Magnetic Field Effects in Biology: A Survey of Possible Mechanisms with Emphasis on Radical-Pair Recombination

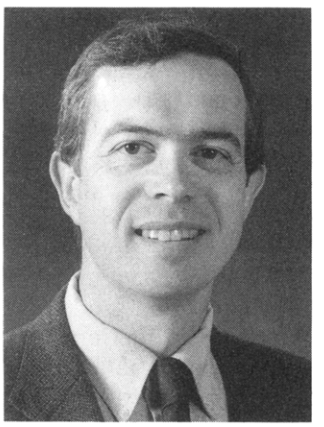
Charles B. Grissom

Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

Received July 27, 1994 (Revised Manuscript Received October 9, 1994)

Contents

I. Introduction 3
A. Producing and Measuring Laboratory Magnetic Fields 4
II. Magnetic Field Effects on Radical Pair Recombination in Biological Systems 4
A. Theory of Magnetic Spin Effects on RP Recombination 5
B. Hyperfine Induced Intersystem Crossing 6
C. Spin Rephasing (Δg Mechanism) Induced Intersystem Crossing 6
D. Singlet–Triplet Energy Level Crossing 7
E. Spin–Orbit Coupling 7
F. Enhanced Recombination by Compartmentalization 7
G. Random Radical Pair Recombination 8
H. Radical Chain Reactions 8
I. Static vs Modulated Magnetic Field Effects on RP Recombination 8
J. Oscillating Magnetic Fields To Induce Changes in RP Recombination 9
K. Radical Pair Recombination in Biological Systems 9
L. Magnetic Field Effects on Thermal (Nonphotochemical) RP Reactions 10
M. Magnetic Field Effects in Catalytic Reactions 10
N. Magnetic Field Effects in Enzymatic Reactions 11
O. Coenzyme B_{12} Photochemistry 14
P. Magnetic Field Effects on B_{12}-Dependent Enzymes 17
Q. Biological and Health Relevance of B_{12} Magnetic Field Effects 19
R. Other Enzymes for Which RP Mechanisms Have Been Considered 20
III. Non-Radical-Pair Magnetic Field Effects in Biological Systems: What Is the Mechanism? 20
A. Magnetoreception 20
B. Melatonin 21
C. Ca^{2+} Binding and Ion Cyclotron (Parametric) Resonance 21
D. Miscellaneous Magnetic Field Effects 22
E. Electric Field Effects 22
IV. Acknowledgments 22
V. References 22



Charles B. "Chuck" Grissom received his B.A. in Biochemistry and Chemistry from the University of California, Riverside in 1981. He completed his Ph.D. in Biochemistry under the direction of W. W. Cleland at the University of Wisconsin—Madison in 1985. He was an NIH Postdoctoral Fellow at Wisconsin in the laboratory of John L. Markley from 1985 to 1987 and at the University of California, Berkeley in the laboratory of Paul A. Bartlett from 1987 to 1989. In 1989, he assumed his position in the Chemistry Department at the University of Utah where his laboratory focuses on magnetic spin effects in enzymatic and chemical reactions. His passion in life is understanding the mechanism of biological catalysts. Outside of the lab, his passions are mountain biking, exploring old uranium mines, and neon art.

I. Introduction

Few areas of research are as controversial, both scientifically and politically, as research into the biological effects of magnetic fields. This area of research began in earnest in the 1960s with the work of Barnothy.[1,2] These early experiments were phenomenological observations: Is a magnetic field beneficial, inconsequential, or deleterious to biological organisms? More than 30 years later, this fundamental question has yet to be answered unambiguously.[3,4]

If the effect was profound and an exogenous magnetic field resulted in the immediate death, mutation, or change in morphology of an organism, no question would remain as to the safety of magnetic fields. Instead, the effects are more subtle. A continuing series of phenomenological studies has provided equivocal evidence for magnetic field effects in biological systems.[3,4] In 1979, intense scientific curiosity and social activism was sparked by the epidemiological study of Werthheimer and Leeper that suggested an increase in childhood cancer for individuals living near electric power lines.[5,6] Beyond this citation classic, no further discussion of epidemiological data will be included in this review. Instead, the purpose of this review is to summarize the evidence for possible biological effects of static and time-varying magnetic fields *within the context of proposed mechanisms*.

In the traditional practice of science, a new field begins with a series of phenomenological observations

0009-2665/95/0795-0003$15.50/0

that cannot be explained within existing paradigms. New hypotheses that describe a fundamental principle are developed and they are adjudicated by experimentation. Further iteration of hypothesis and experiment refine the concept until a new paradigm is accepted by the scientific community. In the study of magnetic field effects on biological systems, phenomenological-based observations that cannot be explained within existing theory have only recently given way to hypothesis-based experimentation.

This article is organized around the principle of hypothesis-based research into biological magnetic field effects. It is intended to serve as an introduction to current issues and to review recent progress toward answering the fundamental question of how magnetic fields interact with biological systems.

This review will focus on those magnetic field effects that can be addressed within the existing paradigms of chemistry and physics in order to encourage further research within this community. In the last section of this review, those biological magnetic field effects that are understood at only a minimal level will be summarized, but not evaluated critically. A broader introduction to the subject and an historical perspective of biological magnetic field effects can be found in several collective books.[7-15]

A. Producing and Measuring Laboratory Magnetic Fields

1. Measuring Magnetic Fields

Magnetic flux density can be measured by a variety of techniques. In common practice, magnetic flux densities greater than 0.05 mT are monitored with a teslameter (gaussmeter) based on a Hall effect transducer, whereas magnetic flux densities less than about 0.2 mT are commonly measured with a fluxgate magnetometer. Both instruments can be calibrated by reference to NMR frequencies. Nearby high-energy RF sources can occasionally lead to incorrect measurements due to instrumental artifacts and proper shielding must be used.

Magnetic field strength (H) is measured in Oersteds, and it decreases rapidly as the distance from the source is increased. Magnetic flux density (B) is measured in Tesla (or Gauss) and it gives the density of lines of magnetic flux per unit area (eq 1). In air, magnetic field strength is closely approximated by magnetic flux density, such that magnetic field strength is commonly specified in units of Tesla (or Gauss).

$$1\ \mathrm{T} = 10000\ \mathrm{G} \qquad (1)$$

2. The Geomagnetic Field

The geomagnetic field is near 0.05 mT, and it does not vary on a time scale relevant to living organisms. It is directional and is best represented as a vector quantity. When required, the geomagnetic field can be subtracted by orienting an experimental apparatus along the geomagnetic north−south axis and applying a suitable bucking current to a small electromagnet or coil.

3. Producing Laboratory Magnetic Fields

Electromagnets with metal cores of high magnetic permeability are the most suitable method of producing static magnetic fields up to 1.8 T. The high permeability metal core allows the lines of magnetic flux to be "concentrated" and spatially confined to produce a substantially higher field than would be achieved with an air gap alone. Furthermore, the high reluctance provided by the high permeability metal attenuates the residual ac ripple that was not filtered out by the constant current dc power supply. This affords adjustable static fields with an immeasurably low electric field gradient and an alternating magnetic field component of less than 0.1 ppm. Magnetic flux densities in the range 1.8−16.5 T are most easily obtained by superconducting magnets. The common ^{1}H NMR spectrometer frequencies of 200, 300, 400, 500, 600, and 750 MHz provide easy access to high magnetic flux densities of 4.4, 6.6, 8.8, 11.0, 13.2, and 16.5 T. In addition to field strength and stability, the homogeneity of the required field must be considered. Typically, the questions related to biological systems are not concerned with extremely narrow field ranges as is required in magnetic resonance spectroscopy. However, magnetic field effects do occur in specific "windows" of field strength and frequency, so a more modest magnetic flux homogeneity of approximately 1% within the area of the experiment is probably sufficient. This criterion of minimal homogeneity may have to be revised if fine features and resonances in biological systems are discovered.

Well-defined alternating magnetic fields can be more problematic to produce and characterize. Paired coils of wire can be oriented orthogonally around the sample area. Helmholtz coils are often employed to achieve magnetic fields of calculatable intensity. An alternating current of the desired frequency and waveform will produce an alternating magnetic field at right angles to the direction of current flow. If timing synchronization is important, it must be remembered that the magnetic field leads the electric field by $\pi/2$. Laminated-core electromagnets (construction similar to a transformer) provide access to small volumes (3−5 cm^3) with flux densities of 0−80 mT at frequencies less than 750 Hz.

Magnetic shielding can be achieved with low-carbon, high-iron steel or special high-permeability mumetal. Laminated shielding typically proves more efficient than thicker, unlaminated material. High efficiency shielding material for sensitive devices such as photomultiplier tubes, ion traps, and light sources are commercially available.

II. Magnetic Field Effects on Radical Pair Recombination in Biological Systems

Magnetic field effects on the rate of radical pair (RP) recombination is the best understood mechanism by which magnetic fields interact with biological systems. However, the health relevance of this mechanism of magnetic field sensitivity is uncertain because the best-known effects only become significant at moderate magnetic flux densities above 1−10 mT. Significant discussion of this mechanism is provided because recent calculations suggest magnetic field effects through changes in RP recombination may even occur at magnetic field strengths near

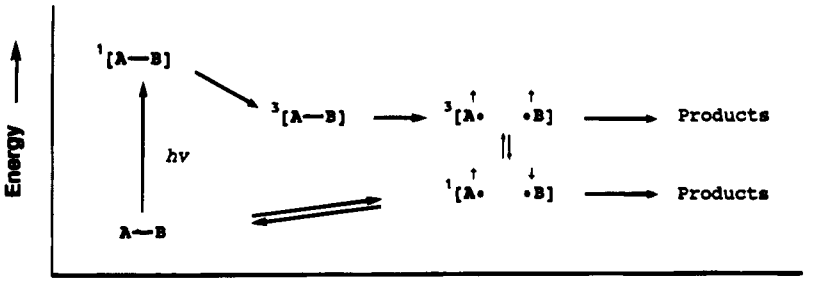


Figure 1. General scheme for generating a triplet radical pair by photolysis.

the intensity of the geomagnetic field.[16-18] Since the yield of excited triplet electronic states in the photosynthetic reaction center was first shown to be magnetic field sensitive in 1977,[19-21] only one other biological system, an enzyme with radical pair intermediates, has been shown to exhibit any magnetic field-dependent parameters.[22] In the following section, the theory of magnetic field effects on radical pair recombination will be summarized and the results of studies on the photosynthetic reaction center and enzymes with RP intermediates will be discussed.

A. Theory of Magnetic Spin Effects on RP Recombination

The most general term that covers the role of magnetic interactions in chemical reactivity is "magnetic spin effect". This includes the influence of an exogenous magnetic field, a so-called "magnetic field effect (MFE)" as well as the effect of an endogenous magnetic field that originates from a non-zero nuclear spin ($I \neq 0$) that has an intrinsic magnetic moment. Because different isotopes have different nuclear magnetic moments, we use the term "magnetic isotope effect (MIE)" to refer to the influence of nuclear spin on the reaction rate, as reflected in the distribution of isotopes in products.

The importance of magnetic spin effects in chemical reactions presents a conceptual problem if only the energy imparted by the field is considered. Thermal reactions that undergo a change from diamagnetic substrates to paramagnetic intermediates, products, or transition states, might be expected to be accelerated by a magnetic field that imparts a stabilizing interaction to the paramagnetic species. In a reaction that undergoes an increase in magnetic susceptibility, $\Delta\chi$, of 10^{-2} cm^3/mol, ΔG becomes more favorable by the trivial amount of 0.0002 kcal/mol in a magnetic field of 1 T.[23,24]

However, even a magnetic field in the range of 1−10 mT can split the Zeeman energy levels of a radical pair and provide an alternate (nonadiabatic) reaction pathway that can change the observed reaction rate or alter the product distribution.[23] The theory behind magnetic spin effects in chemical reactions, especially photochemically produced RP's, has been described with both classical and quantum mechanical formalisms.[23,25-38] The MFE neophyte is directed to several reviews that are especially readable and provide a good introduction to the theory of spin-dependent chemistry.[25,31,39] The physical mechanisms that lead to magnetic spin-dependent chemistry are summarized in the following sections.

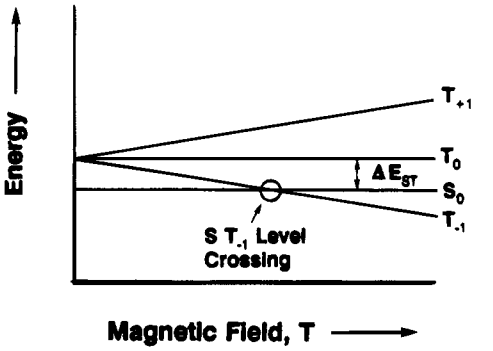


Figure 2. Splitting of triplet energy levels. At $B = 0$, the three triplet states, T_0, T_{+1}, and T_{-1} are energy degenerate. At $B > 0$, T_{+1} and T_{-1} are split to higher and lower energy from S_0 and T_0.

Throughout this article, electron "spin" refers to the total angular momentum of the unpaired electron. Each electron in the RP has a spin quantum number of $\pm^1/_2$.

An isolated molecule with a single unpaired electron can adopt two orientations relative to an external magnetic field. These orientations are with the spin vector aligned parallel or antiparallel to the external field. We call this a "doublet" state by virtue of its having two possible orientations in the magnetic field. The reactivity of a doublet state radical will not be altered by a magnetic field.

In contrast, consider the case of a RP that is produced by homolysis of the A−B bond in the ground state. Prior to dissociation, the two electrons in the σ-bond were spin-paired by virtue of the Pauli exclusion principle. According to the Wigner Spin Conservation Rule, the elements of the RP (denoted by brackets, {}) will retain their original orientation immediately after homolysis, and the singlet RP, with electron spins paired in an antiparallel fashion {A↑ + ↓B}, will prevail.[35] In contrast, if the ground-state starting material absorbs a photon to create the first excited singlet state, S_1, rapid decay to the (usually) lower energy triplet state, T_1, will place the molecule on a dissociative energy surface and the separated triplet RP {A↑ + ↑B} with parallel electron spins will result (Figure 1).

In the absence of a magnetic field, the three triplet spin states, T_{-1}, T_0, and T_{+1}, are energy degenerate. At $B > 0$, T_{-1}, T_0, and T_{+1} are split by the electronic Zeeman interaction energy into three distinct states that are no longer degenerate (Figure 2). The size of the Zeeman splitting is given by eq 2. The term B

$$\Delta E = g\beta B \qquad (2)$$

is the magnetic flux density and β is the Bohr

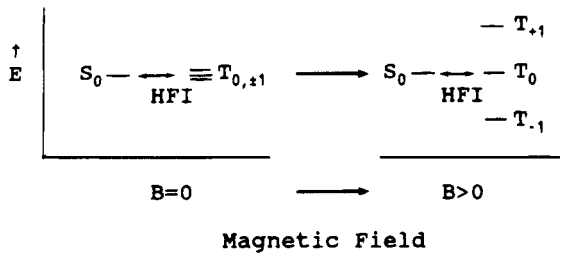


Figure 3. Hyperfine interactions, HFI, promote $S_0 \rightleftharpoons T_{0,\pm1}$ intersystem crossing at $B = 0$. At $B > 0$, HFI can only promote $S_0 \rightleftharpoons T_0$ intersystem crossing and population of the $T_{\pm1}$ sublevels is diminished.

magneton (9.274×10^{-24} J T^{-1}). The value g is the Lande g factor, which is close to 2.00 for the free electron and most organic radicals.

In a rigorous analysis, g is a tensor. In solution, all orientations are averaged by molecular motion such that g becomes isotropic and we can regard it as a scalar quantity. In compartmentalized biological systems or at interfaces, the directionality of g may be very important. The value of g varies slightly as the surrounding nuclear and electronic environment is varied.

The rate of intersystem crossing (ISC) between the singlet and triplet spin states is dependent upon having an accessible energy gap, ΔE_{ST}, and a physical mechanism by which to interconvert the spin states.

B. Hyperfine Induced Intersystem Crossing

Hyperfine interactions (HFI) between the magnetic moment of an unpaired electron and the magnetic moment of a nearby (bonded) nucleus results in a "torque" that promotes flipping of the electron spin. This interaction between the electron and a magnetically active nucleus provides a mechanism to interconvert both electron and nuclear spin quantum numbers in the absence of an external magnetic field. This leads to the NMR-observable phenomenon of chemically-induced dynamic nuclear polarization (CIDNP) and the ESR-observable phenomenon of chemically-induced dynamic electron polarization (CIDEP). *In reactions that are sensitive to the spin angular momentum of the unpaired electron, (e.g., RP recombination), it can lead to changes in the rate of the reaction or the product distribution.* Because the amount of HFI-induced ISC will depend upon the nuclear spin (isotope), this leads to a "magnetic" isotope effect that depends upon the nuclear magnetic moment of the isotope, as well as the mass of the reacting isotope.[28,31,38]

Most hyperfine coupling constants for organic radicals are 1−10 mT. Hyperfine interactions promote mixing of the singlet (attractive) and triplet (dissociative) reaction manifolds of the free energy surface by interconverting the S_0 and T_{-1}, T_0, and T_{+1} states. The three triplet spin states are energy degenerate at $B = 0$, so they are populated to an equal extent (Figure 3). Given sufficient time, an equilibrium distribution between singlet and triplet states (assuming ΔE_{ST} is so small as to be approximately zero) will result in a 25% singlet and 75% triplet spin population.

