Hyperfine structure of the photoexcited triplet state \(^3\)P680 in plant PS II reaction centres as determined by pulse ENDOR spectroscopy

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Abstract

The triplet states in plant photosystem II (PS II), \(^3\)P680, and from chlorophyll \(\text{a}, \(^3\text{Chl} \text{a}\), in organic solution have been investigated using pulse ENDOR combined with repetitive laser excitation at cryogenic temperature with the aim to obtain their hyperfine (hf) structure. The large zero field splitting (ZFS) tensor of \(^3\)P680 enabled orientation selection via the electron spin resonance (EPR) field setting along the ZFS tensor axes. ENDOR spectra have been obtained for the first time also for the in-plane \(X\) - and \(Y\)-orientations of the ZFS tensor. This allowed a full determination of the hf-tensors of the three methine protons and one methyl group of \(^3\)P680. Based on the orientations of the axes of these hf-tensors, a unique orientation of the axes of the ZFS tensor of \(^3\)P680 in the Chl \(\text{a}\) molecular frame was obtained. These data serve as a structural basis for determining the orientation of \(^3\)P680 in the PS II protein complex by EPR on single crystals (see M. Kammel et al. in this issue). The data obtained represent the first complete set of the larger hf-tensors of the triplet state \(^3\)P680. They reflect the spin density distribution both in the highest occupied (HOMO) and lowest unoccupied (LUMO) orbitals. The data clearly confirm that \(^3\)P680 is a monomeric Chl \(\text{a}\) species at low temperature \((T = 10 \text{K})\) used, as has been proposed earlier based on \(D\) - and \(E\)-values obtained from EPR and optically detected magnetic resonance (ODMR) studies. Comparison with the hf data for the cation and anion radicals of Chl \(\text{a}\) indicates a redistribution of spin densities in particular for the LUMO orbital of the triplet states. The electron spin distribution in the LUMO orbital is of special interest since it harbours the excited electron in the excited \(^3\P680\) singlet state, from which light-induced electron transfer proceeds. Observed shifts of hf couplings from individual nuclei of \(^3\)P680 as compared with \(^3\text{Chl} \text{a}\) in organic solution are of special interest, since they indicate specific protein interactions, e.g. hydrogen bonding, which might be used in future studies for assigning \(^3\)P680 to a particular chlorophyll molecule in PS II.

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1. Introduction

In reaction centres (RCs) of photosynthetic organisms, charge separation starts from the photoexcited singlet state of a chlorophyll (Chl) or bacteriochlorophyll (BChl) spe-
detail by a variety of electron spin resonance (EPR) techniques [7–11].

Under physiological conditions, triplet states of the chlorophyll or pheophytin molecules are not observed in the RCs. Chlorophyll triplet states are carefully avoided, since they lead, in the presence of oxygen, via triplet–triplet quenching to formation of singlet oxygen, which in turn leads to cell damage. This is prevented by incorporation of carotenoid molecules in all photosystems, which effectively quench the chlorophyll triplet states, resulting from intersystem crossing, by fast triplet energy transfer. The carotenoid triplet state is too low in energy to enable formation of singlet oxygen and is deactivated by internal conversion to the singlet ground state [12]. However, when the electron transport to the quinone acceptors is blocked by pre-reduction, coherent singlet–triplet mixing in the primary radical ion pair leads to recombination and formation of the triplet state of the primary donor, $^3P$, which is formed (in particular at low temperatures) with high yield [13].

Although $^3P$ is not a functional state during normal electron transfer, its investigation is important for several reasons. (i) Using time-resolved spectroscopy, kinetic studies were performed in order to determine the important triplet energy transfer rates [13–16]. (ii) For understanding the triplet state $^3P$ and its reactions, its electronic structure is of considerable interest [14]. (iii) Formation of $^3P_680$ has been implicated in photo-inhibition, a physiologically significant phenomenon, which at high light intensities leads to a decrease in plant productivity [17]. (iv) $^3P$ exhibits two unpaired electrons, one in the highest occupied molecular orbital (HOMO), the other in the lowest unoccupied molecular orbital (LUMO). The spatial distribution of the LUMO is of particular interest since charge separation occurs from this orbital in the excited singlet state. Since the excited singlet state is short-lived and diamagnetic, information on the electronic distribution of the LUMO is only available via the triplet state [14,18].

The two interacting unpaired electrons in the HOMO and LUMO in $^3P$ lead to a zero field splitting (ZFS) in the spectra, described by two parameters, $D$ and $E$, which have been investigated in great detail by optical detected magnetic resonance (ODMR) [8,13,19] and EPR techniques [14,19,20]. For the bacterial RC the obtained $D$- and $E$-values indicated that $^3P$ is delocalized over the two BChl $a$ molecules which constitute P. In plant PS I ($^3P_{700}$) and II ($^3P_{680}$) the $D$- and $E$-values indicate a localization of the triplet state on one Chl $a$ molecule at low temperature [8,13,14,20,21]. Interestingly, a similar behaviour was found for the respective cation radical states P$^+$ based on their hyperfine (hf) data obtained from electron nuclear double resonance (ENDOR) spectroscopy [9,11,22–27]. The localisation of the triplet states is in contrast to the respective excited singlet states $^1P_{700}$ and $^1P_{680}$, which are known to be excitonically delocalized [28,29]. While excited singlet states interact via excitonic coupling ( Förster mechanism) [30] over large distances, excited triplet states interact via the Dexter mechanism, which requires orbital overlap and hence much closer distances [31].

