TIME-RESOLVED ESR AND CHEMICALLY INDUCED DYNAMIC ELECTRON POLARISATION OF THE PRIMARY REACTION IN A REACTION CENTER PARTICLE OF *RHODOPSEUDOMONAS SPHAEROIDES* WILD TYPE AT LOW TEMPERATURE

A. J. HOFF, P. GAST and J. C. ROMIJN

Department of Biophysics, Huygens Laboratory, State University of Leiden, Leiden, The Netherlands

Received 20 November 1976

1. Introduction

Recently a very fast decaying ESR signal preceding the so-called signal I in plant photosynthesis has been observed [1]. The signal was emissive, which was taken to provide evidence for the occurrence of chemically induced dynamic electron polarisation (CIDEP). Such spin-polarisation effect may give insight into the events preceding the primary charge separation. Because the primary reactions in the bacterial photosystem are much better understood than those of the plant systems [2], we have investigated light-induced paramagnetism of bacterial reaction centers on a time scale of 20–300 μs. At a temperature of 100°K we have found a fast decaying, partly emissive, flash-induced ESR signal, with a complex time- and field-dependency, in particles obtained from *Rhodopseudomonas sphaeroides* wild type by treatment with sodium dodecyl sulphate (SDS). We have attempted to analyse the time-resolved spectra in the framework of current CIDEP theories. We can account for the spectral shape by assuming that the radical pair mechanism is operative, through which the ESR signals of the primary donor–acceptor pair become spin-polarised. This polarisation then decays in a time comparable to the spin-lattice relaxation time. Apparently, by monitoring the time evolution of the light-induced ESR signal one can probe a spin-memory of the much faster events leading to the charge separation.

Abbreviations: SDS, sodium dodecyl sulphate; LDAO, lauryldimethylamine oxide

2. Materials and methods

Reaction center particles were prepared from wild type *R. sphaeroides* by the method described by Slooten [3a]. Briefly, a reaction center fraction was obtained by treatment of chromatophores with sodium dodecyl sulphate. This fraction was purified further by gradient centrifugation in the presence of urea and Triton X-100 at pH 10.0, followed by dialysis against a solution of 0.05 M Tris (pH 8.0) containing 0.01 M $MgCl_2$. The particles have been characterised with respect to their photoactivity under various conditions in a number of investigations using optical methods. They have the same absorption difference spectra as whole cells and particles from *R. sphaeroides* wild type prepared without SDS. The rate of the decay of the state P^+X^- as measured by absorption difference spectroscopy, however, was slower by a factor of 2 at temperatures above 200°K than the decay rate in reaction centers prepared without SDS [2,3d]. For some experiments particles were prepared from the mutant *R. sphaeroides* R-26 (kindly supplied by Dr F. Feher) using LDAO instead of SDS.

ESR spectra were recorded on a Varian E-9 spectrometer, equipped with a Varian variable temperature attachment for temperatures between 100°K and room temperature and an Oxford Instrument helium flow cryostat for temperatures down to 4.2°K. The detector crystal was biased with a reference arm, tuned to the out-of-phase component χ'' of the susceptibility, thus ensuring observation of the absorption. The lock-in detector of the instrument was modified to permit recording ESR signals with an instrumental rise time of 20 μs, using 100 kHz field modulation. All kinetic traces were obtained by accumulating at least 20 times randomly with respect to the phase of the field modulation. This gives the proper slope of the ESR line even at response times close to the inverse of the modulation frequency. The instrumental rise time was determined by observation of the rise of the light-induced triplet ESR signal in reaction centers with reduced primary acceptor. This triplet has an optically determined rise time of 50 ns g-Values were measured using a p-doped silicon marker ($g = 1.9998$) kindly donated by Mr R. A. Isaacson. Actinic light of saturating intensity for the kinetic measurements was provided by a

