Transient Electron Paramagnetic Resonance of the Triplet State of P_{700} in Photosystem I: Evidence for Triplet Delocalization at Room Temperature†

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ABSTRACT: Spin-polarized EPR spectra of the triplet state of P_{700}, the primary electron donor in photosystem I (PS I), have been measured for the first time at room temperature. The measurements were performed on intact PS I from *Synechococcus* sp. after prereduction of all iron–sulfur centers and on vitamin K_1 depleted PS I from *Synechocystis* 6803. The two preparations give similar spectra with a polarization pattern which indicates that the triplet state is formed via recombination of a radical pair. The axial and nonaxial zero-field splitting (zfs) parameters are found to be $|D| = (284 \pm 15) \times 10^{-4}$ cm^{-1} and $|E| = (22 \pm 3) \times 10^{-4}$ cm^{-1}, respectively. The *E*-value is 42% smaller than in monomeric chlorophyll *a*, while the *D*-value is nearly the same. Measurements of the *Synechocystis* 6803 sample at 4.5 K yielded zfs parameters which are identical with those of the chlorophyll monomer, in agreement with previous results. In order to explain this behavior, it is proposed that the triplet excitation is delocalized over the two halves of a chlorophyll dimer at room temperature but appears localized on one half at low temperature. The observed zfs parameters are obtained if (1) the magnetic *z*-axes of the two chlorophylls are collinear, (2) the magnetic *y*-axes (and *x*-axes) of the two chlorophylls make an angle of approximately 55° with each other, and (3) the admixture of charge-transfer states to $^3P_{700}$ is negligible. It is suggested that the orientation within the membrane of the two chlorophylls of P_{700} is similar to that of the special pair in purple bacteria, but that the electronic coupling between the two chlorophylls is weaker in P_{700}. Possible origins of the temperature dependence of the triplet delocalization in P_{700} are discussed, and alternative explanations for the reduced *E*-value at room temperature are considered.

The primary electron donor of photosystem I, P_{700},[1] is made up of chlorophyll *a*, and substantial evidence is available that two excitonically coupled molecules form a "special pair" in analogy to the primary electron donor of purple bacteria [for a recent review, see Sétif (1992)]. However, low-temperature EPR studies of the triplet state of P_{700} yielded zero-field splitting parameters that agree well with those of monomeric Chl *a* (Frank et al., 1979; Rutherford & Mullet, 1981). This is in contrast to purple bacteria, where both zfs parameters, *D* and *E*, of the triplet state of the special pair are considerably smaller than those of the corresponding monomeric BChl's [e.g., Thurnauer (1979) and Levanon and Norris (1982)].

The axial and nonaxial zfs parameters, *D* and *E*, are related to the spatial distribution of the two unpaired electron spins by the following relationships:

$$D = \frac{3}{4}(g\beta)^2\left\langle \frac{r_{12}^2 - 3z_{12}^2}{r_{12}^5} \right\rangle \quad (1)$$

$$E = \frac{3}{4}(g\beta)^2\left\langle \frac{y_{12}^2 - x_{12}^2}{r_{12}^5} \right\rangle \quad (2)$$

where $g\beta$ is the gyromagnetic ratio of the spins, and $\mathbf{r}_{12} = (x_{12},y_{12},z_{12})$ is the vector joining them. The angular brackets imply an average over the electronic wave function. For planar aromatic molecules such as the chlorophylls, the *z*-axis is normal to the molecular plane so that $\langle z_{12}^2\rangle \ll \langle x_{12}^2\rangle,\langle y_{12}^2\rangle$. In such molecules *D* is positive and is inversely related to the average distance between the two unpaired spins, whereas *E* indicates the in-plane asymmetry of the charge distribution.

The reduction of $|D|$ and $|E|$ in purple bacteria mentioned above may be explained by "delocalization" of the triplet state over the two chlorophylls, $BChl_L$ and $BChl_M$, and/or an admixture of the charge-transfer states, $^3(BChl_L^+BChl_M^-)$ and $^3(BChl_L^-BChl_M^+)$. The latter mechanism increases the average distance between the unpaired electrons and hence yields smaller zfs parameters. Delocalization means that either the triplet eigenstate of the pair is a coherent superposition of the individual BChl triplet states or the triplet excitation hops incoherently between $BChl_L$ and $BChl_M$, so that the zfs is determined by the averaged zfs tensor of both BChl triplet states. Generally, this yields a higher "magnetic symmetry" and hence smaller zfs parameters [for reviews, see Levanon and Norris (1982), Budil and Thurnauer (1991), and Angerhofer (1991)].

