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 $9-42^\circ\text{C}$ catalysed by yeast alcohol dehydrogenase. With the aid of thermochemical data for acetone and propan-2-ol the values of $\Delta H = -29.2 \text{kJ/mol} (-6.99 \text{ kcal/mol})$ and $\Delta G^\circ = 22.1 \text{kJ/mol} (5.28 \text{ kcal/mol})$ 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

$$\text{NAD}^+ + \text{propan-2-ol} = \text{NADH} + \text{acetone} + \text{H}^+ \quad (1)$$

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 $25^\circ\text{C}$.

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

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 $K_{1(1)} = 0.219147e^{-5117.13/T}$, obtained by linear regression of ln $K_{1(1)}$ against $1/T$ where $T$ is the absolute temperature.
each dissolved or diluted in 0.65mm-NaH₂PO₄–4.35mm-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 250μg of enzyme in a total volume of 1.3–1.5ml with a final concentration of 4.8mm-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, 1.179g/litre; Na₂HPO₄, 4.30g/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 10–60min 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' = pK₀ − 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 K₁(1) = 7.7 × 10⁻⁹M, in excellent agreement with 7.19 × 10⁻⁹M which was originally reported by Burton & Wilson (1953).

The ethanol–NAD⁺-acetaldehyde equilibrium was also measured in a similar way but by using only 50μg 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 K₂(1) at 25°C is 6.92 × 10⁻¹²M, corresponding to ΔG₂(1) = 63.7kJ/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, [ΔH = −RdlnK/d(1/T)], for which the s.d. was ±1%. R was taken as 8.314433 J·mol⁻¹·K⁻¹. Hence, ΔH₁(1) = 42.5 ± 0.6kJ/mol (10.17 ± 0.15 kcal/mol) at 25°C including an allowance for other errors. Also, ΔG₁(1) = 46.5kJ/mol (11.11kcal/mol; Burton & Wilson, 1953) again at 25°C.

Likewise, from the data of Table 2, ΔH₂(1) = 46.9 ± 1kJ/mol (11.20 ± 0.25 kcal/mol) and ΔG₂(1) = 63.7kJ/mol (15.23 kcal/mol) for the reaction:

\[
\text{NAD}^+ + \text{ethanol} = \text{NADH} + \text{acetaldehyde} + \text{H}^+ \tag{2}
\]

[The subscripts (1), (2) etc. in ΔG⁽1⁾, 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 (ΔHᶠ) 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

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

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Final pH</th>
<th>[NADH] = [acetaldehyde] (μM)</th>
<th>[NAD⁺] = [ethanol] (μM)</th>
<th>10¹²K₂(1) (M)</th>
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</thead>
<tbody>
<tr>
<td>10.6</td>
<td>7.691</td>
<td>82.0</td>
<td>239.5</td>
<td>2.93</td>
</tr>
<tr>
<td>10.6</td>
<td>7.680</td>
<td>96.5</td>
<td>225.1</td>
<td>2.66</td>
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<td>10.6</td>
<td>7.673</td>
<td>107.7</td>
<td>213.8</td>
<td>2.54</td>
</tr>
<tr>
<td>17.35</td>
<td>7.658</td>
<td>91.6</td>
<td>229.9</td>
<td>4.12</td>
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<td>17.35</td>
<td>7.646</td>
<td>109.3</td>
<td>212.2</td>
<td>4.10</td>
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<tr>
<td>20.25</td>
<td>7.635</td>
<td>116.6</td>
<td>196.9</td>
<td>4.93</td>
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<td>34.6</td>
<td>7.605</td>
<td>119.0</td>
<td>202.6</td>
<td>13.4</td>
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<td>34.6</td>
<td>7.584</td>
<td>147.9</td>
<td>173.6</td>
<td>12.7</td>
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<tr>
<td>34.6</td>
<td>7.571</td>
<td>162.2</td>
<td>154.3</td>
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<td>42.8</td>
<td>7.568</td>
<td>133.4</td>
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<td>42.8</td>
<td>7.545</td>
<td>165.6</td>
<td>155.9</td>
<td>19.7</td>
</tr>
<tr>
<td>42.8</td>
<td>7.528</td>
<td>188.5</td>
<td>135.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>
HEAT OF REDUCTION OF NAD\(^+\)

