Model for Magnetic Field Effects on Radical Pair Recombination in Enzyme Kinetics

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ABSTRACT A prototypical model for describing magnetic field effects on the reaction kinetics of enzymes that exhibit radical pair recombination steps in their reaction cycle is presented. The model is an extended Michaelis-Menten reaction scheme including an intermediate enzyme-substrate complex where a spin-correlated radical pair state exists. The simple structure of the scheme makes it possible to calculate the enzyme reaction rate explicitly by combining chemical kinetics with magnetic field-dependent spin kinetics (radical pair mechanism). Recombination probability is determined by using the exponential model. Simulations show that the size of the magnetic field effect depends on relations between different rate constants, such as 1) the ratio between radical pair-lifetime and the rate of magnetic field-sensitive intersystem crossing induced by the hyperfine interaction and the Δg mechanisms and 2) the chemical rate constants of the enzyme reaction cycle. An amplification factor that is derived from the specific relations between the rate constants is defined. It accounts for the fact that although the magnetic field-induced change in radical pair recombination probability is very small, the effect on the enzyme reaction rate is considerably larger, for example, by a factor of 1 to 100. Model simulations enable a qualitative comparison with recent experimental studies reporting magnetic field effects on coenzyme B_{12}-dependent ethanolamine ammonia lyase in vitro activity that revealed a reduction in V_{max}/K_M at low flux densities and a return to the zero-field rate or an increase at high flux densities.

INTRODUCTION

In recent experimental studies Harkins and Grissom (1994) reported magnetic field effects on coenzyme B_{12}-dependent ethanolamine ammonia lyase in vitro activity. Application of magnetic fields around 100 mT magnetic flux density was shown to reduce the V_{max}/K_M kinetic parameter of the enzyme by 25% (K_M, Michaelis-Menten constant). Subsequent investigations revealed that the magnetic field-dependent step is recombination of a transient spin-correlated radical pair that is formed in the reaction cycle of the enzyme ({5′-deoxyadenosyl, cob(II)alamin} radical pair produced by enzyme-induced homolysis of the C-Co bond; Harkins and Grissom, 1995). This is the first study of a magnetic field effect on enzyme activity where radical intermediates are known and an interaction mechanism can be formulated (for a review, see Grissom, 1995). Many enzyme reactions involve free radical-dependent chemistry (Stubbe, 1988, 1989; White, 1991). Therefore, the work of Grissom et al., in combination with other studies on isolated reaction centers (Walleczek, 1995), should inspire future experimental investigations in this research area.

A well-documented example of a magnetic field effect on a biological process is known from photosynthetic reaction centers where photoexcitation leads to the generation of a cation-anion radical pair (for a review see, for example, Hoff, 1981; Boxer et al., 1983). Spin evolution of this radical pair is magnetic field-dependent. In particular, the yield of triplet products is found to be decreased at magnetic flux densities in the range 10–50 mT (Blankenship et al., 1977; Hoff et al., 1977), and to be increased again above 200 mT (Chidsey et al., 1980; Boxer et al., 1982). From the beginning, interpretation of these experimental findings was supported by theoretical modeling (Werner et al., 1978; Haberkorn and Michel-Beyerle, 1979). Relatively simple models—for example, hyperfine interactions were treated within a one-proton model (one representative spin 1/2 nucleus; Haberkorn and Michel-Beyerle, 1979) and spin-lattice relaxation processes were neglected (Werner et al., 1978)—made possible a qualitative comparison with experimental results. These models verified that the observed magnetic field dependence can be explained by the radical pair mechanism (Hoff, 1981; Boxer et al., 1983). At low magnetic flux densities the triplet yield is decreased because of reduced hyperfine interaction-induced intersystem crossing between the singlet and triplet states of the radical pair (separation of $T_{\pm 1}$ states from S,T_0 states due to Zeeman interaction). At high magnetic flux densities the triplet yield is increased again because of the action of the Δg mechanism. (For reviews on the radical pair mechanism see, for example, Gould et al. (1984), Steiner and Ulrich (1989), and McLauchlan and Steiner (1991).)

Although there is a solid theoretical basis explaining magnetic field effects on photosynthetic reaction centers, so far, mathematical analysis of magnetic field influences on enzyme reactions is very limited. Vanag and Kuznetsov (1984) discussed a model in which an enzyme-substrate complex is formed by two reacting paramagnetic species,

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each carrying spin 1/2 at the moment of contact. The enzyme reaction rate was calculated, depending on the spin multiplicity of the transient radical pair. In addition, magnetic field influences on the basis of the relaxation and Δg mechanisms were investigated.

The model by Vanag and Kuznetsov is a first approach to describing magnetic field effects in enzyme reactions; however, application of the model seems to be limited for several reasons. First, hyperfine induced intersystem crossing between singlet and triplet states is not considered. Furthermore, a system of randomly colliding paramagnetic species where both the enzyme and the substrate already have free radical properties (spin 1/2) at contact is not suitable for enzyme processes where the radical pair is generated during the enzyme reaction cycle. The latter situation is encountered, for example, in the reaction mechanism of ethanolamine ammonia lyase (Harkins and Grissom, 1994, 1995). The reaction cycle of cytochrome P-450 enzymes has also been reported to involve the generation of a transient biradical state ($\{(Fe-O)^{2+},{}^{\bullet}R\}$, a radical pair formed by hydrogen abstraction from substrate; Ortiz de Montellano and Stearns, 1987; Atkinson and Ingold, 1993). The previous discussion shows that a theoretical basis accounting for such processes is needed.

In this theoretical study a prototypical model is discussed involving enzyme reactions where a transient spin-correlated radical pair is generated during the enzyme reaction cycle (Eichwald and Walleczek, 1996a). The model is based on an expansion of the well-known Michaelis-Menten mechanism of enzyme kinetics. The expansion consists of an inclusion of intermediate enzyme-substrate complexes where a transient biradical state exists. Recombination probability of the radical pair in the presence of the magnetic field is calculated on the basis of the exponential model (Salikhov et al., 1984; Steiner and Ulrich, 1989). Similar to the early modeling of magnetic field effects in photosynthetic reaction centers, hyperfine interactions are included by taking into account only a spin 1/2 magnetic nucleus, and spin relaxation processes are neglected. Combining the resulting expressions with those for the chemical kinetics makes it possible to obtain an explicit expression for the magnetic field-dependent enzyme reaction rate.

