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Laser-induced photoacoustic calorimetric determination of enthalpy and volume changes in photolysis of 5′-deoxyadenosylcobalamin and methylcobalamin

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Photolysis of 5′-deoxy-5′-adenosylcobalamin (AdoCbl) in neutral aqueous solution and methylcobalamin (MeCbl) in neutral and acid aqueous solution has been investigated using pulsed, time-resolved photoacoustic calorimetry in the temperature range 10–30 °C. The enthalpy changes for the above cobalamins, 129 ± 17, 163 ± 21 and 176 ± 23 kJ mol^{-1}, respectively, are consistent with the values obtained by thermolytic kinetic methods. The reaction volume changes for them, 6 ± 1, 2 ± 0.5 and 5 ± 1 ml mol^{-1}, respectively, are probably due to the conformational changes of the corrin ring and its side chains accompanying Co–C bond cleavage.

Coenzyme B_{12} (5′-deoxy-5′-adenosylcobalamin, abbreviated AdoCbl, Fig. 1) is a cofactor in over a dozen enzymatic reactions, in which 1,2-intramolecular rearrangements of the substrates occur. The essential first step in the catalytic cycle of B_{12}-dependent mutases is the homolytic cleavage of the Co–C bond to produce a 5′-deoxy-5′-adenosyl radical and paramagnetic cobalamin (B_{12r}).[1–4] While isolated AdoCbl undergoes slow thermolysis at ambient temperature (25 °C, $k \approx 10^{-10}$ s^{-1}), the mutases can catalyze Co–C bond cleavage (*e.g.* 25 °C, $k \approx 10^{2}$ s^{-1}) by about 12 orders of magnitude.[5] How these enzymes accelerate rupture of the Co–C bond and achieve such a level is a focus-point in B_{12} chemistry.

Knowledge of the Co–C bond dissociation energy (BDE) of AdoCbl and related organocobalt compounds, and the factors that influence such bond dissociation, may help to understand enzyme-induced bond weakening and dissociation. Since Finke[5] and Halpern[6] accomplished the measurement of the cobalt–carbon BDE of AdoCbl by determining the kinetics of the thermal bond dissociation process, in which radical trap complexes were used to scavenge organic radicals, great efforts have been devoted to the field.[7–10] It has been revealed that there may be an interplay between the *trans* and steric influences of the axial Ado and 5,6-dimethylbenzimidazole (DMBz) ligand, and further, the steric rather than electronic effects modulate the lability of the Co–C bond.[5–11]

Recently, Brown suggested that side chain thermal motions of the corrin ring might be an important source of entropic activation for the homolysis of such complexes.[12] One hypothesis here, that of enzyme-induced corrin 'butterfly' or 'upward' conformational distortion with its resultant corrin ring–adenosyl steric interactions to 'lift' the adenosyl group from cobalt and otherwise distort it along the Co–C bond,[4,11,13] has been accepted by bio-inorganic chemists but remains undemonstrated in the B_{12} enzymes themselves. The recent X-ray crystallographic structure determination for two B_{12}-dependent enzymes, *i.e.* AdoCbl-dependent methylmalonyl-CoA[14a] and MeCbl-dependent methionine synthese,[14b] reveals that the cofactor is bonded in a DMBz base-off form and the cobalt atom is co-ordinated *via* a long bond to a histidine residue from the protein. It has also been proposed that photo-induced homolysis is a consequence of the similar steric strain between

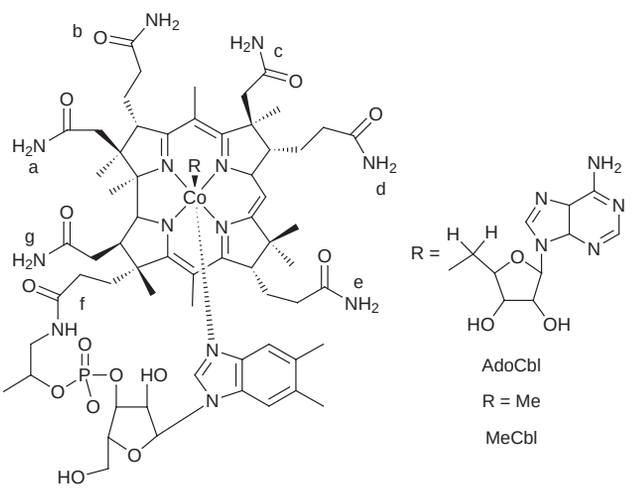

Fig. 1 The structures of 5′-deoxy-5′-adenosylcobalamin and methylcobalamin

the corrin ring and the Ado ligand.[1,15–18] Crystallographic data for various enzyme-free cobalamins suggest that interaction of the corrin ring with axial Ado and DMBz bases causes ring pucker or distortion, *i.e.* upward folding of one side of the corrin ring.[11a,19] However, quantitative data about the volume change accompanying the relaxation process of a sterically strained corrin ring during Co–C bond breaking were not determined.

