Magnetic Field Effects in Adenosylcob(III)alamin Photolysis: Relevance to B_{12} Enzymes

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Abstract: The magnetic field dependence of geminate radical pair recombination following the photolysis of adenosylcob(III)alamin (AdoCbl^{III}) has been studied by continuous-wave photolysis and picosecond laser flash photolysis techniques. The net quantum yield (ϕ) for continuous-wave 514-nm photolysis of AdoCbl^{III} is decreased 2-fold at 80 mT in aqueous 75% glycerol and 1.8-fold at 50 mT in aqueous 20% Ficoll-400. No magnetic field dependence is observed in H_2O. Glycerol is a microviscosigen that enhances geminate radical pair recombination by decreasing diffusion, whereas Ficoll-400 is a macroviscosigen that enhances geminate radical pair recombination by forming "cage" structures in solution. The rate constant for AdoCbl^{III} geminate radical pair recombination following photolysis at 532 nm is increased from 1×10^9 s^{-1} to 4×10^9 s^{-1} at 50 mT in 75% glycerol and from 1×10^9 s^{-1} to 3×10^9 s^{-1} at 80 mT in H_2O, indicating that the true rate for geminate radical pair recombination is being observed and that the rate for geminate radical pair recombination is magnetic field sensitive. The magnetic field-induced increase in the recombination rate constant and the corresponding decrease in net ϕ is consistent with the geminate radical pair being born in the singlet spin state. The picosecond quantum yield, ϕ_{ps}, is 0.7 ± 0.1. Within 2 ns after the photolyzing pulse, 75% of the geminate radical pair population recombines. From nanosecond transient photolysis studies (Chen, E.; Chance, M. R. *J. Biol. Chem.* **1990**, *265*, 12987-12994), a large fraction of the remaining 25% of the radical pair population remains in the solvent cage and recombines on a slower time scale to give an overall fraction of recombination greater than 0.9. The extreme lability of the C-Co bond and the high rate of geminate radical pair recombination makes AdoCbl^{III} well designed to be a transient radical source for enzymatic catalysis. An inherently high rate of geminate radical pair recombination also suggests that the rate of reaction of AdoCbl^{III}-dependent enzymes may be affected by magnetic fields near 50-80 mT at subsaturating substrate concentrations.

Introduction

Nearly all discussions of alkylcob(III)alamin chemistry and reactivity focus on the unusual lability of the C-Co bond.[1] Estimates of the bond dissociation energy of adenosylcob(III)alamin (AdoCbl^{III}) are as low as 31 kcal/mol, and this allows for thermal or photochemical homolysis.[2] The photolysis of (AdoCbl^{III}) is known to produce a geminate radical pair (indicated by brackets) consisting of cob(II)alamin (Cbl^{II}) and the 5′-deoxyadenosyl radical ($^{\cdot}CH_2$-Ado; Chart I).[3] Previous picosecond laser flash photolysis experiments of adenosylcob(III)alamin have shown this to be a reversible process with a *geminate* recombination rate constant of $k_{rec} \approx 1 \times 10^9$ s^{-1} following photolysis.[3a] Recent nanosecond laser flash photolysis studies have probed a slower radical pair recombination that occurs later than 10^{-9} s.[3e,f] Clearly, there are two temporally distinct regimes of recombination: *geminate recombination* that occurs with only minimal separation of the radical pair and before molecular correlation occurs (limited to less than $1-2 \times 10^{-9}$ s), and more general *cage recombination* that is slow enough for molecular motion to be important but still represents events before escape from the solvent cage (up to 10^{-6} s). The transient-state kinetics reported herein are limited to observation of the *geminate* radical pair and the early recombination event.

In a preliminary report, we demonstrated a magnetic field dependence of the steady-state photolysis of methylcob(III)alamin at 248 nm.[4] In this report, we demonstrate a magnetic field dependence of the recombination of the geminate {$^{\cdot}CH_2$-Ado:Cbl^{II}} radical pair (Scheme I). Since an external magnetic field can only alter the rate of a reaction with a biradical or a spin-correlated radical pair intermediate,[5] this observation shows that the {$^{\cdot}CH_2$-Ado:Cbl^{II}} geminate radical pair retains its spin correlation in spite of spin-orbit coupling (SOC) and other processes that would promote electron spin relaxation. This finding is relevant to the chemistry of enzymes that utilize AdoCbl^{III} as a cofactor and may be sensitive to external magnetic fields. This finding also adds to the increasing number of heavy atom systems that exhibit magnetic field-dependent chemistry.[6]

