Thermolysis of the Co–C Bond in Adenosylcorrins. 3. Quantification of the Axial Base Effect in Adenosylcobalamin by the Synthesis and Thermolysis of Axial Base-Free Adenosylcobinamide. Insights into the Energetics of Enzyme-Assisted Cobalt–Carbon Bond Homolysis

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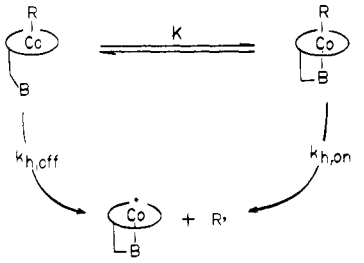
Abstract: A simple one-step synthesis of [adenosylcobinamide]$^+OH^-$ (AdoCbi$^+OH^-$), the base-free derivative of adenosylcobalamin (AdoB$_{12}$), is reported. The purity and identity of this product were verified by HPLC, UV–vis, ^{1}H NMR, FAB–MS, elemental analysis, and TGA. The anaerobic, aqueous thermolysis and photolysis of AdoCbi$^+OH^-$ were then investigated. Product studies establish the photolysis of AdoCbi$^+OH^-$ to proceed exclusively through Co–C bond homolysis. Product and kinetic studies establish that thermolysis of AdoCbi$^+OH^-$ in pH 7.0 aqueous phosphate buffer proceeds via competing homolytic (≥93%) and heterolytic (≤7%) Co–C bond cleavage in a fashion analogous to the thermolysis of AdoB$_{12}$. The temperature dependence of the rate of AdoCbi$^+OH^-$ Co–C bond homolysis is reported from 105.0 to 130.0 °C, yielding $\Delta H^{\ddagger}_h$ = 37.5 ± 1.2 kcal/mol and $\Delta S^{\ddagger}_h$ = 16 ± 3 cal/mol·K. Combining these thermolysis results with other data yields an estimate of 34.5 ± 1.8 kcal/mol for the base-off Co–C BDE (bond dissociation energy) of AdoB$_{12}$, the largest Co–C BDE reported for an alkylcobalt–corrinoid or other alkylB$_{12}$ model compound. Calculated 25 °C rate constants for the thermally induced Co–C bond homolysis of base-off ($10^{-11\pm1}$ s^{-1}) and base-on ($10^{-9\pm1}$ s^{-1}) AdoB$_{12}$ are compared to literature rate constants for enzyme-induced homolysis (≥10^2 s^{-1}), revealing a ≥10^{13} rate enhancement upon going from base-off AdoCbi$^+$ to base-on AdoB$_{12}$ in the holoenzyme. The results also establish that the axial base plays a more minor role than previously thought, at least in the absence of the enzyme, accelerating the AdoB$_{12}$ Co–C bond homolysis by only ≃10^2.

The recent demonstration of the reversible thermal homolysis of the Co–C bond of AdoB$_{12}$ (adenosylcobalamin or B$_{12}$ cofactor, Figure 1)[1,2] has greatly strengthened the case for this step as an essential role of AdoB$_{12}$ in vivo.[3,4] Rate constants for the thermally induced Co–C bond homolysis of AdoB$_{12}$ have been determined in ethylene glycol[1] and in aqueous solution,[2] and an estimate of 30–31 kcal/mol for the Co–C BDE (bond dissociation energy) of AdoB$_{12}$ in the base-on form has been determined.[5] In addition, these earlier studies revealed that the rate of Co–C bond homolysis is ≥10^{10} times faster in the holoenzyme than in the isolated cofactor.

In order to understand this remarkable rate enhancement, our approach has been to identify and quantify "AdoB$_{12}$ localized" components (axial base, steric and electronic character of the alkyl ligand, corrin conformation, cage effects, etc.) that might contribute to the observed ≥10^{10} rate enhancement and then, by difference, to obtain a quantitative idea of the role played by the enzyme. While the participation of the axial base has been widely discussed as a key component in facilitating Co–C bond cleavage,[6-9] this has not been confirmed nor quantified for the cofactor itself, AdoB$_{12}$.

Scheme I



$$(eq\ 1)\quad k_h = \left[\frac{K}{K+1}\right]k_{h,on} + \left[\frac{1}{K+1}\right]k_{h,off}$$

Theoretically, Co–C bond homolysis in AdoB$_{12}$ can occur through both the base-on and base-off forms, Scheme I, so that the observed homolysis rate constant, k_h, may be composite in nature (see eq 1, Scheme I). Studies of cobalt–corrinoids other than AdoB$_{12}$ have shown that alkylB$_{12}$s (axial base present) undergo thermal Co–C bond cleavage much more rapidly than alkylcobinamides (lacking the axial base).[6] The assumption that AdoB$_{12}$ will exhibit similar reactivity greatly simplifies the observed kinetic scheme,[1,2] i.e., that $k_{h,on} \gg k_{h,off}$ so that eq 1 can be simplified to $k_h = (K/(K+1))\cdot k_{h,on}$. However, in order to test the validity of this assumption, a base-off analogue of AdoB$_{12}$ was required.

Herein we report: (a) an improved synthesis and unequivocal characterization of adenosylcobinamide (AdoCbi$^+$), a derivative of AdoB$_{12}$ in which the benzimidazole nucleotide (axial base) is absent, and (b) the results of the anaerobic photolysis and ther-

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(5) (a) A second aqueous, base-on Co–C BDE of 26 ± 2 kcal/mol has been reported for AdoB$_{12}$.[5b] However, this value has been shown to be in error,[2] a conclusion unchanged[1b,2] by a recent report.[5c] (b) Halpern, J.; Kim, S. H.; Leung, T. W. *J. Am. Chem. Soc.* **1984**, *106*, 8317. (c) Geno, M. K.; Halpern, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1052.
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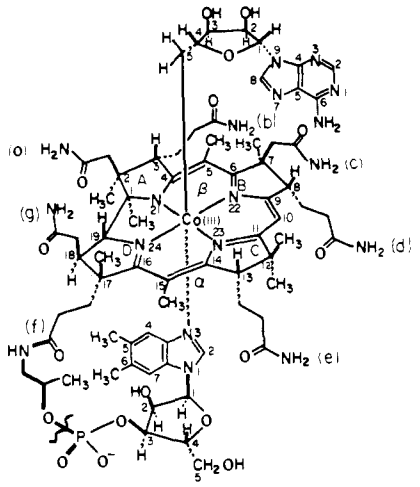

Figure 1. Adenosylcobalamin ($AdoB_{12}$), also known as coenzyme B_{12}. Cleavage at the O–P bond, as indicated by the wavy line, and removal of the axial base yields $AdoCbi^+$. (Reproduced with permission from *B_{12}*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 1.)

molysis of $AdoCbi^+$ in pH 7.0 aqueous phosphate buffer. The effect of the axial base upon $AdoB_{12}$ Co–C5′ bond homolysis is then summarized and discussed as is current thinking about how the B_{12}-dependent enzymes assist Co-C5′ bond cleavage and the energetics involved.