In this paper we report the first EPR spectrum of $^3P_{700}$ at room temperature obtained by time-resolved EPR in the direct

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[1] Abbreviations: BChl, bacteriochlorophyll; $BChl_L$ and $BChl_M$, BChl's constituting the special pair of bacterial reaction centers; Chl *a*, chlorophyll *a*; CAPS, 3-[cyclohexylamino]-1-propanesulfonic acid; *D* and *E*, axial and nonaxial zfs parameters, respectively; EPR, electron paramagnetic resonance; P_{700}, primary electron donor in PS I; $^3P_{700}$, triplet state of P_{700}; PS I, photosystem I; zfs, zero-field splitting.

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detection mode and compare it with the spectrum at 4.5 K measured with the same PS I preparations. Surprisingly, the nonaxial zfs parameter $|E|$ is considerably smaller at room temperature than at low temperature and smaller than that observed in monomeric Chl *a*. In contrast, the axial parameter $|D|$ remains essentially unchanged. This indicates that at room temperature the triplet excitation of $^3P_{700}$ is delocalized over both constituent chlorophylls and that their magnetic *z*-axes are collinear, i.e., their ring planes are parallel, but that their *x*- and *y*-axes are oriented differently.

MATERIALS AND METHODS

Two types of PS I preparations were used in the present study.

(*1*) *Prereduced PS I.* PS I complexes with a chlorophyll concentration of 1.7 mg/mL were prepared from thermophilic cyanobacteria *Synechococcus* sp. according to Witt et al. (1987). The complexes contain all membrane-bound electron acceptors: the primary acceptor A_0, possibly Chl *a*; the secondary acceptor A_1, phylloquinone, also named vitamin K_1; and the iron–sulfur centers F_X, F_B, and F_A. The terminal acceptors F_X, F_B, and F_A were prereduced by anaerobic addition of 10 mg/mL $Na_2S_2O_4$ in the presence of 0.2 M CAPS (pH = 10.5) followed by illumination with continuous white light from a tungsten lamp (≈20 mW/cm²) for 3 min at room temperature. After this pretreatment, flash excitation of the sample generates the secondary radical pair $(P_{700}^+A_1^-)$, which recombines to the triplet state, $^3P_{700}$, with $t_{1/2}$ ≈ 250 ns and a yield close to 100% (Sétif and Bottin, 1989; Sétif & Brettel, 1990). We will refer to this preparation as prereduced PS I.

(*2*) *Extracted PS I.* PS I particles where forward electron transfer beyond A_0 is blocked by extraction of phylloquinone were prepared from mesophyllic cyanobacteria *Synechocystis* 6803 according to Biggins and Mathis (1988). In this preparation $^3P_{700}$ is formed at room temperature via the recombination of the primary radical pair $(P_{700}^+A_0^-)$ with $t_{1/2}$ ≈ 30 ns and a yield of approximately 30% (Biggins & Mathis, 1988). This preparation will be referred to as extracted PS I.

EPR measurements were performed with a modified X-band spectrometer (Bock et al., 1989; Stehlik et al., 1989, and references therein). The sample was pumped through a flat cell using a cyclic flow system for measurements at room temperature and was contained in a standard EPR tube (3-mm i.d.) for variable-temperature measurements using a He-gas flow cryostat. The sample was excited by 532-nm laser pulses of 6-ns duration at a repetition rate of 10 or 20 Hz. The excitation energy was approximately 3 mJ/cm² at the surface of the flat cell or the standard EPR tube. Transient EPR signals were taken either in the direct detection mode without field modulation or with 100-kHz field modulation. The time resolution of the directly detected signals is about 50 ns, which is mainly determined by the response time of the resonator. With 100-kHz field modulation the time resolution is limited to 20 μs. Transient responses at 161 field positions were taken in steps of 0.5 mT to obtain a complete kinetic and spectral data set [for details, see Bock et al. (1989)]. The *D*-value and the *E*-value of the triplet powder spectra were obtained by spectrum analysis either directly from the measured spectrum (see Figure 1) or by simulations.

