One-electron Reduction Potentials of Coenzyme B_{12} and Alkylcobalamins

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Abstract

The one-electron reduction potentials for the alkylcobalamins, R = CH_3, CH_2CH_3, n-propyl, isobutyl, neopentyl and deoxyadenosyl, were examined by differential pulse polarography in 1:1 DMF:H_2O (μ = 0.10 $LiClO_4$) at 24.6 °C. The $E_{1/2}$ values for the couple $RCo + e^- \rightleftarrows RCo^-$ (RCo = alkylcobalamin) were found to be −1.60 V, R = CH_3; −1.54 V, R = CH_2CH_3; 1.55 V, R = n-propyl; −1.48 V, R = isobutyl; −1.38 V, R = neopentyl; −1.35 V, R = deoxyadenosyl versus SCE. $E_{1/2}$ correlates linearly with the Taft steric parameter, E_s; a new E_s value for the deoxyadenosyl functional unit is estimated to be −2.03 from this relationship. A moderate solvent influence was observed by replacing H_2O with D_2O for methylcobalamin ($E_{1/2}$ = −1.68 V versus SCE) and for deoxyadenosylcobalamin (coenzyme B_{12}) (−1.43 V). This suggests that solvation effects are about the same for alkylcobalamins compared to methylcobalamin and therefore do not account for the 0.22 V more negative reduction potential of methylcobalamin. The gradation in $E_{1/2}$ starting with methylcobalamin and continuing through the alkylcobalamin series may reflect changes in the axial ligand distances which modulate the energy of the lowest σ type MO (LUMO) of these complexes.

Introduction

The mechanism of carbon skeleton rearrangements promoted by vitamin B_{12}-requiring enzymes is the object of intensive current research [1]. Within this framework there exists decided differences of opinion regarding the mechanistic route or routes followed in vitamin B_{12}-related chemistry and biochemistry [2–4]. Two current models revolve around the ability of B_{12} or alkylcobalamin analogues to supply an organic free radical of the substrate. Many researchers believe that the rearrangement occurs directly from the organic free radical [4] or from a surface bound radical [1b]. Other researchers have gathered experimental evidence indicating that the rearrangement is mediated by steps involving cobalt-based chemistry. A recent study by Dowd *et al.* indicates that rate of rearrangement of carbon-centered free radical models are five orders of magnitude slower than the vitamin B_{12}-mediated methylmalonate to succinate model rearrangement [5]. The latter cobalt-based rearrangement might involve RCo^- radical ions (RCo = alkylcobalamin). Therefore there is interest in the ease of generation of such chemical species. The polarographic and voltammetric reductions of alkylcobalamins and cobinamides have been studied previously [6–9], but these early studies had to contend with absorption problems on mercury or the inability to detect reversible couples for species such as RCo^- [10]. The electrochemical behavior of vitamin B_{12}, aquocobalamin and methylcobalamin was reviewed in 1983 by Lexa and Saveant [9b], who noted that additional studies on the electrochemistry of alkylcobalamins are desirable. Detailed examination of the electrochemistry of the aquocobalamin complex as a function of pH has been described [9b, 11]. Inferences concerning the anticipated reactivities of the alkylcobalamins have been drawn from these studies, but suitable experiments have not been described in the chemical literature.

The most reliable estimate of a $RCo + e^- \rightleftarrows RCo^-$ couple was obtained for methylcobalamin by Lexa and Saveant [12] who determined a one-electron reversible wave at −1.60 V versus SCE in 1:1 DMF:propanol at −20 °C (μ = 0.10, NBu_4BF_4) [12]. This result differs from earlier polarographic values of $E_{1/2}$ for alkylcobalamins which were determined with a dropping mercury electrode [13]. The latter study reported very similar values of $E_{1/2}$ for all the alkylcobalamins: (R, $E_{1/2}$ versus SCE) CH_3, −1.39 V; CH_3CH_2, −1.37 V; $CH_3CH_2CH_2$, −1.37 V; $HOCH_2CH_2$, −1.39 V; $CH_3OCH_2CH_2$, −1.38 V; deoxyadenosyl, −1.37 V [13].

We undertook to re-evaluate the one-electron reduction potentials of coenzyme B_{12}, methylcobalamin and their ethyl and n-propyl analogues at the glassy carbon electrode where absorption effects are minimized. Additionally the $E_{1/2}$ values for neopentylcobalamin and isobutylcobalamin were determined and are reported here for the first time. The

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neopentyl functionality may be a useful steric and inductive model for alkylcobalamins [14] because alkylcobalamins (except methylcobalamin) possess an additional R′ functionality in the alkyl fragment (CH_2R') which may contribute to steric, inductive and solvation influences in the vicinity of the Co–R bond.

