Effects of Solvent and Ionic Medium on the Kinetics of Axial Ligand Substitution in Vitamin B_{12}. Part III. Reactions of Aquocobalamin and Aquamethylcobaloxime with Sulfur-Coordinating Ligands

SIJBE BALT*, MARTINUS W. G. DE BOLSTER, CAROLINA J. VAN GARDEREN, ALEXANDER M. VAN HERK, KOENE R. LAMMERS and ELISABETH G. VAN DER VELDE

Department of Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

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Abstract

Rate constants for the reactions of aquocobalamin and aquamethylcobaloxime with a series of uncharged sulfur-coordinating ligands were measured in the solvents water and 50 vol% dioxane–water. For both complexes in both solvent systems a linear free energy relationship was found with unit slope, indicating a dissociative mode of activation. With the help of solubility measurements a complete quantitative analysis of solvent effects on the reaction profile could be made. For both cobalt complexes the solvent effects on the reaction profiles are comparable, but in the case of aquocobalamin the kinetic parameters are more influenced by steric factors and hydrogen bonding. From the quantitative analysis of the reactivity of aquocobalamin and aquamethylcobaloxime it is concluded, that for biological reactions where steric effects and/or hydrogen bonding play an important role, aquamethylcobaloxime is not a good model compound for vitamin B_{12}.

Introduction

Our investigations into the reactivity of vitamin B_{12a} so far have comprised the reactions with thiosulfate [1] and thiourea [2] in the solvent mixtures acetonitrile–water [2] and dioxane–water [1, 2]. From these studies it was concluded that a detailed quantitative analysis of solvent effects in terms of initial state and transition state quantities provides essential information on the reaction mechanism and may be used as an additional criterion to select proper model compounds for bioinorganic reactions. The present paper therefore describes such a study of solvent effects for vitamin B_{12a}** and aquamethylcobaloxime***, a well-known model compound for vitamin B_{12} [3]. This study was performed for a series of uncharged sulfur-coordinating ligands in water and 50 vol% dioxane–water. To our knowledge this is the first complete kinetical comparison of a series of related ligands for vitamin B_{12a}. One particular aspect of such a comparison, steric effects, was investigated by Baldwin *et al.* [4] for the coordination of alkylamines to a number of cobalt(III) corrinoids. In this case however, only equilibrium constants were determined.

Experimental

Vitamin B_{12a} in the form of hydroxocobalamin hydrochloride was used as purchased. Aquamethylcobaloxime was prepared according to the method of Schrauzer [5]. Satisfactory analyses for C, H, N, Co and O were obtained. Thiourea (abbreviated as TU, Merck), thioacetamide (TA, Merck), thiosemicarbazide (TSC, Merck), thiocarbohydrazide (TCH, Aldrich), methylthiourea (MTU, Janssen Chimica), dimethylthiourea (DMTU, Aldrich), tetramethylthiourea (TMTU, Fluka) and phenylthiourea (PTU, Fluka) were used without further purification. Dioxane (Baker) was purified as described before [1]. Solutions of aquocobalamin chloride and aquamethylcobaloxime were prepared in degassed, nitrogen saturated, demineralized water and kept in the dark at 4 °C. The solutions were used within 8 hours. The unstable solutions of thiocarbohydrazide were prepared immediately before use. The concentration of the thioacetamide solutions was determined photometrically at 260 nm (ϵ = 11,490 $dm^3\ mol^{-1}\ cm^{-1}$) with a Beckman Acta M IV spectrophotometer. For the other ligands the concentrations were determined by conductometric titration with mercury(II) chloride [6]; in the case of thiocarbohydrazide the titration was performed at 2 °C.

Equilibrium constants were evaluated photometrically by means of the Foster–Hamick–Wardly

*Author to whom correspondence should be addressed.

**Denoted as [Cbl-OH_2]Cl.

***(*a*-aqua-*bcde*-bis[2,3-butanedione dioximato(1−)-N,N',-N'',N''']-*f*-(methyl)cobalt(III), denoted as $CH_3Co(DH)_2H_2O$.