RESULTS

Figure 1 shows results obtained with prereduced PS I particles from *Synechococcus* sp. at room temperature.

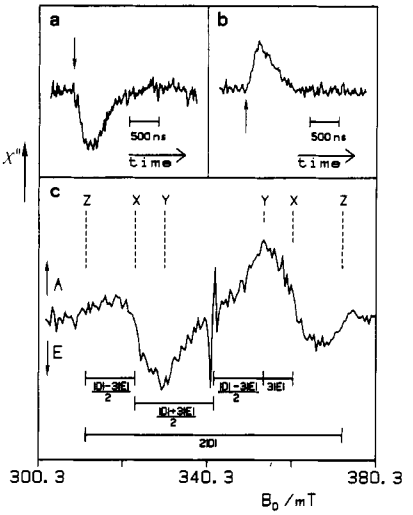

FIGURE 1: Directly directed EPR transients (a, b) and spectrum extracted from the full time/field data set (c) for prereduced PS I complexes from *Synechococcus* sp. at room temperature: microwave frequency, 9.508 GHz (X-band); microwave power, 5 mW; laser repetition rate, 20 Hz. The raw data set contains 161 transients spaced 0.5 mT apart with a digital resolution of 10 ns/point. (a) Averaged emissive transient and (b) averaged absorptive transient in the field region of maximum signal, i.e., 323.5–332.5 and 348.5–357.5 mT, respectively. The position of the laser pulse is indicated by an arrow. (c) Powder spectrum extracted from the full data set by averaging the signal intensity in the time range of 100–400 ns after the laser pulse. X, Y, and Z indicate the resonance positions where the static magnetic field is parallel to the principal axes of the zfs tensor; A = absorption, and E = emission. Characteristic splittings in terms of *D* and *E* values are indicated.

Depending on the magnetic field position, a characteristic transient microwave emission (Figure 1a) or absorption (Figure 1b) is observed. The transients have a rise time which is significantly slower than the instrumental response time of 50 ns, and they decay with a half-time of approximately 300 ns. The spectrum shown in Figure 1c is extracted from the full time/field data set by averaging the signal intensity in the time range from 100 to 400 ns after excitation. Except for the narrow signal around $g = 2$ (339.7 mT), the spectral pattern AEEAAE (E = emission, A = absorption) is characteristic of the light-induced formation of the triplet state of the primary electron donor as observed in various photosynthetic reaction centers. The spin polarization is due to the fact that only the T_0 state is populated. Such a population selection cannot arise from spin-selective intersystem crossing but is a result of triplet formation through charge recombination in a weakly coupled radical pair which originates from a singlet precursor. In the presence of a static magnetic field, singlet–triplet mixing in the radical pair is restricted to the T_0 state and is responsible for the observed polarization pattern [for details, see Thurnauer (1979), Budil and Thurnauer (1991), and Angerhofer (1991) and references therein).

A separate measurement of the central region of the spectrum in Figure 1c has been performed in steps of 0.2 mT and shows a spectrum with a characteristic EAE spin polarization pattern which is attributed to the pair $(P_{700}^+A_1^-)$ (Bock et al., 1989). This spectrum (not shown) rises with the instrumental response time and decays with $t_{1/2}$ ≈ 150 ns in correlation to the rise of the $^3P_{700}$ signal in Figure 1a,b. This is also in satisfactory agreement with results from flash absorption spectroscopy which show a concomittant decay of $(P_{700}^+A_1^-)$ and formation of $^3P_{700}$ with $t_{1/2}$ ≈ 250 ns (Sétif & Brettel, 1990). The decay time of the triplet spectrum ($t_{1/2}$ ≈ 300 ns) is a typical spin–lattice relaxation time at room