If an external magnetic field is applied, the degeneracy between the three triplet spin states is removed by the Zeeman interaction energy that splits T_{+1} and T_{-1} from T_0 equally, but with opposite relative signs (Figure 3). The net result is that HFI induced ISC between S_0 and T_{-1} and T_{-1} is decreased. If the RP was produced in the triplet spin state, the decrease in ISC will decrease population of the singlet state. Since RP recombination usually occurs from the spin-paired singlet state, a net decrease in RP recombination will occur. A net increase in "escape" (nonrecombination) products derived from the triplet RP will be observed. Recombination from the triplet RP is possible, but this yields a high-energy triplet molecule that lies on a dissociative energy surface. This decrease in HFI-promoted ISC can remove up to $^2/_3$ of the enhanced reactivity (throughput) of the magnetically active nucleus.

As an example, consider the photolysis of dibenzyl ketone (DBK).[37−42] Absorption of a photon by DBK produces the first excited singlet state that undergoes rapid ISC to the lowest energy triplet state (dissociative surface). Bond homolysis occurs to produce the triplet RP. The geminate RP partitions between cage escape products (benzyl and benzylcarbonyl radical, the latter of which can undergo decarbonylation) and cage recombination (to produce DBK).

Radical pairs that contain ^{13}C at either of the radical centers will undergo more ISC than radical pairs with ^{12}C at the radical center.[37] Because the majority of photochemically produced radical pairs are borne in the triplet state, an increase in ISC will lead to an increase in the singlet RP population and a corresponding increase in RP recombination. The substrate will be enriched in ^{13}C relative to product. If photolysis is carried out in benzene, the observed isotope effect, k_{12}/k_{13}, is 1.05, thus producing a small enrichment of ^{13}C in starting material.[41,42] However, if the photolysis is carried out in aqueous detergent micelles, k_{12}/k_{13} increases to 1.47 and is beyond the range of reasonable mass-dependent isotope effects.[40] This enormous kinetic isotope effect decreases to 1.12 when an external magnetic flux density of 1.5 T is applied.[37] The external field now splits the T_{-1} and T_{-1} spin states by the Zeeman interaction energy and decreases the HFI induced ISC. The magnetic field dependence of the isotope effect provides a characteristic determinant of the importance of magnetic spin and RP chemistry in any reaction with an unusually large isotope effect.

C. Spin Rephasing (Δg Mechanism) Induced Intersystem Crossing

In addition to electron−nuclear hyperfine interactions, there are other mechanisms that can lead to magnetic field-induced ISC. The spin angular momentum of an electron is quantized as either $+^1/_2$ or $-^1/_2$ relative to an external frame of reference. The spinning electron has a net magnetic moment that is similarly quantized and can occupy only one of two relative orientations. In the presence of an external B-field, the net magnetic moment, described by a vector quantity, will precess at its Larmor frequency given by eq 3. Since we are considering the interac-

$$\omega = g\beta B\hbar^{-1} \qquad (3)$$

tion between two unpaired electrons, it is convenient to express eq 3 as the difference in precession rates for the two unpaired electrons (eq 4). In a homo-

$$\Delta\omega = (g_1 - g_2)\beta B\hbar^{-1} = \Delta g\beta B\hbar^{-1} \qquad (4)$$

nuclear RP, $\Delta g = 0$ and both unpaired electrons will precess at exactly the same frequency. However, if $\Delta g \neq 0$, then the precession rates for the two unpaired electrons will be different. Equation 4 gives the time required for spin evolution to interconvert the S_0 and T_0 states by rephasing of the electron spins.

As an example, consider the organic radical pair $\{CH_3 \cdot CH_3CH \cdot OEt\}$ with g values of 2.00255 and 2.0031, respectively.[44,45] For this RP, $\Delta g = 0.00055$ and $\Delta\omega = 2.07 \times 10^{-8}$ s at $B = 1$ T. If the RP is held close for at least this long, ISC by spin-rephasing can compete with RP separation. In the case of a heteronuclear RP, such as the photolysis product of adenosylcob(III)alamin (or in the active site of a B_{12} enzyme) where one element of the RP is a paramagnetic metal ion, {adenosyl-5′-CH_2 · cob(II)alamin}, $g_1 = 2.00$ and $g_2 = 2.25$,[43] the rate of rephasing (ISC) can be very fast, $\omega = 4.54 \times 10^{-11}$ s at 1 T, and ISC can out compete RP separation or geminate recombination.

D. Singlet–Triplet Energy Level Crossing

In the case of a RP that exists at a well-defined separation distance, r_{sep}, of less than ~10 Å, (i.e. a biradical in which the two unpaired electron spins reside on the same molecule), the Coulombic repulsion between the two electrons will be important in determining how they interact. The exchange integral (or exchange interaction), J, is a measure of this interaction. For two unpaired electrons that are on different atoms and do not "see" each other, $J = 0$ and their ESR signatures will each be doublets. If the two electrons "communicate", either through space, or through a π-bonding interaction, $J > 0$, $\Delta E_{ST} > 0$, and there will be a preference for either the triplet or singlet state. If the exchange interaction is so large (definite preference for S or T) that HFI cannot promote ISC, (the case where $2J > E_{HFI}$), no conversion between the singlet and triplet states will occur at $B = 0$. However, at $B > 0$, the S and T surfaces can cross in a very narrow region of B and r_{sep} to promote ISC (Figure 2). At fields higher than B, exchange–interaction-promoted ISC disappears. The net effect is to produce a narrow window at which ISC is increased.

E. Spin–Orbit Coupling

A radical on an atom of high electron density (sulfur) will tend to have a more anisotropic g value than a radical on an atom of lower electron density (carbon). This is known as spin–orbit coupling (SOC) and it increases in importance as the atomic number increases.[32] SOC is caused by coupling between the electron spin angular momentum and the orbital angular momentum. To a first approximation, this may be regarded as the spinning bonded electrons producing a net torque on the magnetic moment of the unpaired electron. SOC will result in ISC regardless of magnetic field strength. In this sense, it may be regarded as producing a basal degree of ISC that is not influenced by external factors. The relative importance of SOC in a given RP is proportional to the absolute value of the SOC constant for the atom with unpaired electron density: C, 13 cm^{-1}; O, −79 cm^{-1}; Fe^{2+}, −114 cm^{-1}; Co^{2+}, −189 cm^{-1}; S, −184 cm^{-1}; Cl, −545 cm^{-1}; Br, −2194 cm^{-1}; I, 5060 cm^{-1}.[32,46] SOC can lead to the relaxation (randomization) of electron spin intermolecularly, as well as intramolecularly. Photochemists have used "internal heavy atom effects" and "solvent heavy atom effects" to alter the reactivity of spin-correlated RP's by increasing ISC.[29,46-48]

F. Enhanced Recombination by Compartmentalization

Another important aspect of magnetic spin-dependent chemistry is enhanced recombination by compartmentalization of the RP. In a freely diffusing environment, RP separation decreases the likelihood of reencounter through collisions that would otherwise lead to a discrimination based on the spin state of the RP. In the physical organic nomenclature, escape products are favored over cage recombination products. Under these circumstances, no magnetic spin-dependent chemistry is observed. Cage recombination processes can be characterized further as either primary or secondary cage recombination. Primary geminate recombination is limited to 10^{-10} to 10^{-8} s and occurs without intervention of solvent and little or no diffusion. This allows primary geminate recombination to be characterized by the solvent independence of the process. Only systems that do not undergo significant conformational changes or atomic reorganization can undergo primary cage recombination. Recombination in bulk solvent occurs on a much slower time scale, typically 10^{-8}–10^{-4} s. Diffusion and viscosity effects are now important and solvation/desolvation of the RP elements may limit the rate of recombination. The distance between the radical centers is still small enough to define a singlet and triplet surface, however.

In the DBK example above, only a small isotopic enrichment of 5% was observed in benzene, whereas photolysis in the restricted space of a micelle brought the isotope effect up to 47%. A similar increase in the isotope effect is observed when the rate of diffusion is decreased by an increase in solvent viscosity. On going from benzene ($\eta = 0.6$ cP) to dodecane ($\eta = 1.35$ cP) and cyclohexanol ($\eta = 30$ cP), the isotope effect increases from 1.04 to 1.05 and to 1.07, respectively.[37,50]

Enhanced RP recombination by compartmentalization may be important to observing significant RP magnetic field effects in biological systems. A spin-correlated RP that is produced by bond homolysis will experience only a small MFE or MIE if the RP elements are free to diffuse in solution. Although controversial and poorly defined, the cytosolic absolute viscosity may be similar to the viscosity of H_2O (0.69 cP at 37 °C and 1 atm).[51] The cell itself offers a further degree of compartmentalization, but

the volume of a cell is large when considering the mean free path of small molecule diffusion. Interfacial (boundary) effects and organelle compartmentalization are probably the most important restrictions to diffusion in cells.

A RP that is held extremely close will usually have a large ΔE_{ST} that is greater than the interaction energy provided by the magnetic field or HFI, and no increase in ISC will be observed.[52] In contrast, a RP that exists at a large separation distance, r_{sep}, will generally have a small ΔE_{ST} as required for magnetic spin-induced ISC.

G. Random Radical Pair Recombination[53,54]

At equilibrium, with $\Delta E_{ST} \approx 0$, random pairs will exhibit a 3:1 distribution between the triplet and singlet spin states. These random radical pairs of non-geminate origin are sometimes called "F-pairs" to distinguish them from "G-pairs" (geminate pairs). In a solution of low viscosity, encounter events between singlet radical pairs almost always result in reaction, whereas encounter between triplet radical pairs typically will not result in bond formation. The lifetime of the collision complex is too short for ISC after the RP is brought together and before subsequent dissociation. In a more viscous medium or a compartmentalized environment, the RP (of random origin) will stay close enough for ISC to alter its spin state. In the short RP lifetime the reactivity of the 25% of radical pairs that are singlet will be nearly unaffected by magnetic field-dependent ISC, whereas reactivity of the 75% of radical pairs that are triplet will exhibit the greatest dependence on ISC (and magnetic field).[53,54]

H. Radical Chain Reactions

At an infinite separation distance, $\Delta E_{ST} = 0$ and the elements of the RP are characterized as "free radicals" that react independently. If the radical concentration is low, the probability of radical–radical recombination is low, compared to radical–molecule reaction that is more likely. Since only one unpaired electron spin is present, ΔE_{ST} is undefined and there will be no effect of a magnetic field (endogenous HFI or external MFE). This prevents chain propagation in autoxidation and peroxidation reactions of biological interest from being affected by a magnetic field. However, the rate of a radical chain reaction is directly dependent on the concentration of radical species and any decrease in the radical concentration (through decreased initiation or increased termination events) will directly affect the rate. A magnetic field-dependent radical chain reaction involving the peroxyl radical has been demonstrated experimentally.[55]

As an example, consider the autoxidation of a fatty acid. The series of reactions that describe initiation, propagation, and termination are shown in eqs 5–12.[56]

$$R'\text{—}R' \xrightarrow{k_1} 2R'^{\bullet} \quad (5)$$

$$R'^{\bullet} + O_2 \xrightarrow{k_2} R'OO^{\bullet} \quad (6)$$

$$R'OO^{\bullet} + RH \xrightarrow{k_3} R'OOH + R^{\bullet} \quad (7)$$

$$R^{\bullet} + O_2 \xrightarrow{k_4} ROO^{\bullet} \quad (8)$$

$$ROO^{\bullet} + RH \xrightarrow{k_5} ROOH + R^{\bullet} \quad (9)$$

$$2R^{\bullet} \xrightarrow{k_6} RR \quad (10)$$

$$R^{\bullet} + ROO^{\bullet} \xrightarrow{k_7} ROOR \quad (11)$$

$$2ROO^{\bullet} \xrightarrow{k_8} ROOR + O_2 \quad (12)$$

Under steady-state conditions, $d[R^{\bullet}]/dt \approx 0$ and the net rate of lipid peroxidation is described by eq 13.[56]

$$-d[O_2]/dt = (k_1/2k_8)^{1/2} k_5 [RH][R'\text{—}R']^{1/2} \quad (13)$$

The combination of two radical species occurs only in recombination of the initiator radicals (reverse of k_1) and chain termination (k_6, k_7, and k_8). Although k_1 and k_8 appear in the rate expression, the concentration of $R'^{\bullet}$ and $ROO^{\bullet}$ is very low. Radical–radical encounter of these species demands a high concentration of these species to produce a significant rate of recombination. Any magnetic field-induced change in ISC would provide only a small change in the concentration of radical propagators, but this would have a large effect on the rate because each radical can cause multiple chain events. Note that the propagation rate constants, k_2, k_3, k_4, and k_5 only involve one unpaired electron species and will not exhibit a magnetic spin dependence.

I. Static vs Modulated Magnetic Field Effects on RP Recombination

Magnetic field effects on RP recombination originate from the splitting of Zeeman energy levels by static fields. The magnetic vector component of a time-varying magnetic field that is modulated slowly, when compared to the lifetime of the spin-correlated RP and the rate of ISC, has the same net effect.[57,58]

As an example, consider the effect of a magnetic field produced by 60 Hz alternating current. The sign of the B field is unimportant and the absolute value of the field is changing at 120 Hz. If RP recombination is occurring in the time window 10^{-8}–10^{-6} s, then a 60 Hz magnetic field exerts the same instantaneous effect (within 10^{-8}–10^{-6} s) as a static field of equal magnitude.[57,58] The instantaneous field is described by eq 14.[57,58]

$$B = \sqrt{2} B_{rms} |\sin(\theta)| \quad (14)$$

The slow oscillation of a weak magnetic field in the presence of a larger static magnetic field has also been used to increase the signal-to-noise ratio in spectrophotometrically observable reactions.[59,60] In this application, the derivative of the spectrophotometric signal is followed in phase with a weakly modulated magnetic field. A lock-in amplifier allows

a high-precision measurement of the magnetic field effect on the spectrophotometrically observable species. This technique is best suited to reactions that produce a short-lived chemical species that can be probed on a rapid time scale (i.e. fluorescence or luminescence yield). When a stable end-product, P, accumulates, the integrated rate expression that describes [P] as a function of time only weakly reflects the small modulation of d[P]/dt. Nevertheless, in sensitive assays with highly fluorescent products, this technique may offer a superior signal-to-noise ratio. This method of data collection will not work with coupled enzyme assays.

J. Oscillating Magnetic Fields To Induce Changes in RP Recombination[16-18]

The application of perturbation theory treatment to oscillating magnetic fields and RP recombination allows a theoretical consideration of weak oscillating magnetic fields (on the order of 0.003 mT) in the presence of the geomagnetic field of 0.05 mT.[16,17] This perturbation theory approach to the Schrödinger equation suggests that considerable alterations in ISC can be realized at surprisingly low fields that may be relevant to magnetic field bioeffects via changes in RP recombination.[16-18]

K. Radical Pair Recombination in Biological Systems

In sections A–J, the general theory of magnetic field effects on RP recombination rates was developed. In the next sections, this theory is extended to biological systems with RP intermediates. Recent reviews by Scaiano et al.[58] and Walleczek et al.[61] also consider the possibility of biological magnetic field effects via changes in radical-pair recombination.