The results show that the occurrence of significant magnetic field effects depends on specific relations between the chemical rate constants. The effect size of the magnetic field is determined by the ratio between radical pair lifetime and the rate of magnetic field-sensitive intersystem crossing induced by the hyperfine interaction and the Δg mechanisms. The model reproduces experimental findings with ethanolamine ammonia lyase in a qualitative manner.

OUTLINE OF THE MODEL AND BASIC CALCULATIONS

The simplest reaction scheme including an intermediate biradical state is an expansion of the well-known Michaelis-Menten mechanism:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (ES) \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (E^{\bullet\bullet}S) \overset{k_3'}{\rightarrow} E + P, \tag{1}$$

where the forward (backward) rate constants are denoted as $k_i(k_{-i})$, and E, (ES), $(E^{\bullet\bullet}S)$, S, and P stand for enzyme, enzyme-substrate complex, enzyme-substrate complex where a spin-correlated radical pair exists, substrate, and product. Radical pair generation is via k_2, and recombination via k_{-2}. Following Michaelis-Menten kinetics, the reaction rate is

$$v = V_{\max}\frac{[S]}{[S] + K_M}, \tag{2}$$

where $[S]$ denotes substrate concentration, and the kinetic parameters are defined as

$$V_{\max} = \frac{k_3'}{1 + K_2}[E_{\text{total}}], \quad K_M = \frac{(k_3'/k_2)K_1 + K_{n1}K_{n2}}{1 + K_2}, \tag{3}$$

where $K_1 = (k_{-1} + k_2)/k_1$, $K_2 = (k_{-2} + k_3')/k_2$, $K_{ni} = k_{-i}/k_i$ $(i = 1, 2)$, and $[E_{\text{total}}]$ is the total enzyme concentration.

In the above scheme (Eqs. 1–3), magnetic field sensitivity results in an alteration of the radical pair recombination rate via k_{-2}. A second possibility would exist, if product formation via k_3' were to exhibit a requirement for spin correlation, that is, it occurs at different rates depending on the spin multiplicity of the intermediate radical pair. We will not explore this possibility further, but restrict ourselves to the case where magnetic field influences are on the radical pair recombination step via k_{-2}. This situation is encountered, for example, in the reaction mechanism of ethanolamine ammonia lyase where, after substrate binding, enzyme-induced homolysis of the C-Co bond produces the {5′-deoxyadenosyl, cob(II)alamin} radical pair in the singlet state (Harkins and Grissom, 1994, 1995).

To implement magnetic field influences into the model the scheme (Eq. 1) must be expanded by taking into account the spin multiplicity of the radical pair. The following reaction scheme is investigated:

$$\begin{array}{l} E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (ES) \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} {}^{S}(E^{\bullet\bullet}S) \overset{k_3}{\searrow} \\ \qquad\qquad\qquad\qquad \Downarrow\uparrow k_{\text{isc}} \quad (ES)^* \overset{k_4}{\rightarrow} E + P, \\ \qquad\qquad\qquad\qquad {}^{T}(E^{\bullet\bullet}S) \overset{k_3}{\nearrow} \end{array} \tag{4}$$

where the superscript denotes spin multiplicity (S, singlet; T, triplet), and k_{isc} is the magnetic field-dependent rate constant for intersystem crossing between the singlet and triplet states. In the above scheme a short-hand notation, ${}^{T}(E^{\bullet\bullet}S)$, representing all three triplet states $T \rightarrow \{T_{-1}, T_0, T_{+1}\}$, is used (the index refers to the magnetic quantum number). The rate constant k_3 characterizes forward reaction coordinate motion where recombination is not

possible any more (subsequent reencounters of the radical pair are not considered; the exponential model is used—in this case $1/k_3$ is the mean frequency of interradical distance changes; Salikhov et al., 1984). After separation the enzyme-substrate complex is in the $(ES)^*$ configuration. The rate constant k_4 includes all subsequent processes in the enzyme reaction cycle that precede product release. To enable explicit relations in the following calculations the above scheme does not include reverse reactions after radical pair generation via k_2 (that is, reversible product release or back-transition between the $(ES)^*$ configuration and the biradical state).

The magnetic field-dependent reaction rate is

$$v^F = V^F_{max} \frac{[S]}{[S] + K^F_M}, \tag{5}$$

where

$$V^F_{max} = \frac{k_4}{1 + (k_4/k_2)\{1 + (k_2 + k_{-2}/f(k_{isc}))/k_3\}} [E_{total}]$$

$$K^F_M = \frac{(k_4/k_2)\{K_1 + K_{n1}(k_{-2}/f(k_{isc}))/k_3\}}{1 + (k_4/k_2)\{1 + (k_2 + k_{-2}/f(k_{isc}))/k_3\}}, \tag{6}$$

where all symbols retain their previous meaning, and the function $f(k_{isc})$ is defined as

$$f(k_{isc}) = \frac{2k_{isc} + k_3}{k_{isc} + k_3}. \tag{7}$$

Equation 6 reveals that magnetic field influence results in an alteration of the effective rate constant of radical pair recombination, $k_{-2} \rightarrow k_{-2}/f(k_{isc})$, where $f(k_{isc})$ is a monotonically increasing function of k_{isc} in the interval $1 < f(k_{isc}) < 2$.

To obtain an explicit relation for k_{isc}, the reaction rate is expressed in terms of the probability for radical pair recombination:

$$v^F = k_2[(ES)](1 - {}^S P_g), \tag{8}$$

where $[(ES)]$ is the concentration of the intermediate enzyme-substrate complex before radical pair generation. The rate of radical pair generation is $k_2[(ES)]$, and $(1 - {}^S P_g)$ is the probability that the radical pair does not recombine (the superscript S refers to the singlet state; recombination is only allowed from that state).