With the development of time-resolved photoacoustic calorimetry (PAC), it is now feasible to measure the dynamics of enthalpy and volume changes that accompany ligand dissociation and/or molecular movements in photoinduced chemical reactions.[20–22] Since AdoCbl and alkylcobalamins undergo photo-induced Co–C bond homolysis to produce Cob(II)alamin (B_{12r}) and the corresponding alkyl radical, similar to many of the B_{12}-dependent enzyme reactions, we adopted the PAC technique to assess volume changes resulting from the conformational changes in the corrin ring and enthalpy changes in the Co–C bond cleavage process.

Methylcobalamin (MeCbl), another B_{12} coenzyme, is known to be biologically active and essential for human metabolism.[1] We selected this molecule for investigation not only because of its natural occurrence as a cofactor for a methyltransferase

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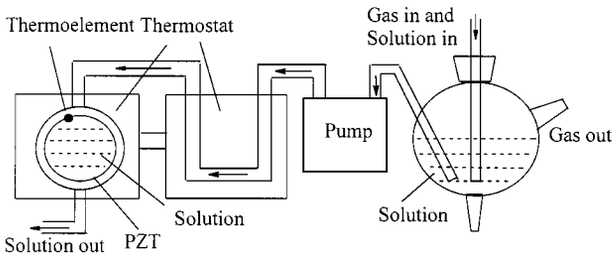


Scheme 1 Flowchart of solution for photoacoustic calorimetry

enzyme but also because CH_3 is a small group and comparative information could be obtained facilitating the investigation into steric interaction and conformational changes of the corrin ring during Co–C bond cleavage.

Experimental

Samples

5′-Deoxy-5′-adenosylcobalamin was obtained from Aldrich and MeCbl prepared according to the literature.[23] For PAC experiments, the concentrations of AdoCbl and MeCbl were 1.5×10^{-5} M in neutral deionized water and in 0.05 M HCl aqueous solution. Potassium dichromate (AR) was used as a calorimetric reference.[21a]

Apparatus and methods

Details for a similar experimental system using pulsed PAC have been described previously.[20–22] In our experiment a Q-switched Nd:YAG laser (Continuum NP70) operating at 10 Hz, 355 nm, pulse width 8 ns, and 15 μJ was used as the excitation source. The laser beam diameter was fixed by a 0.9 mm pinhole, which determined the time resolution to be equal to the travel time of the acoustic wave through the laser beam diameter (about 600 ns in the aqueous solutions). Temperature (10–30 °C) is kept constant within ±0.2 °C by using a thermostat and a thermoelement placed directly into the sample cell. Acoustic waves, an average of the signals from 100–200 pulse excitations, were detected by a 2 MHz PZT cylindrical tube transducer. The receiving area of this transducer is relatively enlarged, compared with a conventional PZT disk, and its receiving face just matches the cylindrical acoustic waveform in the whole circumferential space. The output voltage is amplified by a HP-8847F and recorded on a HP-54510B instrument. The data were transferred to a personal computer where each acoustic wave was normalized to the laser energy measured by a transient radiometer (DigiRad. R-752 and P-444). Sample absorbances range from 0.2 to 0.3 at 355 nm (1 cm path length) and are matched to that of a calorimetric reference. The solutions are argon-saturated before pumping into the sample cell, and flow continuously during photolysis (see Scheme 1).

