21. C. D. Keeling *et al.*, *Geophys. Monogr.* **55**, 165 (1989).
22. V. C. LaMarche Jr., D. A. Graybill, H. C. Fritts, M. R. Rose, *Science* **225**, 1019 (1984); F. I. Woodward, *Nature* **327**, 617 (1987); D. A. Graybill, in *Proceedings of the International Symposium on Ecological Aspects of Tree-Ring Analysis*, G. C. Jacoby and J. W. Hornbeck, Eds. (U.S. Department of Energy, Washington, DC, 1987), pp. 463–474; H. S. Mayeux, H. B. Johnson, H. W. Polley, in *Noxious Range Weeds*, L. F. James, J. O. Evans, M. H. Ralphs, R. D. Child, Eds. (Westview, Boulder, CO, 1987), pp. 62–74; H. W. Polley, H. B. Johnson, B. D. Marino, H. S. Mayeux, *Nature* **361**, 61 (1993); J. Van Der Burgh, H. Visscher, D. L. Dilcher, W. M. Kürschner, *Science* **260**, 1788 (1993). But see also F. Kienast and R. J. Luxmoore, *Oecologia* **76**, 487 (1988); L. J. Graumlich, L. B. Brubaker, C. G. Grier, *Ecology* **70**, 405 (1989); L. J. Graumlich, *ibid.* **72**, 1 (1991).
23. F. A. Bazzaz, *Annu. Rev. Ecol. Syst.* **21**, 167 (1990); C. Korner and J. A. Arnone III, *Science* **257**, 1672 (1992); C. Körner, in *Vegetation Dynamics and Global Change*, A. M. Solomon and H. H. Shugart, Eds. (Chapman & Hall, New York, 1993), pp. 53–70.
24. M. A. Condon, T. W. Sasek, B. R. Strain, *Funct. Ecol.* **6**, 680 (1992).
25. F. E. Putz, *Biotropica* **15**, 185 (1983); E. E. Hegarty and G. Caballé, in *The Biology of Vines*, F. E. Putz and H. E. Mooney, Eds. (C.U.P., New York, 1991), pp. 313–336.
26. C. Darwin, *J. Linn. Soc. London Bot.* **9**, 1 (1867).
27. T. W. Sasek and B. R. Strain, *Weed Sci.* **36**, 28 (1988); *Clim. Change* **16**, 31 (1990).
28. A. H. Gentry, in (*25*), pp. 3–49.
29. F. E. Putz, P. D. Coley, K. Lu, A. Montalvo, A. Aiello, *Can. J. For. Res.* **13**, 1011 (1983).
30. For example, J. A. Taylor and J. Lloyd, *Aust. J. Bot.* **40**, 407 (1992); J. M. Melillo *et al.*, *Nature* **363**, 234 (1993).
31. We thank C. Díaz, C. Grandes, N. Jaramillo, P. Nuñez, K. Johnson, M. Timaná, and R. Vásquez for help in Peru, with logistical support from M. Gunther and M. Morrow (Peruvian Safaris· T1 to T5) and P. Jenson (Explorama Tours. YA); J. Terborgh, R. Foster, P. Nuñez, H. Balslev, J. Korning, D. Neill, W. Palacios, and D. Nicholson for sharing unpublished data; D. Hardin, S. Jennings, A. Moad, and P. Wilkin for additional help; and S. Hubbell, K. Johnson, N. Myers, D. Nicholson, P. Raven, E. Spitznegel, J. Terborgh, and three anonymous reviewers for suggestions that improved the manuscript. Field reseärch supported by the National Science Foundation (BSR-9001051), the World Wildlife Fund-U.S.–Garden Club of America and Conservation International (O.L.P.), and the Pew Charitable Trust and the Mellon Foundation (A.H.G.). We wrote this paper before Al Gentry's death, but he did not have the opportunity to review the final version.