The ansatz, Eq. 8, makes possible the calculation of ${}^S P_g$ by using well-established models known from the theory of radical pair recombination. Here we make use of the exponential model (Salikhov et al., 1984; Steiner and Ulrich, 1989). In this approach the radicals separate after a certain period of time and subsequent reencounters are neglected. This scenario describes the present situation most appropriately, because in general the radicals will not undergo diffusive motion on the intermediate enzyme-substrate complex.

Under steady-state conditions one can show that

$${}^S P_g = \frac{k_{-2}/f(k_{isc})}{k_{-2}/f(k_{isc}) + k_3}. \tag{9}$$

Combining this relation with expressions for ${}^S P_g$ based on the exponential model in the case of a singlet-radical pair precursor with a spin 1/2 magnetic nucleus (see Appendix) makes it possible to calculate the rate constant k_{isc} explicitly. In zero field limit one obtains

$$k_{isc}(0) = 3\left(\frac{k_{-2} + 2k_3}{(k_{-2}/2 + k_3)^2 + (2J + a/2)^2}\right)\left(\frac{a}{4}\right)^2, \tag{10}$$

whereas in the high field limit ($B \gg A_{hfi}$ where B is magnetic flux density and A_{hfi} is the hyperfine interaction constant):

$$k_{isc}(B) = \left(\frac{k_{-2} + 2k_3}{(k_{-2}/2 + k_3)^2 + (2J)^2}\right) \cdot \left(\frac{(\Delta\omega)^2 + (a/4)^2 + \gamma((\Delta\omega)^2 - (a/4)^2)^2}{1 + \gamma((\Delta\omega)^2 + (a/4)^2)}\right)$$

$$\gamma = \frac{1}{k_3(k_{-2} + k_3)}\left(\frac{(k_{-2} + 2k_3)^2}{(k_{-2}/2 + k_3)^2 + (2J)^2}\right), \tag{11}$$

where $2J$ corresponds to the energy splitting induced by the exchange interaction, a is an effective hyperfine interaction constant, and $\Delta\omega = (\mu_B/\hbar)B\Delta g/2$ refers to the Δg mechanism.

The factor 3 in Eq. 10 implies that at zero field three channels for singlet-triplet interconversion are operating ($S \leftrightarrow \{T_{-1}, T_0, T_{+1}\}$). The hyperfine interaction mechanism induces intersystem crossing between these states. In the high field limit only the $S \leftrightarrow T_0$ channel remains, because due to the Zeeman interaction the $T_{\pm 1}$ states are energetically separated from the S, T_0 states. If the g values of the radicals are different, spin rephasing via the Δg mechanism leads to intersystem crossing between the S, T_0 states ($S \leftrightarrow T_0$).

RESULTS AND DISCUSSION

The reaction rate (Eqs. 5 and 6) incorporates relations between rate constants that refer to very different time scales. This is a result of combining processes in the scheme (Eq. 4) related to chemical kinetics and to magnetic field-dependent spin kinetics. The rate constants k_{-2} (radical pair recombination), k_3 (forward reaction coordinate motion), and k_{isc} (singlet-triplet intersystem crossing) represent fast time scales in the nanosecond time domain. The other rate constants are related to the much slower enzyme processes within the enzyme reaction cycle.

Numerical values of k_{-2}, k_3, and k_{isc} can be estimated from experimental studies. Radical pair recombination typically occurs at rates of $k_{rec} = 0.1\text{–}1.0 \cdot 10^9\ s^{-1}$. For example, regen-

eration of the C-Co bond in the reaction mechanism of ethanolamine ammonia lyase ({5′-deoxyadenosyl, cob(II)alamin} radical pair recombination) proceeds at a rate of $k_{rec} = 1 \cdot 10^9$ s^{-1} (Chagovetz and Grissom, 1993). The recombination rate of the $\{(Fe\text{-}O)^{2+},{}^{\bullet}R\}$ radical pair generated in the reaction cycle of cytochrome P-450 enzymes has been estimated to exceed 10^9 s^{-1} (Ortiz de Montellano and Stearns, 1987; Atkinson and Ingold, 1993). Initial spin correlation becomes lost at rates of 10^6–10^8 s^{-1} because of electron spin relaxation processes (Salikhov et al., 1984; Steiner and Ulrich, 1989; McLauchlan and Steiner, 1991). Intersystem crossing induced by the hyperfine interaction mechanism occurs at comparable rates (typical values of the hyperfine coupling constant are 1–10 mT, equivalent to $0.2\text{–}2.0 \cdot 10^9$ rad s^{-1}; Gould et al., 1984; McLauchlan and Steiner, 1991). Finally, the Δg mechanism can also lead to intersystem crossing rates exceeding 10^8–10^9 s^{-1}, if magnetic flux densities are in the 10–100 mT range and, for example, $\Delta g = 0.25$ (this value has been estimated for the enzyme-bound {5′-deoxyadenosyl, cob(II)alamin-radical pair} radical pair; see Grissom, 1995).

The turnover number of the enzyme can be calculated depending on whether the rate-limiting step in the enzyme cycle occurs before or after radical pair generation. If $k_2 \gg k_4$:

$$\left.\frac{V^F_{max}}{[E_{total}]}\right|_{k_2 \gg k_4} = k_4, \tag{12}$$

whereas if $k_4 \gg k_2$:

$$\left.\frac{V^F_{max}}{[E_{total}]}\right|_{k_4 \gg k_2} = \frac{k_2}{1 + (k_2 + k_{-2}/f(k_{isc}))/k_3}. \tag{13}$$

This shows that only in the second case ($k_4 \gg k_2$) a magnetic field effect on V^F_{max} is observed. In the first case ($k_2 \gg k_4$), V^F_{max} is not altered because the net forward flux leading to formation of the intermediate biradical state, $^S(E^{\bullet\bullet}S)$, via k_2 is high, and product release via k_4 is spin-independent. In both situations, however, an influence on V^F_{max}/K^F_M will be observed because the ratio of net forward flux to form product to the net rate of nonproductive substrate dissociation—defined as the forward commitment to catalysis for a substrate (Cleland, 1982)—is altered.

