Magnetic field effect on singlet oxygen production in a biochemical system†

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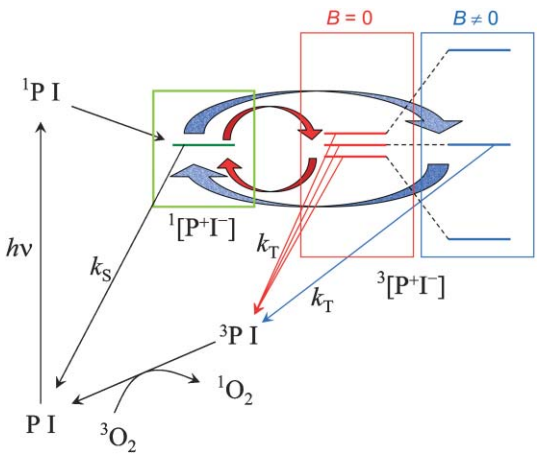
The yield of singlet oxygen sensitized by chemically modified, carotenoidless bacterial photosynthetic reaction centres and the ensuing oxidative damage are both shown to be magnetic field-dependent.

There is a large but mainly inconclusive literature on the effects of weak magnetic fields on living systems.[1] Although the influence of magnetic fields on the rates and product yields of a host of chemical reactions is well documented and can be understood in the framework of the Radical Pair Mechanism (RPM),[2,3] it has so far proved impossible to demonstrate convincingly a potentially damaging biological RPM effect. Here we present proof that a biochemical system, in which the RPM is known to operate, can generate toxic products in amounts that depend on the presence of a relatively weak applied magnetic field.

In plants and photosynthetic purple bacteria, absorption of light by light-harvesting (bacterio)chlorophylls initiates a series of rapid energy transfers that funnel electronic excitation energy into the reaction centre protein complex where it is trapped by the photochemically active pigment P. Elevation of P to an excited singlet state is followed by the transfer of an electron to a second pigment I, thus producing a radical pair $^1[P^+I^-]$ in a singlet state (antiparallel electron spins). This is normally followed swiftly by a second electron transfer to a quinone Q, well before $[P^+I^-]$ can recombine. If Q is extracted or chemically reduced, however, $^1[P^+I^-]$ lives long enough ($\sim$10 ns) to allow either recombination to the singlet ground state or conversion to the triplet state $^3[P^+I^-]$ (parallel electron spins) which can recombine to give an excited triplet state of the primary electron donor, ^{3}P (Scheme 1).

The fraction of the spin-correlated radical pairs that recombine *via* their singlet or triplet states is controlled by the rates of the two electron transfer processes (k_S and k_T in Scheme 1), but also by the extent and frequency of the interconversion of $^1[P^+I^-]$ and $^3[P^+I^-]$ (indicated by curved arrows in Scheme 1), a process that is governed mainly by the electron–nuclear hyperfine interactions and electron Zeeman interactions of the two radicals.[4] As $^1[P^+I^-]$ and $^3[P^+I^-]$ are almost degenerate in zero-field ($B = 0$ in Scheme 1), all three triplet sub-levels become significantly populated in the absence of an applied magnetic field. However, when a strong magnetic field is applied ($B \neq 0$ in Scheme 1), only one of the three sublevels is accessible from the singlet state. Thus, the yield of ^{3}P drops as the applied magnetic field is increased. ^{3}P is potentially dangerous, because it can be quenched by molecular oxygen resulting in the formation of the highly reactive singlet state, $^1O_2$ ($^1\Delta_g$), a species that has been implicated in a variety of biological damage, including lipid peroxidation.[5] In wild-type reaction centres from the photosynthetic bacterium *Rhodobacter* (*Rb.*) *sphaeroides*, $^1O_2$ is not normally formed because ^{3}P is rapidly quenched by a nearby carotenoid molecule; at room temperature the lifetime of ^{3}P is a few hundred nanoseconds.[6] In the carotenoidless mutant R-26, however, it is much longer lived (49 μs[7]), allowing ample time for the formation of $^1O_2$, which is known to attack the reaction centre.[8] Since the yield of ^{3}P depends on the intensity of the applied magnetic field, the amount of $^1O_2$ should also be field-sensitive.

