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Effects of Solvent and Ionic Medium on the Kinetics of Axial Ligand Substitution in Vitamin B$_{12}$.
Part VI. Partial Molar Volumes of some Cobalamin Derivates

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Abstract

Apparent molar volumes ϕ_v, were measured at 25 °C in 0.1 molal $NaClO_4$ for aquocobalamin chloride, methylcobalamin, 5'-deoxyadenosylcobalamin and aquanitrocobaloxime. For the organocobalamins the pH dependence of ϕ_v was studied, and for aquocobalamin and aquanitrocobaloxime the dependence on solvent composition was studied. The base-off forms of the organocobalamins have the same volumes as the base-on forms. The apparent molar volume of aquocobalamin chloride is almost independent of solvent composition in dioxane–water mixtures, but increases dramatically in acetonitrile–water mixtures.

Introduction

In a series of investigations [1–5] into the reactivity of aquocobalamin and model compounds we have attempted to obtain insight into the factors that determine the solvent dependence of the kinetic parameters by separating the solvent effects into initial state and transition state contributions. The transfer Gibbs energies of the reacting compounds in mixed solvents, necessary for this approach, in itself provided interesting information on the solute-solvent interactions [5]. In a study of the solubilities of aquocobalamin and some model compounds the scaled particle theory could be successfully applied to the transfer Gibbs energies. One parameter necessary for these calculations is the solute diameter. Therefore, we determined the molar volumes of aquocobalamin chloride in both acetonitrile–water and dioxane–water mixtures and that of aquanitrocobaloxime in dioxane–water mixtures. Further, these volumes can be used in volume profiles of the axial ligand substitution reactions which we are currently investigating. The study of the volume as a function of pH can give information on the volume of the base-off form of the cobalamin. This is of interest for the pressure dependence of the base-off/

base-on equilibrium constant and also for the factors that determine the size of the cavity in the solvent for the cobalamin. We measured partial molar volumes of the base-on and base-off forms of methylcobalamin and 5'-deoxyadenosylcobalamin.

Experimental

Materials

Aquocobalamin chloride in the form of hydroxocobalamin hydrochloride (abbreviated as [B$_{12}$-H$_2$O]-Cl, Fluka), 5'-deoxyadenosylcobalamin ([B$_{12}$-ad], Fluka), adenosine (ad, Merck), methylcobalamin ([B$_{12}$-CH$_3$], Sigma) and sodium perchlorate (Fluka) were used as purchased. Acetonitrile (ac, Baker) was distilled once prior to use and dried on molsieves. Dioxane (diox, Baker) was purified as described before [1]. Aquanitrocobaloxime (Co(dmg)$_2$-NO$_2$H$_2$O) was prepared according to Tschugaeff [6].

Density Measurements

The molar volumes were evaluated from density measurements carried out with an Anton Paar model DMA 02D densimeter with an external measuring cell. The instrument was calibrated regularly with aqueous NaCl solutions in the range 0–0.25 mol kg^{-1}, using the equation

$$d_1 - d_2 = 1/A\,(T_1{}^2 - T_2{}^2) \tag{1}$$

where d is the solution density, T the oscillation period and A a constant.

With the densities of the NaCl solutions calculated from accurate literature data [7], the value of A for this apparatus was found to be 6.583(3) 10^{-6} s^2 cm^3 g^{-1}. The molal concentrations of the stock solutions of aquocobalamin chloride were accurately determined by potentiometric titration of the chloride anion with AgNO$_3$. The molal concentrations of the solutions of 5'-deoxyadenosylcobalamin and methylcobalamin were calculated from the weight

of the solids, corrected for the amount of water in the solids (determined with a Karl-Fischer titration). The molality of the complex is defined as the number of moles of the complex divided by the weight of the solvent (or solvent mixture) and the sodium perchlorate. The molality of sodium perchlorate was 0.1 mol kg^{-1}. The composition of the solvent mixture with 0.1 molal NaClO$_4$ that was used as solvent was determined by weight and the weight percentage of the organic cosolvent was used to indicate the composition of the solvent mixtures. The measurements were performed relative to a solution of known density, usually water or 0.1 molal NaClO$_4$. The apparent molar volume, ϕ_v, was calculated from the difference in density between the solvent mixture containing 0.1 molal NaClO$_4$ and the same solution containing the complex (eqn. (2)),

$$\phi_v = M/d_r + (d_r - d)\, 10^3/md_r d \qquad (2)$$

where M is the molecular weight of the solute, d_r the density of the solvent (mixture) and d the density of the solution of the complex with molality m in the same solvent (mixture). Both solutions were made at the same time from the same samples of the organic cosolvent because then small differences in the composition of the solvent mixture do not result in errors in the molar volumes, but only in a small inaccuracy in the solvent composition.