To investigate the magnetic field effect on the reaction rate, Eq. 5 is reformulated. Defining $\eta = \eta([S])$:

$$\eta = \left(\frac{k_{-2}}{k_3}\right)\frac{(k_4/k_2)\{[S] + K_{n1}\}}{\{1 + (k_4/k_2) + (k_4/k_3)\}[S] + (k_4/k_2)K_1}, \tag{14}$$

yields

$$\frac{v^F(B)}{v^F(0)} = \frac{1 + \eta/f(k_{isc}(0))}{1 + \eta/f(k_{isc}(B))}, \tag{15}$$

where B denotes magnetic flux density in high field limit.

Equation 15 contains only three effective parameters, namely η, $k_{isc}(0)/k_3$, and $k_{isc}(B)/k_3$. This enables a simple interpretation of the magnetic field-induced change in the reaction rate. At the same time η can be related to the enzyme kinetic parameters; combining Eqs. 6 and 14 yields

$$\frac{(V^F_{max}/K^F_M)(B)}{(V^F_{max}/K^F_M)(0)} = \frac{1 + (K_{n1}/K_1)\{(k_{-2}/f(k_{isc}(0)))/k_3\}}{1 + (K_{n1}/K_1)\{(k_{-2}/f(k_{isc}(B)))/k_3\}} = \frac{1 + \eta([S] \to 0)/f(k_{isc}(0))}{1 + \eta([S] \to 0)/f(k_{isc}(B))}. \tag{16}$$

Thus Eq. 16 is equivalent to Eq. 15 in the limit of low substrate concentration ($[S] \to 0$), that is, under V_{max}/K_M conditions. In experimental investigations the V_{max}/K_M kinetic parameter and its possible magnetic field dependence are usually determined (Hammes, 1982; Harkins and Grissom, 1994; Grissom, 1995), whereas in the simulations it is more convenient to use Eq. 15. The equivalence shown in Eq. 16, however, allows for an easy comparison between numerical results and experimental observations.

In Fig. 1 the magnetic field effect on the reaction rate and on the recombination probability of the radical pair is depicted as a function of magnetic flux density at different

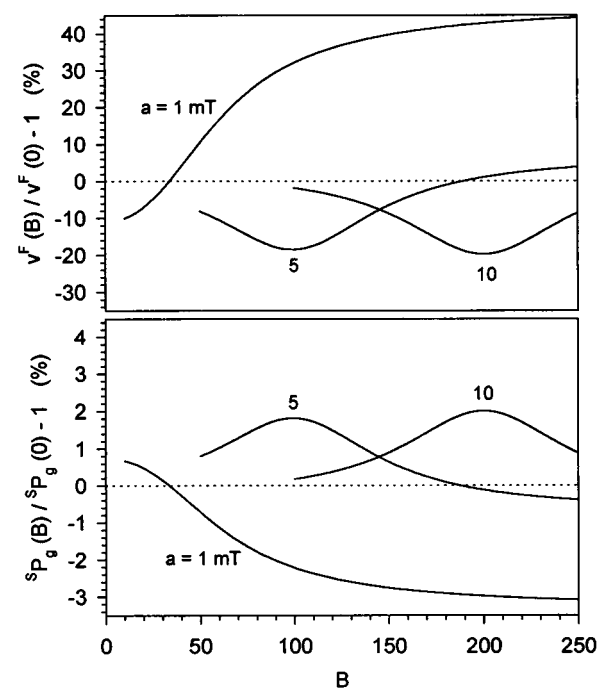

FIGURE 1 Magnetic field effect on the enzyme reaction rate, v^F, and on the recombination probability of the radical pair, ^SP_g, as a function of magnetic flux density. (*Top*) Magnetic field-induced change (Eq. 15) in the reaction rate (in percent) as a function of magnetic flux density, B, in milli-Tesla. (*Bottom*) Magnetic field-induced change in the recombination probability of the radical pair (in percent) calculated with the exponential model (Eqs. 20–23). Different values of the hyperfine interaction constant, a, are used in the simulations (indicated in figure). Note the different scales. To observe the high field limit, $B \gg a$, calculations start at $B = 10$ a. The parameter η depends on k_3. Equation 14 yields $\eta \approx \beta\,(k_{-2}/k_3)$, where $\beta = \beta[S]$ is, to a first approximation, independent of k_3, because $k_4/k_3 \ll 1$ ([S], substrate concentration). Parameters: $k_{-2} = 10^9$ s^{-1}, $k_3 = 0.5 \cdot 10^8$ s^{-1}, $2J = 0.5$ mT, $\Delta g = 0.05$, $\eta = 0.5\,(k_{-2}/k_3)$.

values of the hyperfine interaction constant. At lower magnetic flux densities (for example, for the graph with $a = 5$ mT, $B < 180$ mT) radical pair recombination is increased because of decreased hyperfine interaction-induced singlet-triplet intersystem crossing (separation of $T_{\pm 1}$ states from singlet state). This results in a reduction of the enzyme reaction rate, because the forward flux to form product is decreased. At high magnetic flux densities (for the graph with $a = 5$ mT, $B > 180$ mT) the Δg mechanism compensates for this effect. Reduced radical pair recombination leads to an increase in the reaction rate. Fig. 1 also reveals that the specific combination of the hyperfine coupling constant and the Δg value is the major determinant of the magnetic field effect profile. Specifically, for the one spin 1/2 nucleus model, the value of the magnetic flux density at maximum decrease in the enzyme reaction rate is determined by the ratio of these two parameters.

In Fig. 2 the behavior obtained by decreasing the value of the rate constant k_3, representing forward reaction coordinate motion, is shown. At a rate that is five times slower (compared to the value used in Fig. 1), the effect on the recombination probability of the radical pair is considerably smaller (<0.5%). At the same time the maximum magnetic field-induced decrease in the enzyme reaction rate is larger. This shows that there is no simple, linear relationship between the magnetic field effect on the recombination probability of the radical pair and on the enzyme reaction rate (see also Fig. 4 and discussion below).

