Effects of disorder and motion in a radical pair magnetoreceptor

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A critical requirement in the proposed chemical model of the avian magnetic compass is that the molecules that play host to the magnetically sensitive radical pair intermediates must be immobilized and rotationally ordered within receptor cells. Rotational disorder would cause the anisotropic responses of differently oriented radical pairs within the same cell to interfere destructively, while rapid molecular rotation would tend to average the crucial anisotropic magnetic interactions and induce electron spin relaxation, reducing the sensitivity to the direction of the geomagnetic field. So far, experimental studies have been able to shed little light on the required degree of ordering and immobilization. To address this question, computer simulations have been performed on a collection of radical pairs undergoing restricted rigid-body rotation, coherent anisotropic spin evolution, electron spin relaxation and spin-selective recombination reactions. It is shown that the ordering and motional constraints necessary for efficient magnetoreception can be simultaneously satisfied if the radical pairs are uniaxially ordered with a moderate order parameter and if their motional correlation time is longer than about a quarter of their lifetime.

Keywords: anisotropy; migratory birds; cryptochrome; hyperfine interaction; magnetic compass; radical pair mechanism

1. INTRODUCTION

There is growing evidence that the remarkable ability of birds to detect the direction of the geomagnetic field relies on magnetically sensitive chemical reactions that have radical pairs as transient intermediates. The original idea for this mechanism (Schulten *et al.* 1978; Schulten 1982; Schulten & Windemuth 1986) was inspired by the behavioural properties of the avian magnetic compass (Wiltschko & Merkel 1966; Wiltschko, W. & Wiltschko, R. 1972, 2001; Wiltschko, R. & Wiltschko, W. 2006) and the fact that the rates and yields of radical pair reactions *in vitro* can be altered by the application of external magnetic fields of modest intensity (Steiner & Ulrich 1989; Brocklehurst 2002; Woodward 2002; Timmel & Henbest 2004; Rodgers 2009). To be a source of directional information, a radical pair should recombine spin selectively to form products whose relative yields depend on its orientation with respect to the magnetic field. This, in turn, requires that the radicals experience appropriate anisotropic local magnetic fields, the most probable source of which is the intra-radical coupling of electron and nuclear spins (the ‘hyperfine interaction’; Schulten *et al.* 1978; Efimova & Hore 2009). Ritz *et al.* (2000) proposed that magnetically sensitive radical pairs are produced photochemically in cryptochrome proteins

(Sancar 2003; Lin & Todo 2005; Partch & Sancar 2005) in the retina. To act as directional sensors, these molecules should be spatially ordered at two levels: they should be immobilized and aligned within specialized receptor cells which themselves should be aligned. The bird could then orient itself by comparing the responses from cells at different locations in the retina (Ritz *et al.* 2000). One suggestion is that the transduction of the magnetic compass information might ‘piggy-back’ the visual reception pathway, allowing the bird literally to see the magnetic field as a ‘signal (or visual) modulation pattern’, reflecting the anisotropy of the radical pair reaction (Ritz *et al.* 2000; Wang *et al.* 2006; Zapka *et al.* 2009).

The radical pair mechanism imposes a number of constraints—chemical, magnetic, kinetic, structural and dynamic—that should be satisfied by a viable chemical magnetoreceptor (Rodgers & Hore 2009). Based on *in vitro* precedents and theoretical arguments, together with the growing body of information on the photochemistry of cryptochromes, it seems plausible that these proteins may have many of the required properties. Probably the physico-chemical aspect of the radical pair hypothesis about which least is known is the mechanism and extent of the alignment and immobilization of the magnetoreceptor molecule within the receptor cells. Rotational disorder would cause the anisotropic responses of differently oriented radical pairs within the same cell to cancel one another, reducing the directional sensitivity. Rapid molecular

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rotation would tend to average the anisotropic magnetic interactions that are crucial for compass action and cause the electron spins to relax, with concomitant loss of spin correlation and reduced sensitivity to external magnetic fields (Rodgers & Hore 2009).

So far, experimental studies have been able to shed little light on these crucial matters. Radical pair magnetic field effects have been reported for isotropic solutions of a DNA photolyase (a DNA-repair enzyme, homologous to cryptochrome, with an apparently similar photoreduction reaction; Henbest *et al.* 2008), but it has not yet been possible to produce the oriented protein samples that would be needed to study anisotropic responses to an applied magnetic field. However, measurements of this sort have recently been performed using a model compound structurally quite unlike cryptochrome. A carotenoid–porphyrin–fullerene molecule aligned in a frozen liquid-crystalline solvent at low temperature (193 K) has been shown to be sensitive to the direction of an external magnetic field (3.1 mT) some 60 times stronger than the Earth's (approx. 50 μ T; Maeda *et al.* 2008). Although such experiments provide an important proof of principle that a photochemical reaction can act as a magnetic compass, they have not yet given insight into the degree of immobilization and alignment needed for a radical pair to exhibit a sufficiently sensitive response to an approximately 50 μ T magnetic field at physiological temperatures.

