

## Magnetic field effects on radical pairs photochemically derived from 2,3,6,7-dibenzofluorenone

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### Abstract

2,3,6,7-Dibenzofluorenone triplets react with triethylamine, 2,4,6-trimethylphenol and ascorbyl-palmitate in micellar solutions to yield radical pairs which are sensitive to magnetic field effects whenever separation is inefficient. The three substrates afford three distinctively different scenarios with respect to the confinement of daughter radicals within the micellar phase. Triethylamine and corresponding radicals are soluble in water, which results in rapid entry–exit equilibria with the micellar phase, and negligible magnetic field effects. Conversely, ascorbyl-palmitate and its radicals are virtually insoluble in water, thus being confined to the micellar phase. Application of an external magnetic field attenuates the geminate recombination of radicals, probably through slowing down  $T_+ \leftrightarrow T_0$  and  $T_- \leftrightarrow T_0$  interconversions. The case of 2,4,6-trimethylphenol is intermediate, with the proportion of radicals that escape geminate recombination increasing with increasing applied field. The relevance of these reactions in relation to the use of 2,3,6,7-dibenzofluorenone or molecules of comparable solubility as a triplet probe in biological systems is discussed. © 1998 Elsevier Science S.A.

**Keywords:** 2,3,6,7-Dibenzofluorenone; Magnetic field effects; Radical pairs

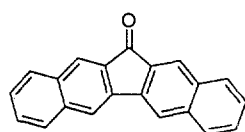
### 1. Introduction

The influence of external magnetic fields on the behavior of radical pairs in supramolecular systems has been the subject of numerous studies during the last two decades. In particular, the behavior of radical pairs in micellar systems is now reasonably well understood and many examples are available in the literature [1–5]. In the last few years, there has been considerable concern in relation to possible health risks caused by environmental magnetic fields [6]. Surprisingly, the possibility that such effects could be due to the influence of environmental fields on free radical processes *in vivo* has only very recently received serious consideration [7–9]. In our own laboratory we have been concerned with establishing the molecular basis for such effects [8,10], and the conditions under which they may be of importance in biological systems. For example, recent work has explored the relationship between AC and DC fields [10], the dynamics of radical pairs incorporated onto proteins and DNA [11], and effects involving biologically relevant substrates such as vitamin E [12] and melatonin [13].

As our studies in systems of more direct relevance to biological and health issues progressed, it became obvious that many of the molecules employed in earlier work lack some of the key characteristics that may be desirable for these studies. For example, very few examples in the literature deal with planar molecules (e.g. xanthone) [14,15] of the type that could best intercalate into the DNA structure. Many of the ketones employed as radical precursors in micelles (such as the widely studied dibenzyl ketone [2,5,16–18]) have such low extinction coefficients that they are totally inadequate at the low concentrations required for biological work. In those systems where the radicals are formed via ketone photoreduction, the resulting ketyl radicals are generally more hydrophilic than the starting ketone and tend to separate rather quickly in the sometimes poor confinement that biomolecules offer to these foreign substrates [11].

During recent work on naphthyl-substituted carbonyl compounds [19], we noted that 2,3,6,7-dibenzofluorenone (DBF) had many desirable properties for this type of research. In this article we report a study of magnetic field effects on the chemistry of radicals derived from DBF in micellar systems; our work aims at providing the molecular basis for a molecule with good potential as a probe for these effects in biological systems.

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DBF

## 2. Experimental

Sodium dodecyl sulfate (SDS) from Fluka was used as received. Cetyl-trimethylammonium chloride (CTAC) was purchased from K and K Labs. 2,3,6,7-Dibenzofluorenone (DBF) was synthesized according to the method of Cook and Preston [20].

The laser flash photolysis facility in our laboratory allows the monitoring of reaction intermediates in the nanosecond and microsecond time scales. In this study, we have used for excitation the pulses from a Lumonics excimer laser (308 nm,  $\sim 6$  ns,  $\leq 60$  mJ pulse<sup>-1</sup>). Our automated system uses a Tektronix 2440 digitizer to capture the signals from the monochromator-photomultiplier detection system. The information was then transferred to a PowerMacintosh computer which provided control and processing software written in the LabVIEW 3.1.1 environment from National Instruments. Other aspects of this instrumentation are similar to those described in earlier reports [21,22]. Magnetic fields were applied with a homemade electromagnet driven by a Xantrex XKW 3000 W DC power supply and is capable of DC fields of up to 6000 G.