The ZFS parameters $D$ and $E$, on which most of the interpretations were so far based, are integral properties of the triplet wave function, and depend on its overall spatial distribution. However, electronic properties, in particular the amount of charge transfer character in a dimeric species, strongly affect the observed $D$- and $E$-values [14,18]. This seriously complicates their interpretation. Additional and more specific information about the electron distribution in the HOMO and LUMO orbitals is obtained from the electron-nuclear hyperfine couplings (hf), from which the distribution of the unpaired electrons of $^3P$ is obtained. In contrast to the large body of EPR and ODMR investigations, yielding $D$- and $E$-values, there are only a few studies on the hf structure of the triplet state $^3P$, which is only resolved by ENDOR spectroscopy [18,32,33]. Pulse ENDOR combined with repetitive laser excitation at low temperatures is well-suited since this method takes advantage of the large spin polarization of $^3P$ being present only in the first few microseconds after the laser pulse. In an earlier pulse ENDOR study on $^3P_{865}$ in RCs of the bacterium Rhodobacter sphaeroides, we could show that by this method hf-tensor components including their signs (relative to that of $D$) can be obtained, which enabled an assignment to specific nuclei and, by comparison with the cation and anion radical of BChl $a$, allowed us to discriminate between hf couplings from the HOMO and LUMO orbitals. Thereby a detailed characterization of $^3P_{865}$ was obtained showing an asymmetric distribution of this triplet state over the two BChl $a$ molecules constituting $^3P_{865}$. In a dimeric species ($P_L P_M$, where $L$ and $M$ indicate the two branches of pigments), a delocalized triplet state is a superposition of four contributions [18]. The two local excitations, $^{3}_P P_L P_M$ and $^{3}_P P_L^+ P_M$, and the two charge transfer states, $^{3}_P (P_L^+ P_M^-)$ and $^{3}_P (P_L^- P_M^+)$. Our data indicated for $^3P_{865}$ a significant charge transfer character ($\approx 20\%$), most probably in favour of $^{3}_P (P_L^+ P_M^-)$ [18].

The primary donor, P680, of plant PS II is of particular interest for several reasons. The origin of the strongly positive oxidation midpoint potential for P680/P680$^{++}$ ($\geq 1.2$ V), necessary for water splitting, is still not understood. It indicates unusual orbital energies, most probably induced by interactions with the protein, that change the electronic properties as compared with Chl $a$ in organic solvents. ENDOR investigations have been performed to explore the hf structure of the cation radical P680$^{+}$. No dramatic changes have been found as compared with Chl $a$ in organic solvents or the cation radical of Chl$\_y$, which acts as donor to P680$^{+}$ in plant PS II [26,27]. However, these studies have also shown that it is not possible to accumulate P680$^{+}$ with low temperature illumination in the presence of external donors, without having contributions from Chl$\_y^+$ and/or other Chl$^-\_$ species. Furthermore, depending on the conditions, also additional carotenoid cation radicals were observed [26,27,34,35].
The triplet state in PS II, \(^3\)P680, is easier to prepare. Interactions with the protein are expected to influence its hf structure, which reflects the spin density distributions both in the HOMO and LUMO orbital. It is however important to note that unlike in bacterial RCs the triplet state, \(^3\)P680 may reside on a molecule different from the cation radical state P680\(^{+}\). A localization of \(^3\)P680 on one of the accessory chlorophylls, Chl\(_{D1}\) or Chl\(_{D2}\), was suggested earlier by van Mieghem et al. [36], based on EPR investigations on oriented plant PS II membrane fragments. Recent EPR work on PS II single crystals corroborates this suggestion and yields additional details on the triplet axes [37]. Interestingly, models for the light-induced charge separation in PS II have been discussed, which are different from those for bRCs and involve Chl\(_{D1}\) as early primary donor, before the positive charge moves on to P680 [38–40].

In this paper we report on a pulse ENDOR investigation of \(^3\)P680 in PSII RCs (also known as the D1D2 cytochrome b559 complex) and the triplet state of isolated chlorophyll, \(^3\)Chl \(a\) in frozen organic solvents, using repetitive laser excitation. The large ZFS enabled single crystal type ENDOR spectra to be recorded, using the EPR field position for selection of molecules with particular orientations in the frozen solution samples for the ENDOR experiment. This study gives the first complete picture of the large hf-tensors, which are important to characterize the unpaired electron distribution in the two triplet states \(^3\)P680 and \(^3\)Chl \(a\). The anisotropic hf-tensor components presented here, for all three canonical orientations of the triplet state, enable an assignment to specific nuclei and thereby allow a determination of the orientation of all three triplet axes, with respect to the molecular axes of \(^3\)P680. This result is a prerequisite for the interpretation of the EPR data obtained for \(^3\)P680 in PS II single crystals [37]. Comparison of the hf data with those of \(^3\)Chl \(a\) in organic solvents clearly confirms that \(^3\)P680 is a monomeric chlorophyll triplet species at \(T = 10\) K. Observed shifts of individual hf couplings different from those in organic solvents indicate specific interactions with the protein.

2. Materials and methods

2.1. Sample preparation

Chl \(a\) was extracted from spinach and dissolved in highly purified deoxygenated 2-methyl tetrahydrofuran (MTHF). Samples (concentration \(\approx 1\) mM) were prepared anaerobically on a high vacuum line in standard EPR quartz tubes (4-mm O.D., 3-mm I.D.), sealed and then quickly frozen in liquid nitrogen to form an optically transparent glass. PS II RCs (D1/D2-cyt b-559) lacking the quinone acceptors were prepared from pea as described previously [41,42] and concentrated to \(\approx 0.5\) mM. Triplet states were generated inside the optically transparent dielectric ring ENDOR resonator (Bruker, ESP 380-1052 DLQ-H) at \(T = 10\) K using repetitive pulse laser excitation; see below [18].

2.2. Transient and pulse EPR

Transient EPR was performed using continuous microwave irradiation and recording transient signals after laser pulse excitation for each field position. The spectrum was obtained by plotting the integrated signal intensity between 0.5 and 1.5 \(\mu s\) versus the magnetic field (Ref. [43], see accompanying paper).

Pulse EPR spectra were recorded using a pulse sequence as outlined in Fig. 1, leaving out the rf pulse. Spectra were obtained by plotting the integrated echo intensity as function of the magnetic field.

