BBA 43219

On the nature of the free radical formed during the primary process of bacterial photosynthesis

In this note we wish to present new evidence suggesting that the photo-induced free radical[1,2] seen in photosynthetic bacterial preparations is associated with the primary photochemical process and that the chemical species involved is oxidized bacteriochlorophyll.

We used two independent experimental approaches. In the first, we measured the kinetics of the light-induced EPR signal and optical absorbance changes at cryogenic temperatures and compared their several kinetic parameters. In the second approach, we compared the EPR parameters of chemically oxidized bacteriochlorophyll *in vitro* with those of the photo-induced signal.

The low-temperature optical absorbance changes were measured with a kinetic spectrophotometer utilizing a Cary 14 monochromator equipped with an optical dewar. EPR measurements were made with a 9-GHz superheterodyne spectrometer[3] designed for low temperature work and modified for pulsed measurements of magnetic relaxation times[4]. The sample was illuminated with rectangular pulses of actinic light obtained with a mechanical shutter. The light-induced signals were fed into a commercial signal-averaging computer synchronized with the flashing actinic light. The light-induced optical and EPR changes remained reversible over many thousands of flashes. The same sample was used for both EPR and optical measurements.

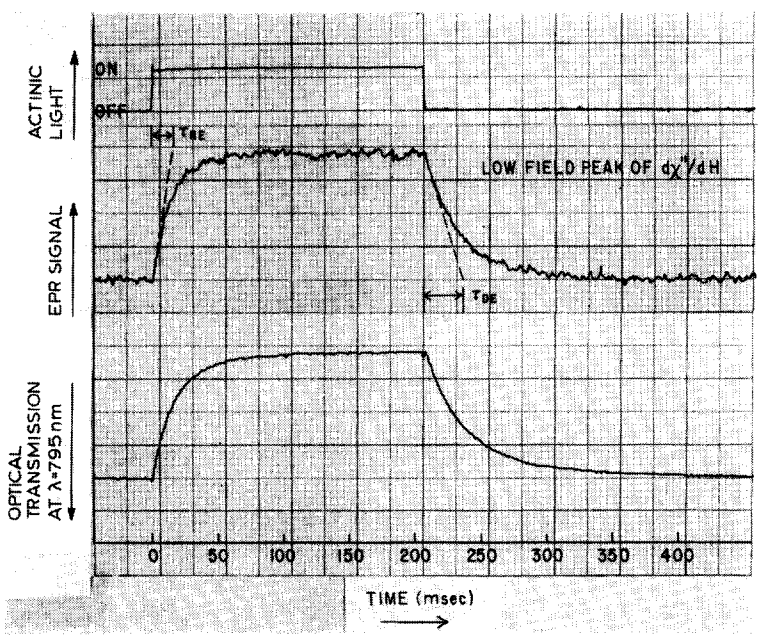

Fig. 1. EPR and optical kinetics of the reaction centers of R-26 mutant of *R. spheroides* at 77°K. The same sample was used for both sets of experiments. Changes in optical transmission were converted to absorbance changes. The rise and decay times were obtained by replotting the data on semilog paper and taking the $1/e$ values. The results for different experimental conditions are summarized in Tables I and II.

Representative kinetic results using the reaction centers of the R-26 mutant of *Rhodopseudomonas spheroides*[5] are shown in Fig. 1. By expanding the time scale near the origin, we have determined that within our time resolution of 0.3 msec, there is no lag between the appearance of the signals and the light flash, in contrast to an earlier report[6] but in agreement with recent work[7]. This finding, in conjunction with the low-temperature conditions which preclude ordinary chemistry, indicates that the free radical is created at or near the active site.

Table I summarizes the data of the optical and EPR decay experiments over a range of temperatures, and Table II shows the results of the rise times at 77°K for different light intensities. At the lowest temperatures the spin life time enters into the EPR kinetics, and a more complicated analysis is required to obtain the relevant kinetic parameters. It should be noted that the rise and decay experiments represent two independent pieces of evidence for the identity of the free radical.