## 3. Results and discussion

2,3,6,7-Dibenzofluorenone is a near-planar aromatic ketone. The photochemistry of DBF is dominated by the excited triplet state, which has  $\pi$ ,  $\pi^*$  character. The triplet, <sup>3</sup>DBF, reacts in homogeneous solutions predominantly by electron abstraction from suitable electron donors. One electron transfer reactions of <sup>3</sup>DBF with triethylamine, ascorbyl-6-palmitate, and 2,4,6-trimethylphenol were studied by laser (308 nm) flash photolysis in acetonitrile at 20°C [19]. The reaction rate constants are summarized in Table 1.

The reactions of <sup>3</sup>DBF generate the DBF radical anion in addition to the substrate radicals. The typical reaction sequence is illustrated in Fig. 1. We note that the DBF radical anion absorbs strongly at 400 nm [19], where the contributions of the absorptions due to other substrate radicals may be neglected. In any event the kinetic analysis for radical pair decay is identical regardless of whether one monitors a single radical, or combined absorptions from both radical pair partners. Similarly, the analysis is also the same for radicals or radical-ions, although in the latter case radical exit probabilities may be more strongly dependent on micellar charge.

Table 1 shows that the quenching rate constants for the reaction of <sup>3</sup>DBF with the donors examined are reasonably fast in acetonitrile solutions. Such rates enable rapid and

Table 1

Reaction rate constants for the quenching of <sup>3</sup>DBF determined by laser (308 nm) flash photolysis in acetonitrile at 20°C

Reactant (D)	$k(^3\text{DBF} + \text{D})^a$ , M <sup>-1</sup> s <sup>-1</sup>
Triethylamine	$4.6 \times 10^{10}$ <sup>b</sup>
2,4,6-Trimethylphenol	$9.2 \times 10^{10}$ <sup>b</sup>
Ascorbyl-6-palmitate	$1.8 \times 10^9$

<sup>a</sup>Accurate to  $\pm 10\%$ .

<sup>b</sup>From Ref. [19].

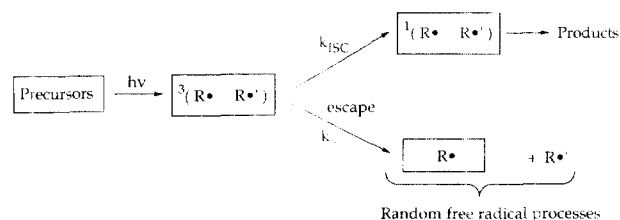


Fig. 1. Schematic representation of the competition between radical pair intersystem crossing and escape.

efficient generation of the triplet radical pairs in micellar solutions for the studies of effects of external magnetic fields (MFE). It is important to realize that DBF is almost completely insoluble in water, which probably applies also to the corresponding ketyl radical. Therefore, external magnetic fields are expected to affect the competition between spin evolution and separation of the radical pair (Fig. 1); separation can only result from escape of donor-derived radicals since the radical from DBF is too hydrophobic to exit. The electron-transfer reactions with water soluble amines, which lead to the generation of charged DBF radical anions and amine radical cations, enable the investigation of MFE on the reactions with rapid escape of one of products. Similar escape characteristics are expected for the <sup>3</sup>DBF reaction with 2,4,6-trimethyl phenol, where the neutral phenoxyl radical can escape from the micelle and separate from the neutral DBF ketyl radical. Conversely, the reaction with water insoluble ascorbyl-6-palmitate will be similar to the recombination of a triplet biradical [23–25], since the transient products cannot escape the micelle.

