Thermochemical Studies of Vitamin B_{12}. Part I. Thermodynamic Parameters for Acid Dissociation of Aquocobalamin (Vitamin B_{12a})

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The following thermodynamic data have been obtained for acid dissociation of aquocobalamin (Vitamin B_{12a}) ⟶ hydroxocobalamin in aqueous solution at 25·0 °C and in an ionic background of 0·1M-NO_3^- ions: pK_a 7·62; ΔG^0 43·45 kJ mol^{-1}; ΔH^0 18·47 kJ mol^{-1}; and ΔS^0 −83·8 J K^{-1} mol^{-1}. These results, obtained by potentiometric methods and direct calorimetry, are discussed. Attention is drawn to the invalidity of previous comparisons of ΔS for free and bound water molecules in this and similar systems.

A NECESSARY prerequisite to a detailed calorimetric investigation of the thermodynamics of reactions of Vitamin B_{12} is accurate knowledge of the equilibrium constant for reaction (1) under precisely specified

aquocobalamin, $[B\text{-}Co\text{-}H_2O]^+ \longrightarrow$ hydroxocobalamin, $[B\text{-}Co\text{-}OH] + H^+$ (1)

conditions. Literature values vary widely (pK_a 6·9—7·8) [1-5] and have been obtained by spectrophotometric methods of uncertain reliability using buffer solutions to control the pH. Buffers, being Lewis bases, are potential ligands which might be expected not only to interact with the metal atom but also, through hydrogen bonding, to be involved with the side chains of the corrin ring. These latter interactions would not be expected to be detected by spectrophotometric methods since their effect on the chromophore is likely to be slight. They would, however, contribute to the enthalpies measured calorimetrically. This, in principle, suggests a method, or methods, of investigating these important side-chain effects and will be dealt with in subsequent papers.

The present series of measurements have been made in a medium (0·1M-NO_3^-) in which such interactions are negligible in order to specifically investigate the aquocobalamin ⇌ hydroxocobalamin equilibrium.† Potentiometric methods are generally to be preferred to spectrophotometric methods for determining ΔG because of the greater inherent accuracy of the former. Direct calorimetric measurement of ΔH is preferable to a derivation *via* the variation of pK with temperature since the latter method assumes ΔH to be constant over the temperature interval used, an assumption which is frequently not justified.

EXPERIMENTAL

Aquocobalamin was a gift from Glaxo Laboratories. The purity was checked by thin-layer chromatography [6] and the material was then used without further purification. Solutions were analysed spectrophotometrically after conversion to the dicyanide (ε 3·04 × 10^4 dm^3 mol^{-1} cm^{-1} at 367 nm).[7]

Instrumentation.—The burette used was a precision syringe type built in this laboratory. It consisted of a Radiometer B104 5 cm^3 all-glass syringe, operated by a Mauser micrometer head with a 25 mm movement. The latter had a digital register displaying to 0·01 mm and an additional Vernier scale. The burette was readable to one part in ten thousand of the total volume delivered and was reproducible on calibration to one part in five thousand (0·001 cm^3). All potentiometric determinations were made as described previously [8] at 25·0 ± 0·05 °C with 0·1M-nitrate ion (as potassium nitrate) as constant ionic background. The glass–reference electrode system (Russell type CMT 74/60) was used in conjunction with a Beckman 'Research' pH meter and was calibrated to read stoicheiometric hydrogen-ion concentrations as previously described.[8]

Spectrophotometric measurements were made using Beckman DK2A ratio recording and Unicam SP 500 manual spectrophotometers.