Steric factors are reputed to destabilize the Co–R bond of alkylcobalamins [15–17] and to influence Co–C bond lengths in vitamin B_{12} model complexes [18]. Steric factors for the alkylcobalamins with CH_2R' units are important enough to induce changes in the Co–C–C angle away from the tetrahedral value, and this may be indicative of changes in hybridization of the carbon bonded to cobalt [19]. Angular distortions weaken the Co–R bonds. Zhu and Kostic carried out Fenske–Hall iterative molecular orbital calculations on a coenzyme B_{12} model system [20]. The model contains the corrin macrocyclic ring together with axial methyl and imidazole ligands to approximate the coenzyme B_{12} core structure. Distortion of the Co–CH_2–R′ angle to 125°, as found in coenzyme B_{12} [21], reduced the Co–C overlap by 43%. Zhu and Kostic concluded that the probable cause of reactivity of coenzyme B_{12} is the *cis* interaction of the bulky axial R group (deoxyadenosyl) with the corrin ring which results in distortion of the R group itself, and weakens the Co–R bond [20].

Alkyl steric effects in cobalamins suggest that conformational changes in the corrin ligand may occur as a function of branching in the alkyl group [15a]. This conformational effect may be the source of the 'triggering' of Co–C bond cleavage produced by the enzyme [10, 15]. It would be predicted that methylcobalamin would be the least hindered and possess the shortest Co–C bond and the greatest 'bending up' of the corrin ring [22]. Indeed, the CH_3 ligand has the shortest Co–C bond among the alkyl cobaloximes and longer bonds are observed for more branched alkyls ($CH_3 < CH_2C(CH_3)_3 < CH(CH_3)_2$) [17, 23]. Therefore, it is surprising that the X-ray structure of methylcobalamin shows little difference from that of coenzyme B_{12} or vitamin B_{12} (CN in place of CH_3) [24]. The angles between the planes of the 'folded' corrin rings differ by only 1.2° (15.8 for R = CH_3; 14.6° for R = deoxyadenosyl) [24]. The Co–C bonds differ slightly (1.99 Å for R = CH_3 and 2.05 Å for R = deoxyadenosyl) but are nearly equivalent within the reported e.s.d.s [24]. Furthermore, the recent X-ray structure of the five-coordinate Co^{II} cobalamin (B_{12r}) shows little difference in the atom positions of the corrin ring compared to coenzyme B_{12} [25]. The Co^{II} center is, however, displaced 0.12 Å below the four corrin N donors. Based on the similarity of the B_{12r} structure to that of coenzyme B_{12}, Krautler *et al.* proposed that the main apoenzyme/coenzyme interaction, which leads to steric assistance of the Co–R bond homolysis, occurs by stabilization of separated B_{12r} and deoxyadenosyl (or alkyl) radical fragments [25]. The similarity of the coenzyme B_{12} structure in the corrin region to that of B_{12r} may lower the activation barrier for the Co–R homolysis since little reorganization of the corrin ring of coenzyme B_{12} is necessary to form B_{12r}. Therefore, all alkylcobalamins would be predisposed to dissociate into the B_{12r},R$^{\bullet}$ radical pair with little change of the large corrin structure. Extended logically this argument would imply nearly independent chemical and structural behaviors for various alkylcobalamins in the absence of the apoenzyme. The issue of the extent of distortion of the corrin region induced upon binding of coenzyme B_{12} to an apoenzyme remains to be determined. Properties which reflect on differences in reactivity of methylcobalamin, coenzyme B_{12} and other alkylcobalamins are therefore of substantial interest.

2,3-Dihydroxypropylcobalamin exists as *R*- and *S*-isomers which have been isolated and crystallized [24b–d]. The *S*-isomer is compromised by hydrogen bonding to the corrin nucleus, but the *R*-isomer is not complicated by hydrogen bonding and shows a carbon–cobalt bond comparable to that of methylcobalamin. We report our findings on the reduction potentials of alkylcobalamins in H_2O:DMF and D_2O:DMF media.