To address this problem, we have performed computer simulations of a collection of radical pairs undergoing restricted rotation, coherent anisotropic singlet $\leftrightarrow$ triplet interconversion and spin-selective recombination reactions. We show that the ordering and motional constraints can be simultaneously satisfied if the radical pairs are uniaxially ordered with a moderate order parameter and if the characteristic motional correlation time is longer than about a quarter of the radical pair lifetime.

2. THEORETICAL METHODS

The aim is to model a molecule containing a pair of simple radicals that have a fixed separation and relative orientation, with uniaxial ordering, undergoing rotational diffusion. As the molecule rotates, the radical pair should undergo coherent spin evolution under the influence of internal and external magnetic interactions, and recombine spin selectively to form distinct products from its singlet (S) and triplet (T) states. The spin relaxation that is an inevitable consequence of rotational modulation of the anisotropic magnetic interactions must also be included. To reveal the underlying physics in the clearest possible way, and for computational tractability, we consider a radical pair containing a single spin- $\frac{1}{2}$ nucleus with an axial hyperfine interaction. The object is to calculate the fractional yield (Φ_S) of the 'singlet product', i.e. the reaction product formed by recombination of the S state, once all radical pairs have recombined. Φ_S must be determined as a function of the direction of the magnetic field vector with respect to the alignment axis (the 'director') as a measure of the directional information

obtainable from the magnetic response of the radical pair. Calculations were performed using a stochastic Liouville equation (SLE; Kubo 1969*a,b*; Freed *et al.* 1971) to describe the spatial and temporal evolution of the spin density operator $\hat{\rho}(\Omega, t)$ of the radical pair

$$\frac{\partial}{\partial t}\hat{\rho}(\Omega, t) = [-i\hat{H}(\Omega) - k + D\Gamma\Omega]\hat{\rho}(\Omega, t), \quad (2.1)$$

in which Ω represents the three Euler angles (α, β, γ) that specify the orientation of the molecule with respect to the laboratory frame. The three terms in the square bracket in equation (2.1) account, respectively, for the spin evolution, the reactivity and the Brownian rotational motion. For examples of the application of the SLE to radical pair reactions, see Evans *et al.* (1973) and Pedersen & Freed (1973). In the first term in equation (2.1), $\hat{H}(\Omega)$ is the commutation superoperator (the 'Liouvillian'; Ernst *et al.* 1987; Mehring & Weber 2001) obtained from the Hilbert-space spin Hamiltonian, $\hat{H}(\Omega)$,

$$\begin{aligned} \hat{H}(\Omega) = \gamma_e B_0 \left[(\hat{S}_{Az} + \hat{S}_{Bz}) \cos \theta + (\hat{S}_{Ax} + \hat{S}_{Bx}) \sin \theta \right] \\ + a\hat{S}_A \cdot \hat{\mathbf{I}} + \frac{\Delta A}{\sqrt{6}} \sum_{m=-2}^2 \mathcal{D}_{m,0}^{(2)}(\Omega) \hat{T}_{2,m}, \end{aligned} \quad (2.2)$$

where $\hat{S}_A, \hat{S}_B$ and $\hat{\mathbf{I}}$ are, respectively, the spin operators for the two electrons (one in each of the radicals, A and B) and a spin- $\frac{1}{2}$ nucleus (e.g. a proton, ^1H) in radical A. Similar expressions can be found in, for example, Muus *et al.* (1977). B_0 is the intensity of the external magnetic field, γ_e the magnetogyric ratio, θ the angle between the field vector and the director, and a and ΔA , respectively, the isotropic and axial anisotropic hyperfine interaction parameters for radical A. ΔA is related to the principal components of the traceless anisotropic hyperfine coupling tensor by $\Delta A = 2A_{ZZ} - (A_{XX} + A_{YY})$. $\hat{T}_{2,m}$ are the irreducible tensor operators