We have studied the magnetic field dependence of the light-induced formation of $^1O_2$ in Q-depleted reaction centres from wild-type *Rb. sphaeroides* and its carotenoidless mutant R-26 suspended in a perdeuterated buffer, saturated with oxygen. Singlet oxygen was monitored *via* its time-resolved near-infrared phosphorescence at 1270 nm[9,10] following flash excitation of the reaction centre at 532 nm.† A signal decaying with a time constant of 43 μs was observed for the R-26 mutant, whereas no long-lived (>15 μs) emission was found for wild-type reaction centres, consistent with the very rapid energy transfer to the carotenoid.[6] The 43 μs signal for the R-26 mutant was assigned to $^1O_2$ because this signal was not observed in the presence of 1 mM sodium azide, an efficient $^1O_2$ quencher. The observed lifetime is somewhat shorter than that reported for $^1O_2$ in D_2O (68 μs)[11] due to the presence of the protein complex, the solvent and residual H_2O in the solution. A quantum yield of 9 $\pm$ 4% was determined using rose bengal as a standard.†



Scheme 1

† Electronic supplementary information (ESI) available: experimental procedures and measurement of $^1O_2$ quantum yield. See http://www.rsc.org/suppdata/cc/b4/b413489c/

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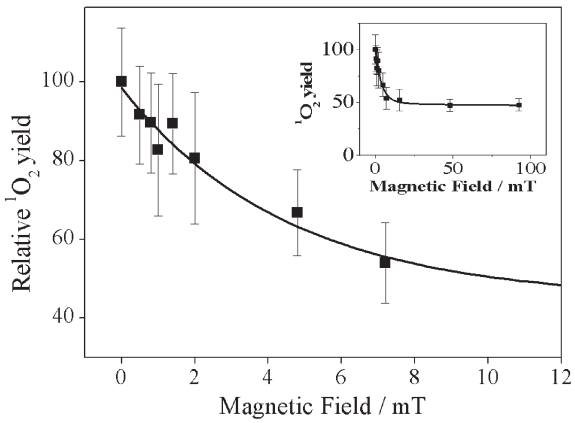

Fig. 1 Relative $^1O_2$ yield in Q-depleted reaction centres from the R-26 mutant from *Rb. sphaeroides* as a function of the applied magnetic field. The inset shows the same measurements made over a wider range of magnetic fields.

Singlet oxygen yields were obtained from the amplitude of mono-exponential fits of the luminescence traces. Fig. 1 demonstrates that a magnetic field of a few mT has a profound effect on the $^1O_2$ yield in reaction centres of the R-26 mutant: a 50% reduction for fields of 20–100 mT and a 10% reduction for 1 mT. The 50% figure corresponds closely to the reduction in ^3P yield observed at similar magnetic field strengths.[7] The field strength, $B_{1/2}$, needed to produce half the limiting change in the yield of $^1O_2$ is 4.6 $\pm$ 0.3 mT, which is very similar to $B_{1/2}$-values found for the yield of ^3P (4.2 mT[7] and 5.7 mT[12]).

Fig. 2 shows the absorption spectra of reaction centres before and after 12,000 laser flashes. Illumination attenuates the absorption bands at 760, 800 and 860 nm belonging to I, the accessory bacteriochlorophyll B, and P, respectively, and results in a slight increase in absorption around 680 nm. These changes are indicative of disruption of the interactions between the chromophores and of changes in the reaction centre structure and provide a measure of the extent of the photodegradation caused by $^1O_2$.[8] The bleaching of the 800 nm band is about 45% smaller in a field of 15 mT than it is in zero field. This finding corroborates the

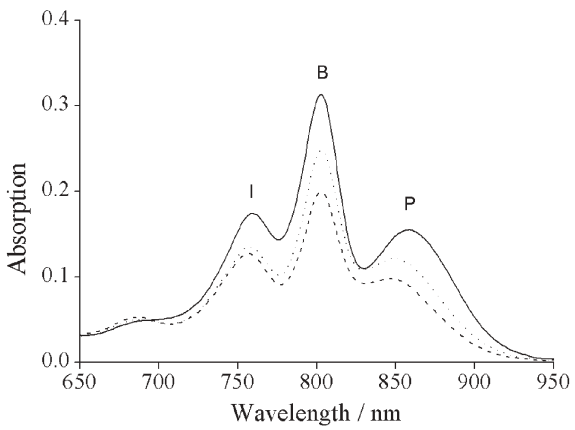

Fig. 2 Absorption spectrum of Q-depleted reaction centres from the R-26 mutant from *Rb. sphaeroides* before (solid) and after (dashed) illumination at zero field. Dotted line: after illumination in a magnetic field of 15 mT.

measurements shown in Fig. 1 and demonstrates directly that a relatively modest magnetic field affords substantial protection for the reaction centre protein against $^1O_2$-induced damage. In wild-type reaction centres the total photobleaching was 80% less than that for the R-26 mutant and no magnetic field dependence was found.