Table I: Zfs Parameters of the Triplet State of Chl *a* and P_{700} Obtained with Different PS I Preparations and Experimental Conditions

sample	T [K]	detection mode[a]	$\|D\|$ [10^{-4} cm^{-1}]	$\|E\|$ [10^{-4} cm^{-1}]	references
Chl *a*[b]	4.5	mod	273 ± 8	40 ± 2	Thurnauer & Norris, 1977
spinach PS I–CP1	4.2	mod	280 ± 5	38 ± 2	Sétif et al., 1982
Synechococcus sp. PS I, prereduced	298.0	dir	284 ± 15	22 ± 3	this work
Synechocystis 6803 PS I, extracted	298.0	dir	279 ± 10	23 ± 2	this work
Synechocystis 6803 PS I, extracted	4.5	dir	284 ± 10	40 ± 3	this work
Synechococcus sp. PS I, strongly prereduced[c]	4.5	mod	289 ± 15	39 ± 2	this work

[a] mod, 100-kHz field modulation; dir, direct detection. [b] In 10–15% pyridine-d_5 in toluene-d_8. [c] Strongly prereduced as described in the text (see Results).

temperature for such species. These results indicate that the spectrum depicted in Figure 1c is attributed to $^3P_{700}$ formed by recombination of the radical ion pair ($P_{700}^+A_1^-$).

From this spectrum we evaluate the axial and nonaxial zfs parameters to be $|D| = (284 \pm 15) \times 10^{-4}$ cm^{-1} and $|E| = (22 \pm 3) \times 10^{-4}$ cm^{-1}. The *D*-value agrees rather well with the values reported in the literature (Frank et al., 1979; Rutherford & Mullet, 1981) for $^3P_{700}$ at 4.2 K, but the observed *E*-value is 42% smaller than reported for low temperature (see Table I).

It could be argued that this striking difference in the *E*-value is related to factors other than the temperature. The precursor of $^3P_{700}$ is the secondary radical pair ($P_{700}^+A_1^-$) in our room temperature measurement, whereas the primary pair ($P_{700}^+A_0^-$) is the precursor at low temperature. Different biological material (PS I from cyanobacteria versus higher plants) and different EPR techniques (direct detection versus field modulation) were used. Therefore we performed several control experiments, which yielded the following results:

(1) Essentially the same zfs parameters (see Table I) are obtained when $^3P_{700}$ is formed at room temperature via recombination of the primary radical pair ($P_{700}^+A_0^-$) in extracted PS I from *Synechocystis* 6803 (Figure 2a)[2] or via recombination of the secondary radical pair ($P_{700}^+A_1^-$) in prereduced PS I from *Synechococcus* sp. (Figure 1).

(2) When measured at 4.5 K with 100-kHz field modulation, the spectrum of $^3P_{700}$ of the same *Synechococcus* sp. preparation (Figure 2c; note that this is a derivative spectrum) shows the same zfs parameters as reported for $^3P_{700}$ in higher plants (see Table I). For these measurements PS I was strongly prereduced by illuminating the sample containing $Na_2S_2O_4$ with strong white light (500 mW/cm^2) for 3 min at room temperature and freezing under illumination. This pretreatment leads to reduction of all iron-sulfur centers and probably double reduction of A_1 so that $^3P_{700}$ is formed via the primary radical pair ($P_{700}^+A_0^-$) (Sétif & Bottin, 1989). Double reduction of A_1 is necessary because ($P_{700}^+A_1^-$) recombines to the singlet ground state of P_{700} at low temperature (Sétif & Bottin, 1989). The presence of a narrow signal at $g = 2$ in Figure 2c indicates that electron transfer beyond A_0 was still functional for a minor fraction of centers.

[2] The narrow signal around $g = 2$ (339.7 mT), which is much weaker than in the prereduced sample (see Figure 1c), is due to the radical pair ($P_{700}^+A_1^-$) in the small fraction of the centers in which the phylloquinone was retained.

