

A study of spin chemistry in weak magnetic fields

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This paper reviews the latest developments in the field of spin chemistry with a particular focus on the effects of weak static and/or oscillating magnetic fields (typically smaller than the average hyperfine coupling) on radical recombination reactions. Anisotropic magnetic field effects and their significance in the debate about potential mechanisms controlling magnetoreception in birds are discussed.

Keywords: spin chemistry; magnetic field effect (MFE); low-field effect (LFE); oscillating magnetic field effect (OMFE); reaction yield detected magnetic resonance (RYDMR)

1. Introduction

The discovery and analysis of nuclear and electron spin polarization phenomena about 40 years ago marked the birth of modern spin chemistry, the study of magnetic field effects on chemical reactions that proceed via radical pair intermediates. These highly reactive species determine the course of a wide variety of photochemical and biochemical processes and are therefore of great interest to chemists, physicists, biochemists and biologists (Steiner & Ulrich 1989; Hoff 1989; Steiner & Wolff 1991; McLauchlan & Steiner 1991; Halliwell & Gutteridge 1995). Investigation of the spin characteristics of these radicals and radical pairs via their behaviour under the influence of static and/or oscillating magnetic fields is one of the most powerful methods of studying these species and facilitates the elucidation of the underlying physics of many radical pair phenomena.

No other spin-correlated radical pair (SCRIP) has been studied as thoroughly as the radical species that form the essential electron transfer chain in the primary steps of photosynthesis. The investigation of the behaviour and lifetime of SCRIPs has led to a better understanding of the crucial initial events of photosynthesis. While the characteristics of this particular radical pair system have been studied intensively by many sophisticated methods (Hoff 1981, 1984, 1989, 1992), in particular magnetic resonance techniques, until recently little was known about the influence of weak static and oscillating magnetic fields (e.g. the Earth's magnetic field) on the behaviour of other radical pairs.

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However, within the last few years research into the effects of *weak* magnetic fields on magnetosensitive reactions has intensified due to the growing interest in the potential risks to human health of electromagnetic fields such as those produced by powerlines and mobile phones (Brocklehurst & McLauchlan 1996 and references therein). According to the World Health Organisation (WHO 1998), ‘It has been estimated that concerns about electromagnetic fields and health are now costing the US economy alone US\$ 1 billion’. The Interaction Association for Cancer Research (IARC), a specialized cancer research agency of WHO, recently classified extremely low frequency (ELF) magnetic fields as ‘possibly carcinogenic to humans, . . . a classification used to denote an agent for which there is limited evidence of carcinogenicity in humans and less than sufficient evidence for carcinogenicity in experimental animals’ (WHO 2001).

Simultaneously, the search for explanations for animal navigation and orientation phenomena has focused increasingly on the existence of a magnetic sense (Wiltschko & Merkel 1966). It has been proposed that in addition to a magnetic compass, animals might also have established a magnetic map, which would command a far higher accuracy of magnetic field sensing than the magnetic compass and most likely would require the animal to use two properties (e.g. the dip angle and the strength) of the Earth’s magnetic field (Edmonds 2001). In any case, the sensitivity of the animal to magnetic field parameters is extraordinary considering that the magnitude of the Earth’s magnetic field is only about 50 μT .

The interaction of SCRPs with magnetic fields presents itself therefore as a possible magnetosensitive mechanism to account for all of the above phenomena. In this paper some of the most recent experimental and theoretical developments in the field of spin chemistry are reviewed with a particular focus on the effect of weak static and/or oscillating magnetic fields on radical recombination reactions. Although the majority of the paper will concentrate on SCRPs in solution, §4 will be devoted to a theoretical discussion of radicals with anisotropic hyperfine interactions in ordered systems.

2. The radical pair mechanism

Radical pairs can be divided into two main groups according to their spin state. *Uncorrelated* radical pairs have electron spins oriented in a random manner and can, for example, result from a random encounter of two radicals in the bulk. Radical pairs created from a molecular precursor, under conservation of the total spin angular momentum, are *spin-correlated* radical pairs, i.e. the electron spins are mutually oriented in a certain way. When their orientation is anti-parallel, the total spin angular momentum quantum number, \mathbf{S} , is zero and the spin state is named after its spin multiplicity, ‘singlet state’ (S). When the spins are aligned parallel, \mathbf{S} equals unity, resulting in three ‘triplet states’ with overall magnetic quantum numbers $M_S = -1, 0, 1$ (T_-, T_0, T_+).

If the radical pair was initially born in a singlet state, certain processes and interactions may cause its spin state to change from singlet to triplet and vice versa. This evolution of the radical pair after its creation is of great significance to the consequent behaviour and reactivity of the radicals and is determined by the following three mechanisms (Salikhov *et al.* 1984).

The Δg mechanism. If neither electron spin couples to any magnetic nuclei, in order for S–T₀ evolution to occur, the electron spins must differ in their g values, i.e. their interaction with the external magnetic field, B . The frequency of the mixing is then given by the difference in precession frequency of the two electron spins, $(g_1 - g_2)\mu_B B \hbar^{-1}$, where g_1 and g_2 are the g values of electron spins \mathbf{S}_1 and \mathbf{S}_2 . This mechanism is only effective in the presence of external magnetic fields and the S–T₀ mixing frequency increases with growing external magnetic field (Salikhov *et al.* 1984).