1. Magnetic Field Effects on the Photosynthetic Reaction Center

The first biological system to be probed by magnetic field effects was the light-harvesting reaction center from the photosynthetic bacterium *Rhodopseudomonas sphaeroides*. This 100000 MW membrane-bound protein (from *R. viridis*), along with its nine prosthetic groups, converts visible light energy into a transmembrane proton gradient that drives ATP synthesis.[62] In close analogy to the photochemical reaction of DBK described above, the absorption of a photon produces the first excited singlet state that irreversibly donates an electron to an acceptor and leads to formation of a compartmentalized radical pair (eq 15).[63,64] In this scheme, P is the primary

$$PIX \xrightarrow{h\nu} P^*IX \rightarrow {}^1\{P^{+\bullet}\,{}^{\bullet-}I\,X\} \rightarrow {}^1\{P^{+\bullet}\,I\,{}^{\bullet-}X\} \quad (15)$$

electron donor (chromophore), I is an intermediate electron acceptor, and X is the first stable electron acceptor.[63] Absorption of a photon by P produces the excited P*IX complex that decomposes to the metastable $P^{+\bullet}$ radical cation and $^{\bullet-}I$ radical anion in about 2.8 ps. In the normal biological reaction, the $^1\{P^{+\bullet}\,{}^{\bullet-}I\,X\}$ radical pair transfers an electron to

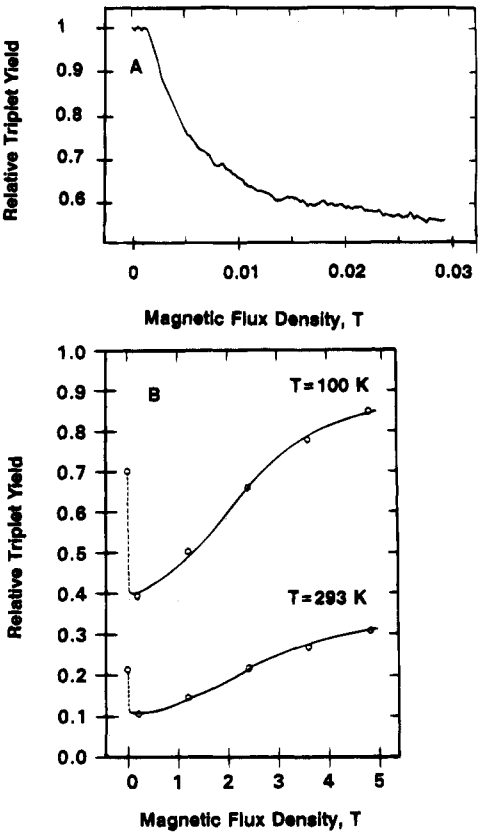


Figure 4. (A) Relative triplet quantum yield of *Rhodopseudomonas sphaeroides* reaction center and (B) absolute triplet quantum yield of *R. sphaeroides* reaction center at high magnetic fields. (A: Reprinted from Moehl, K. W.; Lous, E. J.; Hoff, A. J. *Chem. Phys. Lett.* **1985**, *121*, 22. Copyright 1985 Elsevier Science Publishers B.V. B: Reprinted from ref 68. Copyright 1982 American Chemical Society.)

quinone acceptor X to produce $^1\{P^{+\bullet}\,I\,{}^{\bullet-}X\}$ in a process that requires a minimum of 200 ps. The lower free energy of the $^1\{P^{+\bullet}\,I\,{}^{\bullet-}X\}$ RP makes this an irreversible process. Instead, if the electron acceptor, X, is chemically reduced ($X^{-\bullet}$) to prevent it from accepting an electron, the lifetime of the $^1\{P^{+\bullet}\,{}^{\bullet-}I\,X^{-\bullet}\}$ RP is extended and can undergo recombination (disfavored) or ISC to the triplet RP $^3\{P^{+\bullet}\,{}^{\bullet-}I\}$ that can recombine (by virtue of its prereduced state) to yield $^3P^*IX^{-\bullet}$. This case is described by eq 16.[63,64]

$$P^*IX^{-\bullet} \rightarrow {}^1\{P^{+\bullet}\,{}^{\bullet-I}\}\,X^{-\bullet} \rightarrow {}^3\{P^{+\bullet}\,{}^{\bullet-I}\}\,X^{-\bullet} \rightarrow {}^3P^*IX^{-\bullet} \quad (16)$$

The quantum yield, ϕ_T, of the triplet chromophore, ^3P, decreases by about 50% at 50 mT (Figure 4A).[65,66] As the applied magnetic field increases, the Zeeman interaction energy between the $T_{\pm1}$ levels increases and this causes a decrease in HFI. At $B = 0$ T, HFI mixes S_0–$T_{\pm1,0}$ states, but at magnetic fields greater than zero, only the S_0–T_0 states can interconvert.[64,66] At high magnetic fields greater than 500 mT, ϕ_T begins to increase slowly and attains parity with the $B = 0$ T value at $B \approx 3$ T (Figure 4B).[68,69] At even higher magnetic fields, a net increase in ϕ_T is observed. This linear increase in ISC at high fields is ascribed to the interconversion of the S_0–T_0 spin states via the Δg mechanism.[67-70] In Figure 4B, a

plateau in ϕ_T becomes apparent above $B \approx 5$ T. Although the $\omega = B\beta\Delta g$ term (where $\omega \approx k_{ISC}$) continues to increase, a limiting value of ϕ_T will be reached when $\omega \geq k_T$ (where k_T is the rate constant for formation of $^3PIX^{-\bullet}$).[68] The limit of $\phi_T = k_T/(k_S + k_T)$ and the rate of reaction of the singlet RP, k_S, will be independent of magnetic field.[68] The biphasic dependence of ϕ_T vs magnetic field is characteristic of many magnetic spin-dependent photochemical RP reactions in which HFI *decreases* ISC at low field, and the competing Δg mechanism *increases* ISC at higher fields.

a. Biological Relevance. The thrust of these experiments (*vide infra*) was not to test the harmfulness of magnetic fields toward photosynthetic bacteria and plants. Rather, the significant outcome of these experiments is the absolute proof of the formation of a RP in the early events of photosynthesis. A RP in which ISC and recombination could compete with the forward step had to be created in order to observe a magnetic field-dependent process. The normal reaction pathway is composed of a series of coupled vectorial processes that create an irreversible free energy cascade and drives H^+ pumping. Hence, no magnetic field effect on the photosynthetic quantum yield would be expected in intact and unadulterated photosynthetic systems.

L. Magnetic Field Effects on Thermal (Nonphotochemical) RP Reactions

Magnetic field effects are best described for photochemical reactions where the RP is prepared in the triplet spin state. When RP recombination from the singlet state is being monitored, or there is both a triplet and a singlet product manifold, it is easy to understand how changing the rate of ISC will alter the course of the reaction. Thermally driven homolysis reactions produce a RP that is born in the singlet electronic state. If the RP stays together for $\sim 10^{-10}$ s, geminate recombination is very favorable because it liberates substantial free energy when the covalent bond is formed once again. Furthermore, no electronic spin conversion is required for recombination and only limited atomic motion may have occurred (i.e. incomplete atomic reorganization to the optimal ground-state geometry). These factors make it more difficult to understand how a magnetic field can affect a thermally generated RP.

Magnetic field effects on thermal reactions have received very little attention, although they are the most relevant to possible magnetic field effects on biological reactions through changes in RP recombination. Several thermal reactions do exhibit a marked magnetic field dependence. In refluxing hexane, *n*-butyllithium will react with pentafluorobenzyl chloride to form LiCl and *n*-butylpentafluorobenzene (eq 17).[71] At magnetic fields greater than 20 mT, the

$$F_5PhCl + n\text{-BuLi} \rightarrow {}^1\{F_5Ph^{\bullet}\ n\text{-}Bu^{\bullet}\} \rightarrow n\text{-Bu}, F_5Ph + LiCl \quad (17)$$

amount of *n*-butylpentafluorobenzene (cage product) relative to $(F_5Ph)_2$ (escape product) is increased by 50%.[71] This magnetic field-dependent increase in cage recombination originates from a decrease in HFI that would otherwise populate the triplet spin states and prevent recombination.

A magnetic field effect has also been observed on the thermal decomposition of dilauroyl peroxide.[72] At

$$C_{11}H_{23}COOOCOC_{11}H_{23} \rightarrow {}^1\{C_{11}H_{23}^{\bullet}\ {}^{\bullet}C_{11}H_{23}\} + 2CO_2 \quad (18)$$

$$^1\{C_{11}H_{23}^{\bullet}\ {}^{\bullet}C_{11}H_{23}\} \rightarrow C_{22}H_{46} \quad (19)$$

magnetic fields greater than 150 mT, the cage recombination of the spin-correlated lauroyl radicals is increased by 3–6%.[72] This reaction might appear to be relevant to the chemistry of biological lipid autoxidation, but in fact, the peroxide formed by the reaction of molecular oxygen with an unsaturated lipid is a hydroperoxide (see eqs 5–13). The thermal decomposition of a hydroperoxide will lead to the alkoxyl and hydroxyl radicals as a RP. Except in

$$ROOH \rightarrow {}^1\{RO^{\bullet}\ {}^{\bullet}OH\} \quad (20)$$

$$^1\{RO^{\bullet}\ {}^{\bullet}OH\} \rightarrow ROOH\ (\text{minor}) \quad (21)$$

$$RO^{\bullet} + R'H \rightarrow ROH + R'^{\bullet} \quad (22)$$

$$HO^{\bullet} + R'H \rightarrow H_2O + R'^{\bullet} \quad (23)$$

$$R'^{\bullet} + O_2 \rightarrow R'OO^{\bullet} \quad (24)$$

$$R'OO^{\bullet} + R''H \rightarrow R'OOH + R''^{\bullet} \quad (25)$$

cases of unusual stability, the oxygen-centered radicals are 10–15 kcal/mol less stable than the possible alkyl radicals. This will give the alcohol and H_2O as the stable products of the starting hydroperoxide. The fate of the R'• radical is varied and this radical can participate in free-radical chain chemistry. The possibility of observing magnetic spin-dependent chemistry with •OH is uncertain because of the unquenched orbital angular momentum and the ensuing SOC.

M. Magnetic Field Effects in Catalytic Reactions

A remarkable magnetic field dependence has been observed for the cobalt and manganese catalyzed oxidation of 2,6-di-*tert*-butylphenol (Figure 5).[73,74] The rate of quinone product formation exhibits a biphasic increase to a maximum 50% enhancement at 100 mT (relative to the control rate at 0 T). At higher fields, the rate of product formation decreases and goes below the control rate at 7 T. A radical pair mechanism is proposed in which the magnetic field-dependent (partially) rate-determining step is regeneration of the active Co(II) catalyst and production of the phenolic radical in a spin-correlated step (Figure 6).[74]

This is a landmark result in magnetic field effect studies of catalytic reactions. The reaction has only one product, so a distinct singlet and triplet reaction product manifold does not exist. Perito and Corden point out, "Any process that alters the concentration of the active catalyst (steps a and f; Figure 6), or the phenoxy radical concentration (steps c and f; Figure 6) will affect the reaction rate." Thus, a magnetic

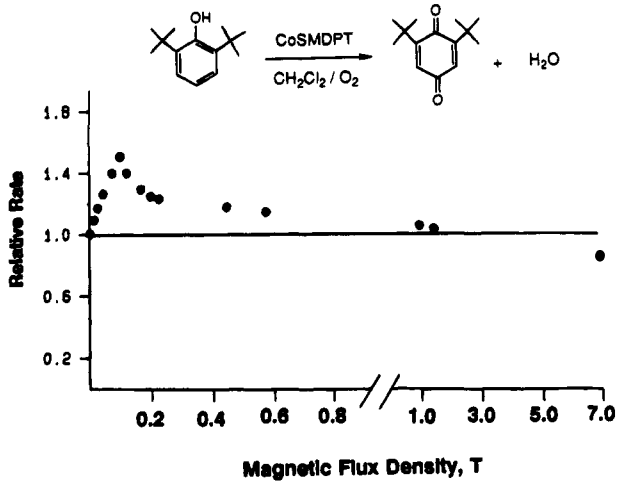


Figure 5. Relative rate of 2,6-di-*tert*-butylbenzoquinone formation vs magnetic field. (Reprinted from ref 74. Copyright 1988 American Chemical Society.)

field can alter singlet RP recombination rates in *catalytic* reactions with only one product. This statement is supported by a theoretical treatment of MFE on the steady-state rate of product formation in a catalytic reaction.[75] Only a transient pair of paramagnetic particles involving either the catalyst or the substrate and catalyst complex is required to invoke a possible magnetic field effect.

N. Magnetic Field Effects in Enzymatic Reactions

1. Literature Reports

Magnetic field effects on enzymatic reactions have long been proposed. The earliest studies of enzymatic reaction rate vs magnetic field were phenomenological in nature, and they were carried out before the theory of MFE on RP recombination was developed. The rate of ribonuclease and cytochrome *c* reductase was independent of applied magnetic field from 0 to 4.8 T.[76] With a few noteworthy exceptions, other negative reports of magnetic field effects on enzymatic reactions followed (Table 1).[77-86]

2. Theory

In 1986, Vanag and Kuznetsov examined the evidence for magnetic spin effects in biological reactions other than the bacterial photosynthetic reaction center.[77] At that time, no significant magnetic field effects on enzymatic reactions had been observed. In 1984, they considered the possibility of MFE on enzymatic reactions through changes in RP chemistry.[87] Their analysis is based on the central paradigms of enzymatic catalysis: (1) product formation can only occur from the enzyme–substrate (ES) complex; (2) the overall conversion of substrate, S, to product, P, can be broken down into a series of discrete unimolecular steps that can be described as a series of microscopic rate constants; and (3) ex-

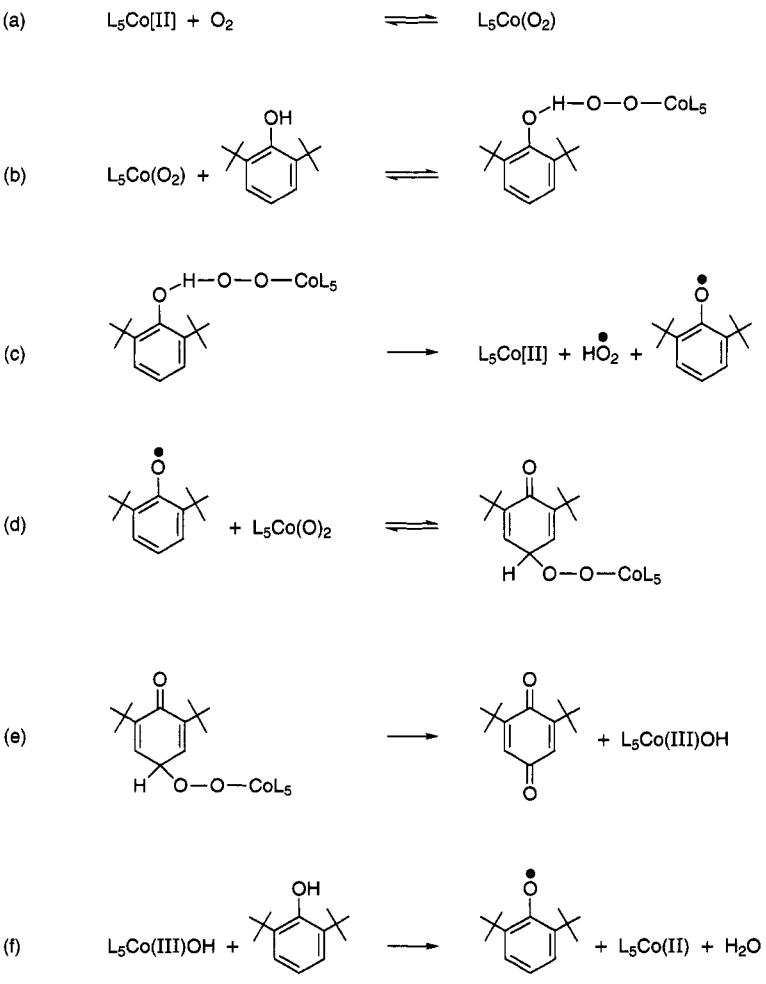


Figure 6. Mechanism of 2,6-di-*tert*-butylphenol oxidation catalyzed by cobalt(II) bis(3-(salicylideneamino)propyl)-methylamine (CoSMDPT). (Reprinted from ref 74. Copyright 1988 American Chemical Society.)

Table 1. Magnetic Field Effects on Enzyme Reaction Rates *In Vitro*[a]

enzyme	catalyzed reaction	induction of MF, T	change in reaction rate, %	experimental error %	year	ref
succinate cytochrome *c* reductase	reduction of cytochrome *c*	5.0	less than experimental error	±10	1965	76
peroxidase	oxidation of *o*-dianidizine with hydrogen peroxide	8.5; 17.0	same	±2	1967	78
tyrosinase	oxidation of L-tyrosine by molecular oxygen	17.0	same	±3−7	1967	78
alcohol dehydrogenase	oxidation of C_2H_5OH, reduction of NAD	1.4	0	±2	1971	79
lactate dehydrogenase	oxidation of lactate, reduction of NAD	1.4	0	±2	1971	79
glutamate dehydrogenase	oxidation of 2-oxoglutarate, reduction of NAD	6.0	−(5−10)	no statistical treatment	1967	80
catalase	degradation of H_2O_2	6.0	+(5−9)	same	1967	80
	liberation of O_2 during degradation of H_2O_2	0.65	+20	±5	1978	81
catalase	degradation of H_2O_2	1.05	0	±2	1989	84
ascorbate oxidase	oxidation of L-ascorbate by O_2	1.05	0	±2	1989	83, 84
bacterial luciferase	$FMNH_2$ decanal oxidation by O_2; light emission	0−1.0	0	±5	1985	82
chymotrypsin	*p*-nitrophenyl ester	0−0.27	0	±1	1990	*b*
staphylococcal nuclease	*p*-nitrophenyl-dTp → *p*-Nphenol (Ca^{2-} req.)	0−0.27	0	±3	1990	*b*
hexokinase	glucose → glucose-6-P (coupled assay with G6PDH/NADP)	0−0.27	0	±<1	1990	*b*
horseradish peroxidase	guaicol; oxidation by H_2O_2	0−0.25	0	±10	1991	*c*
lipoxygenase	linoleate oxidation by O_2	0−0.20	0	±5	1994	85
tyrosine hydroxylase	Tyr, O_2 → L-Dopa	0−0.28	0	±20	1993	*d*
monoamine oxidase B	benzylamine → benzaldehyde + NH_3	0−0.2	0	±3	1993	*e*
ethanolamine ammonia lyase (coenzyme B_{12} requirement)	ethanolamine → acetaldehyde + NH_3 (unlabeled EA)	0−0.25	−25	±5	1994	86
	(deuterated EA)	0−0.25	−60	±5	1994	86

[a] Table adapted from ref 77 (with permission). Entries 1−8 are from ref 77. [b] Grissom, C. B. Unpublished results. Chymotrypsin, staphylococcal nuclease, and hexokinase were used in the author's laboratory as controls for spectrophotometer/electromagnet evaluation. No radical pair intermediate is proposed for these enzymes. G6PDH = glucose-6-phosphate dehydrogenase. [c] Lee, K.; Grissom, C. B. Unpublished results. [d] Hillas, P. J.; Fitzpatrick, P. F.; Grissom, C. B. Unpublished results; supported by NIH GM 47291 to P.F.F. [e] Farmer, D.; Silverman, R. B.; Woo, J. C. G.; Grissom, C. B. Unpublished results; supported by NIH GM 32634 to R.B.S.

trapolation to infinitely low substrate ([S] ≪ K_m) will change the overall kinetics from zero order in S (at saturating substrate where ES predominates) to pseudo-first-order in S (where free E and S predominate). The latter two points are important, since the overall turnover rate of an enzyme may be as slow as a few molecules of product formed per second. If the first irreversible step in the conversion of S to P were slower than about 10^3 s^{-1}, random molecular interactions would cause a loss of spin coherence and no magnetic spin-dependent chemistry would be observed. However, if the microscopic steps of catalysis are considered, the interconversion of these enzyme−substrate complexes occurs with much faster rates. The requirements for a magnetic field-dependent enzymatic reaction, as suggested by Vanag and Kuznetsov,[87] are restated below using the more familiar biochemical formalisms of enzyme kinetics.