The results presented in Figs. 1 and 2 qualitatively resemble the experimentally observed biphasic magnetic field dependence of the V_{max}/K_M kinetic parameter of ethanolamine ammonia lyase. In Fig. 3 the experimental data of the magnetic field effect on coenzyme B_{12}-dependent ethanolamine ammonia lyase in vitro activity reported by Harkins and Grissom (1994) are shown together with a model prediction. The latter was obtained by applying Eq. 16 where $V^F_{max}/K^F_M(0)$ and $\eta([S]\rightarrow 0)$ served as fitting parameters. The former was determined by the experimental value of V_{max}/K_M at $B = 0$, and the latter was adjusted such that the model yields a maximum decrease in enzyme activity at $B \approx 100$ mT. Note that the parameter values fitted are not uniquely defined and other combinations will yield graphs of similar quality.

The parameters that appear in the calculation of the rate constant k_{isc} (Eqs. 10 and 11) in the function $f(k_{isc})$ (Eq. 7) were determined as follows: 1) radical pair recombination rate constant $k_{-2} = 10^9\ s^{-1}$ (Chagovetz and Grissom, 1993); 2) rate constant representing forward reaction coordinate motion $k_3 = 10^8\ s^{-1}$; 3) exchange interaction constant $2J = 0$; 4) effective hyperfine interaction constant $a = 9.5$ mT; 5) $\Delta g = 0.09$. These parameter values yield a recombination probability in the absence of the magnetic field of $P_R(0) \approx 0.85$, which is comparable to the experimental estimate $P_R \geq 0.9$ reported by Chagovetz and Grissom (1993). The exchange interaction was neglected be-

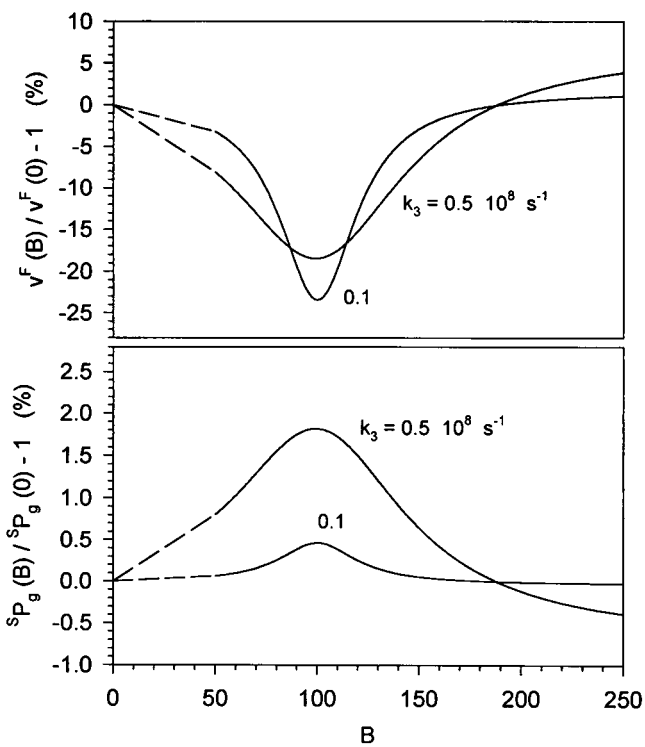

FIGURE 2 Magnetic field effect on the enzyme reaction rate, v^F, and on the recombination probability of the radical pair, ^SP_g, as a function of magnetic flux density. (*Top*) Magnetic field-induced change (Eq. 15) in the reaction rate (in percent) as a function of magnetic flux density, B, in milli-Tesla. (*Bottom*) Magnetic field-induced change in the recombination probability of the radical pair (in percent) calculated with the exponential model (Eqs. 20–23). Different values of the rate constant k_3 representing forward reaction coordinate motion are used in the simulations (indicated in figure). The dashed line extrapolates the behavior for $B \rightarrow 0$. Parameters: $k_{-2} = 10^9\ s^{-1}$, $2J = 0.5$ mT, $a = 5$ mT, $\Delta g = 0.05$, $\eta = 0.5(k_{-2}/k_3)$.

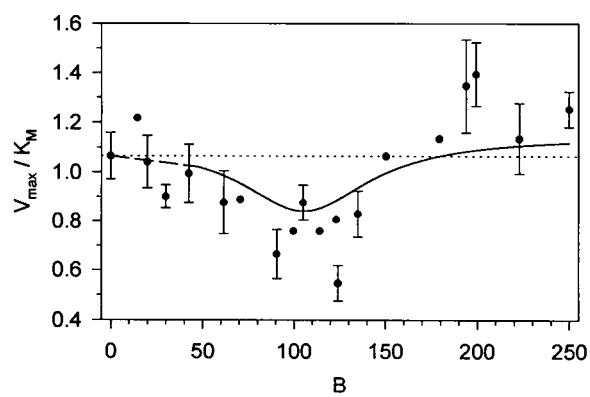

FIGURE 3 Comparison between experimental data and model simulations. (*Data points*) Magnetic field effect on V_{max}/K_M of coenzyme B_{12}-dependent ethanolamine ammonia lyase in vitro activity; data taken from figure 4 *B* in Harkins and Grissom (1994). (*Solid/dashed curve*) Model simulation using Eq. 16. The experimentally obtained value of V_{max}/K_M at $B = 0$ (*dotted line*) and the maximum decrease in enzyme activity at $B = 100$ mT as determined by the model were used as fitting parameters for $V^F_{max}/K^F_M(B = 0)$ and $\eta([S] \rightarrow 0)$ in Eq. 16. The procedure yields $V^F_{max}/K^F_M(0) = 1.065$, $\eta = 9.5$. The value $2J$ of the exchange interaction was set equal to zero; results do not qualitatively change if $2J > 0$. Other parameters: $k_{-2} = 10^9\ s^{-1}$, $k_3 = 10^8\ s^{-1}$, $2J = 0$, $a = 9.5$ mT, $\Delta g = 0.09$.

