ESR and ENDOR of Primary Reactants in Photosynthesis

A.J. Hoff

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

1.1 Basics of photosynthesis

Photosynthesis is the energy conversion process on which ultimately all life on earth depends. Because of this and because it may provide future ways of harnessing the sun's energy, photosynthesis is the most important chemical process on earth. Its basic mechanism is now slowly unravelling, to which ESR and ENDOR techniques have much contributed. The whole of the photosynthetic process is summed up in the reaction

$$2\ H_2X + Co_2 \xrightarrow{h\nu} (CH_2O) + H_2O + 2X,$$

where X stands for O in the case of plants, and may be sulfur or another reduced material in the case of photosynthetic bacteria, and CH_2O is the unit of carbohydrate. The initial photoreaction is represented by $D_1A_1 \xrightarrow{h\nu} D_1^+A_1^-$ which stands for a (quasi) irreversible photoinduced charge separation: D is a donor molecule or complex and A is an acceptor. In real life the charges on D and A are rapidly replaced:

$$D_2D_1^+A_1^-A_2 \rightarrow \begin{matrix} \nearrow D_2^+D_1A_1^-A_2 \\ \searrow D_2D_1^+A_1A_2^- \end{matrix} \rightarrow D_2^+D_1A_1A_2^- \text{ and so on.}$$

The electron that is liberated by the light quantum is finally transported to a biomolecule of high reductive potential (NAD^+ and $NADP^+$ (nicotinamide adenine dinucleotide(phosphate)) in bacterial and plant photosynthesis, respectively). This molecule is then oxidized in the carbohydrate producing cycle of reactions known as the Calvin cycle, named after its discoverer Melvin Calvin.
The charge separation $D\ A \xrightarrow{h\nu} D^+A^-$ is called the primary process of photosynthesis and it is this reaction that will mainly concern us in my lecture.

2 Bacterial photosynthesis

2.1 The primary donor

In bacterial photosynthesis one light reaction takes place, in contrast to plant photosynthesis where two different photoreactions occur. Consequently, bacterial photosynthesis is simpler, and lends itself better to detailed investigations than its plant counterpart.
It has been possible to isolate that part of the photosynthetic machinery in which the primary process, and nothing or little

else proceeds. This part is called the reaction center (RC). It consists of a protein molecule (MW about 70 kD), which contains 4 bacteriochlorophyll (Bchl), two bacteriopheophytin (Bph), one or two quinones, and one iron atom. The illumination of a RC preparation gives rise to a Gaussian ESR signal, of width ~ 9.5 and g-value g = 2.0026, with a risetime < 1 μs. Its decay at room temperature is complex, at low temperature (2 - 100 K) it decays with 30 ms. First discovered by Commoner et al. (1) and Sogo et al. (2), this signal has been the subject of many investigations (see general reference). It has been established that it is due to the oxidized primary donor, D^+.

Model studies on chemically oxidized monomeric bacteriochlorophyll revealed that the signal of D^+ resembled very much the ESR spectrum of $Bchl^+$, except for its linewidth, which was 1.4 times less. This, and similar evidence for plant material, led Norris (3) to the hypothesis that D is a bacteriochlorophyll dimer, on which the unpaired electron is fully delocalized. Subsequent ENDOR (electron-nuclear double resonance) experiments (4,5,6) demonstrated that this was indeed the case. Apparently, only a dimeric structure is able to photoeject an electron. The decay of the light-induced signal at low temperature was attributed to a back reaction in the dark: $D^+A^- \rightarrow D\ A$.

2.2 The "primary" acceptor

The first observation by ESR of A^- is due to Feher (7), who reported a very broad line with a principal g-value of 1.83, which could only be observed at low temperatures. Later work showed that this signal was due to a species with strongly anisotropic g-values: $g_1 = 1.83$, $g_2 = 1.68$. At the time it was attributed to an iron-sulfur protein, possibly a two-iron ferredoxin type. However, in iron-depleted RC the broad signal is replaced by a much narrower one ($\Delta H \sim 8.5$ G) with g = 2.0046. Model studies on chemically reduced ubiquinone, employing both X and Q band ESR spectroscopy, demonstrated that this signal is due to a semiubiquinone: UQ^-. For normal reaction centers Mößbauer studies showed that the Fe retained its valency (Fe^{2+}) upon photoreduction of the acceptor. Thus, the unpaired spin is mainly located on the ubiquinone, but its ESR spectrum is very much broadened by exchange interaction with the electron spin of the iron atom, which is in a (for Fe^{2+}) rather unusual S = 2 state.