Consider a unimolecular enzymatic reaction that converts substrate, S, to product, P:

$$S \rightleftharpoons P \tag{26}$$

Product formation can only occur from the ES complex, so the reaction mechanism can be expanded to include the enzyme catalyst (eq 27). The first step

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \tag{27}$$

is binding of E and S to form ES. Since P can only be formed from ES, $d[P]/dt = k_3[ES]$. Under initial velocity conditions, [P] = 0 and the reverse reaction, conversion of P to S, does not occur. In the conditions of a typical *in vitro* assay, [E] ≪ [S], and the steady-state assumption can be employed to describe [ES]: $d[ES]/dt \approx 0$.

With these assumptions, the kinetic rate expression that describes the rate of product formation takes the form of a hyperbola. This is the Michaelis−Menten equation that describes simple enzymatic catalysis (eq 28).

$$d[P]/dt = V_{max}[S]/(K_m + [S]) \tag{28}$$

Saturation kinetics are observed (Figure 7), such that when E exists only as ES, the maximum rate of catalysis is obtained. We define the maximum catalytic rate as V_{max}, where $V_{max} = k_3[E]_{total}$. The asymptote to the saturation curve is V_{max} and, on an intuitive level, this describes the behavior of the ES complex. No information as to the rate of ES formation is contained in V_{max}. At the other extreme of [S], the tangent to the initial slope of the hyperbola (extrapolated to infinitely low [S]), contains information about each step prior to, and including, the first irreversible step. This includes how tightly S is

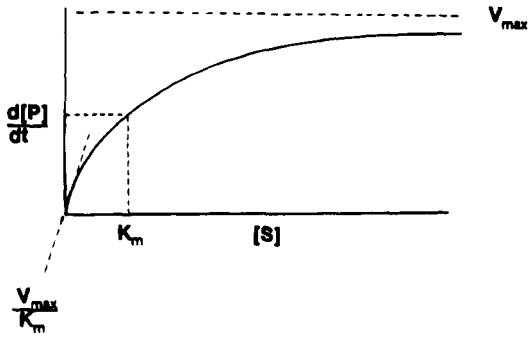


Figure 7. Saturation kinetics observed in enzyme-catalyzed reactions. The kinetic parameter V_{max} is the maximal observed rate when all enzyme exists as the ES complex. The kinetic parameter K_m is the substrate concentration that corresponds to half-maximal V_{max}. The tangent to the observed rate at infinitely low substrate concentration is V_{max}/K_m. This corresponds to the condition when [S] $\ll K_m$ (typically when [S] = $^1/_{10}K_m$ or less).

bound to ES and how fast ES is converted to product (if the conversion of ES to product is the first irreversible step). The tangent to the initial slope is defined as V_{max}/K_m, where $K_m = (k_2 + k_3)/k_1$. The parameter K_m is often called the Michaelis constant. It is *not* always a true dissociation constant, since it also contains k_3. Rather, it is the ratio of the fundamental parameters, V_{max} and V_{max}/K_m. The rate of E + S association is, to a first approximation, determined by the rate of diffusion in solution. Hence, the "tightness of binding" in the ES complex will be largely determined by k_2.

Consider the two extreme cases, where the rate of catalysis (k_3) is either much slower or much faster than the rate of substrate dissociation (k_2). When $k_2 \gg k_3$, $K_m \approx k_2/k_1$ and a true equilibrium of free S and bound S (as ES) is established. This leads to the full expression of any kinetic isotope effect on the chemical step, k_3. In contrast, if $k_2 \ll k_3$, any kinetic isotope effect on k_3 will be minimized by the propensity of all S to go forward through catalysis (k_3) rather than to dissociate (k_2).[88] Under this condition, S is called a "sticky substrate" because once it binds to E, the tendency is to go forward through catalysis rather than to dissociate.[89] The full isotopic discrimination (based on a kinetic isotope effect on k_3) for a sticky substrate will not be observed, as all substrate molecules, regardless of isotopic composition, will tend to go forward through catalysis.

Now, consider the case of a MFE on k_3 rather than a kinetic isotope effect. If the precatalytic step(s) prior to the magnetic field sensitive step are irreversible, all substrate molecules will go forward (given sufficient time for spin randomization). A MFE on catalysis will be masked if the magnetic field sensitive step is preceded by an irreversible step.

Vanag and Kuznetsov suggest that assay conditions in which [S] $\ll K_m$ will produce the largest dependence of observed rate on magnetic field.[87] In practice, rather than follow the rate of single assays at ultralow [S], the value of V_{max}/K_m will yield the same information but with greater accuracy and precision.

The importance of reversibility in the {ES} complex is obvious when eq 27 is expanded to eq 29 to show the ES complex as a radical pair. It is largely

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E^{\bullet}\,{}^{\bullet}S \xrightarrow{k_5} E + P \qquad (29)$$

through k_4 (recombination of {E$^{\bullet}$ $^{\bullet}$S} to {ES}) that the observed rate of the reaction will be magnetic field dependent. If $k_4 = 0$, then {E$^{\bullet}$ $^{\bullet}$S} can only react via k_5 and the chemical transformation step, k_5, may or may not have a requirement for spin correlation.

Generally, enzymes do not allow release of a reactive intermediate, so the {E$^{\bullet}$ $^{\bullet}$S} complex will not undergo diffusive separation. Also, the fidelity of product formation is maintained by high barriers to alternate reaction pathways so that only one product is formed.

If electron spin relaxation in the {E$^{\bullet}$ $^{\bullet}$S} radical pair is fast compared to ISC, the spin-correlated nature of the RP will be lost. Fast relaxation can be promoted by nearby atoms with large SOC (i.e., cys-SH, metal ion, halogen, etc.), or nearby paramagnetic centers (i.e., metal ions) that exert a large magnetic field at the molecular level. In these cases, rapid spin randomization that is independent of an applied external magnetic field will occur.

3. Requirement for Two Unpaired Electrons During Catalysis

Perhaps the most significant caveat to observing MFE in enzymatic reactions is the requirement of having two unpaired electrons (or two paramagnetic species, in the general case) present at the same time during the reaction. Many enzymes with radical intermediates must be activated by two-electron reduction prior to catalysis (there are many excellent reviews on the mechanism of enzymes with radical intermediates).[90,91] Subsequent homolysis of this two-electron reduced species or another group on the enzyme generates the active radical enzyme form that abstracts a hydrogen atom from substrate to initiate catalysis.

a. Ribonucleotide Reductase. As an example, consider the mechanism of ribonucleotide reductase from *Escherichia coli* (Figure 8). Following reductive activation, a radical exists at tyrosine-122 of the B_2 subunit. The stable Tyr-122 radical either abstracts a hydrogen atom from substrate directly,[90,92] or produces another transient radical species[93] that abstracts a hydrogen atom from substrate. S$^{\bullet}$ undergoes subsequent conversion to P$^{\bullet}$, and reverse hydrogen atom abstraction produces P and the regenerated tyrosyl radical (Figure 8). At no time during the reaction (after initial activation) does more than one radical species exist in the active site. The single radical species exists as a doublet, rather than either a singlet or triplet RP. This is tantamount to one round of free radical chain chemistry with an initiation and termination step. *Because only one radical species exists at a time, no MFE on turnover of ribonucleotide reductase is expected.* Although catalytic turnover involves only one radical species, the *activation* step may still involve a RP and exhibit magnetic field dependent recombination.

4. Requirements for MFE on Enzymatic Reactions

In light of the theoretical treatment of MFE on chemical and enzymatic reactions (*vide supra*) and

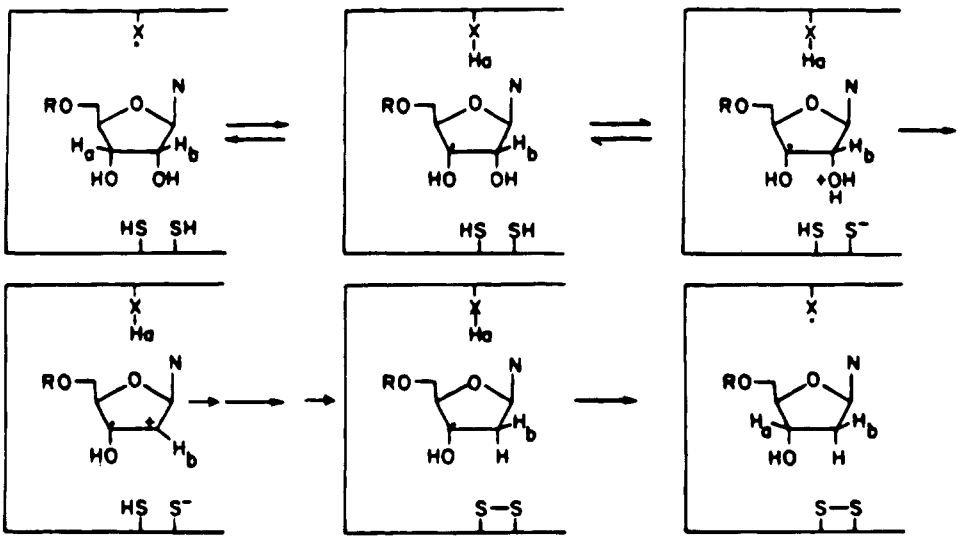


Figure 8. Mechanism of iron-dependent mammalian ribonucleotide reductase. Following the initial enzyme activation step (not shown), only one radical species is present in the enzyme active site. No radical pair exists during turnover of the enzyme. (Reprinted from ref 90. Copyright 1988 American Chemical Society.)

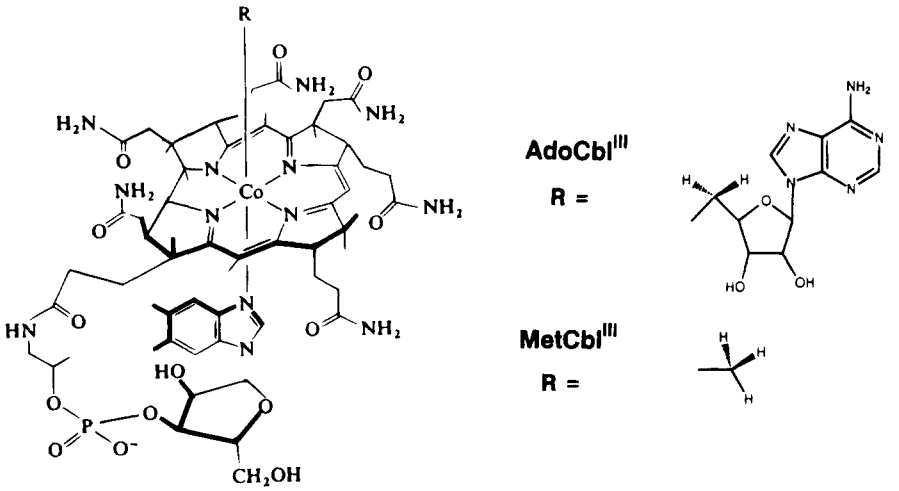


Figure 9. Structure of cob(III)alamin cofactor. (Reprinted from ref 103. Copyright 1993 American Chemical Society.)

the example of ribonucleotide reductase, a set of rules can be formulated that describes the situation that must exist in order to observe a MFE on an enzymatic reaction:

(1) There must be at least one step in the reaction that generates a *pair* of spin-correlated radicals or paramagnetic particles.

(2) The RP must be weakly coupled. A strongly coupled RP (with $\Delta E_{ST} \approx$ large) will not undergo ISC. Similarly, no spin-dependent chemistry is defined for a noncoupled RP ($\Delta E_{ST} = 0$ and r_{sep} = infinite).

(3) A physical mechanism must exist to promote magnetic field-dependent ISC. This may be HFI, Δg mechanism, level crossing, or similar.

(4) The observed rate of the enzymatic reaction must be sensitive to the fraction of ES complex in the active form. Furthermore, this active form must require spin correlation, or be directly convertible to a catalytically inactive complex via a reaction pathway that requires spin correlation (i.e. RP recombination).

(5) The radical pair E$^{\bullet}$ $^{\bullet}$S complex must exist long enough for ISC to compete with other modes of reaction. If the ES complex is too stable (i.e. long-lived), electron spin relaxation by interaction with the enzyme/solvent lattice will remove spin correlation.

(6) Binding steps and conformational changes that precede formation of the enzyme–substrate RP must be reversible (i.e. substrate is "non-sticky").

These stringent requirements suggest that many enzymes with radical intermediates will not satisfy all of the conditions necessary (especially 1 and 4) to produce magnetic field dependent reaction kinetics. An examination of Table 1 will reveal the limited observation of magnetic field dependent reaction kinetics in enzymatic reactions. In the author's laboratory, the unique spin-correlated RP chemistry of one coenzyme B_{12}-dependent enzymatic reaction has been explored, along with the magnetic field dependent photochemistry of the B_{12} cofactor. The next two sections describe these results.

O. Coenzyme B_{12} Photochemistry

In its various forms, vitamin B_{12} is a cofactor for over a dozen enzymatic reactions.[94,95] The common structural element is the macrocyclic corrin ring that holds Co^{3+} in a square-planar coordination geometry (Figure 9). The form found in nutrition supplements

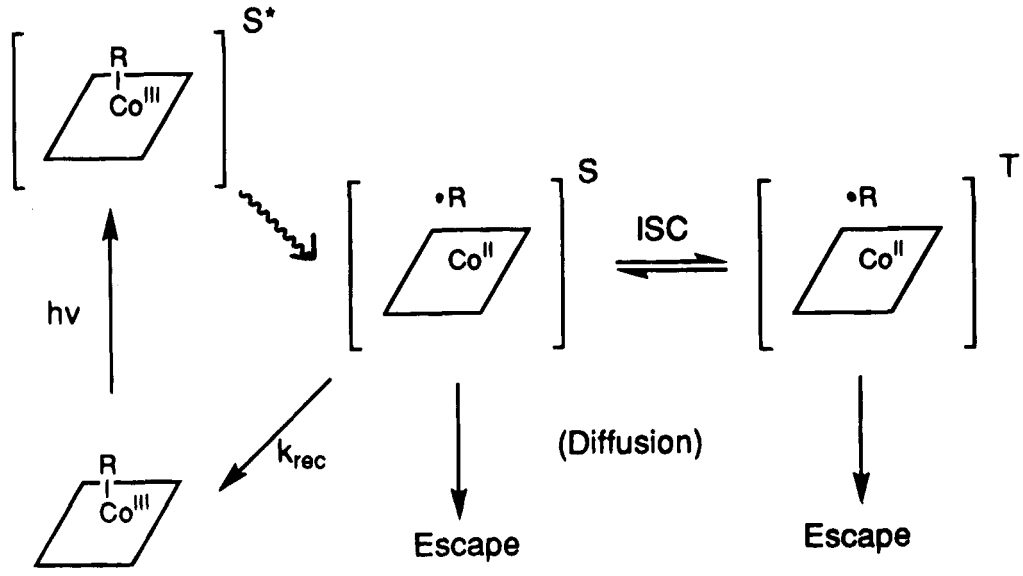


Figure 10. Proposed scheme for the photolysis of alkylcob(III)alamin to produce a spin-correlated radical pair. The singlet and triplet radical pairs can be interconverted by intersystem crossing. Both the singlet and triplet RP can escape the solvent cage. Only the singlet RP can recombine. Whether bond homolysis occurs from the excited singlet or triplet state has not been demonstrated unambiguously.

is vitamin B_{12}, cyanocob(III)alamin; the form that is a cofactor for about a dozen enzymes that catalyze 1,2-migrations is coenzyme B_{12}, adenosylcob(III)-alamin ($AdoCbl^{III}$); and the form that is a cofactor for methyl transferase reactions is methylcob(III)alamin ($MetCbl^{III}$).

1. The Carbon–Cobalt Bond

All biologically active forms of B_{12} have an unusually labile C–Co bond that has a bond dissociation energy as low as 31 kcal/mol (for $AdoCbl^{III}$).[96–98] Visible light below 610 nm will induce homolysis of the C–Co bond to produce a spin-correlated geminate RP consisting of the 5′-deoxyadenosyl radical and cob(II)alamin {$AdoCH_2^{\bullet}$ Cbl^{II}}.[99–103] The C–Co bond in $MetCbl^{III}$ is slightly stronger (37 kcal/mol),[104,105] but the analogous photoproducts are the same: {$CH_3^{\bullet}$ Cbl^{II}}.[99,106,107] Photolysis of the alkylcob(III)alamins and subsequent partitioning between cage recombination and escape is described in Figure 10.