9 August 1993; accepted 22 December 1993

Magnetic Field Effects on B_{12} Ethanolamine Ammonia Lyase: Evidence for a Radical Mechanism

Timothy T. Harkins and Charles B. Grissom*

A change in radical pair recombination rates is one of the few mechanisms by which a magnetic field can interact with a biological system. The kinetic parameter V_{max}/K_m (where K_m is the Michaelis constant) for the coenzyme B_{12}–dependent enzyme ethanolamine ammonia lyase was decreased 25 percent by a static magnetic field near 0.1 tesla (1000 gauss) with unlabeled ethanolamine and decreased 60 percent near 0.15 tesla with perdeuterated ethanolamine. This effect is likely caused by a magnetic field–induced change in intersystem crossing rates between the singlet and triplet spin states in the {cob(II)alamin:5′-deoxyadenosyl radical} spin-correlated radical pair.

More than 20 enzymes are thought to incorporate radical chemistry in the conversion of substrates to products (*1*, *2*). Those enzymes that utilize spin-correlated radical pair intermediates should be sensitive to an applied magnetic field according to the same principles that govern radical pair chemical reactions. This proposal is not new, but it has not been substantiated by experiment until now (*3*). The only other example of a biological system that is sensitive to an applied magnetic field through electron spin selectivity is the triplet yield and emission intensity of the bacterial photosynthetic reaction center (*4*). Through a mechanism other than spin-correlated chemistry, integral membrane enzymes may couple to the electric field vector of an alternating electromagnetic field. This process does not require radical chemistry, and it is limited to membrane-bound proteins that undergo large conformational changes during catalysis (*5*).

The rate or product distribution of chemical reactions that involve geminate radical pair or biradical intermediates can be altered by a magnetic field that increases or decreases intersystem crossing (ISC) rates between the singlet and triplet spin-correlated states (*6*). A geminate radical pair born in the singlet spin state after bond homolysis will readily recombine to reform starting material. If ISC to the triplet spin state occurs, recombination to the starting material is prohibited by the Pauli exclusion principle. This results in a longer radical pair lifetime and an increased forward flux to product (Fig. 1). To allow for electron spin rephasing (ISC), a geminate radical pair must be held spatially close for 10^{-10} to 10^{-6} s. Beyond this time, interactions with solvent and neighboring atoms will lead to spin randomization. Thus, only chemical reactions that occur in this time domain may exhibit a magnetic field dependence through the radical pair spin exclusion mechanism.

The enzyme-substrate (ES) complex formally constitutes a biradical or radical pair if radical character exists on both the substrate and the enzyme or cofactor at some time during the course of the reaction. If ISC occurs in the singlet ES complex to produce a triplet ES complex, the probability of nonproductive radical recombination and dissociation of S from the ES complex is decreased. The result will be an increase in the forward commitment to catalysis for the substrate (*7*). This will increase the kinetic parameter V_{max}/K_m if it occurs before the first irreversible step. If a kinetically slow event that occurs after formation of the radical pair ES intermediate requires the singlet spin state, then increased ISC will populate the three unreactive triplet spin states and V_{max} will be decreased. Conversely, if the triplet spin state is required for product formation, increased ISC will populate the triplet spin state and lead to an increase in V_{max}. These arguments are reversed if the radical pair in the ES complex is born in the triplet state, but this is unlikely in a nonphotochemical system. If the slow step is independent of the spin state of ES, V_{max} will not change, but V_{max}/K_m can still be altered by a change in nonproductive radical pair recombination.

An enzyme that requires coenzyme B_{12} (5′-deoxyadenosylcobalamin) and catalyzes a 1,2 rearrangement was chosen for study because of the ubiquitously proposed mechanism that begins with homolysis of the C–Co bond to yield 5′-deoxyadenosyl radical (·CH_2Ado) and cob(II)alamin (Cbl^{II}) as the initial radical pair (Fig. 2) (*8*). Electron spin resonance (ESR) studies of ethanolamine ammonia lyase (EAL) with the slow substrate L-2-amino-1-propanol show evidence for two radicals (*9*, *10*).