2.3. Pulse ENDOR

Pulse ENDOR experiments were performed on a Bruker ESP 380E pulse EPR spectrometer equipped with a Bruker ESP 360D-P pulse ENDOR accessory, a 500-W ENI A500 radio frequency amplifier, and an Oxford CF935 cryostat for temperature control. Samples were excited inside the optically transparent dielectric ring ENDOR resonator with a frequency doubled Nd:YAG laser (Spectra-Physics, GCR 130) with 8-ns pulses at 532 nm and \(\sim 10\) mJ incident light energy per pulse with a 10-Hz repetition rate. According to the Davies ENDOR scheme [44], weak selective microwave pulses synchronized with the laser pulses were used for preparation (first \(\pi\) pulse 1 \(\mu s\) after the laser pulse) and for detection (echo sequence \(\pi/2, \pi, \sim 3\) \(\mu s\) after the end of the radio frequency \(\pi\) pulse (Fig. 1). The 56- and 112-ns microwave \(\pi/2\) and \(\pi\) pulses correspond to a rotating frame microwave field strength of \(B_1 \approx 0.16\) mT. The 8-\(\mu s\) RF pulse with an rf field strength of \(B_2 \approx 1.5\) mT is a \(\pi\) pulse for protons. A comprehensive description of pulse EPR and ENDOR techniques has been given by Schweiger [44]. Pulse ENDOR spectra were accumulated for \(\approx 15\) h at 10 K.

**Fig. 1.** Pulse scheme for Davies ENDOR on photoexcited triplet states of \(^3\)Chl \(a\) and \(^3\)P680 showing laser, microwave (mw) and radio frequency (rf) ENDOR pulses. \(t = 1\) \(\mu s\), \(T = 12\) \(\mu s\), \(\tau = 250\) ns; pulse durations: laser: 8 ns, mw 90° pulse: 56 ns, mw 180° pulse: 112 ns, rf 180° pulse: 8 \(\mu s\). For other conditions see text.
3. Results

3.1. EPR spectra

Fig. 2A shows the Zeeman splitting of the spin energy levels for the three canonical orientations of a triplet state (magnetic field parallel to the triplet axes $X$, $Y$, and $Z$) for the case of a zero-field splitting parameter $D>0$. For $^3$P680 a positive sign of $D$ is expected, as it is generally the case for $\pi-\pi^*$ triplet states of porphyrins [36]. $^3$P680 is formed by charge recombination from the singlet-born primary radical pair $P^*$. This leads in a magnetic field to exclusive population of the $m_S=0$ sublevel, as indicated by bold lines (Fig. 2A) [8,13,14]. From this scheme the spin polarization pattern in the EPR spectrum is expected to be AEEAAE ($A$=absorption, $E$=emission), proceeding from the low-field $Z_I$, via $X_{II}$,... to $Z_{II}$ at the high-field side. The possible EPR transitions for the three canonical orientations are indicated in Fig. 2A. The transient EPR spectrum of $^3$P680 obtained in frozen PSII RC solution at 10 K is shown in Fig. 2B. The spectrum exhibits the predicted polarization pattern from Fig. 2A. The dotted traces show the simulated powder pattern (sum of all possible orientations) for the $m_S=0$ to $m_S=+1$ transition ($X_I$, $Y_I$, $Z_I$), in absorption, and for the $m_S=0$ to $m_S=-1$ transition, ($X_{II}$, $Y_{II}$, $Z_{II}$), in emission. The observed spectrum is the superposition of both patterns.

In contrast to $^3$P680 the triplet state of Chl $a$ in MTHF is generated by spin–orbit-coupling-induced intersystem crossing from the photoexcited singlet state of Chl $a$. This process leads to a lower triplet yield as compared with $^3$P680 and also to a lower spin polarization since more than one triplet sublevel is occupied [14]. The observed polarization pattern is EAEAEA, starting with the low field $Z_I$ transition (spectrum not shown).

The zero-field splitting parameters obtained for $^3$P680 were $|D|=287 \times 10^{-4} \text{ cm}^{-1} \pm 1\%$ and $|E|=43 \times 10^{-4} \text{ cm}^{-1} \pm 8\%$. For the triplet of $^3$Chl $a$ in MTHF (spectrum not shown) we obtained $|D|=282 \times 10^{-4} \text{ cm}^{-1}$ and $|E|=38 \times 10^{-4} \text{ cm}^{-1}$ with similar errors. The values for both triplet states agree within their error margins and are in good agreement with values reported earlier for both triplet states [13,14,21]. This is in contrast to the case of $^3$P865 in the bacterial RCs, where a strong reduction (ca 30%) of both the $D$- and $E$-values as compared with those of $^3$BChl $a$ in organic solvent was observed, which was attributed to delocalization of the triplet state over two BChl $a$ molecules in $^3$P865 [13,14,20].

Fig. 2C shows the field-swept electron spin echo (ESE) spectrum of $^3$P680 also obtained at $T=10$ K. The spectrum was recorded with the same microwave pulse parameters as used for the ENDOR spectra, except for the missing rf pulse (see below). Comparison with the transient EPR spectrum (Fig. 2B) clearly shows that in the pulse EPR spectrum the intensities of noncanonical orientations (field positions in between the $X$, $Y$, and $Z$-orientations) are diminished (see discussion below).

3.2. Pulse ENDOR spectra

3.2.1. ENDOR spectra from the $Z_I$- and $Z_{II}$-orientations

Pulse ENDOR spectra of $^3$P680 and $^3$Chl $a$ were recorded starting with field settings corresponding to the respective $Z_I$ and $Z_{II}$ EPR transitions (Fig. 3B). Thereby exclusively orientations of $^3$P680 and $^3$Chl $a$ molecules with their respective triplet $z$ axis, $Z$, parallel to $B_0$ were selected, yielding single crystal-like ENDOR spectra. Fig. 3A shows the spin energy levels for this specific case, including hf interaction for one nuclear spin I=1/2 in the high-field limit [46]. The $Z_I$ and $Z_{II}$ EPR transitions are indicated by thin
ENDOR transitions for Z

Gray thin arrows: m field parallel to the Z difference between the two field positions. Z arrows (and AZ negative hyperfine splitting Fig. 3. (A) Spin energy levels of a triplet state showing positive and m spectra are shifted (see shift of the intense narrow line at m connecting the m hf shifted ENDOR line is expected at m side with respect to AZ spectrum (Fig. 3) . Hence, the magnitude and the sign of the microwave k frequency side, in case of nucleus i. In case of A transition (connecting the m hf coupling A Z (i) ; black thick arrows: ENDOR transitions for Z I; black thick arrows: ENDOR transitions for Z II; gray thick arrows: ENDOR transitions for Z II) are directly (i) (relative to the sign of D) are directly

The pulse ENDOR spectra of 3P680 at T = 10 K for the Z I and Z II EPR transition show indeed the expected behaviour (Fig. 3B). A strong, narrow line is observed at vH and, in addition, four broader lines are observed, one shifted to higher and three to lower frequencies. According to Fig. 3A, the low-frequency ENDOR line in the spectrum for the Z I EPR transition results from a positive hf coupling, the high-frequency ENDOR lines from three negative hf couplings. As expected from Fig. 3A, the positions of the ENDOR lines with respect to vH are exchanged, when the Z II EPR transition connecting the m = 0 and m = −1 levels is selected (Fig. 3B). Now the line of the positive hf coupling is shifted to higher frequency and those of the negative hf couplings to lower frequency. The ENDOR spectrum connected to the Z II EPR transition is, as expected from Fig. 3A, in emission, but has been inverted in Fig. 3B for better comparison. The obtained hf couplings A Z (i) are given in Table 1.