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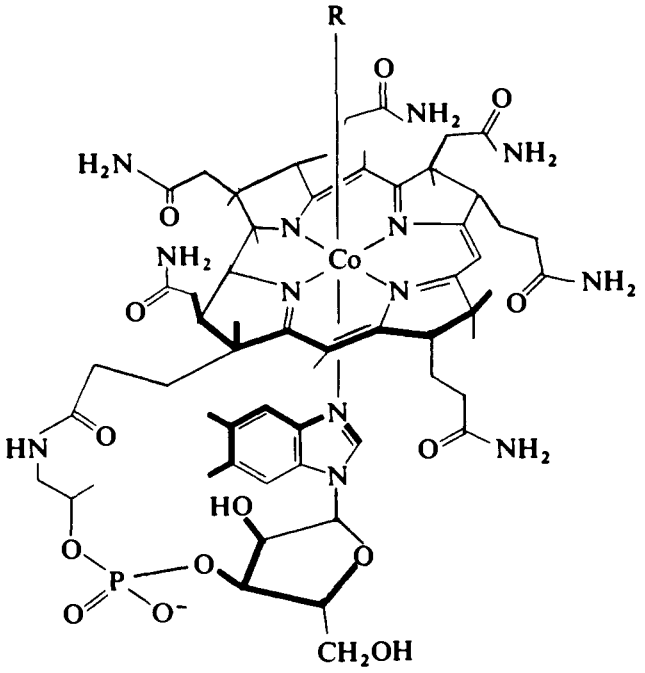
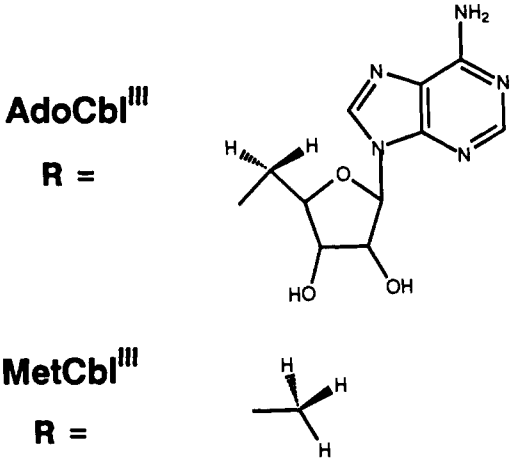
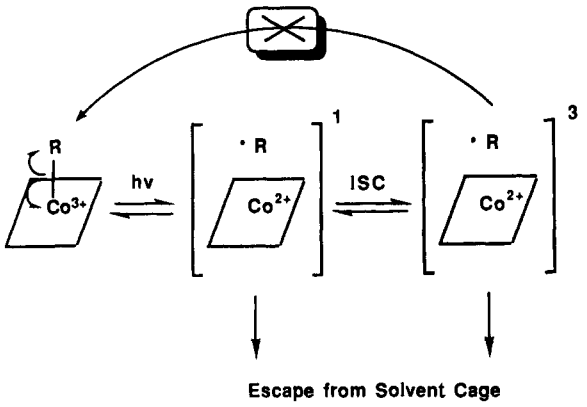
Chart I





Scheme I



Experimental Section

General. Adenosylcob(III)alamin and Ficoll-400 were purchased from Sigma. All other chemicals were of reagent grade or better. All glassware used for the picosecond kinetic studies was pyrolyzed at 600 °C to remove contaminating chromophores. Zero magnetic field flux is defined as the geomagnetic field of 0.05 mT.

Continuous-Wave Photolysis Kinetics. An aqueous solution of 200 μM AdoCbl^{III} and 50 mM potassium *N*-2-(hydroxyethyl)piperazine-*N'*-2-ethanesulfonate (HEPES), pH 7.0, with the indicated viscosigen was deaerated in a sealed 1 cm quartz cuvette by purging with Ar for 40 min immediately prior to photolysis. Continuous-wave UV irradiation was accomplished as described previously.[4,7] The UV lamp intensity was maximal at 248 nm and dropped off sharply, with less than 5% intensity remaining below 210 and above 300 nm. The incident light on the face of the cuvette was 2.1 mW cm^{-2}. Continuous-wave visible light irradiation was accomplished at 514 nm with an Ar^+ laser (Coherent Innova 900). The incident light on the face of the cuvette was reduced to 12 mW cm^{-2} with neutral density filters. The light flux was determined by potassium ferrioxalate actinometry[8] and by a Scientech surface-reading thermopile. The cuvette was placed in a thermostated cell holder at 20 °C in the gap of a GMW Associates electromagnet with 7.5-cm-diameter cylindrical poles. The magnetic field within the area of the cuvette was homogeneous to within 2%, and the long term stability was better than 0.5% as monitored by a transverse Hall probe and a digital teslameter. Absorption spectra from 300 to 600 nm were recorded in 1 s with a diode array spectrophotometer at variable time intervals from 10 s to 2 min (depending upon the fluence of the photolyzing light source) for a total of $3\tau_{1/2}$. Exposure to light during analysis was kept to a minimum. The concentration of AdoCbl^{III} was determined using the measured absorbance at 520 nm and $\epsilon_{520} = 8.4 \times 10^3$ M^{-1} cm^{-1} for AdoCbl^{III} and $\epsilon_{520} = 3.6 \times 10^3$ M^{-1} cm^{-1} for Cbl^{II} to give $\Delta\epsilon_{520} = 4.8 \times 10^3$ M^{-1} cm^{-1} to correct for the contributing absorbance of Cbl^{II} by the method of Chance.[3e,f] The plot of [AdoCbl^{III}] vs time (t) appeared zero order in all cases.