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 \(\Delta H^\circ\) and \(\Delta G^\circ\) 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 & Lange (1951). (-7.31 Vol. (5.30 kcal/mol), mol

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

are taken from the tabulation of Stull et al. (1969), whose \(\Delta H^\circ\) 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 \(\Delta H^\circ\) 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 \(\Delta H^\circ\) = 71.8 kJ/mol (17.16 kcal/mol) and \(\Delta G^\circ\) = 24.4 kJ/mol (5.83 kcal/mol) for the reaction:

Propan-2-ol(aq.) + H\(_2\)(g) → aceton(eaq.) + H\(_2\)(g) (3)

Likewise, \(\Delta H^\circ\) = 77.4 kJ/mol (18.51 kcal/mol) and \(\Delta G^\circ\) = 41.5 kJ/mol (9.93 kcal/mol) for the reaction:

Ethanol(aq.) + acetaldehyde(aq.) + H\(_2\)(g) (4)

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

\[ \text{NAD}^+(\text{aq.}) + H_2(\text{g}) = \text{NADH}(\text{aq.}) + H^+(\text{aq.}) \] (5)

Less certain estimates can also be obtained from reactions (2) and (4). The above values give \(\Delta H^\circ\) = -30.6 kJ/mol (-7.31 kcal/mol) and \(\Delta G^\circ\) = 22.2 kJ/mol (5.30 kcal/mol), but if the \(\Delta H^\circ\) values for acetaldehyde and ethanol selected by Cox & Pilcher (1970) are used \(\Delta H^\circ\) and \(\Delta G^\circ\) become -31.6 and 21.2 kJ/mol respectively. In these calculations, \(\Delta G^\circ\) differs slightly from that used by Burton & Wilson (1953), based on the value of \(K_{\text{eq}} = 1.15 \times 10^{-11} \text{M} \) (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 \(\Delta G^\circ\) 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 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 \(\Delta H^\circ\) agrees well with unpublished calorimetric results of M. Schott & J. M. Sturtevant \(\Delta H^\circ\) = -6.56±0.6 kcal/mol, i.e. -27.4±2.5 kJ/mol as well as with earlier calorimetric data which indicate \(\Delta H^\circ\) = -6.7±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 \(\Delta H^\circ\) (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 \(\Delta G^\circ\) and \(\Delta H^\circ\) values for the reduction of NADP\(^+\) are close to those for the reduction of NAD\(^+\). The difference between the two \(\Delta H^\circ\) values (0.6 kcal) is comparable with probable errors in the comparison. It is, however, in good
agreement with unpublished calorimetric work of
M. Schott & J. M. Sturtevant, who have made two
independent estimates of $\Delta H_{(6)}$ ($-0.92 \pm 0.3$ and
$-1.02 \pm 0.4$ kcal/mol).

$\text{NADPH} + \text{NAD}^+ = \text{NADP}^+ + \text{NADH}$ (6)

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

$\text{NADP}^+ + \text{H}_2 = \text{NADPH} + \text{H}^+$ (7)

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, $\Delta G_{(6)}^0$ and $\Delta G_{(7)}^0$
calculated for several temperatures between 10°C
and 40°C by using the equation $\Delta G^0 = \Delta H - T \cdot \Delta S^0$
and assuming that $\Delta H$ and $\Delta S^0$ are constant within the
temperature range.

I am most grateful to Dr. J. M. Sturtevant, who has
kindly allowed me to quote his unpublished work and to
Miss Jane Bennett, who started the present work as an
undergraduate project.

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