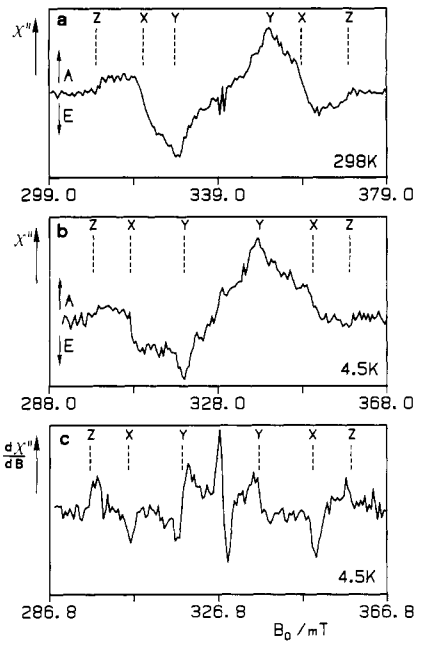

FIGURE 2: EPR spectra extracted from the full time/field data set by averaging a convenient time range after the laser pulse. (a) Extracted PS I complexes from *Synechocystis* 6803 at room temperature in direct detection mode (microwave frequency, 9.506 GHz; microwave power, 10 mW; laser repetition rate, 10 Hz), averaged in the time range of 150–400 ns. (b) Extracted PS I complexes from *Synechocystis* 6803 at 4.5 K in direct detection mode (microwave frequency, 9.137 GHz; microwave power, 1 mW; laser repetition rate, 10 Hz), averaged in the time range of 1.5–4 μs. (c) Strongly prereduced PS I complexes (see Results) from *Synechococcus* sp. at 4.5 K with 100-kHz field modulation (modulation amplitude, 1.6 mT; microwave frequency, 9.132 GHz; microwave power, 0.05 mW; laser repetition rate, 10 Hz), averaged in the time range of 100–800 μs. A = absorption, and E = emission. X, Y, and Z are defined in the caption to Figure 1.

(3) Measurements at 4.5 K in direct detection mode (Figure 2b) yield the same zfs parameters as those measured with 100-kHz field modulation (Figure 2c) at the same temperature (see Table I).

These results [(1), (2), and (3)] confirm that the observed change in *E*-value is a result of the change in temperature.

When we tried to observe the temperature dependence throughout the range from 5 K to room temperature, we had some unexpected difficulties. Between 100 and 200 K the signal-to-noise ratio of the measured transients was rather poor, and the edges and peaks of the spectra extracted from these time/field data sets were less well defined. In this temperature range no reliable zfs parameters could be determined. From 200 to 220 K the Y-peaks and X-edges were observable again, but less clearly than at low or room temperature. Above 220 K the quality factor of the loaded EPR cavity became quite low presumably as a result of increased dielectric absorption. The resulting loss of sensitivity hampered the detection of the triplet signal. This problem is overcome at room temperature by using a flat cell. The values of *D* and *E* are listed in Table II.

DISCUSSION

The results in Tables I and II show that the nonaxial zfs parameter $|E|$ of $^3P_{700}$ agrees with the value for monomeric Chl *a* up to at least 50 K but is reduced by 42% when the temperature is raised to 298 K. The axial parameter $|D|$, on the other hand, remains close to the "monomeric" value at all temperatures studied. This is in contrast to purple bacteria, where $|D|$ and $|E|$ of the special pair triplet state are significantly

Table II: Zfs Parameters Obtained from the Triplet Spectra of Extracted PS I Complexes from *Synechocystis* 6803 at Different Temperatures[a]

T [K]	$\|D\|$ [10^{-4} cm^{-1}]	$\|E\|$ [10^{-4} cm^{-1}]
4.5	284 ± 10	40 ± 3
50	285 ± 5	40 ± 2
200	277 ± 25	30 ± 6
221	290 ± 25	28 ± 8
298	279 ± 10	23 ± 2

[a] The spectra were observed in the direct detection mode; microwave power: 1 mW at 4.5 and 50 K, 5 mW at 200 and 221 K, and 10 mW at 298 K.

smaller than for the corresponding monomeric BChl's over the same temperature range, and $|D|$ increases with temperature (Hoff & Prosuryakov, 1985; Proskuryakov & Manikowski, 1987; Ullrich et al., 1991).

In the following we will (I) propose a model explaining the decreased E-value of $^3P_{700}$ at room temperature and use this model to estimate the relative orientation of the magnetic axes of the two chlorophylls which form P_{700}, (II) relate our room temperature results to the possible orientations of these two chlorophylls in the membrane and compare our results for PS I to those for purple bacteria, and (III) present several explanations for the observed temperature dependence of E.