The hyperfine mechanism. Consider a pair of radicals where only one of the electron spins, \mathbf{S}_1 , couples to a single magnetic nucleus of spin quantum number $I = \frac{1}{2}$ with hyperfine constant a . In high magnetic fields, the magnetic moments of both nucleus and electron, \mathbf{S}_1 , are quantized along the field direction. The magnetic moment of the nucleus acts like an additional local magnetic field on \mathbf{S}_1 . While \mathbf{S}_2 precesses with the unperturbed Larmor frequency, the precession frequency of \mathbf{S}_1 differs from this value by $\frac{1}{2}a$. Hence, the S–T₀ mixing frequency is given by $\frac{1}{2}a$. No S–T_± mixing is induced by this ‘high-field’ mechanism. In low magnetic fields (i.e. fields weaker than or comparable with the hyperfine field), the hyperfine mechanism induces transitions between the singlet and all three triplet levels (for a vector model, see Salikhov *et al.* (1984) and Brocklehurst & McLauchlan (1996)). In contrast to the situation in high magnetic fields, \mathbf{S}_1 now precesses around a total field which is the vector sum of external and hyperfine fields and does not coincide with the external field. Hence, the projection of the electron spin onto the external magnetic field direction changes as a function of time and S–T₊ and S–T_– transitions become possible.

Relaxation. Finally, both spin–spin and spin–lattice relaxation can lead to singlet–triplet interconversion. The former causes the equilibrium value of the projection of the spin angular momentum onto the field direction to be re-established after perturbation and leads to S–T₊ mixing. The latter destroys the phase coherence between the two precessing spins and causes S–T₀ transitions.

The evolution of the radical pair between singlet and triplet states is of crucial importance if singlet and triplet radical pairs undergo different fates on re-encounter. Usually, singlet radical pairs recombine on re-encounter to produce the ground state of the parent molecule while triplet radical pairs diffuse apart to give the radicals. Hence, influencing the efficiency of singlet–triplet mixing through applied magnetic fields can change the amount of singlet recombination product with respect to the yield of free radicals (or triplet recombination product).

3. The effect of weak static magnetic fields

In zero field, singlet–triplet mixing is fast and involves all three triplet substates. When the field is increased, two of the three triplet levels, namely T₊ and T_–, become progressively decoupled resulting in singlet–triplet interconversion being restricted to S and T₀ only. If the radical pair is originally created in a singlet state, this decrease in singlet–triplet mixing at high fields leads to an increase in the yield of the singlet recombination product. This is the ‘normal’ or ‘high’ magnetic field effect (MFE), where ‘high’ indicates the application of static magnetic fields much larger than

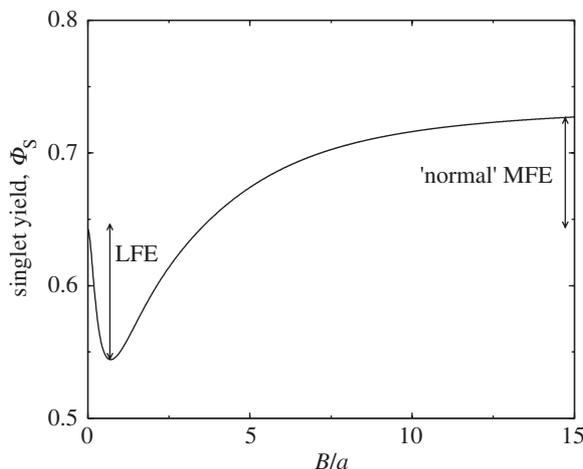


Figure 1. Typical curve for the magnetic field dependence of the singlet yield of a singlet-born radical pair. Magnetic field strength, B , is given in units of the average hyperfine interaction, a , in the system.

the typical hyperfine coupling in the radical pair. The ‘normal’ MFE has been well explored both in solution (for reviews, see Salikhov *et al.* (1984) and Steiner & Ulrich (1989)) and in the solid state, in particular in bacterial reaction centres (Boxer *et al.* 1983; Hoff *et al.* 1985; Steiner & Ulrich 1989; Volk *et al.* 1995). Figure 1 displays schematically a typical MFE curve showing the recombination yield of a singlet-born radical pair as a function of the magnetic field (in units of the hyperfine coupling).

Although the existence of a low-field effect (LFE) opposite in phase to the ‘normal’ MFE was first predicted in 1976 (Brocklehurst 1976) and low-field peculiarities in solution were frequently observed (Anisimov *et al.* 1982; Fischer 1983; Hamilton *et al.* 1989; Tarasov *et al.* 1993; Batchelor *et al.* 1993*a, b*), until recently little attention was focused on the effects of weak applied fields on radical recombination reactions.