$$\left. \begin{aligned} \hat{T}_{2,0} &= \sqrt{\frac{2}{3}}(\hat{S}_{Az}\hat{I}_z - \frac{1}{4}[\hat{S}_{A+}\hat{I}_- - \hat{S}_{A-}\hat{I}_+]), \\ \hat{T}_{2,\pm 1} &= \mp \frac{1}{2}(\hat{S}_{Az}\hat{I}_{\pm} + \hat{S}_{A\pm}\hat{I}_z) \\ \text{and } \hat{T}_{2,\pm 2} &= \frac{1}{2}\hat{S}_{A\pm}\hat{I}_{\pm} \end{aligned} \right\} \quad (2.3)$$

and $\mathcal{D}_{m,0}^{(2)}(\Omega)$ are the Wigner matrix elements

$$\left. \begin{aligned} \mathcal{D}_{\pm 2,0}^{(2)}(\Omega) &= e^{\mp i 2\alpha} \frac{1}{2} \sqrt{\frac{3}{2}} \sin^2 \beta, \\ \mathcal{D}_{\pm 1,0}^{(2)}(\Omega) &= \mp e^{\mp i \alpha} \sqrt{\frac{3}{2}} \sin \beta \cos \beta \\ \text{and } \mathcal{D}_{0,0}^{(2)}(\Omega) &= \frac{1}{2}(3 \cos^2 \beta - 1). \end{aligned} \right\} \quad (2.4)$$

The third Euler angle (γ) is not required here because the hyperfine interaction is assumed to be axial (i.e. cylindrically symmetrical) rather than rhombic.

The g -values of the two radicals are taken as equal to the free electron g -value (2.0023) and g -anisotropy is neglected; both are excellent approximations for organic radicals subject to the very weak magnetic fields ($B_0 \sim 50 \mu\text{T}$) of interest here. The radicals are assumed to be

far enough apart that the electron–electron exchange and dipolar interactions can be ignored and the only relaxation process considered is that which results from the modulation of the hyperfine and Zeeman interactions in radical A. We do not use a Redfield superoperator here partly because relaxation is automatically included in the SLE approach, but also because we wish to cover rotational diffusion rates that fall outside the usual range of validity of Redfield theory (Redfield 1965). Although it is an unrealistic simplification to consider a single magnetic nucleus, this model contains all the important features of a more complex system and allows the essential principles to be extracted.

The matrix representation of $\hat{H}(\Omega)$ has dimension 8 so that the Liouvillian $\hat{\hat{H}}(\Omega)$ has dimension 64. However, this can be reduced to 16 by calculating the spin evolution of radical B analytically, which is possible because the two radicals do not interact with one another and so evolve independently and because the part of $\hat{H}(\Omega)$ describing radical B contains only the isotropic electron Zeeman interaction.

The second term on the right-hand side of equation (2.1) accounts, phenomenologically, for the recombination of the radical pair (Haberkorn 1976), with a first-order rate constant k , assumed to be the same for S and T states. This common simplification (the ‘exponential model’; Kaptein & Oosterhoff 1969; Brocklehurst 1976) is unrealistic but innocuous: it does not obscure the essential physics (Steiner & Ulrich 1989; Timmel *et al.* 1998). We note that an alternative description of spin-selective reactions of radical pairs has recently been proposed (Kominis 2009).

The final term in equation (2.1) accounts for the spin-independent rotational motion of the molecule that contains the radical pair in terms of the isotropic diffusion coefficient D (related to the isotropic rotational correlation time, τ_c , by $D = (6\tau_c)^{-1}$) and the angular part of the diffusion operator, Γ_Ω . Uniaxial molecular ordering is introduced by means of a potential, $U(\Omega)$. The diffusion equation in the absence of spin-dependent processes is (Debye 1942)

$$\frac{\partial(\Omega, t)}{\partial t} = D[\nabla^2 p(\Omega, t) + \nabla \cdot (p(\Omega, t) \nabla U(\Omega)/k_B T)], \quad (2.5)$$

where $p(\Omega, t) d\Omega dt$ is the classical probability density that the molecular orientation lies in the range $(\Omega, \Omega + d\Omega)$ during the time interval $(t, t + dt)$. We choose a generic axially symmetric potential of the form

$$U(\beta) = \varepsilon k_B T \sin^2 \beta \quad (2.6)$$

with energy minima at $\beta = 0^\circ$ and 180° and a barrier of height $\varepsilon k_B T$ at $\beta = \pm 90^\circ$ where ε is a dimensionless energy parameter that defines the depth of the potential well in which the molecules move. The alignment that results is akin to that of molecules in a uniaxial nematic liquid crystal (de Gennes & Prost 1993).

