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

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The rate constants for the reaction of aquocobalamin with thiourea were measured as a function of ionic strength, pH and solvent composition in dioxane–water and acetonitrile–water mixtures. With the help of solubility measurements a complete quantitative analysis of solvent effects on the reaction profile could be made. The transfer Gibbs energy of the initial state strongly depends on solvent composition. Because the transition state and the final state closely follow the initial state, this is not reflected in the rate constants.

For the acetonitrile–water mixtures the transfer enthalpy and transfer entropy were determined and were found to exhibit the familiar compensation effect.

It is concluded that, when the solvent changes, vitamin B_{12} creates its own micro-environment around the active metal site, so that the reactivity is effectively solvent independent. The mechanism of activation is dissociative.

Introduction

The interpretation of the generally extraordinary reactivity of biologically active metal complexes, compared to analogous simple coordination compounds, is not straightforward and many factors seem to be involved [1]. One of these undoubtedly is the structure of the macromolecule containing the metal site. Changes in the tertiary structure of a protein have been shown to play an essential part in the kinetics of exchange reactions at the site of hemoglobin [2].

It is known that solvation may essentially change the tertiary structure of a protein [3]. The solvation of the metal ion and of the direct surroundings of the metal ion can have an even more pronounced effect on the kinetics [4]. In this connection it would be interesting to see what influence these solvation changes have on the reactivity of a metal centre in a biologically active metal complex. The main question then is: Is the reactivity of this centre dependent on solvational changes or does the macromolecule preserve a constant micro-environment, independent of peripheral structural changes.

As the start of an investigation into this problem of transfer of structural changes in biological macromolecules to metal centres, we have reported in part I of this series [5] on the reaction between aquocobalamin and the thiosulfate ion in dioxane–water mixtures. For this system it was shown that kinetic solvent and ionic strength effects were comparable in magnitude. The fact that this system presents an ionic reaction made a quantitative analysis of solvent effects hard to perform. It seemed therefore advantageous to choose an uncharged ligand for studying solvent effects on axial substitution reactions of aquocobalamin. The ligand thiourea appeared to be suitable because its coordination through sulfur assures sufficient stability of its vitamin B_{12} complex and because the electronic absorption spectrum of thioureacobalamin belongs to the so-called 'atypical' spectra [6], making a spectrophotometric monitoring of the reaction possible.

The present paper reports a full and quantitative dissection of solvent effects on the initial state, the transition state and the final state for the reaction of thiourea with aquocobalamin in acetonitrile–water and dioxane–water mixtures. To our knowledge this is the first complete study of this kind for a biologically active metal complex.

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Experimental

Vitamin B_{12a} in the form of hydroxocobalamin hydrochloride (Fluka) was used as purchased. Thiourea (Baker), sodium perchlorate (Fluka) and acetonitrile (Baker) were used without further purification. Dioxane (Baker) was purified as described before [5]. Solutions of aquocobalamin chloride were prepared as before [5]. Thiourea concentrations were determined by conductometric titration with $HgCl_2$ [7]. Spectrophotometric determinations were carried out with a Beckman Acta MIV spectrophotometer, equipped with a thermostatted cell compartment. Equilibrium constants were evaluated by means of the Foster–Hamick–Wardly equation [8]. pH-measurements were done with a Metrohm Herisau E603 pH-meter, equipped with a Metrohm EA 120 combination electrode.

The stopped-flow technique used for monitoring the reactions has been described previously [9]. The rate constants were determined at a wavelength of 562 nm*. The reactions of aquocobalamin with thiourea were done under pseudo first-order conditions at at least four concentrations of thiourea, each in triplicate. No salt was added in the case of the dioxane–water mixtures, whereas the ionic strength was maintained constant at 0.10 mol dm^{-3} (addition of sodium perchlorate) in the kinetic runs in acetonitrile–water. The reactions in the acetonitrile-water mixtures were done at three temperatures, between 278.15 K and 298.15 K, with a temperature accuracy of 0.1 K.