2. Continuous-Wave Photolysis

As a probe of spin correlation in the photochemically produced RP, the continuous-wave quantum yield (ϕ_{CW}) for $AdoCbl^{III}$ and $MetCbl^{III}$ was examined as a function of magnetic field in solvents of varying viscosity (Figures 11 and 12).[103,108] Anaerobic photolysis was carried out by irradiation at 514 nm in the presence of 50 mM buffer and the indicated viscosigen.

In buffered water with a relative viscosity of 1, no magnetic field dependence is observed. In contrast, ϕ_{CW} decreases by up to 50% in the presence of 75% glycerol (a microviscosigen, $\eta/\eta_0 = 30$) and 20% ficoll-400 (a macroviscosigen, $\eta/\eta_0 = 30$). Both viscosigens make RP recombination magnetic field sensitive, but in different ways.

Microviscosigens such as glycerol increase the bulk viscosity (commonly measured with an Ostwald viscosimeter) and decrease the rate of small-molecule diffusion in parallel (Figure 13).[108] In contrast, macroviscosigens such as ficoll-400 (400000 kDa

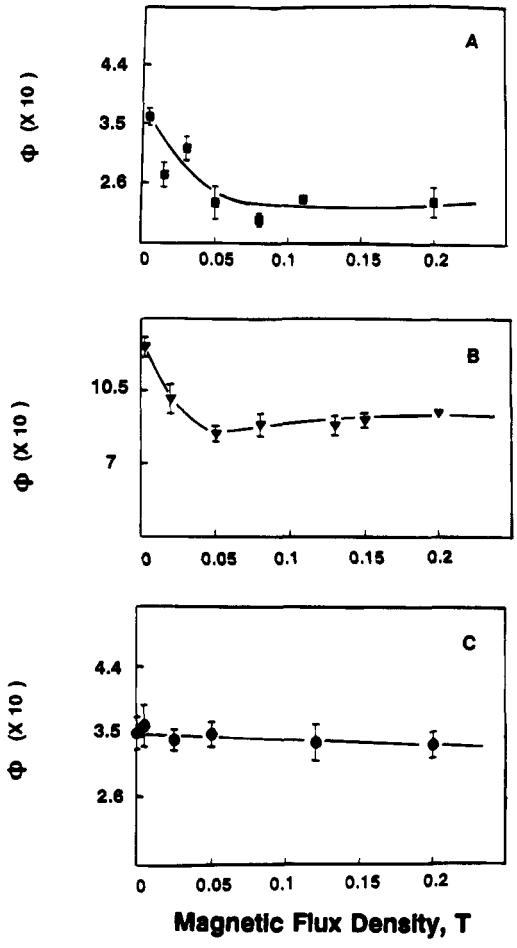


Figure 11. Magnetic field dependence of ϕ_{CW} for anaerobic $AdoCbl^{III}$ photolysis at 514 nm, 20 °C, 200 μM $AdoCbl^{III}$, 50 mM Hepes, pH 7.0 and (a) 75% glycerol ($\eta/\eta_0 = 30$); (b) 20% Ficoll-400 ($\eta/\eta_0 = 30$); (c) buffered H_2O ($\eta/\eta_0 = 1$). The curves represent best-fit empirical lines through the data. (Reprinted from ref 103. Copyright 1993 American Chemical Society.)

copolymer of sucrose and epichlorohydrin) increase the bulk viscosity, but do not significantly change the

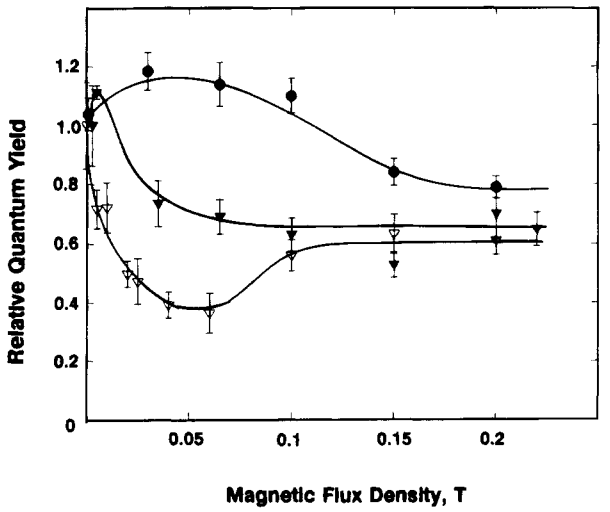


Figure 12. Magnetic field dependence of methylcob(III)-alamin anaerobic photolysis in (●) buffered water, (▽) 20% (w/v) Ficoll-400, and (▼) 75% glycerol. The rate of decomposition of methylcob(III)alamin was determined by monitoring the decrease in absorbance at 520 nm. The curves represent best-fit empirical lines through the data. (Reprinted from ref 108. Copyright 1993 Oldenbourg Verlag GmbH.)

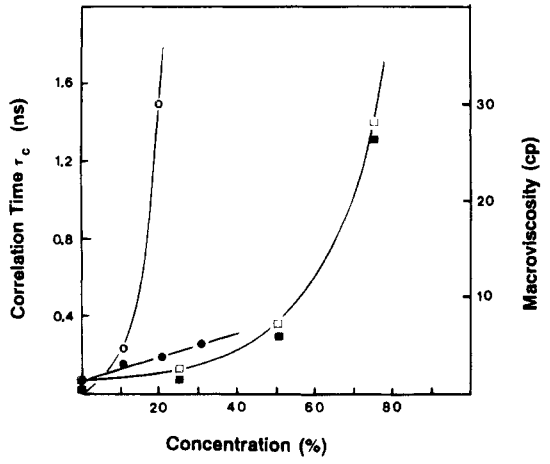


Figure 13. Relative macroviscosity and microviscosity of buffered water, Ficoll-400, and glycerol solutions at 25 °C. The absolute macroviscosity was measured with an ostwald viscosimeter and the relative microviscosity was determined as τ_c (in ns) for 4-(2-iodoacetamido)-TEMPO. Legend: (○) Ficoll-400 macroviscosity, (●) Ficoll-400 microviscosity, (■) glycerol macroviscosity, (□) glycerol microviscosity. (Reprinted from ref 108. Copyright 1993 Oldenbourg Verlag GmbH.)

rate of small-molecule diffusion (Figure 13). Ficoll-400 can still potentiate the magnetic field dependence of RP recombination, but probably through the formation of hydrogen-bonded cage structures produced by the interdigitation of linear polymer strands.[108,109]

3. Laser Flash Photolysis

The magnetic field dependence of $AdoCbl^{III}$ and $MetCbl^{III}$ photolysis was also probed by picosecond laser flash photolysis.[103] In these time regimes, the magnetic spin dependence of geminate primary recombination (10^{-10}–10^{-9} s) can be dissected away from recombination in the bulk solvent (10^{-8}–10^{-4} s). Figure 14 shows the time-dependent disappearance of Cbl^{II} following photodissociation of $AdoCbl^{III}$

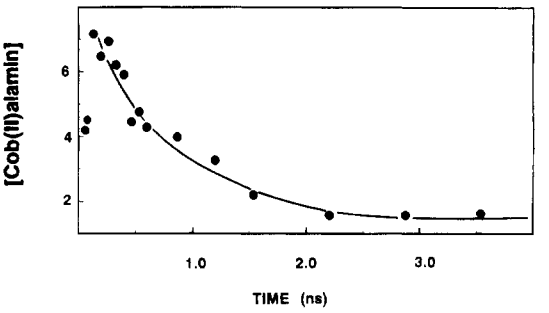


Figure 14. Adenosylcob(III)alamin photolysis. Kinetic trace of [cob(II)alamin] after the 30 ps 532 nm pulse as a function of time as determined by the integrated transient absorbance centered at 470 nm. Conditions are 20 °C, 200 M $AdoCbl^{III}$, 50 mM Hepes, pH 7.0, 75% glycerol. The line is the result of fitting the data to the first-order rate equation. (Reprinted from ref 103. Copyright 1993 American Chemical Society.)

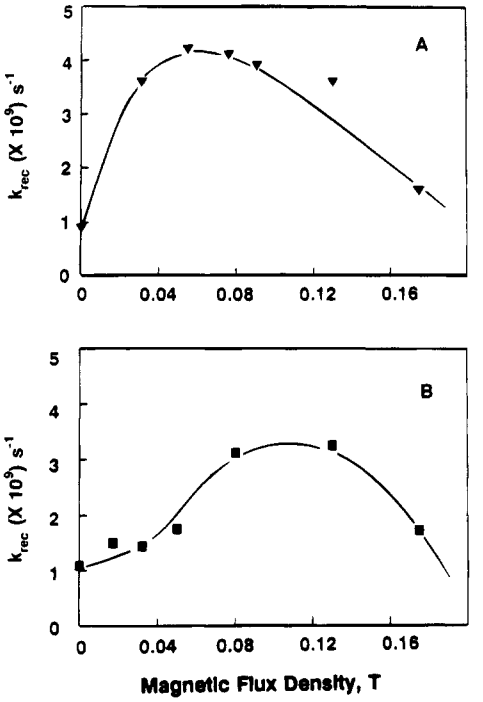


Figure 15. Magnetic field dependence of k_{rec} following photolysis of $AdoCbl^{III}$ at 532 nm, 20 °C, 200 μM $AdoCbl^{III}$, 50 mM Hepes, pH 7.0 and (A) buffered H_2O ($\eta/\eta_0 = 1$) or (B) 75% glycerol ($\eta/\eta_0 = 30$). The curves represent best-fit empirical lines through the data. (Reprinted from ref 103. Copyright 1993 American Chemical Society.)

by a 30 ps laser pulse.[103] The geminate RP {$AdoCH_2^{\bullet}$ Cbl^{II}} exhibits extraordinarily fast recombination with a first-order rate constant of $k_{rec} = 1.0 \times 10^9$ s. True geminate RP recombination is being monitored, since k_{rec} does not vary as the microviscosity increases from $\eta/\eta_0 = 1$ to $\eta/\eta_0 = 30$ (Figure 15). In buffered H_2O, the dependence of k_{rec} on magnetic field is biphasic, with a maximum 3-fold increase in k_{rec} in the range 60–120 mT (Figure 15). In 75% glycerol, a 4-fold increase in k_{rec} is observed at 50 mT. The initial radical pair is formed in the triplet spin state and ISC limits the rate of recombination. In remarkable agreement, if Δg for the RP is ≈ 0.25,[43] then $k_{ISC} \approx 2.2 \times 10^9$ s^{-1} (see section I.C) and $k_{rec} \approx k_{ISC}$. Clearly, the Δg mechanism for spin rephasing must be contributing to the observed magnetic field dependence!

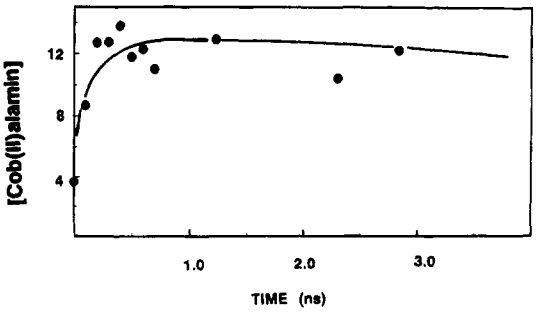


Figure 16. Methylcob(III)alamin photolysis. Kinetic trace of [cob(II)alamin] after the 30 ps 532 nm pulse as a function of time as determined by the integrated transient absorbance centered at 470 nm. Conditions are 20 °C, 200 μM MetCbl^{III}, 50 mM Hepes, pH 7.0, 75% glycerol. (Unpublished result, refs 110 and 111.)

Analogous picosecond laser flash photolysis experiments with MetCbl^{III} show that no recombination occurs in less than 3.5 ns (Figure 16).[110,111] However, the continuous-wave quantum yield for MetCbl^{III} in 20% Ficoll-400 and 75% glycerol exhibits a biphasic magnetic field dependence that is similar to the corresponding data for AdoCbl^{III}. This suggests two different magnetic field-dependent time regimes for RP recombination: (1) primary geminate recombination that occurs in less than 3.5 ns; and (2) secondary recombination that occurs in the bulk solvent at longer times. Because the continuous-wave quantum yield of neither AdoCbl^{III} nor MetCbl^{III} exhibits a significant magnetic field dependence in buffered H_2O of low viscosity, the RP recombination of {AdoCH$_2$$^\bullet$ $^\bullet$Cbl^{II}} observed in picosecond laser flash photolysis experiments must be fast enough such that a 4-fold increase in k_{rec} does not significantly affect the overall fraction of {AdoCH$_2$$^\bullet$ Cbl^{II}} in the primary cage that undergoes recombination. Therefore, the net MFE on ϕ_{CW} must be the result of RP recombination that occurs in bulk solvent.

From the magnetic field dependence of {AdoCH$_2$$^\bullet$ Cbl^{II}} recombination in the photolytically produced RP, it is clear the system can exhibit magnetic spin dependent chemistry. However, recombination from a photolytically produced RP is markedly different than recombination from a thermally produced singlet RP, as would be encountered in B_{12}-dependent enzymatic systems. In the following section, the opportunity to observe magnetic spin-dependent chemistry in B_{12}-dependent enzymes will be considered.

P. Magnetic Field Effects on B_{12}-Dependent Enzymes

All of the reactions that require AdoCbl^{III} involve a 1,2-migration as the key step in catalysis. Many of these 1,2-migrations are thought to occur via a transient radical intermediate that is produced by hydrogen atom abstraction by AdoCH$_2$$^\bullet$. By analogy to radical reactions in organic chemistry, AdoCbl^{III} serves a dual role as the initiator, and perhaps as propagator, of radical chain chemistry (Figure 17).

Because the C−Co bond in AdoCbl^{III} is very weak, very little force is required to homolyze the bond in the active site of an enzyme. The sum of multiple hydrogen bonds to the adenosine moiety and the

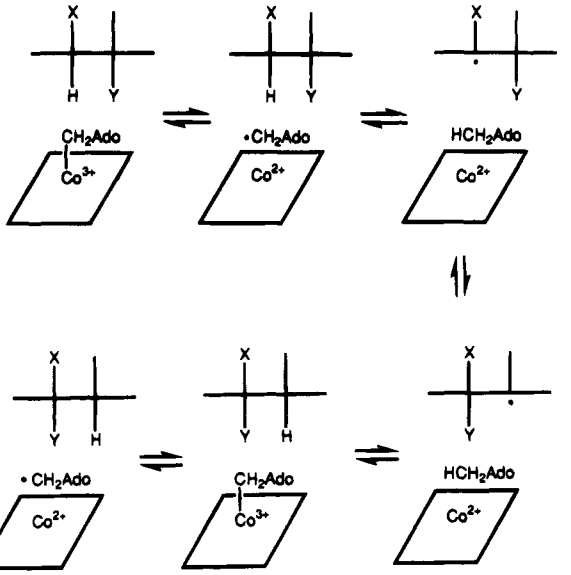


Figure 17. Generalized mechanism of 1,2-group migration in adenosylcob(III)alamin-dependent enzymes.

corrin/benzimidazole moiety could easily overcome a bond dissociation energy of 31 kcal/mol. In effect, the enzyme uses the excess free energy of multiple hydrogen bonding interactions to weaken the C−Co bond such that, on a time-averaged basis, a dynamic equilibrium is established between AdoCbl^{III} and ^{1}{AdoCH$_2$$^\bullet$ Cbl^{II}} (eqs 30 and 31). The RP will be

$$\text{AdoCbl}^{III} \rightarrow {}^1\{\text{AdoCH}_2^\bullet\ \text{Cbl}^{II}\} \quad (30)$$

$$^1\{\text{AdoCH}_2^\bullet\ \text{Cbl}^{II}\} \rightarrow \text{AdoCbl}^{III} \quad (31)$$

produced on the lowest energy (singlet) surface, by virtue of the paired electron interaction that existed in the covalent C−Co bond. Because the rate of an enzyme is proportional to the active form of the enzyme, a decrease in nonproductive recombination will produce an increase in the rate of catalysis.

