Nanosecond Time-Resolved Magnetic Resonance of the Primary Radical Pair State P^F of Bacterial Photosynthesis

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Many reactions of chemical and biochemical interest involve short-lived paramagnetic intermediates. Direct observation of EPR spectra of radicals and radical pairs that live only a few nanoseconds is precluded by difficulties in instrumental response time.[1] The ability to directly measure magnetic parameters of fast radical reactions would prove very useful in elucidating both the structure of the radical intermediates and the mechanistic details of these reactions. We now report direct optical detection of magnetic resonance of the primary radical pair state in bacterial photosynthesis, P^F, which possesses only a 15-ns lifetime.

Photoexcitation of the reaction-center protein from purple photosynthetic bacteria results in rapid (<5 ps) formation of a radical pair, P^F, composed of an oxidized bacteriochlorophyll *a* dimer (P^+), and a reduced bacteriopheophytin a molecule (I^-).[2] If the endogenous quinone molecules in the protein are either removed or chemically reduced prior to excitation, P^F lives for about 15 ns in the absence of an external magnetic field.[3] During this time a fraction of the initially formed singlet population of P^F, $^1[P^+I^-]$, is under the influence of local magnetic fields primarily due to nuclear hyperfine interactions in P^+ and I^-. As a result P^F undergoes radical pair intersystem crossing (RP ISC) to yield $^3[P^+I^-]$.

$^1[P^+I^-]$ decays directly to the singlet ground state P, while $^3[P^+I^-]$ back-reacts to form ^3P, which lives for microseconds before intersystem crossing back to ground-state singlet P. At zero magnetic field the $^1[P^+I^-]$ and $^3[P^+I^-]$ states mix because they are nearly degenerate. Upon application of a strong magnetic field, the resultant T_0 level of $^3[P^+I^-]$ remains nearly degenerate with $^1[P^+I^-]$.[4] In general, both in the presence and in the absence of an external magnetic field the mixed radical pair states are at least slightly paramagnetic during most of the lifetime of P^F. In order to determine the spatial arrangement of P and I that leads to efficient charge separation, it would be extremely useful to obtain a magnetic resonance spectrum of P^F directly. In earlier work we approached this problem by monitoring the yield of ^3P by using RYDMR.[5] However, on the microsecond time scale many features of the spin dynamics of the radical pair are not observable.

Reaction centers were isolated from the R-26 mutant of *Rhodopseudomonas sphaeroides* and depleted of endogenous quinones.[3] A 80–100 μM solution of reaction centers was placed in a standard EPR flat cell in a Varian optical transmission cavity centered between the poles of an electromagnet. The sample remained at ambient temperature (21 °C) throughout the experiment. One-microsecond 9.4-GHz microwave pulses possessing powers up to 20 kW were provided by a magnetron source. The optical absorbance of the sample was monitored with 420-nm light from a flash lamp during the time following a 0.5–1.0-mJ 6-ns 600-nm dye laser pulse. At 420 nm the optical absorbance of P^F is about 7 times larger than that of ^3P. Optical density changes were monitored as a function of time with a 2.5-ns fwhm response photomultiplier. The output of the photomultiplier was recorded with a Tektronix 7912AD digitizer. The entire optical observation was completed within the 1-μs microwave pulse, so that the sample was under constant microwave power during this time. Magnetic field sweep and data acquisition were under computer control.

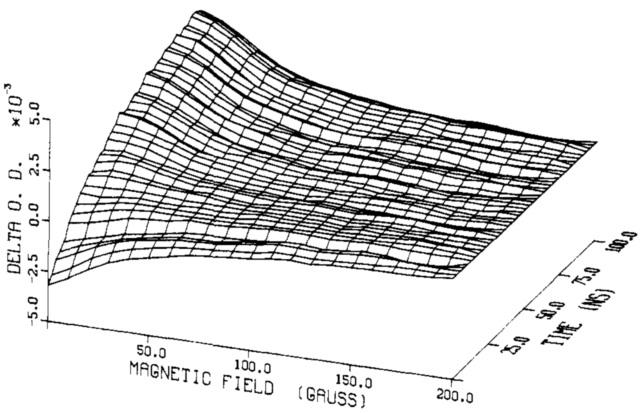

Figure 1. Relative changes in optical density monitored at 420 nm as a function of time and magnetic field for R-26 reaction centers, quinone removed. The depicted changes are normalized relative to the observed absolute optical density change measured at 200 G for each time.

