Adocobalamin (AdoCbl or Coenzyme B_{12}) Co–C Bond Homolysis Radical-Cage Effects: Product, Kinetic, Mechanistic, and Cage Efficiency Factor (F_c) Studies, plus the Possibility That Coenzyme B_{12}-Dependent Enzymes Function as "Ultimate Radical Cages" and "Ultimate Radical Traps"

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Received October 28, 1992

Following an introduction into solvent radical-cage effects and the general use of cage-trapping methods to study solvent-cage effects, the bio-organometallic enzyme cofactor coenzyme B_{12} (adocobalamin; AdoCbl) is studied using the TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxy) nitroxide cage-trapping method. Specifically, the products, kinetics, and mechanism of AdoCbl Co–C thermolysis in ethylene glycol are presented at high [TEMPO], results which establish (after ruling out other conceivable mechanisms and interpretations) a radical-cage effect for AdoCbl in ethylene glycol. The results also allow a limit to be placed on the important fractional cage efficiency factor (F_c) for AdoCbl, $0.4 \leq F_c \leq 1.0$ (where F_c is defined as the ratio of cage recombination to the sum of all competing cage processes, $F_c \equiv k_{-1}/\sum k_{cage}$). The findings suggest possibly important and more general, but little recognized, biological analogs of cage effects in coenzyme B_{12}-dependent enzymes. Also discussed is the quite speculative but novel and especially intriguing idea that B_{12}-dependent enzymes may include protein "ultimate radical cage" and "ultimate radical trap" effects.

Introduction[1]

Following Franck and Rabinowitch's classic 1934 paper,[2] the concept of solvent-caged radical pairs has been widely accepted and investigated by organic chemists over the intervening half-century.[3] However, solvent cage effects in organometallic or inorganic chemistry are little studied if not largely ignored,[4] save a few notable exceptions.[5,6] Especially rare are measurements of the key indicator of cage efficiency, the fractional cage-recombination efficiency,[7] F_c (defined as the ratio of cage recombination to the sum of all competing cage processes, $F_c \equiv k_{-1}/\sum k_{cage}$). A F_c of even 0.5 for example means that a strong cage exists, with 50 out of 100 cage events being cage recombination.

The significance of F_c measurements in organometallic chemistry are severalfold: (i) There is increasing evidence for sizable solvent-cage effects in organometallic radical reactions.[5,8,9,10] (ii) F_c values for bigger, more massive organometallic radicals are predicted (and recently found[9]) to be larger ($F_c \geq 0.5$) than those seen for smaller, purely organic systems[3a] (perhaps not unexpectedly, as the cage is "wholly enthalpic",[3a] with work by the solute against the solvent-cage walls being the essence of the barrier to cage escape). (iii) Cage effect investigations using organometallic compounds should extend the type and range of fundamental studies that are possible (i.e. in comparison to past organic systems), since metal-based radicals can easily be modified or "fine-tuned" electronically and sterically by simple ligand additions or removals (e.g. the base-off $AdoCbi^+$ vs base-on AdoCbl example herein). (iv) A knowledge of F_c is *required* for solution bond dissociation energy (BDE) measurements, a point completely overlooked until recently.[3]

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(1) Abbreviations used herein include the following: AdoCbl (coenzyme B_{12}, adocobalamin, 5′-deoxy-5′-adenosylcobalamin); $AdoCbi^+$ ($AdoCbi^+BF_4^-$, adocobinamide, 5′-deoxy-5-adenosylcobinamide), $Co^{II}B_{12r}$ (cob(II)alamin), TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxy).

(2) Franck, J.; Rabinowitch, E. *Trans. Faraday Soc.* **1934**, *30*, 120.

(3) (a) For a historical account of solvent cage effects in radical chemistry, plus lead references to important subtopics such as CIDNP and magnetic field effects on chemical reactions, see: Koenig, T. W.; Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1499. (b) For the proper reaction coordinate diagram, and associated equations, for obtaining a solution bond dissociation energy free of cage (but not differential solvent) effects, see: Koenig, T. W.; Finke, R. G. *J. Am. Chem. Soc.* **1988**, *110*, 2657 (see also ref 3a). (c) Koenig, T.; Fischer, H. In *Free Radicals*; Kochi, J., Ed.; John-Wiley: New York, 1973; Vol. I.

(4) Major contributions toward understanding the conceptually related area of cage effects in M–L bond *heterolyses* in heme proteins and bioorganic model systems are available. For example, see the following lead references (see also ref 11b): Traylor, T. G.; Magde, D.; Taube, D. J.; Jongeward, K. A.; Bandyopadhyay, D.; Luo, J.; Walda, K. N. *J. Am. Chem. Soc.* **1992**, *114*, 417. Bandyopadhyay, D.; Walda, K. N.; Magde, D.; Traylor, T. G.; Sharma, V. S. *Biochem. Biophys. Res. Commun.* **1990**, *171*, 306 and references therein.

(5) (a) Sweany, R. L.; Halpern, J. *J. Am. Chem. Soc.* **1977**, *99*, 8335. (b) Sweany, R.; Butler, S. C.; Halpern, J. *J. Organomet. Chem.* **1981**, *213*, 487. (c) Nalesnik, T. E.; Orchin, M. *J. Organomet. Chem.* **1981**, *222*, C5. (d) Nalesnik, T. E.; Orchin, M. *Organometallics* **1982**, *1*, 222. (e) Matsui, Y.; Orchin, M. *J. Organomet. Chem.* **1983**, *244*, 369. (f) Connolly, J. W. *Organometallics* **1984**, *3*, 1333. (g) Jacobsen, E. N.; Bergman, R. G. *J. Am. Chem. Soc.* **1985**, *107*, 2023. These authors provide an early example suggesting that cage effects for organometallic radical pairs can be sizeable, even in low viscosity solvents. (h) Garst, J. F.; Bockman, T. M.; Batlaw, R. *J. Am. Chem. Soc.* **1986**, *108*, 1689. (i) Wassink, B.; Thomas, M. J.; Wright, S. C.; Gillis, D. J.; Baird, M. C. *J. Am. Chem. Soc.* **1987**, *109*, 1995. (j) Bullock, R. M.; Samsel, E. G. *J. Am. Chem. Soc.* **1987**, *109*, 6542.

(6) A new method for measuring *photochemical* fractional cage-efficiency factors, F_{cp} (i.e. of photochemically generated radical pairs) has been developed: Covert, K. J.; Askew, E. F.; Grunkemeir, J.; Koenig, T.; Tyler, D. R. *J. Am. Chem. Soc.* **1992**, *114*, 10446.

(7) Aside from our own recent work,[9] only a limited number of measurements or estimates for organometallic bond homolysis F_c values are available; three earlier *estimates* are (see also ref 5) Endicott's and Netzel's F_c estimate of 0.7 ± 0.5 (range 0.2–1.0) for AdoCbl[8] at 25 °C in H_2O and two *estimated*[3a] on the basis of literature data (see ref 3a, p 1512, $Ni(CO)_4$ in benzene, F_c (est.) ≈ 0.5, and p 1501, a trimetallic metal cluster example, F_c (est.) ≈ 0.5 in benzene). See also ref 10.

(8) (a) Endicott, J. F.; Netzel, T. L. *J. Am. Chem. Soc.* **1979**, *101*, 4000. (b) Chen, E.; Chance, M. R. *J. Biol. Chem.* **1990**, *265*, 12987. (c) Chen, E.; Chance, M. R. *Biochemistry* **1993**, *32*, 1480.

(9) Garr, C. D.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 10440.

(10) (a) Gerards, L. E. H.; Bulthuis, H.; de Bolster, M. W. G.; Balt, S. *Inorg. Chim. Acta* **1991**, *190*, 47. (b) This work[10a] reports viscosity-derived values ($F_c = 0.6$ for AdoCbl in ca. 1.6 cP solution and a photochemically derived $F_{c,p} = 0.27$ for $AdoCbi^+$ in ca. 1.6 cP solution) which are based on assumed products (that are now known to be incorrect; see elsewhere[9] and see also the Experimental Section herein for a AdoCbl in glycerol thermolysis control experiment showing that a β-H elimination product is formed for AdoCbl as well as for[9] $AdoCbi^+$). Hence, the associated kinetic equations[10a] need modification, at least conceptually, and the numerical results are, therefore, quantitatively unreliable. [Note, also that photochemical F_{cp} values cannot be directly compared to thermal F_c values; for further discussion see elsewhere (footnote 12 in ref 9).]