Experimental Section

Adenosylcobalamin (≥98%) and SP Sephadex were obtained from Sigma. $Ce(NO_3)_3 \cdot H_2O$ (99.9%) and 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) were obtained from Aldrich. The latter was purified by sublimation (33 °C, water aspirator, mp 39 °C, lit. 37–39 °C[10]). The nucleoside derivatives, 8,5′-anhydroadenosine, 5′-deoxyadenosine, and 5′-deoxy-5′-[2,2,6,6-tetramethyl-1-piperidinyl-1-oxy]adenosine (T-Ado), were obtained as described previously.[3] The pH 7.0 aqueous buffer, H_3PO_4/K_3PO_4, was prepared at a concentration of 0.01 M. Proton NMR spectra were recorded in D_2O solutions at 20 °C on a General Electric QE-300 spectrometer (300 MHz) with use of DSS (3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt) as an internal standard. Visible spectra were recorded in Schlenk cuvettes (1-cm path length Pyrex cuvettes, glass blown onto Teflon needle valves) on a Beckman DU-7 spectrophotometer thermostated to 25.0 °C. HPLC analyses were carried out on a Beckman chromatograph equipped with a Gilson Holochrome variable wavelength UV–vis detector and an Alltech 300 × 4.1 mm Versapack C-18 column (conditions given below). FAB mass spectrometry was provided by the Midwest Center for Mass Spectrometry (Lincoln, NE). Elemental analysis was provided by E + R Microanalytical Laboratory (Corona, NY). TGA (thermal gravimetric analysis) was provided courtesy of Dr. Robert Saxton at Catalytica Associates (Mountain View, CA).

Synthesis of Adenosylcobinamide.[11] A slurry of $Ce(OH)_3$ was freshly prepared from 12.5 g of $Ce(NO_3)_3 \cdot H_2O$ as described by Renz.[12] A suspension of this $Ce(OH)_3$ in 150 mL of H_2O and a stir bar were placed in a 3-necked, 300-mL, round-bottomed flask equipped with a condenser, thermometer, and a pH electrode. The flask was immersed in an 80 °C oil bath, and the pH was adjusted to 8.5 by the dropwise addition of 10% aqueous NH_3. Due to the photolability of the Co–C bond, the rest of this procedure was carried out in a darkened environment (a fume hood equipped with black plastic curtains). With the contents of the flask at 80 °C, 500 mg (0,32 mmol) of $AdoB_{12}$ were added to the slurry, and the resulting mixture was allowed to stir for 70 min. During the first 20 min the pH was checked every 5 min, and 10% aqueous NH_3 was added to maintain the pH between 8 and 9.[12] After 70 min, the mixture was cooled and centrifuged for 5 min at 10 000 rpm, and the supernatant was decanted. The precipitate was washed 3 times by resuspension in 40-mL aliquots of 0.3% aqueous NH_3 followed by centrifugation as above. The combined supernatants were filtered through a fine glass frit, placed in a 1-L separatory funnel, and desalted by phenol extraction.[13] The resulting aqueous solution was reduced in volume to 10 mL by rotoevaporation (water aspirator, ≤40 °C) and introduced onto a 30 × 3 cm column of SP Sephadex which had been previously washed with 4 L of 0.01 M pH 7.0 aqueous phosphate buffer. Upon elution with this buffer, $AdoCbi^+$ was separated from unreacted $AdoB_{12}$ and the dealkylated side products. The unreacted $AdoB_{12}$ starting material (≤10%) eluted first as a tight red band (it is not retained on the column and passes through readily). $AdoCbi^+$ was eluted next as a diffuse brown band and was collected in a 500-mL fraction. The dealkylated side products, $Co^{III}B_{12}$ and/or $Co^{III}Cbi$, were retained at the top of the column.[14] The yellow $AdoCbi^+$ fraction was desalted by phenol extraction.[13] The resulting 50-mL aqueous solution was introduced onto a 10 × 2 cm column of Whatman DEAE cellulose which had been previously charged with OH^- and then washed with 4 L of deionized H_2O. Upon elution with deionized H_2O, a solution of the desired product $AdoCbi^+OH^-$ was obtained. The yellow solution was evaporated to dryness by rotoevaporation (water aspirator, ≤40 °C, in the dark) followed by drying in vacuo (10^{-3} Torr, 12 h, room temperature). The resulting dark brown glass was scraped from the flask and then dried in vacuo (10^{-3} Torr, 24 h, 80 °C) to yield 200–220 mg (0.16–0.18 mmol) of $AdoCbi^+OH^-$ (50–55% yield, <2% corrin impurity by HPLC and ^1H NMR). Visible spectrum (extinction coefficients, M^{-1} cm^{-1}, in parentheses) in pH 7.0 aqueous phosphate buffer at 25.0 °C, found 379 nm (8.4×10^3), 458 (9.3×10^3); lit.[11] for $AdoCbi^+A^-$ in H_2O: 380 (8.2×10^3), 460 (8.6×10^3): ^1H NMR (300 MHz, D_2O, 20 mg/mL, 20 °C, DSS) from 5–9 ppm 5.66 (d, 1 H), 7.02 (s, 1 H), 8.07 (s, 1 H), 8.26 (s, 1 H);[15,16] FAB-MS (dithiothreitol/dithioerythritol matrix, positive ion spectrum) calcd mass for $AdoCbi^+$ $C_{58}H_{84}N_{16}O_{11}Co$ 1239.6; found parent ion at 1239.7. Anal. (for BF_4^- salt)[17] calcd for $C_{58}H_{84}N_{16}O_{11}CoBF_4$: C, 52.49; H, 6.38; N, 16.89; Co, 4.44. Found: C, 52.91; H, 6.63; N, 17.20; Co, 4.48. TGA (BF_4^- salt)[19] shows ≤1.4% weight loss up to 130 °C consistent with the presence of ≤1 H_2O per $AdoCbi^+BF_4^-$. HPLC (C-18 column, isocratic at 0.5 mL/min, 30% CH_3CN:70% 1.0 mM pH 4.5 aqueous acetate buffer) shows a single peak with retention time, 8.9 min.

Anaerobic Photolysis of $AdoCbi^+OH^-$. A solution of 1.0×10^{-4} M $AdoCbi^+OH^-$ in pH 7.0 aqueous phosphate buffer was prepared, and a 2.0-mL sample was placed in a Pyrex Schlenk cuvette. A second sample was prepared which also contained 2.0×10^{-2} M TEMPO. The samples were deoxygenated by bubbling with argon for 20 min, the cuvette was sealed, and initial visible spectra were recorded. The samples were placed at a distance of 30 cm from a 350-W tungsten lamp. During the photolysis, the samples were removed from the light at 3-min intervals, overlaid visible spectra were recorded, and the samples were returned to the light. After 20 min of irradiation, no further change in the visible spectrum was observed, and the final spectrum was that of $Co^{II}Cbi$ (469 nm, 1.1×10^4 M^{-1} cm^{-1}). After recording the final spectra, the samples were opened to air, and nucleoside product yields were determined by HPLC as described previously.[2]

Thermolysis of $AdoCbi^+OH^-$. Both product and kinetic studies were carried out on samples sealed in Schlenk cuvettes containing (5–15) × 10^{-5} M $AdoCbi^+OH^-$ in pH 7.0 aqueous phosphate buffer. The samples were prepared in a Vacuum Atmospheres glovebox (N_2, ≤2 ppm O_2) in solvent deoxygenated by 3 freeze/pump/thaw cycles. During the handling of $AdoCbi^+$ solutions, exposure to light was minimized by working under dim lighting and by wrapping vessels with aluminum foil. Thermolyses were carried out in an oil bath thermostated to ±0.2 °C. The anaerobic thermolysis of $AdoCbi^+$ was studied over a temperature range of 105.0–130.0 °C. In addition, reactions were also carried out in the presence of 1.0×10^{-2} M (100 equiv) and 2.0×10^{-2} M (200 equiv) TEMPO.[1,32c]

The corrin products and reaction progress were monitored by visible spectroscopy. While the thermolyses were carried out in an oil bath, all visible spectra were recorded at 25.0 °C after rapidly cooling the sample to quench the reaction. Nucleoside products were identified and quantified by HPLC in comparison to authentic materials as described previously.[2] Kinetic data were obtained by monitoring the absorbance at both 380 and 469 nm as a function of time. In the presence of ≥1.0×10^{-2} M TEMPO, limiting values of k_{obsd} (±7%) were obtained by linear regression of ln $(A_0 - A_\infty)/(A - A_\infty)$ vs time plots. Initial (≤20% reaction) rate data were used since isosbestic points are lost in the latter stages of reaction due to the subsequent reaction(s) of $Co^{II}Cbi$ (vide infra). Interference from this slow secondary reaction was worse at the lower

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(11) Bernhauer, K.; Müller, O. *Biochem. Z.* **1961**, *335*, 44.
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(17) $AdoCbi^+$ has been prepared as the BF_4^- salt by substituting a DEAE cellulose column charged with BF_4^- (0.5 M $NaBF_4$) instead of OH^-.