A comparison of the pulse ENDOR spectrum for the EPR Z I transition of 3P680 and the triplet state of chlorophyll in MTHF, 3Chl a, is given in the lowest two traces of Fig. 4. Note that the frequency scale gives here the deviation from vH. For 3P680 only one narrow line is observed on the low-frequency side corresponding to one positive A Z (1), but three lines are observed on the high-frequency side, corresponding to three negative A Z (2,3,4) values. Due to the high triplet yield and strong spin polarisation (selective population of the m = 0 level), it was possible to set the EPR field at the outer wing of the Z I edge (Fig. 2B), resulting in a very narrow orientation selection. The spectrum of 3Chl a exhibits broader lines as compared with 3P680. This has probably two reasons. (i) 3Chl a is generated by spin orbit-induced intersystem crossing from the excited singlet state of Chl a, with a lower triplet yield and less selective population of the triplet sublevels as compared with 3P680. Consequently the signals were much smaller as for 3P680. Therefore, spectra with sufficient signal-to-noise ratio could not be obtained at the outer wings of the Z edge, and an EPR field setting close to the maximum of the Z edge had to be chosen, resulting in a broader distribution of orientations contributing to the ENDOR spectrum. (ii) For 3Chl a in frozen glassy organic solution (MTHF), a distribution of slightly different microenvironments is expected, which results in a distribution of the ZFS parameters D and E (ZFS-strain), as well as in a distribution of the hf-tensor values A K>Z, which both causes broadening of the observed ENDOR lines.

For 3Chl a one intense line is observed on the low-frequency side (positive A Z (1)). In addition there is a weak broad shoulder near −5 MHz, which is not observed in

Fig. 3. (A) Spin energy levels of a triplet state showing positive and negative hyperfine splitting A z from one nucleus with spin I = 1/2 for the field parallel to the Z-axis of the ZFS tensor. Black thin arrows: m_s=0 to m_s=1 EPR transition (Z I); black thick arrows: ENDOR transitions for Z I. Gray thin arrows: m_s=0 to m_s=−1 EPR transition (Z II); gray thick arrows: ENDOR transitions for Z II. (B) Pulse ENDOR spectrum of 3P680 for the Z I and Z II EPR field positions (compare Fig. 2). Note that the two ENDOR spectra are shifted (see shift of the intense narrow line at vH) due to the large difference between the two field positions.

black and grey arrows, the ENDOR transitions by bold black arrows (Z I field position) and bold grey arrows (Z II field position). From this scheme, it is clear that the ENDOR transition frequencies are no longer symmetrically spaced about the nuclear Zeeman frequency, vH, as for a doublet state (S=1/2). Instead, a strong line is expected from the m_s=0 manifold at the Larmor frequency, vH, and for the EPR Z I transition (connecting the m_s=0 and m_s=+1 levels), one hf shifted ENDOR line is expected at vH−A Z (i) for each nucleus i. In case of A Z (i)=0 this line occurs on the low-frequency side, in case of A Z (i)<0 on the high-frequency side with respect to vH. When the EPR Z I transition, connecting the m_s=0 and m_s=−1 levels, is driven with the microwave π-pulse, the lines change sides in the ENDOR spectrum (Fig. 3). Hence, the magnitude and the sign of the hf coupling A Z (i) (relative to the sign of D) are directly obtained from the shift of the respective ENDOR line relative to vH. Note that A Z (i) is the hf component along the triplet Z-axis, which is perpendicular to the π-plane. Furthermore, the ENDOR spectrum connected with the m_s=0 to m_s=+1 EPR transition (Z I) is expected to be in absorption, while that connected with the m_s=0 to m_s=−1 EPR transition (Z II) should be in emission [26].
Table 1
Hyperfine couplings [MHz] for the methyl and methine protons of 3P680, 3Chl a, and the cation and anion radical states Chl a⁺⁺ and Chl a⁻⁻.

<table>
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<th>Molecule</th>
<th>Hyperfine component</th>
<th>Assignment</th>
<th>A_X</th>
<th>A_Y</th>
<th>A_Z</th>
<th>A_A</th>
<th>A_B</th>
<th>A_C</th>
<th>A_D</th>
<th>A_E</th>
<th>A_F</th>
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<td></td>
<td></td>
<td>18</td>
<td>1.2</td>
<td></td>
<td>0.3</td>
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a See molecular structure, Fig. 5.
b Principal component of the respective hyperfine tensor, axes collinear with the ZFS axes X, Y, Z. A_X-tensor values of + 9.6 and − 5.4 MHz for 3P680 and + 8.7 MHz for 3Chl a have been reported in Ref. [33].
c See text, Fig. 5 for molecular positions and Fig. 4 for line numbering (in brackets) nd, not determined.
d ENDOR in liquid solution, radical generated electrolytically in 1,2-dimethoxy-ethane (DME).
e ENDOR in liquid solution, radical generated with I₂ in CH₂Cl₂.
f ENDOR in liquid solution, radical generated electrochemically in 1,2-dimethoxy-ethane (DME). A_iso = +1.7 and − 1.53 MHz for β-protons at positions 17 and 18, see text [9,48].

3P680 (see below). Only two lines are clearly resolved on the high-frequency side, A_2(3,4). However, a weak third line, A_3(2), seems to be superimposed on the low-frequency side of the line at − 7 MHz or, alternatively, on the low-frequency side of the line at 10–11 MHz. The obtained hf tensor components A_2(i) are listed in Table 1.