However, in recent years, this particular field of spin chemistry has been investigated in more detail (Saik *et al.* 1995; Stass *et al.* 1995*a, b*; Brocklehurst & McLauchlan 1996; Saik & Lipsky 1997; Sacher & Grampp 1997; Timmel *et al.* 1998; Till *et al.* 1998; Toropov *et al.* 2000; Ritz *et al.* 2000, and references therein). A theoretical investigation of the origin of the LFE (Timmel *et al.* 1998) established that it arises from coherent superpositions of the electron–nuclear spin states in SCRPs in zero field. Once a small magnetic field is applied, some or all of the energy level degeneracies associated with these coherences might be lifted leading to an alteration in the efficiency of singlet–triplet interconversion. Consequently, the formation rate of singlet recombination products as well as free radicals arising from the encounter of triplet radical pairs is modified. The paper established the conditions under which a significant LFE might be observed. It was concluded that the radical pair must (i) recombine spin-selectively to give distinct singlet and triplet products, (ii) live long enough for significant evolution of the electron spins under the influence of the weak applied field to occur, (iii) have radical–radical interactions that are small compared with the average hyperfine coupling in the radical pair. Finally, (iv) relaxation in the radical pair must be slow compared with both spin evolution and radical recombination. Under these conditions it was found that weak static magnetic fields

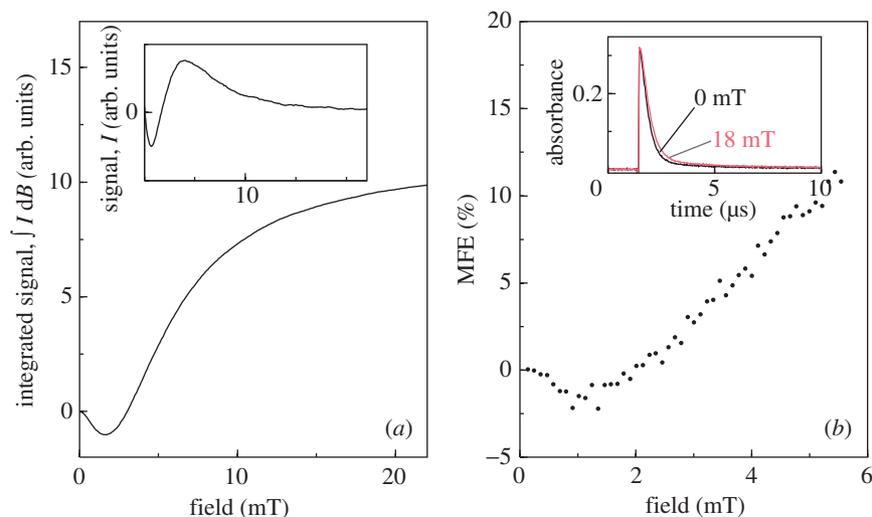


Figure 2. MFE graphs. (a) Singlet recombination of the radical pair $\text{Py-d}_{10}^{-\bullet}/\text{DMA}^{+\bullet}$ in a solution of cyclohexanol and acetonitrile. The inset shows the signal obtained from the phase-sensitive detector (first derivative). (b) MFE for the BP-SDS radical pair recombination as monitored via the absorbance of the protonated ketyl radical (see inset) as a function of applied magnetic field.

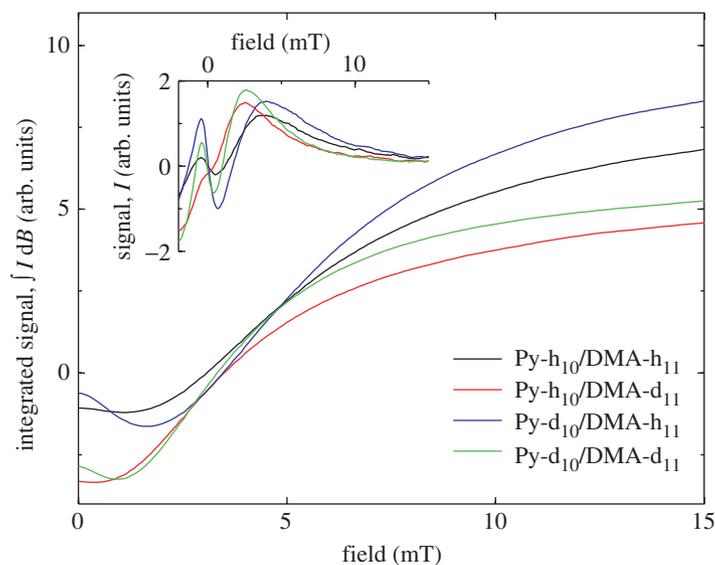


Figure 3. MFE graphs for four different isotopomeric $\text{Py}^{-\bullet}/\text{DMA}^{+\bullet}$ radical pairs in a solution of cyclohexanol and acetonitrile. The inset shows the signal obtained from the phase-sensitive detector (first derivative).

can decrease the singlet yield of a singlet-born radical pair by up to 40%. Provided the long lifetime limit is fulfilled so that $\tau^{-1} \ll a$ (where τ is the radical pair lifetime and a the average radical pair hyperfine coupling), the magnitude of the singlet recombination yield in zero field in excess of its statistical value of one-quarter is

simply three times that in a weak field. For all radical pair systems investigated (Till *et al.* 1998), the LFE was shown to be at least 10% irrespective of the number of magnetic nuclei, their arrangement and distribution between the radicals and whether or not the nuclei are equivalent. It was also shown that the size of the LFE decreases drastically with the rate of radical recombination, interaction between the radicals and spin relaxation.