Results and Discussion

Extrapolation of the apparent molar volume to infinite dilution provides the partial molar volume of the solute (V°). No concentration dependence of the apparent molar volume was found within experimental error in 0.1 molal NaClO$_4$ with concentrations of aquocobalamin between 1×10^{-3} and 1×10^{-2} mol kg^{-1}. Therefore the apparent molar volumes were set equal to the partial molar volumes at concentrations of cobalamin of 3×10^{-3} mol kg^{-1}. The volume of aquocobalamin chloride was found to be 940 ± 3 cm^3 mol^{-1} without added salt and 936 ± 3 cm^3 mol^{-1} in 0.1 molal NaClO$_4$. The volume of methylcobalamin was found to be 944 ± 5 cm^3 mol^{-1} and that of 5$'$-deoxyadenosylcobalamin was 1085 ± 5 cm^3 mol^{-1}. When we correct for the volume of the chloride anion the aquocobalamin cation is some 30 cm^3 mol^{-1} smaller than methylcobalamin.

The benzimidazole group is known to dissociate from the cobalt in methylcobalamin and 5$'$-deoxyadenosylcobalamin on protonation. A protonation constant for methylcobalamin of $-\log K = 2.8$ has been reported [8]; for 5$'$-deoxyadenosylcobalamin, $-\log K = 3.4$ [8] has been reported. We determined a protonation constant for methylcobalamin at 25 °C

in 0.1 molal NaClO$_4$ of $-\log K = 2.84$ spectrophotometrically on the molality scale. To investigate whether the detachment of the benzimidazole group in methylcobalamin causes any significant change in volume, we measured the apparent molar volumes at two pH values, pH = 1 (0.1 molal HClO$_4$) where both methylcobalamin and 5$'$-deoxyadenosylcobalamin are almost exclusively in the base-off form and at pH = 7 where both compounds are mainly in the base-on form. No volume change was observed for methylcobalamin within experimental error ($\phi_v = 944 \pm 5$ cm^3 mol^{-1} at pH = 7; 938 ± 5 cm^3 mol^{-1} at pH = 1). The molar volume of the 5$'$-deoxyadenosylcobalamin also does not change when the pH is changed from 7 to 1; $\phi_v = 1085 \pm 5$ cm^3 mol^{-1} at pH = 7; 1094 ± 5 cm^3 mol^{-1} at pH = 1. This finding is also confirmed by the fact that the base-on/base-off equilibrium of both species is not shifted when a pressure of 600 bar is applied; the extinctions of solutions at several pH values between 1 and 7 are independent of pressures up to 600 bar [9]. Consequently, the detachment of the benzimidazole group is not accompanied by a significant change in volume. This can be explained in two ways: either the displacement of the benzimidazole group on detachment is very small or it does not change the volume of the complete molecule. The molar volume of the complete molecule is determined by the cavity this molecule creates in the solvent. It is possible that within this cavity part of the B$_{12}$ molecule can freely move without changing the size of the cavity. If we compare the sum of the volumes of methylcobalamin and adenosine (169 ± 2 cm^3 mol^{-1}) minus a methyl and a hydroxy group (results in 1075 cm^3 mol^{-1}) with the volume of 5$'$-deoxyadenosylcobalamin, it is clear that the volume of 5$'$-deoxyadenosylcobalamin is even bigger than the sum of the components. This indicates that the acetamide side-chains are pushed aside when the adenosine group is attached, whereas in the aquo and methylcobalamin the side chaines are bent more towards the axial ligands. The crystal structure data [8, 10] confirm these assumptions and also explain why aquocobalamin is even smaller than methylcobalamin. In aquocobalamin the side chains form hydrogen bonds with the coordinated water molecule and therefore bend even more inwards.

We measured the partial molar volumes of aquocobalamin chloride in both acetonitrile–water and dioxane–water mixtures. The results are given in Table I. The volumetric behaviour in either mixture is very different; an increase of volume of 25 percent is found going from water to 70 wt.% acetonitrile–water, whereas in dioxane–water mixtures a slight decrease is found, followed by an increase after 30 wt.% dioxane.