(I) On the basis of the agreement of the zfs parameters of P_{700} at low temperature with those of monomeric Chl *a*, it has been proposed that (a) the triplet excitation is localized on a single chlorophyll or (b) the excitation is shared by two chlorophylls whose magnetic axes are collinear and that $^3P_{700}$ lacks charge-transfer character (Frank et al., 1979; Rutherford & Sétif, 1990; Sétif, 1992).

We propose as a straightforward interpretation of our room temperature results that the triplet excitation is delocalized by fast hopping between the two chlorophylls and that the magnetic z-axes are collinear. In such a case the spin Hamiltonian is averaged at sufficiently rapid hopping rates. However, because the z-axes are assumed parallel, this averaging does not affect the value of D. In contrast, the value of E is smaller than in monomeric chlorophyll if the x- or y-axes are not parallel. For symmetric hopping one obtains

$$|E^*| = |E| \cos \alpha \qquad (3)$$

where E^* and E refer to the dimeric (excitonic) and monomeric triplet states, respectively, and α is the angle between the magnetic x- or y-axes in the dimer. Inserting the measured values of E at 298 and 4.5 K for $|E^*|$ and $|E|$, respectively, yields a value of 55° for α. It is important to stress that eq 3 and hence the estimated angle are only valid if the triplet excitation of P_{700} at room temperature is completely and symmetrically delocalized and lacks charge-transfer character (Clarke et al., 1976; Hägele et al., 1978).

(II) Now we will discuss our results in relation to the possible structure and orientation of P_{700} within the thylakoid membrane. The magnetic z-axis of Chl *a* is known to be perpendicular to the molecular plane (Thurnauer & Norris, 1977). Thus the interpretation of the zfs parameters presented above suggests a nearly parallel orientation of the two chlorophyll ring planes. Since the magnetic z-axis of the (localized) triplet state at low temperatures lies in the plane of the thylakoid membrane (Rutherford & Sétif, 1990), the ring planes of both chlorophylls should be nearly perpendicular to the membrane, as is the case for the special pair in purple bacteria (Deisenhofer et al., 1984; Allen et al., 1987). In the latter species the two BChl's are related to one another by an approximate 2-fold symmetry axis perpendicular to the membrane (Deisenhofer et al., 1984; Allen et al., 1987). Assuming the same symmetry for P_{700}, the estimated angle $\alpha = 55°$ between the magnetic y-axes of the two chlorophylls would imply that each of these axes is tilted out of the membrane by either 27.5° or 62.5°. The latter value agrees well with the tilt of 60–70° reported for the magnetic y-axis of the (localized) triplet state of P_{700} at low temperature (Rutherford & Sétif, 1990). However, the main optical bleaching due to the oxidation of P_{700} (around 700 nm) is polarized almost parallel to the membrane (Breton et al., 1975) so that the Q_y transition moments of the individual chlorophylls (generally assumed to be parallel to the molecular y-axis connecting pyrrole rings I and III)[3] should also be roughly parallel to the membrane. The magnetic y-axis of Chl *a* in vitro coincides within ±35° with the Q_y transition moment (Thurnauer & Norris, 1977) so that the orientation of the optical bleaching is in better agreement with our alternative tilt angle of 27.5°. Thus, the geometry of P_{700} may be similar to that of the special pair in purple bacteria, where the molecular y-axes of both BChl's are tilted approximately 20° out of the membrane (Deisenhofer et al., 1984; Allen et al., 1987). It should be pointed out that the zfs parameters of the triplet states of the two species show different behaviors. The orientation of the principal axes of the triplet state of the special pair in *Rhodobacter sphaeroides* indicates that the triplet excitation is almost symmetrically delocalized with 63% on the L half of the dimer and 37% on the M half (Norris et al., 1989). In addition the triplet state is also estimated to have 13% charge-transfer character. The delocalization alone leads to a reduction of $|D|$ by 2% and $|E|$ by 21%. Thus the observed 17% reduction in D is mostly determined by the charge-transfer character of the triplet state, whereas the reduction in E contains a significant contribution from the delocalization of the excitation. In PS I, the D-value is not significantly reduced compared to monomeric Chl *a* even at room temperature. Thus a charge-transfer contribution is not present in $^3P_{700}$, which may indicate that the electronic coupling between the two chlorophylls is weaker than in bacterial reaction centers. This is supported by the small exciton splitting reported for the excited singlet state of P_{700} [Schaffernicht & Junge, 1981; for discussion of deviating results, see Sétif (1992)], and it is in agreement with the suggestion that the substitution of the acetyl group on ring I in bacteriochlorophyll by a vinyl group as in Chl *a* could have an influence on the interactions in a chlorophyll special pair (Rutherford, 1986).