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equation [7]. In the case of thiocarbohydrazide a consecutive reaction was observed. Therefore the differences in absorption as monitored on the stopped-flow apparatus were used. The stopped-flow technique used for monitoring the reactions has been described previously [8]. The extinction scale was calibrated before use, so absolute extinctions could be measured. Rate constants were determined at 560 nm in the case of aquocobalamin and at 445 nm in the case of aquamethylcobaloxime*. All reactions were done under pseudo first-order conditions at at least four ligand concentrations between 10^{-3} and 4×10^{-1} mol dm^{-3}. The ionic strength was maintained constant at 0.10 mol dm^{-3} by addition of sodium perchlorate. Solubilities of the ligands and complexes were determined in water and 50 vol% dioxane–water with the aid of a specially designed solubility tube (Fig. 1). This apparatus has been designed for measurements on small volumes (0.5 ml). After four hours stirring the saturated solutions were filtered off by pushing down the inner tube (1) (the inner tube is fitted with a glass filter). At the moment the glass filter comes into contact with the saturated solution the latter is pressed up from the outer tube (4) through the glass filter (2) into the inner tube (1) because of the built up pressure. This apparatus has the advantage that relatively small amounts of the expensive compounds are needed and that saturated solutions are filtered at the same temperature at which the solubility measurements are done. Furthermore this method gives almost no losses, because, after the sample (10–200 μl) has been removed from the inner tube with a microsyringe, the remainder of the solution flows back when the inner tube is pulled up. With this apparatus the solubility of vitamin B_{12a} in water at 25 °C was found to be 8.0×10^{-2} mol dm^{-3}; this value is identical with that reported by Fendler *et al.* [9].

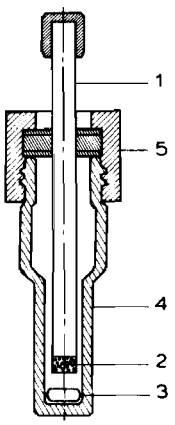

Fig. 1. Solubility tube for measurements on small volumes. 1 Inner tube, 2 Sintered glass filter, 3 Stirring bar, 4 Outer tube, 5 Rotulex cap.

All measurements were done at a temperature of 25.0 ± 0.1 °C.

Results and Discussion

Spectrophotometric and NMR measurements showed that in dioxane–water mixtures dioxane and perchlorate do not coordinate to aquocobalamin or aquamethylcobaloxime. Further the observed rate of the reaction of both compounds with thiourea (a representative sulfur-coordinating ligand) was found to be independent of pH for the region used ($5 < pH < 6$). Therefore the reaction is as follows:

$$M\text{–}H_2O + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M\text{–}L + H_2O \qquad (1)$$

in which M denotes the methylcobaloxime or the cobalamin moiety and L the uncharged sulfur-coordinating ligand. The pseudo first-order rate constant (k_{obsd}) is given by

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

In all cases a plot of k_{obsd} *versus* ligand concentration was found to be linear. From these plots k_1, the second-order ligation rate constant, and k_{-1}, the first-order aquation rate constant, could be determined. Whenever the intercept was too small to yield accurate values of k_{-1}, it was determined from k_{obsd} and the photometrically determined equilibrium constant K:

$$K = \frac{[M\text{–}L]}{[M][L]} = \frac{k_1}{k_{-1}} \qquad (3)$$

For thioacetamide and thiocarbohydrazide a slow consecutive reaction was observed. The spectral changes observed for this slow reaction are dependent on the oxygen content of the solution and are similar to those observed for the redox reaction between l-cysteine and aquocobalamin [10]. The spectral band that appears at 475 nm is associated with the formation of cobalt(II) corrinoids [10]. Because the rates of these reactions are at least four orders of magnitude smaller than the rates of ligand substitution, these reactions do not interfere with the determination of the rate constants. In Table I the solubilities, rate constants and equilibrium constants are presented. From this it can be seen that aquamethylcobaloxime reacts almost as fast as aquocobalamin and that almost all rate constants decrease in going from water to 50 vol% dioxane–water. For the reactions of aquocobalamin with

*Complete kinetic data are available on request.