2.3 The intermediary acceptor

Recently, it was found by Dutton and coworkers (8) and by Shuvalov and Klimov (9), that under conditions of high light intensity in preparations of the photosynthetic bacterium Chromatium vinosum in a reducing environment a new ESR signal appeared, with g = 2.0035, ΔH = 8.5 G which was accompanied by a doublet with spacing of about 50 G. This signal was identified to be due to an intermediary acceptor, I, which accumulated under the conditions used: cyt D I $A^- \xrightarrow{h\nu}$ cyt $D^+I^-A^- \rightarrow$ cyt$^+$D I^-A^-. Electron donation from the cytochrome to D^+ prevents the back reaction between D and I. By comparison with ESR and optical spectra of bacteriopheophytin (Bph) I^- was suggested to be a Bph^- molecule.

Apparently, in normal photosynthesis Bph (of which two molecules are found in purified RC's) serves as an intermediary electron acceptor, which lives for only about 250 ps, as demonstrated by picosecond laser spectroscopy. A paramagnetic exchange interaction of I^- with A^- is part of the RC results in the splitting of the signal of I^-.

2.4 The triplet state

When the primary acceptor is chemically reduced, forward electron transport stops at I^-, so that eventually the electron can fall back into the hole:

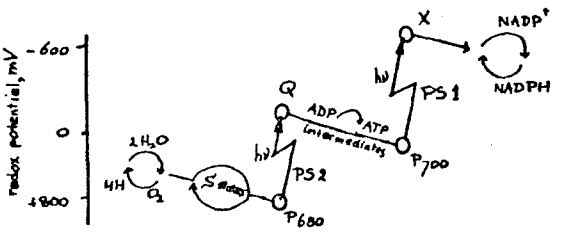
$$D^+I^-A^- \begin{cases} \rightarrow D\ I\ A^- \\ \rightarrow D^*I\ A^- \xrightarrow{1\ ns} D\ I\ A^- \\ \xrightarrow{10\ ns} D^TI\ A^- \xrightarrow{110\ \mu s} D\ I\ A^- \end{cases}$$

where the asterisk denotes the first excited singlet state, and T indicates the excited triplet state. The halftimes are derived from fast optical spectroscopy. The triplet state of the donor is relatively long-lived. It was first observed by Dutton and Leigh (10), and exhibits a most unusual spin-polarization with some of the lines inverted, which cannot be unobtained by the spin-orbit coupling mechanism for triplet generation. It probably results from spin-dephasing in the radical pair D^+I^- before recombination to D^TI takes place. Support for this mechanism has come from experiments on the dependence of the yield of the triplet state as a function of the strength of an applied magnetic field (11,12) and from the observation of spin-polarized doublet ESR signals in bacterial RC (13-15). Recently, the technique of electron spin resonance in zero magnetic field has been applied to the bacterial triplet, yielding accurate values of the zero field splitting parameters $|D|$ and $|E|$ and of the molecular radiationless decay rates k_x, k_y and k_z (16,17). An application of exciton theory has been published (18) which uses the measured values of monomeric and RC triplet state parameters to devise a geometric structure for the dimeric bacteriochlorophyll molecule. Thus, the triplet state D^T is important as a probe into the detailed geometric layout of the primary reactants within the reaction center.

3. Plant photosynthesis

3.1 Introduction

As already mentioned, in plants nature has devised a two-step mechanism of photosynthesis:



The two steps are called photosystems 1 and 2 (PS 1 and PS 2), respectively. They both consist of a light-induced charge separation followed by secondary reactions. The result after many successive steps is the transport of an electron from water to $NADP^+$, generating the high reductive power catalyst NADPH, whereas in-between a number of high-energetic ATP molecules are formed.The two photosystems can be structurally separated by biochemical techniques, but up to date no highly purified reaction centers have been prepared.