Experimentally, LFEs have been observed frequently in recent years. In order to prolong the geminate lifetime of the pair, either the radicals are studied in micro-reactors such as micelles (Turro *et al.* 1988; Tarasov *et al.* 1989, 1993; Shkrob *et al.* 1991; Eveson *et al.* 2000) or ionic radical pairs are created whose Coulombic attraction opposes the radicals' separation. Radical ion pairs can be produced by radiolysis of hydrocarbon solutions (Anisimov *et al.* 1982; Stass *et al.* 1995*a, b*; Saik *et al.* 1995; Saik & Lipsky 1997) or photolysis of a solution of donor-acceptor pairs in polar solvents (Batchelor *et al.* 1993*b*; Sacher & Grampp 1997). Figure 2 displays typical MFE curves for such systems. Figure 2*a* shows the magnetic field dependence of the recombination reaction of a photochemically produced ionic radical pair consisting of a (deuterated) pyrene anion (Py-d₁₀⁻) and a dimethylaniline cation (DMA⁺) in a solution of cyclohexanol and acetonitrile. The recombination of the radicals from the singlet state produces a fluorescing exciplex. Consequently, the singlet recombination yield of this reaction can be measured conveniently via the fluorescence intensity of the exciplex. The applied magnetic field is audiofrequency modulated and the exciplex fluorescence passed through a photomultiplier in series with a phase-sensitive detector demodulating the signal at the same frequency. Consequently, only the magnetic field sensitive component of the fluorescence signal is detected and the signal-to-noise ratio greatly increased. As a result, the signal from the phase-sensitive detector corresponds to the first derivative of the fluorescence intensity with respect to the applied field, as shown in the inset of figure 2*a*. Integration of this signal results in the MFE spectrum shown in figure 2*a* with the LFE centred around 1.5 mT. The low-field effect in figure 2*a* is at its maximum value (*ca.* 10% of the MFE) around 1.5 mT, which, as predicted, is considerably smaller than the average hyperfine coupling in the radical pair system.

Figure 2*b* shows the MFE obtained inside a micelle for a system of neutral radicals created in a flash photolysis experiment. Benzophenone (BP) is used as the photoactive molecule which, in its excited triplet state, hydrogen abstracts from the alkyl chain of the surfactant, sodium dodecyl sulphate (SDS), to produce a neutral radical pair. The inset in figure 2*b* displays the absorption decay of the ketyl radical at 555 nm as a function of time in zero field and in the presence of an 18 mT field. The MFE is obtained by integrating the graphs between convenient time limits so as to minimize the contribution from other components such as the BP triplet state which has a lifetime of less than 400 ns in micellar solutions (Scaiano & Abuin 1981). The resulting MFE spectrum shows a pronounced LFE centred at *ca.* 1.2 mT. The magnitude and position of the LFE are strongly dependent on which surfactant is used (Tarasov *et al.* 1993) and on parameters that influence the size and stability of the micelle as well as the diffusion coefficient within the micro-reactor such as solution temperature and salt concentration (Eveson *et al.* 2000). It was concluded that the maximum LFE is expected for radical pair systems for which the escape rate from the micro-reactor is of the same order of magnitude as the recombination rate of the two radicals.

According to our theoretical studies (Till *et al.* 1998), the magnitude of the LFE should be independent of the number and arrangement of magnetic nuclei on the radicals. However, this statement is only true in the long lifetime limit and was derived using the exponential model (Kaptein & Oosterhoff 1969; Closs *et al.* 1970; Lawler & Evans 1971) to take into account spin kinetics. In this somewhat crude but convenient model, radical diffusion is ignored and both singlet and triplet radical pairs decay with first-order kinetics and a rate constant k to form recombination products and free radicals, respectively. Spin relaxation is ignored. Furthermore, the model does not allow for multiple encounters of the pair and assumes that spin motion and diffusion can be separated. Considering the shortfalls of the model, it is therefore not surprising that it is limited in predicting the size of the LFE quantitatively. Figure 3 shows the LFEs obtained for the four isotopomers of $\text{Py}^{\cdot-}/\text{DMA}^{\cdot+}$ in a solution of cyclohexanol and acetonitrile. Perdeuteration of one or both of the radicals results in a reduction of the proton hyperfine couplings by a factor of approximately 6.5 according to the ratio of the gyromagnetic ratios $\gamma_{\text{H}}/\gamma_{\text{D}}$ and a change in the nuclear spin quantum number ($I_{\text{H}} = \frac{1}{2}$, $I_{\text{D}} = 1$). The consequent magnetic isotope effect on the recombination yield of the radical pair is clearly demonstrated in figure 3 as the position, shape and magnitude of the LFE in the four radical pairs differ greatly. The LFE is most pronounced for the $\text{Py-d}_{10}^{\cdot-}/\text{DMA-h}_{11}^{\cdot+}$ and $\text{Py-d}_{10}^{\cdot-}/\text{DMA-d}_{11}^{\cdot+}$ systems with average hyperfine couplings of 0.23 mT/3.0 mT and 0.23 mT/1.6 mT, respectively, where the average hyperfine coupling of radical K to n nuclei of spin quantum number I is defined as $\langle a_K \rangle = \sqrt{\sum_i^n a_{Ki}^2 I_i(I_i + 1)}$ (Schulten & Wolynes 1978). When the protonated pyrene constitutes the anionic radical, namely for $\text{Py-h}_{10}^{\cdot-}/\text{DMA-h}_{11}^{\cdot+}$ and $\text{Py-h}_{10}^{\cdot-}/\text{DMA-d}_{11}^{\cdot+}$ with respective hyperfine couplings of 0.92 mT/3.0 mT and 0.92 mT/1.6 mT, the LFEs are visibly reduced. Similar results are found for the pyrene/aniline, pyrene/phenylaniline and pyrene/diphenylaniline systems. Furthermore, perdeuteration of the electron donor (ED = pyrene, anthracene, ethylcarbazole) also leads to an increased LFE in the ED-dicyanobenzene systems (using the three isomers of the dicyanobenzene). It therefore seems that in all exciplex systems used in these experiments the LFE is large when only one of the radicals exhibits significant hyperfine couplings, while the second radical is fully deuterated and hence, has very small hyperfine couplings. These data are of course in some disagreement with the theoretical prediction that the magnitude of the LFE is independent of the number or distribution of hyperfine couplings in the radical pair. However, one should remember that these conclusions were drawn within the framework of the exponential model imposing the long lifetime limit, assuming equal rate constants for singlet and triplet reactions and ignoring relaxation. Although the theoretical results provided us with analytical expressions facilitating a better understanding of the origin of the LFE, any quantitative interpretation of experimental data clearly requires more-sophisticated methods, such as the recent discussion of Hansen & Pedersen (2002). In their model, the relative motion of the radicals is described as free diffusion and a steady-state approach is applied to the stochastic Liouville equation. For a radical with a single spin- $\frac{1}{2}$ nucleus, analytical expressions for the quantum yield were obtained. However, the model needs to be extended to more realistic radical pairs with a larger number of hyperfine couplings before any of the above data can be modelled satisfactorily.