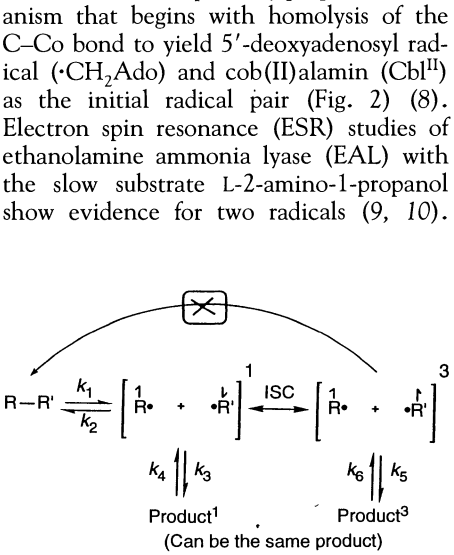

Fig. 1. Effect of a magnetic field on radical pair recombination rates in a chemical reaction.

Department of Chemistry, University of Utah, Salt Lake City, UT 84112, USA.

*To whom correspondence should be addressed.

There is a cob(II)alamin signal and another signal (probably an organic radical) that share a weak exchange interaction and are separated by ~6 Å (*9*, *10*). Estimates of the C–Co bond dissociation energy are as low as 31 kcal/mol (*11*, *12*).

We chose EAL (E.C. 4.3.1.7) for study because it is one of the best characterized B_{12}-requiring enzymes (*10*, *13*). It catalyzes a 1,2 shift of the amine group either to produce a hydrolytically unstable carbinolamine or acetaldehyde and ammonia (Fig. 3). EAL from *Salmonella typhimurium* was produced in *Escherichia coli* containing the overexpressed gene, isolated, and assayed spectrophotometrically as described (*14*, *15*). The magnetic field dependence of the kinetic parameters was determined with a spectrophotometer having an electromagnet in the cell compartment (*16*).

The dependence of the kinetic parameters V_{max} and V_{max}/K_m for ethanolamine on magnetic flux density is shown in Fig. 4, A and B, respectively (*17*). The kinetic parameter V_{max} was invariant up to 0.25 T. This is not surprising because V_{max} is limited by product release (*18*). The kinetic parameter V_{max}/K_m decreased by up to 25% and reached a minimum value at 0.1 T. This is consistent with the {·CH_2Ado:Cbl^{II}} radical pair being produced in the singlet spin state after C–Co homolysis, and a magnetic field–induced decrease in ISC that would otherwise populate the $T_{\pm1}$ spin states. This increase in the singlet radical pair population enhanced recombination and led to an overall decrease in V_{max}/K_m. As the field increased beyond 0.1 T, V_{max}/K_m began to increase and reached the initial value seen at 0 T.

The parameter V_{max} with perdeuterated ethanolamine is invariant with magnetic field. The magnetic field–dependent decrease in V_{max}/K_m with perdeuterated ethanolamine (Fig. 4C) is even greater than the decrease in V_{max}/K_m observed with unlabeled ethanolamine. A minimum was reached at 0.15 T with perdeuterated ethanolamine rather than at 0.1 T as with unlabeled ethanolamine. The deuterium isotope effect is a combination of primary and secondary kinetic isotope effects because the ethanolamine is perdeuterated. At 0 T, $^{D}V_{max} = 6.8 \pm 0.2$ and $^{D}V_{max}/K_m = 5.4 \pm 0.4$ (mean ± SE) as determined from our data.

The difference in the magnitude and position of the maximum magnetic field–induced change in V_{max}/K_m with unlabeled and deuterated ethanolamine is surprising if only the recombination of the initial {·CH_2Ado:Cbl^{II}} radical pair is being affected. The radical pairs in all of the steps that follow C–Co homolysis will, in theory, possess the same spin correlation as in the initial {·CH_2Ado:Cbl^{II}} radical pair (Fig. 3). Interactions with atoms in the active site will tend to randomize the spin state of the correlated radical pair beyond 10^{-6} s and render a magnetic field effect on subsequent steps unlikely.

There are several possible explanations for the difference in magnetic field dependence for unlabeled and deuterated ethanolamine. First, in processing the deuterated ethanolamine, the 5′-deoxyadenosyl radical abstracts D· from C-2 of ethanolamine. Subsequent turnover of the cofactor would now involve the dissociation and recombination of a heterogeneous population of 5′-CH_2, 5′-CHD, and 5′-CD_2 on the 5′-deoxyadenosine cofactor.