There is only one previous study reporting hf values A_2(i) from 3P680 and 3Chl a [33]. In that work a different technique, transient ENDOR, was used, which is more sensitive to smaller couplings. Several small hf couplings were observed in Ref. [33], which are difficult to observe with pulse ENDOR used in the present study. Also some of the large couplings have been observed by these authors (see Table 1, caption). The two A_2(i) values reported for 3P680 agree within the error margins with the A_2(1) and A_2(2) values of our study. The two larger negative values A_2(3,4) found in our work were, however, not reported in Ref. [33]. The positive A_2(1) value observed in the previous study for 3Chl a [33] is somewhat larger than the value in this paper. This could be due to the different solvent used. None of the negative A_2(2,3,4) values were observed in Ref. [33]. The data presented in Table 1 represent the first complete set of the large hf couplings for 3P680 and 3Chl a, which reflect the major spin densities in the HOMO and LUMO orbitals of the respective triplet state.

3.2.2. ENDOR spectra from the X₁ and Y₁ orientations of 3P680
Pulse ENDOR spectra of 3P680 were also measured for the other canonical orientations where the magnetic field \( B_0 \) is parallel to the zero field axes X and Y (EPR transitions X₁ and Y₁). The obtained ENDOR spectra for these EPR transitions are shown in Fig. 4, upper two traces. For the X₁ spectrum three high-frequency lines 2, 3, 4, resulting from negative A_2(2,3,4) values, are observed, which are significantly shifted to lower frequencies as compared with the spectrum for the Y₁ EPR transition. There is also a small shift of the low-frequency line 1, resulting from a positive A_2(1) value. Interestingly there is an additional inverted line (in emission) on the high-frequency side at \( \approx 10 \) MHz. Closer inspection of Fig. 2B shows that the X₁ position in the EPR...
spectrum corresponds to an edge of the powder pattern of the absorptive \( m_S = 0 \) to \( m_S = -1 \) transition, and hence good orientation selection along \( X \) is achieved for the ENDOR lines connected with this EPR transition, which are expected to be in absorption (compare Fig. 3A). This is reflected in the narrow lines of the ENDOR spectrum. However, at the \( X \) position in the EPR spectrum (Fig. 2B) there is also a contribution expected from the emissive \( m_S = 0 \) to \( m_S = -1 \) transitions, from orientations in the \( Z-Y \) and \( Z-X \) plane not too far from \( Z_{II} \). The ENDOR lines connected to this EPR transition are expected to be in emission. Indeed, an emissive line, marked with an asterisk, is observed on the high-frequency side in the \( X \) ENDOR spectrum (Fig. 4). Since for this “\( Z_{II} \)” transition the high- and low-frequency ENDOR lines are exchanged (see Fig. 3B) this line should correspond to the positive coupling \( A(1) \). The value of the hf coupling from this line agrees remarkably well with the \( A(1) \) value observed in the \( Z_I \) and \( Z_{II} \) spectra. The emissive high-frequency ENDOR line in the \( X \) ENDOR spectrum is surprisingly narrow in view of the fact that the emissive EPR part at the \( X \) EPR field position does not correspond to a canonical orientation but to a distribution of noncanonical orientations both in the \( Z-Y \) and \( Z-X \) plane (Fig. 2B). This is due to the small anisotropy of the \( A(1) \) hf tensor which leads only to small shifts of the ENDOR line 1 in the \( X_I, Y_I \), and \( Z_I \) ENDOR spectra. Similar emissive ENDOR lines from \( m_S = 0 \) to \( m_S = -1 \) EPR transitions are also expected from the negative hf couplings of lines 2, 3, and 4. These should appear on the low-frequency side of the \( X_I \) ENDOR spectrum. The corresponding hf tensors \( A(2,3,4) \) have, however, much larger anisotropy (see below), which broadens these emissive lines on the low-frequency side beyond detection.

The \( Y_I \) field position (Fig. 4, trace 2) is expected to pick up not only the \( Y \)-orientation, but also a distribution of orientations, both from the \( m_S = 0 \) to \( m_S = +1 \) and the \( m_S = 0 \) to \( m_S = -1 \) transitions. Consequently the observed lines from anisotropic couplings are broader than those obtained at the other orientations. Two of the three high-frequency lines corresponding to negative \( A_I(3,4) \)-values are shifted to considerably higher frequencies. The third line, from \( A_I(2) \), has very low intensity and is shifted close to \( v_{II} \) (see discussion below). Again there is an additional emissive line marked with an asterisk on the high-frequency side resulting from the positive coupling from the \( m_S = 0 \) to \( m_S = -1 \) EPR transition (Fig. 2B). The low-frequency line at \( \approx -10 \) MHz (positive \( A_I(1) \)) is still narrow and close to its position in the \( Z_{II} \) spectrum. Several very weak additional features are observed on the low-frequency side (see below).

It is obvious from Fig. 4 that the negative hf couplings exhibit a large anisotropy, while the anisotropy for the positive hf coupling is small. This explains why in the \( X_I \)- and \( Y_I \)-spectra the emissive lines from the \( m_S = 0 \) to \( m_S = -1 \) EPR transition, which are observed for the positive hf coupling \( A(1) \), are not observed for the negative hf tensors \( A(2,3,4) \). All anisotropic hf tensor components obtained, \( A_{X,Y,Z}(i) \), are collected in Table 1.

For the in vitro triplet \( ^3 \)Chl \( a \), no pulse ENDOR spectra were recorded from the \( X_I \) and \( Y_I \)-orientations. This was a consequence of the lower triplet yield and much lower spin polarisation for \( ^3 \)Chl \( a \), which is generated by spin–orbit-coupling-induced intersystem crossing (see above). In particular for the \( X_I \) and \( Y_I \)-orientation only very small signal intensities were observed already in the pulse EPR spectra (data not shown), making it impossible to obtain pulse ENDOR spectra for these orientations. However, we expect the anisotropy of the respective hf tensors for \( ^3 \)Chl \( a \) to be similar to those determined for \( ^3 \)P680, since both triplet states originate from monomeric chlorophyll species. We also note that a sound comparison of the spin density distribution of both triplet states is obtained already from the pulse ENDOR spectra for the \( Z_I \) and \( Z_{II} \)-orientations (see above). The triplet \( Z \)-axis is perpendicular to the molecular plane in both cases, and is therefore the same molecular axis in both chlorophyll species. Furthermore, this axis is expected to be also a principal axis of the observed hf tensors for the CH3 and methine protons in both triplet states. Hence, changes of the spin density distribution of \( ^3 \)Chl \( a \) and \( ^3 \)P680 should be directly reflected in the hf values \( A_I(i) \) for the respective nuclei \( (i) \).