cause it does not significantly modulate the model prediction as long as its value is smaller than the hyperfine interaction constant. The effective hyperfine interaction constant was obtained by using the semiclassical approximation developed by Schulten and Wolynes (1978). In this approach hyperfine couplings from all magnetic nuclei contribute to an effective hyperfine interaction constant that is defined as (Schulten and Wolynes, 1978; see also Salikhov et al., 1984; Steiner and Ulrich, 1989)

$$A^2_{\text{hfi,eff}} = \frac{1}{3}\sum_i I_i(I_i + 1)A_i^2, \qquad (17)$$

where the sum extends over all magnetic nuclei with nuclear spin I_i and hyperfine interaction constant A_i. Following a procedure proposed by Grissom et al. (Grissom, private communication), an effective hyperfine interaction constant $A_{\text{hfi, eff}} = 9.5$ mT is obtained by taking into account in Eq. 17 the $I = 7/2$ spin of the cobalt nucleus and the $I = 1$ spin of one of the nitrogen atoms. Finally, the Δg value is adjusted to yield a minimum in the enzyme reaction rate at $B \approx 100$ mT.

The model prediction shown in Fig. 3 does not entirely reproduce the experimentally observed effect size in $V_{\max}/K_M$ as a function of magnetic flux density, which appears to be greater than in the simulations. This deviation can partially be attributed to the simplicity of the model in comparison to the complex reaction mechanism of ethanolamine ammonia lyase. For example, the enzyme reaction cycle includes a reversible formation of the initial biradical state (Harkins and Grissom, 1994, 1995). In the reaction scheme (Eq. 4) this would correspond to the transition $(ES)^* \rightarrow (E^{\bullet\bullet}S)$. If this process takes place after initial spin correlation is lost, which is likely to be the case because it occurs at a later stage of the reaction cycle, then the biradical state could be regenerated in any spin multiplicity, thus resembling a random radical pair encounter. In this scenario the triplet $T_{\pm 1}$ states will also become populated. Under high field conditions these are energetically decoupled from the singlet state, leading to a decrease in the overall radical pair recombination rate. This behavior will contribute to the observed magnetic field effect on enzyme activity. (To keep calculations general and to obtain the exact relation, Eq. 9, between radical pair recombination probability, ${}^S P_g$, and the rate constant for intersystem crossing, k_{isc}, the scenario described here is not explicitly included in the model.)

Model predictions reproduce, however, the biphasic magnetic field dependence of the enzyme reaction rate. Therefore, the simulations suggest, as has been assumed by Grissom et al. before (Harkins and Grissom, 1994; Grissom, 1995), that both the hyperfine interaction mechanism and the Δg mechanism contribute to the observed magnetic field dependence of ethanolamine ammonia lyase activity.

The experimentally observed magnetic field-induced decrease in $V_{\max}/K_M$ is larger with perdeuterated ethanolamine (60% decrease at 150 mT; Harkins and Grissom, 1994). This behavior has been interpreted as resulting from an increase in the fraction of enzyme that exists in the complex with the initial radical pair (due to a primary isotope effect on one of rate constants of the enzyme reaction cycle; see Grissom, 1995, for further details). In the model simulations the magnitude of the magnetic field-induced decrease in the reaction rate is larger at a lower k_3 value (Fig. 2). A lower k_3 value is related to a slower separation of the radical pair, thus increasing the fraction of enzyme in the biradical state. In this limited sense a first comparison between model simulations and the isotope experiment can be made. However, a more quantitative interpretation regarding the effect size and the value of the magnetic flux density at maximum effect necessitates additional information regarding the magnetic interactions (for example, hyperfine interactions and site of the isotope effect).

The function $f(k_{\text{isc}})$ (Eq. 7) reveals that the rate constants k_3 and k_{isc} must be of similar magnitude to observe a magnetic field effect on the reaction rate. If forward reaction coordinate motion is too fast ($k_3 \gg k_{\text{isc}}$), there is not enough time to interconvert the singlet and triplet states. On the other hand, if it is slow ($k_3 \ll k_{\text{isc}}$), recombination is likely to occur. In both cases there is no competition between coherent spin motion and forward reaction coordinate motion and, consequently, no field effect will be observed. The behavior is illustrated in Fig. 4, where the magnetic field effect on the reaction rate and on radical pair recombination probability is depicted as a function of k_3. For the

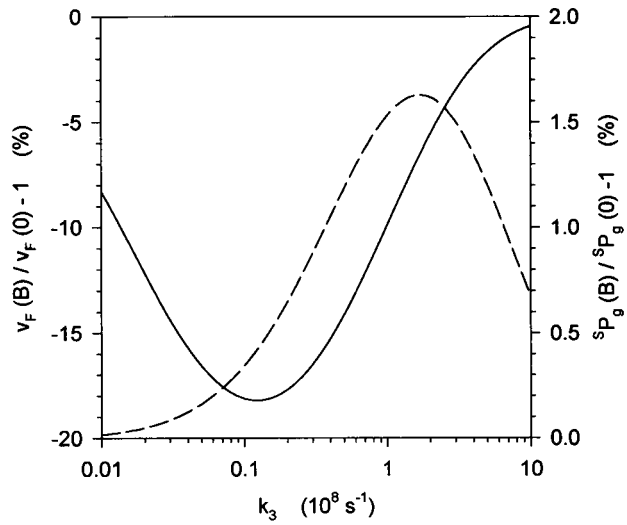

FIGURE 4 Magnetic field effect on the enzyme reaction rate, ν^F, and on the recombination probability of the radical pair, ${}^S P_g$, as a function of the rate constant k_3 representing forward reaction coordinate motion. (*Solid line*) Magnetic field-induced change (Eq. 15) in the reaction rate (in percent) as a function of the parameter k_3. Within the exponential model $1/k_3$ is the mean frequency of interradical distance changes. (*Dashed line*) Magnetic field-induced change in the recombination probability of the radical pair (in percent) calculated with the exponential model (Eqs. 20–23). Note the different scales. Parameters: $k_{-2} = 10^9$ s^{-1}, $2J = 0.5$ mT, $a = 2$ mT, $\Delta g = 0.05$, $\eta = 0.5$ (k_{-2}/k_3), $B = 30$ mT

parameter values used, the rate of intersystem crossing under no field conditions is $k_{isc}(0) = 0.7 \cdot 10^8\ s^{-1}$ (at $k_3 = 0.5 \cdot 10^8\ s^{-1}$). The value of k_{isc} is decreased to k_{isc} (30 mT) $= 0.25 \cdot 10^8\ s^{-1}$ (at $k_3 = 0.5 \cdot 10^8\ s^{-1}$). The figure also shows that the maximum in the effect size is at different k_3 values for the reaction rate and the recombination probability. Therefore, a change in radical pair lifetime induced by isotopic substitution, for example, will have different consequences regarding the size of the magnetic field effect on these two variables.