Results and Discussion

We recognized that the rapid bond dissociation of the reduced alkylcobalamin radical ions would eliminate reversible cyclic voltammetric (CV) waves except at high scanning rates. These sweep rates were not accessible with our instrumentation (IBM 225 Electrochemical Analyzer). In order to circumvent this problem we obtained both CV and differential pulse polarograms (DPP). The CV waves of the alkylcobalamins exhibited a single reduction wave forming RCo^{-} in the negative potential region, positive of the solvent reduction ramp. The reoxidation wave for $CoR^{-} \rightarrow RCo$ was absent as anticipated for the rapid bond dissociation of CoR^{-} into B_{12s}-(Co^{I}) and R$^{\bullet}$. Reduction waves for the aqua $Co^{II/I}$ and $Co^{III/II}$ couples were absent for purified samples of the alkylcobalamins (R = CH_3, CH_2CH_3, CH_2CH_2-CH_3, isobutyl, neopentyl, deoxyadenosyl) on the first reductive sweep of either the CV of DPP voltammograms. The waves of the aquocobalamin species, formed following dissociation of RCo^{-}, are clearly present in the reverse oxidation cycle of the CV voltammograms.

The DPP technique has the advantage of determining the electrochemically reversible $E_{1/2}$ value from the peak potential of the DPP wave [26]. This is true even for systems which possess theoretically

reversible electrochemical waves, but which are complicated by a chemical step subsequent to electron transfer at the electrode surface. With the alkylcobalamins, the rapid dissociation of $R^{\cdot}$ from the reduced radical anion RCo^{-}, which liberates B_{12s}, is not a problem for the determination of the $E_{1/2}$ values as long as the $RCo + e^{-} \rightleftarrows RCo^{-}$ couple is theoretically reversible. The criteria for reversibility is met when the DPP wave has a width at half-height of *c.* 90 mV.

We carried out studies of the $RCo + e^{-} \rightleftarrows RCo^{-}$ couples in 1:1 DMF:H_2O at 25 °C with 0.10 M $LiClO_4$ as the ionic strength control. Lexa and Saveant reported previously that mixing DMF 1:1 with H_2O lowers the activity of H_2O sufficiently that one can obtain reliable electrochemical data with methylcobalamin prior to the solvent reduction ramp at *c.* −1.8 V versus SCE [12]. A study of the methylcobalamin complex was undertaken to reproduce the results of Lexa dn Saveant under our conditions. The results are shown in Fig. 1 for a 3.59×10^{-3} M solution of methylcobalamin. All electrochemical data were obtained on Ar purged samples which were examined in the dark in order to prevent photochemical cleavage of the Co–R bonds. The DPP wave for the reduction sweep exhibits one wave at −1.60 V versus SCE confirming the value of Lexa and Saveant. The DPP width at half-height is 100 mV, indicative of a nearly reversible couple at 25 °C. The CV wave shows only the reduction wave of RCo (R = CH_3) on the reduction sweep without the oxidation counterpart; waves for the $Co^{I/II}$ and $Co^{II/III}$ steps are seen on the reoxidation sweep. These species are absent in the purified CH_3Co initial CV scan.

The same procedures were applied to coenzyme B_{12} and the neopentylcobalamin complex. Initial CV scans in the +0.60 to −1.0 V region show that both preparations were free of aquated species (aquocobalamin). However, well defined waves for the $RCo + e^{-} \rightleftarrows RCo^{-}$ couple are determined at −1.35 V versus SCE for 3.93×10^{-3} M coenzyme B_{12} and −1.38 V versus SCE for 5.19×10^{-3} M neopentylcobalamin (Figs. 2 and 3). A separate study of the authentic B_{12a}, aquocobalamin complex, at 4.66×10^{-3} M is shown in Fig. 4 (pH = 6.78). Both the $Co^{II/III}$ and $Co^{II/I}$ waves are readily detected by CV and DPP at −0.07 and −0.91 V versus SCE in good agreement with reported values at pH 7.1 of −0.03 and −1.07 V (determined at the DME), μ = 0.02 with KCl [13] and with −0.04 and −0.89 V (Hg–Au minielectrode), μ = 0.50 with KCl [11].

A pH-dependence study (not shown) was made for the neopentylcobalamin complex. Adjustment of the pH from 7.0 to 2.94 with HCl, followed by elevation of the pH to 12.02 with NaOH and returned to pH 7.02, produced $E_{1/2}$ values of −1.38 (pH =

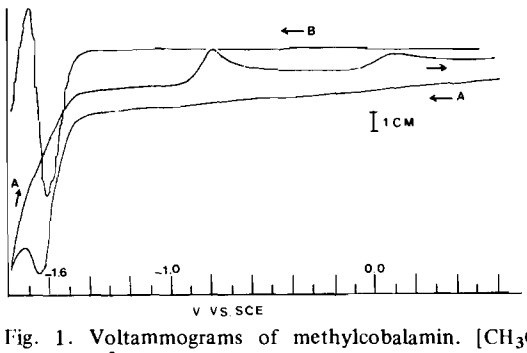

Fig. 1. Voltammograms of methylcobalamin. [CH_3Co] = 3.59×10^{-3} M, μ = 0.10 $LiClO_4$, 1:1 DMF:H_2O, T = 24.6 °C, pH = 7.62. (A) CV sweep 50 mV/s, *y* axis 10 μA/cm; (B) DPP sweep 40 mV/s, *y* axis 0.25 μA/cm.