3.2 Photosystem 1

3.2.1 The primary donor

When plant material (leaves, or chloroplasts, i.e. intracellular structures containing the whole photosynthetic apparatus) is exposed to light, a ESR signal is generated (signal I) that much resembles that of the bacterial donor, viz. g = 2.0026 and Gaussian lineshape (19). Its linewidth is about 7.5 G, 1/1.4 times that of monomeric oxidized Chl a. Here also, ENDOR experiments demonstrated that the signal is due to an oxidized dimeric chlorophyll a molecule 3,4 . It can be shown that signal I is due to the donor of photosystem 1, P700, by means of selective irradiation (far-red light (λ > 700 nm) only excites PS 1) and by the use of various inhibitors. Thus, it appears that the donor of PS 1 resembles much the primary donor of photosynthetic bacteria, be it that Chl a instead of Bchl is used.

3.2.2 The primary acceptor

Half a decade ago it was found (20) that concurrently with signal I an ESR spectrum was induced by light, that showed characteristics of a reduced ferredoxin spectrum, viz. g = 2.05, 1.95, 1.86. This spectrum could also be generated by chemical reduction with dithionite ($Na_2S_2O_4$). The light saturation curve and its redox behaviour showed that it was an acceptor which was reduced at the same time as P700 was oxidized. It was therefore regarded to be the primary acceptor of PS 1. However, later work (21) revealed that another ferredoxin type of spectrum could be induced by light,with g-values of 2.05, 1.92 and 1.89. At low temperatures these signals are generated irreversibly,as is $P700^+$. Recently it was found (22,23) that when the two ferredoxin type centers (called A and B) were pre-reduced by long treatment with dithionite, a reversible signal appeared with g-values of 2.05, 1.83 adn 1.78, together with an almost completely reversible induction of $P700^+$. From this finding it was concluded that this species, X, represented the "true" primary acceptor. In non-treated material, photoreduction of A and/or B is irreversible at low temperatures, and so is P700 oxidation, but if A and B are reduced prior to illumination, the electron remains on X and can fall back to P700 via a tunneling back reaction. Thus, schematically:

$$P700\ X\ A\ B \xrightarrow{h\nu} P700^+X^-A\ B \begin{cases} \rightarrow P700^+X\ A^-B \\ \rightarrow P700^+X\ A\ B^- \end{cases}$$

and $P700\ X\ A^-B^- \xrightarrow{h\nu} P700^+X^-A^-B^- \rightarrow P700\ X\ A^-B^-$. The g-values of X^- are similar to those of ferredoxin type molecules. Quite

possibly it may be a reduced 2 Fe - 2 S cluster having magnetic interactions with A^-B^-, but this has as yet to be proven.

3.2.3 An intermediary acceptor

Very recently evidence has become available that between P700 and X another acceptor is situated (24-26). The optical spectrum is interpreted as being due to the anion of a Chl a dimer (25). From the ESR spectrum, however, it appears that it is a monomeric chlorophyllous species (27).

3.3 Photosystem 2

3.3.1 The primary donor

At room temperature, the primary donor, P680, of PS 2 is so quickly reduced after charge separation by a secondary donor (in less than 1 μs) that no ESR signal of $P680^+$ can be detected. However, at low temperatures, re-reduction is sufficiently slow to permit observation of a transient on top of the light-saturated signal I of PS 1. This transient is abolished by treatment with agents that reduce all components of PS 2, indicating that it indeed originates from its primary donor. Its spectral shape and g-value are identical to $P700^+$, suggesting that also P680 is a chlorophyll dimer.

3.3.2 The primary acceptor

So far no reports have appeared claiming the observation of an ESR signal of Q, the primary acceptor of PS 2. From optical work it can be inferred that it is a plastoquinone molecule (28). The reasons for its non-observability by ESR spectroscopy may be severe line broadening due to complexation with a transition metal, analogous to the quinone,iron complex in the bacterial photosystem.