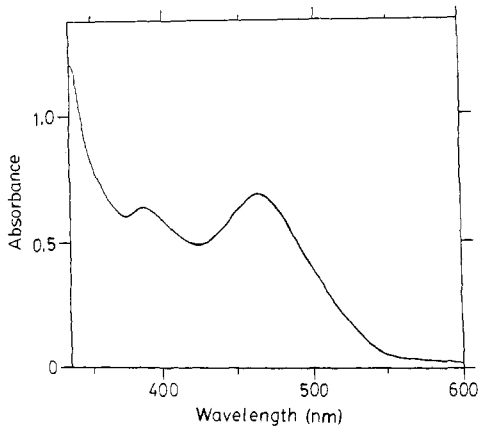

Figure 2. Visible spectrum of $AdoCbi^+OH^-$ (7.5×10^{-5} M) in pH 7.0 aqueous phosphate buffer at 25 °C.

temperatures where longer reaction times were required.

$Co^{II}Cbi$ Stability Controls. Two samples, one with and one without TEMPO, were prepared and photolyzed as described above for the photolysis of $AdoCbi^+$. The resulting $Co^{II}Cbi$ samples were heated in a 110.0 °C oil bath for a period of 24 h in the dark. Periodically the samples were removed from the bath, spectra were recorded (after cooling to 25.0 °C), and the samples were returned to the bath. Overlaying the visible spectra revealed that, in both samples, $Co^{II}Cbi$ engaged in a slow reaction(s) as evidenced by an increase in absorbance from 380–480 nm. These spectral changes are not consistent with the formation of $H_2O(HO)Co^{III}Cbi$ which exhibits maxima at 349, 499, and 519 nm,[18] The nature of this reaction(s) was not investigated further.

Nucleoside Stability Controls. The stability of the nucleoside products 8,5′-anhydroadenosine, 5′-deoxyadenosine, and T-Ado to hydrolysis was investigated under the reaction conditions used for $AdoCbi^+OH^-$ thermolysis. Three solutions, each containing 1.0×10^{-4} M of one nucleoside in pH 7.0 aqueous phosphate buffer under N_2, were sealed in Schlenk cuvettes in the glovebox and heated for 24 h at 110.0 °C, HPLC analysis revealed no reaction for 8,5′-anhydroadenosine and ≤2% hydrolysis of either 5′-deoxyadenosine or T-Ado.

Results

(A) Synthesis of $AdoCbi^+OH^-$. Alkylcobinamides, a class of compounds which includes $AdoCbi^+$, are typically prepared via the reaction of an alkylhalide with Co^ICbi.[6,19] $AdoCbi^+$ has been synthesized in this manner by alkylation with 5′-tosyl-5′-deoxyadenosine[15] or 2′,3′-isopropylidene-5′-tosyl-5′-deoxyadenosine.[20] This method of synthesis requires the preparation of a Co^ICbi precursor (usually $(CN)_2Cbi$ obtained from the cerous hydroxide hydrolysis of CNB_{12}),[12,21] and the overall yields in these multistep syntheses ($CNB_{12} \rightarrow (CN)_2Cbi \rightarrow \rightarrow AdoCbi^+$) are low.

A search of the literature yielded a more direct route. In 1961, Bernhauer and Müller accomplished the synthesis of $AdoCbi^+$ by heating $AdoB_{12}$ in a slurry of $Ce(OH)_3$,[11] The reaction mixture was fractionated by electrophoresis and revealed the presence of $AdoCbi^+$, $H_2OB_{12}^+$, and $(H_2O)_2Cbi^{2+}$. $AdoCbi^+$ prepared in this manner exhibits the same UV–vis, paper chromatographic, and electrophoretic properties as $AdoCbi^+$ obtained from *P. shermanii*.[11]

We have modified and improved this one-step synthesis by increasing the scale by 100-fold, employing a longer reaction time at a lower temperature to improve the yield, using freshly prepared, washed $Ce(OH)_3$,[12] maintaining a pH range of 8–9,[12] and using a column chromatographic separation. Typical $AdoCbi^+OH^-$ yields are 50–55%; the rest of the theoretical yield is lost to unreacted starting material (5–10%), dealkylated side products (20–25%), and manipulative losses (10–15%). After desalting, $AdoCbi^+$ is readily separated from the crude reaction mixture by cation exchange chromatography on SP Sephadex. Both the OH^- and BF_4^- salts of $AdoCbi^+$ have been prepared by a final pass through an anion exchange column,[17]

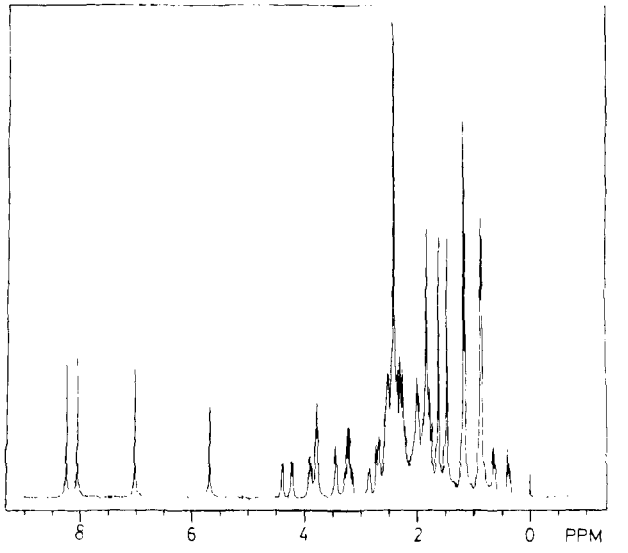

Figure 3. 300-MHz ^1H NMR spectrum of $AdoCbi^+OH^-$ (15 mg/mL) in D_2O at 20 °C with DSS added as a reference.

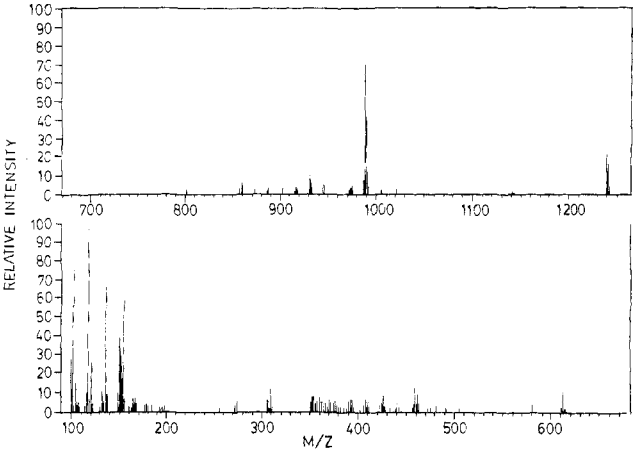

Figure 4. Positive ion FAB-MS of $AdoCbi^+OH^-$ (dithiothreitol/dithioerythritol matrix).