(III) In the following we will consider some possible reasons for the striking difference between the values of E observed at room and low temperatures. One might propose the trival explanation that the triplet state observed at low temperature resides on a monomeric chlorophyll apart from P_{700}. A candidate for the low-temperature trap may be an accessory chlorophyll close to P_{700} or even the primary electron acceptor A_0. The triplet excitation might be transferred rapidly from $^3P_{700}$ to the monomeric chlorophyll, so that only the latter is detected by standard EPR at low temperature. We have not observed any indication of such a triplet transfer at 4.5 K within the available time resolution of 50 ns. An even faster triplet transfer to a monomeric chlorophyll at low temperature seems unlikely in view of the absence of such a transfer at

[3] An angle of 5° between the Q_y transition moment and the molecular y-axis of zinc chlorophyllide has been estimated from fluorescence measurements in synthetic zinc chlorophyllide substituted hemoglobin (Moog et al., 1984).

room temperature during at least a few hundred nanoseconds. Another possibility would be that charge recombination in the pair ($P_{700}^{+}A_0^{-}$) directly populates the triplet state of A_0. However, this appears unlikely, as the main bleaching due to formation of $^3P_{700}$ at liquid helium temperature is centered at 697 nm (Den Blanken & Hoff, 1983), whereas the bleaching due to the reduction of A_0 is centered around 690–693 nm (Shuvalov et al., 1986; Mathis et al., 1988). Therefore we assume that the room- and low-temperature spectra are both due to the triplet state of P_{700}.

The temperature dependence of the E-value can be explained as a result of changes in the P_{700} triplet state. At room temperature the E-value is reduced compared to monomeric Chl *a*, which suggests that the triplet excitation is delocalized over the two halves of the dimer. At low temperatures no such reduction is observed, and thus we propose that the excitation becomes localized. There are several mechanisms by which this could occur. One simple possibility is that the rate with which the excitation hops from one half of the dimer to the other becomes slow on the time scale of the experiment. The effect on the spectrum of such a change in the hopping rate can be described in analogy to the line broadening and coalescence phenomena for two interconverting spins at different resonance frequencies (Abragam, 1961). On the basis of this usual exchange model we calculated EPR-triplet spectra of Chl *a* dimers with parallel ring planes and $\alpha = 50°$ for various hopping rates. A "monomeric" E-value, as experimentally observed at low temperature, is obtained if the hopping rate is below $10^7\ s^{-1}$. In the case where the hopping rate exceeds $10^8\ s^{-1}$ the simulation yields the "averaged" value E^* as defined in eq 3. At intermediate hopping rates the simulated spectra show broadened features at the X- and Y-resonance positions.

Another possible mechanism is that the excitation hops rapidly but asymmetrically between the two halves of the dimer. In this case eq 3 becomes

$$|E^*| = |E|[1 - (4p_1p_2 \sin^2 \alpha)]^{1/2} \quad (4)$$

where p_1 and p_2 are the equilibrium populations of the excitation of the two chlorophylls. In order to explain the observed "monomeric" E-value at low temperatures, p_1 or p_2 must be essentially 0, i.e., the energy difference, ΔE, between the two localized triplet states must well exceed kT at those temperatures. With increasing kT, the delocalization becomes more symmetric. It is not possible to separate the contributions of p_1, p_2, and α to the reduction of $|E|$. However, if $kT \gg \Delta E$, the delocalization will be symmetric, i.e., $p_1 = p_2 = {}^1/_2$. Because the hopping is assumed to be fast, this mechanism predicts spectra which are described by an averaged Hamiltonian at all temperatures and thus no broadening should occur.

Our difficulties in measuring the triplet spectra between 50 and 200 K could be due to line broadening as predicted by the first mechanism (the transition from slow to fast hopping in this temperature range). However, these difficulties also could be due to other effects, such as temperature gradients or other inhomogeneities in the sample.