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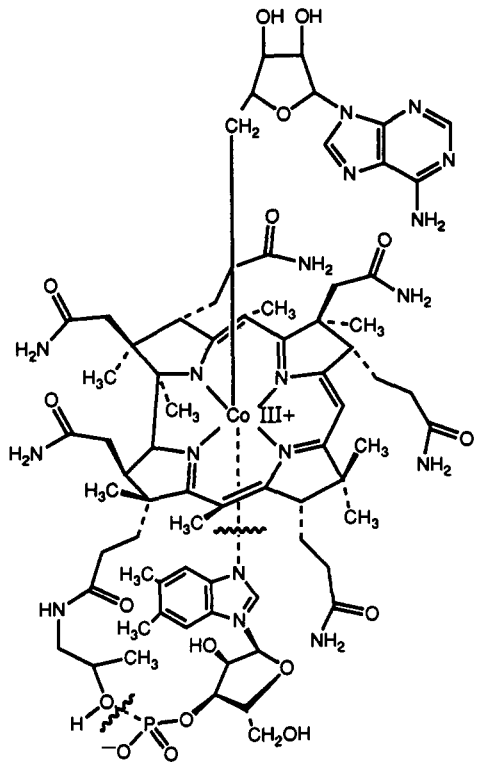


Figure 1. 5′-Deoxy-5′-adenosylcobalamin (AdoCbl). The symbol ⟿ denotes the locations of chemical cleavage during synthesis[14] of axial-base-free AdoCbi$^+$ BF_4^- from AdoCbl.

In addition, cage effects in important bio-organometallic systems such as coenzyme B_{12} or its derivatives, Figure 1, have received only limited attention.[4,9,11] This is despite quite suggestive indications that protein-imposed analogs of solution-cage effects may be operative in enzymes employing radical mechanisms.[8b,12,13,14]

Historically, several methods have been utilized to detect the presence of caged radical pairs:[3c] direct, real time detection using laser flash photolysis;[4,8] stereochemical scrambling experiments;

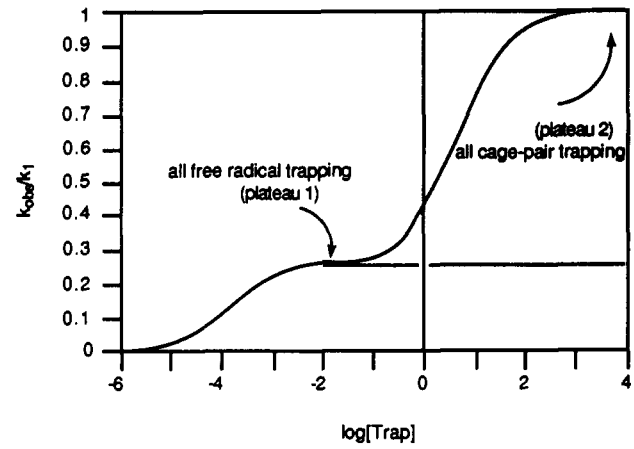
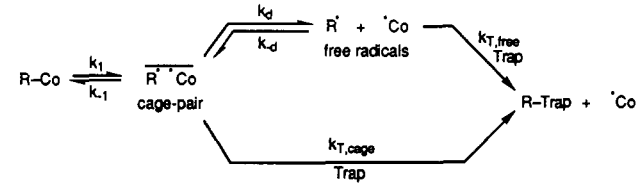


Figure 2. Qualitative plot of the *expected* dependence of k_{obs}/k_1 as a function of log [Trap]. In practice, the exact shape of the curve is dependent on the factors which determine the fractional cage efficiency factor, F_c, such as the solvent and its viscosity, the specific caged-pair, the trap employed, the temperature, and the pressure.

Scheme I. Generalized Phenomenological Model of Cage-Pair Formation from a R–Co Precursor, Followed by Competing Cage-Pair Trapping of Diffusive Cage Escape



viscosity rate dependence investigations;[6,10] crossover experiments; CIDNP effects; and cage-pair scavenging or trapping methods.[3c] Each method has its own advantages and disadvantages, but one of the simplest and often quite effective methods is cage-pair trapping.

Recently we showed that caged-pair trapping, using the nitroxide stable free radical TEMPO,[1] is a simple yet powerful method applicable to alkyl B_{12} complexes such as base-off AdoCbi$^+$.[9] It is useful to consider here the general case of cage-pair trapping of an alkylcobalt complex, Scheme I, and the expected (as well as the at least partially observed[9]) plot of the kinetics as a function of [Trap], Figure 2. Four distinctly different regions are expected in an experiment monitoring k_{obsd}/k_1 vs [trap] from low to very high [Trap] (approaching 1.0 M or greater), Figure 2. First, at low [Trap] where free radical trapping is rate determining (k_{-d}[˙Co(II)] $\gg$ $k_{T,free}$[Trap]), freely diffusing R˙ is trapped outside the cage in competition with R˙ recombination with ˙Co(II); k_{obsd}/k_1 increases with increasing [Trap] in this initial region, Figure 2. Second, at plateau no. 1 in Figure 2, there is still no cage-pair trapping, but diffusion out of the cage (k_d) is rate-determining and there is no diffusive cage return, $k_{T,free}$[Trap] $\gg$ k_{-d}[˙Co(II)], so that k_{obsd}/k_1 is independent of [Trap]. In the third region, the k_{obsd}/k_1 again becomes dependent on [Trap], as the Trap concentration is now high enough that some molecules of the solvent cage are actually Trap molecules; in this region $k_{T,cage}$[Trap] is $\geq k_d$. Fourth, at very high [Trap], a large fraction of the molecules forming the solvent cage are trap and all the caged R˙ is trapped within the cage, in principle even before any internal, "geminate" cage recombination can occur. Homolysis, k_1, is now rate determining, $k_{T,cage}$[Trap] $\gg$ k_{-1}, so that $k_{obsd} = k_1$ at plateau no. 2, Figure 2. In practice, however, this second plateau is difficult to reach, even with very high concentrations of very efficient traps.[3c]

Herein we report the use of the TEMPO nitroxide radical-trapping method to investigate radical-cage effects during the Co–C bond thermolysis of AdoCbl in the moderately viscous, cage-inducing solvent ethylene glycol. The product, kinetic, and

(11) (a) There are only three such studies that we are aware of, including Endicott's and Netzel's important 1979 preliminary communication,[8a] a reinvestigation thereof,[8b] and a very recent (albeit quantitatively unreliable)[10] viscosity-dependent study of adocobamides. (b) For a recent symposium which includes papers on radical cage effects in metallo-biochemistry, see: *J. Inorg. Biochem.*, in press (abstracts from The Sixth International Conference on Bioinorganic Chemistry, August 22–27, 1993, University of California, San Diego, Ca.; see sessions I–V on "Reactive Intermediates, Fast and Ultrafast Kinetics").

(12) (a) Toraya, T.; Ishida, I. *Biochemistry* **1988**, *27*, 7677. (b) Brown, K. L.; Brooks, H. B.; Behnke, D.; Jacobsen, D. W. *J. Biol. Chem.* **1991**, *266*, 6737. (c) Others have indirectly alluded to, or in the case of Chance's work have directly mentioned, the possibility of cage effects in B_{12}-dependent proteins. Specifically, Kraütler has noted that the Co–C bond in AdoCbl "may be labilized largely by way of apoenzyme (and substrate) induced separation of homolysis fragments, made possible by strong binding of both separated fragments to the protein".[23b] Chance has insightfully and directly noted the possibility for cage effects, remarking "our results suggest that control of geminate recombination may be important"[8b] ...and... "one of the enzyme's functions is to separate the radicals in order to prevent rebinding".[8b] Very recently, Chance has noted that B_{12} enzymes likely function "...as sophisticated cages".[8c] (d) Marzilli's review concisely notes evidence which we interpret as fully consistent with a enzyme radical-cage effect (i.e. with the protein in the "ultimate trap" conformation; see the text): "Photolysis of enzyme-bound alkylcobalamins is much more difficult than for the free cobalamin.[28,348,578] In fact, some alkylcobalamins such as the long-chain adenylalkylcobalamins did not photolyze at all when bound to the (B_{12}-binding) enzyme.[348,578]" (References refer to Toscano's and Marzilli's review.) Toscano, P. J.; Marzilli, L. G. *Prog. Inorg. Chem.* **1984**, *31*, 105 and see p 152. (e) See also refs 33–36.