Solubilities of aquocobalamin chloride and thiourea were determined as described before [10]. The solubility measurements in the acetonitrile-water mixtures were done at four temperatures, between 293.15 K and 308.15 K; those in the dioxane–water mixtures at one temperature of 298.15 K, with a temperature accuracy of 0.1 K.

Calorimetric measurements were done with a LKB 8700 precision calorimetric system having a isoperibol calorimeter, at a temperature of 298 K. Experimental details have been described [11]. The heat leakage correction during a calorimetric experiment was calculated by the Regnault–Pflaunder method [12]. The corrections for the enthalpy of dilution to infinite dilution in water calculated from the Debye–Hückel formula [13] are negligible, so the experimental values are equal to the standard values at infinite dilution.

Results and Discussion

The observed rate of the reaction between aquocobalamin (further denoted as $(Cbl\text{-}OH_2^+)$ and thiourea (tu) is pH dependent at pH values above 6. A plot of the observed rate constant (at 0.21 mol dm^{-3} tu) *versus* pH (Fig. 1) shows two inflection points, suggesting that two acid–base equilibria are involved. Thiourea is known not to react with acid in the studied pH region (1–12.6) [14], and no acid dissociation constant has been reported. The reaction scheme explaining the pH dependence will be:

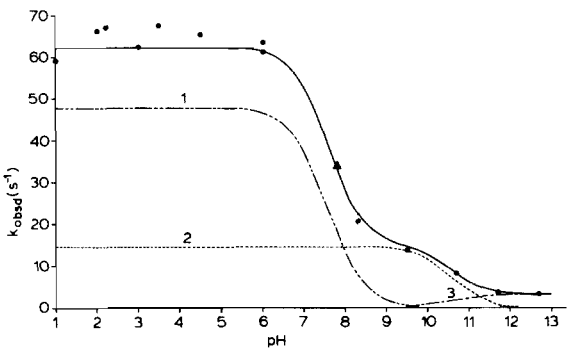

Fig. 1. pH dependence of the observed rate constant for the approach to equilibrium between vitamin B_{12} and thiourea. The solid line was calculated from eqn. 2. The contributions from k_1, k_{-1} and k_{-2} are given by lines (1), (2) and (3) respectively. Estimated standard deviation (E.S.D.): 5%. ▲ Sodium phosphate buffer.

$$\begin{array}{ccc} (Cbl\text{-}OH) + tu & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & (Cbl\text{-}tu)^+ + OH^- \\ K_I \uparrow\downarrow H^+ & & K_w \uparrow\downarrow H^+ \\ (Cbl\text{-}OH_2)^+ + tu & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & (Cbl\text{-}tu)^+ + H_2O \\ H^+ \uparrow\downarrow K_{III} & & H^+ \uparrow\downarrow K_{II} \\ (Cbl\text{-}OH_2)^+ + tu(-H)^- & \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} & (Cbl\text{-}tu(-H)) + H_2O \end{array} \qquad (1)$$

K_I, K_{II} and K_{III} are the acid dissociation constants of aquocobalamin, thioureacobalamin and thiourea, respectively. The conjugate base of tu is formulated as $tu(-H)^-$. It is known that hydroxocobalamin is relatively inert towards ligand substitution [15, 16], so k_2 will be small. Obviously the kinetics of the k_2, k_{-2} equilibrium are mathematically indistinguishable from those of the k_3, k_{-3} equilibrium. So the kinetics cannot discriminate between the two possibilities. The approach to equilibrium of the reactions in scheme (1) was studied under pseudo first-order conditions (more than tenfold excess of thiourea), starting from aquocobalamin and thiourea in acid media and from the product, *in situ* generated thioureacobalamin, for pH > 8. Assuming the acid–base reactions to be rapidly established equilibria and leaving out the k_3, k_{-3} equilibrium, the observed

*All kinetic data are available on request.

first order rate constant (k_{obsd}) for attainment of equilibrium is given by:

$$k_{obsd} = \frac{k_1[tu]}{1 + K_I[H^+]^{-1}} + \frac{k_2[tu]}{1 + [H^+]K_I^{-1}} + \frac{k_{-1}}{1 + K_{II}[H^+]^{-1}} + \frac{k_{-2}K_w[H^+]^{-1}}{1 + K_{II}[H^+]^{-1}} \quad (2)$$