These $AdoCbi^+$ salts have been characterized by a variety of techniques, The visible spectrum of the $AdoCbi^+$ cation, shown in Figure 2, agrees with the literature spectra for this compound.[11,15] As is the case for a variety of $alkylB_{12}$/alkylCbi pairs,[22] the 400–800-nm region of the spectrum is superimposable upon the visible spectrum of $AdoB_{12}$ in 0.1 M H_3PO_4 (i.e., the visible spectrum of protonated, and thus base-off, $AdoB_{12}$). The 300-MHz ^1H NMR spectrum in D_2O, shown in Figure 3, is the cleanest, impurity-free spectrum of $AdoCbi^+OH^-$ observed to date and is in general agreement with literature spectra,[15,16] Several of the resonances have been assigned—including the downfield adenosyl resonances at 8.26 (H-2), 8.07 (H-8), and 5.66 (H-1′) ppm and the single downfield corrin resonance at 7.02 (C-10) ppm. The two upfield peaks, at 0.39 and 0.65 ppm, correspond to the diasteriotopic 5′-methylene protons; 2-D NMR studies should allow a full assignment of all ^1H NMR peaks, as has recently been accomplished for $AdoB_{12}$,[23a] and are in progress,[23b] The ^1H NMR

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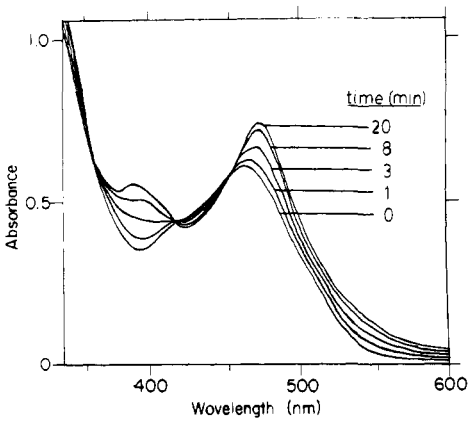
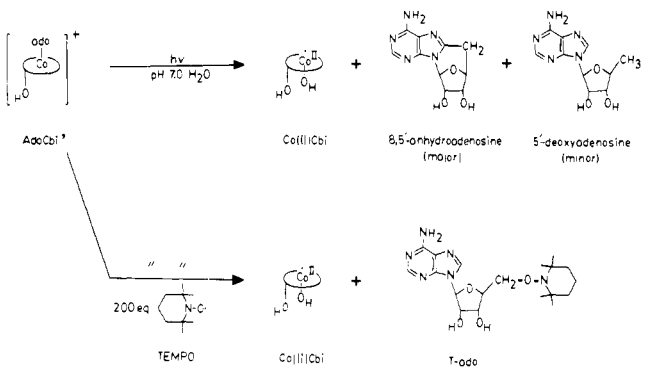


Figure 5. Superimposed visible spectra of the anaerobic photolysis of AdoCbi$^+$OH$^-$ in pH 7.0 aqueous phosphate buffer with 200 equiv of TEMPO present. Isosbestic points are apparent at 350 and 409 nm.

Scheme II



establishes the presence of the adenosyl moiety and shows that the benzimidazole nucleotide has been removed.[23c]

The positive ion mass spectrum of AdoCbi$^+$OH$^-$, given here because it is the first mass spectrum of an alkylcobinamide,[24] is shown in Figure 4. The parent ion at 1239,7 corresponds to the calculated mass of 1239.6 for a five-coordinate AdoCbi$^+$ cation, $C_{58}H_{84}N_{16}O_{11}Co$, a rare example of a five-coordinate alkylcorrin complex,[25] in this case in the gas phase. The large peak at 989.5 corresponds to the calculated mass for a four-coordinate Co^{II}Cbi$^+$ cation, $C_{48}H_{72}N_{11}O_8Co$. Elemental analysis of AdoCbi$^+$BF$_4^-$ is consistent with the proposed composition, and TGA reveals that there are ≤ 1 H_2O per AdoCbi$^+$BF$_4^-$ after drying.

(B) Anaerobic Photolysis of AdoCbi$^+$OH$^-$. The photolysis of AdoCbi$^+$OH$^-$ was examined initially, as this method of Co–C bond cleavage proceeds quantitatively via a homolytic mechanism (vide infra) providing a valuable comparison for subsequent thermolysis studies. The photolysis (350-W tungsten lamp) of anaerobic solutions of AdoCbi$^+$OH$^-$ in pH 7.0 buffer with 200 equiv of TEMPO shows no further changes in the visible spectra after 20 min, Figure 5. The final spectrum is that of Co^{II}Cbi (λ_{max} = 469 nm, $\epsilon_{max} = 1.1 \times 10^{-4}$ M^{-1} cm^{-1}). HPLC analysis of the nucleoside products shows that (97 ± 4)% T-Ado and (3 ± 1)% 5′-deoxyadenosine are formed (Scheme II). In the absence of TEMPO, small deviations from isosbestic behavior are detected, and HPLC analysis of the nucleoside products finds (93 ± 4)% 8,5′-anhydroadenosine and (7 ± 2)% 5′-deoxyadenosine (Scheme II). The production of 5′-deoxyadenosine coupled with the isosbestic point aberrations in the absence of TEMPO suggest that H· abstraction from the corrin is occurring, although this hypothesis remains to be confirmed.

The AdoCbi$^+$ photolysis results are summarized in Scheme II. As with the photolysis of AdoB$_{12}$,[26] the photolysis of AdoCbi$^+$ serves as a clean, low-temperature route to the homolysis products Co^{II}Cbi and the 5′-deoxyadenosyl radical. The nucleoside radical then either undergoes intramolecular cyclization to yield 8,5′-anhydroadenosine (major) or H· abstraction (apparently from the corrin) to yield 5′-deoxyadenosine (minor). In the presence of 200 equiv of TEMPO, the nucleoside radicals are scavenged to yield T-Ado.

(C) Anaerobic Thermolysis of AdoCbi$^+$OH$^-$. Anaerobic solutions of AdoCbi$^+$OH$^-$ in pH 7.0 buffer were thermolyzed in the dark (105.0–130.0 °C), both in the presence and absence of TEMPO. In both cases, isosbestic points at 350 and 309 nm are observed during the initial (≤20%) reaction, the same two isosbestic points observed for the photolytic conversion of AdoCbi$^+$ to Co^{II}Cbi. As the thermolysis proceeded, deviations in these isosbestic points developed. In separate control experiments, it was shown that prolonged exposure of Co^{II}Cbi to the extreme (≥105 °C) conditions required for AdoCbi$^+$ thermolysis results in slow increases in absorbance from 380 to 480 nm, i.e., these deviations are due to a subsequent reaction of the Co^{II}Cbi.

The isosbestic points establish Co^{II}Cbi as the initial corrin product of AdoCbi$^+$ thermolysis. While Co^{II}Cbi is the expected product of Co–C bond homolysis, these visible spectral results do not, by themselves, exclude other *initial* pathways such as heterolysis to either Co^{I}Cbi or Co^{III}Cbi. For example, Co^{I}Cbi, like Co^{I}B$_{12}$, is unstable under anaerobic, aqueous conditions and reduces H_2O to form H_2 and Co^{II}Cbi,[6d] Likewise, Co^{III}Cbi, like Co^{III}B$_{12}$,[2] is unstable to heating in anaerobic, aqueous solution and yields a product having the same visible spectrum as Co^{II}Cbi.[6d]

The preferred pathway(s) of thermal Co–C bond cleavage was established through the analysis of the nucleoside products (identified and quantified by HPLC comparison to authentic materials).[2] In the absence of TEMPO, three products account *quantitatively* for the nucleoside yield, e.g., at 110.0 °C the products are (90 ± 4)% 8,5′-anhydroadenosine, (4 ± 2)% 5′-deoxyadenosine, and (6 ± 2)% adenine. The first two nucleosides are the same two products formed during the anaerobic, aqueous photolysis of AdoCbi$^+$ (i.e., they are the expected products of Co–C bond homolysis). The third product, adenine, is the characteristic product of Co–C bond heterolysis.[2]