Data analysis

According to previous studies,[20–22] the acoustic signal (S) results from expansion or contraction, *i.e.* the volume changes of the irradiated sample [equation (1) where the parameter K is a

$$S = K\Delta V \tag{1}$$

function of the instrument response]. There are two contributions to the overall volume changes. One is derived from the thermally induced volume change in the solution, ΔV_{th}, which is related to the thermal expansion coefficient (β) of the solvent and the heat capacity (C_p) of the solution. The other may arise from the volume change between the products and reactants, ΔV_r [equation (2), where ρ is the density of the solution, and ΔE

$$S = K(\Delta V_{th} + \Delta V_r) = K[(\beta/C_p\rho)\Delta E + \Delta V_r] \tag{2}$$

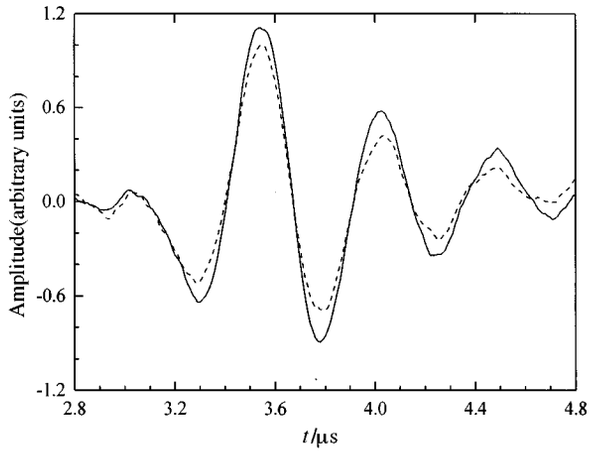


Fig. 2 Photoacoustic signal of $K_2Cr_2O_7$ (– – –) and 5′-deoxy-5′-adenosylcobalamin (——) in neutral aqueous solution at 10 °C (both are 1.5×10^{-5} M)

is the thermal energy released to the medium upon decay]. A compound ($K_2Cr_2O_7$) is used as the calorimetric reference. It converts the photon energy entirely into heat with no reaction volume change, *i.e.* $\Delta V_r = 0$. Therefore $S_{ref} = K(\beta/C_p\rho)E_{h\nu}$. The ratio of the acoustic wave amplitudes of the sample to the calorimetric reference is then defined as φ, expression (3), thus

$$\varphi = S/S_{ref} = (\Delta E/E_{h\nu}) + \Delta V_r/[(\beta/C_p\rho)E_{h\nu}] \tag{3}$$

giving equation (4). It is assumed that ΔE and ΔV_r are

$$E_{h\nu}\varphi = \Delta E + \Delta V_r C_p\rho/\beta \tag{4}$$

independent of temperature. The intercept and slope of the linear plot of $E_{h\nu}\varphi$ *vs.* $C_p\rho/\beta$ at different temperatures yields ΔE and ΔV_r, respectively. However, the quantum yield Φ of the photo-induced chemical reaction must be taken into account for the evaluation of ΔH and ΔV_R. Therefore, the overall enthalpy and volume changes for a reaction are determined according to equations (5) and (6), respectively.

$$\Delta H = (E_{h\nu} - \Delta E)/\Phi \tag{5}$$

$$\Delta V_R = \Delta V_r/\Phi \tag{6}$$

Results

The photoacoustic signals for both AdoCbl and $K_2Cr_2O_7$ (reference) in neutral aqueous solution at 10 °C are shown in Fig. 2. The relationship between the signals and the absorbed energy is linear within the range studied (Fig. 3), which eliminates the possibility of multi-photon effect in solutions. The values of ΔE and ΔV_r for each complex are obtained through equation (6). Plots of $E_{h\nu}\varphi$ *vs.* $C_p\rho/\beta$ are shown in Fig. 4. Values of β, C_p and ρ at different temperatures are according to the literature.[24] The quantum yield values are 0.23 for base-on AdoCbl, 0.35 for base-on MeCbl and 0.50 for base-off MeCbl respectively (see Discussion).[15–18] From equations (6) and (7), the enthalpy and reaction volume changes are obtained. The associated enthalpies, Co–C BDEs and volume changes are given in Table 1. For comparison the data from thermolysis experiments are also listed.

Discussion

Reaction mechanism

Alkylcobalamins exhibit a temperature-dependent axial-base equilibrium, *i.e.* the DMBz group is either co-ordinated to Co^{III} or not.[1b] The photochemical reactions in the system include

Table 1 Data from photolysis of 5′-deoxy-5′-adenosylcobalamin and methylcobalamin by PAC

	AdoCbl	MeCbl (base-on)	MeCbl (base-off)
ΔV_r/ml mol^{-1}	6 ± 1	2 ± 0.5	⩾5 ± 1
ΔH/kJ mol^{-1}	129 ± 17	163 ± 21	⩾176 ± 23
BDE/kJ mol^{-1}	129 ± 17	163 ± 21	⩾176 ± 23
$\Delta H^{\ddagger}$/kJ mol^{-1} [a]	138 ± 8 [b]	171 ± 13 [c]	
BDE/kJ mol^{-1} [a]	125 ± 8 [b]	155 ± 13 [c]	

[a] Data obtained from thermolysis. [b] See ref. 5(*b*). [c] In ethane-1,2-diol, see ref. 9(*b*).

