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DETERMINATION OF THE PRIMARY REACTIONS OF PHOTOSYNTHESIS FROM TRANSIENT ESR SIGNALS

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1. Introduction

One possible source of information about the primary reactions of photosynthesis is a light-induced transient ESR signal. Such transient ESR signals have been observed to have an anomalous intensity distribution where parts of the spectrum may be in emission. Both polarized triplet spectra [1,2] and polarized doublet spectra [3–6] have been observed. The present discussion is limited to doublet spectra. The anomalous polarization of the radicals is known as CIDEP and presently two mechanisms can give rise to CIDEP:

(i) The radical pair mechanism;
(ii) The triplet mechanism.

The radical pair mechanism seems to be the important mechanism for photosynthesis [5,6] and is the only one considered here. This mechanism is well understood for radicals in solution and has been put into a quantitative theory [7]. However, this theory is not directly applicable to the kind of processes involved in photosynthesis and since the proper modifications remained to be performed the full potential of the experimental spectra as a source of information for discriminating between the conceivable primary processes of photosynthesis has not been appreciated.

Here we give a simple graphical and analytical method to interprete transient ESR doublet signals observed from photosynthetic systems. Emphasis is placed on the possibility of discriminating between various conceivable primary processes. For example, one might ask whether one of the observed radicals is derived from an intermediate unobserved radical and if so can the g-factor of the intermediate radical be determined so that a chemical characterization of the species may be facilitated.

2. The method

It is assumed that the observed transient ESR spectrum is a superposition of the polarized doublet spectra of two radicals. These radicals are called the primary donor P^+ and the primary acceptor X^-. Due to spin-lattice relaxation, the experimental spectrum must be recorded shortly ($\sim T_1$ s) after the flash that induces the signal. If the full time-dependence of the spectrum is observed the spectrum of interest is that with maximum polarization.

The equilibrium line shape function (i.e., the distribution of resonance fields) of a radical R is denoted by $L_R(H)$. This function is assumed normalized, i.e., $\int L_R(H)\,dH = 1$. The average resonance field $H_0(R)$ of radical R is defined as:

$$H_0(R) = \int H\, L_R(H)\, dH \qquad (1)$$

and is equal to the centerfield of a symmetric spectrum. Many randomly-oriented systems have Gaussian lineshapes:

$$L(H) = (\pi \Delta H_{pp}^2/2)^{-\frac{1}{2}} \exp[-2(H-H_0)^2/\Delta H_{pp}^2] \qquad (2)$$

which are characterized by the centerfield H_0 and the width ΔH_{pp} (the peak-to-peak distance of the first derivative line). The graphical illustrations given below are based on Gaussian lineshapes. However, the corresponding spectra for arbitrary lineshapes are easily calculated by the analytic expressions (3) and (4).

That the experimental spectra may be analyzed rather simply is due to the fact that the most polarized (in the following called maximum polarized) spectra of the individual radicals can have two possible form only. These spectral forms are denoted S and D and are displayed in fig.1 and 2, respectively. The spectral forms are shown in the absorption mode as well as in the first derivative absorption mode. Notice that in the absorption mode the spectral form (S or D) is obtained by turning the appropriate letter (S or D) 90° degrees. The spectral form D is associated with a 'dynamic' radical, i.e., a radical that is derived from another radical by a transfer reaction (e.g., an electron transfer). A dynamic radical is thus a secondary radical. The spectral form S is associated with a 'static' radical, i.e., a radical that is not derived from another radical. A static radical is thus one of the two original radicals formed after charge separation. This characterization of the radicals, whose ESR signals are observed, is obviously important for the process of classifying the primary events following charge separation. Another aspect which is inevitably associated with this process is that of assigning the ESR signals to specific chemical species. This assignment is based on the determination of the g-factors and the lineshapes of the radicals. This information also follows from the analysis.

The form of the radical spectra S and D may be understood by the following comments (a rigorous proof is given in [8]). The polarization is created in two radicals which are so close that an exchange interaction between them exist. It is known (see, e.g., [7]) that the polarization depends on the nuclear states of the radicals and that the sum of the integrated polarizations of both radicals is zero in high magnetic fields. Moreover, the integrated (or net) polarization of either radical is zero if and only if the two radicals have identical g-factors; the integrated polarization of a radical increases with increasing g-factor difference. For systems without diffusional motion of the radicals, as the one treated here, the polarization depends linearly upon the difference in ESR-resonance fields between the radicals in the specified spin states, see e.g., [5,7,8]. All these dependencies are satisfied by eq. (3):