(13) (a) Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1984**, *23*, 3041. (b) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 4820. (c) Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469. Therein the estimate of AdoCbl's Co–C BDE is 30.1 kcal/mol $\leq$ AdoCbl BDE $\leq$ 34.5 kcal/mol.

(14) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012.

mechanistic studies (i) establish the existence of a cage effect for this bio-organometallic cofactor in ethylene glycol by exhibiting three of the four regions depicted in Figure 2, (ii) provide independent and incontrovertable product evidence for a cage (cage-induced β-H elimination) for AdoCbl in the higher viscosity solvent glycerol, and, perhaps most importantly, (iii) suggest possible biological analogs of these solution cage effects in B_{12}-dependent proteins, speculative but intriguing possibilities that are termed "ultimate radical cage" and "ultimate radical trap" effects.

Experimental Section

AdoCbl (Sigma) was stored at 0 °C and used as received. All other materials, instrumentation, and sample preparations are identical to previously published, detailed procedures.[9,13,14] A recent paper,[9] several earlier papers,[13,14] and a dissertation[15] are available for further experimental details.

Product Studies. These were determined by HPLC, as previously described.[9,13,14] Conditions employed were as follows: PRP-1 semi-preparative C_{18} column (Hamilton), λ = 260 nm detection, 2 mL/min flow, 10% CH_3CN/90% H_2O for all nucleosides except Ado-TEMPO, which is retained on the column under these conditions (30% CH_3CN/70% H_2O was used instead). All nucleosides were identified and quantitated by comparison to authentic samples and their HPLC detector response factors (obtained from linear plots of peak area vs concentration of nucleoside); see Results for a summary of the products. No detectable adenine (i.e. ≤1%), a product of Co–C heterolysis, is seen in ethylene glycol; this contrasts AdoCbl thermolysis in H_2O where 3% adenine is seen at 110 °C and 10% adenine is detectable at 85 °C.[13b]

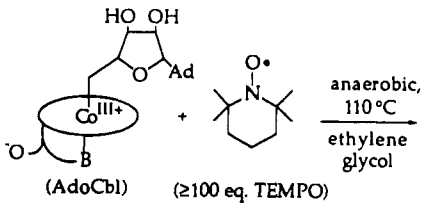
Kinetic Studies. Kinetic runs were carried out as previously reported[9,13,14] by immersing Schlenk cuvettes containing ca. 1×10^{-4} M AdoCbl in purified[9] ethylene glycol in an oil bath (110.0 ± 0.2 °C) for predetermined time periods. Thermal quenching was achieved by transferring the cuvettes to a 20 °C water bath. After equilibrating the sample for >10 min at 25.0 °C inside the UV–visible spectrophotometer, the growth of the $Co^{II}B_{12r}$ product at 555 nm[9] (not 520 nm as before[13]) was monitored (the sample was referenced to ethylene glycol containing an identical [TEMPO]). An isosbestic point at 583 nm confirmed the clean thermal conversion of AdoCbl to $Co^{II}B_{12r}$. Endpoints were obtained by photolysis of the Schlenk cuvettes for ca. 30 min with a 350-W tungsten lamp placed 20 cm away. The photolysis isosbestic points are identical to the thermal isosbestic points listed under Results (Product Studies). This confirms the identical nature of the corrin products in both reactions and, therefore, establishes the validity of using *photolysis* end points in the *thermolysis* kinetics.

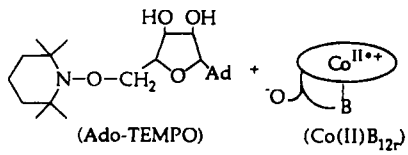
Control Experiment Aimed at Demonstrating Whether or Not AdoCbl Gives a Cage-Induced β-H Elimination Product in Higher Viscous Solvents. In our recent studies of base-off AdoCbi$^+$, the new β-H elimination product 4′,5′-didehydroadenosine [9-(5-deoxy-β-D-*ethryo*-penta-4-enofuranosyl)-adenine] was seen as a major product.[9] However, in these studies and for AdoCbl in 110 °C ethylene glycol, this β-H elimination product is not seen (as summarized under Results). Hence, the control experiment below was done in the more viscous solvent glycerol, primarily to establish whether or not this pathway exists for AdoCbl and, thus, whether or not it needed to be included in our mechanistic scheme, Scheme II.

AdoCbl (8.5×10^{-5} M) was thermolyzed in neat glycerol (Baker) with and without TEMPO (1.7×10^{-2} M). The organic products were quantified by HPLC exactly as described in our prevous paper;[9] in the absence of TEMPO they consisted of 71% 8,5′-anhydroadenosine, 21% 5′-deoxyadenosine, *5% 4′,5′-didehydroadenosine*, and 2% of the heterolysis product adenine. With 1.7×10^{-2} M TEMPO, the organic product distribution is 74% Ado-TEMPO, 16% 8,5′-anhydroadenosine, *4% 5′-deoxyadenosine, 4% 4′,5′-didehydroadenosine*, and 2% of the heterolysis product adenine. Clearly, the β-H elimination product 4′,5′-didehydroadenosine can be formed even for AdoCbl in sufficiently viscous solvents.

Results

Products. The AdoCbl nucleoside thermolysis products at 110 °C (in ethylene glycol) were determined for the range of [TEMPO] covered by the kinetic studies, from 0.0 to 0.02 M TEMPO.[13] *In the absence of TEMPO*, two nucleoside are formed: 8,5′-anhydroadenosine (ca. 77%) and 5′-deoxyadenosine (ca. 23%). These expected homolysis products account for 100 ± 5% of the nucleoside products[13] and are fully consistent with the previously reported product studies of Hay.[16] From 100 equiv of TEMPO (ca. 0.01 M) up to the maximum [TEMPO] studied (0.91 M), *all of the Ado• nucleoside radical is trapped as Ado-TEMPO* (100 ± 5%). This demonstrates that homolysis of the Co–C bond occurs exclusively and quantitatively as summarized in eq 1. Confirming this conclusion, when the AdoCbl





(1)

absorbances were monitored at the following wavelength ranges (and for the indicated [TEMPO]), the following isosbestic points were maintained: from 330 to 600 nm (for 0.0–0.1 M [TEMPO]), isosbestic points were maintained at 583, 487, 391, and 338 nm (lit. values[13] 581, 487, 390, 337); from 500 to 600 nm (for 0.12–0.30 M [TEMPO]), an isosbestic point at 583 nm was maintained; from 540 to 600 nm (for 0.43–0.91 M [TEMPO]), an isosbestic point at 583 nm was maintained. (Representative spectra documenting this isosbestic behavior are provided as supplementary material.)

Kinetic Studies. A crucial difference from all but one[9] of our previous kinetic studies[13,14] is that the disappearance of AdoCbl (λ_{max} = 520 nm) was monitored at 555 nm, somewhat off of its absorbance maximum. This, however, *increases* the precision of the kinetic studies (rather than decreasing them, as one might guess) since interference from TEMPO's competing absorbance is minimized at 555 nm [$\epsilon(AdoCbl)_{555} = 5.6 \times 10^3$ $M^{-1}cm^{-1}$, $\epsilon(TEMPO)_{555} = 1.05$ M^{-1} cm^{-1}]. The key point here is that TEMPO is used in high, up to nearly 1.0 M, concentrations in these studies, while the [AdoCbl] is ca. 1×10^{-4} M; hence, the absorbance of TEMPO is significant and must be minimized by using the 555-nm wavelength (and by subtracting the remaining TEMPO absorbance by employing a reference cell with the identical [TEMPO] as used in the cuvette containing the AdoCbl; see the Experimental Section).

Kinetics were followed over 1.0–4.2 half-lives, Table I, and first-order rate constants (±2–11%, Table I) were obtained by linear regression of $\ln[Abs_\infty/(Abs_\infty - Abs_t)]$ kinetic data. The 23 new kinetic runs done as part of this work are tabulated in Table I and plotted in Figure 2. Note that Table I and Figure 2 also include the 15 *low [TEMPO]* (only) data of Hay,[16b] included to allow the reader to judge the reproducibility of the kinetic data (which is quite good).[16] We also plotted the data in different ways (i.e. a $[trap]^{1/2}$ dependence)[3c,17] in order to test for the possibility of distinguishing so-called 1° vs 2° radical pairs, but these plots (available as supplementary material) were inconclusive.