TABLE I. Solubilities (S) and Reaction Parameters in Water and 50 vol% Dioxane–Water for the Reactions of Aquocobalamin and Aquamethylcobaloxime with a Series of Uncharged Sulfur-coordinating Ligands.

L	Water						50 vol% Dioxane–Water							
		[Cbl–OH_2]Cl[a]			$CH_3Co(DH)_2H_2O$[b]			[Cbl–OH_2]Cl[a]			$CH_3Co(DH)_2H_2O$[b]		
	S(M)	k_1 (M^{-1} s^{-1})	k_{-1} (s^{-1})	K (M^{-1})	k_1 (M^{-1} s^{-1})	k_{-1} (s^{-1})	K (M^{-1})	S(M)	k_1 (M^{-1} s^{-1})	k_{-1} (s^{-1})	K (M^{-1})	k_1 (M^{-1} s^{-1})	k_{-1} (s^{-1})	K (M^{-1})
TU	2.0	223	15	15	113	2.0	57	2.3	51	5	10	44	1.14	39
MTU	1.9	153	6.3	24	98	1.0	98	1.5	37	1.7	22	41	0.5	82
DMTU	4.1	106	42	2.5	100	1.9	53	7.4	25	6.7	3.7	38	0.7	54
TMTU	0.21	0.55	0.16	3.4	107	0.88	122	0.96	0.1	0.08	1.3	24	2.6	9
PTU	–[e]	–	–	–	–	–	–	–	32	6.9	4.6	34	0.8	43
TA	1.7	174	39	4.5	102	5.4	18.9	0.23	59	17.4	3.4	43	1.1	39
TSC	0.14	180	12.5	14.4	119	1.5	79	0.30	47	2.4	19.6	70	0.57	123
TCH	0.058	100	0.15	670	94	0.74	127	0.106	19	0.03[d]	630[e]	42	0.22	190

[a]Concentration between 5×10^{-5} mol dm^{-3} and 2×10^{-4} mol dm^{-3}. [b]Concentration 5×10^{-4} mol dm^{-3}. [c]Thermodynamic equilibrium constant. [d]Derived from thermodynamic equilibrium constant and k_{obsd}. [e]No measurements possible because of the low solubility of phenylthiourea in water.

thiosulfate [1] and thiourea [2] a linear free energy relationship has been found earlier upon varying the solvent composition. In both cases the L.F.E.R. has a slope of approximately 1, indicating that both reactions have a dissociative character. In Fig. 2 the L.F.E.R. for the reactions of aquocobalamin and aquamethylcobaloxime in water and 50 vol% dioxane–water (upon varying the ligand) are shown. From this Figure it can be seen that the ligand tetramethylthiourea does not follow the general behaviour. The linear free energy relationships all have unit slopes. This implies that for both complexes the reactions have dissociative character.

Aquocobalamin

The kinetic parameters for aquocobalamin are clearly influenced by steric factors. In the case of tetramethylthiourea k_1 is very small and for dimethylthiourea and thiocarbohydrazide k_1 is also relatively small. These steric effects are reflected in the equilibrium constants. The range of equilibrium constants with sulfur-coordinating ligands for aquocobalamin is much smaller than that found for the coordination of alkylamines to aquocobalamin [4]. In the latter case the distance between the bulky groups and the corrin ring is smaller. Phenylthiourea yields an equilibrium constant between methylthiourea and dimethylthiourea, which implies that the phenyl ring is directed away from the corrin ring.

It is remarkable that, although tetramethylthiourea suffers from severe steric hindrance (as inferred from k_1), it has a small k_{-1} value. A possible explanation for this phenomenon is that the ligand tetramethylthiourea is so bulky that it experiences a large energy barrier when passing the acetamide side chains, both on entering the first coordination sphere and on leaving it. The fact that the decrease in rate for both k_1 and k_{-1} is of the same order of magnitude, when compared for instance with dimethylthiourea, supports this explanation. For the ligand thiocarbohydrazide, k_{-1} is also very small. However k_1 has a normal value, so steric hindrance cannot be the explanation here. It seems likely, that the possibility of hydrogen bonding between the (amide) hydrogen atoms of the ligand and the oxygen or nitrogen atoms of the acetamide side chains of aquocobalamin is the important factor in this case.