Furthermore, it might be of huge significance to include relaxation in the modelling of the above spectra (for a discussion of relaxation phenomena in weak and

zero magnetic field, see Fedin *et al.* (2003) and references therein) as the hyperfine coupling is altered through perdeuteration replacing an $I = \frac{1}{2}$ by an $I = 1$ nuclear spin, hence introducing quadrupolar nuclei which might contribute significantly to the relaxation of the radical pair. Additionally, as the singlet yield of this radical reaction is monitored via the exciplex fluorescence of the singlet recombination product, mass isotope effects on the Franck–Condon overlap between the ground and excited states might also be of some importance. However, their impact on data obtained using field-modulation techniques should be outweighed by the clearly significant magnetic isotope effects.

4. Anisotropic hyperfine couplings

As demonstrated in the last section, the effect of weak static magnetic fields on chemical reactions proceeding via radical pair intermediates is now well understood in terms of its theoretical origins and has been shown experimentally in a variety of systems. These results further strengthen the hypothesis that the radical pair mechanism (RPM) might be involved in the ability of birds to sense the geomagnetic field, as originally suggested by Schulten & Windemuth (1986). However, in order for the RPM to emerge as a viable alternative to other magnetosensitive mechanisms such as those based on magnetite particles and magnetosensitive light transduction pathways, the magnetosensitivity of the radical pair reactions needs to be anisotropic so as to provide the necessary source of orientation information. In a theoretical paper, Ritz *et al.* (2000) recently showed that a model radical pair subject to a 50 μT field and containing an anisotropic hyperfine coupling can produce significantly anisotropic singlet yields and hence provided a proof of the principle that the RPM could serve as a geomagnetic compass.

Detailed results for LFEs on a one-proton radical pair with axial and biaxial hyperfine tensors for a variety of radical pair lifetimes was presented by Timmel *et al.* (2001*b*). In agreement with other theoretical work (Timmel *et al.* 1998; Ritz *et al.* 2000), the recombination yield and hence the size of the MFE was shown to depend on the recombination rate relative to the size of the hyperfine couplings. It was furthermore observed that energy level crossings lead to sharp resonances in the singlet yield in analogy to the behaviour of long-lived radical pairs with many equivalent isotropic hyperfine couplings (Anisimov *et al.* 1982; Shushenko *et al.* 1983, 1985; Stass *et al.* 1995*b*; Canfield *et al.* 1996). The magnitude and position of these resonances was determined analytically. In the case of a purely axial symmetry of the hyperfine space, a maximum LFE (in the long lifetime limit) of 22% was found, about half that for the isotropic case (40%). The LFE in the presence of a single rhombic hyperfine coupling was found to be completely quenched.

A highly justified criticism of the model systems was their lack of a realistic number of hyperfine couplings with both axial and rhombic hyperfine anisotropy as would be expected for radical pairs generated by photoinduced electron transfer from cofactors in a protein. Cintolesi *et al.* (2003) therefore calculated the anisotropies of the singlet recombination yield of a multinuclear radical pair including up to eight magnetic nuclei using a model avian photomagnetoceptor consisting of a redox active cofactor flavin adenine dinucleotide (FAD), and a tryptophan amino acid. This system was based on a photoinduced radical pair produced in *Escherichia coli* DNA photolyase (Deisenhofer 2000), a member of a class of enzymes which are highly homologous