(15) Garr, C. D. Ph.D. Dissertation, University of Oregon, 1993.

(16) (a) At low (≤0.1 M) [TEMPO] the products, as well as kinetics, have been independently measured by two different researchers[13,15,16b] over a period of ca. 4 years. *All* results are in agreement within experimental error, demonstrating a high degree of reproducibility and, therefore, confidence in at least the low [TEMPO] data reported herein. (b) Hay, B. P. Ph.D. Dissertation, University of Oregon, 1986.

Table I. Reaction of AdoCbl with Varying [TEMPO] Trap (110 °C; Ethylene Glycol)[a]

[TEMPO] (mM)	log [TEMPO]	rate[b] (10^{-4} s^{-1})	half-lives
0.87[c]	−3.0605	0.95(1)	3.0
0.87[c]	−3.0605	1.00(3)	1.0
1.57	−2.8041	1.11(3)	2.3
1.80[c]	−2.7447	1.14(3)	3.6
1.80[c]	−2.7447	1.05(4)	1.0
2.02	−2.6946	1.14(1)	2.0
2.02	−2.6946	1.15(1)	1.6
2.02	−2.6946	1.16(2)	2.1
2.31	−2.6364	1.13(2)	2.2
3.04	−2.5171	1.18(1)	2.4
4.04	−2.3936	1.20(2)	2.2
4.04	−2.3936	1.22(2)	2.0
4.08	−2.3893	1.20(2)	2.5
4.08	−2.3893	1.23(2)	2.5
6.03	−2.2197	1.21(2)	2.6
7.96	−2.0991	1.20(1)	2.6
10.00	−2.0000	1.21(1)	2.7
12.80	−1.8928	1.24(2)	1.7
19.00[c]	−1.7212	1.18(2)	2.2
19.00[c]	−1.7212	1.18(3)	2.2[d]
22.00[c]	−1.6576	1.18(3)	2.2[d]
22.00[c]	−1.6576	1.17(3)	2.2
38.00[c]	−1.4202	1.14(5)	2.8
64.00	−1.1938	1.20(6)	0.6
78.00[c]	−1.1079	1.19(2)	2.7[d]
78.00[c]	−1.1079	1.20(1)	2.7
103.00[c]	−0.9872	1.16(2)	2.5
103.00[c]	−0.9872	1.17(2)	2.5[d]
119.00[c]	−0.9244	1.27(3)	2.9[d]
119.00[c]	−0.9244	1.27(3)	2.9
145.00[c]	−0.8386	1.32(2)	2.9
145.00[c]	−0.8386	1.31(2)	2.9[d]
296.00[c]	−0.5287	1.50(3)	3.6[e]
296.00[c]	−0.5287	1.46(2)	3.6
430.00[c]	−0.3665	1.59(3)	3.6[d]
597.00[c]	−0.2240	1.86(6)	4.2[d]
900.00[c]	−0.0458	1.89(2)	3.6[d]
910.00[c]	−0.0410	1.72(3)	3.3[d]

[a] Followed at 520 nm unless otherwise indicated. [b] The error in the least significant digit is given in parentheses. [c] Data from this work (the other 15 entries, from the previous work of Hay,[13a,c,16b] are included here and in Figure 3 to allow the reader to judge the reproducibility of the kinetic data). [d] Followed at 555 nm. [e] Followed at 540 nm.

Discussion

Cage-Pair Mechanism (Scheme II). The minimum mechanism needed to account for the results obtained herein, plus the relevant literature,[9,13,14,18] is presented in Scheme II. This proposed mechanism is fully consistent with and fortified by the body of prior literature cited above, including the important fact that preliminary evidence for a cage for AdoCbl, in the *weaker cage solvent H_2O* (i.e. vs the present more viscous ethylene glycol), was previously reported as part of laser flash-photolysis studies.[8]

In Scheme II, AdoCbl base-on and base-not-on (i.e. base-off plus tuck-in[19b]) species are in rapid equilibria,[19] but only base-on AdoCbl contributes to Co–C homolysis within experimental error (see Table I elsewhere[14]). The tuck-in form[19b] of AdoCbl has been included in Scheme II for the sake of completeness and to be consistent with the literature but is thought to be base-off-like in its reactivity[19] so that its contribution to the kinetics of Co–C homolysis is also generally assumed to be negligable.[19c] Hence the caged [Ado• •$Co^{II}B_{12r}$] radical pair is formed from homolysis of base-on AdoCbl;[13] the caged pair can either recombine to form the reactant or proceed to product by three known routes: (i) The cage pair can diffuse into the bulk solvent to form free radicals, where the Ado• radical is then quickly trapped to form Ado–TEMPO (plateau 1, Figures 3). (ii) Cage pair trapping can occur at [TEMPO] greater than ca. 0.1 M (where the trap becomes an integral component of the molecules composing the solvent cage), leading to a concomitant increase in the observed rate (Figure 3, the rise in $k_{h,obsd}$ past 10^{-1} M TEMPO). (iii) An in-cage β-hydrogen elimination can occur, resulting in the formation of 4′,5′-didehydroadenosine.

The β-H elimination pathway is *not* present for AdoCbl in moderate viscosity solvents (110 °C; ethylene glycol, 1.73 cP)[20a] since no 4′,5′-didehydroadenosine is detected among the nucleoside products. However, in a control experiment designed to see if this pathway could be "turned on" by the use of a more viscous (stronger cage) solvent, AdoCbl was thermolyzed in neat glycerol, viscosity ca. 10 cP[20b] at 110 °C (or roughly 8 times that of 110 °C ethylene glycol). As detailed in the Experimental Section, HPLC analysis of the organic nucleoside products revealed 4 ± 1% 4′,5′-didehydroadenosine. The in-cage, β-H elimination pathway shown in Scheme II is, therefore, correct and can be enhanced by going to higher viscosity, stronger cage-effect solvents like glycerol.

Consideration of Conceivable Alternative Mechanisms. An important issue is whether or not mechanisms other than a cage-pair trapping pathway can account for the observed rise in $k_{h,obsd}$ vs [TEMPO] seen in Figure 3 at ≥0.1 M TEMPO. In radical-trapping kinetic studies such as the present work, it is always conceivable that the "trap" is *inducing*[21] the observed reaction instead simply of *reporting* on an existing pathway. However, the evidence summarized below is consistent only with TEMPO serving as the expected innocent radical trap, at least in the present studies.[21]

First and foremost, one needs to recall that a solvent cage has been detected for AdoCbl by flash-photolysis studies;[8] hence, a rise in $k_{h,obsd}$ at high [TEMPO] as seen herein is the expected (but heretofore undemonstrated) result. Moreover, the literature of cage chemistry[3-6] effectively predicts a cage process for large organometallics like AdoCbl, even in moderate viscosity solvents. Second, excellent evidence for the presence of a solvent cage effect is provided by the demonstration that we could *induce* the *known* cage β-H elimination product[9] 4′,5′-didehydroadenosine by going to the more viscous, stronger cage solvent glycerol. Third,

(17) It is of fundamental interest to solution cage chemistry to try to determine the order or dependence, that is the $[trap]^x$ dependence, when cage-pair trapping is occurring (i.e. ≥0.1 M TEMPO in Figure 2); is the early prediction[17a] of a half-order dependence, $[trap]^{1/2}$, seen? In the present work, our own attempt to rigorously determine the dependence on the $[trap]^x$ showed an inability of the data to distinguish $[TEMPO]^{1/2}$ vs $[TEMPO]^1$ plots (provided as supplementary material). Historically, the lack of such a dependence caused Hammond[17b] and then Koenig[3c] to abandon cage models that included distinguishable 1° and 2° pairs. (a) Noyes, R. M. *J. Am. Chem. Soc.* **1955**, *77*, 2042. Noyes, R. M. *J. Am. Chem. Soc.* **1956**, *78*, 5486. Noyes, R, M. *J. Chem. Phys.* **1961**, *65*, 763. (b) Watts, H. P.; Hammond, G. S. *J. Am. Chem. Soc.* **1964**, *86*, 911. See also footnote 14 therein pointing out that the half-order dependence is itself an approximation [the result of a series-expansion of a double integral *truncated after the first term*].