Eqn. (2) was fitted to the values of k_{obsd}, measured at a thiourea concentration of 0.21 mol dm^{-3}, by means of a weighted non-linear least-squares program. The following values were obtained: k_1 = 226 mol^{-1} dm^3 s^{-1}; k_{-1} = 15 s^{-1}; k_{-2} = 8200 mol^{-1} dm^3 s^{-1}; $K_I = 2.6 \times 10^{-8}$ mol dm^{-3}; $K_{II} = 2.6 \times 10^{-11}$ mol dm^{-3}. Because under the conditions used the k_2 term does not contribute appreciably to the total rate, no accurate value for k_2 was obtained from the fit. Therefore k_2 was calculated from the relation:

$$\frac{k_1 \cdot k_2 K_w}{k_{-1} k_{-2} K_I} = 1 \quad (3)$$

to be 0.05 mol^{-1} dm^3 s^{-1} (found from the fit: 0.02 mol^{-1} dm^3 s^{-1}). The experimental rate constants are compared to the calculated pH profile in Fig. 1. The average fitting error is 4.5%.

The rate constant k_{-2} is very large in comparison to k_{-1}. Generally it is found that changing the charge on the entering ligand has a much smaller effect on the rate constants of reactions of cobalamins [16, 17]. So we also tried to interpret the kinetics using the k_3, k_{-3} part of scheme (1). This gave of course the same results for k_1, k_{-1}, K_I and K_{II}, and a value for k_{-3} of 3.1 s^{-1}. It follows from k_{-1} and k_{-3} that the release of thiourea occurs five times faster than the release of deprotonated thiourea. This factor of five for k_{-1}/k_{-3} is of the same order as that found for the interaction of aquocobalamin with azide [17].

The acid dissociation constant of thiourea is not known, but with the use of the following equation:

$$\frac{k_1 k_{-3} K_{II}}{k_{-1} k_3 K_{III}} = 1 \quad (4)$$

we can set a reasonable upper limit to the pK_{III} value by assuming that $tu(-H)^-$ reacts by no more than a factor of 50 faster than tu. This factor of 50 comes from comparing neutral and charged entering ligands [16]. In this case pK_{III} should be <13. Although pK_{III} has not been measured in water we can make an estimate of it by comparing related compounds. Urea has a pK value of 14.3 [18]. Going from thioacetamide to acetamide, the pK value is lowered by one unit [19]. This would mean that the pK value of thiourea is one unit lower than the pK value of urea, *i.e.* about 13. Also in liquid ammonia it is found that thiourea has a lower pK value than urea [20].

The value for K_{II} is comparable to the value of 1.6×10^{-11} mol dm^{-3} found for *trans*-anilinebis-[2,3-butanedione dioximato(1−)]thioureacobalt(III) nitrate, a model compound for vitamin B_{12} [21]. The value of K_I agrees well with previously found values of 2.5×10^{-8} mol dm^{-3} [22] and 1.6×10^{-8} mol dm^{-3} [23].

At pH values below 6 eqn. (2) reduces to:

$$k_{obsd} = k_1[tu] + k_{-1} \quad (5)$$

Plots of k_{obsd} *versus* thiourea concentration in aqueous solutions at different pH values below 6 were found to be linear, giving as average value for k_1 and k_{-1} 224 mol^{-1} dm^3 s^{-1} and 15 s^{-1} respectively (K_I = 15 mol^{-1} dm^3). These values agree well with the values obtained from the fit over the complete pH range.

From the value of K_I it follows that in aqueous solution at a pH below 5.6 less than 1% of vitamin B_{12} is present as hydroxocobalamin. When dioxane or acetonitrile were added, the concentration of hydroxocobalamin (determined spectrophotometrically) decreased. It can thus be concluded that in the unacidified solvent mixtures deprotonation of aquocobalamin can be neglected.