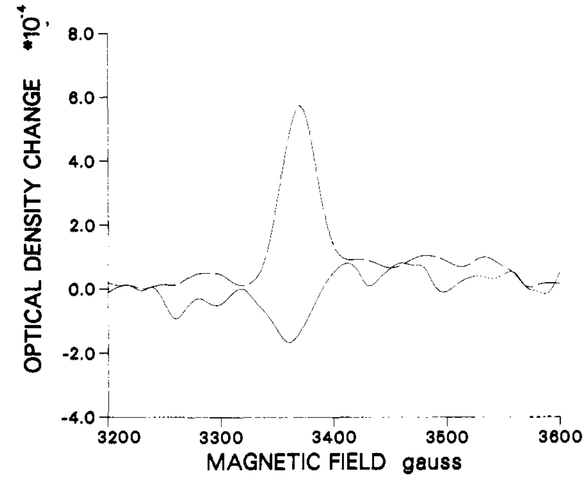

Figure 2. Magnetic resonance spectra of P^F at 5 ns (—) and 200 ns (---) following the laser pulse; microwave power = 1 kW. Optical density changes at 420 nm are measured. The depicted changes are normalized relative to the observed absolute optical density change measured at each time off resonance.

Figure 1 shows the time evolution of optical density changes at 420 nm as a function of both time and magnetic field. Immediately following the laser pulse during the lifetime of P^F the optical density change monitors the total P^F concentration.[3] The data show that the amount of P^F surviving at a given time during its lifetime increases as the field increases. At longer times (>50 ns) most of P^F has decayed, and the optical density change at 420 nm monitors ^3P alone. In this time regime we observe a decrease in ^3P optical density as has been obtained previously.[6] These results are a consequence of the fact that application of a magnetic field splits the three triplet sublevels of $^3[P^+I^-]$, leaving only T_0 nearly degenerate with $^1[P^+I^-]$. Thus, RP ISC to T_{+1} and T_{-1} is greatly slowed, resulting in a decrease in the population of ^3P and an increase in the population of P^F.

Two states possessing only partial triplet character result from mixing $^1[P^+I^-]$ and T_0 in P^F. In order to observe resonance in

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(5) Reaction yield detected magnetic resonance: (a) Bowman, M. K.; Budil, D. E.; Closs, G. L.; Kostka, A. G.; Wraight, C. A.; Norris, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3305–3307. (b) Norris, J. R.; Bowman, M. K.; Budil, D. E.; Tang, J.; Wraight, C. A.; Closs, G. L. *Ibid.* **1982**, *79*, 5532–5536.

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P^F, microwave transitions must occur between these mixed states and T_{+1} and T_{-1}. This requires that a substantial H_1 field be present during the lifetime of P^F. In Figure 2 magnetic resonance spectra of P^F are presented. The signal 200 ns after the laser pulse results from monitoring the ^3P population, which increases upon application of 1-kW microwaves at resonance. However, 5 ns after the laser pulse we directly observe the resonance signal of P^F. This signal shows that the P^F population decreases upon application of resonant microwaves. Since resonant microwaves increase the amount of $^3[P^+I^-]$ at the expense of $^1[P^+I^-]$, the observed decrease in P^F yield (the sum of $^1[P^+I^-]$ and $^3[P^+I^-]$ concentrations) at resonance shows that the decay rate of $^1[P^+I^-]$ to ground state, k_S, is significantly slower than that of $^3[P^+I^-]$, k_T, to ^3P. If $k_S > k_T$, then the P^F yield would increase at resonance. Moreover, if $k_S = k_T$, no P^F resonance would appear. It is important to note that the resonance observed by monitoring ^3P 200 ns after the laser flash would appear regardless of whether $k_S > k_T$, $k_T > k_S$, or $k_S = k_T$. Appearance of this signal only requires that resonant microwaves increase the amount of $^3[P^+I^-]$ leading to ^3P.

Earlier experiments concerning magnetic field effects alone have concluded that k_S is probably smaller than k_T.[3,7] In addition, several quantum mechanical models of magnetic field effects on the yield of ^3P have examined a wide variety of k_S and k_T values and have generally supported the notion that $k_S < k_T$.[8] Within the constraints of the radical pair model our data demonstrate directly that $k_S < k_T$. In conclusion, the ability both to monitor and to control the dynamics of radical pair populations directly should prove very useful in determining structure and mechanism in electron-transfer reactions especially in important biological systems such as photosynthesis.