4. Discussion

4.1. Assignment of hf tensor components of \( ^3 \)P680 and \( ^3 \)Chl \( a \)

For the triplet state in bacterial RCs, we have shown previously that an assignment of the observed hf tensors to specific nuclei can be achieved by comparing the components \( A_I(i) \) with the isotropic hf coupling constants from the respective cation and anion radicals \([18,47–49]\). This is justified by the following reasons. (i) For an \( \alpha \)-proton, directly attached to a carbon atom of the \( \pi \)-system, this tensor component is expected to be close to the isotropic value \( A_{iso} = 1/3(A_x + A_y + A_z) \). The calculated values for an isolated C–H fragment are \( 0.5 \times A_{iso} \), \( 1.5 \times A_{iso} \), and \( 1 \times A_{iso} \) for the \( A_x, A_y \), and \( A_z \)-tensor components, respectively, with \( z \) being parallel to the \( \pi \)-orbital of the carbon carrying a \( \pi \)-spin density and \( x \) being parallel to the C–H bond and perpendicular to \( z \) (note that in Fig. 5 the indices in \( A_{X,Y,Z} \) indicate the directions of the ZFS tensor, which agree with this definition only for positions 10 and 20. For position 5 \( A_X \) and \( A_Y \) are exchanged with respect to this definition). \( A_{iso} \) scales with the \( \pi \)-spin density and has a value of \( -60 \) MHz for a spin density of 1 \([49]\). Hence, \( A_Z \) should be always close to \( A_{iso} \). For \( \beta \)-protons, which are one bond away from the \( \pi \)-system, in particular for those from rotating methyl groups, only a small anisotropy of about 10% of \( A_{iso} \) is expected, i.e. the tensor values are close to \( A_{iso} \). Hence, for both types of protons the \( A_I(i) \) values should be close to the respective \( A_{iso} \) values. (ii) In the simple Hückel approximation, the hf coupling for a given nucleus of a triplet state is expected to be the average of
that observed in the respective cation (singly occupied HOMO) and anion radical (singly occupied LUMO), \( (A^+(3P) = 1/2[(A(P^+)) + A(P^-))] \) [49] because in the triplet state both orbitals, the HOMO and LUMO are singly occupied. This approximation gives a rough qualitative picture of the expected hf values, which reflect the unpaired spin density distribution.

Table 1 lists the major isotropic hf coupling constants of Chl \( a^{+} \) and Chl \( a^{-} \) obtained from previous work [47,48]. In the cation radical all larger couplings have positive values and result from the CH3 protons at positions 2, 7, and 12, and from the \( \beta \)-protons 17 and 18 of ring D (see Fig. 5 and Table 1). In the anion radical, on the other hand, large negative couplings are observed for the methine protons 5, 10, and 20, but also large positive values for the CH3 protons 2 and 12. Therefore, the negative \( A_A(\beta) \)-values in the triplet states are assigned to the methane protons. They result from unpaired spin density in the LUMO orbital [18]. Based on its small anisotropy the positive coupling \( A_A(\beta) \) is assigned to protons of a rotating methyl group.

From comparison with the Chl \( a^{+} \) and Chl \( a^{-} \) data, it is unexpected that only one narrow line corresponding to one positive \( A_A(\alpha) \)-value is observed in \( ^3P680 \). However, the same effect, only one positive hf coupling, was observed earlier for the bacterial triplet states, \( ^3BChl \ a \), in organic solvent, and \( ^3P865 \) in bacterial RCs [18]. Both CH3 groups at positions 2 and 12 (Fig. 5) exhibit reasonably large hf couplings in the cation and anion radical. The coupling from the CH3 group at position 2 is smaller than that from position 12, in particular in the cation radical (Table 1). Hence, two different couplings would be expected from these two methyl groups in the triplet state. A possible explanation for the missing ENDOR line of the protons from the methyl group at position 2 could be that this group shows a restricted rotation at the low temperature used \( (T=10\ \text{K}) \), probably due to steric effects caused by the neighboring vinyl group (Fig. 5). Rotation of CH3 groups is known to be a major source of nuclear (proton) spin relaxation at low temperature [50]. Small nuclear relaxation rates could prevent reestablishment of equilibrium populations between the laser pulses, thereby diminishing the ENDOR amplitudes. Similar effects of weak and broadened ENDOR lines from methyl protons at low temperatures have been reported earlier for bacteriochlorophyll cation radicals [51]. The coupling of the third CH3 group in Chl \( a \) at position 7 exhibits in the cation radical a small positive value, and is in the anion radical close to zero. Consequently only a small coupling would be expected for the triplet state. We therefore assign \( A_A(\alpha) \) in \( ^3P680 \) to the CH3 group at position 12. This is in line with the relative magnitudes of the hf-tensor components \( A_X, Y, Z \). The largest value \( A_X \) is expected for the \( C-CH3 \) bond direction, which is consistent with an assignment to position 12, but not consistent with an assignment to position 7 (see below).

It should be noted that the used Davies pulse ENDOR technique is sensitive for large hf couplings, but less sensitive for very small couplings, and ENDOR line intensities from transitions in the \( m_S=+1 \) and \( m_S=-1 \) manifolds decrease as they approach the Zeeman frequency \( \nu_H \). This is a consequence of the band width of the first microwave \( \pi \)-pulse, which does not allow selective excitation of EPR transitions, which are separated by hf couplings smaller than this band width (Fig. 3A, and Ref. [44]). This effect is reflected in the intensity pattern of the high-frequency lines in the \( Z_1 \) spectrum (Fig. 3B). This, however, does not hold for the ENDOR transition in the \( m_S=0 \) manifold, which occurs at \( \nu_H \) and is very narrow because it experiences no hf shift (Fig. 3A). Small hf couplings can be detected by other pulse ENDOR techniques like Mims-ENDOR [44]. Such additional experiments were not performed in view of the extended accumulation times \( (\approx 15\ \text{h}) \) necessary for recording one ENDOR spectrum. Furthermore, several small hf couplings for \( ^3P680 \) have been reported in an earlier paper [33]. The goal of this investigation was to obtain all of the large hf couplings, which reflect the majority of the spin density distribution.