The nuclear spin and nuclear magnetic moment for protium and deuterium are different: for ^{1}H, $I = 1/2$ and $\mu_N = 1.79$ (in units of the nuclear magneton, 5.05×10^{-27} JT^{-1}), and for ^{2}H, $I = 1$ and $\mu_N = 0.86$. This difference should be inconsequential in C–Co bond homolysis, but

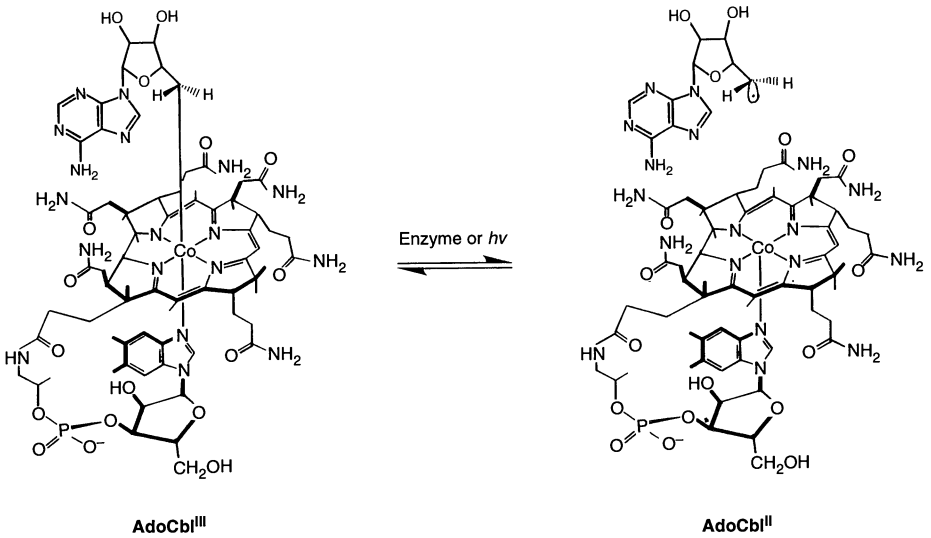

Fig. 2. Homolysis of the C–Co bond by an enzyme or a photon with energy *hv* in adenosylcob(III)alamin. The C–Co bond is one of the weakest organometallic bonds known. Estimates of the bond dissociation energy are as low as 31 kcal/mol (*11*).