Aquamethylcobaloxime

From Table I it can be seen that steric effects are not operative for the reactions of this model compound. The range of rate constants is much smaller than that observed for aquocobalamin. The aquation rate constant for tetramethylthiourea in 50 vol% dioxane–water is larger than in water. This deviating behaviour will be explained below.

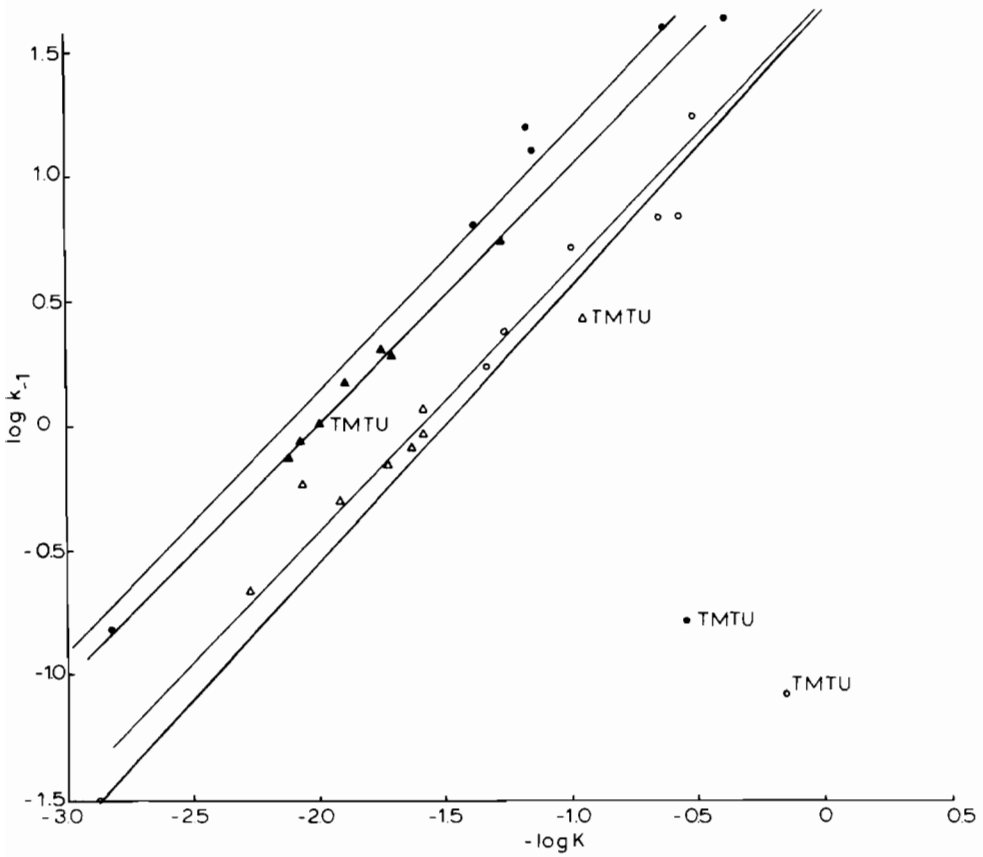

Fig. 2. Linear free energy relationship for the reactions of aquocobalamin and aquamethylcobaloxime with a series of uncharged sulfur-coordinating ligands in the solvent systems water and 50 vol% dioxane–water. Aquamethylcobaloxime in water ▲, in 50 vol% dioxane–water △. Aquocobalamin in water ●, in 50 vol% dioxane–water ○.

Influence of Solvent Composition

A dissection of solvent effects on initial state, transition state and final state, can be made when kinetic measurements are combined with solubility measurements [11]. In Fig. 3 the transfer Gibbs energies for the initial state, transition state and final state are shown for the reactions of aquocobalamin and aquamethylcobaloxime.