Picosecond Photolysis Kinetics. Laser flash photolysis in the picosecond time regime was carried out at the NIH Center for Fast Research Kinetics at the University of Texas, Austin. Photolysis at 532 nm was accomplished with a frequency-doubled Nd:YAG mode-locked laser (Quantel YG402) with a pulse length of 30 ps. The light incident on the face of the cuvette was 20 mW per pulse. Transient absorbance measurements in the range 350–800 nm were accomplished with a variable time-delayed continuum light source produced by soft phase modulation of a portion of the 1064-nm beam.[10] The absorption spectra were collected with a reference channel to subtract the spectral and temporal variations of light source intensity. Absorption spectra before the photolyzing pulse were collected periodically to balance the sample and reference channels. The time range of 0–3.5 ns after the photolyzing pulse was available for study with a maximum resolution of 6 ps. Each time delay value was averaged over 300 or 400 laser pulses. Aqueous solutions of 200 μM AdoCbl^{III} with 50 mM HEPES, pH 7.0, and the indicated viscosigen were purged with Ar for 40 min immediately prior to and during photolysis. Sample solutions were pumped through a 1-cm-path length, 2-mL-volume quartz flow cuvette at 10 mL/min without recycle. The flow cuvette was held between the poles of a GMW Associates electromagnet with 4.5-cm cylindrical poles and square pole faces. The magnetic field flux was determined as specified above. The appearance of Cbl^{II} in transient absorption spectra was monitored by integrating the absorption peak centered at 470 nm using the computer program FASTUTAH written by C. B. G. For ϕ_{ps} calculations, the concentration of Cbl^{II} was determined using $\epsilon_{476} = 9.2 \times 10^3$ M^{-1} cm^{-1} and AdoCbl^{III} $\epsilon_{476} = 6.5 \times 10^3$ M^{-1} cm^{-1}, which gives $\Delta\epsilon_{476} = 2.7 \times 10^3$ M^{-1} cm^{-1} to correct for the presence of [Cbl^{III}] by the method of Chance.[3e,f] The disappearance of [Cbl^{II}] from the photolysis of AdoCbl^{III} was first order in appearance and was fitted to [Cbl^{II}] = [Cbl^{II}]$_0$ exp($-k_{rec}t$) by nonlinear methods.

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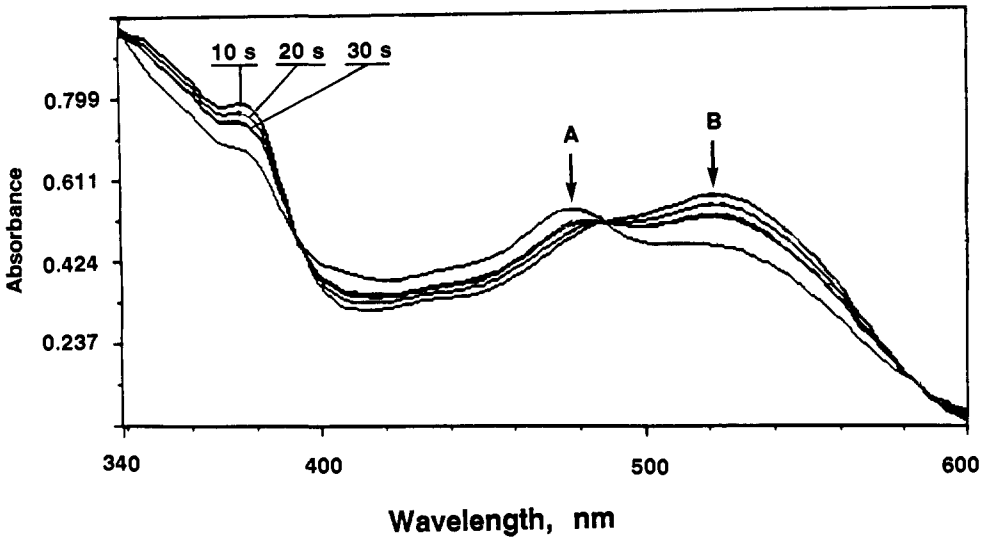

Figure 1. Anaerobic continuous-wave photolysis of AdoCbl^{III} at 514 nm, 20 °C, 100 μM AdoCbl^{III}, 50 mM HEPES, pH 7.0. Time of irradiation of traces starting from top spectrum at 350 nm: 10, 20, 30, and 60 s. Arrows A and B indicate Co^{II} and AdoCbl^{III} absorption maxima, respectively.