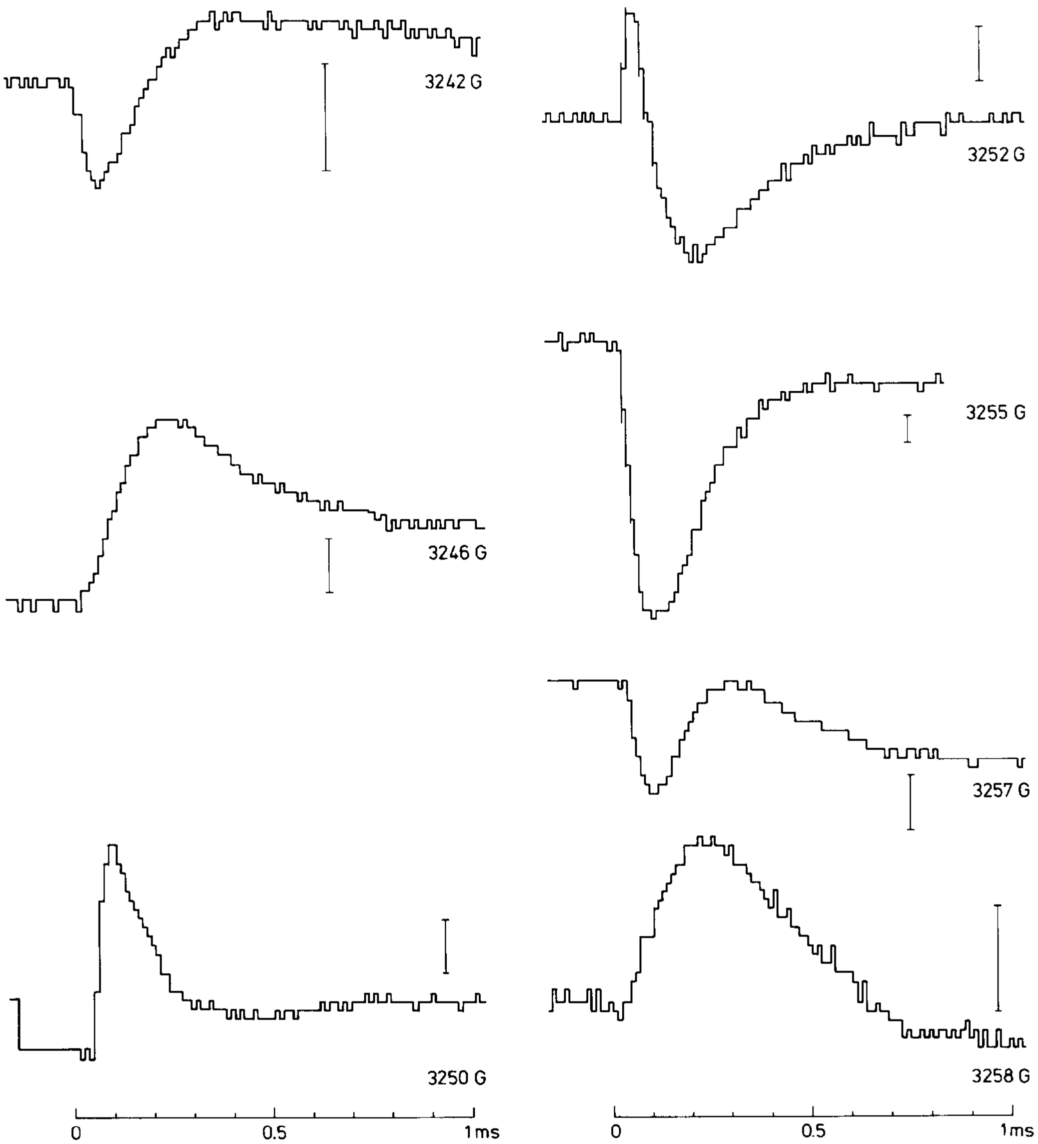


Fig.1. Kinetics of flash-induced ESR signal in reaction center particles of *R. sphaeroides* wild type at 100°K. Traces are selected from data taken with 1 Gauss spacing. Field modulation 2.5 Gauss, power 1 mW; $g = 2.0040$ corresponds to $H = 3253$ Gauss. Vertical bars indicate gain. The signals were accumulated for 20–100 scans.

Xenon flash lamp (duration of the flash 2 μs at one-third of the peak). Great care was taken to avoid flash artefacts [4]. A number of control experiments was carried out using a Chromatix dye laser (pulse width 1 μs). The observed kinetics proved to be independent of the light source. All kinetic data were obtained using a Datalab DL102 signal averager (minimal dwell time 4 μs per channel).

3. Results

The light-induced steady-state ESR signal was a slightly asymmetric gaussian with a g-value of 2.0040 ± 0.0002. The spectrum resembled the one obtained from iron-depleted reaction centers of *R. sphaeroides* R-26 [5] and *R. rubrum* [6]. All these preparations were treated with SDS. Although some non-haem iron is present in our particles, in reduced particles at 4.2°K, no signal corresponding to the $g = 2.0$, 1.82 and 1.68 ESR signal ascribed to the iron–ubiquinone complex [7,8] was detected and only the triplet spectrum ascribed to the state P^TX^- [8,9] was observed in the light minus dark runs. It is believed, therefore, that in our particles the SDS treatment has removed the iron bound to the acceptor, i.e., ubiquinone as such is the primary acceptor.