Upon carrying out the reactions in the presence of 100 equiv of TEMPO the yield of adenine was unaffected, while the yields of 8,5′-anhydroadenosine (≤5%) and 5′-deoxyadenosine (≤2%) were decreased with a corresponding increase in the yield of T-Ado. Control experiments showed that these yields are not complicated by subsequent reactions of the products since the

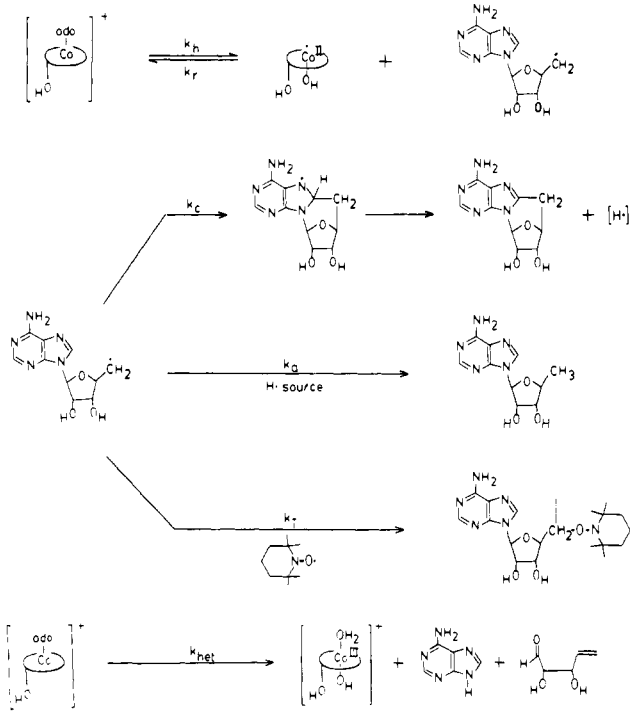
(23) (a) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285. Bax, A.; Marzilli, L. G.; Summers, M. F. *J. Am. Chem. Soc.* **1987**, *109*, 566. (b) Collaboration with L. Marzilli and co-workers in progress. (c) The free 5,6-dimethylbenzimidazole nucleoside, α-ribazole, exhibits downfield resonances at 6.17, 7.36, 7.34, and 8.25 ppm in Me$_2$SO-d_6.[23d] In AdoB$_{12}$, the 5,6-dimethylbenzimidazole nucleotide moiety exhibits downfield resonances at 6.24, 6.26, 6.95, and 7.16 ppm in D_2O.[23a] The absence of any extra peaks in the downfield region of the AdoCbi$^+$OH$^-$ spectrum shows that the axial base has been removed. (d) Brown, K. L.; Hakimi, J. M.; Nuss, D. M.; Montejano, Y. D.; Jacobsen, D. W. *Inorg. Chem.* **1984**, *23*, 1463.

(24) For FAB-MS studies of cobalamins and other corrins, see: (a) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *Nature (London)* **1981**, *293*, 270. (b) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *Biomed. Mass Spectrom.* **1981**, *8*, 492. (c) Schiebel, H. M.; Schulten, H.-R. *Biomed. Mass Spectrom.* **1982**, *9*, 354 and references therein. (d) Schwartz, H.; Eckart, K.; Taylor, L. C. E. *Org. Mass Spectrom.* **1982**, *17*, 458.

(25) While five-coordinate species have been proposed, few authentic examples of either five-coordinate alkylcobalt–corrins[25a] or alkylB$_{12}$ model compounds[8a,25b] are known. (a) Pratt, J. M. In *B$_{12}$*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 1, Chapter 10. See also: Firth, R. A.; Hill, H. A. O.; Mann, B. E.; Pratt, J. M.; Thorp, R. G.; Williams, R. J. P. *J. Chem. Soc.* **1968**, 2419. (b) Marzilli, L. G.; Summers, M. F.; Bresciani-Pahor, N.; Zangrando, E.; Charland, J.-P.; Randaccio, L. *J. Am. Chem. Soc.* **1985**, *107*, 6880. (c) The exact axial ligation of AdoCbi$^+$, e.g., five-coordinate or six-coordinate (H_2O, phosphate) under the conditions of the present thermolysis study, is unknown. However, higher temperatures such as those used herein will favor the five-coordinate form, the species seen by FAB mass spectroscopy. Furthermore, the rates and activation parameters for thermolysis of five-coordinate vs weakly solvated six-coordinate forms of AdoCbi$^+$ are not expected to be greatly different.

(26) The anaerobic photolysis of AdoB$_{12}$ in aqueous solution results in the quantitative formation of 8,5′-anhydroadenosine and Co^{II}B$_{12}$.[26a,b] (a) Hogenkamp, H. P. C. *J. Biol. Chem.* **1963**, *238*, 477. (b) Hogenkamp, H. P. C. In *B$_{12}$*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 1, pp 296–300.

Scheme III



$$\text{(eq 2)}\quad \frac{-d\,[\text{AdoCbi}^+]}{dt} = k_{obs} = k_h\left[\frac{k_c + k_o[\text{H}\cdot\text{ source}] + k_T[\text{TEMPO}]}{k_c + k_o[\text{H}\cdot\text{ source}] + k_T[\text{TEMPO}] + k_r[\text{Co(II)Cbi}]}\right] + k_{het}$$

nucleoside products are stable toward hydrolysis under the reaction conditions (≤2% hydrolysis after 24 h at 110 °C), These TEMPO scavenging results demonstrate that the 5′-deoxyadenosyl radical is an intermediate in the formation of 8,5′-anhydroadenosine and 5′-deoxyadenosine but that adenine is formed via a separate, heterolysis pathway. A stoichiometric amount (≤7%) of Co^{III}Cbi (one Co^{III}Cbi per adenine) was not detected; Co^{II}Cbi is the only corrin product observed. However, this is the expected result given the known ability of Co^{III}Cbi to self-reduce upon heating, giving a visible spectrum identical with that of Co^{II}Cbi,[6d]

In summary, these nucleoside product studies require parallel kinetic pathways, homolysis to Co^{II}Cbi and the 5′-deoxyadenosyl radical, and heterolysis to Co^{III}Cbi and adenine (and, by mass balance, 2,3-dihydroxy-4-pentenal).[2] At pH 7.0 and over the temperature range of 105.0–130.0 °C, homolysis dominates (≥93%), The minimum mechanism required to account for the observed product, nitroxide scavenging, and kinetic results is shown in Scheme III. These $AdoCbi^+$ results are completely analogous to our previous findings of parallel homolysis and heterolysis pathways for the thermolysis of $AdoB_{12}$ in aqueous solution.[2]

In the presence of ≥100 equiv of TEMPO, first-order plots for the disappearance of $AdoCbi^+$ are linear during the first 20% of reaction. Doubling the concentration of TEMPO did not change the rate of reaction, demonstrating the absence of bimolecular reactions between TEMPO and $AdoCbi^+$ and establishing that Co–C bond cleavage (and not TEMPO trapping) is rate limiting when ≥100 equiv of TEMPO is present. Under these rate-limiting conditions, values of k_{obsd} (±7%) were measured at 5 °C intervals from 105.0 to 130.0 °C.[27] A $\ln(k_{obsd}/T)$ vs $1/T$ plot and least-squares analysis of the data yielded $\Delta H^{\ddagger}_{obsd} = 37.3 \pm 1.2$ kcal/mol and $\Delta S^{\ddagger}_{obsd} = 15.8 \pm 2.8$ cal/mol·K. After corrections for 7% heterolysis at 105.0 °C and 6% heterolysis at 130.0 °C, activation parameters for the Co–C bond homolysis of this base-off (possibly five-coordinate)[25c] $AdoB_{12}$ analogue are obtained—37.5 ± 1.5 for $\Delta H^{\ddagger}_{h,off}$ and 16 ± 3 cal/mol·K for $\Delta S^{\ddagger}_{h,off}$.[28]

(27) The k_{obsd} values (s^{-1}, ±7%) and temperatures (°C, ±0.2 °C) are, respectively, as follows: 5.6×10^{-6}, 105.0; 1.2×10^{-5}, 110.0; 2.2×10^{-5}, 115.0; 3.7×10^{-5}, 120.0; 7.6×10^{-5}, 125.0; 1.5×10^{-4}, 130.0.