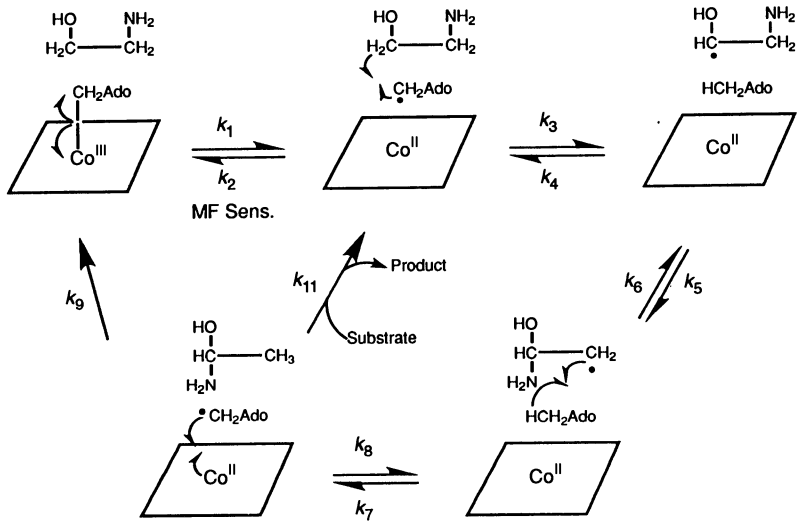

Fig. 3. Proposed reaction mechanism for EAL. Enzyme-induced homolysis of the C–Co bond produces the 5′-deoxyadenosyl radical and cob(II)alamin in the singlet spin state. The 5′-deoxyadenosyl radical abstracts H· from C-2 of ethanolamine to generate the initial substrate radical. The amine group migrates to form the carbinolamine radical that abstracts H· from 5′-CH_3-adenosine to produce the hydrolytically unstable carbinolamine product and regenerate the 5′-deoxyadenosyl radical. Under V_{max} conditions, the enzyme always has ethanolamine bound and the {·CH_2Ado:Cbl^{II}} radical pair does not have to recombine between turnover (k_{11} includes product dissociation and substrate binding before {·CH_2Ado:Cbl^{II}} recombination occurs. Under V_{max}/K_m conditions, recombination of the {·CH_2Ado:Cbl^{II}} radical pair (k_9) is more likely. This would begin the catalytic cycle with the transient {·CH_2Ado:Cbl^{II}} radical pair in the singlet spin state. MF Sens., magnetic field sensitivity.

perhaps significant to maintaining the correlated spin state of the radical pair before recombination occurs. Protium, with its greater nuclear magnetic moment, will promote ISC and spin relaxation of the 5′-deoxyadenosyl radical to a greater extent than will deuterium with its smaller nuclear magnetic moment. This is an expression of the magnetic isotope effect, and it is in addition to the expression of any mass isotope effect on the reaction (*19*).

Another possible explanation for the observed difference in magnetic field dependence with unlabeled and deuterated ethanolamine is that deuteration increases the barrier to H(D)· abstraction from substrate. This increases the fraction of enzyme in the $\{\cdot CH_2Ado:Cbl^{II}\}$ radical pair state and allows for greater recombination.

We have demonstrated a magnetic field–dependent decrease in the net quantum yield, ϕ, and a corresponding increase in the geminate pair recombination rate of the $\{\cdot CH_2Ado:Cbl^{II}\}$ radical pair produced by photolysis of adenosylcob(III)alamin in the absence of enzyme (*20*). When 532-nm light (53.8 kcal/mol) is used to homolyze the C–Co bond, the radical pair recombines with a rate constant (k_{rec}) of $1 \times 10^9\ s^{-1}$. In a $B = 0.05$ T (500 G) magnetic field, k_{rec} increases to $4 \times 10^9\ s^{-1}$ (*20*). At magnetic field flux densities greater than 0.12 T, k_{rec} begins to decrease to yield an overall biphasic shape to the magnetic field dependence (*21*). A demonstrated magnetic field dependence to $\{\cdot CH_2Ado:Cbl^{II}\}$ recombination in photolytically produced radical pairs provides precedence for modulating $\{\cdot CH_2Ado:Cbl^{II}\}$ recombination in B_{12}-dependent enzymatic reactions.

In summary, coenzyme B_{12}–dependent EAL exhibits a magnetic field dependence similar to the magnetic field–dependent recombination of the $\{\cdot CH_2Ado:Cbl^{II}\}$ radical pair produced by photolysis. This supports the proposed radical mechanism for EAL and other coenzyme B_{12}–dependent enzymes. This is an example of a magnetic field effect on an enzymatic reaction with known radical intermediates.

