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A study of the cage mechanism for the homolytic cleavage of the cobalt–carbon bond in alkylcobalamins using pressure effects*

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Abstract

The effect of pressure on the rate of the anaerobic thermal fission of the Co–C bond in alkylcobalamins (RB_{12}, R = adenosyl, methyl, isopropyl and neopentyl) and $[adenosylcobinamide]^+OH^-$ has been determined in water and 1-propanol/water mixtures. The volumes of activation for homolysis are discussed in terms of the cage model and solvent dependence. It is shown that homolysis at high temperatures (370–390 K) can be described by a negligible cage efficiency and a large volume of activation. At lower temperatures the cage efficiency increases. The type of group R has hardly any influence on the size of the volume of activation for homolysis, which is constant at about 20 cm^3 mol^{-1}. The results are compared with those obtained earlier for organocobalt models of coenzyme B_{12}. It is shown that pressure effects can be used as mechanistic probes to identify reactions in which the radicals have the opportunity to escape from the cage.

Introduction

Recently, we have studied the effect of pressure on the rates of the thermal homolytic fission of the cobalt–carbon bond in alkylcobalamins [1] and some organocobalt model compounds for coenzyme B_{12} [2, 3]. The observed volumes of activation were discussed in terms of the cage model and solvent dependence. For the model compounds it was concluded that for both non-aqueous and aqueous systems cobalt–carbon bond homolysis is characterized by a very efficient cage, a small intrinsic volume of activation and a solvent dependent observed volume of activation that can be quite large. For both coenzyme B_{12} and methylcobalamin a low cage efficiency and a large volume of activation for homolysis were found. This was explained in terms of solvent viscosity and the fact that alkylcobalamins are rather voluminous molecules.

We subsequently investigated the effect of viscosity on the rates of thermal and photolytical fission of the cobalt–carbon bond in coenzyme B_{12} in water/glycerol mixtures [4]. This afforded a more complete analysis of the cage model allowing the determination of the fractional cage efficiency and the Co–C bond dissociation energy.

It seemed interesting to study the thermal homolytic fission of the cobalt–carbon bond more fully for a number of alkylcobalamins in mixtures of solvents that show large differences in the change of viscosity with pressure, expected to be found back in the volume of activation for diffusion [2, 3].

The present paper therefore describes the effect of pressure on the rate of the Co–C bond fission for methylcobalamin, isopropylcobalamin, neopentylcobalamin, adenosylcobalamin and adenosylcobinamide in water and 1-propanol/water mixtures.

Experimental

Materials

Solvents were purchased as analytical grade, if necessary dried by routine methods, and deoxygenated by three freeze/pump/thaw cycles.

Adenosylcobalamin (Fluka), aquocobalamin (Fluka), methylcobalamin (Sigma) and the radical trap tempo (Aldrich) were used without further purification. $[Adenosylcobinamide]^+OH^-$ was prepared according to Hay and Finke [5]. The purity was tested by UV–Vis, ^1H NMR and FAB-MS spectroscopy. Neopentyl- and isopropylcobalamin were synthesized using the following procedure [6]. To a stirred solution of 0.20 g (1.5×10^{-4}

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*The following abbreviations are used in this paper: RB_{12} = alkylcobalamin; Ado = deoxyadenosyl; Me = methyl; Pr^i = isopropyl; Np = neopentyl; B_{12r} = Cob(II)alamin; tempo = tetramethylpiperidine 1-oxide; $[AdoCbi]^+OH^-$ = $[adenosylcobinamide]^+OH^-$.

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mol) aquocobalamin in 1–2 ml water 0.050 g $NaBH_4$ (1.4×10^{-3} mol) was added in portions under N_2 atmosphere. The solution was stirred until the colour had changed to black–brown. At this point 3.0×10^{-4} mol alkyliodide or bromide was added and the solution was stirred for 5 min. Then 0.2 ml acetone was added to destroy the remaining $NaBH_4$ and the solution was diluted with 10.0 ml glacial acetic acid and poured out in 200 ml of diethyl ether. The precipitant was filtered off and washed with diethyl ether and acetone. It was dried at reduced pressure at room temperature (yield 75%). The purity of the compounds was checked by TLC using 1-propanol/glacial acetic acid/water 10:3:7 as diluent. The compounds were identified from their UV–Vis spectra [6–8] in neutral and acidic medium.