Table I. Values of $k_{h,on}$, $k_{h,off}$, and K for $AdoB_{12}$ in Neutral Aqueous Solution

temp (°C)	$k_{h,on}$[a] (s^{-1})	$k_{h,off}$[b] (s^{-1})	K[c] ([on]/[off])
85	1×10^{-5}	3×10^{-7}	3.8
90	3×10^{-5}	6×10^{-7}	3.4
95	5×10^{-5}	1×10^{-6}	3.0
100	9×10^{-5}	3×10^{-6}	2.7
105	2×10^{-4}	5×10^{-6}	2.5
110	3×10^{-4}	1×10^{-5}	2.3
115	5×10^{-4}	2×10^{-5}	2.1
120	9×10^{-4}	4×10^{-5}	1.9
125	2×10^{-3}	7×10^{-5}	1.7
130	3×10^{-3}	1×10^{-4}	1.6

[a] Calculated from $\Delta H^{\ddagger}_{h,on} = 33 \pm 2$ kcal/mol and $\Delta S^{\ddagger}_{h,on} = 11 \pm 3$ cal/mol·K.[2] [b] Calculated from $\Delta H^{\ddagger}_{h,off} = 37.5 \pm 1.5$ kcal/mol and $\Delta S^{\ddagger}_{h,off} = 16 \pm 3$ cal/mol·K, this work. [c] Calculated from $\Delta H = -5.6 \pm 0.8$ kcal/mol and $\Delta S = -13 \pm 3$ cal/mol·K.[2]

Discussion

The values of $k_{h,off}$ obtained in this study are compared to values of $k_{h,on}$, calculated from $AdoB_{12}$ thermolysis data,[2] in Table I, This table also includes values of K, the base-on/base-off equilibrium constants for $AdoB_{12}$.[2] It can be seen that over the temperature range used for $AdoB_{12}$ thermolysis (85.0–110.0 °C), the rate constants for base-off homolysis are 50–30 times smaller than the rate constants for base-on homolysis. Moreover, the rate and equilibrium constants are such that $(K/(K+1))\cdot k_{h,on} \gg (1/(K+1))\cdot k_{h,off}$ (see Scheme I), In the worst case (at 110.0 °C) the results predict that only 1.4% of the thermal Co–C bond homolysis of $AdoB_{12}$ proceeds through the base-off form. These results clearly justify the earlier assumption[1,2] that the base-off form does not participate within experimental error during the thermolysis of $AdoB_{12}$.

It has been shown that a reasonable estimate of a Co–C BDE can be obtained from kinetic data for Co–C bond homolysis in solution.[7a,29] For alkyl(pyridine)cobaloximes in toluene, Co–C BDEs (determined via a thermochemical cycle) were found to be ≃2 kcal/mol lower than $\Delta H^{\ddagger}_h$ values (determined via kinetics).[7a,29] This 2 kcal/mol difference was attributed to the enthalpic barrier to diffusive flow in toluene, $\Delta H^{\ddagger}_{\eta} = 1.5$ kcal/mol.[30] In water, the enthalpic barrier to diffusive flow is slightly larger, 2.3 kcal/mol (85.0–110.0 °C) and 2.0 kcal/mol (105.0–130.0 °C).[31] Hence, a slightly larger corrective term is applied to aqueous data—Co–C BDE ≃ $\Delta H^{\ddagger}_h$ – (3 ± 1 kcal/mol).[31c] Application of this *approximate*[30b] equation to aqueous $AdoB_{12}$ data yielded a base-on Co–C BDE of 30 ± 2 kcal/mol[2] and now yields a value of 34.5 ± 1.8 kcal/mol for the base-off Co–C BDE of $AdoB_{12}$. These data reveal that removing the trans axial base strengthens the Co–C bond in this molecule by 4.5 ± 2.7 kcal/mol.

When compared to other alkyl–cobalt bonds, the stability of the adenosyl–cobalt bond is apparent. Not only do $AdoB_{12}$ and $AdoCbi^+$ exhibit the largest $\Delta H^{\ddagger}_h$ values known for an $alkylB_{12}$ (20–27 kcal/mol)[6a] or an $alkylCbi^+$ (27–32 kcal/mol),[6a] respectively, but also when compared to all literature values for Co–C bond homolysis activation enthalpies (17–35 kcal/mol),[6a,7,29,32]

(28) Assuming that $k_h \simeq$ (fraction of radical products)·k_{obsd}, the best fit line through the $\ln(k_{obsd}/T)$ vs $1/T$ plot was adjusted at the end points.

(29) Halpern, J.; Ng, F. T. T.; Rempel, G. L. *J. Am. Chem. Soc.* **1979**, *101*, 7124.

(30) (a) Dobis, O.; Pearson, J. M.; Szwarc, M. *J. Am. Chem. Soc.* **1968**, *90*, 278. (b) The significance of radical cage effects in the interpretation of this barrier will be dealt with elsewhere: Hay, B. P.; Finke, R. G.; Koenig, T. W. *Polyhedron*, in press.

(31) Calculated from $\ln(\eta)$ vs $1/T$ plots[31a] with use of literature viscosity data[31b] and Andrade's law ($\ln \eta = \ln A_{\eta} + E_{\eta}/RT$) and noting $\Delta H^{\ddagger}_{\eta} = E^{\ddagger}_{\eta} - RT$. (a) Glasstone, S.; Laidler, K. J.; Eyring, H. *Theory of Rate Processes*; McGraw-Hill: New York, 1941. (b) Innes, K. K. *J. Phys. Chem.* **1956**, *60*, 817. (c) The equation, Co–C BDE ≃ $\Delta H^{\ddagger}_h$ – (3 ± 1) kcal/mol, is the same as that used previously even for the more viscous solvent, ethylene glycol, which exhibits a slightly larger $\Delta H^{\ddagger}_{\eta} \simeq 4.4$ kcal/mol over the temperature range of 90–120 °C.[2] The importance of, and uncertainties introduced by, cage effects are such that a more precise correction is not possible at this time.[30b]

$AdoCbi^+$ exhibits the largest $\Delta H^{\ddagger}_h$ value measured to date, 37,5 kcal/mol.

Since the homolysis of the Co–C bond is thought to be the essential, and perhaps the only, role of the $AdoB_{12}$ cofactor,[3,4] it is of special significance to compare the rates of thermally induced Co–C bond homolysis to literature rates of enzyme-induced Co–C bond homolysis.[33,34] The addition of substrate to the $AdoB_{12}$–enzyme (holoenzyme) complex triggers a rapid Co–C bond homolysis. Upon monitoring the appearance of $Co^{II}B_{12}$, first-order rate constants for this enzyme-induced, substrate-triggered homolysis have been measured in two enzyme systems. For diol dehydrase at 5 °C, Co–C bond homolysis exhibits a rate constant of $\geq 2 \times 10^2$ s^{-1}.[33a] For ethanolamine deaminase at 25 °C, a similar value of 3×10^2 s^{-1} is observed.[33b] On the basis of this data, we assign a 25 °C value of $\geq 10^2$ s^{-1} for the rate constant for enzyme-assisted $AdoB_{12}$ Co–C bond homolysis.