The calorimeter used was an L.K.B. Batch Microcalorimeter (type 10700-2C) and was calibrated both electrically and by measuring the heat of formation of water. The two calibration procedures were consistent. The calorimeter is a twin-differential instrument and records the difference in heat output between a reaction cell and a matched blank cell. Each cell is divided into two compartments and mixing of the reagents is effected by rotating the complete cell block. A detailed description of the instrument has been published elsewhere.[9] In a typical experiment the calorimeter was charged with reagents as follows:

Reaction cell

Compartment (1)		Compartment (2)	
2·5 cm^3	*ca.* 10^{3}M-aquocobalamin 0·1M-potassium nitrate	1·8 cm^3	0·01M-potassium hydroxide 0·1M-potassium nitrate

Blank cell

2·5 cm^3	0·1M-potassium nitrate	1·8 cm^3	0·01M-potassium hydroxide 0·1M-potassium nitrate

The sources of heat output were: (reaction cell) (*a*) heat of dilution of aquocobalamin, (*b*) heat of dilution of potassium hydroxide, (*c*) heat of reaction aquocobalamin +

† 1M = 1 mol dm^{-3}.

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[9] I. Wadsö, *Acta Chem. Scand.*, 1968, **22**, 927.

OH^- ⟶ hydroxocobalamin + water, (*d*) heat of formation of water due to pH change of solution, and (*e*) mechanical heat effects due to the mixing operation; (blank cell) (*a*) heat of dilution of potassium hydroxide and (*b*) mechanical heat effects. By operating the calorimeter in a differential mode the heat of dilution of the potassium hydroxide solution was automatically allowed for, as was most (but not necessarily all) of the mechanical heating effect. Separate experiments were carried out to determine the heat of dilution of aquocobalamin and the excess of mechanical heat effects due to imperfect matching of the cell geometries. The corrections for the heat of formation of water were calculated on the basis of the measured pH change and the known value for the molar heat of formation of water.

RESULTS

p*K of Aquocobalamin.*—This was determined by titrating a solution of aquocobalamin (2·0 cm^3, *ca.* 10^{-3}M) with potassium hydroxide solution (0·01—0·1M) at 25·0 °C in the presence of 0·1M-NO_3^-. The titration cell was continuously purged with dioxygen-free dinitrogen, purified by passage through gas wash bottles containing successively 2M-chromium(II) chloride to remove traces of dioxygen, 2M-sodium hydroxide to remove carbon dioxide, and finally 0·1M-potassium nitrate to ensure that the vapour pressure of water in the gas was the same as that in the titration cell to preclude evaporation or condensation effects which are important with the small volumes used. The degree of protonation of hydroxocobalamin, $\bar{n}_H$, at any titration point was calculated from equation (2),[8] where c_H and c_A

$$\bar{n}_H = (c_H - [H^+] + K_w/[H^+])/c_A \qquad (2)$$

are the total concentrations of hydrogen ions and hydroxocobalamin, $[H^+]$ is the concentration of free hydrogen ions, and K_w is the ionic product of water. Calculation of the p*K* from 20 points in the range $0·2 < \bar{n}_H < 0·8$ gave a value of 7·62 ± 0·02 at 25·0 °C and I = 0·1M-NO_3^-; this gives ΔG^0 = +43·45 kJ mol^{-1}.

Enthalpy of Dissociation.—The molar enthalpy change for reaction (3) was calculated from the corrected heat

$$[B\text{–}Co\text{–}H_2O]^+ + OH^- \longrightarrow [B\text{–}Co\text{–}OH] + H_2O \qquad (3)$$

outputs from the calorimeter as described in the Experimental section. This enthalpy change, $\Delta H_{subs.}^0$, is related to that ($\Delta H_{diss.}^0$) for the dissociation reaction (1) as in equation (4), where $\Delta H_f^0(H_2O)$ is the molar enthalpy of

$$\Delta H_{diss.}^0 = \Delta H_{subs.}^0 - \Delta H_f^0(H_2O) \qquad (4)$$

formation of water from H^+ and OH^- under the reaction conditions, *i.e.* 25·0 °C and 0·1M-NO_3^-. The results are summarised below:

p*K*	ΔG^0/kJ mol^{-1}	ΔH^0/kJ mol^{-1}	ΔS^0/J K^{-1} mol^{-1}
7·62 ± 0·02	+43·45 ± 0·1	+18·47 ± 0·1[a]	−83·8 ± 1

[a] Mean of seven determinations.