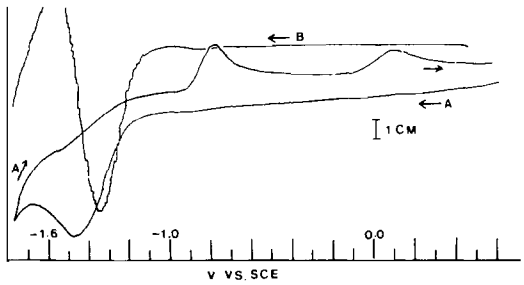

Fig. 2. Voltammograms of coenzyme B_{12}. [coenzyme B_{12}] = 3.93×10^{-3} M; all other settings as in Fig. 1 except *y* axis on the DPP curve is at 0.10 μA/cm; pH = 8.03.

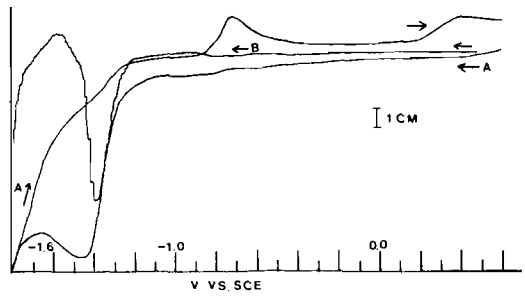

Fig. 3. Voltammograms of neopentylcobalamin. [$(CH_3)_3CH_2Co$] = 5.19×10^{-3}; all other settings as in Fig. 1; pH = 7.62.

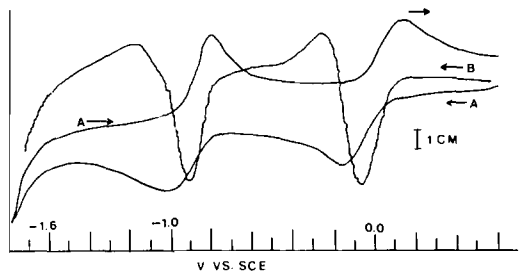

Fig. 4. Voltammograms of aquacobalamin. [$(H_2O)Co$] = 4.66×10^{-3} M; all other settings as in Fig. 2; pH = 6.78.

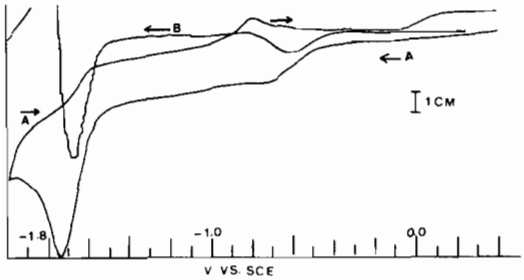

Fig. 5. Voltammograms of methylcobalamin in D_2O:DMF. $[CH_3Co] = 3.70 \times 10^{-3}$ M, $\mu = 0.10$ Bu_4NClO_4; all other settings as in Fig. 1; pD = 8.06.

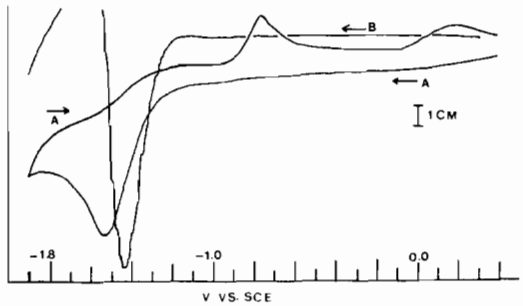

Fig. 6. Voltammograms of coenzyme B_{12} in D_2O:DMF. [coenzyme B_{12}] = 4.00×10^{-3} M, $\mu = 0.10$ Bu_4NClO_4, pD = 8.03; all other settings as in Fig. 2.

6.78), −1.36 (pH = 2.94), −1.49 (pH = 12.02) and −1.42 (pH = 7.02) V for the $RCo + e^- \rightleftarrows RCo^-$ couple. Above pH = 2.94 the couple is nearly pH-independent unless high concentrations of OH^- are available to displace or deprotonate the axial benzimidazole; this results in a negative shift in the reduction potential and a slow recovery for recoordination of the axial base on return to pH ~ 7.