The $AdoCbi^+$ activation parameters reported herein permit the calculation of a 25 °C base-off $AdoB_{12}$ rate constant of $10^{-11 \pm 1}$ s^{-1}. Our previously determined base-on $AdoB_{12}$ activation parameters yield a 25 °C base-on rate constant of $10^{-9 \pm 1}$ s^{-1}.[2] Comparison of these extrapolated solution rate constants to those reported in the enzyme environment show a $\geq 10^{13}$ overall rate enhancement, corresponding to a $\Delta\Delta G^{\ddagger}$ lowering of ≥ 17.5 kcal/mol, upon going from the isolated base-off cofactor to the base-on cofactor in the holoenzyme, a solution half-life of 2200 years compared to an enzyme half-life of a few milliseconds! A search of the literature revealed that other authentic examples of enzymatic rate enhancements of $\geq 10^{13}$ for the cleavage of a single bond are rare.[35,36] However, we note that the $\Delta\Delta G^{\ddagger}$ lowering of ≥ 17.5 kcal/mol is at least plausible when one notes the ≥ 10 surface binding/hydrogen bonding sites[36b] (many of which may have multiple hydrogen bonds)[36c] in the large and complex $AdoB_{12}$ cofactor, an average cofactor-enzyme $\Delta\Delta G^{\ddagger}$ lowering of only ≤ 1.5 kcal/mol per binding site is required (after subtraction of the $\Delta\Delta G^{\ddagger} \simeq 2.7$ kcal/mol effect of the axial base, vide infra). This number is very close to the average hydrogen bond energy, $\simeq 1.4$ kcal/mol, found in a recent study of biological hydrogen-bonding energetics[36c] (although it is unclear what fraction of this binding energy can be utilized for catalysis).

The extrapolated $AdoCbi^+$ and $AdoB_{12}$ 25 °C rate constants also permit quantitation of the widely discussed,[6-9] Co–C5′ bond

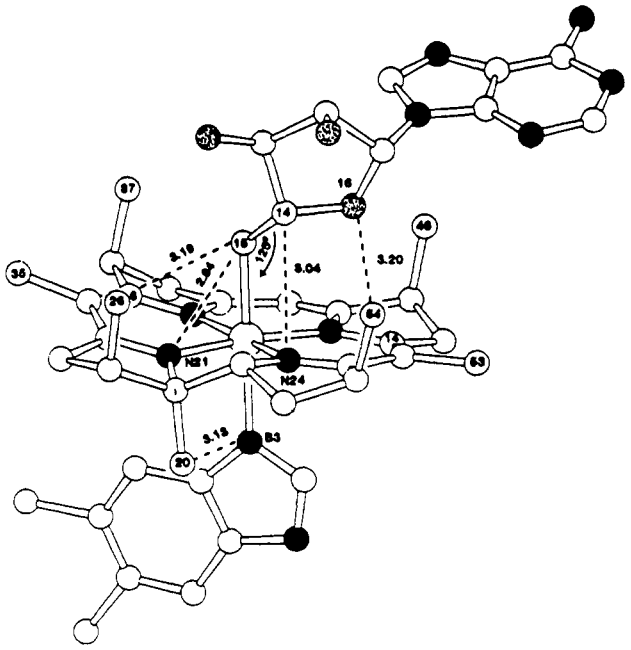


Figure 6. Selected nonbonded contacts between the adenosyl and corrin ring in $AdoB_{12}$. (Most of the side chains, the propanolamine, ribose, and phosphate have been removed for clarity; reproduced with permission from Glusker's review.[39b]) Further enzyme-induced "upward" conformational flexing is thought to facilitate Co–C5′ bond homolysis as discussed in the text, although this has not been proven for the enzyme·cofactor complex.

weakening effect of the axial 5,6-dimethylbenzimidazole. The axial base accelerates the Co–C5′ bond homolysis by ca. 10^2 at 25 °C (and 50- and 30-fold at 85 and 110 °C, respectively), much less than the total enzyme-induced acceleration of $\geq 10^{13}$. Enzymic studies using $AdoB_{12}$ modified at both the ribose and base portions of the appended nucleotide (Figure 1) support our finding of a relatively small effect of the axial base. Base-free $AdoCbi^+$ is reported to be partially active by two separate research groups[37a] (others report it as inactive[37b,c]). Moreover, alkylation at the benzimidazole N3 nitrogen (Figure 1, preventing coordination to cobalt) produces a cofactor that still has half of its activity.[37c] This and other evidence strongly suggests that the nucleotide is more involved in *binding* the cofactor to the enzyme than in the Co–C cleavage process.[37a,d]

A significant finding, then, is that of an enzymic rate enhancement of $\geq 10^{11}$ over and above the ca. 10^2 effect of the axial base. Clearly, and as noted earlier, the protein must account for the majority of this rate acceleration, i.e., the intrinsic binding energy of the protein to both the cofactor and the substrate almost surely must result in substantial Co–C lengthening, weakening,[33b,34] and thus catalysis,[38] Indeed, it is known that, following $AdoB_{12}$ uptake by the apoprotein, a slow step occurs which has been ascribed to a protein conformational change.[33b] Furthermore, it is known that partial (albeit slow) racemization from this conformation occurs at the $AdoB_{12}$ C5′ carbon (stereospecifically labeled with deuterium),[34] It follows, then, that this reactive holoenzyme conformation, presumably the resting state of the catalytic cycle, contains a weakened Co–C bond.

The question that naturally arises is exactly how does the enzyme weaken the $AdoB_{12}$ Co–C bond? Unfortunately, there

(32) (a) Gjerde, H. B.; Espenson, J. H. *Organometallics* **1982**, *1*, 435. (b) Tsou, T. T.; Loots, M.; Halpern, J. *J. Am. Chem. Soc.* **1982**, *104*, 623. (c) Finke, R. G.; Smith, B. L.; Mayer, B. J.; Molinero, A. A. *Inorg. Chem.* **1983**, *22*, 3677. (d) Bakac, A.; Espenson, J. H. *J. Am. Chem. Soc.* **1984**, *106*, 5197. (e) Ohgo, Y.; Orisaku, K.; Hasegawa, E.; Takeuchi, S. *Chem. Lett.* **1986**, 27. An interesting feature of this work is that substitution of an alkyl ligand by an electron-withdrawing methoxycarbonyl group increases $\Delta H^{\ddagger}$ from 31 to 35 kcal/mol.

(33) (a) Valinsky, J. E.; Abeles, R. H.; Fee, J. A. *J. Am. Chem. Soc.* **1974**, *96*, 4709. (b) Hollaway, M. R.; White, H. A.; Joblin, K. N.; Johnson, A. W.; Lappert, M. F.; Wallis, O. C. *Eur. J. Biochem.* **1978**, *82*, 143.

(34) Gaudemer, A.; Zylber, J.; Zylber, N.; Baran-Marszac, M.; Hull, W. E.; Fountoulakis, M.; König, A.; Wölfe, K.; Rêtey, J. *Eur. J. Biochem.* **1981**, *119*, 279.

(35) (a) Enzymatic rate enhancements in the range of 10^{10-14} are well-known.[38] However, these generally involve substrate binding, thereby overcoming a bimolecular component compared to the same reaction in the absence of the enzyme. Estimates of this bimolecular component[38] fall in the range of 10^{5-9}, generally[35b] leaving factors of $<10^{11}$ for comparison to the present example. (b) A 10^{10}–$10^{12.5}$ "unimolecular" enzymic rate enhancement vs that in solution for the S_N1 hydrolysis of glycosyl pyridinium ions has been documented.[35c] The $\Delta G^{\ddagger}$ lowering of $\simeq 13.5$ kcal/mol at 25 °C is achieved by a $\Delta H^{\ddagger}$ lowering of 21 kcal/mol working against a *less favorable* $\Delta S^{\ddagger}$ of 25 cal/mol·K. We are indebted to Professor S. Withers, University of British Columbia, for bringing this example to our attention. (c) Jones, C. C.; Sinnott, M. L.; Souchard, I. J. L. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1192.