Influence of the Ionic Medium

The effect of the ionic medium on the rate constants was studied by adding various amounts of sodium perchlorate. At a pH below 6, the rate in water and in the solvent mixtures was found to be independent of ionic strength ($0 < I < 0.4$ *M*). At higher pH values the observed rate constant was found to depend on the ionic strength. For instance at a sodium hydroxide concentration of 5×10^{-4} mol dm^{-3}, the value of k_{obsd} was found to vary between 8.5 s^{-1} (I = 0) and 12.8 s^{-1} (I = 0.8 mol dm^{-3}). Because at high pH ionic reactions are involved, this is not unexpected [5]. Moreover, the acid–base equilibrium constants will also be influenced by the ionic strength, which in turn will have an influence on k_{obsd}. It can be concluded that at pH values below 6 the system cobalamin–thiourea is well suited for studying solvent effects on axial substitution reactions of aquocobalamin.

Nature of the Solid in the Solubility Measurements

In solubility measurement undertaken to determine transfer functions a constant composition of the solid in equilibrium with the solution is essential [24]. It has been suggested [25] that the drying

TABLE I. Rate Constants for the Formation (k_1) and Dissociation (k_{-1}) of Thiourea–Cobalamin and Solubilities (S) of Aquocobalamin Chloride and Thiourea as a Function of Solvent Composition and Temperature.

Acetonitrile–Water							
Vol. % cosolvent	T (K)[a]	S (mol dm^{-3})[b] $(Cbl\text{-}OH_2)Cl$	T (K)[a]	S (mol dm^{-3})[c] tu	T (K)[a]	k_1 (mol^{-1} dm^3 s^{-1})[d]	k_{-1} (s^{-1})[e]
0	293.15	7.73	298.15	1.95	278.15	28.2	1.25
	298.15	7.98	303.15	2.30	288.15	84	4.8
	303.15	8.53	308.15	2.70	298.15	223	15.2
	308.15	9.08	313.15	3.24			
2.5	298.15	6.05	298.15	2.03	298.15	227	12.4
5	298.15	5.44	298.15	2.12	298.15	220	12.7
7.5	298.15	5.12	298.15	2.21	298.15	212	11.5
10	293.15	4.51	298.15	2.29	278.15	23.8	0.99
	298.15	4.98	303.15	2.65	288.15	71	4.1
	303.15	5.43	308.15	2.98	298.15	187	13.9
	308.15	6.54	313.15	3.54			
15	298.15	5.03	298.15	2.49	298.15	188	9.0
20	293.15	4.79	298.15	2.66	278.15	20.2	0.84
	298.15	5.34	303.15	3.01	288.15	57	3.6
	303.15	6.00	308.15	3.47	298.15	165	10.7
	308.15	6.98	313.15	3.84			
30	293.15	5.11	298.15	2.85	278.15	17.1	0.72
	298.15	5.65	303.15	3.27	288.15	50	3.1
	303.15	6.56	308.15	3.69	298.15	142	9.2
	308.15	7.55	313.15	4.04			
40	293.15	4.43	298.15	3.05	278.15	15.9	0.63
	298.15	5.09	303.15	3.35	288.15	47	2.4
	303.15	5.87	308.15	3.78	298.15	142	6.9
	308.15	6.89	313.15	4.14			
50	293.15	3.04	298.15	3.06	278.15	14.9	0.68
	298.15	3.57	303.15	3.34	288.15	41	2.7
	303.15	4.40	308.15	3.79	298.15	128	7.9
	308.15	5.62	313.15	4.13			
60	293.15	1.66	298.15	2.89	278.15	14.4	0.73
	298.15	1.95	303.15	3.18	288.15	41	2.5
	303.15	2.28	308.15	3.53	298.15	122	7.9
	308.15	2.87	313.15	3.81			
70	293.15	0.66	298.15	2.52	278.15	13.9	0.80
	298.15	0.80	303.15	2.77	288.15	41	2.7
	303.15	0.94	308.15	3.17	298.15	112	8.1
	308.15	1.10	313.15	3.63			
80	293.15	0.114	298.15	1.96	278.15	14.0	0.70
	298.15	0.134	303.15	2.23	288.15	40	2.5
	303.15	0.153	308.15	2.54	298.15	114	6.7
	308.15	0.189	313.15	2.80			