Methods and measurements

Details about the experimental setup have already been described [1, 2]. The measurements were carried out photometrically at 376 nm and at temperatures of 374.6 and 380.6 K ($AdoB_{12}$), 473 nm and 388.4 K ($[AdoCbi]^+OH^-$), 376 nm and 388.2 K (MeB_{12}), 351 nm and 303.2 K (Pr^iB_{12}) and 473 nm and 314.7 K (NpB_{12}). The cobalamin concentration was varied between 5×10^{-5} and 10×10^{-5} M. The solutions were kept at pH = 8.0 using a 0.02 M tris(hydroxymethyl)aminomethane/perchloric acid buffer. Excess tempo (10^{-2} M) was used as radical scavenger; in the case of NpB_{12} 2.5×10^{-2} M was used.

Reactions were followed for at least three half-lives and at least two independent runs were made. The mean deviation of the fit of the observed absorbance as function of time to a first-order rate law in all cases better than 2%.

The reproducibility of the rate constants is better than 5%. The accuracy of the temperature was better than 0.2 K. Independence of the measured rates on the nature of the buffer (0.02 M tris(hydroxymethyl)aminomethane/perchloric acid buffer and 0.01 M trikalium phosphate/phosphoric acid buffer), tempo concentration (in the range of $5–25\times10^{-3}$ M) and pH (in the range 7.0–8.5) was checked.

From the pressure dependence of the rate constants (5–12 measurements in the range of 0.1 to 150 MPa) the volume of activation was calculated with a weighted linear least-squares regression routine on the basis of the formula: $\ln k=\ln k_0$- $(\Delta V^{\#}_{obs}/RT)P$ [9]. All plots of $\ln k_{obs}$ versus pressure showed a linear dependence within the measured pressure range. Addition of a second-order term gave no significant increase in accuracy of the fit on the basis of a F-test [10].

Results and discussion

It is well established [11] that in the presence of a radical trap, the reaction scheme for the cobalt–carbon band dissociation in alkylcobalamins can be described by eqns. (1) and (2).

$$\mathrm{RB_{12}(base\text{-}off)} \overset{K}{\rightleftarrows} \mathrm{RB_{12}(base\text{-}on)} \tag{1}$$

$$\mathrm{RB_{12}(base\text{-}on)} \underset{k_c}{\overset{k_1}{\rightleftarrows}} \underset{\text{cage pair}}{\overline{[\mathrm{R}\cdot\cdot\mathrm{B_{12}}]}} \xrightarrow{k_d} \underset{\text{trapped radical}}{[\mathrm{R}\cdot]} + \mathrm{B_{12r}} \tag{2}$$

K is defined as $K=[RB_{12}(\text{base-on})]/[RB_{12}(\text{base-off})]$ and, as the base-off form may be protonated, is pH dependent. The scheme given is simplified by the general and recently established assumption [6,12–14] that for thermal reactions the base-on form (RB_{12}(base-on), where the pendant 5,6-dimethylbenzimidazole ligand of the coenzyme is axially coordinated to Co) is much more reactive (≈100 times) then the base-off [5] form. The observed rate constant for the disappearance of RB_{12} will then be given by [15, 16]

$$k_{obs}=\frac{K}{1+K}\times k_1\times\frac{k_d}{k_c+k_d} \tag{3}$$

The difference in the barriers for cage recombination and escape can be described by the fractional cage efficiency, F_c, defined as $F_c=k_c/(k_c+k_d)$ [17–19].

If the cage efficiency is large due to $k_c \gg k_d$, the volume of activation will be given by [15]

$$\Delta V^{\#}_{obs}=RT\partial\ln[(1+K)/K]/\partial P+\Delta V^{\#}_1 - \Delta V^{\#}_c + \Delta V^{\#}_d \tag{4}$$

If the cage efficiency is negligible due to $k_c \ll k_d$, the volume of activation will be given by

$$\Delta V^{\#}_{obs}=RT\partial\ln[(1+K)/K]/\partial P+\Delta V^{\#}_1 \tag{5}$$

Assuming that besides the diffusion barrier no extra barrier to escape from the cage exists, and eqn. (4) is valid, $\Delta V^{\#}_d$ will be equal to the pseudo volume of activation for diffusion, $\Delta V^{\#}_{\eta}$ (also called the transport contribution), given by [20]: $\Delta V^{\#}_d=\Delta V^{\#}_{\eta}=RT\partial\ln\eta/\partial P$. For 1-propanol/water mixtures, the viscosity has been measured in dependence of composition, temperature and pressure [21]. From these data $\Delta V^{\#}_{\eta}$ can be obtained. The observed activation parameters and the fitted $\Delta V^{\#}_{\eta}$ values are listed in Table 1.