Viscosity Measurements. The macroviscosity of reaction solutions was measured by an Ostwald viscosimeter. Relative macroviscosity (η/η_0) is shown relative to H_2O at the same temperature. It is generally accepted that the rate of diffusion of small and compact molecules varies inversely with solution microviscosity.[11] The microviscosity was estimated by measuring the molecular correlation time, τ_c, for the spin-labeled compound 4-(2-iodoacetamido)-2,2,6,6-tetramethyl-1-piperidinyloxy–(free radical) by ESR line width analysis[12] as previously described.[4,7] The measurement of τ_c of a small paramagnetic probe molecule has been used successfully to measure the relative microviscosity in other heterogeneous solutions.[13]

Results and Discussion

Continuous-Wave Photolysis at 514 nm. Figure 1 shows the absorption spectrum for AdoCbl^{III} following anaerobic photolysis for the indicated time. The distinct isosbestic points indicate a clean conversion of AdoCbl^{III} to Cbl^{II} over this time without significant intermediates or side reactions. There was no indication of aquocob(III)alamin or hydroxocob(III)alamin formation.[14] Figure 2 shows the continuous-wave quantum yield (ϕ_{CW}) as a function of magnetic field flux (**B**) in 75% (v/v) glycerol in H_2O ($\eta/\eta_0 = 30$), 20% (w/v) Ficoll-400 in H_2O ($\eta/\eta_0 = 30$), and H_2O ($\eta/\eta_0 = 1$). In glycerol and Ficoll-400, ϕ_{CW} decreases by nearly 2-fold as **B** is increased, whereas ϕ_{CW} is nearly invariant with **B** (within experimental error) in H_2O.

In viscous solvents, separation of the radical pair is restricted by a slower rate of diffusion than in H_2O, and a magnetic field dependence of the reaction is observed.[15] This is easily understood for microviscosigens such as glycerol in which the rate of molecular diffusion is inversely proportional to the macroviscosity measured by the Ostwald viscosimeter.[4] The viscosity dependence of the magnetic field effect in the macroviscosigen, Ficoll-400, arises not from restricted diffusion (molecular diffusion is decreased only 2.7-fold in 20% Ficoll-400 relative to the rate in H_2O)[4] but rather from less escape from the "cage" structure formed by Ficoll-400.[16] In H_2O, separation of the radical pair occurs quickly and

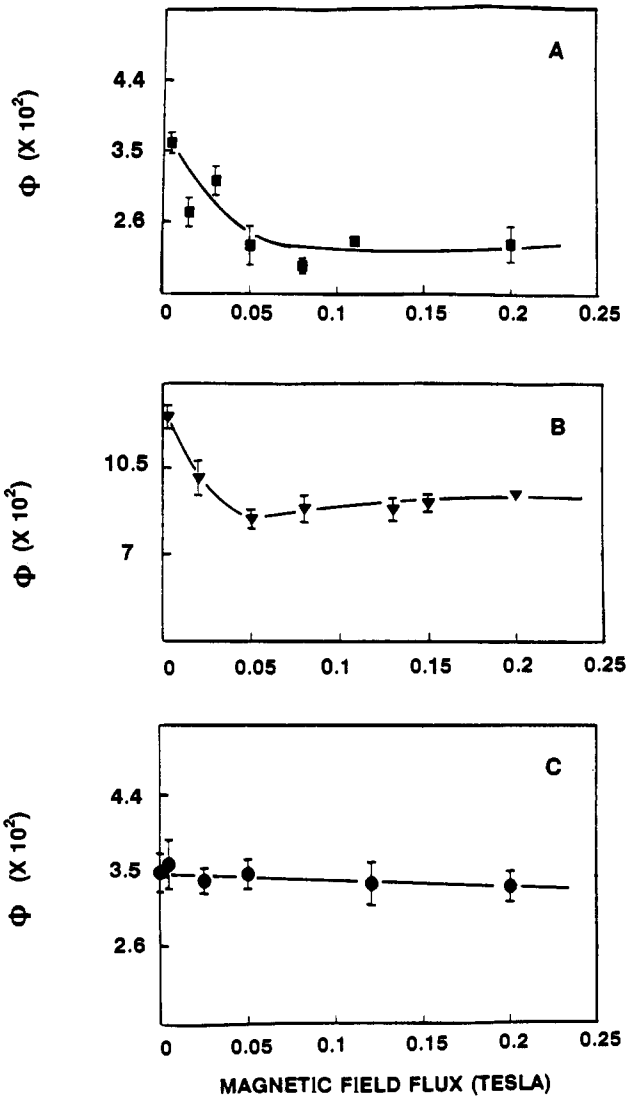

Figure 2. Field dependence of ϕ_{CW} for anaerobic AdoCbl^{III} photolysis at 514 nm, 20 °C, 200 μM AdoCbl^{III}, 50 mM HEPES, pH 7.0, and (A) 75% glycerol ($\eta/\eta_0 = 30$), (B) 20% Ficoll-400 ($\eta/\eta_0 = 30$), and (C) buffered H_2O ($\eta/\eta_0 = 1$). The curves represent best-fit empirical lines through the data.