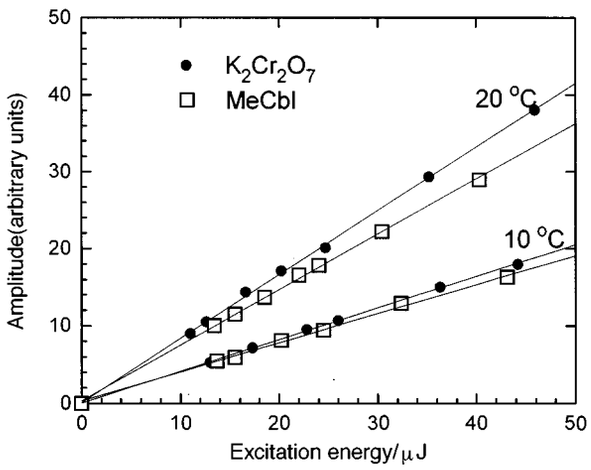


Fig. 3 The relationship between signal amplitude and excitation energy of $K_2Cr_2O_7$ and MeCbl (both are 1.5×10^{-5} M)

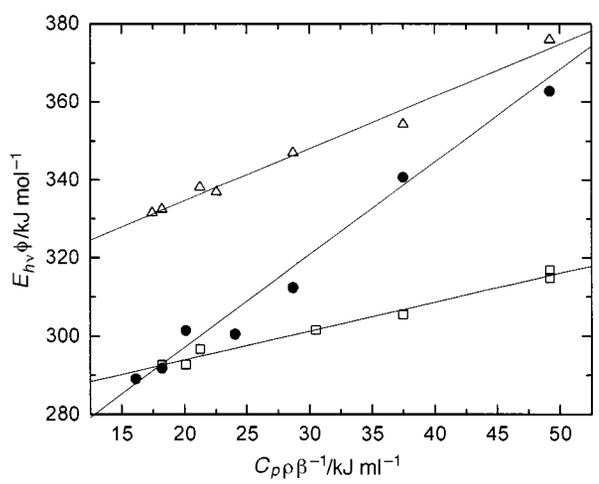
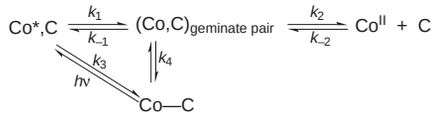


Fig. 4 Plots of $E_{h\nu}\varphi$ *vs.* $C_p\rho/\beta$ for AdoCbl (△) and MeCbl (□) in neutral aqueous solution, and MeCbl (●) in 0.05 M HCl aqueous solution (all are 1.5×10^{-5} M)

Co–C bond dissociation of both the six-co-ordinate base-on form and the five-co-ordinate unprotonated base-off species. Studies of the temperature-dependent axial-base equilibrium indicate that base-on forms are predominant for AdoCbl and MeCbl in neutral aqueous solution at room-temperature (*ca.* 95 and 97% at 25 °C).[5b,9b] Acidification of MeCbl produces base-off MeCbl where the DMBz group is not co-ordinated to Co^{III} and is protonated. The pK_a of DMBz in MeCbl is 2.7. In 0.05 M HCl aqueous solution, the base-off MeCbl is *ca.* 96%.[25] Therefore, this study only considers the signal contribution from the base-on form of AdoCbl and MeCbl in neutral solution or the base-off form of MeCbl in acidic solution.

A mechanism for photolytic cleavage of the Co–C bond is proposed in Scheme 2.[15]

As Chen and Chance indicated,[15] upon photolysis of AdoCbl and MeCbl, the excited Co*,C state can either relax to the