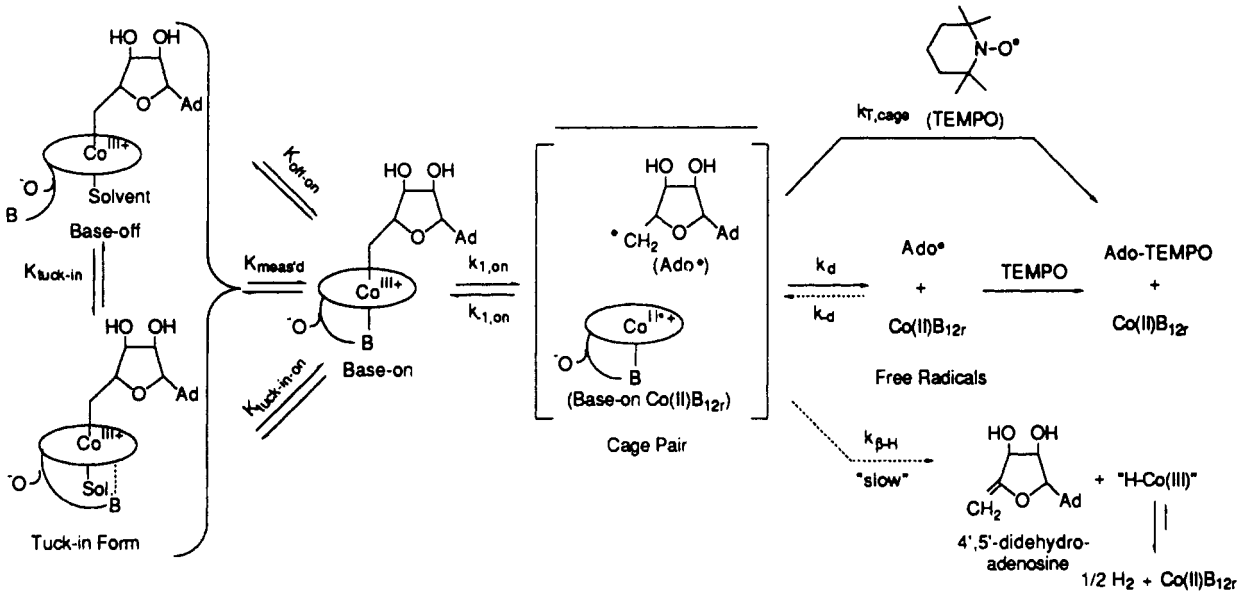
(18) For example, earlier evidence requires that all Co–C bond cleavage events (at ≤0.1 M TEMPO, at least) are Co–C homolysis. The evidence here includes[13] the following: (i) the inverse Co^{II} B_{12r}-dependence exhibited prior to reaching a zero-order TEMPO trap-dependence; (ii) the finding that, upon reaching a zero-order dependence in TEMPO trap, all of $AdoB_{12}$'s 5′-deoxyadenosyl forms Ado–TEMPO, the expected TEMPO-trapped, Ado• radical product.

(19) (a) It is known that axial base-on, base-off and tuck-in-forms[19b] exists and that these equilibria are facile, especially at the higher temperatures required for AdoCbl Co–C bond cleavage.[19c] (b) Brown, K. L.; Peck-Siler, S. *Inorg. Chem.* **1988**, *27*, 3548. Brown, K. L.; Evans, D. R. *Inorg. Chim. Acta* **1992**, *197*, 101. Note that in Brown's literature "K_{Co}" is used for $K_{off\text{-}on}$ and "K_H" for $K_{tuck\text{-}in}$. (c) Martin, B. D.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 585 and references therein. (d) The base-off forms are written as 6-coordinate, solvent-bound forms, although they are conceivably 5-coordinate,[19e-f] especially at higher temperatures. (e) See ref 14 and especially footnote 25 therein. (f) Wirt, M. D.; Chance, M. R. *J. Inorg. Biochem.* **1993**, *49*, 265.

(20) (a) Thomas, L. H.; Meatyard, R.; Smith, H.; Davis, G. H. *J. Chem. Eng. Data* **1979**, *24*, 161. (b) Segur, J. B.; Oberstar, H. E. *Ind. Eng. Chem.* **1951**, *43*, 2117.

(21) For example, as expected (and as discussed in a footnote elsewhere[26b]), TEMPO• is *not* a suitable "trap" for a *paramagnetic*, d^5 $Ru(OEP)CH_3$ complex: Seyler, J. W.; Fanwick, P. E.; Leidner, C. R. *Inorg. Chem.* **1992**, *31*, 3699.

Scheme II. Proposed Minimum Mechanism for the Anaerobic Thermolysis of AdoCbl (110 °C; Ethylene Glycol) in the Presence of TEMPO Radical Trap[a]



[a] A pre-equilibrium between the base-on and base-not-on (base-off and tuck-in)[19a] species is followed by AdoCbl base-on Co–Co homolysis to give a caged [Ado• •Co(II)B_{12r}] radical pair. The caged radical pair can either be trapped by TEMPO in the cage (at ≥0.1 M [TEMPO]), undergo an in-cage β-H elimination reaction, or diffuse out of the cage. Note that the tuck-in form is included but is thought to be base-off-like in its reactivity and thus not a contributor to the Co–C bond homolysis process[19] (or its microscopic reverse, cage recombination).

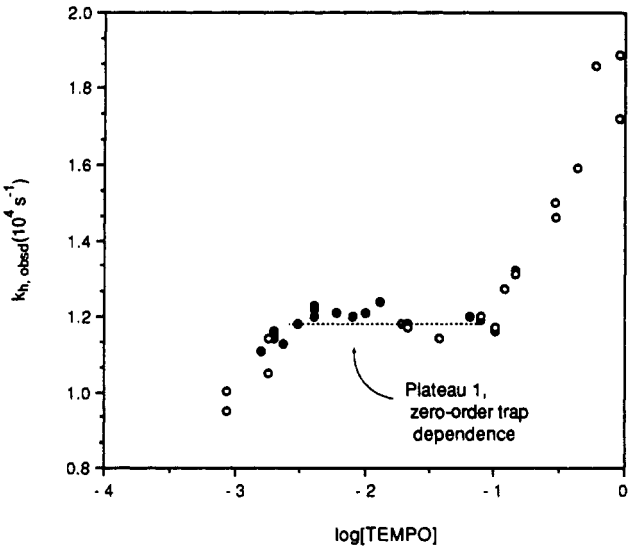


Figure 3. Observed rate constant for the anaerobic thermolysis of AdoCbl (110 °C; ethylene glycol) as a function of log [TEMPO] trap concentration. Error bars (2–11%; available in Table I) are omitted for clarity (○ = this work, ● = previous low [TEMPO] data, included here for comparative purposes[13a,c,16b]).

of all the alternative mechanisms we considered[22] (and ruled out) in the closely related case of AdoCbi$^+$,[9] only one applies or needs to be considered here for AdoCbl (see supplementary material elsewhere[9] if further details are desired). The only "reasonable" alternative mechanism for AdoCbl plus high [TEMPO] is a S_H2 type bimolecular displacement of the nitroxide attacking the 5′ position of the adenosyl in AdoCbl. However, the S_H2 mechanism would require an unprecedented and improbable bimolecular reaction of a *stable* nitroxide on a *neopentyl-like* adenosyl carbon center; moreover, a S_H2 mechanism is completely at odds (and unable to explain) the formation of the β-H elimination product 4′,5′-didehydroadenosine. The TEMPO nitroxide is, therefore, not involved in inducing Co–C homolysis in AdoCbl but instead is simply "reporting" cage-pair trapping (as previously found[9] for base-off AdoCbi$^+$).

Note that we cannot rule out mechanisms where cage-pair trapping occurs in part (or even completely) by the paramagnetic TEMPO• reacting with •Co^{II}Cbl. This, however, does little to change the inherent usefulness of the TEMPO cage-pair trapping method.