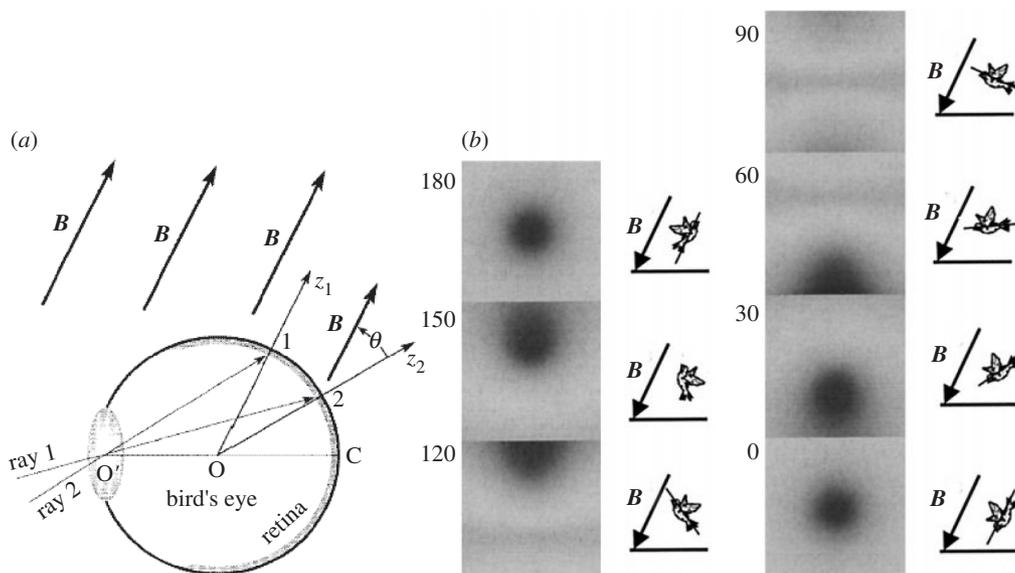


Figure 4. (a) Eye model used for the calculation of visual modulation patterns. The receptor molecules are oriented normal to the retina surface (directions z_1 and z_2) thus forming different angles with the direction of the magnetic field vector. (b) Visual modulation patterns through the geomagnetic field ($50 \mu\text{T}$) for a bird facing a uniformly grey screen looking into different directions at the angles 0° , 30° , 60° , 90° , 120° , 150° , 180° with respect to the magnetic field vector. The patterns are evaluated assuming radical pair receptors with anisotropic hyperfine couplings arranged in the eye model on the left. The schematics next to the modulation patterns indicate the corresponding direction in which a bird would fly in Urbana Champaign (geomagnetic field inclination of 68°). (Reproduced with kind permission from Ritz *et al.* (2000). Copyright (2000) Biophysical Society.)

to cryptochromes (see below) and contain the same FAD cofactor. The anisotropic response of the reaction yield subject to a magnetic field of *ca.* $50 \mu\text{T}$ was found to be dominated by two nitrogen nuclei in the flavin radical which have near-axial hyperfine couplings with almost collinear principal axes. It was also demonstrated that the anisotropy of the product yield depends little on the rate of radical reaction in the range $1\text{--}5 \mu\text{s}$.

Having established that *anisotropic* effects of weak static magnetic fields on SCRP reactions can *in principle* be detectable, the question arises as to how these primary magnetosensitive radical pair reactions might, after suitable amplification, be coupled to the nervous system. Ritz *et al.* (2000) developed a model to illustrate how a field-dependent radical pair process could affect processes involved in visual information transfer. They assumed that the radical pair process affects the sensitivity of light receptors in the eye resulting in a response pattern that varies over the hemisphere of a bird's eyeball, itself modelled as a pinhole camera (see figure 4a). The patterns that describe the view as a function of head orientation, calculated on the base of this eye model, are depicted in figure 4b.

Amongst a variety of other results discussed in detail in Ritz *et al.* (2000), the modulation pattern obtained clearly shows that the radical-pair-based compass is an inclination compass (as the 0° and 180° orientation produce the same pattern)

in agreement with experimental results in a variety of studies on migratory birds (Wiltschko & Wiltschko 1996). The Ritz model finally provides two proposals as to how the primary processes described above might be amplified to facilitate an RPM-driven magnetosensitivity. The first and more obvious suggestion proposes the existence of a radical-pair-based photoreceptor involved in the visual pathway whose reaction yield can be directly influenced by a magnetic field. The second hypothesis suggests that a *neurotransmitter* is part of a radical pair system or a decay product of the radical reaction. The former hypothesis would allow amplification of the MFEs through the processes involved in vision while in the latter the amplification would depend on the number of neuroreceptors. According to Ritz *et al.* cryptochromes, a recently discovered class of vertebrate photoreceptors, in connection with an as yet undiscovered second cofactor could be part of the magnetoreception system (see also Sancar 2003).

5. The effect of weak oscillating magnetic fields

The previous sections described how relatively weak static magnetic interactions influence the yields and rates of chemical reaction proceeding via radical pair intermediates. These discussions include the effects that 50 Hz fields produced by electrical appliances or overhead powerlines would impose on such RPM-controlled systems as the radical pairs live for only a small fraction of the 50 Hz oscillation period (a radical pair lifetime of 1 μ s would correspond to a 5×10^{-5} th part of a full 50 Hz cycle). Any *resonant* effects of time-dependent fields are expected to occur in the range of the hyperfine interaction frequency, i.e. for C-centred organic radicals with predominantly proton and nitrogen hyperfine couplings, between *ca.* 1 and 100 MHz.

The effects of magnetic fields oscillating at frequencies corresponding to hyperfine splittings in the radical pairs have been shown both theoretically and experimentally to change the yield of chemical reactions (Timmel & Hore 1996; Woodward *et al.* 1997; Stass *et al.* 2000; Timmel *et al.* 2001*a*). A decrease in product yield of up to 25% was predicted theoretically in the long lifetime limit for one-proton radical pairs and in analogy with the effects of weak static magnetic fields, the largest effects were predicted to occur for systems with singlet–triplet interconversion rates far faster than the rate of reaction of the radical pair. Canfield *et al.* (1994, 1995, 1996) developed perturbation theory methods to calculate the influence of multiple oscillating magnetic fields and applied them to radical pairs involved in coenzyme B₁₂ systems.

