Effects of Solvent and Ionic Medium on the Kinetics of Axial Ligand Substitution in Vitamin B_{12}. Part IV. The Reaction between Aquocobalamin and the Thiocyanate Ion in Acetonitrile–Water Mixtures

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Abstract

The rate constants for the reaction of aquocobalamin with the thiocyanate ion were measured as a function of ionic strength and solvent composition in acetonitrile–water mixtures. The reaction is described by a two-step mechanism: the ligation reaction, where the most stable isomer (S-bonded) is formed and the isomerisation reaction (S-bonded to N-bonded thiocyanate). For the ligation reaction a full quantitative analysis of solvent effects could be performed, whereas for the isomerisation reaction only qualitative observations were made. The equilibrium constant for the isomerisation (S-bonded/N-bonded) is large and does not change with the solvent composition. It is found that the transfer Gibbs energies of activation for the ligation reaction are the same as found for the ligand thiourea. The absence of a solvent effect on the isomerisation reaction is a further example of the ability of vitamin B_{12} to create its own micro environment.

Introduction

Our investigations into the reactivity of vitamin B_{12} and model compounds have so far comprised reactions with several sulfur-coordinating ligands in the solvent mixtures dioxane–water [1, 2, 3] and acetonitrile–water [2]. From these studies it was shown that the quantitative analyses of the solvent effects on the rate profile can give essential information on the reaction mechanism and can be used as an additional criterion to select proper model compounds [3].

So far we have only studied the substitution reactions of vitamin B_{12a} and aquamethylcobaloxime. We have now extended our studies to the closely related isomerisation reactions. In 1966, Randall and Alberty [4] studied the kinetics of the reaction between thiocyanate and aquocobalamin. A few years later Thusius [5] reinvestigated this system, using the T-jump and stopped-flow technique and observed three relaxations. The fastest relaxation is common to a number of other cobalamins and is probably associated with a rapid equilibrium between two conformers. The other two relaxations were assigned to the ligation reaction, followed by an isomerisation reaction. The isomerisation was thought to be the reaction from the S-bonded to the N-bonded isomer of thiocyanate. However, no direct evidence was presented that it was not the reverse linkage isomerisation reaction (from isothiocyanate to thiocyanate). We are interested in the way the solvent composition influences the rate constants for this isomerisation reaction and how this compares with the same reaction for model compounds [6]. The study of the solvent effects on the isomerisation reaction is interesting because this type of reaction usually takes place between the first and second coordination sphere and is not directly associated with the bulk solvent mixture, as in the case of the axial ligand substitution. We measured the rate constants for the ligation and isomerisation.

Experimental

Vitamin B_{12a} in the form of hydroxocobalamin hydrochloride (Fluka) and sodium thiocyanate (Baker A.R.) were used as purchased. Acetonitrile (Baker A.R.) was distilled once prior to use. Tetraphenylarsonium thiocyanate was prepared by mixing equal amounts of saturated aqueous solutions of tetraphenylarsonium chloride (Fluka) and sodium thiocyanate. The salt precipitated immediately as white needles, which were washed with water. Analysis for the thiocyanate ion gave 13.05% (13.14% calculated). Solutions of aquocobalamin chloride were prepared as before [1]. Concentrations of solutions of sodium thiocyanate, tetraphenylarsonium thiocyanate and aquocobalamin chloride were determined by potentiometric titration with silver nitrate.

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Equilibrium constants were determined by a photometric titration in a thermostatted cell, in which the solution of aquocobalamin chloride in the appropriate mixture was held. The thiocyanate solution was added with a Metrohm Herisau E457 microburette and the changes in absorption were monitored with a Zeiss M4 QIII photometer at 560 nm. The equilibrium constants were evaluated from the photometric data by means of the Rose-Drago equation [7]. The stopped flow technique used for monitoring the reactions was described previously [8]. The ligation and hydrolysis reactions were followed at a wavelength of 5601 nm; the isomerisation reactions were followed at 500 nm†. The reactions of aquocobalamin with the thiocyanate ion were done under pseudo first-order conditions at at least five concentrations of thiocyanate. All solutions had an ionic strength of 0.10 M (addition of sodium perchlorate).