Figs. 1, 2, and 4 reveal that whereas radical pair recombination is probability altered by at most a few percent in the presence of the magnetic field, the effect on the reaction rate is considerably larger. Indeed, the effect size of the magnetic field-induced change in the reaction rate is determined by the parameter η. Combining Eqs. 9 and 15 yields

$$\frac{v^F(B)/v^F(0) - 1}{{}^SP_g(B)/{}^SP_g(0) - 1} = - \left(\frac{\eta}{f(k_{isc}(0))}\right) \frac{1 + (k_{-2}/k_3)/f(k_{isc}(B))}{1 + \beta(k_{-2}/k_3)/f(k_{isc}(B))}, \tag{18}$$

where $\beta = (k_4/k_2)\ \{[S] + K_{m1}\}/\{\ (1 + (k_4/k_2) + (k_4/k_3))[S] + (k_4/k_2)K_1\} < 1$.

The right-hand side of Eq. 18 is linearly dependent on the parameter η, showing that η can be regarded as an "amplification" factor. The kinetic features inherent to the enzyme reaction cycle thus provide a basis for amplifying small initial changes in the recombination probability of the radical pair. As an example, in the simulations shown in Fig. 4 radical pair recombination probability is changed by 1.6% at most; however, the enzyme reaction rate is reduced by up to 18%, depending on k_3. These results show that interpretation of the experimental results reported by Grissom et al. has to be based on both the radical pair mechanism as a primary physical coupling mechanism of the magnetic field and on the secondary biological mechanism related to enzyme kinetics. In Fig. 5 the dependence of the size of the magnetic field effect on η is shown. The parameter range of η can be estimated, depending on substrate concentration:

$$\eta([S] \to 0) = \left(\frac{k_{-2}}{k_3}\right)\frac{k_{-1}}{k_{-1} + k_2}, \qquad \eta([S] \ll K_1) = \left(\frac{k_{-2}}{k_3}\right)\frac{k_4/k_2}{1 + k_4/k_2 + k_4/k_3}. \tag{19}$$

At low substrate concentrations (that is, under V_{max}/K_M conditions), η is determined by the ratio k_{-2}/k_3, reduced by the factor $k_{-1}/(k_{-1} + k_2)$. If radical pair recombination via k_{-2} is faster than forward reaction coordinate motion via k_3, and radical pair generation via k_2 is not too fast (compared to the rate constant k_{-1}), one might expect values of $\eta = 1$–100. In this case a considerable magnetic field effect will be observed. At high substrate concentrations similar arguments hold; however, a considerable change in the reaction rate will only occur if the ratio k_4/k_2 is sufficiently high.

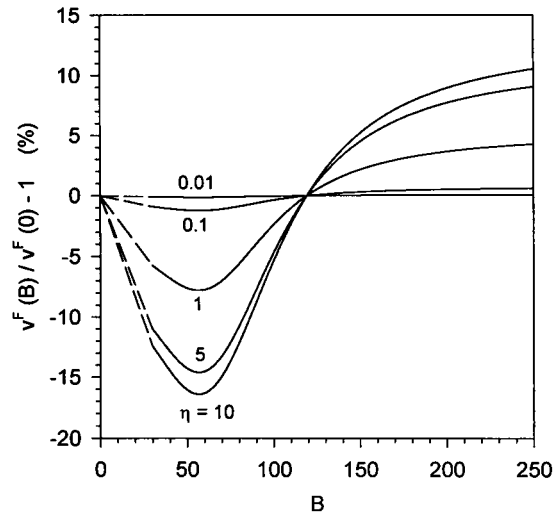

FIGURE 5 Magnetic field effect on the enzyme reaction rate, v^F, as a function of magnetic flux density and in dependence on the amplification factor η. Magnetic field-induced change (Eq. 15) in the reaction rate, v^F (in percent), as a function of magnetic flux density, B, in milli-Tesla. Different values of the parameter η are used in the simulations (indicated in figure). The dashed line extrapolates the behavior for $B \to 0$. The parameter η includes all rate constants of the enzyme reaction scheme (Eq. 4) and depends on substrate concentration (see Eq. 14 for definition). The figure shows that the effect size of the magnetic field is determined by η. Parameters: $k_{-2} = 10^9\ s^{-1}$, $k_3 = 0.5 \cdot 10^8\ s^{-1}$, $a = 3$ mT, $2J = 0.5$ mT, $\Delta g = 0.05$.

SUMMARY AND OUTLOOK

In this theoretical study a prototypical model accounting for magnetic field effects in enzyme reactions is discussed. Magnetic field influence is exerted on the recombination probability of a transient radical pair that is generated within the enzyme reaction cycle.

The model consists of an expansion of the Michaelis-Menten mechanism where an intermediate biradical state on the enzyme-substrate complex is taken into account, including the spin multiplicity of the radicals. The specific structure of the scheme (Eq. 4) is the simplest form of an enzyme reaction scheme that makes it possible to explicitly calculate a magnetic field-dependent reaction rate. An advantage of this scheme is that under steady-state conditions an exact relation, Eq. 9, between the recombination probability of the radical pair, ${}^SP_g$, and the rate constant k_{isc} for intersystem crossing between the singlet and triplet states, is obtained. This makes it possible to solve the problem of the chemical kinetics first, and then calculate k_{isc} by referring to quantum mechanically based models for determining ${}^SP_g$, like the exponential model used in our simulations.