Scheme 2

ground state ($k_3 \approx 3 \times 10^{11}$ s^{-1}), or undergo cleavage to form a geminate pair ($k_1 \approx 3 \times 10^{12}$ s^{-1}).[26] Although thermolysis of cobalamin can also form a geminate pair, the rate is low enough (≈10^{-10} for AdoCbl and ≈10^{-13} s^{-1} for MeCbl at 25 °C)[5b,9b] to be negligible. As discussed elsewhere, there is a solvent 'cage' for the radical pair.[15,16,27] The caged geminate pair can either recombine to form the reactant or diffuse into the solvent to form free radicals. For AdoCbl and MeCbl geminate recombination rates in H_2O ($k_4 \approx 10^9$ s^{-1}) are competitive with cage escape ($k_2 \approx 1.8 \times 10^9$ s^{-1}).[16,28] This means that Co^{II} species and the free radical are the final products for our experimental time windows of *ca.* 100 ns. In the system, there are some radical side reactions, including hydrogen abstraction by the carbon radical from the solvent and/or the corrin ring ($k = 10^2$–10^4 s^{-1}),[15b] dimerization ($k \approx 10^{10}$ s^{-1}), cyclization ($k = 10^4$–10^5 s^{-1} for Ado) and diffusional recombination, *etc.*[5,15] Recombination of the methyl (10^9 M^{-1} s^{-1}) and 5′-deoxy-5′-adenosyl (10^8–10^9 M^{-1} s^{-1}) radicals with Co^{II} is found to be near the diffusion-controlled limit in H_2O.[5,15] For the sample concentrations of about 10^{-5} M, the concentration of the free radicals should be much less than 10^{-5} M. Our investigations on photo-dissociation of the Co–C bond are not contaminated by artefactual side reactions.

Quantum yields

Quantum yields for AdoCbl and MeCbl have been investigated by several groups.[14–17] Using nanosecond laser pulse with difference absorption spectra, Chen and Chance reported in 1990 that the nanosecond (ns) quantum yield of AdoCbl is 0.23 ± 0.04 and that there is no significant change in the quantum yield at two wavelengths (355 and 532 nm).[15a] Later, with a continuous-wave (CW) laser they measured the CW anaerobic quantum yield of AdoCbl ($\Phi = 0.20 \pm 0.03$, at 442 nm), which agrees with the ns quantum yield within experimental error.[15b] The measured CW anaerobic quantum yield of MeCbl ($\Phi = 0.35 \pm 0.03$, at 442 nm)[15b] is in agreement with its investigation by Pratt and Whitear that gave a quantum yield of *ca.* 0.30 with no wavelength dependence.[16] However, Endicott and Netzel obtained quantum yields of 0.16 ± 0.05 for MeCbl and 0.09 ± 0.035 for AdoCbl by a picosecond flash photolysis technique.[17] This result is consistent with that of Taylor *et al.*[18] where they based their yields on continuous photolysis with O_2 scavenging. Chen and Chance suggested that the lower quantum yields reported by Endicott's group are probably due to a calculation from the single wavelength absorbance changes at the Co^{II} peak absorbance and not taking into consideration the spectral overlap of various absorptions. Therefore, we adopt Chen's quantum yields, 0.23 for AdoCbl and 0.35 for MeCbl. However, as there is only a qualitative quantum yield for base-off MeCbl reported by Pratt[16] and Taylor,[18] we use $\Phi \leqslant 0.5$ in our calculations.

Enthalpy change and Co–C bond dissociation energy (BDE)

The enthalpy changes of photolysis for AdoCbl and MeCbl measured by PAC are given in Table 1. Since the apparent ΔH is a net enthalpy change from an excited state to forming a Co^{II} species and a free radical, which are homolytic decomposition products of the Co–C bond within our experimental time-scale, the values of ΔH are equal to those of Co–C BDE themselves, and most of the error in the measurement (10–15%) reflects the uncertainty in the quantum yield data. Besides, data for thermolysis for AdoCbl and MeCbl determined by kinetic methods at 80–110 °C are also listed in Table 1. As discussed by Finke *et al.*

the enthalpy changes are contributed to from two components: (a) dissociation of the Co–C bond forming a geminate pair and (b) diffusion of the geminate pair from the 'cage' to solvent forming a free radical.[27,29] The Co–C BDE is given by subtracting 13 kJ mol^{-1} from the activation enthalpy.[5b] It has been found that our Co–C BDE values obtained from the PAC method at 10–30 °C are consistent with the previously literary values for thermolysis dissociation determined by kinetic methods at 80–110 °C. Furthermore, for base-off MeCbl, there is no enthalpy change reported to date and it is even difficult to obtain by the kinetic methods. We have for the first time estimated the enthalpy change and Co–C BDE of base-off MeCbl by the PAC method to be $\geqslant 176 \pm 23$ kJ mol^{-1}. It is higher than that for the base-on form as expected.