A study of the $RCo + e^- \rightleftarrows RCo^-$ couple for R = CH_3 was made in D_2O (Fig. 5) at pD values of 8.06, 12.10, 4.97, 2.12, 6.14 in the specified order. The reduction wave shifts from −1.60 V versus SCE in H_2O to −1.68 V in D_2O. This potential remained constant for all pD values $\geqslant$ 4.97. At pD = 2.12 ~ 90% of the complex is present as a complex reducible at −1.15 V; 10% at −1.68 V. Return to pD 6.14 recovered 100% of the complex as the initial species reducible at −1.68 V. Protonation of the axial benzimidazole, and its dissociation are inferred from the pD = 2.12 behavior. The pK_a for protonation of the axial benzimidazole is 3.2 for the aquocobalamin complex [11, 27]. The distribution of forms at pD 2.12 implies a similar pK_a of 3.1 for protonation of the axial benzimidazole of methylcobalamin.

The influence of D_2O on the reduction potential of methylcobalamin is of interest in regard to its $E_{1/2}$ relative to other alkylcobalamins. D_2O produces a negative shift of *c.* 0.08 V. Since the molecular orbital which receives the electron is the same, it would appear that D_2O is less good at solvating either RCo or RCo^- than H_2O, but that the greatest difference involves the solvation shell of the RCo^- radical ion. The influence is small (1.84 kcal/mol), but close to the 1.0 kcal difference between H_2O and D_2O in stabilizing lyonium and lyate ions of these solvents [28].

It is tempting to ascribe the influence of the $E_{1/2}$ shift in D_2O of methylcobalamin to changes in H-bonding and solvation in the vicinity of the Co–CH_3 bond. However, the shift in potential by 0.08 V to a more negative value must be explained by a more general solvation phenomena. The $E_{1/2}$ for the B_{12} coenzyme was also investigated in 1:1 D_2O:DMF (Fig. 6). The wave again shifts by 0.08 V from −1.35 V SCE for the 1:1 H_2O:DMF solvent to −1.43 V in 1:1 D_2O:DMF at pD 8.03. Therefore, the influence of D_2O on the $E_{1/2}$ value of reduction of alkylcobalamins is similar for the more substituted CH_2R' moiety (deoxyadenosyl in the coenzyme B_{12} case) to that of methylcobalamin. A pD study at sequential values of 8.03, 12.24, 4.12, 2.07 and 6.01 determined one-electron potentials of −1.43, −1.46, −1.46, −1.35 and −1.46, respectively, for the wave. The experiment at pD 2.07 showed substantial waves at −1.58 and −1.82 V; both were removed and the wave at −1.46 V reappeared upon return to pD 6.01. These changes in the $E_{1/2}$ value of coenzyme B_{12} parallel those described above for the neopentyl complex and illustrate the protonation and loss of the benzimidazole group at low pH; the latter process is reversible upon return to physiological pH.

Our results show that the methylcobalamin reduction potential is more negative than that of deoxyadenosylcobalamin and confirm the potential of −1.60 V versus SCE of Lexa and Saveant [12]. It has generally been believed that the reduction potentials of other alkylcobalamins are insensitive to the nature of R as the axial donor [10, 13]. (Extreme electronic alteration by substituted groups is known to alter the reduction potential; for example, the peak reduction CV wave for R = CF_3 is about 0.29 V more positive than for R = CH_3 [29].) Accordingly, a re-examination of the CoR reduction potentials for R = CH_2CH_3 and R = $CH_2CH_2CH_3$ and the determination of this couple for R = isobutyl were carried out. The DPP voltammograms established the $E_{1/2}$ values for reduction of RCo as −1.54 V, R = CH_2CH_3; −1.55 V, R = $CH_2CH_2CH_3$; −1.48 V, R = $CH_2CH(CH_3)_2$. The CV and DPP waves for 5.0×10^{-3} M in solutions are shown in Fig. 7 at pH = 8.67 for the n-propyl and Fig. 8 at pH = 7.70 for the isobutyl derivatives. $Ru(NH_3)_6^{3+}$ was added as its chloride salt to the n-propylcobalamin and isobutylcobalamin solutions as an additional calibration marker. The reversible $Ru(NH_3)_6^{2+/2+}$ couple is detected by the waves at −0.19 V versus SCE as well as the RCo reduction waves at −1.55 V (R =