So far, our approach does not take into account paramagnetic relaxation processes that lead to a reduction in the magnitude of the magnetic field effect. Mechanisms leading to

electron spin relaxation involve, for example, anisotropic hyperfine and g tensors (Steiner and Ulrich, 1989; Khudyakov et al., 1993; Wu et al., 1995). In organic radical pairs paramagnetic relaxation is usually on the order of 10^6 s^{-1} and thus is much slower than coherent spin motion (Salikhov et al., 1984; Steiner and Ulrich, 1989). However, large spin-orbit coupling in systems with unquenched electronic orbital angular momentum can induce fast paramagnetic relaxation (Khudyakov et al., 1993; Wu et al., 1995). In particular, in transition metal complexes with a large nuclear charge and symmetrical ligand structures, spin-orbit coupling is effective (Khudyakov et al., 1993). Many enzyme systems belong to this group, an example being coenzyme B_{12}-dependent ethanolamine ammonia lyase. Other potentially magnetic field-sensitive enzymes in this category are those involving iron complexes like, for example, cytochrome P-450s, peroxidases, and catalases. The latter systems have been tested for magnetic field dependence; however, no conclusive results have been obtained so far (Grissom, 1995; Walleczek, 1995).

Although strong spin-orbit coupling will quench the magnetic field effect, it can also induce such effects (Khudyakov et al., 1993). The reason is that besides leading to an anisotropic g factor, it also causes the g value to differ substantially from the free electron value. This means that a spin-correlated radical pair where one of the partners is subjected to strong spin-orbit coupling will exhibit a high Δg value. Thus the Δg mechanism will become an important mechanism of spin rephasing, even at very low magnetic flux densities in the milli-Tesla range (Khudyakov et al., 1993).

The preceding discussion stresses that more experimental evidence is needed before general conclusions can be drawn regarding the possibility of magnetic field effects on reactions involving other transition-metal enzymes. So far our model simulations suggest that the magnetic field effect on coenzyme B_{12}-dependent ethanolamine ammonia lyase in vitro activity can be interpreted in a qualitative manner on the basis of the hyperfine and the Δg mechanisms.

Additional experimental evidence is also needed with regard to the lower limit of magnetic field effects on enzyme reactions. Our simulations show that the kinetic features of the enzyme reaction cycle itself provide a basis for amplifying small initial changes in the recombination probability of the radical pair. This means that even subtle alterations in radical pair recombination kinetics induced by low-intensity magnetic fields (for example, $B \approx 1$–10 mT) might lead to measurable effects on enzyme activity.

The reaction schemes used in our model (Eqs. 1 and 4) are based on simple Michaelis-Menten kinetics. So far they do not include cooperative reaction steps. Inclusion of such processes in future investigations would be a meaningful generalization of the present model, because cooperativity is a common way of controlling enzyme function (Hammes, 1982; Neet, 1995; Eichwald and Walleczek, 1996b). The involvement of cooperative steps in enzyme kinetics might provide an additional source for the amplification of small initial changes in the recombination probability of the radical pair.

The question of whether the radical pair mechanism can be linked to magnetic field effects in biological systems has been raised in several publications recently (Walleczek and Budinger, 1992; Grundler et al., 1992; Scaiano, 1994; Canfield et al., 1994; Grissom, 1995; Walleczek, 1995; Brocklehurst and McLauchlan, 1996; Eichwald and Walleczek, 1996b). This proposal is not new; for example, as early as 1978 Schulten et al. suggested a biomagnetic sensory mechanism based on the radical pair mechanism to explain the ability of biological organisms to orient themselves in the geomagnetic field (Schulten et al., 1978). The challenge remains, however, to conclusively establish the link between the primary event (magnetic field influence on radical pair recombination kinetics) and the secondary effect on the biological system. To further explore this relationship, the following recommendations are in order (Walleczek, 1995). Future work should include 1) the development and application of reliable methods for the detection of magnetic field effects in biochemical and related in vitro reactions with higher resolution (1% or better), which should enable systematic studies, even at very low magnetic flux densities ($B \approx 1$–10 mT); 2) the determination of physicochemical parameters regarding relevant biomolecular systems such as transition metal enzymes (data concerning, for example, hyperfine interaction couplings, Δg values, radical pair lifetimes, and relaxation time constants); and 3) the development of theoretical models describing magnetic field effects on radical pair recombination that take into account the biological context. The present study contributes to the latter aspect.

APPENDIX

Here we show expressions for the recombination probability ${}^{S}P_g$ that have been derived on the basis of the exponential model for radical pair recombination (see Salikhov et al., 1984). It is assumed that the radical pair is generated in the singlet state and that a single spin 1/2 magnetic nucleus exists. Under these conditions one obtains in the zero field limit

$$ {}^{S}P_g(0) = \left(\frac{k_{-2}}{k_{-2}+k_3}\right)\left(\frac{1+3C_1(a/4)^2}{1+3C_2(a/4)^2}\right), \tag{20} $$

where

$$ C_1 = \frac{1}{k_3}\frac{2k_3+k_{-2}}{(k_3+k_{-2}/2)^2+(2J+a/2)^2}, \tag{21} $$

$$ C_2 = \frac{2k_3+k_{-2}}{k_3+k_{-2}}C_1. $$

In the high field limit:

$$ {}^{S}P_g(B) = \left(\frac{k_{-2}}{k_{-2}+k_3}\right)\left(\frac{1+(C_1'+C_2')\Omega_s+C_1'C_2'\Omega_d^2}{1+2C_2'\Omega_s+(C_2')^2\Omega_d^2}\right), \tag{22} $$

where $\Omega_{s,d} = (\Delta\omega)^2 \pm (a/4)^2$ and

$$C_1' = \frac{1}{k_3}\frac{2k_3 + k_{-2}}{(k_3 + k_{-2}/2)^2 + (2J)^2}, \quad C_2' = \frac{2k_3 + k_{-2}}{k_3 + k_{-2}} C_1'. \qquad (23)$$

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