For an applied magnetic field to have a damaging effect in this context, it would need to *promote* the formation of $^1O_2$. There are two ways in which a weak magnetic field (< 1 mT) could cause such an increase. First, there is the "Low Field Effect" (LFE) which has opposite phase to the effects reported here and occurs for fields smaller than the average hyperfine interactions in the radical pair.[3,13] Second, and similar in appearance to the LFE, is the "2*J* resonance" that arises from energy-level crossings at field strengths that match the radical pair's exchange interaction.[14] That neither is observed here is due to the short lifetime of the radical pair and, in the case of the LFE, to the presence of the exchange and dipolar interactions between the two electron spins. A 2*J* resonance in the yield of ^3P has been found for *Rb. sphaeroides*,[15] but only at temperatures below 0 °C. In very strong magnetic fields (>5 T), the triplet yield becomes larger than in the absence of an applied field as a result of the difference in Zeeman interactions of the two radicals.[16] The size of this effect and the field at which it occurs are determined by the difference in the two *g*-values, which is quite small for P^+ and I^- but can be much higher for other radical pairs, such that relatively modest fields could cause the photosensitised $^1O_2$ yield to rise above that in zero field.

These effects are not necessarily restricted to reaction centres or indeed to $^1O_2$ produced by photosensitisation. For example, $^1O_2$ is formed during lipid peroxidation by the self reaction of peroxyl radicals (the Russell mechanism), a process which could, conceivably, show RPM effects.[17]

Based on the evidence at present, there does not appear to be a strong likelihood of physiologically significant changes in cellular functions or of long term mutagenic effects arising from magnetic field-induced variations in free radical concentrations or fluxes. Extraordinary changes in metabolic rates are perhaps not to be expected given the efficiency of homeostatic buffering processes, at least in healthy cells, and the existence of protection mechanisms against toxic by-products analogous to that afforded by the carotenoid in wild-type reaction centres.

In summary we have demonstrated that the yield of singlet oxygen photosensitized by chemically modified, carotenoidless bacterial photosynthetic reaction centres and as a consequence the stability of the reaction centre protein are strongly magnetic field-dependent. We believe this to be the first clear demonstration that a biochemical system, in which the Radical Pair Mechanism is known to operate, can generate toxic products in amounts that depend on the presence of a relatively weak applied magnetic field.

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Notes and references

1 A. Lacy-Hulbert, J. C. Metcalfe and R. Hesketh, *FASEB J.*, 1998, **12**, 395–420; A. Ahlbom, N. Day, M. Feychting, E. Roman, J. Skinner, J. Dockerty, M. Linet, M. McBride, J. Michaelis, J. H. Olsen, T. Tynes and P. K. Verkasalo, *Br. J. Cancer*, 2000, **83**, 692–698; S. Greenland, A. R. Sheppard, W. T. Kaune, C. Poole and M. A. Kelsh, *Epidemiology*, 2000, **11**, 624–634.

2 C. B. Grissom, *Chem. Rev.*, 1995, **95**, 3–24; B. Brocklehurst, *Chem. Soc. Rev.*, 2002, **31**, 301–311.

3 B. Brocklehurst and K. A. McLauchlan, *Int. J. Radiat. Biol.*, 1996, **69**, 3–24.

4 A. J. Hoff, P. Gast, R. van der Vos, J. Vrieze, E. M. Franken and E. J. Lous, *Z. Phys. Chem.*, 1993, **180**, 175–192.

5 B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, 1999, pp. 311–312.

6 R. J. Cogdell and H. A. Frank, *Biochim. Biophys. Acta*, 1987, **895**, 63–79.

7 C. E. D. Chidsey, L. Takiff, R. A. Goldstein and S. G. Boxer, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 6850–6854.

8 J. Tandori, E. Hideg, L. Nagy, P. Maroti and I. Vass, *Photosynth. Res.*, 2001, **70**, 175–184.

9 A. A. Krasnovsky, *Photochem. Photobiol.*, 1979, **29**, 29–36.

10 M. Kasha and A. U. Khan, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6047–6049.

11 R. Schmidt, *J. Am. Chem. Soc.*, 1989, **111**, 6983–6987.

12 M. H. Vidal, P. Setif and P. Mathis, *Photosynth. Res.*, 1986, **10**, 347–354.

13 C. R. Timmel, U. Till, B. Brocklehurst, K. A. McLauchlan and P. J. Hore, *Mol. Phys.*, 1998, **95**, 71–89.

14 W. Lersch and M. E. Michel-Beyerle, *Chem. Phys.*, 1983, **78**, 115–126.

15 J. R. Norris, C. P. Lin and D. E. Budil, *J. Chem. Soc., Faraday Trans 1*, 1987, **83**, 13–27.

16 R. A. Goldstein, L. Takiff and S. G. Boxer, *Biochim. Biophys. Acta*, 1988, **934**, 253–263.

17 S. Miyamoto, G. R. Martinez, M. H. G. Medeiros and P. Di Mascio, *J. Am. Chem. Soc.*, 2003, **125**, 6172–6179.