Interestingly a broad weak feature at \( \approx -5 \) to \(-6\ \text{MHz} \) corresponding to one or more additional positive hf couplings is observed in the \( Z_1 \) spectrum of \( ^3P680 \). However, the \( Y_1 \) spectrum of \( ^3P680 \) shows a few weak features at about \(-8 \) to \(-6\ \text{MHz} \). These could correspond to the “missing” hf couplings from the CH3 group at position

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**Fig. 5.** Molecular structure of Chl \( a \) with numbering scheme. Orientation of the \( Q_Y \) transition dipole axis from Ref. [58]. The orientations of the ZFS tensor (\( X \) and \( Y \)) and the hyperfine tensors of protons at positions 5, 10, and 20 (\( A_X \) and \( A_Y \)) were obtained from the ENDOR data (see text). Z-axis is perpendicular to the molecular plane.
2, and/or from the β-protons at 17 and 18 (Fig. 5 and Table 1, caption). The low intensity of these signals, however, is not under-
stood. It could be due to slow relaxation rates for these β-protons as referred to above for $^3$P680. These features may be more pronounced in $^3$Chl a than in $^3$P680 due to a broader distribution of orientations contributing to the Z$_\text{E}$-ENDOR spectrum of $^3$Chl a (see below). Due to their weak intensities these features are not further discussed in this paper. Their corresponding hf values would, however, fit into the general picture of the strong hf couplings, since they have comparable magnitude in $^3$Chl a and $^3$P680.

The obtained absolute values of the couplings, $|A_{\alpha}(i)|$, in particular those of the negative couplings, are significantly larger than the expected average of the cation and anion value of Chl a (Table 1). This behaviour was observed already earlier for the triplet states in the bacterial RC, $^3$P865, and for $^3$BChl a [18]. Apparently, there is a consid-
erable redistribution of spin densities in the triplet state orbi-
tals, both for the chlorophyll molecules in organic solvent and in the RCs, as compared with the respective cation and anion orbitals, leading to high spin densities with LUMO character on the methine positions. This observation urges for theoretical calculations on a more sophis-
ticated quantum chemical level, which, to our knowledge, were so far not performed on these chlorophyll triplet states.

4.2. Comparison of $^3$P680 and $^3$Chl a

It is obvious from Fig. 4 and from Table 1 that the respective $A_{\alpha}(i)$-values of $^3$P680 and $^3$Chl a show a fairly good agreement. This is in contrast to the bacterial RC, where the couplings in $^3$P865 were significantly decreased by more than a factor of 2 and the respective lines were split as compared with $^3$BChl a [18]. This clearly indicated an asymmetric delocalisation over two BChla molecules. For the plant PS II triplet state, $^3$P680, investigated here, the positive coupling changes from 7.4 MHz in $^3$Chl a to an even larger value of 9.5 MHz in $^3$P680. The two larger negative couplings, $-7.8$ and $-10.0$ MHz, of $^3$P680 are close to the respective values of $^3$Chl a. The line of the third negative coupling, $A_{\alpha}(2)$, is not clearly resolved in the spectrum of $^3$Chl a, however, there is a weak low-frequency shoulder with a corresponding $A_{\alpha}(2)$-value of $-6.2$ MHz on the $A_{\alpha}(3)$-line at 7 MHz (Fig. 4). Alternatively, the third line may be hidden under the weak shoulder of the $A_{\alpha}(4)$-
line at 10–11 MHz. The sum of these three negative couplings is remarkably similar for $^3$Chl a and $^3$P680 in the case of the former assignment.

Comparison with the respective $A_{\alpha}(i)$-values of $^3$Chl a corroborates the view obtained from comparison of the $D$-
and $E$-values that at low temperature ($T=10$ K), $^3$P680 is a monomeric $^3$Chl a species in the PS II complexes investig-
ated here. It also shows that there are small shifts of the individual $A_{\alpha}(i)$-values, which we attribute to specific inter-
actions with the protein, e.g. hydrogen bonding to the 13$^1$
carbonyl group (Fig. 5). MTHF used as solvent for $^3$Chl a is not expected to form hydrogen bonds. Hydrogen bonding to this keto group is known to enhance the hf coupling of the adjacent CH$_3$ group at position 12 in the cation radical [52]. Previous transient EPR studies on $^3$P680 by other authors indicated a possible delocalisation at higher temperatures [53,54]. Pulse ENDOR experiments, which we tried at higher temperature, were, however, not successful because of a sharp decrease of signal intensity at elevated temperatures.

4.3. Assignment of the ZFS- and hf-tensor axes in the Chl a molecular frame

The EPR field settings $X_I$, $Y_I$, and $Z_I$ (Fig. 2B) correspond to orientations, where the respective axes of the ZFS tensor, $X$, $Y$, and $Z$, are parallel to the magnetic field. Hence, evaluation of the respective ENDOR spectra should give information on the relative orientations of the hf tensor axes and the ZFS axis system. For the α-protons at the methine positions, strongly anisotropic hf tensors are expected with the smallest tensor component $(0.5 \times A_{\text{iso}})$ along the C–H bond, the largest component $(1.5 \times A_{\text{iso}})$ in the molecular plane, perpendicular to the C–H bond, and the third intermediate component $(1 \times A_{\text{iso}})$ perpendicular to the plane (see above).

The $Z$-axis of the ZFS tensor of a $\pi-\pi^*$ triplet state like a chlorophyll molecule is perpendicular to the molecular $\pi$-
plane [45]. Therefore, the $Z_I$ and $Z_{II}$ ENDOR spectra originate from molecules oriented with their molecular planes perpendicular to the magnetic field. This corresponds to a principal orientation of the hf tensor of the methine protons, $A_Z$, which is reflected in the narrow lines observed in the ENDOR spectra (Fig. 4). The hf components $A_{\alpha}(i)$ obtained (Table 1) are thus a principal value of the tensor for the respective methine proton.