[a]Temperature accuracy of 0.1 K. [b]E.S.D.: 2%. [c]E.S.D.: 1%. [d]E.S.D.: 278.15 K: 3%; 288.15 K: 3.5%; 298.15 K: 4%. [e]E.S.D.: 278.15 K: 4%; 288.15 K: 6%; 298.15 K: 8%.

of aquocobalamin in the solid state leads to the formation of hydroxocobalamin. Other reactions in the solid state have also been reported [16]. Diffuse reflectance and infrared spectra proved to be uninformative as to the nature of the solid in the present case. However, we found the enthalpy of solution (ΔH_{sol}) to be more informative. We determined this value calorimetrically and compared it to the values obtained from the measurements of the temperature dependence of the solubility (+17 kJ mol^{-1} in water). First the enthalpy of solution of the commercially available 'hydroxocobalamin hydrochloride' (Fluka) was measured. This compound, by differential thermogravimetric analysis, contains 9.9% water. The calorimetrically determined enthalpy of solution is −28 kJ mol^{-1}. The

TABLE II. Rate Constants for the Formation (k_1) and Dissociation (k_{-1}) of Thioureacobalamin and Solubilities (S) of Aquocobalamin Chloride and Thiourea as a Function of Solvent Composition and Temperature.

Dioxane–Water (298.15 K)[a]				
Vol. % cosolvent	S (mol dm^{-3})[b] $(Cbl\text{-}OH_2)Cl$	S (mol dm^{-3})[c] tu	k_1 (mol^{-1} dm^3 s^{-1})[d]	k_{-1} (s^{-1})[d]
0	8	1.9(5)	214	16.4
8	8[e]	2.4	161	11.3
20	8[e]	2.1		
30	7.8	1.9		
40	8.4	2.2	64	5.2
50	8	2.2	51	5.0
60	3.5	2.1		
70	1.3	2.1	23	3.5
80	0.3	2.1[f]	7.5	1.7

[a]Temperature accuracy 0.1 K. [b]E.S.D.: 2%. [c]E.S.D.: 5%. [d]Until 40 vol.%; E.S.D.: 5%; above 40 vol %: 10%. [e]Interpolated value. [f]Extrapolated value.

heat release on solution seemed to be composed of an endothermic and an exothermic part. This can be explained by assuming that part of the solid is hydroxocobalamin hydrochloride and part is aquocobalamin chloride. The negative enthalpy of solution of the former compound is largely due to the enthalpy of solution of hydrogen chloride (ΔH_{sol} (HCl) = −75 kJ mol^{-1} [26]).

After drying the solid an even more exothermic value of −61 kJ mol^{-1} was found. Addition of a small known amount of water to the commercial product before dissolution, after correcting for the amount of aquocobalamin chloride already dissolved, gave a value of 14 kJ mol^{-1}, which agrees well with the value obtained from the solubility measurements. This latter observation can be understood by assuming that in the presence of water hydroxocobalamin hydrochloride is turned into aquocobalamin chloride.

Further the solubility product of the cobalamin determined at four different concentrations of chloride ion (0.08–0.15 mol dm^{-3}) at pH = 5.5 and T = 298.15 K using the formula $K_s = [(Cbl\text{-}OH_2)^+][Cl^-]$ for the calculation was found to be constant within 10% ($K_s = 6.6 \times 10^{-3}$ mol^2 dm^{-6}).

In conclusion it seems that the commercially available 'hydroxocobalamin hydrochloride' contains 10% water and consist of a *circa* 50:50 mixture of aquocobalamin chloride and hydroxocobalamin hydrochloride. Wetting of this compound produces the aquocobalamin and drying the hydroxocobalamin.

We also tried to determine the enthalpy of solution in the mixed aqueous solvents. Qualitatively the picture was the same as in pure water (*i.e.* sign reversal of ΔH_{sol} on wetting of the commercial product. However, quantitative data could not be obtained due to the fact that calculated corrections are less reliable in mixed solvents. Nevertheless it seems that in the solubility measurements in the mixed solvents enough water is present to convert all hydroxocobalamin hydrochloride into aquocobalamin chloride and so the nature of the solid remains the same when going from water to the mixed solvents.