Solubilities of tetraphenylarsonium thiocyanate were determined as described before [3].

Infrared measurements in solution were performed with a Perkin-Elmer 580 B spectrophotometer. Spectra were measured in a BaF_2 cell with a path length of 0.0025 cm. Integrated intensities were calculated as described previously [9]. NMR spectra were recorded on a WM-250 Bruker spectrometer. The ^{59}Co NMR spectra were measured at a frequency of 59.73 MHz and the ^{14}N NMR spectra at a frequency of 18.09 MHz.

Results and Discussion

The reaction of aquocobalamin (denoted as (Cbl–OH_2)$^+$) with thiocyanate takes place in two steps [5]. The first step is assumed to be the ligation reaction of thiocyanate, probably bound through sulfur. The second step is the linkage isomerisation from thiocyanate to isothiocyanate. The corresponding reaction scheme will be:

$$(\text{Cbl–OH}_2)^+ + \text{SCN}^- \underset{+H_2O}{\overset{-H_2O}{\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}}} (\text{Cbl–SCN}) \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (\text{Cbl–NCS}) \qquad (1)$$

Although it has always been assumed that the sulfur-bound thiocyanate complex is formed first and is the most stable, it has never been proved. The only indication is that in the solid complex thiocyanate is bound through sulfur [10]. In order to obtain information concerning this problem, infrared and NMR spectra of a solution containing 0.05 M NaSCN and 0.05 M (Cbl–OH_2)Cl were measured. The integrated intensities of the CN-stretching frequency have characteristic values depending on the mode of coordination of the thiocyanate group [11]. The value for N-bonded thiocyanate is approximately 10×10^4 dm^3 mol^{-1} cm^{-2}, while for S-bonded thiocyanate this value is 3×10^4 dm^3 mol^{-1} cm^{-2} [11]. The CN-stretching frequency for a solution of the complex between aquocobalamin and thiocyanate (0.05 M) was found at 2112 cm^{-1}; this is in the same range as observed for a series of cobaloximes (2100–2138 cm^{-1}) [12]. The integrated intensity was found to be 3×10^4 dm^3 mol^{-1} cm^{-2}. This value shows that in solution thiocyanate is mainly bound through sulfur. No signal was observed that could be assigned to the N-bonded thiocyanate. For the same solution we tried to measure the ^{59}Co and ^{14}N NMR spectra, but failed to observe any signal in either case; this is probably caused by line broadening.

As described previously [5], above a concentration of 0.03 M NaSCN the reaction between SCN$^-$ and aquocobalamin in water takes place in two discrete steps. The first step is accompanied by a relatively large spectral change at 560 nm ($\Delta\epsilon_{560}$ = 3000 dm^3 mol^{-1} cm^{-1}) whereas the second step is accompanied by only a small change in absorbance ($\Delta\epsilon_{500}$ = 150 dm^3 mol^{-1} cm^{-1}). From a comparison of absorbance changes for this reaction with spectra of complexes of aquocobalamin with several other sulfur and nitrogen-coordinating ligands (phenylisothiocyanate, thiosulfate and azide), we conclude that the first step is indeed the formation of the more stable sulfur-bound thiocyanate complex and the second step the isomerisation to the N-bonded isomer. For this system scheme (1) applies. If the two steps are well separated the observed rate constants for the first and second step are (pseudo first-order conditions with [SCN$^-$] > 0.03 M in water):

$$k_{obsd}(1) = k_1[\text{SCN}^-] + k_{-1} \qquad (2)$$

$$k_{obsd}(2) = k_2\left(\frac{k_1[\text{SCN}^-]}{k_1[\text{SCN}^-] + k_{-1}}\right) + k_{-2} \qquad (3)$$

In the case that the two steps coalesce (pseudo first-order conditions and [SCN$^-$] < 0.03 M in water):

$$k_{obsd}(3) = k_1[\text{SCN}^-] + k_{-1}\left(\frac{k_{-2}}{k_2 + k_{-2}}\right) \qquad (4)$$

When the rate of aquation of the thiocyanate complex generated *in situ* is measured, while the reversible reaction is suppressed by adding base [1], the rate constant for the aquation reaction is given by:

$$k_{obsd}(4) = k_{-1}\left(\frac{k_{-2}}{k_2 + k_{-2}}\right) \qquad (5)$$

† A complete set of kinetic data is available on request.