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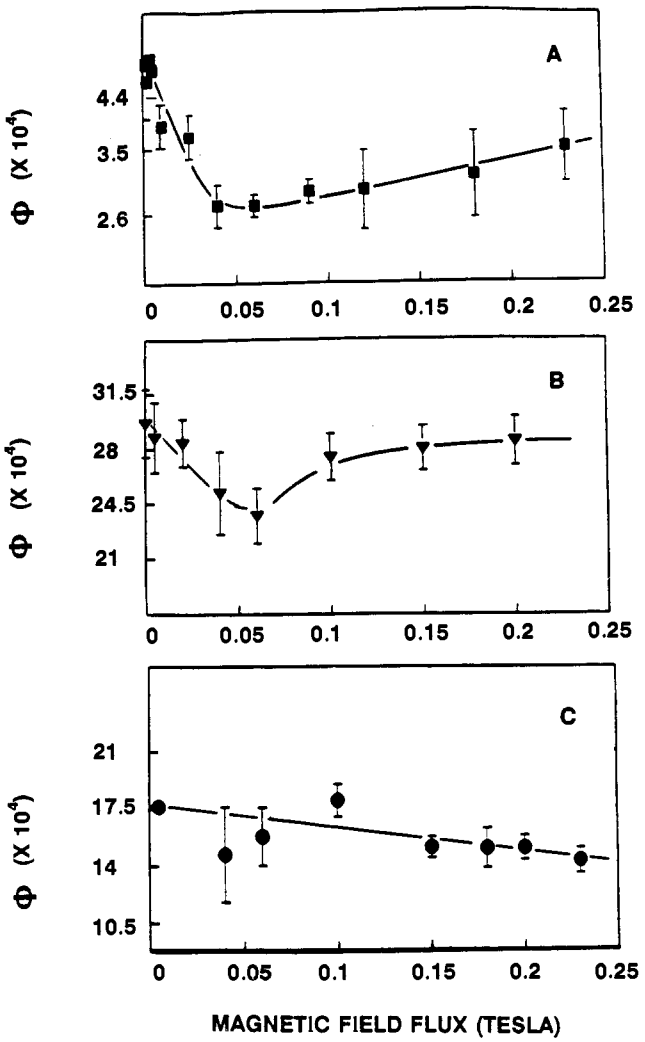


Figure 3. Field dependence of ϕ_{CW} for AdoCbl^{III} photolysis at 248 nm, 20 °C, 200 μM AdoCbl^{III}, 50 mM HEPES, pH 7.0, and (A) 75% glycerol (η/η_0 = 30), (B) 20% Ficoll-400 (η/η_0 = 30), and (C) buffered H_2O (η/η_0 = 1). The curves represent best-fit empirical lines through the data.

ϕ_{CW} is nearly independent of **B** within the error of the experiment. The absolute value of ϕ_{CW} is larger in Ficoll-400 than in either glycerol or H_2O. This is likely due to radical reaction with Ficoll-400. In previous studies, we observed an increase in ϕ_{CW} for cyanocob(III)alamin photolysis in low glycerol concentrations that did not significantly increase η/η_0 but did provide an increase in abstractable H·, HO·, or alkyl· sources over H_2O.[7] At high glycerol concentrations, the viscosity increase is significant, and enhanced recombination dominates to decrease ϕ.

Continuous-Wave Photolysis at 248 nm. In order to investigate the spin dynamics of the radical pair produced with an excess of energy over that required to break the C–Co bond, the magnetic field dependence of steady-state photolysis at 248 nm was investigated (Figure 3). Again, ϕ_{CW} for AdoCbl^{III} photolysis is definitively magnetic field sensitive only in glycerol and Ficoll-400. However, the absolute value of ϕ_{CW} is 2 orders of magnitude lower for photolysis at 248 nm compared to photolysis at 514 nm. Because the apical benzimidazole and adenosine moieties provide chromophores that absorb strongly at 248 nm but not at all at 514 nm, these chromophores are expected to absorb considerable energy at 248 nm but dissipate that energy through vibrational modes that do not efficiently lead to photolysis. The photo

(16) Ficoll-400 is a linear polymer that exhibits nonglobular hydrodynamic properties (Lavrenko, P. N.; Mokryukova, O. I.; Didenko, S. A. *Vysokomol. Soedin.* **1986**, *28A*, 517–524.) Molecules of Ficoll-400 are believed to interdigitate and form "cages" defined by a highly hydrogen-bonded matrix.