The results presented in Tables I and II clearly indicate the identity of the

TABLE I

COMPARISON OF OPTICAL AND EPR DECAY TIMES AS A FUNCTION OF TEMPERATURE

Experimental conditions: *R. spheroides* R-26 reaction centers suspended in 50% (v/v) glycerin; sample thickness 1 mm; $A_{890\,nm} = 0.7$ at 1.7°K; Actinic light source, $I_{max} = 10^5$ ergs·cm^{-2}·sec^{-1}; $\lambda = 900$ nm (Bausch and Lomb B-3 (NIR) interference filter); measuring beam intensity, 50 ergs·cm^{-2}·sec^{-1}. Rms errors are quoted.

Temp. (°K)	*Optical decay time,* τ_{DO} *(msec)* ($\lambda = 795$ *nm*)*	*EPR decay time,* τ_{DE} *(msec)* *(low-field peak)*	*Electron spin life time,* T_S*** *(msec)*
1.7	28 ± 4	36 ± 5**	27 ± 3
4.2	—	31 ± 5	13 ± 5
77	30 ± 2	30 ± 4	<1

* Similar times were obtained for $\lambda = 805$, 880, and 1242 nm.

** When the magnetic relaxation rate is comparable to the chemical decay rate, the EPR decay kinetics are slowed down and become non-exponential. This number is the result of a theoretical fit (details to be published elsewhere).

*** The spin life time T_S was obtained from the recovery of the EPR signal after the application of a saturating microwave pulse. Note that the value of T_S is affected by the "chemical process" (*i.e.*, the life time of the free radical) and is therefore not identical to the spin lattice relaxation time T_1.

Note: In addition to the tabulated fast times, we have observed that a small fraction of both the optical and EPR signal decays with a time constant of 3.5 ± 0.5 sec.

TABLE II

COMPARISON OF OPTICAL AND EPR RISE TIMES

Experimental conditions: temp., 77°K; other conditions are as described in Table I.

Light intensity (%) (100% = 10^5 *ergs·cm*$^{-2}$·*sec*$^{-1}$)	*Optical rise time,* τ_{RO} *(msec)*	*EPR rise time,* τ_{RS} *(msec)*
100	12 ± 1	15 ± 2
50	17 ± 2	18 ± 3
21	23 ± 2	20 ± 5
8.1	28 ± 2	30 ± 5

TABLE III

COMPARISON OF g VALUES AND LINE WIDTHS OF THE OXIDIZED BACTERIOCHLOROPHYLL EPR SIGNAL WITH THE LIGHT-INDUCED EPR SIGNAL FROM WHOLE CELLS OF *R. rubrum*

Experimental conditions: $\nu_e = 9$ GHz; temp., 77°K (the same parameters are found for *R. rubrum* at room temperature); bacteriochlorophyll in methanol–glycerin (1:1, v/v) was oxidized with iodine in an evacuated system and transferred to the EPR cuvette in the same closed system.

Species	*Electronic g value*	ΔH^* (*gauss*)	$\frac{\Delta H_{normal}}{\Delta H_{deuterated}}$ **
Oxidized bacteriochlorophyll			
normal	2.0025 ± 0.0001	12.8 ± 0.5	2.4 ± 0.2
deuterated	2.0026 ± 0.0001	5.4 ± 0.3	
R. rubrum			
normal	2.0026 ± 0.0001	9.5 ± 0.5	2.3 ± 0.2
deuterated	2.0026 ± 0.0001	4.2 ± 0.3	

* Total width between inflection points of $d\chi''/dH$. All line shapes were gaussian.

** For broadening due to protons only, the theoretically predicted ratio is 4. For broadening due to protons and to four nitrogen nuclei having a hyperfine interaction of the form $AI \cdot S$ the above data are consistent with A approximately equal to 1 gauss.

free radical with the species undergoing the light-induced optical changes[8,*]. These optical changes have been associated with the oxidation of the specialized bacteriochlorophyll P_{870} by several authors[5,9,10–13] and implicated in the primary process[14,15].

The constancy of the decay times over the large temperature range sets an upper limit on the apparent activation energy of approx. 0.2 wave number (0.6 cal/mole), and is consistent with a tunneling process of the unpaired electron[**].

We have obtained similar kinetic results with whole cells and chromatophores of *Rhodospirillum rubrum* and its G-9 mutant, *R. spheroides*, and *Chromatium*. We feel therefore that the results reported here are general for the purple photosynthetic bacteria.