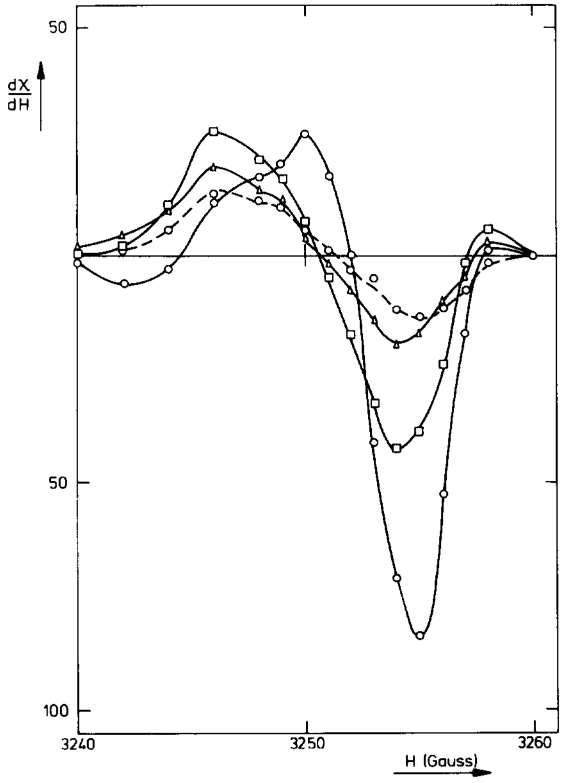


Fig.2. Time resolved ESR spectra derived from the kinetic traces. (-○-) 30, (-□-) 90, (-△-) 200 and (- -○- -) 2000 μs after flash.

Figure 1 shows a few typical kinetic traces at various field values obtained at 100°K. The amplitude of the signal after about 400 μs corresponds to the amplitude of the light-induced ESR signal obtained with continuous illumination of saturating intensity. It is clearly seen that the ESR signal before reaching its 'steady-state' value after about 400 μs shows enhanced absorption and/or emission. In fig.2 spectra are presented for various times after the flash. These spectra are constructed from kinetic traces such as displayed in fig.1. It is seen that the field-dependency of the fast ESR signal is complex and that it does not correspond to a simple inversed or enhanced 'steady-state' line. Apparently, one or more species are present on this fast time scale, that do not correspond to the P^+X^- state as measured in steady-state equilibrium. The kinetics of the ESR signal were found to depend on microwave power incident on the cavity, being faster with increasing power. No other fast, flash-induced resonances were observed in the range of 0–6000 G. At 100°K we have extensively looked for fast decaying signals around $g = 2$ in whole cells and chromatophores of *R. sphaeroides* wild type and in a reaction center preparation from its mutant R-26 made by LDAO treatment. In all cases we only found the familiar donor signal, which grew with our instrumental rise time and decayed with a time constant of 20–30 ms. Hence, the above described fast signals seem to be confined to SDS treated material.