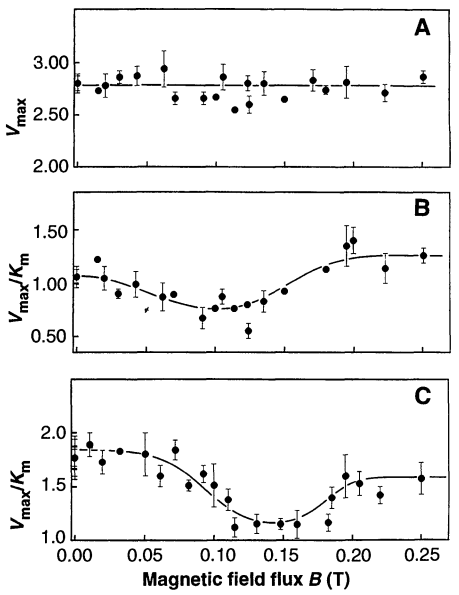

Fig. 4. Magnetic field dependence of (**A**) V_{max} with unlabeled ethanolamine, (**B**) V_{max}/K_m with unlabeled ethanolamine, and (**C**) V_{max}/K_m with [1,1,2,2,]-d_4-ethanolamine. Each assay contained 100 mM Hepes (pH 7.48), 5 μM adenosylcob(III)-alamin, and EAL at 25°C. Each data point represents the kinetic parameter derived by fitting observed d[P]/d*t* versus [ethanolamine] data to $d[P]/dt = V_{max}[S]^n/K_m + [S]^n$ by nonlinear methods (P, product; S, substrate). The Hill number, *n*, varied only slightly between 0.75 to 0.85. To keep the measured rates with deuterated and unlabeled substrates similar, 8.59-fold more EAL was used in assays with deuterated ethanolamine than in assays with unlabeled ethanolamine. This yields an observed kinetic isotope effect of $^DV_{max} = 6.8 \pm 0.2$ and $^DV_{max}/K_m = 5.4 \pm 0.4$ (mean ± SE) at 0 T.