TABLE I. Partial Molar Volumes[a]

Compound	Medium	V°	Compound	Medium	V°
[B_{12}-H_2O]Cl	H_2O	940(4)	[B_{12}-H_2O]Cl	5% ac	969(4)
[B_{12}-H_2O]Cl	0.1 molal $NaClO_4$	936(4)	[B_{12}-H_2O]Cl	10% ac	965(4)
[B_{12}-ad]	H_2O	1079(4)	[B_{12}-H_2O]Cl	15% ac	969(5)
[B_{12}-ad]	0.1 molal $NaClO_4$	1085(4)	[B_{12}-H_2O]Cl	20% ac	1008(5)
[B_{12}-ad]	0.1 molal $HClO_4$	1094(4)	[B_{12}-H_2O]Cl	30% ac	1037(5)
[B_{12}-CH_3]	0.1 molal $NaClO_4$	944(4)	[B_{12}-H_2O]Cl	40% ac	1062(5)
[B_{12}-CH_3]	0.1 molal $HClO_4$	938(6)	[B_{12}-H_2O]Cl	50% ac	1120(5)
ad	H_2O	170(1)	[B_{12}-H_2O]Cl	60% ac	1175(6)
ad	0.1 molal $NaClO_4$	169(1)	[B_{12}-H_2O]Cl	70% ac	1189(6)
$Co(dmg)_2NO_2H_2O$	H_2O	216(3)	[B_{12}-H_2O]Cl	20% diox	915(8)
$Co(dmg)_2NO_2H_2O$	0.1 molal $NaClO_4$	209(3)	[B_{12}-H_2O]Cl	40% diox	916(5)
$Co(dmg)_2NO_2H_2O$	0–80% diox[b,c]	210(8)	[B_{12}-H_2O]Cl	60% diox	916(5)
			[B_{12}-H_2O]Cl	75% diox	927(8)

[a]Units cm^3 mol^{-1}. [b]Abbreviations: ac = acetonitrile, diox = dioxane. [c]Average over five different mixtures.

We also measured the partial molar volumes of aquanitrocobaloxime, a model compound of vitamin B_{12} in dioxane—water mixtures. Because of the smaller volume of this compound and the low solubility [5], the differences in density $(d - d_r)$, are very small and therefore the apparent molar volumes are inaccurate. We found no clear solvent dependence on the volume of aquanitrocobaloxime (ϕ_v average in the mixtures 210 cm^3 mol^{-1} ± 5%).

The volume of aquocobalamin chloride can also be estimated from the crystal structure of cyanocobalamin [11]. If the unit cell volume is corrected for the volume occupied by the crystal water molecules, a volume of 960 cm^3 mol^{-1} remains. This volume is a reasonable estimate, because the four molecules of cyanocobalamin are roughly placed in a hexagonal close packing and the 88 water molecules in the unit cell fill up the void space between the four molecules. When we compare this volume with the partial molar volume of aquocobalamin, it is clear that the cavity for aquocobalamin in water is comparable in size to the space it occupies in the crystal structure and that not many water molecules have interpenetrated the structure of aquocobalamin.

The large difference in volumetric behaviour for aquocobalamin between the mixtures is unexpected. First of all, the transfer Gibbs energies, enthalpies and entropies are comparable in both mixtures [2–5]. Furthermore, in both cases the solvent compressibility increases on going from water to the organic cosolvent ($\beta(H_2O) = 0.46 \times 10^{-4}$ atm^{-1}, β-(dioxane) = 0.6 $\times 10^{-4}$ atm^{-1}; β(acetonitrile) = 1.08 $\times 10^{-4}$ atm^{-1}) [12]. An increase in solvent compressibility is expected to give a decrease in partial molar volume [12], which is contrary to the increase of partial molar volume found for the acetonitrile-water mixtures.

Other factors that could influence the partial volume are the size of the aquocobalamin and

solute—solvent interactions. The size of the solvent cavity is mainly determined by the solute particle size in combination with the solvent compressibility (if solute—solvent interactions are unimportant) and cannot be the factor that causes the great differences between the two solvents. The size of the aquocobalamin cation can of course change with the solvent composition, but an increase of 25% is unlikely to be caused by conformational changes in the molecule. The only factor left is the interaction between solute and solvent. The increase in volume from 940 cm^3 mol^{-1} to 1190 cm^3 mol^{-1} corresponds to an increase in cavity radius from 7.2 to 7.8 Å. At this moment we have no reasonable explanation for this effect, but it is clear that these differences in the two solvent systems are not reflected in other thermodynamic parameters of aquocobalamin in acetonitrile—water and dioxane—water mixtures.

Acknowledgements

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