Experimentally, oscillating MFE (OMFE) spectra at zero static field have so far only been observed for radical ion pairs in solution. However, the spectra that are obtained using this OMFE technique give a fingerprint of the system investigated since resonances in the singlet yield occur centred around the average hyperfine couplings of each radical, as demonstrated in figure 5 and Woodward *et al.* (2001).

Static field measurements on the other hand report mainly on the average hyperfine coupling of the two radicals and hence provide less insight into the radical pair physics. Indeed, the OMFE spectra show clearly that the change in the singlet yield of the radical recombination reaction after deuteration of one or both of the radicals is mainly a magnetic isotope effect and unequivocally distinguish it from the more common mass isotope effects. Their interpretation therefore advances any discussion of the LFE of isotopomers as presented in the previous section. The OMFE allows the identification and characterization of paramagnetic species without the need for

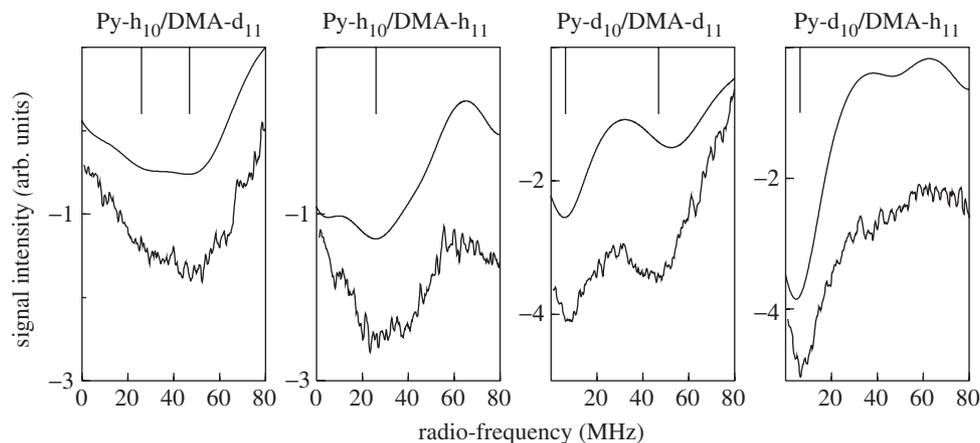


Figure 5. Calculated and experimental oscillating magnetic field effect spectra for the four isotopomeric $\text{Py}^{\cdot-}/\text{DMA}^{\cdot+}$ ionic radical pairs as a function of the frequency of the applied magnetic field (of strength $300 \mu\text{T}$). The vertical lines indicate the average hyperfine couplings of the radicals: $\langle a \rangle(\text{Py-h}_{10}^{\cdot-}) = 26 \text{ MHz}$, $\langle a \rangle(\text{Py-d}_{10}^{\cdot-}) = 6.4 \text{ MHz}$ and $\langle a \rangle(\text{DMA-d}_{11}^{\cdot+}) = 47 \text{ MHz}$. The average hyperfine coupling of $\text{DMA-h}_{11}^{\cdot+}$ of 84 MHz cannot be shown on this scale.

the application of strong static magnetic fields and hence can be understood as a kind of optically detected (zero field) electron paramagnetic resonance (ZF-EPR).

6. The effect of combined weak static and oscillating magnetic fields

While probing the characteristics of SCRPs using optically detected ZF-EPR is a recent technique, time-dependent magnetic fields oscillating at the frequency of the Zeeman splitting produced by static magnetic fields have long been used to study radical recombination yields, a technique known as reaction yield detected magnetic resonance (RYDMR). Traditionally, RYDMR experiments are performed in the presence of strong static fields so as to energetically isolate the T_+ and T_- levels from the singlet level. Consequently, the interconversion between the singlet and the T_+ and T_- levels is impeded altering the yield of singlet recombination product. When resonant microwave radiation is applied, reconnecting the T_+ and T_- levels with the $S-T_0$ manifold, the efficiency of singlet-triplet interconversion is boosted and the singlet recombination rate increases correspondingly.

Very few experimental RYDMR studies (Moehl *et al.* 1985) have been performed at fields weaker than 100 mT . Usually, the static fields applied are quite strong (Norris *et al.* 1982; Lersch & Michel-Beyerle 1989; Batchelor *et al.* 1992) and, consequently, the applied static magnetic field provides a quantization axis for the spin system. This is *not* the case for the low-field (LF) RYDMR experiments presented here. As shown theoretically (Morozov & Doktorov 1991*a,b*; Morozov *et al.* 1991), informative spectra can be obtained both in parallel and in perpendicular orientation of the two applied fields. LF RYDMR experiments and simulations were recently presented by Woodward *et al.* (2002) using static magnetic fields not exceeding 2.7 mT in orthogonal orientation to a radio-frequency field of amplitude $300 \mu\text{T}$ swept from 1 to 80 MHz . The ionic SCRPs investigated are derived from deuterated pyrene and 1,3-dicyanobenzene and, as before, the recombination yield is detected via the fluor-

escence of the singlet exciplex. These experiments explore the transition from zero static field (where the electron spins are quantized in the molecular frame of the rotating molecules) to high magnetic field where the electron spins are quantized along the direction of the applied static field. The size of the MFE signals obtained increases significantly between zero field and 2.7 mT applied static field. Although the spectrum obtained at the highest static field is mainly determined by the Zeeman splitting resonance, several features, such as the shoulders on the Zeeman peak are observed whose width and shape has been shown to be dependent on the radical pair's magnetic parameters (such as the hyperfine coupling). LF RYDMR therefore presents itself as a highly sensitive probe for testing the involvement of the RPM in chemical and biochemical processes.