Influence of Solvent Composition

In Part I [5] of this series we have already shown that dioxane does not coordinate to cobalamin in dioxane-water mixtures. Spectrophotometric measurements showed that in the case of acetonitrile also coordination in the mixtures can be excluded. Because an initial state transition state dissection of solvent effects on reactivity requires thermodynamic as well as kinetic information, both solubilities and rate constants were determined in the solvent mixtures. The rate constants k_1 and k_{-1} were evaluated by fitting eqn. 5 to the observed rate constants. In Table I and Table II values for the rate constants are listed, together with the solubilities of aquocobalamin chloride and thiourea in acetonitrile-water (Table I) and dioxane–water mixtures (Table II).

The solubility measurements show that up to 60 vol % cosolvent the solution of vitamin B_{12} is progressively stabilized and that above 60 vol % a destabilization sets in. This makes it highly probable that vitamin B_{12} is preferentially solvated by water. It follows that eqn. 5 can be used in the solvent mixtures without explicitly taking the change of water activity into account [27]. In the case of the acetonitrile–water mixtures the solubility

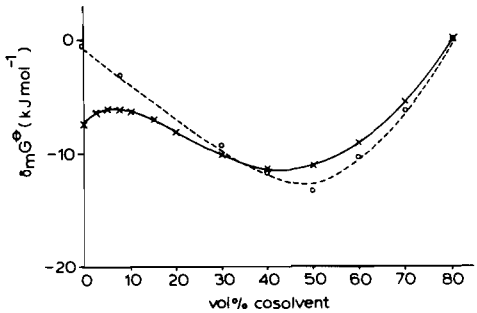

Fig. 2. Transfer Gibbs energy of the aquocobalamin cation in acetonitrile–water mixtures (×) and dioxane–water mixtures (○) at 298.15 K. E.S.D.: 0.5 kJ mol^{-1}.

of both compounds was plotted *versus* the solvent composition for each temperature. The shape of the plots appeared to be hardly affected by the temperature. This indicates that the structure of the solvent mixture does not change appreciably with temperature in the temperature range used. This legalizes the calculation of entropy and enthalpy contributions from the temperature dependence of the solubilities and rate constants.

From the kinetic measurements the ratio k_1/k_{-1} can be calculated, and should be equal to the thermodynamically evaluated equilibrium constants. This indeed is the case within experimental error (15%).

In Fig. 2 the transfer Gibbs energy of the aquocobalamin cation in acetonitrile–water and dioxane–water mixtures is depicted. Interestingly, the transfer Gibbs energy of the aquocobalamin cation for the acetonitrile–water mixtures shows a maximum at 6 vol % acetonitrile, which is also mirrored in the kinetics. In addition a minimum at 40–50 vol % cosolvent is present. The enthalpy of mixing for acetonitrile and water also shows a minimum at 6 vol % [28]; It was suggested that this is caused by hydrogen bond formation between acetonitrile and water at low acetonitrile concentrations [29]. Our results are in agreement with this suggestion. Obviously a less structured solvent mixture (40–50 vol % cosolvent) stabilizes the vitamin B_{12} skeleton and the extra destabilization at low acetonitrile concentrations (6 vol %) is caused by an increase of the structure of the solvent mixture. At high concentratins of acetonitrile and dioxane the stability of the aquocobalamin cation clearly decreases. This implies that vitamin B_{12} needs to form intermolecular hydrogen bonds to dissolve, as can be deduced from its solubility behaviour in various solvents [25].