With this definition,

$$\Gamma_\Omega = \cot \beta \frac{\partial}{\partial \beta} + \frac{\partial^2}{\partial \beta^2} + \frac{1}{\sin^2 \beta} \frac{\partial^2}{\partial \alpha^2} + \frac{1}{k_B T} \left[\frac{dU}{d\beta} \frac{\partial}{\partial \beta} + \frac{dU}{d\beta} \cot \beta + \frac{d^2 U}{d\beta^2} \right]. \quad (2.7)$$

Although we model the radical pair as a rigid entity with no internal mobility, a functional compass sensor would still be feasible if one radical were free to move, provided the other were sufficiently ordered and immobilized and had suitably anisotropic hyperfine interactions (see supporting information in Rodgers & Hore (2009)). This possibility has recently been aired in the context of a putative flavin-superoxide (O_2^-) radical pair in cryptochrome (Hogben *et al.* 2009; Ritz *et al.* 2009; Solov'yov & Schulten 2009).

The singlet product yield Φ_S is calculated as

$$\Phi_S = k \int_0^\infty \text{Tr}\{\hat{P}^{S\dagger} \hat{\rho}(\Omega, t)\} dt d\Omega = k \int \text{Tr}\{\hat{P}^{S\dagger} \hat{\sigma}(\Omega, 0)\} d\Omega. \quad (2.8)$$

The corresponding fractional triplet yield, Φ_T , is given by $\Phi_S + \Phi_T = 1$. $\hat{P}^S$ is the singlet projection operator, $\hat{\sigma}(\Omega, s)$ is the Laplace transform of $\hat{\rho}(\Omega, t)$ with Laplace variable s . $\hat{\sigma}(\Omega, s)$ is obtained by Laplace transformation of equation (2.1)

$$[(s + k)\hat{E} + i\hat{H}(\Omega) - D\Gamma_\Omega] \hat{\sigma}(\Omega, s) = \hat{\rho}(\Omega, 0), \quad (2.9)$$

where $\hat{E}$ is the identity superoperator and the initial condition is a pure singlet state with an equilibrium distribution of orientations

$$\hat{\rho}(\Omega, 0) = \frac{1}{2} \hat{P}^S p(\Omega, 0) = \frac{\frac{1}{2} \hat{P}^S e^{-U(\Omega)/k_B T}}{\int e^{-U(\Omega)/k_B T} d\Omega}. \quad (2.10)$$

The form of $p(\Omega, 0)$ is illustrated in figure 1 for $\varepsilon = 2.5$. For smaller ε , the polar plot is more nearly spherical; for larger values, it is narrower and elongated along the z -axis. The equilibrium order parameter, S , is calculated as (de Gennes & Prost 1993)

$$S = \frac{1}{2}(3 \cos^2 \beta - 1), \quad (2.11)$$

where the angled brackets indicate an average over the equilibrium distribution, $p(\Omega, 0)$. Some values of S and the corresponding mean angular deviations are given in table 1.

To solve equation (2.9), a second-order centred derivative finite difference technique was used to discretize α ($0 \leq \alpha < 2\pi$) and $q = \cos \beta$ ($0 \leq \beta < \pi$) on uniform grids with spacings $\delta\alpha$ and δq , respectively. In this way, the diffusive motion can be written in terms of a transition probability matrix $\mathbf{W}$ coupling $p(\alpha, q, t)$ with $p(\alpha \pm \delta\alpha, q \pm \delta q, t)$

$$\frac{dp(\alpha_i, q_j, t)}{dt} = D \sum_{k,l} W_{ij,kl} p(\alpha_k, q_l, t). \quad (2.12)$$

The approach is essentially similar to Pedersen and Freed's treatment of the radical pair mechanism of

Figure 1. A representation of the model radical pair that is the subject of the calculations presented here. A molecule (red sphere) contains two radicals (red ellipsoids) with fixed relative orientation and separation. It undergoes rotational diffusion in three dimensions subject to an aligning potential, $U(\beta)$, represented by the blue surface which is a polar plot of the probability distribution of molecular orientations at thermal equilibrium for a potential energy parameter, $\varepsilon = 2.5$. The yield of the reaction product formed from the singlet state of the radical pair, Φ_S , is calculated as a function of the direction of the applied magnetic field, B_0 (green arrow), relative to the alignment axis, z .

electron spin polarization with the substitution of rotational for translational diffusion and the inclusion of anisotropic interactions (Pedersen & Freed 1973). Thus, equation (2.9) becomes a matrix equation in an N -dimensional space,

$$[(s + k)\mathbf{E} + i\mathbf{L} - D\mathbf{W}]\boldsymbol{\sigma}(s) = \boldsymbol{\rho}(0), \quad (2.13)$$

where $\mathbf{E}$ and $\mathbf{L}$ are square matrices corresponding to $\hat{E}$ and $\hat{H}$ and $\boldsymbol{\sigma}(s)$ and $\boldsymbol{\rho}(0)$ are vectors corresponding to $\hat{\sigma}(\Omega, s)$ and $\hat{\rho}(\Omega, 0)$. N is the product of the number of grid points ($n_\alpha \times n_q$) and the dimension of the spin space of radical A ($=16$). Values of Φ_S , converged to plotting accuracy, were obtained using $n_\alpha = 24$ and $n_q = 48$.