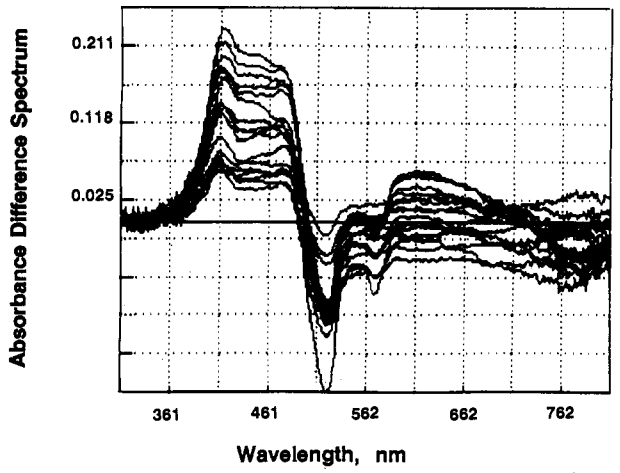


Figure 4. Transient absorbance difference spectra for AdoCbl^{III} photolysis at 532 nm, 24 °C, 200 μM AdoCbl^{III}, 50 mM HEPES, pH 7.0, in H_2O. Each spectrum is the average of 300–400 pulses with the AdoCbl^{III} background spectrum subtracted. Top trace at 461 nm is 30 ps after the light pulse, and each spectrum below is taken at increasing time after photolysis. The incident energy at 532 nm is 20 mW over 0.8 cm^2. The transient absorbance was detected with a collinear light path of 1 cm.

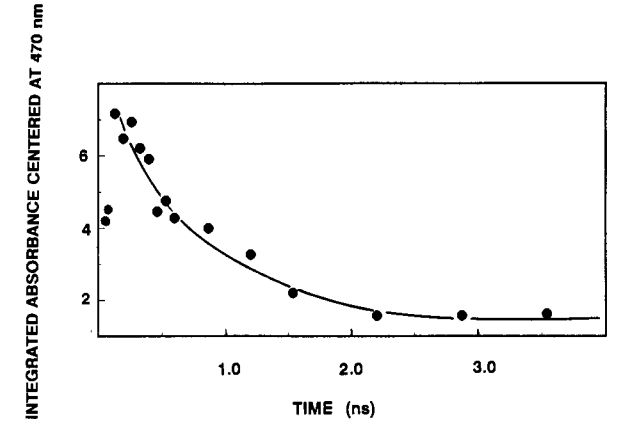


Figure 5. Kinetic trace of [cob(II)alamin] after the 30-ps, 532 nm pulse as a function of time as determined by the integrated transient absorbance centered at 470 nm. Conditions are 20 °C, 200 μM AdoCbl^{III}, 50 mM HEPES, pH 7.0, 75% glycerol. The line is the result of fitting the data to the first order rate equation (see text).

chemically-active transitions are the $\pi \rightarrow \pi^*$ transitions of the corrin ring that absorb at higher wavelength.[3c]

Picosecond Flash Photolysis of AdoCbl at 532 nm. Figure 4 shows transient absorption spectra recorded at increasing times following a 30-ps laser pulse at 532 nm. The transient increase in [Cbl^{II}] was monitored near 470 nm as described in the Experimental Section. Below 390 and above 700 nm, the light flux present in the interrogating beam was not sufficient to determine the absorbance accurately. This is reflected in the greater noise at the extremes of the spectrum. The absorption spectrum near 532 nm was also unreliable because of saturation of the sample and reference detectors. Examination of each temporally distinct absorption spectrum showed no evidence for the formation of any spectrally distinct species other than cob(II)alamin. Early investigations also showed no evidence for an excited state complex prior to bond scission.[3a]

Cob(II)alamin formation occurred within the 30-ps photolyzing pulse and decayed with pseudo-first-order kinetics (a typical kinetic trace for AdoCbl^{III} is shown in Figure 5). The first-order rate constant, k_{rec}, for {'CH_2-Ado:Cbl^{II}} geminate recombination as a function of **B** in H_2O and 75% glycerol is shown in Figure 6. In contrast to steady-state photolysis experiments, k_{rec} in both H_2O and 75% glycerol solutions increases with increasing **B**. The insensitivity of k_{rec} to solution microviscosity confirms that k_{rec}