Volume changes

A reaction volume change in solution has two components,[20–22] (a) the solvation volume change associated with property changes in the surrounding medium, such as polarity, electrostriction, and dipole interactions, *etc*., and (b) the intrinsic volume change related to the size of the molecules or ions, for example, formation or destruction of empty space that is too small to be occupied by solvent molecules.

Previous crystallographic[19,30,31] and 2-D NMR[32] investigations of cobalamins indicate that the main molecular movements associated with the cleavage of the Co–C bond for alkylcobalamins include, (a) the diffusion of the organic radical into the solvent from the 'cage', (b) upward movement of the corrin ring, (c) the shifts of the corrin-ring side chains. During photolysis of AdoCbl and MeCbl, the volume changes attributed to the medium reorganization[20–22] invoked by the molecular movements as mentioned above should be presented. According to the X-ray diffraction investigation on five-co-ordinated B_{12r},[31] the conformation of the corrin ring is upward with a folding angle 16.3°, which is larger that in AdoCbl (13.3°) and similar to that in MeCbl (15.8°). The solvent molecule near the axial co-ordination site is *ca*. 3.42 Å from the center in B_{12r},[31] which may form a small empty space, not occupied by the solvent molecule, around Co^{II}. Therefore, in the process of Co–C bond cleavage, it can contribute to the positive volume changes.

Our results have revealed that reaction volume changes for base-on AdoCbl (6 ± 1 ml mol^{-1}) is larger than for base-on MeCbl (2 ± 0.5 ml). Since the estimated radii for the Ado and Me radical are 6 and 1.1 Å, respectively, it is reasonable that the methyl radical is less interrupted by a solvent cage. Further, as discussed above, adenosyl is a bulky group, while methyl is small.[11,15,19,32a] Therefore conformational changes accompanying the cleavage of the Co–C bond and cage escape in AdoCbl should be larger than those in MeCbl. This gives rise to the reaction volume changes, both the solvational and the intrinsic volume changes for AdoCbl are larger.

Unfortunately, there is no single-crystal diffraction structural information for base-off B_{12r}. We cannot make structure comparison between base-off MeCbl and its photolytic product base-off B_{12r}. In the base-off species, as the bulky DMBz is not co-ordinated to the cobalt atom, the folding angle would be markedly decreased, and also the corrin ring, as well as its side chain, in the base-off form is much more flexible than that in the base-on form.[9a,10b,11] It suggests that with the dissociation of the Me group, there is much more conformational change for base-off MeCbl than for base-on MeCbl. This might contribute to the larger reaction volume change in photolysis of base-off MeCbl ($\geqslant 5 \pm 1$ ml mol^{-1}).

Conclusion

This paper reports photoacoustic calorimetry studies in the temperature range 10–30 °C, used to measure the energy changes for photo-induced Co–C bond homolysis of two kinds of coenzyme B_{12}. The resultant Co–C BDE values for AdoCbl and MeCbl in neutral water (base-on forms) are in good agreement with those obtained by kinetic methods at a higher temperature range (80–110 °C). Moreover, the Co–C BDE value of MeCbl in acid solution (base-off form) has also been determined by the PAC method, which would be larger than that of the base-on form and probably have been difficult to obtain by the kinetic method.

This is the first time that the quantitative structural volume changes for the photolysis of two kinds of cobalamin derivatives using temperature-dependent PAC studies in neutral and acidic solution have been obtained. These volume changes were suggested to be due to conformational changes of the corrin ring and its side chains accompanying the cleavage of the Co–C bond. The values of such volume changes are in the following order: base-on AdoCbl > base-on MeCbl and base-off MeCbl > base-on MeCbl. They reflect that the extent of the conformational changes depends on the bulkiness of the upper (α) ligand of the corrin or the co-ordination of the lower (β) nucleotide loop DMBz base with cobalt. The results give evidence about the flexing of the corrin ring, and the steric interactions between the corrin ring and the Ado group, which have been suggested to be important in enzyme-induced distortion and lability of the Co–C bond.