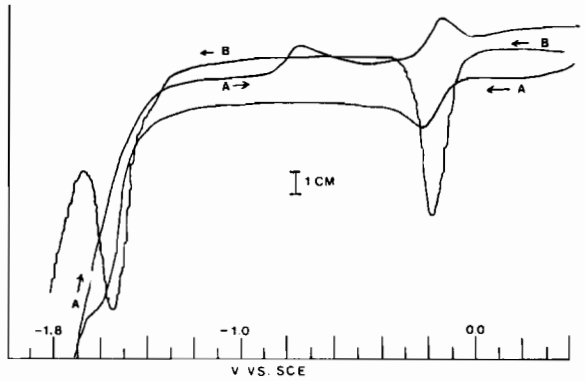

Fig. 7. Voltammograms of n-propylcobalamin. $[CH_3CH_2CH_2Co] = 5.0 \times 10^{-3}$ M; $\mu = 0.10$ $LiClO_4$, 1:1 DMF:H_2O, T = 24.6 °C, pH = 8.67. (A) CV sweep 50 mV/s, y axis 10 μA/cm; (B) DPP sweep 40 mV/s, y axis 5 μA/cm; $[Ru(NH_3)_6]Cl_3$ added as an internal standard.

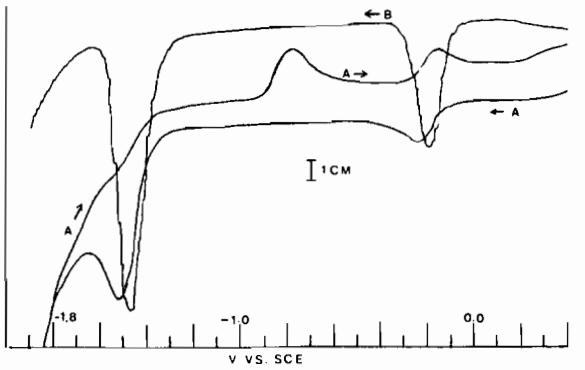

Fig. 8. Voltammograms of isobutylcobalamin. $[(CH_3)_2CHCH_2Co] = 5.0 \times 10^{-3}$ M, pH = 7.70; all other settings as in Fig. 7.

n-propyl, Fig. 7) and −1.48 V (R = isobutyl, Fig. 8). The presence of $Ru(NH_3)_6^{3+}$ did not produce oxidation of the alkylcobalamins; CV and DPP waves obtained prior to the addition of the $[Ru(NH_3)_6]Cl_3$ standard were identical except for the absence of the $Ru^{III/II}$ calibration couple.

These results show the $RCo + e^- \rightleftarrows RCo^-$ couples are sensitive to the nature of the alkyl group and that a gradient of potentials spanning 0.25 V is observed upon changing the alkyl group from R = CH_3 to R = deoxyadenosyl. Grate and Schrauzer observed that the log of the rate constant for decomposition of alkylcobalamins correlates linearly with the steric bulk of the R group [15a]. The $-E_{1/2}$ for the RCo reduction potentials determined in our current study are plotted against Taft's steric parameter, $-E_s$ (Fig. 9) [30]. An excellent linear correlation (r^2 = 0.999) is obtained for R = methyl, n-propyl, isobutyl and neopentyl; the point for R = ethyl deviates only modestly from the least-squares line of slope 0.113 ± 0.016 and intercept 1.58 ± 0.014 V for $-E_{1/2}$ of a

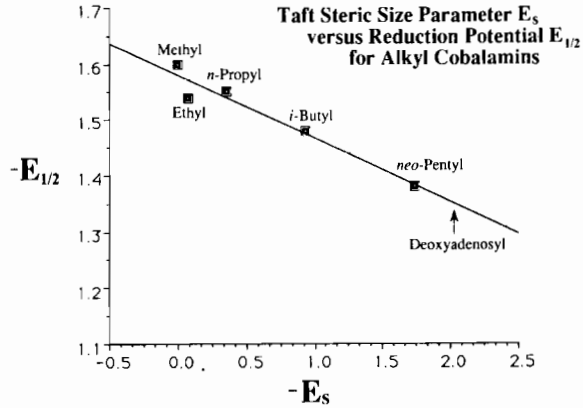

Fig. 9. Correlation of alkylcobalamin $E_{1/2}$ potentials with the Taft steric parameter E_s.