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Asymmetric Synthesis via Acetal Templates. 4. Reactions with Silylacetylenic Compounds. Formation of Chiral Propargylic Alcohols

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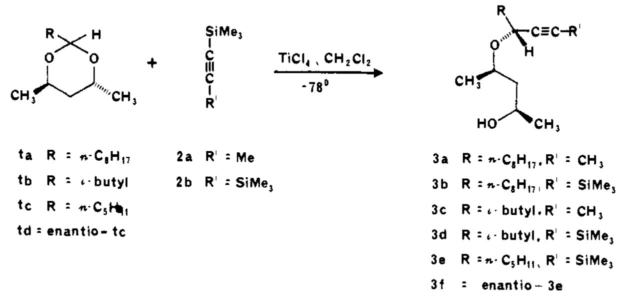
Recently we disclosed[1] the titanium tetrachloride catalyzed coupling of some chiral acetals, e.g., **1a**, with allyltrimethylsilane.[2] The products were formed diastereoselectively, and removal of the chiral auxiliary afforded optically active homoallylic alcohols, ee 65-74%. The coupling results (diastereomeric ratios up to 88:12) are at least qualitatively consistent with the Bartlett model[1] for the transition state of a related, but more selective (ratio 92:8), intramolecular reaction.[3]

As part of a program aimed at exploring the acetal reaction with various nucleophiles, we now report on the coupling with (trimethylsilyl)acetylenic compounds.[4] From these experiments a methodology has emerged for the highly enantioselective production of secondary propargylic alcohols. In addition, this study promised to shed some light on whether the diastereoselectivity of the process is enhanced by the presence of a substituent (the trimethylsilyl group, in the present instance) located at the reacting site of the nucleophilic partner.[5]

Scheme I



Scheme II

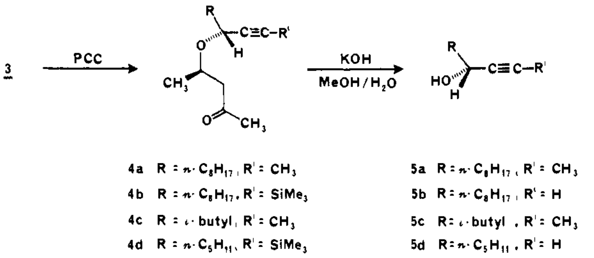


Table I. Results of the Transformation Shown in Scheme I

	1			3[8b,9]		
entry	acetal[7,8b,9]	% yield	2, silylacetylene	coupled product	% yield	diastereomeric ratio[10]
1	1a	96	2a	3a	81	93:7
2	1a	96	2b	3b	98	96.5:3.5
3	1b	91	2a	3c	94	86:14
4	1b	91	2b	3d	85	94:6
5	1c	95	2b	3e	91	96.5:3.5
6	1d	96	2b	3f	90	96:4

Table II. Results of the Transformation Shown in Scheme II

		4[8a,9]		5[8b,9a]			
entry	3, coupled product	ketone	% yield	propargylic alcohol	% yield	$[\alpha]^{23}{}_D$ (*c*, solvent), deg	% ee[11]
1	3a	4a	99	5a	95[9b]	+8.6 (1.0, CCl_4)	87
2	3b	4b	97	5b	92	+14.3 (1.0, Et_2O)	90
3	3c	4c	93	5c	90	+10.2 (4.9, $CHCl_3$)	70
4	3e	4d	97	5d	88	+19.3 (1.0, Et_2O)	90
5	3f	4e	98	5e	91	-20.3 (1.0, Et_2O)	92

The couplings of the acetal **1** with the silylacetylenes **2**[6] to give **3** (Scheme I, Table I) were performed as described for the reactions with allyltrimethylsilane,[1] except that with the (trimethylsilyl)propyne (**2a**) reactions (Table I, entries 1 and 3) a

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(1) Paper 3: Bartlett, P. A.; Johnson, W. S.; Elliott, J. D. *J. Am. Chem. Soc.*, in press.

(2) The first example of this type of condensation was reported by McNamara andKishi: McNamara, J. M.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 7371-7372.

(3) Johnson, W. S.; Harbert, C. A.; Ratcliffe, B. E.; Stipanovic, R. D. *J. Am. Chem. Soc.*, **1976**, *98*, 6188-6193.

(4) Lewis acid catalyzed acylations and alkylations of silylacetylenes are well-known. See inter alia: Fleming, I. "Comprehensive Organic Chemistry", Barton, D. H. R., Ollis, W. D., Eds.; Pergamon Press: Oxford, 1979; Vol. 3, p 613. Birkofer, L.; Ritter, A.; Uhlenbrauck, H. *Chem. Ber.* **1963**, *96*, 3280-3288.

(5) Such a substituent was originally suggested as being responsible for the asymmetric induction observed on cyclization of the (2*R*,3*R*)-butanediol acetal of 5,9-dimethyldeca-(5*E*)-9-dienal, the proposed effect being due to nonbonded interactions between the C-7 group and one of the chiral centers.[3]

(6) Available from Aldrich Chemical Co.

(7) Prepared by the conditions described in ref 1.