Most of the simulations presented below were obtained using $a = 1$ mT and $\Delta A = 3$ mT, which are reasonably typical of the larger hyperfine coupling parameters encountered for ^1H and ^{14}N nuclei in the flavin and tryptophan radicals thought to constitute the magnetosensitive radical pair in cryptochrome. For example, calculations using density functional theory show that the largest hyperfine anisotropy in the neutral flavin radical occurs for the N5 nitrogen ($\Delta A = 3.1$ mT; $a = 0.43$ mT) and in the tryptophan cation radical for N1 ($\Delta A = 2.3$ mT; $a = 0.32$ mT). B_0 was $50 \mu\text{T}$ in all calculations (comparable to the Earth's magnetic field) and the lifetime of the radical pair, k^{-1} , was $1 \mu\text{s}$ in most calculations. As argued elsewhere (Rodgers & Hore 2009), $1 \mu\text{s}$ is probably close to the optimum lifetime on the basis that there is little to gain in terms of detection sensitivity and much to lose (from relaxation) if the lifetime is significantly longer.

Table 1. Order parameters (S was calculated for different values of the potential energy parameter, ε , using equation (2.11) and $p(\Omega, 0)$ from equation (2.10). $\langle\beta\rangle$ is the mean angular deviation from the z -axis, in degrees, calculated as: $\int_0^{\pi/2} \beta \sin \beta e^{-U(\beta)/k_B T} d\beta / \int_0^{\pi/2} \sin \beta e^{-U(\beta)/k_B T} d\beta$).

ε	0	0.5	1	2.5	3.486	5	∞
S	0	0.0696	0.144	0.371	0.5	0.646	1
$\langle\beta\rangle$	57	54	51	40	34	27	0

Shorter lifetimes result in reduced magnetic responses. Rotational correlation times in the range $1 \text{ ps} \leq \tau_c \leq 100 \mu\text{s}$ (i.e. from very fast to very slow) were used.

The singlet yield was also calculated for the case of an entirely static, perfectly ordered ($\varepsilon = \infty$), non-relaxing radical pair, as described by Timmel *et al.* (2001) and Cintolesi *et al.* (2003).

3. RESULTS

For comparative purposes, we start with an approximate analytical result for the singlet yield Φ_S^{approx} of a perfectly ordered, completely static, non-relaxing radical pair, valid in the limits $k \ll \gamma_e B_0$ and $\gamma_e B_0$ much smaller than the energy-level separations produced by the hyperfine interaction (Timmel *et al.* 2001; Cintolesi *et al.* 2003)

$$\Phi_S^{\text{approx}} = \frac{7}{24} + \frac{1}{24}(3 \cos^2 \theta - 1). \quad (3.1)$$

Φ_S^{approx} varies from 0.375 when $\theta = 0^\circ$ to 0.250 when $\theta = 90^\circ$. The isotropic and anisotropic contributions to Φ_S^{approx} are 0.292 ($=7/24$) and 0.042 ($=1/24$), respectively.

Equation (3.1) may be compared with the numerical calculations described above for a static radical pair, by choosing a rotational correlation time ($\tau_c = 100 \mu\text{s}$) much longer than the radical pair lifetime ($k^{-1} = 1 \mu\text{s}$) (figure 2a). The anisotropic part of Φ_S indeed varies as $3 \cos^2 \theta - 1$ and the simulation for $\varepsilon = \infty$ (obtained numerically without perturbation theory assumptions; Timmel *et al.* 2001; Cintolesi *et al.* 2003) agrees well with equation (3.1). As the degree of ordering (i.e. ε and therefore S) is reduced, the amplitude of the reaction yield anisotropy decreases because the responses of radical pairs with different orientations tend to cancel one another. Under the conditions of figure 2a, the simulated values of Φ_S for finite ε are found empirically to be given by

$$\Phi_S \approx \frac{7}{24} + \frac{S}{24}(3 \cos^2 \theta - 1), \quad (3.2)$$

which is identical to equation (3.1) except for the scaling of the anisotropic part by the order parameter S .