Calculation of the Fractional Cage Efficiency Factor, F_c, for AdoCbl in 110 °C Ethylene Glycol. Given the data in Table I and Figure 3, a lower limit (plus the physical upper limit of 1.0) can be placed on the fractional cage-recombination efficiency factor, F_c. The detailed kinetic derivations and lower limit calculations are provided as supplementary material. The result is a fractional cage-recombination efficiency for AdoCbl of $0.4 \geq F_c \geq 1$ in the moderate viscosity (1.73 cP)[20] solvent 110 °C ethylene glycol. Note that the higher the F_c, the stronger the cage effect.

Comparison of the F_c Values for Base-on AdoCbl vs Base-off AdoCbi$^+$. Previously, our analogous cage-pair trapping studies of AdoCbi$^+$ provided a cage efficiency factor of $0.94 \leq F_c \leq 1$ (also in 110 °C ethylene glycol), an unprecedentedly strong solvent cage in comparison to all available F_c values in similar viscosity solvents.[9] In other words, in the case of AdoCbi$^+$, at least 94 of every 100 cage events are Ado• plus •Co$^{II}B_{12r}$ recombination, despite a more facile competing β-H elimination reaction for AdoCbi$^+$ that also occurs within the cage.[9] The minimum calculated F_c values for AdoCbl (≥0.4) and AdoCbi$^+$ (≥0.94; under identical experimental conditions) *suggest* (but do not prove) that base-on AdoCbl *may* exhibit a significantly weaker solvent cage than does axial-base-free AdoCbi$^+$ at the same [TEMPO]. Unfortunately, a rigorous conclusion cannot be reached presently due to the greater than or equal to signs; that is for AdoCbl, $0.4 \geq F_c \geq 1$, and for AdoCbi$^+$, $0.94 \leq F_c \leq 1$.

(22) In the case of AdoCbi$^+$, we already considered and ruled out explanations involving the following: (i) A TEMPO-induced solvent viscosity change[9] (TEMPO was shown not to cause any measureable change in the viscosity of ethylene glycol;[9] hence, such an explanation can be ruled out for *all* aklylcobamide thermolyses); (ii) a hypothetical axial-bound-nitroxide induced rate enhancement (note, then, that such a mechanism becomes even more implausible for AdoCbl in comparison to AdoCbi$^+$, as any putative TEMPO axial-base adduct would be in lower concentration due to the competing presence of the appended 5,6-dimethylbenzimidazole axial-base which occurs naturally in AdoCbl); (iii) a S_H2 reaction at the 5′ Ado carbon was also ruled out for AdoCbi$^+$. For additional details, see the supplementary material available elsewhere.[9]

(In the supplementary material a plot is provided that gives a better, visual intuition for how the size of F_c is exhibited in a $k_{h,obsd}$ vs log [TEMPO] plot.)

Even though the experimental evidence for a weaker cage for base-on AdoCbl vs base-off AdoCbi$^+$ is not definitive at this time, it should be noted that one *expects* a weaker cage for AdoCbl on the basis of the following literature. The result of an X-ray single crystal structural analysis[23b] confirms[24] that the axial-base is coordinated in $Co^{II}B_{12r}$; it also reveals that the Co(II) atom moves 0.12 Å toward the axial base yielding a Co(II) atom *that is quite hindered sterically (literally on the wrong side of the corrin plane) for Ado$^\bullet$ plus $^\bullet Co^{II}B_{12r}$ recombination.* This surely results in a smaller k_{-1} value and, thus, a smaller F_c value ($F_c \equiv k_{-1}/\sum k_{cage}$) for base-on AdoCbl vs base-free AdoCbi$^+$. Confirming the idea that base-off $Co^{II}Cbi^+$ should undergo faster recombination with Ado$^\bullet$, two single X-ray crystallographic structural investigations of *axial-5,6-dimethylbenzimidazole-base-free* Co(II)cobyrinates[25] show some greater corrin flexibility[26] and thus presumably a greater exposure of Co(II) out of the plane and toward an incoming Ado$^\bullet$. This should result in a significantly greater k_{-1} and F_c value for the axial-base-free form $Co^{II}Cbi^+$, a prediction fully consistent with the large, relatively precise F_c value of 0.94 $\leq F_c < 1.0$ for AdoCbi$^+$.

It will be important, however, to expand upon the structural ideas and initial kinetic studies for AdoCbl presented above by (i) fast kinetic measurements of k_{-1} for $Co^{II}B_{12r}$ and $Co^{II}Cbi^+$ and (ii) X-ray crystallographic, EXAFS, and molecular modeling structural studies of $Co^{II}Cbi^+$ in, ideally, a more *distorted, nonplanar, more thermolysis transition-state-like conformation* (one that is required by microscopic reversibility to be equivalent to the transition state for recombination).

Refinement of AdoCbl's Solution Co–C Bond Dissociation Energy (BDE). The evidence presented herein (and elsewhere[8,10]) for a cage effect for AdoCbl reveals that previous methods for the derivation of solution BDEs,[27] which rely on a gas-phase reaction coordinate in which a solvent cage cannot possibly be accounted for, are oversimplified and must be abandoned.[3a] The proper equations for converting a solution $\Delta H^{\ddagger}_{obsd}$(solu) measurement into a solution BDE (*assuming* that differential solvation effects can be neglected[3a]) have been described; a key equation is provided[3a] in the form of eq 2. Note that *a measurement of F_c*

$$\text{BDE} \approx \Delta H^{\ddagger}_{obsd}(\text{solu}) - F_c \Delta H^{\ddagger}_{\eta} \qquad (2)$$

is an absolute requirement[3a] for obtaining a BDE from a solution $\Delta H^{\ddagger}_{obsd}$(solu). Using the $F_c \geq 0.4$ reported herein, plus published values[13a,c] for AdoCbl in ethylene glycol of $\Delta H^{\ddagger}_{obsd}$(solu) = 34.5 ± 0.8 kcal/mol and $\Delta H^{\ddagger}_{\eta}$ = 4..4 kcal/mol (the enthalpy for viscous flow for ethylene glycol in the range 90–110 °C), one can refine significantly the earlier estimate of AdoCbl's Co–C BDE[13c] as done in eqs 3a–c. Note also that in the proper equation, eq

$$\text{AdoCbl BDE} \approx 34.5 - (\geq 0.4)4.4 \text{ kcal/mol} \qquad (3a)$$

$$30.1 \text{ kcal/mol} \leq \text{AdoCbl BDE} \leq 32.7 \text{ kcal/mol} \qquad (3b)$$

$$\text{AdoCbl BDE} = 31.4 \pm 1.5 \text{ kcal/mol} \qquad (3c)$$

2 above, $\Delta H^{\ddagger}_{\eta}$ of ca. 4.4 kcal/mol is used to approximate $\Delta H^{\ddagger}_{d}$, the enthalpy for *diffusion out through the solvent cage walls* and into solution (see[3a] eqs 11 and 11a). Hence the assignment of this enthalpic barrier by others to "the solvent dependence of the activation enthalpy of the *reverse recombination process*"[27a] is a conceptually backward and confusing notion that also must be abandoned. (This confusion again results from the improper adaptation of a gas-phase reaction coordinate to solution kinetic measurements; for further discussion, see elsewhere.[3a])

A Discussion of the Possibility That B_{12}-Dependent Enzymes Function as Protein "Ultimate Radical Cages" and "Ultimate Radical Traps". The findings that large bio-organometallic cofactors such as AdoCbl and AdoCbi$^+$ exhibit sizable cage effects even in moderate viscosity solutions raises the logical and intriguing question "Can B_{12}-cofactor-dependent enzymes take advantage of perhaps much larger *protein-based analogs of solution radical-cage effects?*" The idea here, one schematically illustrated in Figure 4, is that a B_{12}-binding protein could easily sit in a conformation with an enzyme-weakened Co–C bond (evidence for this exists[28]) but a conformation where any Co–C homolysis events *in the absence of substrate* lead only to radical recombination (AdoCbl Co–C bond re-formation). In this mode, and drawing analogy to the solution cage, the "viscous" protein (with its large barrier to "viscous flow"; i.e., its large $\Delta H^{\ddagger}_{\eta}$) could serve as the "ultimate radical trap", *effectively enforcing cage recombination nearly 100% of the time for a F_c of near 1.0.* (Interestingly, in this conformation the protein would be providing a unimolecular analog of the solution, bimolecular "persistent Co(II) radical effect" recently reported.[29])

Then, once substrate binds providing additional enzyme·substrate·B_{12} "intrinsic binding energy",[14,30] one can imagine a protein conformational change that moves the enzyme-bound[31] Ado$^\bullet$ effectively off of the normal Co---C vector (or, possibly, still roughly along the Co–C axis, but some 7–8 Å above the corrin as Glusker's detailed modeling and graphics suggest).[32]

(23) (a) A crystal structure of $Co^{II}B_{12r}$ has been reported[23b] as has the EXAFS[23c] and X-ray edge spectroscopy[23d] of $Co^{II}B_{12r}$. (b) Kräutler, B.; Keller, W.; Kratky, C. *J. Am. Chem. Soc.* **1989**, *111*, 8936. (c) Sagi, I.; Wirt, M. D.; Chen, E.; Frisbie, S. M.; Chance, M. R. *J. Am. Chem. Soc.* **1990**, *112*, 8639. (d) Wirt, M. D.; Sagi, I.; Chen, E.; Frisbie, S. M.; Lee, R.; Chance, M. R. *J. Am. Chem. Soc.* **1991**, *113*, 5299.