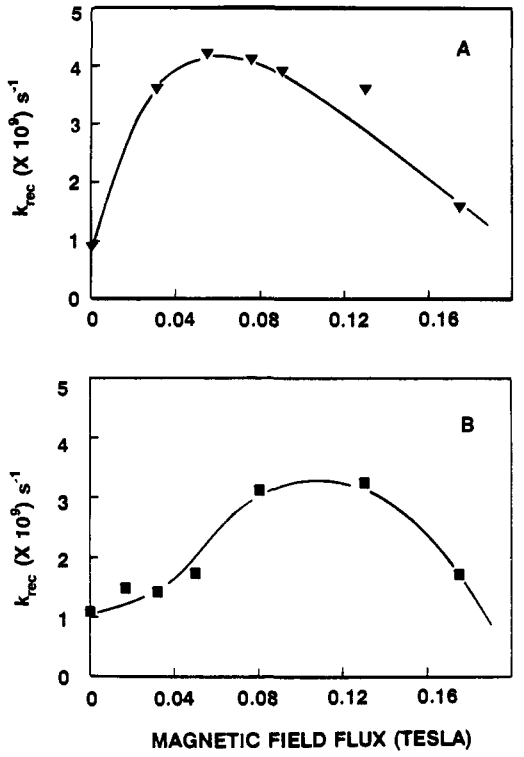
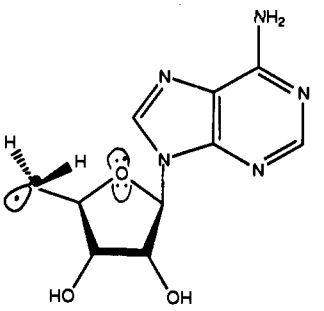


Figure 6. Field dependence of k_{rec} following photolysis of AdoCbl^{III} at 532 nm, 20 °C, 200 μM AdoCbl^{III}, 50 mM HEPES, pH 7.0, and (A) buffered H_2O ($\eta/\eta_0 = 1$) and (B) 75% glycerol ($\eta/\eta_0 = 30$). The curves represent best-fit empirical lines through the data.

is indeed the rate constant for true geminate radical pair recombination. At **B** = 0, the value of $k_{rec} = (1.1 \pm 0.06) \times 10^9$ s^{-1} observed in our experiments is in agreement with the recombination rate constant of $k_{rec} = (1.3 \pm 1.1) \times 10^9$ s^{-1} determined by earlier picosecond laser flash photolysis studies of AdoCbl^{III}.[3a] The higher pulse repetition rate afforded by the mode-locked Nd:YAG laser used in our studies allowed more signal averaging and reduced the error from 100% to 5%.

The transient quantum yield (ϕ_{ps}) we observe is 0.7 ± 0.1. This is compared to $\phi_{ps} = 0.36$ reported for AdoCbl^{III} photolysis from previous transient absorption studies in which the contributing absorbance from Cbl^{III} was not accounted for.[3a] Our value is 3-fold higher than the transient quantum yield of 0.23 determined by a 10-ns laser flash to initiate photolysis, followed by kinetic data acquisition in the microsecond time domain.[3e] In Figure 5, the integrated absorbance decays from a maximum integrated absorbance of 7 to about 1.8. Because Figure 5 is based on a difference spectrum (Figure 4), the integrated absorbance should return to 0 if complete recombination occurs on this time scale. Approximately 75% of the {$^{\cdot}CH_2$-Ado:Cbl^{II}} geminate radical pair population recombines during the 2 ns following photolysis. This leaves 25% of the {$^{\cdot}CH_2$-Ado:Cbl^{II}} radical pair population either to recombine on a slower time scale or to escape from the solvent cage. The slower recombination events of this fraction of the total radical pair population have been studied by Chen and Chance.[3e] In agreement with this statement, 0.25 of 0.7 (our value for the picosecond quantum yield, ϕ_{ps}) is 0.18, which is close to the nanosecond quantum yield of 0.23 observed by Chance.[3e] Since the quantum yield for continuous-wave photolysis is low ($\phi_{CW} = 0.13$, cf. Figure 2), the total recombination of {$^{\cdot}CH_2$-Ado:Cbl^{II}} in both the picosecond and nanosecond time regimes must be at least 0.9. This high fraction of recombination agrees with the high cage recombination efficiency, F_c, of >0.94 for the axial-base-off 5′-deoxyadenosylcobinamide radical pair in ethylene glycol.[17]

Chart II



The unusually high rate of *geminate* recombination on the picosecond time scale even in the absence of viscosigen may be caused by the pyramidal geometry of the 5′-deoxyadenosyl radical. In alkyl radicals with significant σ-acceptor or π-donor ligands, a pyramidal geometry is maintained and recombination is favored.[18,19] In the case of the 5′-deoxyadenosyl radical, we propose a slightly pyramidal geometry for the 5′-radical to minimize unfavorable interactions of the singly-occupied p-orbital of the 5′-carbon with the lone pair electrons of the ribofuranose ring oxygen (Chart II). In addition, hyperconjugation to the C-4′ H will favor a pyramidal geometry. This geometry allows the p-orbital on carbon to interact with the σ^* antibonding orbital of C–O.[19,20]

Spin State of the Radical Pair. The increase in k_{rec} and the net decrease in ϕ as a function of **B** are consistent with the geminate radical pair being born in the singlet spin state. A magnetic field-induced decrease in ISC leads to a decrease in the population of triplet spin states and therefore an increase in recombination.