We now look at radical pairs undergoing restricted rotational motion using the method outlined in §2. Figure 2b shows the effect on Φ_S of increasing the diffusion rate. Both the isotropic and anisotropic contributions decrease as τ_c is reduced from $100 \mu\text{s}$ to 10 ns . For $\tau_c = 10 \text{ ns}$, $\Phi_S \approx 0.25$, irrespective of the direction of the magnetic field. This statistical value for the singlet yield implies that the spin relaxation induced by rotational modulation of the anisotropic

Figure 2. Calculated dependence of the singlet yield, Φ_S , on the angle, θ , between the magnetic field vector and the radical pair alignment axis. (a) Variation of $\Phi_S(\theta)$ with the height of the alignment barrier, ε , for $B_0 = 50 \mu\text{T}$; $a = 1 \text{ mT}$; $\Delta A = 3 \text{ mT}$; $k = 10^6 \text{ s}^{-1}$; $\tau_c = 100 \mu\text{s}$. (b) Variation of $\Phi_S(\theta)$ with the rotational correlation time, τ_c , for $B_0 = 50 \mu\text{T}$; $a = 1 \text{ mT}$; $\Delta A = 3 \text{ mT}$; $k = 10^6 \text{ s}^{-1}$; $\varepsilon = 5$.

magnetic interactions in the radical pair is rapid enough to destroy its spin correlation before it recombines.

Focusing now on the part of Φ_S that contains directional information, we calculate the anisotropy $\Delta\Phi_S = \Phi_S^{\text{par}} - \Phi_S^{\text{per}}$ as a function of τ_c , where Φ_S^{par} and Φ_S^{per} are, respectively, the values of Φ_S when the magnetic field is parallel ($\theta = 0^\circ$) and perpendicular ($\theta = 90^\circ$) to the director. Figure 3a shows $\Delta\Phi_S$ for $1 \text{ ps} \leq \tau_c \leq 100 \mu\text{s}$ and the default set of parameter values (see caption). Not surprisingly, the reaction yield anisotropy is more pronounced the larger the degree of ordering. As already noted (figure 2b), $\Delta\Phi_S$ is significant for slow diffusion ($\tau_c > 1 \mu\text{s}$) and becomes smaller as τ_c decreases towards 10 ns as a result of spin relaxation. However, $\Delta\Phi_S$ rises again as τ_c is reduced beyond 10 ns. In the fast motion limit (where $\Delta A\tau_c \ll 1$), the anisotropic hyperfine interaction is motionally averaged over the distribution of molecular orientations, resulting in an effective interaction $\Delta A_{\text{eff}} = S\Delta A$, i.e. the true value scaled by the order parameter. In this limit, the magnetic response of the radical pair may be calculated by assuming no motion and perfect ordering, using ΔA_{eff} in place of ΔA . Such calculations agree very well with the $\tau_c = 1 \text{ ps}$ data in figure 3a.

The importance of spin relaxation may also be seen in figure 3b, which shows data calculated for radical pairs with a lifetime (k^{-1}) of 10 μs instead of 1 μs . With more time available for it to act, the attenuating

Figure 3. Calculated dependence of the anisotropy of the singlet yield, $\Delta\Phi_S$, on the rotational correlation time. (a) Variation of $\Delta\Phi_S(\tau_c)$ with the height of the alignment barrier, ε , for $B_0 = 50 \mu\text{T}$; $a = 1 \text{ mT}$; $\Delta A = 3 \text{ mT}$; $k = 10^6 \text{ s}^{-1}$. (b) Variation of $\Delta\Phi_S(\tau_c)$ with the height of the alignment barrier, ε , for $B_0 = 50 \mu\text{T}$; $a = 1 \text{ mT}$; $\Delta A = 3 \text{ mT}$; $k = 10^5 \text{ s}^{-1}$. (c) Variation of $\Delta\Phi_S(\tau_c)$ with the anisotropy of the hyperfine interaction, ΔA , for $B_0 = 50 \mu\text{T}$; $a = 1 \text{ mT}$; $k = 10^6 \text{ s}^{-1}$; $\varepsilon = 5$.

effect of relaxation is now much more pronounced and the minimum in $\Delta\Phi_S$ is broader. Relaxation effects become less important when the anisotropy of the hyperfine interaction is reduced. As ΔA decreases, the minimum in $\Delta\Phi_S$ becomes narrower and shallower (figure 3c).