(36) (a) It should be noted that the full $\geq 10^{13}$ activation results only when comparing the Co–C bond cleavage of $AdoB_{12}$ in solution, $AdoB_{12} \rightarrow$ Ado· + Co(II)·, vs the step from the reactive holoenzyme conformation, (Ado- - -B_{12})·Enz·substrate → (Ado·Co(II)·)·Enz·substrate. Restated, each Ado–B_{12} needs the full $\geq 10^{13}$ activation only once to enter the catalytic cycle. Subsequent turnovers with fresh substrate should proceed from the activated Ado- - -B_{12}·Enz in what is essentially a chain reaction. (b) This is perhaps best seen by examining the view of the cofactor provided in Figure 12 of Lenhert, P. G. *Proc. Roy. Soc. A* **1968**, *303*, 45. (c) Fersht, A. R.; Shi, J.-P.; Knill-Jones, J.; Lowe, D. M.; Wilkinson, A. J.; Blow, D. M.; Brick, P.; Carter, P.; Waye, M. M. Y.; Winter, G. *Nature (London)* **1985**, *314*, 235; see Table II.

(37) (a) Toraya, T.; Fukui, S. In *B_{12}*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 2, Chapter 9. Toraya, T.; Fukui, S. *Adv. Chem. Ser.* **1980**, *191*, 139. See, also: Zagalak and Pawelkiewicz (Zagalak, B.; Pawelkiewicz, J. *Acta Biochim. Pol.* **1964**, *11*, 49) who report $AdoCbi^+$ active for propanediol (but inactive for glycerol or ethylene glycol) as a substrate. (b) See ref 20b, Table IV. (c) Yakusheva, M. I.; Poznanskaya, A. A.; Pospelova, T. A.; Rudakova, I. P.; Yurkevick, A. M.; Yakovlev, V. A. *Biochim. Biophys. Acta* **1977**, *484*, 216. See: p 229. (d) For methylmalonyl-CoA mutase the evidence suggests, as with diol dehydratase,[37a] that the axial base is more involved in binding $AdoB_{12}$ to the enzyme: Rêtey, J. In *B_{12}*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 2, Chapter 13.

is a complete lack of X-ray crystallographic structural information for any of the B_{12}-dependent enzymes at this time. Generally speaking, however, one might expect the protein to weaken the Co–C bond by binding best to the transition state for $AdoB_{12}$ Co–C5′ bond homolysis[38] (i.e., to $Co^{II}B_{12r}$ and Ado· rather than to the $AdoB_{12}$ ground state, since the former more closely resembles the homolysis transition state by Hammond's postulate). Unfortunately, there also currently is no single crystal diffraction structural information for base-on $Co^{II}B_{12r}$,[39,40]

Despite the absence of exact protein-cofactor or even $Co^{II}B_{12r}$ structural information,[40] one theory has slowly emerged as the most accepted idea of how the B_{12}-dependent enzymes might best assist Co-C5′ cleavage. However, this theory involves a *ground-state distortion* and is known as the enzyme-induced "butterfly" or "upward" conformational distortion theory.[39b] It involves corrin ring "upward" flexing and corrin ring-adenosyl steric interactions to "lift" the adenosyl group from cobalt and otherwise distort it along the Co---C5′ bond homolysis reaction coordinate as shown in Figure 6. Evidence for this picture derives from three sources; from precise structural information for the $AdoB_{12}$ cofactor and other corrinoids,[39b,c,41] from studies of B_{12} models, and from investigations of the enzymic affinity and activity of a number of more than 66 chemically modified forms of $AdoB_{12}$.[20b,37a,42] These latter studies clearly show that enzyme binding of the cofactor's "upper", "lower", and side chain groups (Figure 1) is necessary for catalysis. Key cofactor variables in the conformational distortion picture are the following: the corrin's flexibility[39b,c,43,44] and its butterfly conformation;[43,45] the role of the axial base in inducing this conformation[7b,43,45] (and the suggested importance of a long Co---N bond length for homolysis[25b,45,46]); the role of the flexible corrin-ring b, d, and e side chains (see Figure 1) in achieving the butterfly (or other necessary) conformations;[43] the abnormality of the Co–C_α–C_β bond angle (124.0 ± 0.5°) of $AdoB_{12}$ in comparison to other alkylcobalamins, as recently stressed by Golding,[41b] and the susceptibility of the Co–C BDE to angular distortions as stressed by Marzilli[45] and, more recently, by Lipscomb.[9] However, only when structural data is available for one or more of the B_{12}-dependent proteins can we hope to know with certainty, and at the molecular level, exactly how the protein labilizes the Co-C5′ bond.

Finally, it is worth noting that the expected significant role of the protein is consistent with our[4a,b] and Golding's[41b] previous studies of the putative role of the cofactor in the substrate rearrangement step (the so-called cobalt participation or nonparticipation question)[4a,b] for diol dehydrase and with Halpern's probe of the cobalt-participation question for methylmalonyl-CoA mutase,[3b] All the evidence points to the protein's, and not the cofactor's, role in the rearrangement step and in the stereochemistry of the B_{12}-dependent reactions.[4c] The presence of a relatively weak Co–C bond for homolysis is the only—although key—role of the cofactor identified to date.

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Registry No. $AdoCbi^+OH^-$, 27056-34-4; $AdoB_{12}$, 13870-90-1; Co, 7440-48-4.

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(40) One goal of our own efforts is to begin to fill these gaps.

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(43) See, for example: Figures 6, 12, 13, and 14 in ref 39b. (Figure 14 is reproduced herein with permission as Figure 6.)

(44) Compared to a corrin, a porphyrin has been shown to be rather inflexible. Geno, M. K.; Halpern, J. *J. Am. Chem. Soc.* **1987**, *109*, 1238.

(45) (a) An upwardly bent (or butterfly) conformation with unusually long Co–C and cobalt–axial base bond lengths (and often distorted Co–C_α–C_β angles) in B_{12} model complexes with bulky axial bases has been convincingly demonstrated by Marzilli, Randaccio, and co-workers. (b) Marzilli, L. G.; Toscano, P. J.; Randaccio, L.; Bresciani-Pahor, N.; Calligaris, M. *J. Am. Chem. Soc.* **1979**, *101*, 6754. (c) Randaccio, L.; Bresciani-Pahor, N.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1980**, *102*, 7372. (d) Randaccio, L.; Bresciani-Pahor, N.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1981**, *103*, 6347. (e) Bresciani-Pahor, N.; Randaccio, L.; Toscano, P. J.; Marzilli, L. G. *J. Chem. Soc., Dalton Trans.* **1982**, 567. (f) Bresciani-Pahor, N.; Calligaris, M.; Nardin, G.; Randaccio, L. *J. Chem. Soc., Dalton Trans.* **1982**, 2549. (g) Summers, M. F.; Toscano, P. J.; Bresciani-Pahor, N.; Nardin, G.; Randaccio, L.; Marzilli, L. G. *J. Am. Chem. Soc.* **1983**, *105*, 6259. (h) Summers, M. F.; Marzilli, L. G.; Bresciani-Pahor, N.; Randaccio, L. *J. Am. Chem. Soc.* **1984**, *106*, 4478. (i) Bresciani-Pahor, N.; Randaccio, L.; Zangrando, E.; Summers, M. F.; Ramsden, J. H., Jr.; Marzilli, P. A.; Marzilli, L. G. *Organometallics* **1985**, *4*, 2086.

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