. Sturtevant, who have made two independent estimates of $\Delta H_{(6)}$ (-0.92 ± 0.3 and -1.02 ± 0.4 kcal/mol).

Taking $\Delta H_{(6)}$ as -0.95 kcal/mol, $\Delta H_{(7)} = -6.04$ kcal/mol (-25.3 kJ/mol) for the reaction

Also, Engel & Dalziel (1967) give $\Delta G_{(6)}^0 = -0.71$ kcal/mol and so, $\Delta G_{(7)}^0 = 5.28 + 0.71 = 5.99$ kcal/mol (25.1 kJ/mol).

The values arrived at in the present paper are summarized in Table 4. In addition, $\Delta G_{(5)}^0$ and $\Delta G_{(7)}^0$ are calculated for several temperatures between 10° and 40°C by using the equation $\Delta G^0 = \Delta H - T \cdot \Delta S^0$ and assuming that ΔH and ΔS^0 are constant within the temperature range.

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