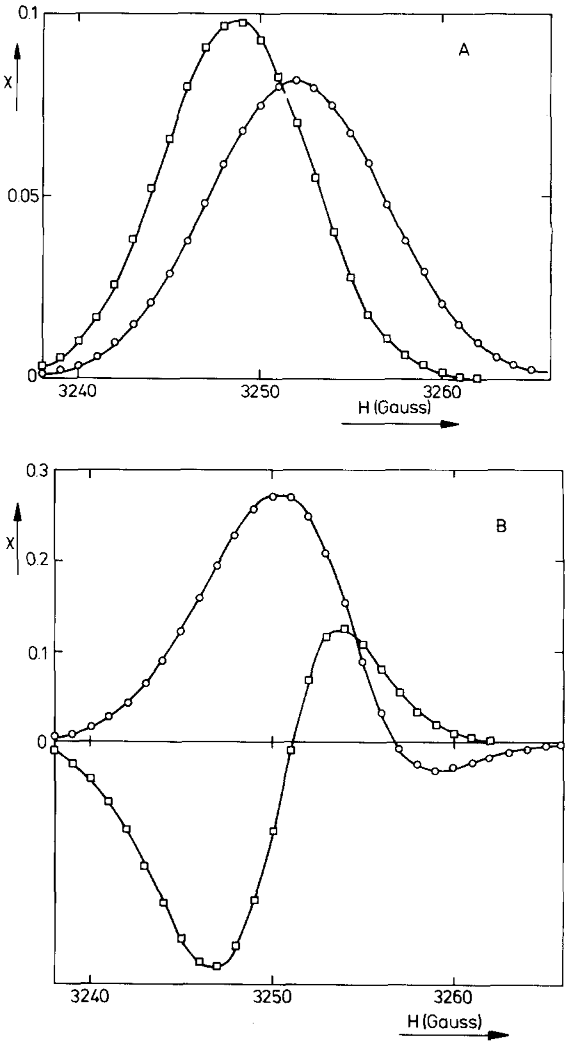
4. Discussion

A natural question is how the photoactivity of SDS treated material relates to the in vivo photosynthetic mechanism. All available evidence points to the fact that, although they apparently lack quinone-bound iron, the particles behave, with regard to the primary charge separation, identically with whole cells, not only with respect to their absorption difference spectra, but also to their optical kinetic behaviour on the μs and slower time scale (R. van Grondelle, private communication) with the exception of the temperature-dependence of the back reaction [3d].

The observation of spin-polarisation (fig.1) suggests an explanation of the observed phenomena in the framework of chemically induced dynamic polarisation (CIDEP). In recent years, ample evidence has been accumulated that a non-Boltzmann distribution of spins can be generated via various magnetic interactions, whenever a radical pair is generated from a singlet state [10,11]. In the act of charge separation, the hole (P^+) and the expulsed electron form a radical pair with correlated spins. When the radicals come apart, their spins will sort themselves out in a way which depends on local magnetic fields. Although the removal of the iron apparently does not impair the primary act, it does greatly influence the magnetic properties of the reduced acceptor. It has been concluded that the iron has a spin-exchange interaction with the reduced ubichinone [6,10] and with the recently found intermediate reactant, bacteriopheophytin [13]. Thus, it is not unreasonable that the absence of the paramagnetic iron results in quite different spin statistics, i.e., in a polarisation of either one, or both, of the donor and acceptor. Since some of the kinetic traces show both enhanced absorption and emission at different times, more than one polarised species must be involved. We therefore have attempted to explain our kinetics curves by assuming that both P^+ and X^- are spin-polarised. The state P^+X^- is presumably generated in less than 200 ps [9] but its spin-memory has a much longer decay time, of the order of the spin-lattice relaxation time (several hundred μs at this temperature). At present, two mechanisms are thought to be responsible for the spin-polarisation: the triplet mechanism (TM) and the radical pair mechanism (RPM) [10,11]. In the TM the polarisation is directly transmitted from the triplet precursor to the emerging radicals. In the latter, polarisation is generated by a difference in resonance frequency (because of different g-values, or different hyperfine fields) which causes a loss of phase. One can discriminate between the TM and the RPM by observing the polarisation of both radicals: for the TM they should have the same polarity (i.e., emissive or absorptive) whereas for the RPM they have opposite polarity. We were unable to construct spectra such as those of fig.2 by taking P^+ and X^- radicals and have them both emissively or absorptively polarised. In contrast, assuming the RPM operative, and taking P^+ absorptively and X^- emissively polarised, spectra were simulated (fig.3) which fairly closely resemble the experimental spectra. The polarisation was taken to be proportional according to

$$P \propto \frac{1}{2}\ [(g_1 - g_2)\beta H + \Sigma_i A_{1i} m_i - \Sigma_i A_{2i} m_i]$$

where g_1 (electronic g-value) and A_{1i} (hyperfine coupling constant) refer to P^+ and g_2 and A_{2i} to X^-; m_i are the nuclear-spin quantum numbers of nuclei within a given radical [11].