In principle the base-on/base-off pre-equilibrium is pressure dependent. It is therefore necessary to correct k_{obs} to obtain the 'real' homolytic rate constant. We performed this correction for $AdoB_{12}$ and NpB_{12} by studying their visible spectra as a function of pressure and temperature. From the temperature dependence it is possible to calculate $\Delta H°$ and $\Delta S°$ at atmospheric pressure as described by Hay and Finke [11]. For $AdoB_{12}$

TABLE 1. Volume of activation for the cobalt–carbon bond dissociation of alkylcobalamins in 1-propanol/water mixtures

Compound	Solvent	T (K)	$\Delta V^{\#}_{obs}$ (cm^3 mol^{-1})	$\Delta V^{\#}_{obs}$(corr.)[a] (cm^3 mol^{-1})	$\Delta V^{\#}_{\eta}$[b] (cm^3 mol^{-1})
$AdoB_{12}$	water	380.6	19.0 ± 0.2	20.5 ± 0.5	0.2
	20% 1-propanol	380.6	17.7 ± 0.2	21.1 ± 0.4	5.4
	50% 1-propanol	374.6	16.7 ± 0.4	21.0 ± 0.6	10.5
	80% 1-propanol	380.6	17.7 ± 0.5	23.1 ± 0.8	19.2
NpB_{12}	water	314.7	16.4 ± 0.1	21.7 ± 1.0	0.2
	20% 1-propanol	314.7	18.8 ± 0.1	23.8 ± 0.7	3.0
	35% 1-propanol	314.7	21.4 ± 0.1	27.6 ± 0.5	5.6
	50% 1-propanol	314.7	23.1 ± 0.1	29.1 ± 1.0	8.2
	65% 1-propanol	314.7	28.0 ± 0.1	32.4 ± 0.5	11.3
	80% 1-propanol	314.7	29.9 ± 0.1	35.9 ± 0.4	14.8
MeB_{12}	water	388.2	18.2 ± 0.2		
$[AdoCbi]^{+}OH^{-}$	water	388.4	18.5 ± 0.2		
$Pr^{i}B_{12}$	water	303.2	1.2 ± 0.8		
	20% 1-propanol	303.2	1.3 ± 1.3		
	40% 1-propanol	303.2	0.1 ± 0.1		
	50% 1-propanol	303.2	-0.6 ± 1.0		
	60% 1-propanol	303.2	-0.1 ± 0.7		
	80% 1-propanol	303.2	-0.2 ± 0.9		
	90% 1-propanol	303.2	0.0 ± 0.3		

[a] Corrected for the base-on/base-off pre-equilibrium (see text). [b] Fitted (see text).

we adjusted the fit procedure [11, 22] to a ten-parameter fitting procedure with the restriction that $\epsilon_{base\text{-}on}$ and $\epsilon_{base\text{-}off}$ are independent of the solvent mixtures. The resulting $\Delta H°$ and $\Delta S°$ for each solvent mixture allow K to be calculated at all temperatures. However, due to the instability of NpB_{12} at higher temperatures the above procedure could not be followed for this compound. Instead, to determine K at the temperature used to measure the reaction rate, an acidified solution of NpB_{12} was warmed up, adjusted to pH=8 and its absorbance measured. Using the absorbance coefficients of $AdoB_{12}$ for the pure base-on and base-off form it was possible to determine K.

Now $\Delta V°$ can be obtained by fitting to eqn. (6), which describes the absorbance of a solution of an alkylcobalamin as a function of pressure.

$$A_{obs} = \frac{[RB12]_{tot}(\epsilon_{base\text{-}off} + \epsilon_{base\text{-}on}\, K \exp(-\Delta V°/RT)(P-0.1))}{1 + K \exp(-\Delta V°/RT)(P-0.1)} \tag{6}$$