The apparent equilibrium constant K_{app} is given by:

$$K_{app}(1) = \frac{k_1}{k_{-1}}\left(\frac{k_2 + k_{-2}}{k_{-2}}\right) \quad (6)$$

By combination of the equilibrium constants as given by eqn. (6) and the kinetic results obtained at high thiocyanate concentrations (eqn. (2) and eqn. (3)), all rate constants can in principle be determined. Another way to achieve this is the combination of kinetic results obtained at low and high thiocyanate concentrations (eqns. (2), (3) and (4)). Further, if k_{-1} is not too small compared to $k_1[SCN^-]$ and k_2 is not too small compared to k_{-2}, k_2 and k_{-2} can be obtained from the ligand concentration dependence of $k_{obsd}(2)$ (eqn. (3)). All these methods were tried but in no instance could accurate values be obtained for k_2 and k_{-2}. This is because k_{-1} is very small compared to $k_1[SCN^-]$ and also the quotient k_2/k_{-2} is very small (probably smaller than 0.1) [5]†.

The conclusion is that from these data no accurate values for k_2 and k_{-2} can be inferred. However, from the fact that the infrared spectrum in solution shows no signal of nitrogen-bonded thiocyanate we can place an upper limit of 0.05 on the equilibrium constant K_2 ($=k_2/k_{-2}$). From these observations it is possible to simplify eqns. (3) to (6): $k_{obsd}(2) = k_{-2}$; $k_{obsd}(3) = k_{obsd}(1)$; $k_{obsd}(4) = k_{-1}$ and $K_{app}(1) = k_1/k_{-1} = K_1$.

We also measured the rate constants in mixtures of acetonitrile and water going from 0 to 80 vol% acetonitrile. We obtained k_1 from the slope of the plot of $k_{obsd}(1)$ *versus* $[SCN^-]$ over the whole range (both when the two steps coalesce and are well separated). The values for k_{-1} were obtained in three different ways. In the first place from the intercept of the plot of $k_{obsd}(1)$ *versus* $[SCN^-]$, secondly from the hydrolysis reaction and thirdly by the combination of the slope of eqn. (2) and the equilibrium constant. The hydrolysis rate constants $k_{obsd}(4)$ are only accurate in mixtures containing no more than 50 vol% acetonitrile, because above these values acetonitrile decomposes under the influence of the added base. The intercepts of plots according to eqn. (2) gave only inaccurate values for k_{-1}. All three values for k_{-1} are equal within the estimated experimental errors, as expected if the simplifications made above are valid. The difference in absorbance resulting from the isomerisation reaction does not change when the solvent composition changes. Because the spectrum of aquocobalamin is almost independent of solvent composition in acetonitrile–water mixtures, this implies that there are no large changes in the isomerisation equilibrium constant. This means that the simplifications mentioned above are valid for the whole range of acetonitrile–water mixtures.

The rate of hydrolysis was measured as a function of added concentration of base (0.001–0.002 M NaOH). Only for solutions containing less than 50 vol% acetonitrile was the rate found to be independent of the amount of added base. The rate of hydrolysis and the rate of isomerisation were both found to be independent of the thiocyanate concentration (0.05–0.09 M). The ligation reaction is dependent on ionic strength; when the ionic strength is increased from 0.1 to 0.5 M, $k_{obsd}(1)$ at 0.1 M NaSCN decreases by a factor of three. The isomerisation rate constant $k_{obsd}(2)$ was found to be independent of the ionic strength (0.1–0.5 M).