Acknowledgements

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References

1 (*a*) D. Dolphin (Editor), *B_{12}*, Wiley, New York, 1982; (*b*) J. M. Pratt, *Inorganic Chemistry of Vitamin B_{12}*, Academic Press, New York, 1972.
2 J. Halpern, *Science*, 1985, **227**, 869.
3 R. G. Finke, in *Molecular Mechanisms in Bioorganic Processes*, eds. C. Bleasdale and B. T. Golding, The Royal Society of Chemistry, Cambridge, 1990, p. 244; R. G. Finke, D. A. Schiraldi and B. J. Mayer, *Coord. Chem. Rev.*, 1984, **54**, 1.
4 L. G. Marzille, in *Bioinorganic Catalysis*, ed. J. Reedijk, Marcel Dekker, New York, 1993.
5 (*a*) R. G. Finke and B. P. Hay, *Inorg. Chem.*, 1984, **23**, 3041; 1985, **24**, 1278; (*b*) B. P. Hay and R. G. Finke, *J. Am. Chem. Soc.*, 1986, **108**, 4820.
6 J. Halpern, S.-H. Kim and T. W. Leung, *J. Am. Chem. Soc.*, 1984, **106**, 8317; 1985, **107**, 2199.
7 R. J. Blau and J. H. Espenson, *J. Am. Chem. Soc.*, 1985, **107**, 3530.
8 S.-H. Kim, H. L. Chen, N. Feilchenfeld and J. Halpern, *J. Am. Chem. Soc.*, 1988, **110**, 3120; M. K. Geno and J. Halpern, *J. Am. Chem. Soc.*, 1987, **109**, 1238.
9 (*a*) B. P. Hay and R. G. Finke, *J. Am. Chem. Soc.*, 1987, **109**, 8012; (*b*) B. D. Martin and R. G. Finke, *J. Am. Chem. Soc.*, 1990, **112**, 2419; 1992, **114**, 585; (*c*) M. D. Waddington and R. G. Finke, *J. Am. Chem. Soc.*, 1993, **115**, 4629.
10 (*a*) K. L. Brown and H. B. Brooks, *Inorg. Chem.*, 1991, **30**, 3420; (*b*) K. L. Brown and D. R. Evans, *Inorg. Chem.*, 1994, **33**, 6380.
11 (*a*) V. B. Pett, M. N. Liebman, P. Murray-Rust, K. Prasad and J. P. Glusker, *J. Am. Chem. Soc.*, 1987, **109**, 3207; (*b*) D. W. Christianson and W. N. Lipscomb, *J. Am. Chem. Soc.*, 1985, **107**, 2682.
12 K. L. Brown, D. R. Evans, S. Cheng and D. W. Jacobsen, *Inorg. Chem.*, 1996, **35**, 217; K. L. Brown, S. Cheng and H. M. Marques, *Inorg. Chem.*, 1995, **34**, 3038; K. L. Brown, H. B. Brooks, D. Behnke and D. W. Jacobsen, *J. Biol. Chem.*, 1991, **266**, 6737; K. L. Brown, X. Zou and D. R. Evans, *Inorg. Chem.*, 1994, **33**, 5713.
13 J. Halpern, *Pure Appl. Chem.*, 1983, **55**, 1059; T. Toraya and A. Ishida, *Biochemistry*, 1988, **27**, 7677; J. M. Pratt, *Pure Appl. Chem.*, 1993, **65**, 1513; M. D. Waddington and R. G. Finke, *J. Am. Chem. Soc.*, 1993, **115**, 4629.
14 (*a*) F. Mancia, N. H. Keep, A. Nakagawa, P. F. Leadlay, S. McSweeney, B. Rasmussen, P. Bösecke, O. Diat and P. R. Evans, *Structure*, 1996, **4**, 339; (*b*) C. L. Drennan, S. Huang, J. T. Drummond, R. G. Matthews and M. L. Ludwing, *Science*, 1994, **266**, 1669.