While a wide variety of biological processes are known to involve radicals (Halliwell & Gutteridge 1995; Stubbe & van der Donk 1998) other biochemical mechanisms have been proposed to involve radicals and possibly radical pairs such as the avian compass mentioned above (Ritz *et al.* 2004, and references therein). Devising a highly sensitive technique that allows the involvement of the RPM in the magnetosensitive processes to be unequivocally distinguished from any other effects caused by the application of the magnetic field (such as tissue heating) is therefore of huge importance.

Our recent experimental work, depicted in figure 6, shows the strong dependence of the LF RYDMR spectra on the angle between the two applied fields. The parallel (0°) and perpendicular (90°) spectra are very different indeed, with the former showing an increase in the singlet yield just below the field corresponding to the Zeeman resonance and the latter depicting a significant decrease in the singlet yield at a field just above the Zeeman splitting. The spectra at intervening angles show features of both the parallel and the perpendicular orientation without being linear combinations of the two. The 45° spectrum includes both emissive and absorptive features and at this orientation of the fields the spectra are most sensitive to a change in angle. It emerges that LF RYDMR spectra show a great potential for the investigation of SCRPs in weak fields. Not only is their magnitude larger than either the LFE or the OMFE but their sensitivity to the orientation between the fields can be used to distinguish an RPM-mediated magnetic field dependence from almost any other magnetosensitive effect. The orientation dependence should be a unique feature of magnetic resonance induced changes in the singlet recombination yield, while all other mechanisms are likely to have to rely on the presence of magnetic fields rather than their specific orientation. It has therefore been proposed that LF RYDMR experiments should be used to determine whether the avian compass is indeed due to the RPM (Ritz *et al.* 2004). In their very recent experiment, Ritz *et al.* showed that the ability of a bird to navigate in the geomagnetic field is destroyed when an additional weak radio-frequency field is applied. The effect was shown to be dependent on the orientation of the geomagnetic field with respect to the applied oscillating magnetic field. The birds were not disoriented when the axes of oscillating field and geomagnetic field coincided. This study hence strongly suggests a magnetic compass based on the RPM.

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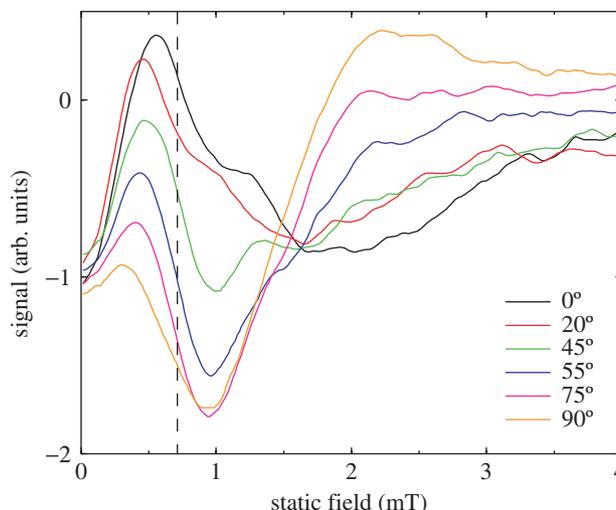


Figure 6. MFE spectra for the radical reaction of $\text{Py-d}_{10}^{+}/1,3\text{-DCB}^{-}$ in cyclohexanol and acetonitrile. The oscillating magnetic field has a frequency of 20 MHz and an amplitude of 300 μT . The static field varies between 0 and 4 mT. The orientation of the two fields is fixed at 0° , 20° , 45° , 55° , 75° and 90° , respectively. The applied oscillating magnetic field is modulated at 389 Hz and the singlet exciplex fluorescence signal is passed through a photomultiplier tube and demodulated in a phase-sensitive detector. The dashed vertical line indicates the magnetic field corresponding to the Zeeman resonance of 20 MHz.

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Christiane Timmel was born in Zwickau, Germany, in 1971. In 1994, she graduated from the University of Dresden with a first-class Diploma in Chemistry. She worked under the supervision of Professor Peter Hore at the University of Oxford first as a Visiting student and later as a DPhil student during which time she was awarded a Florey EPA scholarship by The Queen's College. In 1998 Dr Timmel was elected the Beale Fellow in Chemistry at St Hilda's College, Oxford, and took up a Royal Society Dorothy Hodgkin Fellowship. She currently holds a Royal Society University Research Fellowship and was elected to a Tutorial Fellowship at St Hilda's College in 2003. Dr Timmel's research interests are focused on the effects of magnetic fields on chemical and biological systems. These range from the investigation of magnetic field effects on radical recombination reactions and paramagnetic convection phenomena to the application of electron paramagnetic resonance to the study of biological and biochemical systems.

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