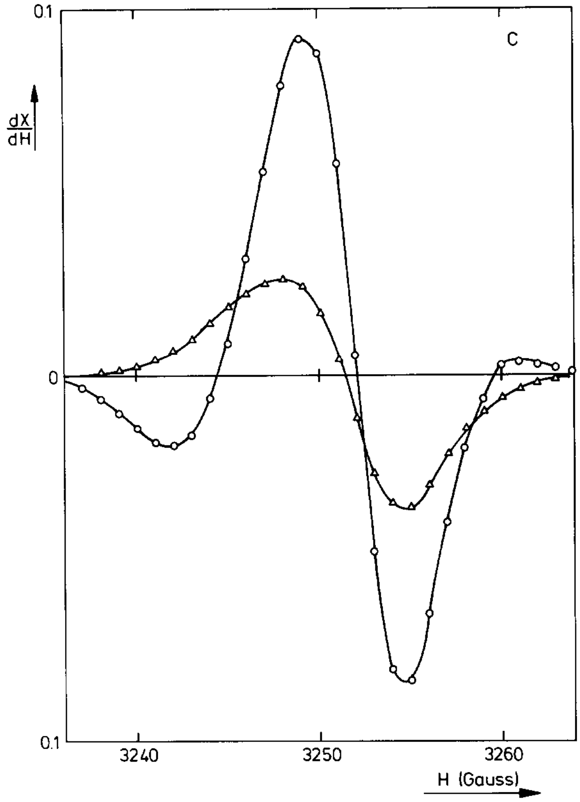


Fig.3. Spectra calculated for the spin-polarised radical pair P^+X^- as indicated in text. ΔH_{pp} for P^+ and X^- 9.7 and 8.1 Gauss, respectively; $g(P^+) = 2.0026$, $g(X^-) = 2.0046$. (a) Calculated P^+ and X^- absorption spectra. (b) Spin-polarised absorption spectra derived from (a) as indicated in text, polarisation factor, 6. (c) Derivatives of summed spin-polarised P^+ and X^- spectra. (-o-) Polarisation factor 6 and (-△-) polarisation factor 3.

The concept of spin-polarisation through a radical pair mechanism in the solid state is, although unusual, not wholly unreasonable. Radical separation takes place in the primary act, and it may well occur by electron hopping, generating a time-dependent exchange interaction between the members of the pair. In fact, the RPM has been invoked by Thurnauer et al. [14] to explain the anomalous polarisation of the triplet state that can be generated (via a back reaction between P^+ and an intermediate I^-) in reaction centers in which the primary acceptor is chemically reduced. Recently, evidence has been obtained that the triplet yield is dependent on the strength of an applied magnetic field (Parson, private communication) supporting this hypothesis.

A somewhat better fit between constructed and experimental spectra was obtained by admixture of polarisation that varies non-linearly with the hyperfine coupling constants. This means that some $S-T_{-1}$ mixing might occur, suggesting that the electron resides for a finite time on an intermediate molecule having during that time a relatively large exchange interaction with P^+.

Spin-polarisation provides a unique way to probe, via the spin-memory of the systems, the molecular mechanism of photochemical reactions that occur much faster than the spin-lattice relaxation time. Further work on an even faster time scale will hopefully lead to fuller understanding of the role of intermediates in the primary reaction.

Acknowledgements

The authors are indebted to Miss M. F. Klunder and Mr A. H. M. de Witt for technical assistance. One of us (A.J.H.) is grateful for discussions with Drs W. Parson and T. J. Sschaafsma. This work was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organisation for the Advancement of Pure Research (ZWO).

References

[1] Blankenship, R., McGuire, A. and Sauer, K. (1975) Proc. Natl. Acad. Sci. USA 72, 4943–4947.

[2] Feher, G. and Okamura, M. Y. in: The photosynthetic Bacteria (Clayton, R. K. and Sistrom, W. R. ed) in the press.

[3] (a) Slooten, L. (1972) Biochim. Biophys. Acta 256, 452–466.
(b) Slooten, L. (1972) Biochim. Biophys. Acta 275, 208–218.
(c) Slooten, L. (1973) Biochim. Biophys. Acta 314, 15–27.
(d) Romijn, J. C. and Amesz, J. (1976) Biochim. Biophys. Acta 423, 164–173.

[4] Bolton, J. R. and Warden, J. T. (1973) in: Creation and the Detection of the Excited State (Ware, W. R. ed) Vol. 2, Ch. 2, pp. 63–97, Marcel Dekker, New York.

[5] Loach, P. A. and Hall, R. L. (1972) Proc. Natl. Acad. Sci. USA 69, 786–790.

[6] Feher, G., Okamura, M. Y. and McElroy, J. D. (1972) Biochim. Biophys. Acta 267, 222–226.

[7] Feher, G. (1971) Photochem. Photobiol. 14, 373–387.
[8] Dutton, P. L., Leigh, J. S. and Reed, D. W. (1973) Biochim. Biophys. Acta 292, 654–664.
[9] Dutton, P. L., Kaufmann, K. J., Chance, B. and Rentzepis, P. M. (1975) FEBS Lett. 60, 275–280.
[10] Dobbs, A. J. (1975) Molec. Phys. 30, 1073–1084.
[11] Atkins, P. W. and Evans, G. T. (1976) Adv. Phys. Chem. 35, 1–29.
[12] Feher, G., Isaacson, R. A., McElroy, J. D., Ackerson, L. C. and Okamura, M. Y. (1974) Biochim. Biophys. Acta 368, 135–139.
[13] Tiede, D. M., Prince, R. C., Reed, G. H. and Dutton, P. L. (1976) FEBS Lett. 67, 301–304.
[14] Thurnauer, M. C., Katz, J. J. and Norris, J. R. (1975) Proc. Natl. Acad. Sci. USA 72, 3270–3274.