4. DISCUSSION

We have used two parameters to characterize the disorder and motion of a molecule containing a magnetoreceptive radical pair: the order parameter S (determined here by the potential energy parameter, ε) and the correlation time τ_c (the average time the molecule would take to rotate through approximately one radian in the absence of an aligning potential). Figures 2 and 3 show that the reaction yield anisotropy, $\Delta\Phi_S$, in a 50 μT applied magnetic field can be quite severely attenuated when S is substantially less than unity and/or when τ_c is neither very large nor very small.

The dependence of $\Delta\Phi_S$ on S is easily understood: reducing the degree of molecular alignment within a static array of radical pairs causes their individual anisotropic responses to the magnetic field to interfere destructively. Without alignment ($S = 0$), this cancellation would be complete and no directional information would be available.

The effects of restricted rotational motion are more subtle but can be understood using elementary spin relaxation arguments familiar from magnetic resonance spectroscopy. Considering an anisotropic magnetic interaction of magnitude $\Delta\nu$ (in Hz), modulated by rotational motion with correlation time τ_c , two limiting cases can be identified: slow ($\Delta\nu\tau_c \gg 1$) and fast ($\Delta\nu\tau_c \ll 1$) motion. In the slow motion limit, the anisotropic interaction is not motionally averaged and the relaxation rate is $R \approx \tau_c^{-1}$. In the fast motion limit, the anisotropic interaction is averaged and the relaxation rate becomes $R \approx (\Delta\nu)^2\tau_c$. (The argument here is essentially identical to that used conventionally to describe the effects of slow and fast exchange processes on magnetic resonance spectra.) The maximum relaxation rate occurs when $\Delta\nu\tau_c \approx 1$, giving $R_{\max} \approx R_{\text{slow}} \approx R_{\text{fast}} \approx \Delta\nu$. Taking $\Delta A = 3$ mT, as above, we have $\Delta\nu \approx \gamma_e \Delta A / 2\pi \approx 10^8 \text{ s}^{-1}$ and therefore $R_{\max} \approx 10^8 \text{ s}^{-1}$ when $\tau_c \approx 10$ ns. Such an electron spin relaxation rate—approximately 100 times faster than the recombination of the radical pair—is sufficient to destroy the electron spin correlation and any magnetic field effect (figure 3a, $\tau_c \approx 10$ ns). For larger or smaller values of τ_c , the relaxation is slower than $R_{\max}$ and the reduction in $\Delta\Phi_S$ is less acute. These simple arguments also shed light on the dependence of $\Delta\Phi_S$ on ΔA . For example, when $\Delta A = 0.5$ mT instead of 3 mT, one predicts $R_{\max} \approx 2 \times 10^7 \text{ s}^{-1}$ at $\tau_c \approx 60$ ns, and indeed the dip in $\Delta\Phi_S$ (figure 3c) is shifted to longer τ_c and is less pronounced, consistent with the retention of a greater fraction of the spin correlation during the lifetime of the radical pair.

To draw quantitative conclusions about the range of acceptable S and τ_c values, one would need to know (among other things) how large a reduction in $\Delta\Phi_S$ could be tolerated before the ability of the radical pair to act as a magnetoreceptor became seriously compromised. This is currently unrealistic given the paucity of information on matters such as signal transduction and amplification, the number of magnetoreceptor molecules per cell, the total number of receptor cells, and so on. We therefore adopt the rough-and-ready measure that radical pair magnetoreception is unlikely to be viable if, individually, the effects of disorder and motion are such as to reduce $\Delta\Phi_S$ by more than about 50 per cent from the value expected for perfect ordering and complete immobilization. The 50 per cent figure, of course, is completely arbitrary but it does at least allow one to get an impression of how ordered and immobilized the arrays of cryptochrome molecules might need to be to act as a magnetoreceptor.

Looking first at the order parameter, $\Delta\Phi_S$ in the limit of slow diffusion is approximately proportional to S under the conditions of figure 2a (see also equation (3.2)). The 50 per cent condition is therefore satisfied for $S > 0.5$, which corresponds to a potential energy

parameter $\varepsilon > 3.5$ and a mean angular deviation of $< 34^\circ$ (table 1). Winklhofer (2009) has come independently to a similar conclusion using a model of disorder without molecular motion. To put this in context, $\varepsilon = 3.5$ means that the barrier to end-over-end rotation of the molecule ($\varepsilon N_A k_B T$) at 35°C is approximately 9 kJ mol^{-1} (somewhat less than the strength of a typical hydrogen bond). A sense of the range of thermally accessible orientations can be obtained from the angle at which $U(\beta) = k_B T$, i.e. $\beta = \sin^{-1}(1/\sqrt{\varepsilon})$, which equals approximately 30° when $\varepsilon = 3.5$. An order parameter of approximately 0.5 is therefore not a tight constraint on the ordering of the magnetoreceptor molecules.