1. B_{12} Ethanolamine Ammonia Lyase

One of the most intensely studied B_{12}-dependent enzymes is ethanolamine ammonia lyase. The enzyme catalyzes the conversion of ethanolamine to acetaldehyde and ammonia (eq 32) in bacteria (the best described sources are *Clostridium* and *Salmonella*).

$$CH_2OHCH_2NH_3^+ \rightarrow CH_3CHO + NH_4^+ \quad (32)$$

The catalytic mechanism is illustrated in Figure 18.[22,112,113] The catalytic cycle begins with binding of substrate to the enzyme−cofactor complex, followed by homolysis of the C−Co bond, to generate enzyme-bound ^{1}{AdoCH$_2$$^\bullet$ Cbl^{II}} RP in the singlet spin state. Nonproductive RP recombination of ^{1}{AdoCH$_2$$^\bullet$ Cbl^{II}} competes with forward catalytic throughput that occurs by 5′-deoxyadenosyl radical abstraction of a hydrogen atom from ethanolamine to generate the substrate radical. 1,2-Migration (rearrangement) occurs to form the product radical that is trapped by reverse H$^\bullet$ donation from 5′-deoxymethyladenosine. This hydrolytically unstable

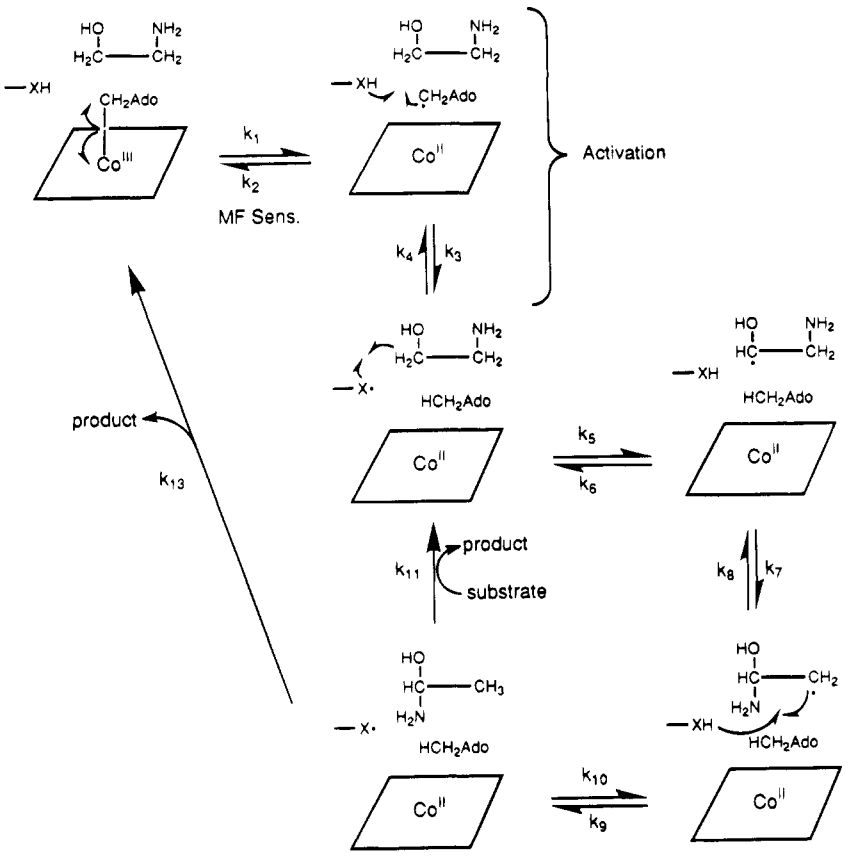


Figure 18. Reaction mechanism for ethanolamine ammonia lyase. Enzyme-induced homolysis of the C−Co bond produces the 5′-deoxyadenosyl radical and cob(II)alamin in the singlet spin state. The 5′-deoxyadenosyl radical abstracts H• from C-2 of ethanolamine to generate the initial substrate radical. The amine group migrates to form the carbinolamine radical that abstracts H• from 5′-methyladenosine 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 {$AdoCH_2$• Cbl^{II}} radical pair does not have to recombine between turnover (k_{11} includes product dissociation and substrate binding before {$AdoCH_2$• Cbl^{II}} recombination occurs. Under V_{max}/K_m conditions, recombination of the {$AdoCH_2$• Cbl^{II}} radical pair (k_9) is more likely. This would begin the catalytic cycle with the transient {$AdoCH_2$• Cbl^{II}} radical pair in the singlet spin state. (Reprinted from ref 22. Copyright 1994 American Association for the Advancement of Science.)

carbinolamine can be released into solution or decompose to the ultimate products of acetaldehyde and ammonia.

The magnetic field dependence of V_{max} and V_{max}/K_m for ethanolamine ammonia lyase is shown in Figure 19.[22] The kinetic parameter V_{max} is independent of applied magnetic field up to 250 mT, whereas V_{max}/K_m exhibits a decrease of 25% at 100 mT.[22]

Under conditions of saturating substrate (expressed by the kinetic parameter V_{max}), product dissociation is followed immediately by the binding of another substrate molecule that is poised for the next round of catalysis.[22] If step k_{13} is circumvented, k_{11} and k_5 should out compete k_2 and k_4. The net effect is no dependence of V_{max} on magnetic field.[22]

In contrast, under conditions of less-than-saturating substrate (expressed by the kinetic parameter V_{max}/K_m), product dissociation occurs via k_{13} and the "resting" state of the enzyme−cofactor complex is restored.[22] Subsequent turnover must start with homolysis of the C−Co bond to produce the spin-correlated {$AdoCH_2$• Cbl^{II}} RP in the singlet state. Only the singlet ^{1}{$AdoCH_2$• Cbl^{II}} RP can undergo nonproductive recombination via k_2. If HFI that normally populates the triplet RP is disfavored by the application of a magnetic field, then k_2 will increase and cause a net decrease of the forward flux through the first irreversible step. This will be expressed as a decrease in V_{max}/K_m.[22] At higher magnetic fields, the Δg mechanism becomes significant and increases ISC, thus producing the biphasic magnetic field dependence that is observed. Alternatively, a specific S_0-T_{-1}-level crossing might be responsible for the dip in V_{max}/K_m. Further experiments at higher magnetic flux density are required to decide which explanation is correct.

Deuteration of ethanolamine produces a larger 60% decrease in V_{max}/K_m at 150 mT.[22] Replacement of H with D introduces a primary isotope effect on k_5. A decrease in k_5 will increase the fraction of enzyme that exists as the E−S−{$AdoCH_2$• Cbl^{II}} complex prior to hydrogen atom abstraction from substrate. The recombination rate constant, k_2, will remain unchanged and the net flux via k_2[E−S−{$AdoCH_2$• Cbl^{II}}] will increase. Thus, a greater magnetic field effect on k_2 is expressed.[22]

The magnetic field dependence of enzyme-bound {$AdoCH_2$• Cbl^{II}} recombination has been verified by stopped-flow kinetic studies.[112,113] The apparent first-order rate constant for Cbl^{II} formation can be determined by monitoring Cbl^{II} formation on the enzyme. In this experiment, ethanolamine ammonia lyase is placed in one syringe and ethanolamine and $AdoCbl^{III}$ is placed in the other syringe. The solutions are

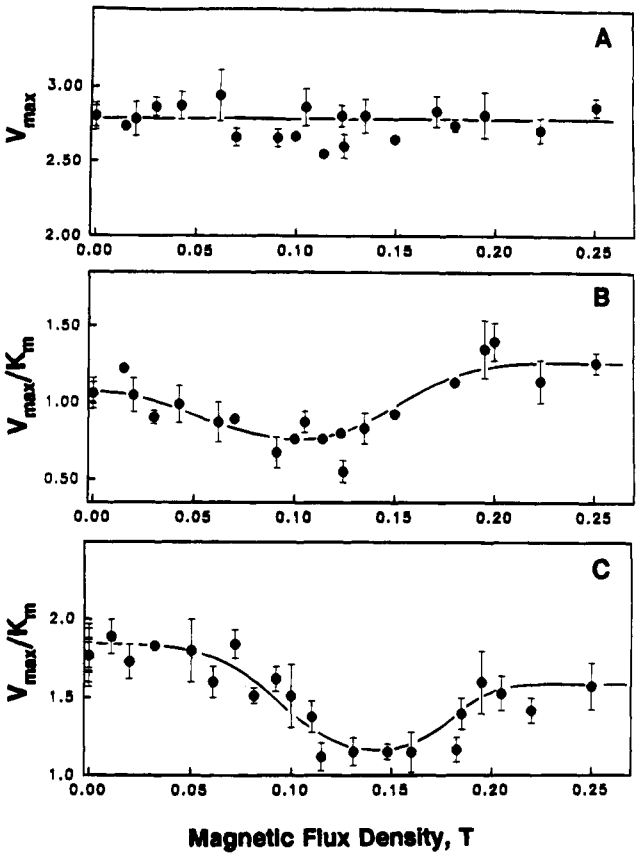


Figure 19. Ethanolamine ammonia lyase. Magnetic field dependence of (A) V_{max} with unlabeled ethanolamine, (B) V_{max}/K_m with unlabeled ethanolamine, and (C) V_{max}/K_m with 1,1,2,2-D_4-ethanolamine. Each assay contained 100 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethane sulfonic acid (Hepes) pH 7.48, 5 μM adenosylcob(III)alamin, and ethanolamine ammonia lyase at 25 °C. Each data point represents the kinetic parameter derived by fitting observed d[P]/dt vs [ethanolamine] data to d[P]/dt = V_{max}·[S]^n/K_m + [S]^n by nonlinear methods. The Hill number, n, varied only slightly between 0.75 and 0.85. In order to keep the measured rates with deuterated and unlabeled substrates similar, 8.59-fold more EAL enzyme 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 ± 0.2 and $^DV_{max}/K_m$ = 5.4 ± 0.4 at 0 T. (Reprinted from ref 22. Copyright 1994 American Association for the Advancement of Science.)

rapidly mixed and the rate of Cbl^{II} formation on the enzyme is monitored as a function of magnetic field. Figure 20 shows the rate of Cbl^{II} formation vs magnetic field.[112,113] This result unambiguously identifies {$AdoCH_2$· Cbl^{II}} recombination as the magnetic field sensitive step in ethanolamine ammonia lyase. No deuterium isotope effect on the rate of Cbl^{II} formation is observed and the magnetic field dependence of Cbl^{II} formation is independent of isotopic composition of ethanolamine.

Q. Biological and Health Relevance of B_{12} Magnetic Field Effects

Other B_{12} enzymes that catalyze a 1,2-migration via initial hydrogen atom abstraction might be expected to exhibit a similar magnetic field effect. Coenzyme B_{12}-dependent 1,2-migrations appear to be most important in bacterial metabolism, although mammals have an absolute requirement for B_{12} to

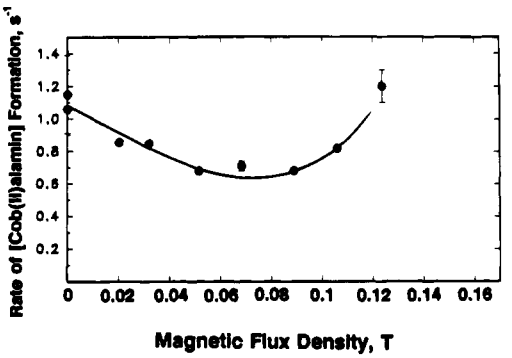


Figure 20. Ethanolamine ammonia lyase stopped-flow kinetic study. The magnetic field dependence of the first-order rate of appearance of Cbl^{II} with unlabeled ethanolamine is shown. Standard error bars may be smaller than the plotted symbol. Identical rates are observed with unlabeled and deuterated ethanolamine. (Reprinted from refs 112 and 113.)

thrive. Without sufficient B_{12}, humans develop pernicious anemia. The enzyme methylmalonyl-CoA mutase is noteworthy as an $AdoCbl^{III}$-dependent enzyme that is found in mammals.[114]

Methionine synthase is a $MetCbl^{III}$-dependent methyl transferase that is found in bacteria and mammals.[115] It is important in the conversion of homocysteine to methionine in the cycling of the potent methylating agent, S-adenosylmethionine (SAM). However, radical chemistry via Co(II) is *not* implicated in the mechanism of B_{12}-dependent methionine synthase.[116,117] Methyl transfer in methionine synthase appears to occur from the 2-electron reduced Co(I) form of methylcobalamin. In view of the absence of geminate recombination in the photochemically produced {CH_3· Cbl^{II}} RP (*vide supra*), methyl radical appears to be an unwieldy and uncontrollable molecule that is too dangerous to generate in a biological setting. It is safer to transfer a one carbon unit as the cation, rather than the radical. The evidence for the importance of Co(I)alamin *in vivo* is compelling.[116,117] Nitrous oxide, or laughing gas, is a potent inactivator of Co(I) processes *in vitro* and is known to deplete B_{12} stores in humans who are chronically exposed. Acute megaloblastic anemia and other diseases associated with B_{12} deficiency can be induced with nitrous oxide. Because enzymatic processes involving Co(II) and Co(III) are unaffected, at least some of the B_{12}-dependent processes that are important for human health will be insensitive to magnetic field on the basis of RP recombination considerations.

A caveat to minimizing the biological importance of magnetic field effects on B_{12} reactions in animals lies in the unusual photoreactivity of methylcob(III)alamin. Direct methylation of DNA by $MetCbl^{III}$ has been reported.[118] Considering the high photochemical quantum yield for $MetCbl^{III}$ photodissociation, this may be an adventitious process that is light dependent. Tissue becomes increasingly transparent to light above 600 nm (Figure 21)[119] and the possibility of photochemical production of CH_3· *in vivo* and the magnetic field-induced alteration of {CH_3· Cbl^{II}} recombination cannot be summarily discounted. The magnetic flux density that is currently known to alter B_{12}-dependent processes is far greater than the

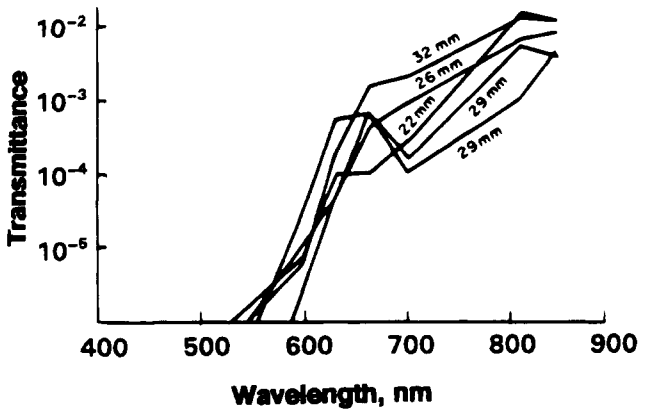


Figure 21. Spectral transmittance through human abdominal wall. Thickness = 22–32 mm. (Reprinted from ref 119. Copyright 1981 American Society for Photobiology.)

environmental magnetic flux densities that are of concern to human health.

R. Other Enzymes for Which RP Mechanisms Have Been Considered

Enzymes with radical mechanisms are numerous.[90] Of these, only a small subset can be considered radical pair mechanisms that may exhibit magnetic spin-dependent ISC. Besides enzymes that use $AdoCbl^{III}$ to initiate radical chemistry, the cytochrome P-450 family of enzymes is an obvious place to look for spin-correlated RP intermediates that can partition between nonproductive recombination and forward catalytic throughput.[120–122] In this reaction, an activated $(Fe-O)^{3+}$ species abstracts a hydrogen atom from substrate to produce the $\{(Fe-O)^{2+}:{}^{\bullet}R\}$ radical pair. RP recombination has been estimated to exceed 10^9 s^{-1}.[122] The symmetrical ligand environment of the heme may offer an additional advantage in minimizing the otherwise highly anisotropic electronic environment of the iron that would strongly couple to the RP and promote ISC through magnetically insensitive SOC. Lipoxygenase, an enzyme with a nonheme iron center for which a similar RP mechanism has been proposed, does not exhibit magnetic spin-dependent chemistry.[85]

III. Non-Radical-Pair Magnetic Field Effects in Biological Systems: What Is the Mechanism?

In this section, some of the more common reports of biological magnetic field effects that occur by mechanisms other than changes in RP recombination will be surveyed. It is in these research areas where theory and chemical intuition does not yet exist, that scientists at the interface between mathematics, physics, chemistry, biology, and the clinical sciences can have their greatest impact. A complete treatment of all proposed mechanisms of biological magnetic field effects is beyond the scope of this review and would require several volumes of this journal.

A. Magnetoreception

The evidence that some organisms—from bacteria to vertebrates—can sense and respond to the geomagnetic field, is undeniable (Table 2).[123–141] Birds, sea turtles, tuna, salmon, and whales can sense the geomagnetic field and use it for migratory navigation. Honeybees, hornets, and mollusks can use the geomagnetic field for systematic food acquisition, and magnetotactic bacteria and salamanders alter their swimming patterns in response to changes in the environmental magnetic field.

Table 2. Organisms with Magnetite

organism	behavior or proposed function	light dependent[a]	ref(s)
algae	directional		123
bacteria, *Spirillium*	flagellar propulsion		124, 125
bees, honey	food location		126–128
hornets	food location		129
humans	unknown		142, 143
mollusks, marine	systematic feeding		130
pigeons, homing	migration	yes	131–135
salamander	directional swimming	yes	137
salmon	migration		138
tuna	directional swimming		139
turtles	migration	yes	140
whales	migration		141

[a] The light dependence is unspecified if unknown.

Magnetoreception is the only biological magnetic field effect that is demonstrably of consequence to the organism. Furthermore, magnetoreception is generally thought of as being distinct from the magnetic field-induced changes in chemistry discussed previously. In magnetoreception, the gross magnitude of the field is always near 0.05 mT (depending on latitude) and the sign of the field, north vs south, is important.