3.3.3 An intermediary acceptor

Evidence is now accumulating that also in PS 2 a transient acceptor is found (29,30). From optical and ESR spectroscopy it transpires that it is a pheophytin monomer (29,31).

General reference
A.J. Hoff (1979) Applications of ESR in photosynthesis, Physics Reports 54, 75-200.

1. Commoner, B., Heise, J.J. and Townsend, J. (1956) Proc. Natl. Acad. Sci. USA 42, 710.
2. Sogo, P., Jut, M. and Calvin, M. (1959) Rad. Res. Suppl.I, 511.
3. Norris, J.R., Uphaus, R.A., Crespi, H.L. and Katz, J.J.(1971) Proc. Natl. Acad. Sci. USA 68, 625.
4. Feher, G., Hoff, A.J., Isaacson, R.A. and McElroy, J.D. (1973) Biophys. J. 13, 61.
5. Norris, J.R., Druyan, M.E. and Katz, J.J. (1973) J. Am.Chem. Soc. 95, 1680.
6. Feher, G., Hoff, A.J., Isaacson, R.A. and Ackerson, L.C. (1975) Ann. N.Y. Acad. Sci. 244, 239.
7. Feher, G. (1971) Photochem. Photobiol. 14, 373.

8. Dutton, P.L., Kaufmann, K.J., Chance, B. and Rentzepis, P.M. (1975) FEBS Lett. 60, 275.
9. Shuvalov, V.A. and Klimov, V.V. (1976) Biochim. Biophys. Acta 440, 587.
10. Dutton, P.L., Leigh,J.S. and Seibert, M. (1972) Biochem. Biophys. Res. Commun. 46, 406.
11. Blankenship, R.E., Schaafsma, T.J. and Parson, W.W. (1977) Biochim. Biophys. Acta 461, 297.
12. Hoff, A.J., Rademaker, H., van Grondelle, R. and Duysens, L.N.M. (1977) Biochim. Biophys. Acta 460, 547.
13. Hoff, A.J., Gast, P. and Romijn, J.C. (1977) FEBS Lett. 73, 185.
14. Gast, P. and Hoff, A.J. (1979) Biochim. Biophys. Acta 548, 520.
15. Hoff, A.J. and Gast, P. (1979) J. Phys. Chem. 83, 3355.
16. Clarke, R.H., Connors, R.E.,Norris, J.R. and Thurnauer, M.C. (1975) J. Am. Chem. Soc. 97, 7178.
17. Hoff, A.J. (1976) Biochim. Biophys. Acta 440, 765.
18. Clarke, R.H., Connors, R.E. and Frank,H.A. (1976) Biochem. Biophys. Res. Commun.71, 671.
19. Commoner, B., Townsend, J. and Pake, G.E. (1954) Nature 174, 689.
20. Malkin, R. and Bearden, A.J. (1971) Proc. Natl. Acad. Sci. USA 68, 16.
21. Evans, M.C.W., Telfer, A. and Lord, A.V. (1972) Biochim. Biophys. Acta 267, 530.
22. Evans, M.C.W., Shira, C.K., Bolton, J.R. and Cammack, R. (1975) Nature 256, 668.
23. McIntosh, A.R. and Bolton, J.R. (1976) Biochim. Biophys. Acta 430, 555.
24. Sauer, K., Mathis, P., Acker, S. and Van Best, J. (1978) Biochim. Biophys. Acta 503, 120.
25. Shuvalov, V.A., Dolan, E. and Ke, B. (1979) Proc. Natl. Acad. Sci. USA 76, 770 - 773.
26. Shuvalov, V.A., Ke, B. and Dolan, E. (1979) FEBS Lett.100,5.
27. Heathcote, P. and Evans, M.C.W. (1980) FEBS Lett. 111, 381.
28. Van Gorkom, H.J. (1974) Biochim. Biophys. Acta 347, 439.
29. Klimov, V.V., Klevanik, A.V. and Shuvalov, V.A. (1977) Biochim. Biophys. Acta 546, 248.
30. Rademaker, H., Hoff, A.J. and Duysens, L.N.M. (1979) Biochim. Biophys. Acta 546, 248.
31. Baltimore, B. and Malkin, R. (1980) in the press.