The reactions of <sup>3</sup>DBF were investigated in aqueous solutions of CTAC and SDS micelles. CTAC is a cationic detergent, which forms spherical micelles at concentrations  $\leq 0.05$  M [26]. The aggregation number is  $93 \pm 4$ , which means that at 0.05 M CTAC the effective 'concentration' of micelles is 0.54 mM. Given the approximate molecular dimensions of the probes used, DBF ( $d \sim 14$  Å), 2,4,6-trimethylphenol ( $d \sim 7$  Å), and even ascorbyl-6-palmitate ( $d \sim 27$  Å) can be easily accommodated within the hydrophobic interior of the CTAC micelle. SDS micelles are somewhat smaller. Although the 'concentration' of micelles, 1.5 mM (0.1 M SDS and aggregation number [26]  $67 \pm 2$ ), is higher than in aqueous CTAC, the diameter of the micelle is only ca. 30 Å. This may be important for the reactions with ascorbyl-6-palmitate, especially since the ascorbyl moiety ( $pK_1 = 4.17$ ;

$pK_a = 11.57$ ) is hydrophilic and would tend to be closer to the hydrated polar head groups in the surfactant.

The reaction of  $^3\text{DBF}$  with triethylamine was studied in both aqueous CTAC and SDS. The rate constant was found to depend linearly on the concentration of the amine, in the range from 0 to 10 mM triethylamine, giving  $k = 4.5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ , which is in line with the value in acetonitrile [19]. The linear dependence of the  $^3\text{DBF}$  decay on the bulk concentration of amine indicates a fast entry/exit equilibrium for triethylamine. No magnetic field effect was observed on the behavior of the radicals, and the long lifetime for the DBF ketyl ( $\geq 50 \mu\text{s}$ ) is indicative of the loss of its reaction partner. In other words, in the time scale monitored ( $> 20 \text{ ns}$ ) there are no spin correlated radical pairs in the system. This is not unusual; in a related example, the photolysis of 1,1-diphenylacetone leads to diphenylmethyl and acetyl radicals. The latter exits the micelle very rapidly and results in the absence of magnetic field effects [27].

In contrast to triethylamine, 2,4,6-trimethylphenol is practically insoluble in water, and are thus resides predominantly in the micellar phase. The reaction of the  $^3\text{DBF}$  with 2,4,6-trimethylphenol generates a triplet radical pair, whose decay depends on the external magnetic field. Fig. 2 illustrates the decay of the triplet radical pair monitored at 400 nm both in the absence of an external magnetic field and at 3000 G. The observations compare well with those for other ketone-phenol systems [12].

Clearly, the decay of the triplet radical pair consists of two parts. The initial, faster decay (620 ns in Fig. 2) corresponds mainly to the geminate recombination of the radicals within the micellar cage. The slower component, which decays over longer times (hundreds of microseconds), corresponds to the subsequent reactions of the phenoxyl radicals which escape the micelles. Fig. 2 shows that the rate of the geminate recombination is affected by the magnetic field. Gradual shutting down of the spin evolution from triplet sublevels  $T_{-1}$  and  $T_{+1}$  with the increasing external field leads to the higher proportion of the radicals escaping the micellar cage, as illustrated by the increase in residual absorbance. Figs. 3 and 4 illustrate the decrease in the geminate recombination rate and the concomitant increase in the escape of the radicals in aqueous CTAC and SDS micellar solutions.

Kinetic analysis of the traces in Fig. 2 lead to a two component decay that can be adequately fit with a combination of first and second order process; the fit of the latter in terms of absorbance yields  $2k/\varepsilon\ell$ , where  $k$  is the second order rate constant,  $\varepsilon$  the extinction coefficient of the transient at the monitoring wavelength, and  $\ell$  the optical path (0.7 cm in our case). For the first order component we obtain first order rate constants of  $1.61 \times 10^6$  and  $0.58 \times 10^6 \text{ s}^{-1}$  in the absence and presence of a field, respectively. The decrease is due to the slow down of intersystem crossing processes (Fig. 1). For the second order (slow) decay we obtain  $2k/\varepsilon\ell$  values of  $0.38 \times 10^6$  and  $0.21 \times 10^6 \text{ s}^{-1}$  in the absence and presence of a field, respectively. The magnetic field effect on the bimolecular decay (involving random radical encounters) has a