REFERENCES AND NOTES

1. T. T. Harkins and C. B. Grissom, a preliminary report of the work herein was presented at the American Society of Biochemistry and Molecular Biology meeting, San Diego, CA, 31 May 1993.
2. J. Stubbe, *Biochemistry* **27**, 3893 (1988); *Annu. Rev. Biochem.* **58**, 257 (1989).
3. V. K. Vanag and A. N. Kuznetsov, *Izv. Akad. Nauk UZB. SSR Ser. Biol.* **2**, 215 (1988); K. Hummel, K. Martl, M. G. Martl, *J. Mol. Catal.* **54**, 11 (1989).
4. A. J. Hoff, H. Redemarker, R. Van Grondelle, L. N. M. Duysens, *Biochim. Biophys. Acta* **460**, 547 (1977); R. E. Blankenship, *Acc. Chem. Res.* **14**, 163 (1981); S. G. Boxer, C. E. D. Chidsey, M. G. Roelofs, *J. Am. Chem. Soc.* **104**, 1452 (1982).
5. B. Robertson and R. D. Astumian, *Biochemistry* **31**, 138 (1992); J. C. Weaver and R. D. Astumian, *Science* **247**, 459 (1990); H. V. Westerhoff, T. Y. Tsong, P. B. Chock, Y. Chen, R. D. Astumian, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4734 (1986).
6. For reviews on magnetic field effects on chemical reactions see N. J. Turro, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 609 (1983); K. M. Salikhov, *Chem. Phys.* **82**, 163 (1983); U. E. Steiner and T. Ulrich, *Chem. Rev.* **89**, 51 (1989); C. Doubleday, N. J. Turro, J. F. Wang, *Acc. Chem. Res.* **22**, 199 (1989); H. Hayashi, in *Photochemistry and Photophysics*, J. F. Rabek, Ed. (CRC Press, Boca Raton, FL, 1990), vol. 1, p. 59; U. E. Steiner and H. Wolff, in *ibid.*, vol. 4, p. 1; K. A. McLauchlan and U. E. Steiner, *Mol. Physics* **73**, 241 (1991).
7. W. W. Cleland, in *CRC Crit. Rev. Biochem.* **13**, 385 (1982). The forward commitment to catalysis for a substrate is defined as the ratio of net forward flux to form product relative to the net rate of substrate dissociation from the central ES complex.
8. For a review of B_{12} chemistry, see: J. M. Pratt, *Chem. Soc. Rev.* **14**, 161 (1985); D. Dolphin, Ed., B_{12} (Wiley, New York, 1991) vols. 1 and 2; Z. Schneider and A. Stroinski, *Comprehensive* B_{12} (de Gruyter, Berlin, 1987).
9. K. L. Schepler, W. R. Dunham, R. H. Sands, J. A. Fee, R. A. Abeles, *Biochem. Biophys. Acta* **397**, 510 (1975).
10. B. M. Babior, in B_{12}, D. Dolphin, Ed. (Wiley, New York, 1991), chap. 10.
11. R. G. Finke and B. P. Hay, *Inorg. Chem.* **23**, 3041 (1984).
12. B. P. Hay and R. G. Finke, *J. Am. Chem. Soc.* **108**, 4820 (1986); *ibid.* **109**, 8012 (1987); J. Halpern, *Science* **227**, 869 (1985); ———, S. H. Kim, T. W. Leung, *J. Am. Chem. Soc.* **106**, 8317 (1984); M. K. Geno and J. Halpern, *J. Chem. Soc. Chem. Commun.*, (no. 14), (1987), p. 1052.
13. B. M. Babior, *J. Biol. Chem.* **244**, 449 (1969); D. A. Weisblatt and B. M. Babior, *ibid.* **246**, 6064 (1971); M. R. Hollaway *et al.*, *Eur. J. Biochem.* **82**, 143 (1978); P. Diziol, H. Haas, J. Retey, S. W. Graves, B. M. Babior, *ibid.* **106**, 211 (1980); R. J. O'Brien, J. A. Fox, M. G. Kopczynski, B. M. Babior, *J. Biol. Chem.* **260**, 16131 (1985).
14. L. P. Faust and B. M. Babior, *Arch. Biochem. Biophys.* **294**, 50 (1992).
15. B. H. Kaplan and E. R. Stadtman, *J. Biol. Chem.* **243**, 1787 (1968).
16. The experimental apparatus consisted of a Beckman DU monochromator with an electromagnet in the space that the cell compartment would normally occupy. The focal position of the light exiting the monochromator was extended by achromatic quartz optics and focused into a standard 1-cm light path cuvette housed in a brass block between the poles of the electromagnet. Thermostatted water at 8 liter/min was passed through the cuvette holder to maintain temperature control. A Hall probe and digital teslameter were used to monitor the magnetic field flux during the experiment. In this configuration, the magnetic field flux within the cuvette was adjustable up to 0.25 T, and it was homogeneous to ±2% in the assay volume.
17. As a control, the kinetic parameters of the nonradical enzymes hexokinase, staphylococcal nuclease, and chymotrypsin were determined at magnetic field flux densities up to 0.25 T. None of the control enzymes exhibited a magnetic field dependence to either V_{max} or V_{max}/K_m. The lack of an observed effect with these control enzymes demonstrates that the instrument was free of adventitious magnetic field effects on the photomultiplier tube or inductive or conductive heating effects from the magnet. Hexokinase coupled to glucose-6-phosphate dehydrogenase and nicotinamide adenine dinucleotide phosphate reduction makes an excellent probe for thermal effects because of its large temperature dependence. A change in cuvette temperature of 0.02°C produced a discernible change in d*A*/d*t* at the most sensitive setting of the spectrophotometer.
18. The kinetic parameter V_{max} does not vary with an increase in solution viscosity. A manuscript describing the complete kinetic characterization of EAL from *Salmonella typhimurium* is in preparation (T. T. Harkins and C. B. Grissom).
19. For reviews on magnetic isotope effects on chemical reactions, see: N. J. Turro and B. Kraeutler, in *Isotopes in Organic Chemistry*, E. Buncel and C. C. Lee, Eds. (Elsevier, Amsterdam, 1984); A. L. Buchachenko and I. V. Khudyakov, *Acc. Chem. Res.* **24**, 177 (1991); and the references cited in (*6*).
20. A. M. Chagovetz and C. B. Grissom, *J. Am. Chem. Soc.* **115**, 12152 (1993).
21. A biphasic dependence of reaction rate versus magnetic flux density is often observed. This is the sum of competing physical mechanisms that increase or decrease ISC at $B > 0$. Typically, hyperfine interactions (HFI) lead to ISC at $B = 0$ by interconverting the $S \leftrightharpoons T_{0\pm1}$ spin states. At $B > 0$, only the $S \leftrightharpoons T_0$ spin states can be interconverted by HFI, and ISC is decreased. At higher fields, the difference in Lande *g*-factors (Δg) for the two unpaired spins becomes important, and the term $\Delta g \beta B$ becomes significant and leads to an increase in ISC. The HFI and Δg mechanisms always have opposite effects and thus produce a biphasic dependence of reaction rate or product yield on magnetic field flux.

22. Supported by a grant from the National Institutes of Environmental Health Sciences (ES05728). We wish to thank B. M. Babior and L. Faust for the gift of the cloned and overexpressed EAL from *S. typhimurium*.

1 October 1993; accepted 17 December 1993