In Table III, we compare g values and line widths of the light-induced EPR signal from whole cells of *R. rubrum* with those of chemically oxidized bacteriochlorophyll *in vitro*. Both normal and fully deuterated species were used. The identity of the g values, similarity of the line shapes, and the narrowing upon deuteration suggests that the light-induced free radical arises from bacteriochlorophyll. The line width differences can be ascribed to different environments of the bacteriochlorophyll *in vitro* and in the photosynthetic tissue.

The present results show that the photoactive species giving rise to the low-temperature EPR and optical changes are identical, and we conclude that this species is oxidized P_{870}. We infer from the nature of the kinetics at low temperatures that it is associated with the primary step of bacterial photosynthesis.

The question of the primary acceptor remains unanswered. Although we have seen other EPR signals in bacterial preparations, we have yet to establish their kinetic behavior before attributing them to the primary acceptor.

* J. R. BOLTON, D. W. REED and R. K. CLAYTON (personal communication) have arrived at the same conclusion from different, room-temperature experiments.

** This mechanism was first proposed in the context of photosynthesis by DEVAULT AND CHANCE[16] who set an upper limit of 80 cal/mole on the activation energy. Later DEVAULT, PARKES AND CHANCE[17] lowered this limit to 4 cal/mole.

We would like to thank Dr. R. K. CLAYTON for providing us with an inoculate of the R-26 mutant, Dr. J. J. KATZ for sending us the deuterated material, Dr. M. D. KAMEN and his group for stimulating and helpful discussions, and Drs. J. R. BOLTON and R. K. CLAYTON for discussing their results and conclusions concerning the light-induced EPR signal. This work was supported by Public Health Service Grant GM-13191, National Institutes of Health. One of us (D.C.M.) would like to acknowledge the support of a Guggenheim Fellowship.

University of California, San Diego, La Jolla, Calif. (U.S.A.)

JAMES D. MCELROY
GEORGE FEHER

U.C.S.D. and The Rockefeller University, New York, N.Y. (U.S.A.)

DAVID C. MAUZERALL

1 B. COMMONER, J. J. HEISE AND J. TOWNSEND, *Proc. Natl. Acad. Sci. U.S.*, 42 (1956) 710.
2 P. SOGO, M. JOST AND M. CALVIN, *Radiation Res. Suppl.*, 1 (1959) 511.
3 G. FEHER, *Bell System Tech. J.*, 36 (1957) 449.
4 R. ISAACSON, *J. Sci. Instr. (J. Phys. Series E)*, 1 (1968).
5 D. W. REED AND R. K. CLAYTON, *Biochem. Biophys. Res. Commun.*, 30 (1968) 471.
6 B. COMMONER, D. H. KOHL AND J. TOWNSEND, *Proc. Natl. Acad. Sci. U.S.*, 50 (1963) 640.
7 J. R. BOLTON, K. COST AND A. W. FRENKEL, *Arch. Biochem. Biophys.*, 126 (1968) 383.
8 P. A. LOACH AND D. L. SEKURA, *Photochem. Photobiol.*, 6 (1967) 381.
9 L. M. N. DUYSENS, W. J. HUISKAMP, J. J. VOS AND J. M. VAN DER HART, *Biochim. Biophys. Acta*, 19 (1956) 188.
10 J. C. GOEDHEER, *Biochim. Biophys. Acta*, 38 (1960) 389.
11 W. J. VREDENBERG AND L. M. N. DUYSENS, *Nature*, 197 (1963) 355.
12 P. A. LOACH, G. M. ANDROES, A. F. MAKSIM AND M. CALVIN, *Photochem. Photobiol.*, 2 (1963) 443.
13 R. K. CLAYTON, W. R. SISTROM AND W. S. ZAUGG, *Biochim. Biophys. Acta*, 102 (1965) 341.
14 W. ARNOLD AND R. K. CLAYTON, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 769.
15 W. W. PARSON, *Biochim. Biophys. Acta*, 153 (1968) 248.
16 D. DEVAULT AND B. CHANCE, *Biophys. J.*, 6 (1966) 825.
17 D. DEVAULT, J. H. PARKES AND B. CHANCE, *Nature*, 215 (1967) 642.

Received October 17th, 1968