This equation was fitted by variation of the parameters $\Delta V°$, $\epsilon_{base\text{-}on}$ and $\epsilon_{base\text{-}off}$. In the case of $AdoB_{12}$ a six-parameter fitting procedure and in the case of NpB_{12} an eight-parameter fitting procedure was used, with the restriction that the absorbance coefficients are independent of the solvent mixtures used. Using $\Delta V°$ and $K(P=0.1, T)$ the observed rate constant can be corrected:

$$k_{corr} = k_{obs}\, \frac{K(P, T) + 1}{K(P, T)}$$

with

$$K(P, T) = K(P=0.1, T) \exp(-\Delta V°(P-0.1)/RT) \tag{7}$$

From the pressure dependence of k_{corr}, $\Delta V^{\#}_{obs}$(corr.) was calculated; values are listed in Table 1. It is obvious from Table 1 that for $AdoB_{12}$ $\Delta V^{\#}_{obs}$(corr.) is solvent independent, whereas for NpB_{12} it is clearly solvent dependent. The value obtained for $AdoB_{12}$ agrees with that obtained in ethylene glycol [1] and confirms its solvent independence. The same holds for MeB_{12} in ethylene glycol and water. It follows thus that for $AdoB_{12}$ and MeB_{12} the situation is best described by a negligible cage efficiency and a rather large volume of activation for cobalt–carbon bond homolysis. From the fact that $[AdoCbi^{+}]OH^{-}$ shows a comparable volume of activation, it can be concluded that the pendant 5,6-dimethylbenzimidazole axial base does not influence this parameter. For NpB_{12} the case is different: in Fig. 1 we have plotted $\Delta V^{\#}_{obs}$(corr.) versus the calculated value of $\Delta V^{\#}_{\eta}$. Because $\Delta V^{\#}_{c}$ is small but negative [16], the 'real' volume of activation for homolysis ($\Delta V^{\#}_{1}$) for NpB_{12} can be estimated from eqn. (4) to be 20 ± 2 cm^3 mol^{-1}. Obviously, the lower temperature and consequently higher solvent viscosity [4] employed in these measurements favours a higher cage efficiency. It is remarkable that the type of R group has hardly influence on the size of the volume of activation for homolysis; a value of about 20 cm^3 mol^{-1} is found. This value agrees with the strongly positive volumes of activation (15–25 cm^3 mol^{-1}) reported by Swaddle and co-workers [23, 24] for some simple organometallic cobalt(III) and chromium(III) complexes in aqueous solution and ex-

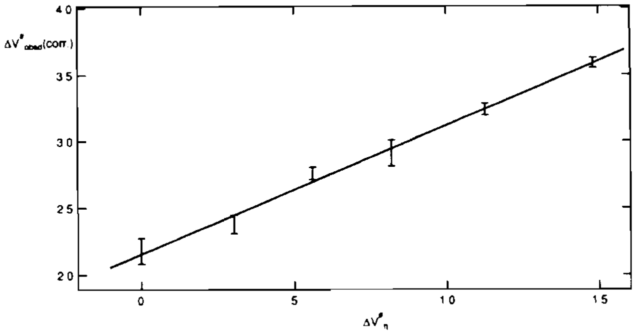

Fig. 1. The observed volume of activation for cobalt–carbon bond dissociation of neopentylcobalamin as a function of the volume of activation for diffusion ($\Delta V^{\#}_{\eta}$) in 1-propanol/water mixtures at 314.7 K. Values are in cm^3 mol^{-1}.

plained by the authors from desolvatation of the cage pair on separation. In contrast, organocobalt models that we have measured [2, 3] show a small intrinsic volume of activation. This behaviour mimicks C–C homolysis for which the work of Neuman [16] indicates that the transition states for homolysis are in the order of 2 to 3% larger in volume than the ground state radical initiators. The large volume of activation found for alkylcobalamins also reflects the occupation of antibonding d-orbitals and the resulting volume increase [1, 25]. The results for the model compounds may perhaps be explained by their small size compared with the voluminous macromolecules [1]. There is a complete parallel between organocobalt models [2] and NpB_{12} in terms of cage efficiency and solvent dependence, but not in $\Delta V^{\#}_{1}$.

For isopropylcobalamin, which decomposes by way of β-H elimination [6, 12], $\Delta V^{\#}_{obs}$ is practically zero. This indicates that the elimination of propylene from isopropylcobalamin either occurs in a direct concerted mechanism, or β-H transfer takes place within the lifetime of the caged radical pair without the formation of free isopropyl radicals. β-H elimination preceded by homolysis is less likely then a direct concerted mechanism, because in the former case a large positive volume of activation (the volume of activation for homolysis) can be expected.

As a final conclusion we may state that for these macromolecules thermal β-H elimination is characterized by a ΔV^{*} close to zero and Co–C bond homolysis by large positive ΔV^{*} values. The cage efficiency of the reaction is strongly dependent on the viscosity of the solvent and consequently on the temperature. To investigate the subject further photolytically induced homolysis at low temperature of the coenzyme B_{12} itself will be studied under pressure.

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