1. Biogenic Magnetite

Biogenic magnetite (crystals of Fe_3O_4) may be responsible for magnetoreception. It has been isolated from most of the organisms listed in Table 2. In honeybees, magnetite is sequestered in iron granules that are about 0.6 μm in diameter.[128] Each iron granule is further composed of magnetite crystals, with the largest having a diameter of about 10 nm.[128] Electron microscopy has revealed innervation of the cells containing the iron granules, thereby suggesting these granules are the primary site for magnetic field signal transduction. One hypothesis envisions these magnetite particles as behaving like bar magnets.[130] In a side-by-side orientation, their poles will repel each other and the size of the composite iron granules will be enlarged. In an end-to-end alignment, their poles will be aligned and the iron granules will collapse to a more compact size. This change in size could be transmitted to the rudimentary nervous system. Alternatively, the magnetosomes (the general term for subcellular organelles containing particles of magnetite) may be placed in a rigid cellular matrix (cytoskeleton) with their magnetic moments aligned. The sum of the torque exerted on the individual microsomes by an external magnetic field is sufficient to rotate the cell and impart a signal to the organism. Either mechanism of magnetoreception and signal transduction is sufficient to explain the behavioral response of honeybees and many other magnetoreceptive organisms.

a. Migratory Navigation. In some organisms, most notably, birds, sea turtles, and salamanders, there is an obligate role for long-wavelength (red) light in setting the organism's biological magnetic compass.[134,137,140] When loggerhead sea turtles hatch, the direction of light they first see "sets" their magnetoreceptive device to allow them to navigate successfully to feeding grounds.[140] When the initial light comes from the east (geomagnetic and geographical east), the turtles swim toward the east, even in the absence of continued irradiation. When the initial light comes from the west (geomagnetic and geographical west), the turtles always begin swimming toward the west.

i. Light-Dependent Magnetoreception. A combination of photoreception and magnetoreception has long been proposed for migratory navigation in birds.[144] Recently, a proposal not involving magnetite, but rather optical pumping of a retinal pigment, has been put forth to account for magnetoreception in the Australian silvereye.[134-137] In this hypothesis, the excited state of the pigment is the ultimate magnetoreceptor.[144] Only wavelengths below 616 nm are effective in allowing magnetoreception.[134] A candidate for the avian magnetoreceptor is the retinal pigment rhodopsin.[144]

2. Magnetotactic Bacteria

Spirillium is a magnetotactic bacterium that also contains magnetite.[124,125] The direction and intensity of flagellar motion changes in response to the application of a magnetic field. The reason for bacterial magnetotaxis is unclear, but it may be analogous to bacterial chemotaxis that is aimed at food acquisition. In this case, trace metal ions may be the desired substance.

3. Thermal Noise Constraints on Magnetoreception

A magnetosome that is held loosely enough to sense and transmit changes in the geomagnetic field vector will also experience the small random effects of Brownian motion.[145] The net torque experienced by the magnetosome complex must be in excess of the thermal noise limit, generally expressed as kT, where k is Boltzmann's constant and T is the absolute temperature. In single magnetosomes, the magnetic moment may be as large as $25kT$, but the magnetic moment in an average magnetosome is probably on the order of kT.[145] In large assemblies with aligned magnetic moments, the net magnetic moment may be as high as $5000kT$. Such a system would have no difficulty responding to the geomagnetic field vector of about 0.05 mT magnitude.[145] Coherent sensing by an array of magnetosomes, or even better, an array of cells containing magnetosomes, further increases the signal-to-noise ratio, just as multiple sampling of a weak signal is used in laboratory measurements. The signal-to-noise ratio of a signal that is sampled by M detectors will increase by the square root of M.

Because much of the concern of biological MFE is related to electric powerline and electrical device exposure, it is useful to consider the effect of a small alternating field in the presence of the geomagnetic field.[145,146] To transmit the time-dependent information from an alternating magnetic field, a magnetite-based magnetic field sensor must be free to rotate. This precludes a large fixed-array from sensing an alternating magnetic field as an intact entity.[145,146] Thus, we must consider the effect of an alternating magnetic field on individual magnetosomes with magnetic moments not much larger than kT. At this level of organization, the effect of a 60 Hz alternating magnetic field of 0.005 mT RMS, in the presence of the static geomagnetic field of 0.05 mT, will be inconsequential.[145,146] Thus, small 60 Hz powerline magnetic fields probably have little effect on magnetite-based magnetoreception that is designed to sense the direction of static magnetic fields near 0.05 mT.[145,146] The underlying assumptions of absolutely free rotation of the microsome and the consequential lack of coherence is not canonical. Without these assumptions, the same type of calculation allows for the sensing of a magnetic field as low as 0.001 mT by biogenic magnetite.[147] Adjudication of these different conclusions is beyond the scope of this review.

B. Melatonin

There is a large body of evidence that serum levels of the hormone melatonin can be decreased by exposure to slowly pulsed static magnetic fields.[148-155] In what appears to be the rate-limiting step of melatonin biosynthesis, *N*-acetylserotonin is converted to melatonin by acetylserotonin methyltransferase (EC 2.1.1.4) in the pituitary. Melatonin controls circadian rhythms, sleep, mood, and physical and mental performance. Beneficial oncostatic properties have been ascribed to melatonin, such that decreased serum melatonin levels are considered undesirable and may be oncopotentiative.[148] In animals and humans, serum melatonin levels increase during darkness (especially during sleep).[150] Considerable evidence indicates this nocturnal increase in melatonin can be suppressed by exposure to weak magnetic fields.[148,152,154,155] Exposure of the same subjects to light during the same nocturnal hours had the same effect as exposure to pulsed magnetic fields.[148,154] Remarkably, rats that were acutely blinded were insensitive to magnetic fields.[154] This observation indicates the magnetic field-induced decrease in melatonin levels may require light and suggests that retinal magnetosensitivity may be involved.[154] Although many research groups have observed the magnetosensitivity of melatonin levels, other research groups have failed to observe a magnetic field-induced decrease in melatonin.[149,151] At MRI relevant magnetic fields near 1.5 mT (with the application of gradient pulses for imaging), no perturbation in the normal nocturnal increase in melatonin was observed in humans.[149] This negative result may be caused by the unusually high magnetic field, or it may be caused by exposure of the subjects in the dark. There is evidence that induced electric currents may be more important than the magnetic field component in inducing changes in pineal gland metabolism.[153]

C. Ca^{2+} Binding and Ion Cyclotron (Parametric) Resonance

1. Ion Cyclotron Resonance

One of the more controversial theories to account for MFE at the cellular level involves the specific

interaction of an oscillating magnetic field and a static magnetic field at a frequency that corresponds to a specific energy level of a metal ion cofactor. In 1985, Liboff applied the equations of ion cyclotron resonance to the binding of Ca^{2+} by biological molecules.[156,157] Ion cyclotron resonance theory describes the frequency-specific absorption of electromagnetic energy by ions in a weak magnetic field and the path circumscribed by these ions. This theory was attractive because it predicted a "window effect" by which only certain frequencies (near the 50−60 Hz powerline frequency) would lead to the resonant absorption of energy by an unhydrated metal ion such as Ca^{2+}.[158−160] At these frequencies, the resonant path of oscillation was greater than 1 m, and this lead to a proposed "dampening" of the oscillation by solvent molecules or active site binding. Reports of experimental data that support and refute the concept of ion cyclotron resonance effects on ion binding and transport have appeared.[161−164]

a. Ion Parametric Resonance. The ion cyclotron resonance model was refined by Lednev within the broader and more general concept of ion parametric resonance.[165,166] According to the theory of ion parametric resonance, many physical parameters that describe the interaction of an ion with its environment will change, including the binding of an ion to a macromolecular ligand. Errors in the original application of ion parametric resonance to biological systems have been corrected.[167−169] Recent data on changes in neurite outgrowth from PC-12 (nervous system) cells supports the window effects and resonance conditions predicted by ion parametric resonance theory.[170] The percent of neurite outgrowth (NO) following stimulation of PC-12 cells with nerve growth factor was determined under 45 Hz resonance conditions for the following ions: Ca^{2+}, Fe^{3+}, Mn^{4+}, V^{4+}, Mg^{2+}, Li^{+}, and H^{+}. Up to a 70% decrease in neurite outgrowth was observed as the magnitude of the ac field was changed from 0.005 mT (RMS) to 0.02 mT (RMS).[170] Under nonresonance conditions, no change in neurite outgrowth was observed.[170] These data support specific predictions of the ion parametric resonance model. As with any measurement involving the visual quantification of cell morphology, these results await duplication and verification in other laboratories.

Ion parametric resonance offers a mechanism that specifically predicts frequency window effects near 50−60 Hz. As with any global theory that tries to explain disparate observations, more experimental results are needed to evaluate its relevance to biological magnetic field effects.

D. Miscellaneous Magnetic Field Effects

The focus of this review has been biological magnetic field effects for which plausible chemical mechanisms have been put forth. In addition to the studies summarized herein, there are numerous phenomenological observations of magnetic field effects for which a satisfactory mechanistic explanation remains elusive. Noteworthy among these are reports of a magnetic field-induced change in cell surfaces,[171] alteration of bacterial growth rates,[172] enhanced biocide activity against biofilm-sequestered bacteria,[173] induction of cellular transcription,[174] increase in c-*myc* transcription,[175] change in transcription of other genes,[176−178] increase in ornithine decarboxylase gene expression,[179−181] and similarity to heat shock-induced changes in protein distribution.[182] The reader is directed to the primary literature cited above (*vide infra*) for lead references.

E. Electric Field Effects

In this review, the effect of the electric field vector that accompanies an alternating magnetic field has been ignored. Because the cell membrane is an efficient dielectric that is only a few micrometers thick, even a modest potential of 1 V, dropped over a distance of 1 μm, is amplified to a voltage drop (electric field strength) of 1×10^6 V/m! There are demonstrated biological effects of oscillating electric fields that appear to depend upon voltage amplification across the cell membrane. Among these, is electroconformational coupling of the dipole moment of membrane proteins and enzymes to the alternating electric field vector.[183−188] Electroconformational coupling has been demonstrated for the transport of ions by the membrane-spanning (Na,K)-ATPase.[189−191]

IV. Acknowledgments

A portion of this work was supported by a grant from the National Institute of Environmental Health Sciences (ES 05728) to C.B.G. The author thanks the generous efforts of reviewers in the physical, chemical, and biological sciences.

V. References

(1) 1. Barnothy, M. F., Ed. *Biological Effects of Magnetic Fields*; Plenum Press: New York, 1969; Vol. 2.
(2) Barnothy, M. F., Ed. *Biological Effects of Magnetic Fields*; Plenum Press: New York, 1964.
(3) Frey, A. H. *FASEB J.* **1993**, *7*, 272.
(4) Bennett, W. R. *Phys. Today* **1994**, April, 23.
(5) Werthheimer, N.; Leeper, E. *Am. J. Epidemiol.* **1979**, *109*, 273.
(6) Silverman, C. In *Biological Effects and Medical Applications of Electromagnetic Energy*; Gandhi, O. P., Ed.; Prentice Hall: Englewood Cliffs, NJ, 1990; p 414.
(7) Blank, M., Ed. *Electricity and Magnetism in Biology and Medicine*; San Francisco Press: San Francisco, 1993.
(8) Gandhi, O. P., Ed. *Biological Effects and Medical Applications of Electromagnetic Energy*; Prentice Hall: Englewood Cliffs, NJ, 1990.
(9) Lin, J. C., Ed. *Electromagnetic Interaction with Biological Systems*; Plenum Press: New York, 1989.
(10) Carstensen, E. L. *Biological Effects of Transmission Line Fields*; Elsevier: New York, 1987.
(11) Blank, M., Findl, E., Eds. *Mechanistic Approaches to Interactions of Electric and Electromagnetic Fields with Living Systems*; Plenum Press: New York, 1987.
(12) Polk, C.; Postow, E. *CRC Handbook of Biological Effects of Electromagnetic Fields*; CRC Press: Boca Raton, FL, 1986.
(13) Dutta, S. K.; Millis, R. M., Eds. *Biological Effects of Electropollution; Brain Tumors and Experimental Models*; Information Ventures, Inc: Philadelphia, 1986.
(14) Tenford, T. S., Ed. *Magnetic Field Effects on Biological Systems*; Plenum Press: New York, 1978.
(15) U.S. Congress, Office of Technology Assessment. *Biological Effects of Power Frequency Electric and Magnetic Fields—Background Paper*, OTA-BP-E-53; U.S. Government Printing Office: Washington, DC, 1989.
(16) Canfield, J. M.; Belford, R. L.; Debrunner, P. G; Schulten, K. *J. Chem. Phys.* **1994**, *182*, 1.
(17) Canfield, J. M.; Belford, R. L.; Debrunner, P. G; Schulten, K. *Abstracts Book*, Third International Converence on Magnetic Spin Effects in Chemistry and Related Phenomena, Chicago, IL, Sept 25−30, 1994.
(18) Canfield, J. M.; Belford, R. L.; Debrunner, P. G; Schulten, K. Submitted for publication.
(19) Blankenship, R. E.; Schaafsma, T. J.; Parson, W. W. *Biochem. Biophys. Acta* **1977**, *461*, 297.