The observed isomerisation rate constant $k_{obsd}(2)$ decreases when acetonitrile is added (Fig. 1). Because

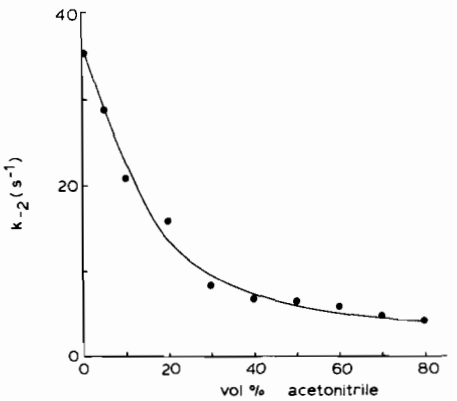

Fig. 1. The rate constant for the isomerisation reaction of isothiocyanatocobalamin to thiocyanotocobalamin (k_{-2}) as a function of solvent composition.

the isomerisation equilibrium constant does not change, k_2 must decrease in the same way. Interestingly, the hydrolysis rate constant k_{-1} shows similar behaviour (Table I). For the calculations of the transfer values [13] we used the values of k_{-1}, obtained by combination of the slope of the plot of $k_{obsd}(1)$ *versus* $[SCN^-]$ and the equilibrium constant K_1. Further we used the values for k_1 and the solubility products of vitamin B_{12a} [2]. Transfer values of SCN^- were calculated from the solubility products of tetraphenylarsonium thiocyanate with the help of the transfer values of the tetraphenylarsonium anion [14] and the TATB assumption [15]. For the calculations of the transfer functions we used as previously 80 vol% cosolvent as the reference point [1, 2]. In Fig. 2 the transfer values of initial state, transition state and final state are shown. All three states show the previously observed maximum [2] in transfer Gibbs energy at approximately 5 vol% acetonitrile. This maximum was ascribed to an increase in

† The determination of the quotient k_2/k_{-2} made by Thusius is very inaccurate because kinetic data at 25 °C are combined with an equilibrium constant ($K_{app}(1)$) at 'room temperature'.

TABLE I. Equilibrium Constant K_1, Rate Constants for the Formation (k_1) and Dissociation (k_{-1}) of Thiocyanatocobalamin and Solubility Product (S^2) of Tetraphenylarsonium Thiocyanate as a Function of Solvent Composition.[a]

Vol% acetonitrile	K_1 (M^{-1})	k_1 (M^{-1} s^{-1})	k_{-1} (s^{-1})	S^2 (M^{-2})
0	1980(60)	3240(80)	1.64(0.06)	$1.7(0.2)10^{-6}$
5	2400(100)	3380(130)	1.40(0.08)	$3.2(0.4)10^{-6}$
10	2900(200)	3120(30)	1.08(0.07)	$8.4(0.8)10^{-6}$
20	3940(250)	2440(50)	0.62(0.04)	$2.6(0.2)10^{-4}$
30	4000(150)	2180(50)	0.55(0.03)	$2.5(0.3)10^{-3}$
40	4880(200)	1800(60)	0.37(0.02)	$2.6(0.2)10^{-2}$
50	5760(400)	1620(30)	0.28(0.02)	$4.8(0.1)10^{-2}$
60	8170(700)	1540(30)	0.19(0.02)	$10.9(0.3)10^{-2}$
70	9080(300)	1580(30)	0.17(0.01)	$16.8(0.5)10^{-2}$
80	14700(500)	1670(30)	0.11(0.01)	$19.4(0.6)10^{-2}$

[a] e.s.d.s in parenthesis.

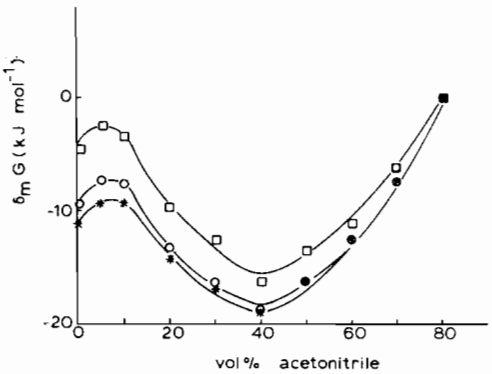

Fig. 2. Transfer Gibbs energy for the initial state (○), transition state (*) and final state (□) for the reaction of aquocobalamin with thiocyanate in acetonitrile–water mixtures at 298.15 K. Estimated standard deviations: 0.8 kJ mol^{-1} (i.s.), 0.9 kJ mol^{-1} (t.s.) and 1.0 kJ mol^{-1} (f.s.).