$$\mathrm{I}\,(H)_{\mathrm{S}} = (H_0 - H)\, L_{\mathrm{S}}\,(H) \qquad (3)$$

which expresses the polarized spectrum of a static radical in terms of its equilibrium lineshape $L_{\mathrm{S}}\,(H)$ and the average resonance field of the other radical, cf. eq. (1). As expected one end of the spectrum is in emission and the other end in absorption. The absorption mode signal is zero at $H = H_0$. Since $-2x \exp(-x^2) = (\mathrm{d}/\mathrm{d}x)\exp(-x^2)$ it follows that for a Gaussian lineshape the spectrum given by eq. (3) can be written as a linear combination of the equilibrium spectrum and its first derivative. This is illustrated in fig.1 and may be helpful for interpretation purposes. The parameter h_0 in fig.1 determines the linear combination and is proportional to the difference between the g-factors (or H_0 values) of the two original radicals. In most cases $h_0 \ll 1$ and the spectral form S thus looks like slightly perturbed first derivative Gaussian line, cf. fig.1. The actual value of h_0 is given below in table 1 for various primary reactions.

There is a very important difference between the polarization of a static radical and of a dynamic radical which has not been appreciated before. The electron spin polarization of a dynamic radical is obtained by a transfer reaction involving no nuclear spins. Consequently, the polarization of the dynamic radical shows no dependence upon the nuclear spin states (or field) and the spectral form D thus becomes proportional to the lineshape function; the proportionality constant being equal to the average (i.e., net) polarization of the precursor radical. The maximum polarized spectrum of a dynamic radical may thus be written as:

$$\mathrm{I}\,(H)_{\mathrm{D}} = -[H_0 - H_0(\mathrm{S})]\; L_{\mathrm{D}}\,(H) \qquad (4)$$

where H_0 is the average resonance field of the precursor radical for the dynamic radical under study. The parameter H_0 (S) is the average resonance field of the other observed radical (e.g., if the spectrum of X^- is of type D then $H_0\,(\mathrm{S}) = H_0\,(\mathrm{P}^+)$. Figure 2 illustrates a D-spectrum. The term in the brackets in

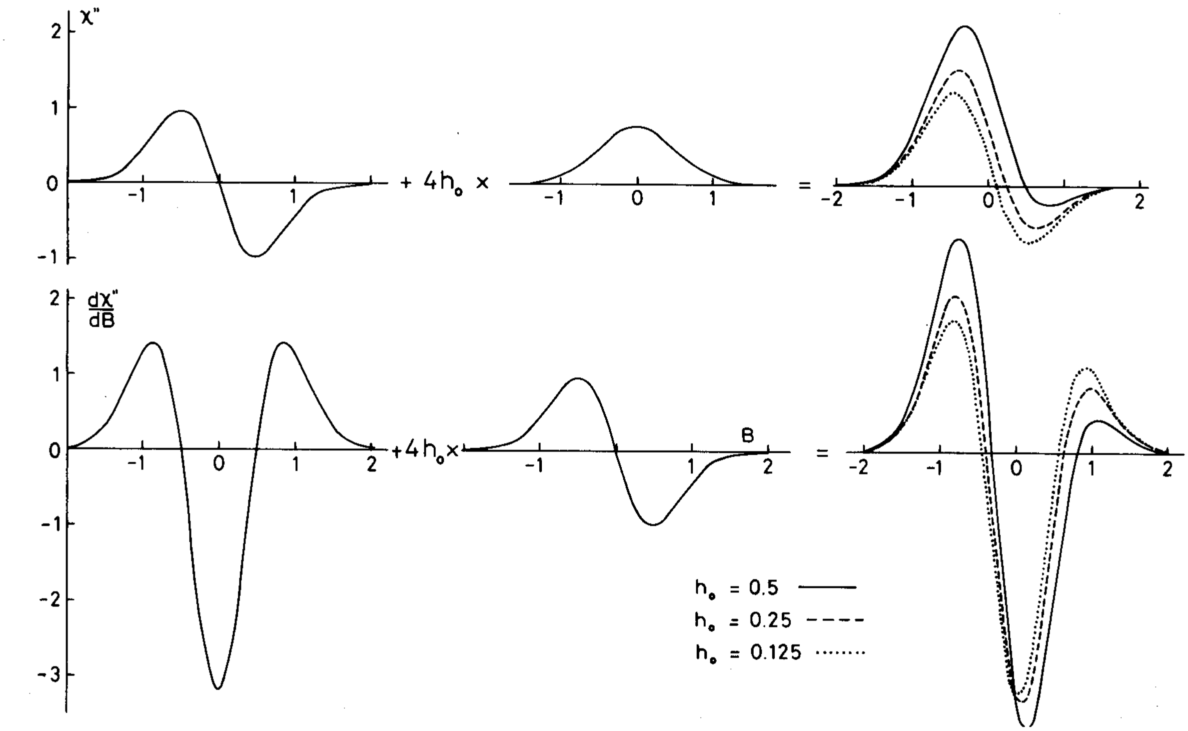

Fig.1. The spectral form S of a 'static' radical for various values of the parameter h_0. The absorption mode spectrum is shown to the right in the upper figure and is equal to a linear combination of the first derivative lineshape and the lineshape (assumed Gaussian) of the radical. The first derivative absorption mode spectrum is shown in the lower figure and is derived from the upper figure by differentiation. The peak-to-peak width is used as unit for the magnetic field B in all figures.