(24) (a) Lexa, D.; Savéant, J.-M. *J. Am. Chem. Soc.* **1978**, *100*, 3220. (b) Lexa, D.; Savéant, J.-M. *Acc. Chem. Res.* **1983**, *16*, 235.

(25) (a) Kräutler, B.; Keller, W.; Hughes, M.; Caderas, C.; Kratky, C. *J. Chem. Soc., Chem. Commun.* **1987**, 1678. This paper shows a structure of [heptamethyl–Co^{II}cobyrinate]$^+ClO_4^-$. (b) A structure of the [Co^{II}–I–Co^{II}]$^+I^-$ complex, [(heptamethyl–Co^{II}cobyrinate)$_2$I]$^+I^-$, has also appeared, see: Glusker, J. P. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, Chapter 3.

(26) (a) The flexibility of the corrin ring, often refered to as the "butterfly effect" or "upward corrin conformational distortion theory" for the AdoCbl Co–C homolysis step, is well-known and generally thought to be dependent on the axial-base. For lead references, see elsewhere Glusker's chapter[25b] as well as elsewhere.[26b-d] (b) Waddington, M. D.; Finke, R. G. *J. Am. Chem. Soc.* **1993**, *115*, 4629. (c) Marzilli, L. *Bioinorganic Catalysis*; Reedijk, J., Ed.; Marcel Dekker: New York, in press. (d) Bresciani-Pahor, N.; Forcolin, M.; Marzilli, L. G.; Randaccio, L.; Summers, M. F.; Toscano, P. J. *Coord. Chem. Rev.* **1985**, *63*, 1. (e) Brown, K. L.; Brooks, H. B. *Inorg. Chem.* **1992**, *30*, 3420.

(27) (a) Halpern, J. *Pure Appl. Chem.* **1979**, *51*, 2171. (b) Geno, M. K.; Halpern, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1052. (c) See also the discussion in references and footnotes 24a–k provided elsewhere.[3a]

(28) (a) Evidence is available that the Co–C bond in the AdoCbl-enzyme *substrate-free* complexes is weakened enough to undergo slow racemization, $\leq 10^{-7}$ the rate of turnover [although one worries about the effects of trace light photohomolysis in such difficult studies, even though this work is by experts who specifically state that the stereospecifically deuterated AdoCbl-enzyme incubation studies (but not the isolation and workup?) were done "in the dark"].[28b] (b) Gaudemer, A.; Zylber, J.; Zylber, N.; Baran-Marszac, M.; Hull, W. E.; Fountoulakis, M.; König, A.; Wölfle, K.; Rétey, J. *Eur. J. Biochem.* **1981**, *119*, 279.

(29) Daikh, B. E.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 2938.

(30) For a brief discussion of this intrinsic binding energy and protein folding see pp 258–259 of the following: Finke, R. G. In *Molecular Mechanisms of Bioorganic Processes*; Bleasdale, C., Golding, B. T., Eds.; The Royal Society of Chemistry: Cambridge, England, 1990.

(31) For a presentation of the B_{12} enzyme "bound radical mechanism," see a 1984 review: Finke, R. G.; Schiraldi, D. A.; Mayer, B. J. *Coord. Chem. Rev.* **1984**, *54*, 1.

(32) In some intriguing molecular modeling and graphics studies, Glusker has found that if adenine is enzyme-bound parallel to the plane of the corrin (as found in AdoCbl's X-ray structure), and if a rotation about the C(8)–N(9)–C(1′)–O(1′) glycosidic bond occurs, that it then puts the Ado 5′-$CH_2^\bullet$ carbon radical ca. 7.4 Å above the corrin plane; see Figures 8–10 and supplementary material Figure S in: Pett, V. B.; Liebman, M. N.; Murray-Rust, P.; Prasad, K.; Glusker, J. P. *J. Am. Chem. Soc.* **1987**, *109*, 3207.

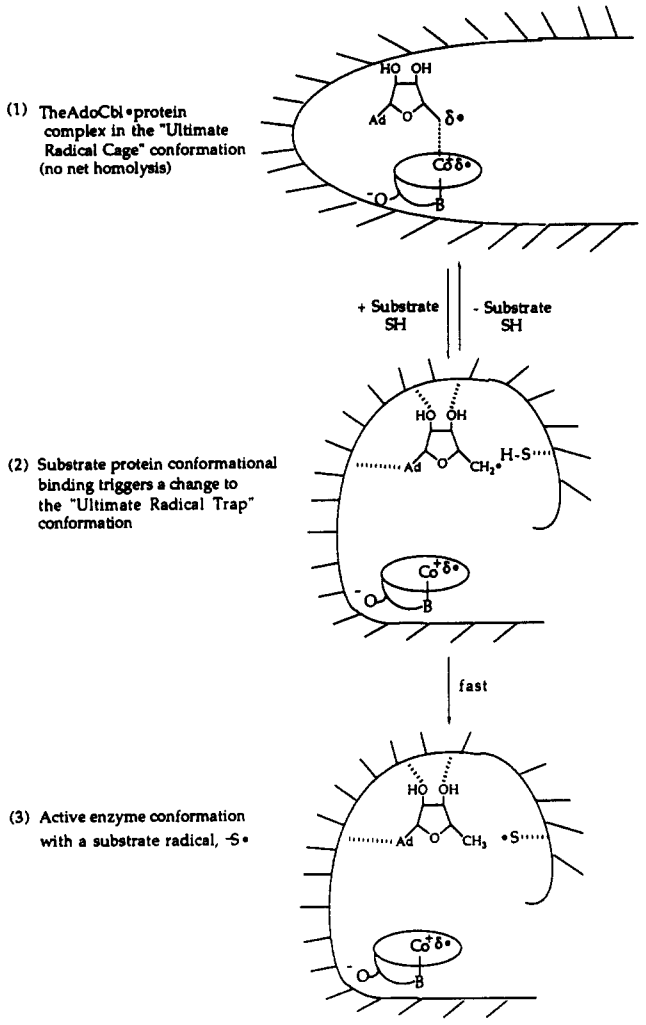


Figure 4. Schematic of the possibility that B_{12}-dependent proteins act as the "ultimate radical cage" and, alternatively, once substrate is bound and a protein conformational change occurs, the "ultimate radical trap". In step 1, the Co–C bond is weakened in enzyme-bound AdoCbl, but any homolysis events lead only to recombination. In step 2, substrate binds and then a precedented protein conformational change[31] occurs, presumably separating Ado• and •$Co^{II}B_{12r}$ radicals while also placing substrate,[32] –S–H (or possibly a protein side-chain, –X–H" group[34]), in direct contact with the Ado• radical. In this latter "ultimate radical trap" mode, each Co–C homolysis event leads to separation of the Ado• and •$Co^{II}B_{12r}$ radicals and thus to the generation of a substrate radical, —S•.