DISCUSSION

Our value of −83 J K^{-1} mol^{-1} for the standard entropy change for dissociation of aquocobalamin [reaction (1)] is surprisingly close to that of −79 ± 8 J K^{-1} mol^{-1} obtained by Hanania and Irvine,[3] considering that their value was obtained from equilibrium-constant measurements at only two temperatures. It is tempting to compare this value with that for the dissociation of a 'free' water molecule (78 J K^{-1} mol^{-1}), and indeed Hanania and Irvine [3] state 'for ferrimyoglobin, vitamin B_{12} factor B, and aquocobalamin, $\Delta S^\ominus$ of ionisation (of the metal-bound water molecule) is approximately constant and equal to the entropy of ionisation of a free water molecule' [reaction (5)]. They and others have

$$H_2O + H_2O \longrightarrow H_3O^+ + OH^- \qquad (5)$$

concluded from this that the effect of co-ordination on the ionisation of this bound water molecule is entirely an enthalpy effect. However, the validity of such a comparison is questionable since numerical values of the thermodynamic parameters are dependent on arbitrarily assigned standard-state conditions and the arbitrarily chosen units in which concentrations are expressed. The thermodynamic parameters on which we base our comparisons ought to be those which are characteristic of the species under investigation and not subject to the arbitrary conventions of thermodynamics.

Gurney [10] introduced the term 'unitary' to describe those contributions to the entropy change which are characteristic of the reaction under investigation and 'cratic' to describe those contributions which arise merely from the change in the number of solute particles in solution (irrespective of what they are) concomitant with the ionisation process. It is the *unitary* part of the entropy change which is of interest and for aqueous solutions this is given by equation (6),[10] where Δn is the increase in the number of solute species present.

$$\Delta S_{unit.}^0 = \Delta S^0 - \Delta nR \ln 55·5 \qquad (6)$$

If we are comparing a series of reactions of the same stoicheiometry type, Δn is constant and a comparison of ΔS^0 for these reactions has some validity. However, reaction (1), for which $\Delta n = 1$, does not belong to the same stoicheiometry type as (5), since in the latter reaction there are no solute species on the left-hand side of the equation and $\Delta n = 2$. An additional and more important distinction between reactions (1) and (5) is that they belong to different charge types. The aquocobalamin, factor B, and ferrimyoglobin reactions are all of the charge type 1+,0 ⟶ 0,1+, whereas reaction (5) is of the type 0,0 ⟶ 1+,1−. Laidler [11] pointed out that entropy changes are extremely sensitive to the charges on reactants and products since these changes are largely due to the orientation of solvent molecules around solute ions. If one therefore wishes to compare entropy changes associated with the ionisation of free and metal-bound water in these systems, then the reference reaction should not be equation (5) but rather the reaction of the same charge type, *i.e.* (7)

$$H_3O^+ + H_2O \longrightarrow H_2O + H_3O^+ \qquad (7)$$

for which ΔS^0 is clearly zero. Alternatively, one may compare the reverse of reaction (5) with (3).

[10] R. W. Gurney, 'Ionic Processes in Solution,' McGraw-Hill, 1953.

[11] K. J. Laidler, *Canad. J. Chem.*, 1956, **34**, 1107.

The significant entropy changes corrected for cratic effects are summarised below:

Reaction	Δn	ΔS°/ J K^{-1} mol^{-1}	$\Delta S_{unit.}^{\circ}$/ J K^{-1} mol^{-1}
(1)	1	184	−117
(7)	0	0	0
(3)	−1	−6	+27
Reverse of (5)	−2	+78	+144

It seems clear that dissociation of a proton from the metal-bound water in aquocobalamin is associated with an unusually large and unfavourable entropy change compared to dissociation of a proton from H_3O^+; a reflection of solvation effects. Previous statements that the change in pK for water on co-ordination is entirely an enthalpy effect are therefore misleading.

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