Table 1
Types of spectra for various primary reactions[a]

Primary reactions		P^+X^-	$P^+I^-X \rightarrow P^+IX^-$	$P^+I^-X \rightarrow P^+IX^-$
Spectrum of P^+		S	S	D
Spectrum of X^-		S	D	S
h_0 [b]	(P^+)	$H_0(X^-) - H_0(P^+)$	$H_0(I^-) - H_0(P^+)$	$H_0(I^+) - H_0(X^-)$
	(X^-)	$H_0(P^+) - H_0(X^-)$		
H_0	(P^+)	$H_0(X^-)$	$H_0(I^-)$	$H_0(I^+)$
	(X^-)	$H_0(P^+)$		

[a] The average resonance field H_0 (R) of a radical R is related to average g-factor g (R) by the resonance condition: $h\nu = g(\mathrm{R})\beta H_0(\mathrm{R})$, where ν is the microwave frequency, h Planck's constant and β the Bohr magneton

[b] The dimensionless value of h_0 is obtained by dividing the indicated value by $\Delta H(\mathrm{S})$, the peak-to-peak width of the equilibrium spectrum of the static radical. If both radicals are of type S then $\Delta H(\mathrm{S}) = \Delta H(X^-)$ when h_0 (P^+) is considered and $\Delta H(\mathrm{S}) = \Delta H(P^+)$ when h_0 (X^-) is considered

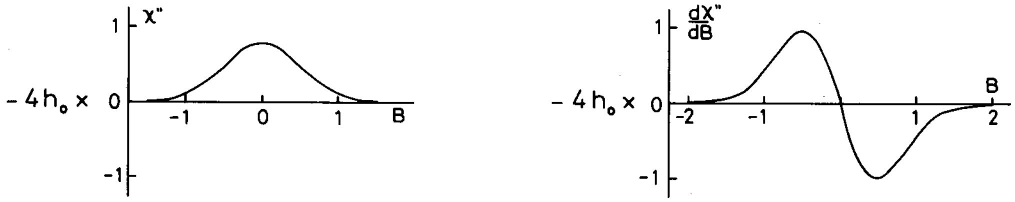

Fig.2. The spectral form D of a 'dynamic' radical. The spectrum is simply equal to the lineshape function of the radical multiplied by $-4h_0$.

eq. (4) is proportional to h_0 introduced earlier, cf. fig.2.

The observed spectrum is a superposition of the two maximum polarized spectra of P^+ and X^-. The form of this spectrum depends on the type of the spectra (S or D) of the radicals and of the value of H_0 (or h_0). Different primary reactions give rise to different types of superposed spectra (SD, DS and SS) and to different values of h_0. These dependences are summarized in table 1. For the purpose of illustration a SD spectrum is displayed in fig.3.

The primary reactions indicated in table 1 are the reaction following the formation of the charge transfer state and leading to the observed radical pair state P^+X^-. The processes following the primary reactions of table 1 are processes that deplete the radical pair state P^+X^-. These may be decay processes to lower laying neutral states or they may be secondary electron transport processes. A few words of explanation to table 1 seems adequate. In the first column the superposed spectrum consists of two S-type spectra. There is no primary reaction since there is no reaction arrow; this means either that the radical pair state P^+X^- is the initial radical pair state or that no polarization is developed in the initial state, which in turn can be due to a very short lifetime or a very large (or very small) exchange interaction. This case, where both radical spectra are of the same type,