For the other orientations, $X_I$ and $Y_I$, the situation is less obvious. The $X_I$ position corresponds to an edge of the powder pattern of the absorptive $m_S=0$ to $m_S=+1$ transition and hence to a good orientation selection of the triplet molecule along the $X$ axis. But it is not obvious whether this orientation corresponds to a principal axis of the methine proton hf-tensor. Closer inspection of the high-
frequency ENDOR lines of the methine proton lines of the $X_I$ and $Z_I$ spectra shows that they have the same line width of $\approx 0.5$ MHz. An estimate of the orientation distribution selected for the $X_I$ spectrum indicates that angular deviations from $\pm 5^\circ$ up to $\pm 10^\circ$ from the ZFS tensor $X$-axis contribute to the $X_I$ ENDOR spectrum. In this case much larger line widths, up to 2 MHz, are estimated from the respective $A_X(3,4)$ and $A_Y(3,4)$ values in the case of signif-
icantly deviating hf- and ZFS-axes systems. We therefore conclude that the orientation selected for $X_I$ is close to a principal axis orientation for all three methine protons, 5, 10, 20, within approximately $\pm 10^\circ$.

The $Y_I$ ENDOR spectrum (Fig. 4) is expected to pick up intensities from a distribution of orientations both from the absorptive and the emissive EPR transitions (Fig. 2B).
Hence, anisotropic broadening is expected in this ENDOR spectrum. Nevertheless, two well-resolved absorptive lines from methine protons are observed on the high-frequency side at about 12 and 15 MHz, which are approximately 50% broader as compared with the $Z_4$ and $X_6$ spectra. It has been observed in previous transient EPR and pulse EPR studies on porphyrin triplet states that anisotropic electronic $T_2$ relaxation time gives rise to intensity patterns in the pulse-EPR spectra, which deviate from those calculated for equal contributions from all orientations. In particular, the intensities along the ZFS principal axes were enhanced as compared with other orientations [55]. The field-swept electron spin echo (ESE) spectrum of $^3$P680 (Fig. 2C) clearly shows such reduced signal intensities for noncanonical orientations. This effect was explained by shorter $T_2$ relaxation times for reduced signal intensities for noncanonical orientations. This modulation is expected to have both similar values, which should be smaller than that for $X$. This is indeed observed (compare the low-frequency sides of the $X$, $Y$ and $Z$-ENDOR spectra in Fig. 4; for values see Table 1). This behaviour of the hf-tensor components $A_{X,Y,Z}(2)$ further corroborates the assignment to the methyl group at positions 12 (or 2) and not to position 7 (see above).

5. Conclusion

In this work, time-resolved pulse ENDOR combined with repetitive laser excitation at cryogenic temperature was used to obtain the hf structure from the short-lived triplet states $^3$P680 in plant photosystem II and $^3$Chl $a$ in a frozen organic solvent. The data obtained represent the first complete set of the larger hf tensor values from all three canonical orientations for $^3$P680 and from the $Z$-orientation for $^3$Chl $a$. They indicate a redistribution of spin densities, in particular in the LUMO orbitals of $^3$Chl $a$ and $^3$P680, as compared with the cation and anion radicals from Chl $a$. This deviation of the triplet state hf couplings from those expected from comparison with the cation and anion radicals is not understood at present and is a challenge for future theoretical studies on these triplet states using advanced quantum chemical methods.

Comparison of the hf data for $^3$Chl $a$ and $^3$P680 clearly confirms that $^3$P680 is a monomeric Chl $a$ species at low temperature ($T = 10$ K), as has been proposed earlier based on $D$- and $E$-values obtained from EPR and ODMR studies [8,14,15,20]. Beyond that, shifts of the hf couplings of individual nuclei were observed, which are of particular interest since they indicate specific protein interactions, e.g. hydrogen bonding to the keto group in ring E.

The ENDOR spectra, obtained here for the in-plane $X$- and $Y$-orientations of the ZFS tensor, enabled a full determination of the hf tensors of the three methine protons and one CH$_3$ group of $^3$P680. Based on the orientations of these
h_\text{f} tensor axes, the orientations of the in-plane axes of the ZFS tensor were determined. They were found to agree well with previously reported angles between the electronic Q_y transition dipole and the ZFS X- and Y-axes, obtained from optical experiments [56,57]. In the present work a unique determination of the orientations of the ZFS tensor axes, X and Y, of 3P680 in the Chl a molecular axis system was achieved, which was not possible in earlier studies, where X- and Y-axes in Fig. 5 could be exchanged. This result is a prerequisite for obtaining the molecular orientation of 3P680 in the PS II protein structure from EPR studies on PS II single crystals (M. Kammler et al., this issue [37]).

The spin (and electron) distribution obtained for the LUMO orbital of 3P680 is of particular interest, since light-induced electron transfer starts from this orbital in the excited singlet state of the primary donor. However, for PS II the identity and nature of the primary donor is still under debate. Recently models have been discussed, which involve Chl_{D1} as primary donor before the positive charge moves to one of the two Chl molecules (P_{D1} and P_{D2}) called “P680” [38,40,59,60]. The EPR experiments on 3P680 in oriented membrane fragments [36] and on 3P680 in PS II single crystals [37] demonstrate that this triplet state may indeed reside on one of the two accessory chlorophylls, Chl_{D1} or Chl_{D2}. In this model, 3P680 may reside indeed on the same molecule, which acts as very early primary electron donor. The small but significant shifts of hf values of 3P680 as compared with those of Chl a are most probably induced by specific interactions with the protein, e.g. hydrogen bonding, which could be further investigated by pulse ENDOR on Chl a using different solvents, which form hydrogen bonds, e.g. to the 13\textsuperscript{1} keto group (Fig. 5). In this way, it may be possible in future studies to use site-directed mutagenesis to assign the triplet state, 3P680, specifically to one of the two chlorophyll molecules, Chl_{D1} and Chl_{D2}, in the photosystem II structure.

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References