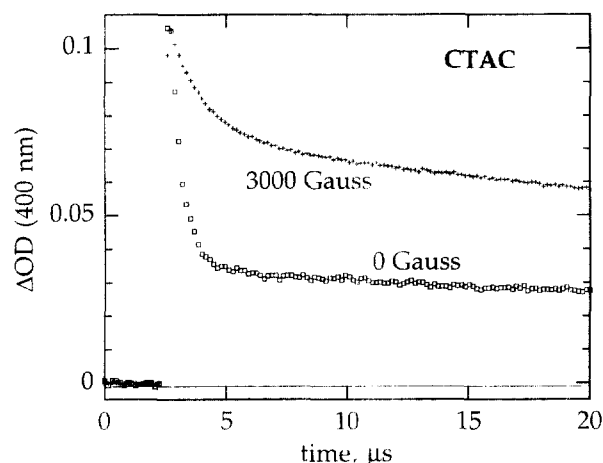


Fig. 2. Decay of the triplet radical pair from DBF and 2,4,6-trimethylphenol in aqueous CTAC solution. Laser (308 nm) flash photolysis of  $10 \mu\text{M}$  DBF and 5 mM 2,4,6-trimethylphenol solubilized in  $\text{N}_2$ -saturated aqueous solution of 50 mM CTAC.

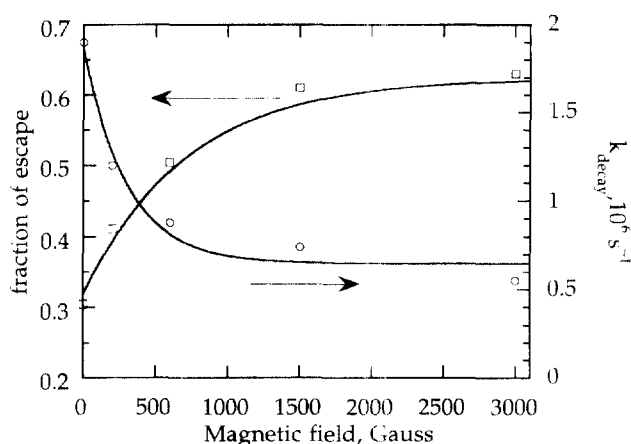


Fig. 3. Magnetic field effects on the decay of the radical pair from DBF and 2,4,6-trimethylphenol in aqueous CTAC. Experimental conditions are the same as in Fig. 2.

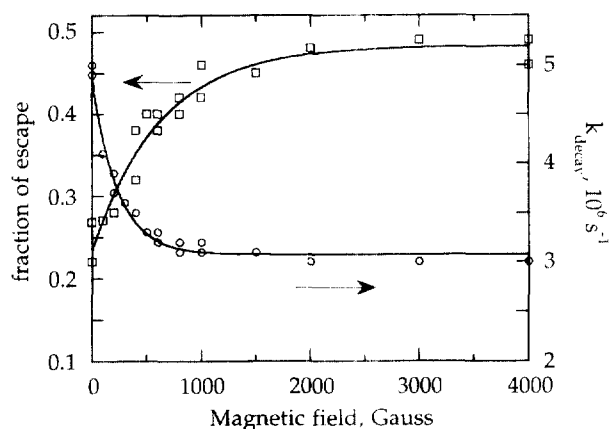


Fig. 4. Magnetic field effects on the decay of the radical pair from DBF and 2,4,6-trimethylphenol in aqueous SDS. Laser (308 nm) flash photolysis of  $10 \mu\text{M}$  DBF and 5 mM 2,4,6-trimethylphenol solubilized in nitrogen-saturated aqueous solution of 0.1 M SDS.

similar origin to that of the geminate process and is well documented in the literature [28].