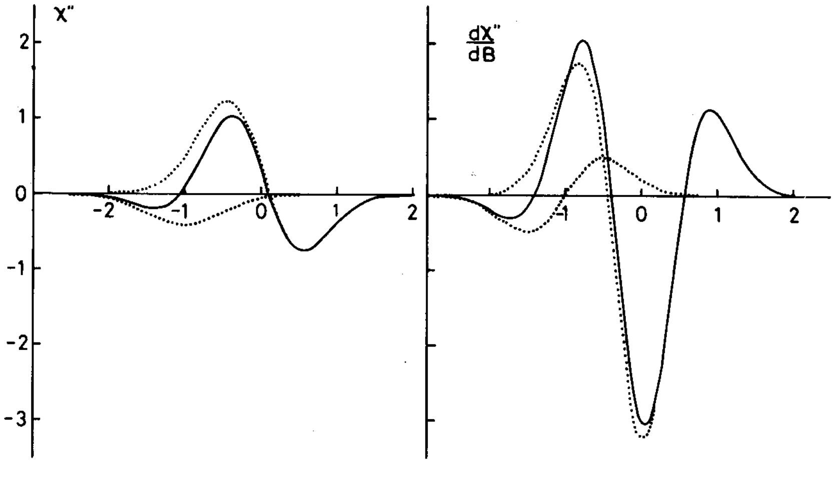

Fig.3. A maximum polarized spectrum of type SD. The spectrum, shown by the full line, is a superposition of the spectra of two radicals. These spectra, which are shown by dotted lines, are a D spectrum centered at −1 and an S spectrum centered at zero. The radicals are assumed to have the same spectral width.

requires two values for h_0 and H_0. In the other cases, the two radicals have different types of spectra and only one common value of h_0 (or H_0) is needed. In the second column the primary reaction is an electron transfer from an intermediate radical I^- to the primary acceptor X^-; the average field of I^- can be determined by h_0 (or H_0). In the third column the reaction is a hole (missing electron) transfer; again the intermediate radical I^+ can be characterized by its average resonance field which is determined through h_0 (or H_0). Other combination of primary reactions may be constructed from those given.

The absolute phase of the spectrum is not important for a determination of the primary processes as discussed here. Two spectra differing only by a sign change of I (*H*) are considered identical in this context. There is thus no need to indicate the absolute sign of I (*H*) on the ordinate axis. The information contained in the phase of the spectrum is discussed in [8].

3. Discussion

The method is directly applicable to spectra recorded for times shorter than T_1 after the light flash. These maximum polarized spectra are solely determined by the CIDEP polarization. However, the polarization decays towards the equilibrium polarization due to spin lattice relaxation. After $\sim 5T_1$ the spectrum has the form expected for an equilibrium system. If this 'equilibrium spectrum' can be observed, the widths and average fields of the radicals P^+ and X^- can be determined and these are, of course, useful for an identification of the radicals. The various degrees of freedom left for a construction of the maximum polarized spectrum are then: the value of H_0 (or h_0) and the types (static or dynamic) of the radicals. If the relaxed spectrum cannot be observed then all parameters must be determined from the maximum polarized spectrum. In general this may be possible. In fact a rough estimate of the parameters may often be performed simply by looking at the experimental spectrum and keeping fig.1 and 2 in mind, cf. fig.3. It may be checked whether an experimental spectrum can be considered a maximum polarized spectrum. This is the case if the integration value of the absorption mode spectrum is negligible compared to the integration value of only the positive (i.e., absorptive) parts of the spectrum. Relaxation effects (i.e., departures from a maximum polarized spectrum) can be corrected for by adding to the constructed maximum polarized spectrum the equilibrium spectra of P^+ and X^-, both with an integrated intensity equal to one half of the integration value of the experimental spectrum. This correction is adequate when P^+ and X^- have similar values of T_1 only, cf. [8].

The time dependence of the signal for a fixed field may show both a maximum and a minimum (or more generally two extrema), cf. [4]. This indicates that the two radicals P^+ and X^- have different values of T_1. Consequently, the maximum polarized spectra of the radicals are obtained at different times. Thus at no time does the observed spectrum correspond to a superposition of two maximum polarized single radical spectra. The method may still be applied, however, if the extremal values occur at well separated times t_1 and t_2 for some field values. For any field value the observed signal at time t_1 may then be approximately ascribed to the radical with the smallest T_1 and the signal at time t_2 may be ascribed to the other radical. The maximum polarized single radical spectra thus obtained may be analysed by the method. When the values of T_1 are not different enough to permit the maximum polarized spectra of the radicals to be determined by this approximate procedure, then the complete time dependence of the observed spectrum must be considered, cf. [8].

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References

[1] Dutton, P. L., Leigh, J. S. and Seibert, M. (1972) Biochem. Biophys. Res. Commun. 46, 406–413.

[2] Thurnauer, M. C., Katz, J. J. and Norris, J. R. (1975) Proc. Natl. Acad. Sci. USA 72, 3270–3274.

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

[4] McIntosh, A. R. and J. R. Bolton (1976) Nature 263, 443–445.

[5] Hoff, A. J., Gast, P. and Romijn, J. C. (1977) FEBS Lett. 73, 185–190.

[6] Dismukes, G. C., McGuire, A., Blankenship, R. and Sauer, K. (1978) Biophys. J. 21, 239–256.

[7] Freed, J. H. and Pedersen, J. B. (1976) Adv. Mag. Res. 8, 1–84.

[8] Pedersen, J. B. (1969) to be published.