The number of magnetic field-sensitive radical pair reactions involving heavy atoms is increasing.[6] A recent report focused on the magnetic field-dependent photochemistry of $Co^{III}(NH_3)_5X^{2+}$ (X = Cl, Br) complexes.[6b] A small decrease in quantum yield ($\phi_B/\phi_0 \approx 0.90$) was observed with a minimum near **B** = 0.25 T. The larger magnetic field modulation we have observed in AdoCbl^{III} photolysis probably reflects the lower electron density at Co(III) in the corrin-liganded system (as indicated by the low C–Co bond energy) and the concomitant decrease in SOC. In addition to the nonadiabatic radical pair recombination effects that are modulated at low fields (less than 0.5 T), higher fields in the range 1–9 T can alter the thermodynamics of reactions involving paramagnetic substrates, transition states, or products typically encountered in electron-transfer reactions involving transition-metal ions.[6d] These effects are important only at high magnetic fields and are not discussed herein.

Relevance to B_{12} Enzymes. Adenosylcob(III)alamin is an obligate cofactor to over a dozen enzymatic reactions that involve 1,2 migrations.[1b] Its catalytic role is believed to be a transient source of unpaired electron density to abstract H· from the substrate and thereby promote a 1,2 migration of groups on the substrate to the more thermodynamically stable radical intermediate. This key step is followed by a reversal of the hydrogen atom abstraction step to regenerate the adenosylcob(III)alamin cofactor and complete the catalytic cycle. Reversibility of the C–Co homolysis step is dependent upon maintaining spin correlation in the {$^{\cdot}CH_2$-Ado:Cbl^{II}} radical pair. However, forward progress through H· abstraction does not require the singlet state. The $^{\cdot}CH_2$-Ado in either the singlet or the triplet

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radical pair spin state can abstract H· from substrate. The 1,2 migration of the substrate radical must maintain spin correlation and will, in theory, produce a product radical that has the same spin as the initial substrate radical.

Recombination of {·CH_2-Ado:Cbl^{II}} to form adenosylcob(III)-alamin does not necessarily have to occur from the end of one catalytic cycle to the beginning of another catalytic cycle. Under saturating substrate (S) conditions where the enzyme (E) exists primarily as the ES complex and very little free E exists in solution, the radical pair state of the enzyme-bound {·CH_2-Ado:Cbl^{II}} can be maintained. This would remove the step that we have shown to be dependent on an external magnetic field. This observation suggests that any magnetic field effect on a B_{12}-dependent enzymatic reaction is likely to be expressed only on the kinetic parameter V_{max}/K_m (the pseudo-first-order rate constant for the combination of E and S). It is unknown whether the other steps involving radical intermediates (i.e., H· abstraction, 1,2 migration, and H· donation) occur fast enough ($>10^6$ s^{-1}) to produce radical pairs that have lifetimes that are adequate for ISC to occur but not so long that spin correlation is lost.[21] Our laboratory has recently shown that V_{max}/K_m for $AdoCbl^{III}$-dependent ethanolamine ammonia lyase (EC 4.3.1.7) is decreased 50% by a 80-mT magnetic field.[22]

Conclusions

The recombination of geminate radical pairs produced by alkylcob(III)alamin photolysis is increased by a magnetic field in the range of 50–80 mT. About 75% of the {·CH_2-Ado:Cbl^{II}} geminate radical pair population recombines within 2 ns following photolysis, and a large fraction of the remaining 25% recombines before escape from the solvent cage.[3e] The radical pair is produced in the singlet spin state.

The balance between lability of the C–Co bond in $AdoCbl^{III}$ and the reactivity of the resulting {·CH_2-Ado:Cbl^{II}} geminate radical pair toward recombination makes $AdoCbl^{III}$ a "safe" source for transient radicals in enzymatic reactions. The short lifetime of the reactive ·CH_2-Ado radical does not allow for significant diffusion (and unwanted side reactions) even in aqueous solution in the absence of an enzyme.

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Registry numbers supplied by the authors: Adenosylcob(III)alamin, 13870-90-1; glycerol, 56-81-5; Ficoll-400, 26873-85-8.

(21) A modification of this general reaction scheme has been proposed in which the role of the radical pair produced by homolysis of AdoCbl is to produce an intermediate radical on the enzyme that is the ultimate abstractor of H· from the substrate. The only radical pair step would then be the initial homolysis of the C–Co bond, and the subsequent chemistry would more closely resemble a free radical process.

(22) Harkins, T. T.; Grissom, C. B., submitted for publication. A preliminary report of this observation was presented at the ACS Biological Division meeting, San Diego, CA, June 1–3, 1993.