In CTAC micelles the proportion of escape radicals increases from about 30% to 60% by application of a magnetic field. An almost exactly opposite trend is observed for the rate constant of the geminate recombination of radicals. The steepest increase is in the magnetic field range from 0–1000 G. At higher magnetic fields, that is >2000 G, the escape rate constant and the proportion of escape radicals level off. The maximum increase in the proportion of escape radicals with the magnetic field is somewhat smaller in SDS (from 0.23 to 0.48) than in CTAC solutions (from 0.32 to 0.62). However, the field required to induce one half of the maximum effect is similar (430 G for SDS and 500 G for CTAC), suggesting that the same mechanism is operative.

The steady decline and leveling off of the rate constant for the observable radical decay may be intuitive, but is not in accord with a simple kinetic model in which intersystem crossing from sublevels  $T_+$  and  $T_-$  slow down and eventually shut off, while  $T_0$  remains unchanged. Fig. 5a and b. Such a mechanism predicts that the experimental decay rate constant will follow single exponential behavior at zero field, slow-down and become biexponential (or even more complex) at intermediate fields, and eventually become single exponential, with the same rate constant as at zero field, when the field is sufficiently high to totally shut off intersystem crossing from the  $T_+$  and  $T_-$  sublevels. This type of behavior has been confirmed experimentally [12]. That observed in Figs. 3 and 4 can be rationalized by a similar mechanism, but where the sublevels  $T_+$  and  $T_-$  interconvert with  $T_0$  in the time scale in which the evolution of the spin correlated radical pair takes place. This is illustrated in Fig. 5c, and has been observed in some bromine substituted thiophenes [29].

Ascorbyl-6-palmitate is an oil-soluble synthetic antioxidant, which on account of its long aliphatic tail, is practically insoluble in water. Since the oxidation of ascorbyl-6-palmitate to a resonance stabilized ascorbate radical does not affect the hydrophobic palmitate tail, it is expected that the radical will also be insoluble in water. Consequently, neither radical in the triplet radical pair generated by the DBF triplet oxidation of ascorbyl-palmitate is expected to escape the micelle in the time scale required for spin evolution (Fig. 6). Note the small amount of residual absorbance in the absence of a field. This is unlikely to reflect radical exit from the micelle, given the size and hydrophobicity of the reactants. Most likely, this residual is the result of some photoionization of DBF, leading to its cation and a hydrated electron. The latter would exit rapidly from the negatively charged SDS micelle. Similar processes are well characterized in other systems [30–32]. It is very likely that a small degree of photoionization also occurs with other donors (e.g. phenols), but this small contribution is hidden by the much more important radical exit, which would then be slightly overestimated in those systems (vide supra).

The influence of the external magnetic field on the decay kinetics (almost exclusively geminate recombination) of the

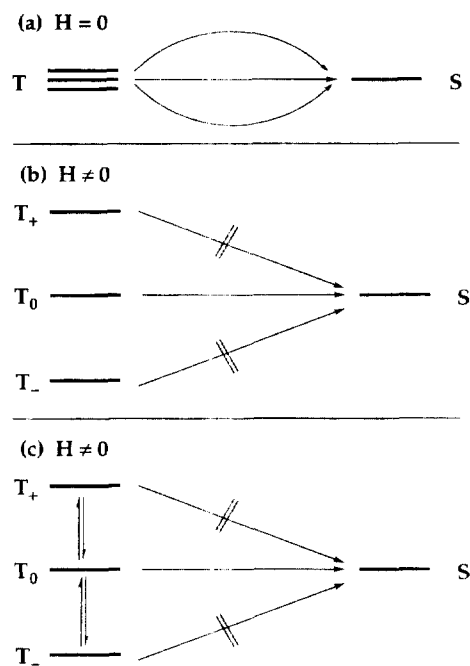


Fig. 5. Schematic representation of Zeeman splitting of the triplet sublevels upon application of an external magnetic field. Note that intersystem crossing from  $T_+$  and  $T_-$  has been shut-off in (b), and that interconversion of the triplet sublevels is permitted in (c). At  $H=0$  [see (a)] the three triplet sublevels and the singlet level are assumed to be degenerate.