From the rate constants and solubilities transfer functions were calculated [9, 10, 30]. As a reference for the transfer functions the mixture consisting of 80 vol % cosolvent was used. This has the advantage that differences in the parameters between the

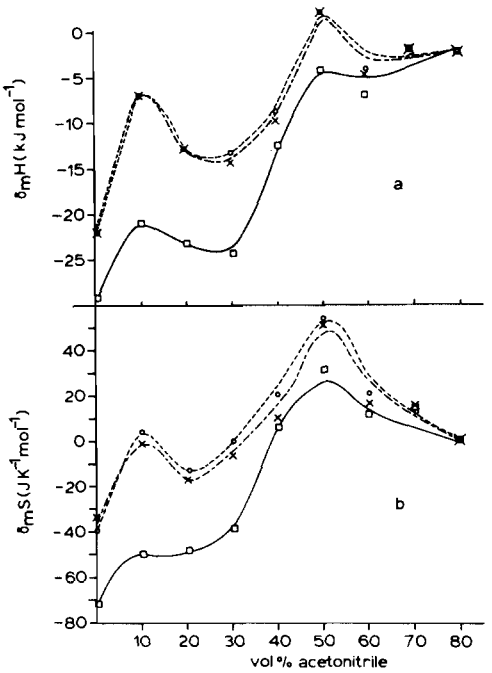

Fig. 3. Transfer enthalpy (a) and transfer entropy (b) for the initial state (×), transition state (○) and final state (□) for the reaction of aquocobalamin with thiourea in acetonitrile–water mixtures, E.S.D.: $\delta_m H^{\ominus}$: 5 kJ mol^{-1} (i.s. and t.s.), 7 kJ mol^{-1} (f.s.). $\delta_m S^{\ominus}$: 16 J K^{-1} mol^{-1} (i.s.), 18 J K^{-1} mol^{-1} (t.s.) and 24 J K^{-1} mol^{-1} (f.s.).

solvent mixtures and pure water are more obvious. We used values for the transfer Gibbs energy of the chloride ion as given by Kundu *et al.* [31] for the acetonitrile–water mixtures and that given by Wells [32] for the dioxane–water mixtures. Transfer enthalpies of the chloride ion in acetonitrile–water mixtures were interpolated from the calorimetrically determined values of Cox *et al.* [33]. The transfer entropies for the chloride ion were then calculated from these data and the transfer Gibbs energies. The transfer parameters were used to calculate thermodynamic transfer functions (denoted as $\delta_m X$) of the initial state (i.s.), the transition state (t.s.) and the final state (f.s.):

$$\delta_m X^{\ominus}(\text{i.s.}) = \delta_m X^{\ominus}((\text{Cbl-OH}_2)^+) + \delta_m X^{\ominus}(\text{tu}) \quad (6)$$

$$\delta_m X^{\ddagger}(\text{t.s.}) = \delta_m X^{\ominus}(\text{i.s.}) + \delta_m \Delta X_1^{\ddagger} \quad (7)$$

$$\delta_m X^{\ominus}(\text{f.s.}) = \delta_m X^{\ddagger}(\text{t.s.}) - \delta_m \Delta X_{-1}^{\ddagger} \quad (8)$$

In Fig. 3 the transfer enthalpies (3a) and transfer entropies (3b) for the three states are shown as a function of the composition of the acetonitrile–water mixture. This Figure shows that in contrast to the large total changes that the individual para-

TABLE III. Enthalpies and Entropies of Activation for the Formation and Dissociation of Thioureacobalamin.

Vol % acetonitrile	$\Delta H_1^{\ddagger}$ (kJ mol^{-1})[a]	$\Delta H_{-1}^{\ddagger}$ (kJ mol^{-1})[b]	$\Delta S_1^{\ddagger}$ (J K^{-1} mol^{-1})[c]	$\Delta S_{-1}^{\ddagger}$ (J K^{-1} mol^{-1})[d]
0	69	85	32	61
10	69	89	30	77
20	70	87	31	68
30	70	88	32	68
40	73	82	40	45
50	71	84	34	54
60	71	80	33	40
70	70	78	28	33
80	70	77	28	30

E.S.D.: [a]2kJ mol^{-1}; [b]3kJ mol^{-1}; [c]6 J K^{-1} mol^{-1}; [d]11 J K^{-1} mol^{-1}.

meter functions undergo on changing the composition of the solvent, the transfer values of the three states (initial state, transition state and final state) stay close together. Apparently the macromolecule does not transfer the large overall solvation changes to the coordination site. In addition second-sphere solvation of the coordination site is not changed with the solvent composition, as we argued above. This also is important for the interchange step in the familiar Eigen–Wilkins two-step mechanism [34]. These factors can be taken together to mean that vitamin B_{12} creates a fairly constant micro-environment at the metal site. Finally, a change in mechanism does not occur, as the picture of Fig. 3 (nearly identical transfer functions for initial and transition state, with a small change for those of the final state) implies a linear free energy relationship. The LFER has slope 1: the transition state follows the aquo-complex plus freely solvated incoming ligand [35, 36]. A consistently dissociative activation is thereby strongly indicated.