cobalamin having a steric constant of E_s of 0.00. An interesting outcome of the linear relationship of $E_{1/2}$ versus E_s is that the steric parameter for deoxyadenosyl as the R functional group may be estimated. $-E_s$ for deoxyadenosyl is found to be 2.03 (Fig. 9) on Taft's scale of steric impact for organic groups. The closest substituent on Taft's list which provides a comparable steric effect is $(C_2H_5)_2CH$ with $-E_s$ of 1.98 [30]. It is difficult to ascribe the correlation of $E_{1/2}$ versus E_s solely to the influence of the size of the R group in the alkylcobalamins because the inherent increase in electron release to the carbon attached to cobalt parallels the complexity and branching of the organic group. Taft noted that for the smaller R groups E_s parallels a parameter E_σ. The latter is supposed to account for electronic release through induction within an R group. However, the ability of units at greater distances to influence the overall effect of an R group should level off for effects dominated by induction. The steady decrease in $E_{1/2}$ versus E_s for the isobutyl and neopentyl derivatives argues that steric factors are the major influence in modulating the energy of the LUMO for the alkylcobalamin series.

A somewhat similar correlation has been made by Costa *et al.* in the reduction potentials of alkyl substituted cobaloximes, $Co(DH)_2(H_2O)R$ [31a]. A plot of the $E_{1/2}$ value for the $Co^{III/II}$ potentials is linear in the Co–R distance for four complexes (R = CH_3, $CHCH_2$, $CH_2C(CH_3)_3$ and $CH(CH_3)_2$). Costa *et al.* noted that the Co–R distance follows the steric bulk of the R group as supported by X-ray data on the series as described by Bresciani-Pahor *et al.* [31b].

The most negative $E_{1/2}$ value of methylcobalamin and progressively less negative $E_{1/2}$ values for those of other alkylcobalamins is consistent with a parallel structural variations among the alkylcobalamins. The study of Marzilli *et al.* shows only minor differences, particularly in regard to the corrin rings, between the

structure of methylcobalamin and coenzyme B_{12} (R = deoxyadenosyl) in the solid state [24]. There are, however, differences in the Co–C and Co–benzimidazole axial bonds with *c.* 0.05 Å longer bonds for coenzyme B_{12}. An electron added to RCo should enter an orbital having significant d_{z^2} metal character. An MO treatment of Salem *et al.* yielded the calculated result that the lowest unoccupied levels in alkylcobalamins are a corrin level, π_8, and a σ level generated by the antibonding combination of d_{z^2} and the σ orbital of the axial R group [32]. These levels are close in energy. The energy of σ relative to π_8 depends on the extent of mixing with the σ level of the alkyl ligand and on estimates placed on the interaction with the corrin π_7 MO. An earlier MO treatment of Schrauzer *et al.* found the d_{z^2}-based MO slightly below π_8 in energy as the LUMO in coenzyme B_{12} and in cobaloxime models [33]. Therefore, a theoretical basis has been established for a sensitivity in the energy of the lowest unfilled, metal-based orbital of alkylcobalamins to the overlap with the σ alkyl orbital and hence the distance between Co and R. The shorter bonds in methylcobalamin should raise this energy level relative to other alkylcobalamins having longer Co–C and Co–benzimidazole bonds. This reasoning has been applied previously to the homolytic bond dissociation process in a computational model for coenzyme B_{12} by Mealli *et al.* [34]. The prediction of the dependence of the MO energies on the axial bond distances has been shown [34]. However, the correctness of the conclusion that these factors are the source of the difference in the $E_{1/2}$ value of methylcobalamin with other alkylcobalamins must await additional structural studies of other alkylcobalamins. The gradient in $E_{1/2}$ for the other alkylcobalamins with that of coenzyme B_{12} suggests that the more substituted alkylcobalamins should have Co–R and Co–benzimidazole bonds intermediate between those in coenzyme B_{12} and methylcobalamin. In the absence of special hydrogen bonding influences of an alkyl group toward peripheral corrin ring substituents, all the known Co–C distances for alkylcobalamins are within 0.09 Å and the axial Co–N distances vary by about 0.21 Å [24b]. (The *S*-isomer of 2,3-dihydroxypropylcobalamin is known to possess abnormally long Co–N (axial) and Co–C bonds of 2.36 and 2.08 Å [24c, d], but this alkyl cobalamin is strongly distorted by peripheral group hydrogen bonding.) Since the observed Co–R and Co–N(axial) bond differences are already small between these two extremes, it may be that the solid state structures are altered by packing effects which minimize differences in the bond distances in the solid state from those present when these species are solvated. Thus there may be greater structural differences for alkylcobalamins in solution than is suggested by X-ray data. Christianson and Lipscomb have carried out self-consistent-field (SCF) calculations on a distorted octahedral Co^{III} complex possessing four in-plane N donors (three ammonias and one amide) and axial CH_3 and NH_3 donors [35]. This system approximates the stereoelectronic environment of coenzyme B_{12}. With bond distances assigned in the model complex to be those of the coenzyme B_{12} structure, the SCF energy of the molecule was studied by sequential variations in the Co–CH_3 and Co–N(axial) distances and then with the upward 'puckering' of the in-plane donors. These SCF calculations reveal a rather soft minimum over the range of Co–C distances of 1.95 to 2.15 Å and Co–N(axial) distances of 1.98 to 2.20 Å. Thus the calculated energy of the molecule is not particularly sensitive to changes in the axial bond distances [35], even though the energy of the σ based LUMO is sensitive to these changes [34]. The effect of puckering of the in-plane donors was small. Christianson and Lipscomb concluded that if a corrinoid distortion is catalytically important in B_{12}-dependent mechanisms, the influence of a corrinoid distortion is strictly transmitted by steric/conformational means rather than electronic changes in alkylcobalamins. These calculations are consistent with the concept that energy minimizing adjustments in forming alkylcobalamin solid state structures may occur with changes in the axial bond positions from those adopted for the solution species. It is possible that a chemical method, such as the determination of the $E_{1/2}$ value of each complex, would prove to be more indicative of the steric influence of the R group than is found for Co–R distances in the solid state.