These mechanisms having been proposed, it is important to discuss their origin. An increase in the triplet hopping rate with temperature could be due to (i) an activation barrier associated with a change of nuclear coordinates of the chlorophylls and the surrounding protein, necessary to achieve a conformation which is more favorable for the transfer of the triplet excitation from one chlorophyll to the other; or (ii) an increase of the orbital overlap between the two chlorophylls with increasing temperature. The latter effect could, for example, result from transient rearrangements induced by the formation of $^3P_{700}$. This would be more important at higher temperature because of higher flexibility of the protein matrix and surrounding solvent.

A difference in the energies of the two localized triplet states could be due to differences in the protein environments of the two chlorophylls constituting P_{700}. With a ΔE of only about 0.01 eV, the triplet excitation would be well localized on the lower lying chlorophyll for $T \leq 50$ K; at room temperature $|E^*|$ predicted in eq 4 would already be very close to the limit of symmetric delocalization (eq 3).

An activation barrier as in (i) has been proposed to explain the temperature dependence of downhill transfer of the triplet excitation from the special pair to a carotenoid in *Rb. sphaeroides* (Frank et al., 1983). However, triplet hopping between identical molecules in molecular crystals like anthracene or naphthalene has been reported to be essentially temperature independent (Haarer & Wolf, 1970). This may be relevant for triplet hopping in P_{700} if the two localized triplet states are isoenergetic. An energy difference is not in agreement with a low-temperature resonance Raman study (Moënne-Loccoz et al., 1990) in which it is reported that two distinct chlorophylls are "Raman bleached" upon formation of $^3P_{700}$. This indicates that the asymmetry of the repartition of the triplet excitation is limited (Moënne-Loccoz et al., 1990). It could not be excluded, however, that one of the chlorophylls remains in the ground state but is Raman bleached because of perturbation of its Soret levels by the unpaired electrons located on the other chlorophyll. It is not clear at present whether the temperature dependence of the E-value is continuous or discontinuous, i.e., stepwise. The mechanisms discussed above and their possible origins are consistent with both types of behavior.

So far the discussion has been based on the assumption that the reduced E-value of $^3P_{700}$ at room temperature is due to triplet delocalization over a pair of chlorophylls. Alternatively, one might assume that the triplet excitation is localized on one chlorophyll throughout the temperature range studied and that $|E|$ is reduced because of thermal population of a low-lying vibronically excited triplet state of the same chlorophyll. Such a mechanism, as established for triplet excitations in molecular crystals [see Fujara and Vollmann (1984) and references therein], has been proposed to explain the weak temperature dependence of $|D|$ and $|E|$ in bacterial reaction centers (Ullrich et al., 1991). However, in contrast to the latter species, either the magnetic axes orientations or the E-value of the vibronically excited state in $^3P_{700}$ would have to be drastically different from the vibronic ground state. Another possibility which cannot be excluded is that the spatial spin distribution of the localized triplet state changes as a function of temperature as a result of structural changes, e.g., due to a phase transition. In order to explain the experimental results, such a change would have to reduce the value of E by more than 40%.

A study of the complete temperature dependence of the EPR spectra of $^3P_{700}$ will provide additional information which will help to elucidate the origin of the change in $|E|$. We are presently trying to improve the signal-to-noise ratio of our measurements in order to obtain spectra of sufficient quality to perform such an analysis in the intermediate temperature range (100–280 K).

Concluding Remarks. The zfs of $^3P_{700}$ measured for the first time at room temperature is considerably reduced compared to that of monomeric Chl *a*, indicating delocalization of the triplet excitation over a chlorophyll dimer. This

interpretation of the spectra leads to the conclusion that the two halves of the dimer are nearly coplanar and that their magnetic *x*- or *y*-axes form an angle of about 55° (125°) with respect to each other, which can also be taken as an estimate of the relative orientation of the molecular *x*- or *y*-axes. An improved electron density map from X-ray diffraction of PS I crystals is expected in the near future (Witt et al., 1992; Krauss et al., 1993). It will then be possible to test whether our structural proposals for P_{700} agree with this X-ray structure.

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