TABLE II. Transfer Gibbs Energies of Activation for the Formation Reactions of Thiocyanatocobalamin and Thioureacobalamin as a Function of Solvent Composition.[a]

Vol% acetonitrile	$\delta_m\Delta G_1^{\ddagger}$ (SCN^-) (kJ mol^{-1})	$\delta_m\Delta G_1^{\ddagger}$ (TU) (kJ mol^{-1})[b]
0	−1.6(0.1)	−1.6(0.2)
5	−1.7(0.1)	−1.6(0.2)
10	−1.5(0.1)	−1.2(0.2)
20	−0.9(0.1)	−0.9(0.2)
30	−0.6(0.1)	−0.5(0.2)
40	−0.2(0.1)	−0.5(0.2)
50	+0.1(0.1)	−0.2(0.2)
60	+0.2(0.1)	−0.1(0.2)
70	+0.2(0.1)	+0.1(0.2)
80	0	0

[a] e.s.d.s in parenthesis. [b] Ref. 2.

solvent structure at low acetonitrile contents [16], which destabilises vitamin B_{12}. The overall pattern is the same as found for the reaction of aquocobalamin with thiourea [2]. The final state lies further above the initial state (compared with thiourea), probably because the charges are cancelled in the final state. This stabilises the final state relatively in the mixtures and destabilises it in water (clearly seen from the enormous increase in the equilibrium constant K_1 in the acetonitrile–water mixtures). The transfer Gibbs energies of activation ($\delta_m\Delta G_1^{\ddagger}$) are equal within experimental error (Table II), which indicates a common dissociative step, not influenced by the entering ligand. This conclusion was reached before [3] for the reactions of several sulfur-coordinating ligands with aquocobalamin in water and 50 vol% dioxane–water. For the isomerisation reaction a complete analysis of solvent effects on the reaction profile cannot be made, because the values for k_2 cannot be assessed accurately. Qualitatively it can be said, that because k_2 and k_{-2} show the same solvent dependence (the equilibrium constant does not change), the solvent dependence of the transfer Gibbs energy of the S-bonded and N-bonded isomer is very similar. The transition state for the isomerisation reaction is also very similar (in its behaviour towards solvent variations) to the transition state of the ligation reaction, because both k_2 and k_{-2} show the same solvent dependence as k_{-1}. Both observations are indications of the ability of vitamin B_{12} to create its own micro environment. The initial state for the aquation reaction is the same as the initial state for the isomerisation reaction. For both reactions (which have the same leaving group) the activation of the molecule consists of bond breaking to some extent. This will probably differ for both reactions, but is not expected to cause large differences in the activation parameters. Therefore no conclusions can be drawn concerning the detailed reaction mechanism. For model compounds, like the cobaloximes, the equilibrium ratio of the two isomers depends on the solvent [17, 18]. The interaction of

the solvent with the uncoordinated sulfur or nitrogen atom is then sufficiently large to influence the relative stability of the two isomers. For the system acetonitrile–water we expect the S-bonded isomer to be favoured in the water-rich mixtures [19]. The fact that a solvent dependence of the equilibrium ratio of the two linkage isomers is not observed can be explained as follows. The solvent effect will only appear when other ligational effects are in balance for the two isomers [18]. In the case of vitamin B_{12} the S-bonded isomer is favoured. However, as shown by the observations of the isomerisation reaction, small amounts of the N-bonded isomer are present. Therefore small effects are expected which should influence the absorbance differences caused by the isomerisation reaction. That these effects are not observed can only be explained by the presence of the cobalamin moiety, which screens the bound ligand from strong interactions with the solvent.

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