Whether there are significant differences between the structure of the corrin region in solution from the structure it has in the solid state was recently addressed by detailed NMR experiments [36]. The presence or absence of the axial benzimidazole ligand does not appear to influence the structure in the corrin portion of coenzyme B_{12} [36]. 2D NMR methods have shown that the corrin structure remains constant for the base-on and base-off forms of coenzyme B_{12} in solution [36]. This implies that the corrin region remains rigid and does not respond with a release of strain when the axial benzimidazole ligand is off. One must then conclude that any differences in the structures of various alkylcobalamins must represent the steric effect of the R group in making contact with the corrin portion of the molecule rather than differences in 'folding-up' of the corrin region. This would account for the linear response of the $E_{1/2}$ value (and the energy of the metal-based σ MO) to the Taft steric parameter, E_s. Although the overall SCF energy of the RCo complexes is relatively insensitive to the Co–R distance [35], the energy of the σ LUMO is quite sensitive to the Co–R distance [34]. The electrochemical data determined in our current study imply that the Co–R

distance is intimately influenced by the steric bulk of the alkyl group in alkylcobalamins. This effect need not involve changes in the corrin position and is consistent with a rigid structure for the corrin portion of coenzyme B_{12} in the base-on and base-off forms [36]. The presence of the apoenzyme could contribute additional assistance to weakening the Co–R bond including steric perturbations by folding up the corrin portion of coenzyme B_{12}. Our study shows that significant differences in reactivity within a series of alkylcobalamins can be observed in the absence of any change in the extent of corrin puckering.

Experimental

Electrochemical studies were performed on an IBM 225 electrochemical analyzer operating in the cyclic voltammetry and differential-pulse modes. A glassy-carbon working electrode, Pt-wire auxiliary electrode and sodium chloride saturated calomel (SSCE) as reference were employed. The electrolyte solution was 0.10 M $LiClO_4$; $T = 24.6$ °C. Suitable standard one-electron reversible calibration waves ($E_{1/2} = 0.072$ V versus NHE) were recorded using $[Ru(NH_3)_6]Cl_3$ in 0.10 M NaCl aqueous solution and were shown to be identical with the same waves in the 1:1 DMF:H_2O, $\mu = 0.10$ $LiClO_4$ medium. Sweep rates of 50 mV/s (CV) and 40 mV/s (DPP) were used in obtaining voltammograms as described previously [37]. The glassy-carbon surface was rigorously cleaned after a series of voltammograms was obtained for each alkylcobalamin. Cleaning steps included treatment with H_2O_2 in 1.0 M HCl, polishing with alumina followed by an H_2O rinse, and or overnight soaking in 1.0 M HCl.

The alkylcobalamins were prepared by standard methods used in former studies [38]. The product alkylcobalamins were chromatographed on acid-washed cellulose, eluting with water. Purity was further established by a single spot on thin layer chromatograms on cellulose developed by the solvent mixture n-butyl alcohol:acetic acid:water (10:3:7). The purified alkylcobalamins were mixed 1:1 with DMF. Ionic strength control was then achieved by adding 3.30 M $LiClO_4$. Total electrochemical cell volume was 15.0 ml. The chromatography solvent was removed with vacuum distillation prior to addition of D_2O for the D_2O:DMF studies (see text). NBu_4ClO_4, added as a known weight of the solid to achieve $\mu = 0.10$, served as the supporting electrolyte for the D_2O:DMF experiments.

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