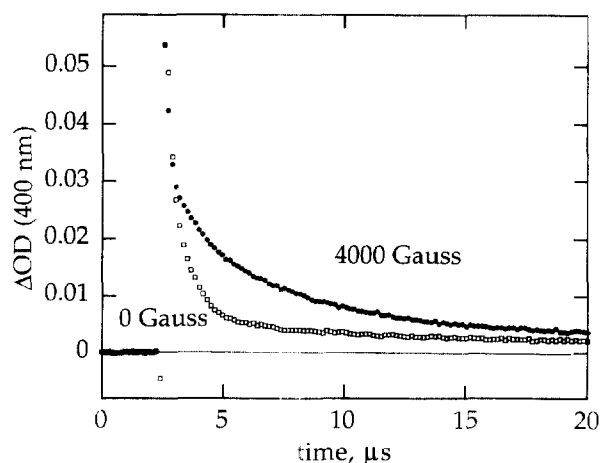


Fig. 6. The decay of the triplet radical pair from DBF and ascorbyl-6-palmitate following laser (308 nm) flash photolysis of 10  $\mu$ M DBF and 5 mM ascorbyl-6-palmitate solubilized in nitrogen-saturated aqueous solution of 0.1 M SDS.

DBF ketyl and ascorbyl-6-palmitate radicals in aqueous SDS and CTAC is presented in Fig. 7.

The rate constant for geminate recombination decreases with the increase in magnetic field. There are however indications—particularly in SDS—that a small decline continues even at relatively high fields. It is very unlikely that this reflects influence of the field on the  $T \leftrightarrow S$  interconversion. More probably, the decline is caused by a slow down of  $T_+ \leftrightarrow T_0$  and  $T_- \leftrightarrow T_0$  interconversions at high fields.

The steepest decrease is in the range from 0 to 1000 G, similar to the behavior of the reaction of DBF with 2,4,6-

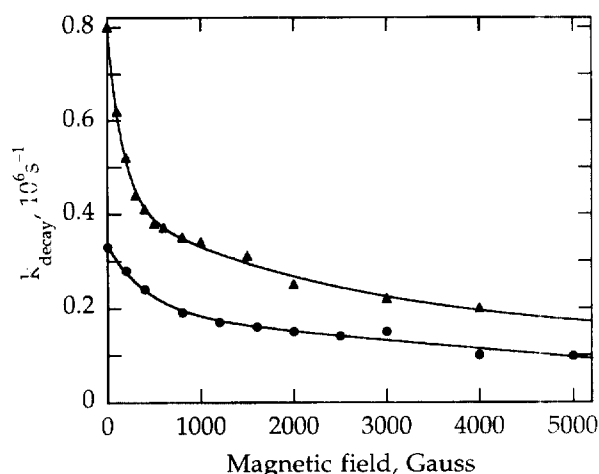


Fig. 7. Magnetic field effects of the decay the triplet radical pair from DBF and ascorbyl-6-palmitate in aqueous SDS and CTAC solutions. Laser (308 nm) flash photolysis of 10  $\mu$ M DBF and 5 mM ascorbyl-6-palmitate in nitrogen-saturated aqueous solution of 0.1 M SDS ( $\blacktriangle$ ) or 50 mM CTAC ( $\bullet$ ).

trimethylphenol, where the field should influence readily the  $T \leftarrow S$  evolution. However, there is a significant difference between the geminate recombination of the DBF ketyl and ascorbyl-6-palmitate radicals in SDS and CTAC micelles. The maximum and minimum rate constants differ by a factor of  $\sim 2$ , e.g.  $8 \times 10^5 \text{ s}^{-1}$  in SDS vs.  $3.3 \times 10^5 \text{ s}^{-1}$  in CTAC. It might be suggested that the different behavior of the geminate radical pair in SDS and CTAC solutions originates from the different charges of the micellar head groups. Positively charged CTAC will stabilize ascorbyl radical anions thus slowing down their decay, whereas the opposite is true for the negatively charged SDS.

#### 4. Conclusion

DBF has the potential for applications in biological systems in cases where radical pair formation is anticipated to result from electron transfer interactions (as opposed to hydrogen abstraction). The near-planar shape and size of DBF will probably make it a good candidate for DNA intercalation.

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