(20) Hoff, A. J.; Rademaker, H.; Van Grondelle, R.; Duysens, L. N. M. *Biochem. Biophys. Acta* **1977**, *460*, 547.
(21) Parson, W. W. Conference on the Primary Electron Transport and Energy Transduction in Photosynthetic Bacteria, 1977; Abstract MA 1, Brussels.
(22) Harkins, T. T.; Grissom, C. B. *Science* **1994**, *263*, 958.
(23) Steiner, U. E.; Ulrich, T. *Chem. Rev.* **1989**, *89*, 51.
(24) Chen, C. P.; Heinsohn, R. J.; Mulay, L. N. *J. Phys. Soc. Jpn.* **1968**, *25*, 319.
(25) Hayashi, H. In *Photochemistry and Photophysics*; Rabek, J. F., Ed.; CRC Press: Boca Raton, 1990; Vol. I, Chapter 2.
(26) Steiner, U. E.; Wolff, H.-J. In *Photochemistry and Photophysics*; Rabek, J. F., Ed.; CRC Press: Boca Raton, 1991; Vol. IV, Chapter 1.
(27) Salikhov, K. M.; Molin, Y. N.; Sagdeev, R. A.; Buchachenko, A. A. L. *Spin Polarization and Magnetic Effects in Radical Reactions*; Elsevier: Amsterdam, 1984.
(28) Schulten, K.; Staerk, H.; Weller, A.; Werner, H.-J.; Nickel, B. *Z. Phys. Chem. Neue Folge* **1976**, *101*, 371.
(29) McLauchlan, K. A.; Steiner, U. E. *Mol. Phys.* **1991**, *73*, 241.
(30) Werner, H.-J.; Staerk, H.; Weller, A. *J. Chem. Phys.* **1978**, *68*, 2419.
(31) Turro, N. J. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 609.
(32) Khudyakov, I. V.; Serebrennikov, Y. A.; Turro, N. J. *Chem. Rev.* **1993**, *93*, 537.
(33) Sagdeev, R. Z.; Salikhov, K. M.; Molin, Y. M. *Russ. Chem. Rev. (Usp. Khim.)* **1977**, *46*, 569.
(34) Salikhov, K. M. *Chem. Phys.* **1983**, *82*, 163.
(35) Buchachenko, A. L. *Russ. Chem. Rev. (Usp. Khim.)* **1976**, *45*, 375.
(36) Turro, N. J.; Kraeutler, B. In *Diradicals*; Borden, W. T., Ed.; Wiley: New York, 1982; p 259.
(37) Turro, N. J.; Kraeutler, B. *Acc. Chem. Res.* **1980**, *13*, 369.
(38) Buchachenko, A. L. *Russ. Chem. Rev.* **1993**, *62*, 1073.
(39) Leffler, J. E. *An Introduction to Free Radicals*; Wiley: New York, 1993.
(40) Turro, N. J.; Kraeutler, B. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: Amsterdam, 1984; Vol. 6, Chapter 3.
(41) Buchachenko, A. L. *Russ. J. Phys. Chem.* **1977**, *51*, 1445.
(42) Turro, N. J.; Kraeutler, B. *J. Am. Chem. Soc.* **1978**, *100*, 7432.
(43) Schepler, K. L.; Dunham, W. R.; Sands, R. H.; Fee, J. A.; Abeles, R. H. *Biochim. Biophys. Acta* **1975**, *397*, 510.
(44) Fessenden, R. W.; Schuler, R. H. *J. Chem. Phys.* **1963**, *39*, 2147.
(45) Norman, R. O. C.; Pritchett, R. J. *Chem. Ind.* **1965**, 2040.
(46) Richards, W. G.; Trivedi, H. P.; Cooper, D. L. *Spin Orbit Coupling in Atoms*; Clarendon Press: Oxford, 1981; p 17.
(47) Turro, N. J. *Modern Molecular Photochemistry*; University Science Books: Mill Valley, CA, 1991; p 191.
(48) Koziar, J. C.; Cowan, D. O. *Acc. Chem. Res.* **1978**, *11*, 334.
(49) Levin, P. P.; Kuzmin, V. A. *Chem. Phys.* **1992**, *162*, 79.
(50) Pines, A.; Sterna, L. Lawrence Berkeley Laboratory, Annual Report, 1977.
(51) Luby-Phelps, K.; Mujumdar, S.; Mumumbar, R. B.; Ernst, L. A.; Galbraith, W.; Waggoner, A. S. *Biophys. J.* **1993**, *65*, 236.
(52) Zimmt, M. B.; Doubleday, C.; Turro, N. J. *J. Am. Chem. Soc.* **1985**, *107*, 6726.
(53) Cozens, F. L.; Scaiano, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 5204.
(54) Margulis, L. A.; Khudyakov, I. V.; Kuzmin, V. A. *Chem. Phys. Lett.* **1986**, *124*, 483.
(55) Buchachenko, A. L.; Fedorov, A. V.; Yasina, L. L.; Galimov, E. M. *Chem. Phys. Lett.* **1984**, *103*, 405.
(56) Huyser, E. S. *Free Radical Chain Reactions*; Wiley: New York, 1970; p 54.
(57) Scaiano, J. C.; Mohtat, N.; Cozens, F. L.; McLean, J.; Thansandote, A. *Bioelectromagnetics* **1994**, *15*, 549.
(58) Scaiano, J. C.; Cozens, F. L.; McLean, J. *Photochem. Photobiol.* **1994**, *59*, 585.
(59) McLauchlan, K. *Phys. World* **1992**, January, 41.
(60) Hamilton, C. A.; Hewitt, J. P.; McLauchlan, K. A.; Steiner, U. E. *Mol. Phys.* **1988**, *65* 423.
(61) Grundler, W.; Kaiser, F.; Keilmann, R.; Walleczek, J. *Naturwissenschaften* **1992**, *79*, 551.
(62) Diesenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michl, H. *Nature* **1986**, *318*, 618.
(63) van den Brink, J. S.; Manikowski, H.; Gast, P.; Hoff, A. J. *Biochem. Biophys. Acta* **1994**, *1185*, 177.
(64) Hoff, A. J. *Photochem. Photobiol.* **1986**, *43* 727.
(65) Hoff, A. J.; Redemarker, H.; van Grondelle, R.; Duysens, L. N. M. *Biochem. Biophys. Acta* **1977**, *460*, 547.
(66) Blankenship, R. E.; Schaafsma, T. J.; Parson, W. W. *Biochem. Biophys. Acta* **1977**, *461*, 297.
(67) Werner, J.-J.; Schulten, K.; Weller, A. *Biochem. Biophys. Acta* **1978**, *502*, 255.
(68) Boxer, S. G.; Chidsey, C. E. D.; Roelfs, M. G. *J. Am. Chem. Soc.* **1982**, *104*, 1452.
(69) Boxer, S. G.; Chidsey, C. E. D.; Roelfs, M. G. *J. Am. Chem. Soc.* **1982**, *104*, 2674.
(70) Chidsey, C. E. D.; Roelfs, M. G.; Boxer, S. G. *Chem. Phys. Lett.* **1980**, *74*, 113.
(71) Sagdeev, R. V.; Molin, Y. N.; Salikhov, K. M.; Leshina, T. V.; Kamha, M. A.; Shein, S. M. *Org. Magn. Reson.* **1973**, *5*, 603.
(72) Tanimoto, Y.; Nishino, M.; Itoh, M. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 3365.
(73) Perito, R. P.; Corden, B. B. *J. Am. Chem. Soc.* **1987**, *109*, 4418.
(74) Perito, R. P.; Corden, B. B. *J. Am. Chem. Soc.* **1988**, *110*, 3472.
(75) Vanag, V. K.; Kuznetsov, A. N. *Sov. J. Chem. Phys.* **1986**, *3*, 2498.
(76) Maling, J. E.; Weissbluth, M.; Jacobs, E. E. *Biophys. J.* **1965**, *5*, 767.
(77) Vanag, V. K.; Kuznetsov, A. N. *Izv. Akad. Nauk. SSSR Ser. Biol.* **1988**, *2*, 215.
(78) Rabinovitch, B.; Maling, J. E.; Weissbluth, M. *Biophys. J.* **1967**, *7*, 187.
(79) Müller, K; Haberditzl, W.; Pritze, B. *Z. Phys. Chem.* **1971**, *278*, 185.
(80) Haberditzl, W. *Nature* **1967**, *213*, 5071.
(81) Vainer, L. M.; Podoplelov, A. V.; Leshina, T. V.; Sagdeev, R. Z.; Molin, Y. N. *Biofizika* **1978**, *23*, 234.
(82) Petushkov, V. N. *Biofizika* **1985**, *30*, 349.
(83) Ghole, V. S.; Damle, P. S.; Thiemann, W. H.-P. *Z. Naturforsch.* **1986**, *41c*, 355.
(84) Hummel, K.; Martl, K.; Martl, M. G. *J. Mol. Catal.* **1989**, *54*, L1.
(85) Hwang, C.-C.; Grissom, C. B. *J. Am. Chem. Soc.* **1994**, *116*, 795.
(86) Harkins, T. T.; Grissom, C. B. *Science* **1994**, *263*, 958.
(87) Vanag, K. K.; Kuznetsov, A. N. *Biofizika* **1984**, *29*, 23.
(88) Cook, P. F. *Enzyme Mechanism from Isotope Effects*; CRC Press: Boca Raton, FL, 1991.
(89) Cleland, W. W. *CRC Crit. Rev. Biochem* **1982**, *13*, 385.
(90) Stubbe, J. *Biochemistry* **1988**, *27*, 3893.
(91) Stubbe, J. *Annu. Rev. Biochem.* **1989**, *58*, 257.
(92) Stubbe, J. *J. Biol. Chem.* **1990**, *265*, 5329.
(93) Nordlund, P.; Sjöberg, B. M.; Eklund, H. *Nature* **1990**, *345*, 593.
(94) Dolphin, D. *B_{12}*; Wiley: New York, 1986; Vols. 1–2.
(95) Schneider, Z.; Stroiński, A. *Comprehensive B_{12}*; de Gruyter: Berlin, 1987.
(96) Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469.
(97) Koenig, T. W.; Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1499.
(98) Halpern, J.; Kim, S.-K.; Leung, T. W. *J. Am. Chem. Soc.* **1984**, *106*, 8317.
(99) Endicott, J. F.; Netzel, T. L. *J. Am. Chem. Soc.* **1979**, *101*, 4000.
(100) Krautler, B.; Stepanek, R. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 62.
(101) Chen, E.; Chance, M. R. *J. Biol. Chem.* **1990**, *265*, 12987.
(102) Chen, E.; Chance, M. R. *Biochemistry* **1993**, *32*, 1480.
(103) Chagovetz, A. M.; Grissom, C. B. *J. Am. Chem. Soc.* **1993**, *115*, 12152.
(104) Martin, B. D.; Finke, R. G. *J. Am. Chem. Soc.* **1990**, *112*, 2419.
(105) Martin, B. D.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 585.
(106) Endicott, J. F.; Ferraudi, G. J. *J. Am. Chem. Soc.* **1977**, *99*, 243.
(107) Pratt, J. M.; Whitear, B. R. D. *J. Chem. Soc.* **1970**, 252.
(108) Grissom, C. B.; Chagovetz, A. M. *Z. Phys. Chem.* **1993**, *182S*, 181.
(109) Lavrenko, P. N.; Mokryukova, O. I.; Didenko, S. A. *Vysokomol. Soedin.* **1986**, *28A*, 517.
(110) Lott, W. B.; Chagovetz, A. M.; Grissom, C. B. Presented at the American Chemical Society Fall National Meeting, Washington, DC, Aug 23, 1994.
(111) Lott, W. B.; Chagovetz, A. M.; Grissom, C. B. *J. Am. Chem. Soc.*, in press.
(112) Harkins, T. T.; Grissom, C. B. Presented at the American Chemical Society Fall National Meeting, Washington, DC, Aug 23, 1994.
(113) Harkins, T. T.; Grissom, C. B. *J. Am. Chem. Soc.* **1995**, *117*, 566–567.
(114) Cannata, J. J. B.; Focesi, A.; Mazumda, R.; Warner, R. C.; Ochoa, S. *J. Biol. Chem.* **1965**, *240*, 3249.
(115) Banergee, R. V.; Matthews, R. G. *FASEB J.* **1990**, *4* 1450.
(116) Drummond, J. T.; Matthews, R. G. *Biochemistry* **1994**, *33*, 3732.
(117) Drummond, J. T.; Matthews, R. G. *Biochemistry* **1994**, *33*, 3742.
(118) Pfohl-Leszkowicz, A.; Keith, G.; Dirheimer, G. *Biochemistry* **1991**, *31*, 8045.
(119) Wan, S.; Parrish, R.; Anderson, R.; Madden, M. *Photochem. Photobiol.* **1981**, *34*, 679.
(120) Groves, J. T.; McClusky, G. A.; White, R. E.; Coon, M. J. *Biochem. Biophys. Res. Commun.* **1978**, *81* 154.
(121) Guengerich, F. P.; McDonald, T. L. *Acc. Chem. Res.* **1984**, *17* 9.
(122) Ortiz de Montellano, P. R.; Stearns, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3415.
(123) Torres de Araujo, F. F.; Pires, M. A.; Frankel, R. B.; Bicudo, C. E. M. *Biophys. J.* **1985**, *50*, 375.
(124) Blakemore, R. *Science* **1975**, *190*, 377.
(125) Frankel, R. B.; Blakemore, R. P.; Wolfe, R. S. *Science* **1979**, *203*, 1355.
(126) Martin, H.; Lindauer, M. *J. Comp. Physiol. A.* **1977**, *122*, 145.
(127) Kirschvink, J. L.; Kobayashi Kirschvink, A. *Am. Zool.* **1991**, *31*, 169.
(128) Li, C.-W.; Hsu, C.-Y. *Science* **1994**, *265*, 95.
(129) Kisliuk, M.; Ishay, J. *Experientia* **1977**, *33*, 885.

(130) Lohmann, K. J.; Willows, A. O. D. *Science* **1987**, *235*, 331.
(131) Walcott, C.; Green, R. P. *Science* **1974**, *184*, 180.
(132) Walcott, C.; Gould, J. L.; Kirschvink, J. L. *Science* **1979**, *205*, 1027.
(133) Leask, M. J. M. *Nature* **1977**, *267*, 144.
(134) Wiltschko, W.; Munro, U.; Ford, H.; Wiltschko, R. *Nature* **1993**, *364*, 525.
(135) Nowak, R. J. *J. NIH Res.* **1993**, *5*, 55.
(136) Phillips, J. B.; Noore, F. R. *Behav. Ecol. Sociobiol.* **1992**, *31* 89.
(137) Phillips, J. B.; Borland, S. C. *Nature* **1992**, *359*, 142−144.
(138) Quinn, T. T.; Brannon, E. L. *J. Comp. Physiol. A.* **1982**, *147*, 547.
(139) Walker, M. M. *J. Comp. Physiol. A.* **1984**, *155*, 673.
(140) Lohmann, K. J.; Fittinghoff Lohmann, C. M. *J. Exp. Biol.* **1994**, *190*, 1.
(141) Kirschvink, J. L.; Dixon, A. E.; Westphal, J. A. *J. Exp. Biol.* **1986**, *120*, 1.
(142) Kirschvink, J. L.; Kirschvink, A. K.; Woodford, B. *Ann. Intl. Conf. IEEE Eng. Med. Biol. Soc. Abstr.* **1990**, *12*, 1089.
(143) Kirschvink, J. L.; Kobayashi-Kirschvink, A.; Woodford, B. J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7683.
(144) Leask, M. J. M. *Nature* **1977**, *267*, 144.
(145) Adair, R. K. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2925.
(146) Adair, R. *Bioelectromagnetics* **1993**, *14*, 1.
(147) Polk, C. *Bioelectromagnetics* **1994**, *15*, 261.
(148) Reiter, R. J.; Richardson, B. A. *FASEB J.* **1992**, *6*, 2283.
(149) Schiffman, J. S.; Lasch, H. M.; Rollag, M. D.; Flanders, A. E.; Brainard, G. C.; Burk, D. L. *J. Magn. Reson. Imag.* **1994**, *4*, 7.
(150) Sandyak, R.; Tsagas, N.; Anninos, P. A.; Derpapas, K. *Int. J. Neuroscience* **1992**, *65*, 61−8.
(151) Lee, J. M.; Stormshak, F.; Thompson, J. M.; Thinesen, P.; Painter, L. J. *Biol. Reprod.* **1993**, *49*, 857.
(152) Liburdy, R. P.; Sloma, T. R.; Sokolic, R.; Yaswen, P. *J. Pineal Res.* **1993**, *14*, 89.
(153) Lerchl, A.; Nonaka, K. O.; Reiter, R. J. *J. Pineal Res.* **1991**, *10*, 109.
(154) Olcese, J.; Reuss, S.; Vollrath, L. *Brain Res.* **1985**, *333*, 382.
(155) Liburdy, R. P.; Sokolic, R.; Yaswen, P. In *Electricity and Magnetism in Biology and Medicine*; Blank, M., Ed.; San Francisco Press: San Francisco, 1993; p 398.
(156) Liboff A. R. In *Interactions Between Electromagnetic Fields and Cells*; Chiabrera, A., Nicolini, C., Schwan, H. P., Eds.; Plenum Press, New York, 1985; p 281.
(157) McLeod, B. R.; Liboff, A. R. In *Mechanistic Approaches to Interactions of Electric and Electromagnetic Fields with Living Systems*; Blank, M., Findl, E., Eds.; Plenum Press, New York, 1987; p 97.
(158) Durney, C. H.; Rushforth, C. K.; Anderson, A. A. *Bioelectromagnetics* **1988**, *9*, 315.
(159) Halle, B. *Bioelectromagnetics* **1988**, *9*, 381.
(160) Sandweiss, J. *Bioelectromagnetics* **1990**, *11*, 203.
(161) Liboff, A. R.; Parkinson, W. C. *Bioelectromagnetics* **1991**, *12*, 77.
(162) Blackman, C. F.; Benane, S. G.; Kinney, L. S.; House, D. E.; Joines, W. T. *Radiat. Res.* **1982**, *92*, 510.
(163) Durney, C. H.; Kaminski, M.; Anderson, A. A.; Bruckner-Lea, C.; Janata, J.; Rappaport, C. *Bioelectromagnetics* **1992**, *13*, 19.
(164) Bruckner-Lea, C.; Durney, C. H.; Janata, J.; Rappaport, C.; Kaminski, M. *Bioelectromagnetics* **1992**, *13*, 147.
(165) Lednev, V. V. *Bioelectromagnetics* **1991**, *12*, 71.
(166) Podgoretskii, M. I. *Sov. Phys.—Usp. (Engl. Transl.)* **1964**, *6*, 682.
(167) Lednev, V. V. Personal communication.
(168) Lednev, V. V. In *Electricity and Magnetism in Biology and Medicine*; Blank, M., Ed.; San Francisco Press: San Francisco, 1993; p 550.
(169) Blanchard, J. P.; Blackman, C. F. *Bioelectromagnetics* **1994**, *15*, 217.
(170) Blackman, C. F.; Blanchard, J. P.; Benane, S. G.; House, D. E. *Bioelectromagnetics* **1994**, *15*, 239.
(171) Goodman, E. M.; Sharpe, P. T.; Greenebaum, B.; Marron, M. T. *FEBS* **1986**, *199*, 275.
(172) Moore, R. L. *Can. J. Microbiol.* **1979**, *25*, 1145.
(173) Benson, D. E.; Grissom, C. B.; Burns, G. L.; Mohammad, S. F. *J. Am. Soc. Artificial Internal Organs* **1994** *40*, M371.
(174) Goodman, R.; Bassett, C. A.; Henderson, A. S. *Science* **1983**, *220*, 128.
(175) Lin, H.; Goodman, R.; Shirley-Henderson, A. *J. Cell. Biochem.* **1994**, *54*, 281.
(176) Phillips, J. L.; Haggren, W.; Thomas, W. J.; Ishida-Jones, T.; Adey, W. R. *Biochem. Biophys. Acta* **1992**, *1132*, 140.
(177) Phillips, J. L.; Haggren, W.; Thomas, W. J.; Ishida-Jones, T.; Adey, W. R. In *Electricity and Magnetism in Biology and Medicine*; Blank, M., Ed.; San Francisco Press: San Francisco, 1993; p 497.
(178) Goodman, R.; Henderson, A. S. *Bioelectrochem. Bioenerg.* **1991**, *25*, 335.
(179) Byus, C. V.; Pieper, S. E.; Adey, W. R. *Carcinogenesis* **1987**, *8*, 1385.
(180) Byus, C. V.; Kartun, K.; Pieper, S. E.; Adey, W. R. *Cancer Res.* **1988**, *48*, 4222.
(181) Mattsson, M.-O.; Rehnholm, U.; Mild, K. H. In *Electricity and Magnetism in Biology and Medicine*; Blank, M., Ed.; San Francisco Press: San Francisco, 1993; p 500.
(182) Blank, M.; Khorkova, O.; Goodman, R. In *Electricity and Magnetism in Biology and Medicine*; Blank, M., Ed.; San Francisco Press: San Francisco, 1993; p 528.
(183) Westerhoff, H. V.; Tsong, T. Y.; Chock, P. B.; Chen, Y.-D.; Astumian, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4734.
(184) Astumian, R. D.; Robertson, B. *J. Chem. Phys.* **1989**, *91*, 4891.
(185) Robertson, B.; Astumian, R. D. *Biophys. J.* **1990**, *58*, 969.
(186) Markin, V. S.; Tsong, T. Y.; Astumian, R. D.; Robertson, B. *J. Chem. Phys.* **1990**, *93*, 5062.
(187) Weaver, J. C.; Astumian, R. D. *Science* **1990**, *247*, 459.
(188) Robertson, B. R.; Astumian, R. D. *Biochemistry* **1992**, *31*, 138.
(189) Serpersu, E. H.; Tsong, T. Y. *J. Biol. Chem.* **1984**, *259*, 7155.
(190) Blank, M.; Soo, L. *Biochem. Bioenerg.* **1990**, *24*, 51.
(191) Blank, M.; Soo, L. *Biochem. Bioenerg.* **1989**, *22*, 313.

CR940164W