15 (*a*) E. Chen and M. R. Chance, *J. Biol. Chem.*, 1990, **265**, 12 987; (*b*) E. Chen and M. R. Chance, *Biochemistry*, 1993, **32**, 1480 and refs. therein.
16 J. M. Pratt and B. R. S. Whitear, *J. Chem. Soc. A*, 1971, 252.
17 J. Endicott and T. Netzel, *J. Am. Chem. Soc.*, 1979, **101**, 4000.
18 R. Taylor, L. Smucker, M. L. Hanna and J. Gill, *Arch. Biochem. Biophys.*, 1973, **156**, 521.
19 P. G. Lenhert and D. C. Hodgkin, *Nature* (*London*), 1961, **192**, 937; P. G. Lenhert, *Proc. R. Soc. London, Ser. A*, 1968, **303**, 45; H. F. J. Savage, P. F. Lindley, J. L. Finney and P. A. Timmins, *Acta Crystallogr.*, *Sect. B*, 1987, **43**, 296.
20 K. S. Peters and G. J. Snyder, *Science*, 1988, **241**, 1053; C. L. Norris and K. S. Peters, *Biophys. J.*, 1993, **65**, 1660; K. S. Peters, T. Watson and T. Logan, *J. Am. Chem. Soc.*, 1992, **114**, 4276; J. A. Westrick, K. S. Peters, J. D. Ropp and S. G. Sligar, *Biochemistry*, 1990, **29**, 6741; J. A. Westrick, J. L. Goodman and K. S. Peters, *Biochemistry*, 1987, **26**, 8313.
21 (*a*) S. E. Braslavsky and G. E. Heibel, *Chem. Rev.*, 1992, **92**, 1381; (*b*) J. L. Habib-Jiwan, A. K. Chibisov and S. E. Braslavsky, *J. Phys. Chem.*, 1995, **99**, 10 246; (*c*) I. Yruela, M. S. Churio, T. Gensch, S. E. Braslavsky and A. R. Holzwarth, *J. Phys. Chem.*, 1994, **98**, 12 789; (*d*) M. S. Churio, K. P. Angermund and S. E. Braslavsky, *J. Phys. Chem.*, 1994, **98**, 1776; (*e*) P. J. Schulenberg, W. Gärtner and S. E. Braslavsky, *J. Phys. Chem.*, 1995, **99**, 9617; (*f*) M. E. Van Brederode, T. Gensch, W. D. Hoff, K. J. Hellingwerf and S. E. Braslavsky, *Biophys. J.*, 1995, **68**, 1101; (*g*) J. L. Habib-Jiwan, B. Wegewijs, M. T. Indelli, F. Scandola and S. E. Braslavsky, *Recl. Trav. Chim. Pays-Bas.*, 1995, **114**, 542.
22 R. R. Hung and J. J. Grabowski, *J. Am. Chem. Soc.*, 1992, **114**, 351; J. L. Goodman and M. S. Herman, *Chem. Phys. Lett.*, 1989, **163**, 417; M. S. Herman and J. L. Goodman, *J. Am. Chem. Soc.*, 1989, **111**, 1849.
23 D. Dolphin, *Inorg. Synth.*, 1982, **20**, 152.
24 R. C. Weast (Editor), *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL, 67th edn., 1986–1987.
25 D. Dolphin, A. W. Johnson and R. Rodrigo, *R. Ann. N. Y. Acad. Sci.*, 1964, **112**, 590.
26 P. A. Anfinrud, C. Hans and P. M. Hochstrasser, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 8387; J. L. Martin, A. Migus, C. Poyart, Y. LeCarpentier, R. Astier and A. Antonetti, *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 173.
27 T. W. Koenig, B. P. Hay and R. G. Finke, *Polyhedron*, 1988, **7**, 1499.
28 T. T. Tsou, M. Loots and J. Halpern, *J. Am. Chem. Soc.*, 1982, **104**, 623.
29 C. D. Garr and R. G. Finke, *J. Am. Chem. Soc.*, 1992, **114**, 10 440; *Inorg. Chem.*, 1993, **32**, 4414.
30 M. Rossi, J. P. Glusker, L. Randaccio, M. F. Summers, P. J. Toscano and L. G. Marzilli, *J. Am. Chem. Soc.*, 1985, **107**, 1729.
31 B. Kräutler, W. Keller and C. Kratky, *J. Am. Chem. Soc.*, 1989, **11**, 8936.
32 (*a*) M. F. Summers, L. G. Marzilli and A. Bax, *J. Am. Chem. Soc.*, 1986, **108**, 4285; (*b*) A. Bax, L. G. Marzilli and M. F. Summers, *J. Am. Chem. Soc.*, 1987, **109**, 566; (*c*) T. G. Pagano, P. G. Yohannes, B. P. Hay, J. R. Scott, R. G. Finke and L. G. Marzilli, *J. Am. Chem. Soc.*, 1989, **111**, 1484; (*d*) T. G. Pagano, L. G. Marzilli, M. M. Flocco, C. Tsai, H. L. Carrell and J. P. Glusker, *J. Am. Chem. Soc.*, 1991, **113**, 531; (*e*) Y. W. Alelyunas, P. E. Fleming, R. G. Finke, T. G. Pagano and L. G. Marzilli, *J. Am. Chem. Soc.*, 1991, **113**, 3781; (*f*) A. Calafat and L. G. Marzilli, *J. Am. Chem. Soc.*, 1993, **115**, 9182.

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