In most cases, the transition state is less stabilized than the initial and final states. The same observation was made for the reaction of aquocobalamin with thiourea in dioxane-water and acetonitrile-water mixtures. For a given ligand, the transfer Gibbs energies of the inital and final state do not show much difference. An exception to this general behaviour is tetramethylthiourea. For the reactions of this ligand a relatively small stabilization is found for the final state, which even results in an increase of the aquation rate constant for the model compound, when going from water to dioxane–water. This can be explained by the loss of hydrophobic interactions, when tetramethylthiourea is bound to cobalt. Thioacetamide however is destabilized in 50 vol% dioxane–water, as can be inferred from its solubility behaviour (Table I). The final state is therefore more stabilized than the initial and transition states, in which thioacetamide is freely solvated.

A dissociative mode of activation implies that the transfer Gibbs energy of activation for the ligation reaction should be independent of the entering ligand. Fig. 3 shows that this is indeed the case. For aquamethylcobaloxime the average value (2.3 kJ mol^{-1}) is smaller than the average value for aquocobalamin (3.6 kJ mol^{-1}).

Conclusions

From the quantitative analyses of the reactivity of aquocobalamin and the model compound aquamethylcobaloxime it can be concluded that for both complexes the mode of activation is essentially the same. Further, the solvent effects on the reaction profiles are comparable. Nevertheless there exists a difference in behaviour as a result of the

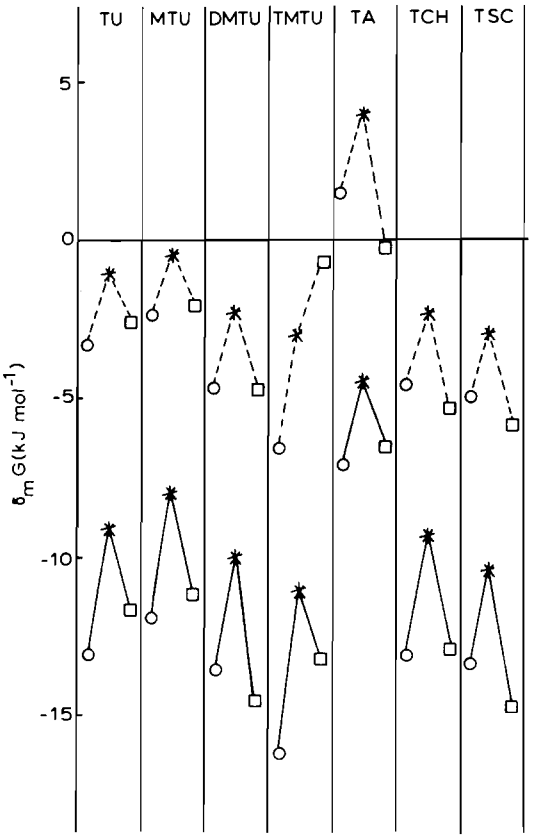

Fig. 3. Transfer Gibbs energies (for the transfer from water to 50 vol% dioxane–water) for the reaction profiles of aquocobalamin (——) and aquamethylcobaloxime (– – –). Initial state ○, transition state ∗, final state □.

fact that kinetic parameters of vitamin B_{12} are clearly influenced by steric factors and hydrogen bonding. This is caused by the presence of acetamide side chains in aquocobalamin, that are missing in the model compound.

In the past several views have been advanced regarding the question whether the cobaloximes are appropriate model compounds [12–14]. Criteria used to select proper model compounds were for example the electrochemical behaviour of these compounds [12], the strength of the Co–C bond [13] and the kinetic behaviour of the cobaloximes [14]. We added additional criteria in terms of the influence of the solvent composition and the behaviour with respect to a series of related ligands. With the help of these criteria it can therefore be said that for biological reactions where steric effects and/or hydrogen bonding play an important role aquamethylcobaloxime in our view is not a good model compound for vitamin B_{12}.

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