In any event, the result of such a *known* substrate-induced protein-conformational change[33] could be the "ultimate radical trap", Figure 4. In this conformation, the protein could serve to completely separate the enzyme-bound Ado• and •$Co^{II}B_{12r}$ and to move Ado• closer to the substrate (or possibly toward a –X–H radical chain H-transfer site; see elsewhere for a discussion of intriguing but poorly understood possibility[34]). In this putative "ultimate radical trap" mode, the protein would achieve a F_c of near 0. We note in this regard that there are already numerous examples in the B_{12} enzyme literature of rate effects on Co–C cleavages in B_{12}-dependent enzymes that are poorly understood but perhaps now best interpreted as protein cage effects[12] (i.e. "best interpreted" until definitive experimental evidence for or against such protein cage effects is forthcoming). Moreover, there exists a whole class of protein motions, the so-called *protein low-frequency motions*,[35] that would seem to be ideal for use in such putative protein conformational changes between the "ultimate radical cage" and the "ultimate radical trap" conformations. It should also be noted that protein cage effects can in principle account for a sizable piece of the enzymic 10^{12} activation of AdoCbl's Co–C bond that we discovered sometime back now.[36,37] Hence, and *although clearly very speculative at this time*, the "ultimate radical cage" and "ultimate radical trap" hypothesis is fully consistent with a body of experimental

(33) (a) Stop flow kinetic experiments provide good evidence for a relatively slow (2–5 s^{-1}) conformational change when AdoCbl and substrate (in one syringe) are added to protein (in a separate syringe) in a stop-flow experiment. If, however, in a separate stop-flow experiment the protein and AdoCbl are premixed in one syringe and then stop-flow-mixed with substrate (placed in a separate syringe), the rate of $Co^{II}B_{12r}$ formation is now 100-fold faster, 300 s^{-1}. Holloway, M. R.; White, H. A.; Joblin, K. N.; Johnson, A. W.; Lappert, M. F.; Wallis, O. C. *Eur. J. Biochem.* **1978**, *82*, 143. (b) The variable O_2 sensitivity of different $AdoB_{12}$ derivatives, and the protection against O_2 afforded by bound substrate, have been interpreted as evidence for a long (weakened) Co–C form when $AdoB_{12}$ is bound to the enzyme: Toraya, T.; Ishida, A. *J. Biol. Chem.* **1991**, *266*, 5430.

(34) (a) Alternatively, if the bound substrate (–S–H; Figure 4) is instead an enzyme side-chain "—X–H" site as it is known in the literature,[34b,c] Ado• H-abstraction from such a enzyme amino acid side chain would instead produce a —X• protein side-chain radical, leading to a novel biological radical-chain mechanism, an idea originally presented by Cleland,[34b] thoroughly discussed by Stubbe,[34c] and for which the available evidence has been reviewed elsewhere.[30] (b) Cleland, W. W. *CRC Crit. Rev. Biochem.* **1982**, *13*, 385. (c) Stubbe, J. *Mol. Cell Biochem.* **1983**, *50*, 25.

(35) One can ask are there likely to be protein motions of the type and time scale suitable for such a substrate-induced conformation change? The literature strongly suggests that, indeed, there is a whole class of protein motions, the so-called protein low-frequency motions, that would seem to be ideal for the required conformational changes. The well-studied protein BPTI (bovine pancreatic trypsin inhibitor) illustrates the possibilities here.[35a] Typically, proteins have many low-frequency modes, 20–200 cm^{-1}, corresponding to timescales of ca. 0.6–6 ps, and the number of such low-frequency modes is large, roughly 3 times the number of amino acids! Frequencies ≥ ca. 200 cm^{-1} are mostly due to localized movements of protein side chains, while those ≤ ca. 120 cm^{-1} correspond more to the protein behaving as a "continuous body", in collective "shear", "torsional", or "pulse" modes, for example.[35] Root-mean-square displacements of ca. 0.2–2.5 Å are possible. Rather clearly, the time scale and distances of such fascinating low-frequency protein motions are in the range that could very plausibly be key for proteins serving as "ultimate cages" or "ultimate traps". (a) Gō, N.; Noguti, T.; Nishikawa, T. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3696. (b) Debrunner, P. G.; Frauenfelder, H. *Ann. Rev. Phys. Chem.* **1982**, *33*, 283 and references therein. (c) Frauenfelder, H.; Wolynes, P. G. *Science* **1985**, *229*, 337. (d) Gō, N.; Noguti, T. *Chem. Scr.* **1989**, *29A*, 151. (e) Genberg, L.; Richard, L.; McLendon, G.; Miller, R. J. D. *Science* **1991**, *251*, 1051. (f) Peticolas, W. L. In *Methods in Enzymology*; Hirs, C. H. W., Timasheff, S. N., Eds.; Academic: New York, 1979; Vol. 61, Part H, p 425.

(36) An important conclusion from our 1984 study of the thermal homolysis of AdoCbl's Co–C bond is that AdoCbl binding enzymes are enhancing the rate of Co–C homolytic cleavage, in comparison to the rate of AdoCbl Co–C homolysis *in enzyme-free solution*, by an enormous and previously unappreciated factor[13a] of ca. 10^{12}. This is as large any enzymic enhancement on record for the cleavage of a single bond (see elsewhere for documentation of this point[14]). In essence, then, a state-of-the-art problem in protein folding was discovered, raising the following question: How does a protein bind a large metallocofactor like AdoCbl so that the intrinsic binding energy is converted not to heat but instead to productive catalysis along the reaction coordinate (of Co---C homolysis, in this case)? The present studies raise, therefore, one final question: Can protein cage effects account for some piece, perhaps the remaining unaccounted for 10^6, of this remarkable 10^{12} rate enhancement?[37] After a bit of reflection, it is clear that if the AdoCbl•protein•*substrate* complexes' F_c were very small and essentially $F_c = 0$, and if the AdoCbl•protein (no substrate) F_c were larger (e.g. 0.999999), then an apparent Co–C homolysis rate slowing of ca. 10^6 could occur in comparing the AdoCbl•protein holoenzyme complex *with and without substrate*. Although this example is contrived for the sake of illustration, it is consistent with the $\leq 10^{-7}$ slower Co–C cleavage rate (actually, a racemization rate[28]) of the AdoCbl•protein complex *in the absence of substrate*. Restating this hypothetical case, if one assumes a $F_c = 0$ in the "ultimate trap" conformation, then the apparent rate decrease, for a protein F_c of 0.99, 0.9999, or 0.999999, is a slowing of the apparent Co–C homolysis by 10^2, 10^4, and 10^6, respectively, in the "ultimate cage" conformation. (A short kinetic proof of this perhaps obvious point is provided in the supplementary material.)

(37) Elsewhere we have investigated the full range of chemical models for this Co–C activation step, the prototype of which currently is the sterically distorted case of NpCbl (Np = neopentyl), where 10^6 of the enzymic 10^{12} rate enhancement is seen (in comparing NpCbl to AdoCbl) in this purely chemical model.[26b] Note that an as yet unknown piece of this 10^6 rate enhancement is *presumably* due to the weaker cage (smaller F_c) of NpCbl vs AdoCbl (although the exact F_c for NpCbl needs to be measured).

data—both enzymic and chemical—and thus deserves close experimental scrutiny.[38]

Future Studies

Additional goals for future research that are both needed and likely to prove productive include (i) unequivocal demonstration of the *protein cage*, measurement of the protein's F_c under conditions with and without substrate, (ii) measurement of a more precise AdoCbl F_c value, and (iii) measurement of a F_c value for important reference alkylcobalamins such as neopentylCbl.[36] These and other studies are planned and will be reported in due course.

(38) Experiments based on this hypothesis, specifically experiments probing whether or not sizable cage effects are present in MeB_{12} binding proteins (and thus whether or not the hypothesis extends to the MeB_{12} proteins), are in progress. Matthews, R. Private communication.

Acknowledgment. We thank Matthias Pohl for helpful comments and proofreading. Support was provided by NIH Grant DK 26214. Support from the U.S. Department of Education, Graduate Assistance in Areas of National Need Program, to C.D.G. is also gratefully acknowledged.

Supplementary Material Available: Kinetic equations for Scheme II, text and equations describing the calculation of the fractional cage recombination efficiency, F_c, figures showing representative spectra documenting the isosbestic points seen during the thermolysis of AdoCbl with high [TEMPO] in ethylene glycol, first-order and half-order TEMPO-dependence graphs, a plot providing a better, visual intuition for how the size of F_c is exhibited in $k_{h,obsd}$ vs log [TEMPO], and text and equations deriving the "ultimate trap" contributions to the enzyme-induced rate enhancement (8 pages). Ordering information is given on any current masthead page.