The values of the enthalpy and entropy of activation are given in Table III. For both the formation and dissociation of thioureacobalamin the enthalpy of activation is relatively large and the entropy of activation is positive. This again is in agreement with the dissociative character of the reaction [37].

Because of the frequently encountered compensation effect [38] the transfer Gibbs energy is much less affected by the solvent composition than the transfer enthalpy and transfer entropy. In Fig. 4 these transfer Gibbs energies of the three states are shown as a function of the composition of the acetonitrile–water mixture (4a) and the dioxane–water mixture (4b). The transfer Gibbs energy changes are larger for dioxane–water than for acetonitrile–water. With respect to their thermodynamic excess functions of mixing, the two solvent mixtures belong to a different class [39]: dioxane–water is a typically aqueous mixture, whereas acetonitrile–water is a typically non-aqueous mixture [39]. The larger solvent effects for the dioxane–water mixtures in our view can be explained by the larger change in dielectric constant for the dioxane–water mixture. In both mixtures the change in transfer Gibbs energy for all three states is mainly determined by the vitamin B_{12} skeleton as found before for the reaction of thiosulfate with aquocobalamin in dioxane–water mixtures [5]. As we observed before

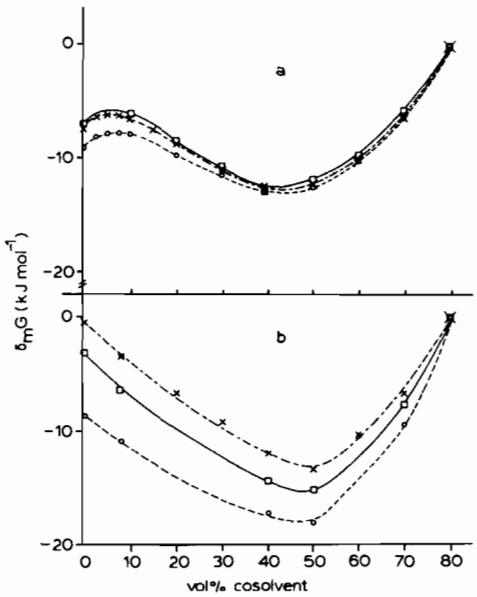

Fig. 4. Transfer Gibbs energy for the initial state (×), transition state (○) and final state (□) for the reaction of aquocobalamin with thiourea in acetonitrile–water mixtures (a) and dioxane–water mixtures (b) at 298.15 K. E.S.D.: Acetonitrile–water mixtures: 0.40 kJ mol^{-1} (i.s.), 0.45 kJ mol^{-1} (t.s.) and 0.53 kJ mol^{-1} (f.s.). Dioxane–water mixtures: 0.5 kJ mol^{-1} (i.s.), 0.6 kJ mol^{-1} (t.s.) and 0.7 kJ mol^{-1} (f.s.).

the water-rich media are clearly those in which the substitution reactions of cobalamin go fastest [5], although the differences are small.

In conclusion, it can be said that solvent effects on the kinetics of axial substitutions at vitamin B_{12} are small. This may be taken as an indication of the ability of vitamin B_{12} to create its own chemical micro-environment. The solvent effects found indicate dissociative activation.

Finally it can be remarked that a study of solvent effects provides essential information on the reaction mechanism. We present the tentative opinion that a detailed quantitative analysis of solvent effects in terms of initial state and transition state quantities should be used as a criterion to select proper model compounds for bio-inorganic reactions.

We are currently investigating this possibility for the vitamin B_{12} system.

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