The restrictions placed by the 50 per cent condition on the rotational correlation time are somewhat more complex in that they depend on the chosen values of ΔA and k . As argued in §2, $\Delta A = 3$ mT is an approximate upper limit on the hyperfine anisotropy for a ^1H or ^{14}N nucleus and $k^{-1} = 1 \mu\text{s}$ is probably close to the optimum radical pair lifetime. These values therefore give conservative estimates of the range of allowable values of τ_c . It is apparent from figure 2a that, when $S = 0.5$, the 50 per cent condition is met when either $\tau_c > 250$ ns or $\tau_c < 300$ ps (for $k = 10^6 \text{ s}^{-1}$). If ΔA is smaller than 3 mT then correlation times somewhat shorter than 250 ns or longer than 300 ps would be acceptable.

The condition $\tau_c < 300$ ps seems somewhat implausible in that it implies a molecule with M_r less than about 500 Da (or even smaller for a solvent more viscous than pure water). Although it is an intriguing possibility that a partially aligned small molecule undergoing rapid restricted rotational diffusion might have the magnetic properties conducive to effective geomagnetoreception, it seems unlikely that such an entity could satisfy all the other chemical, kinetic and structural constraints required of a viable magnetoreceptor (Rodgers & Hore 2009).

The other condition, $\tau_c > 250$ ns (or less, for $\Delta A < 3$ mT), seems more promising. The correlation time of a monomeric cryptochrome undergoing free rotational diffusion in pure water may be estimated using the Stokes–Einstein equation (Cavanagh *et al.* 2007). For a globular protein ($M_r = 50$ – 80 kDa) in water (viscosity, $0.89 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$) at 35°C one obtains $\tau_c = 20$ – 30 ns, i.e. close to the values at which relaxation effects are predicted to be most severe (figure 3). However, only a 10-fold reduction in diffusion rate (to $\tau_c = 200$ – 300 ns) would be needed to ensure that $\Delta\Phi_S$ is more than half the value it has when $\tau_c \gg 1 \mu\text{s}$. The required increase in τ_c would be less than 10-fold for $\Delta A < 3$ mT or for a multinuclear radical pair in which partial cancellation of the effects of the various anisotropic hyperfine tensors would result in an effective overall hyperfine anisotropy smaller than that of any individual nucleus (Cintolesi *et al.* 2003).

In light of the known variations in the strength of the Earth's field over geological time, we comment briefly here on the behaviour of our model compass in a magnetic field substantially smaller than $50 \mu\text{T}$. Compared with figure 3a ($50 \mu\text{T}$), the reaction yield anisotropy, $\Delta\Phi_S$, at $5 \mu\text{T}$ is smaller by a factor of

approximately 2.3 in the slow diffusion limit ($\tau_c = 100 \mu\text{s}$) and the minima caused by rapid relaxation are somewhat broader (the point at which $\Delta\Phi_S$ drops to half its value in the static limit changes from approx. 250 ns at 50 μT to approx. 600 ns at 5 μT). Qualitatively, the dependence on τ_c and ε is little changed from figure 3a. Although it would be imprudent to generalize on the basis of a single set of calculations, this may suggest that a radical pair compass could be relatively robust to geological fluctuations in the geomagnetic field intensity.

To conclude, perfect molecular ordering and complete immobilization, as in a crystal at low temperature, are not requirements for efficient radical pair magnetoreception. It would be acceptable to have radical pairs that are uniaxially ordered with a moderate order parameter, that undergo medium-amplitude orientational fluctuations with respect to the director and whose motional correlation time is longer than about a quarter of their lifetime.

Finally, how might the above conditions ($S > 0.5$ and $\tau_c > 250 \text{ ns}$) be realized for a cryptochrome *in vivo*? Rather than make possibly ill-informed speculations, we offer just one suggestion in the hope that it may inspire others better qualified than ourselves to come up with other proposals. By analogy with the outer segments of the rod neurons responsible for visual transduction, we imagine stacks of membranous discs contained within ordered receptor cells in the retina. Embedded within the membranes would be large, aligned proteins in the manner of the visual receptor protein, rhodopsin. A magnetosensitive cryptochrome specifically bound to such a protein would share its orientational ordering and motional restriction and so could have the properties necessary for efficient magnetoreception.

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