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Carotenoid triplet detection by time-resolved EPR spectroscopy in carotenopyropheophorbide dyads

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Abstract

Carotenoid triplets play a photoprotective role in natural photosynthesis. The main process of carotenoid triplet formation is known to be triplet–triplet energy transfer from chlorophyll triplets. The structural requirements for high transfer yields are still a matter of discussion and the presence of competitive triplet formation pathways has not been excluded. Transient EPR measurements of triplet states formed by photoexcitation allow detection of the initial spin polarization. This pattern derives from the mechanism of triplet formation. In the case of triplet–triplet energy transfer, if the condition of spin angular momentum conservation is fulfilled, simulation of the EPR spectra gives information about the donor–acceptor mutual orientation. We describe transient EPR experiments on two artificial photosynthetic dyads, consisting of a carotenoid covalently-linked to a free-base or zinc substituted pyropheophorbide moiety and we discuss the results in terms of possible dyad conformations. © 1997 Elsevier Science S.A.

Keywords: Carotenoids; Porphyrins; Dyads; Triplets; TREPR

1. Introduction

Carotenoids act as light-harvesting pigments and as photoprotective agents in photosynthetic organisms. Their role as antenna pigments is fulfilled by absorption of photons in the blue-green spectral region followed by rapid singlet–singlet energy transfer to chlorophyll pigments. The photoprotective role involves the direct quenching of singlet oxygen or the quenching of chlorophyll triplets which could sensitize the formation of singlet oxygen. The process of chlorophyll triplet quenching is considered to be triplet–triplet energy transfer from chlorophyll to carotenoid [1,2].

Energy transfer has been widely studied in photosynthetic organisms, including both bacteria and higher plant systems [1–3]. In parallel, investigations of artificial photosynthetic systems have been carried out on molecular dyads consisting of carotenoid polyenes covalently linked to porphyrin and chlorophyll derivatives. These artificial systems can mimic both the antenna and photoprotective functions [4].

The mechanisms of singlet–singlet and triplet–triplet energy transfer between carotenoids and chlorophyll pigments are not clearly understood and are still a matter of debate, although a variety of optical studies have been performed on natural and artificial systems. Very few optical studies of triplet–triplet transfer managed to find a correlation between the carotenoid triplet formation and the chlorophyll triplet disappearance kinetics. In the case of artificial dyads, the correlation has been recognized only if the system is characterized by very slow triplet–triplet transfer rates (in the microsecond range) [5,6]. In natural systems, the carotenoid triplet rise times have been measured in the nanoseconds range [7,8], but no triplet chlorophyll kinetics on the same time-scale have been reported.

In the present study a different approach, time-resolved EPR, has been used to give further insight into the mechanism of triplet–triplet energy transfer, in particular the structural requirements for high transfer yield in photosynthetic systems.

Transient EPR measurements of photoexcited triplet states allow detection of the initial spin polarization of the triplet sublevels. Generally, the spin-polarization pattern derives from the mechanism of triplet formation, which can be direct ISC, ISC followed by triplet–triplet energy migration, singlet fission or the radical-pair mechanism.

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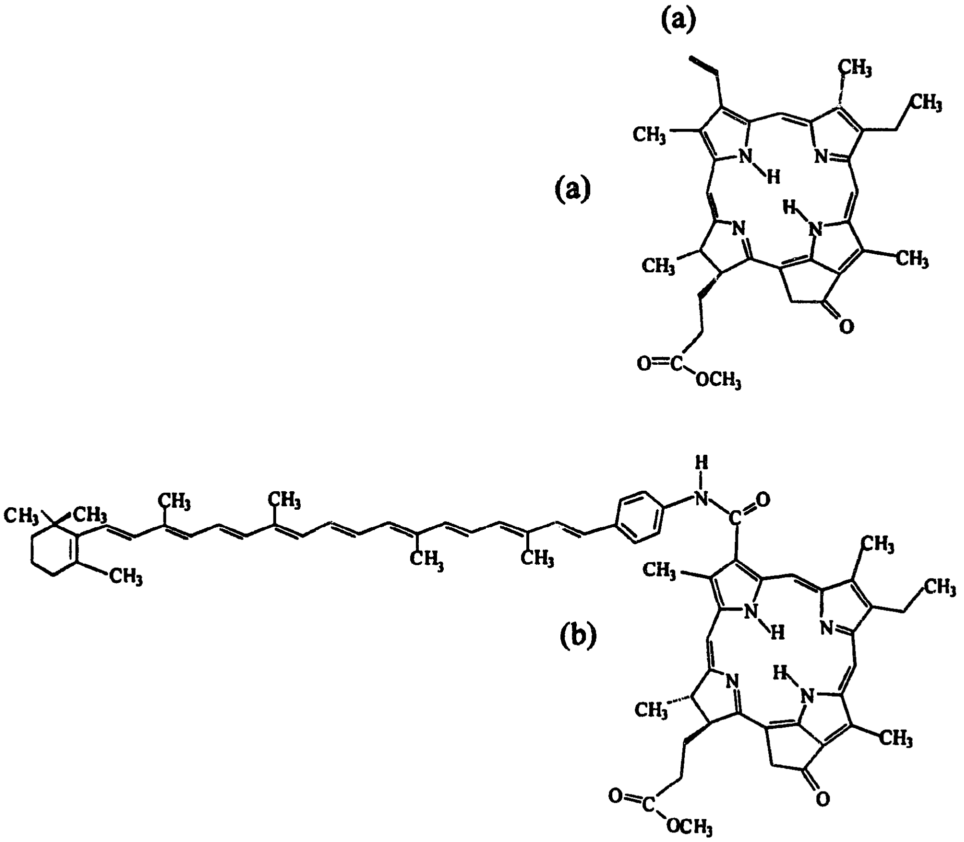

Fig. 1. Molecular structure of (a) free-base pyropheophorbide and (b) free-base carotenopyropheophorbide. The zinc pyropheophorbide and the zinc carotenopyropheophoride have the same molecular structure as the corresponding free-base compounds except for the substitution of the central protons with zinc.

It has been demonstrated that the spin angular momentum is conserved during the transfer process and the relative populations of the triplet acceptor sublevels are proportional to the squares of the projections of the donor principal magnetic axes on the acceptor magnetic axes. It is therefore possible to recognize the conformational requirements for efficient triplet–triplet energy transfer. Many time-resolved EPR studies have been made on spin conservation during intermolecular and intramolecular triplet–triplet energy transfer in the presence of an external magnetic field [9–12].

In this study, we performed transient EPR on two artificial photosynthetic dyads, consisting of a carotenoid polyene covalently-linked to a free-base or zinc substituted pyropheophorbide moiety (see Fig. 1). The two dyads, differing in the ISC population properties of the pheophorbide donor moiety, are suitable artificial systems to examine the conservation of the spin angular momentum during the energy transfer between the porphyrin and carotenoid chromophores and to investigate the structural effect on the transfer mechanism.

2. Experimental

Time-resolved EPR spectra were obtained using an X-band EPR spectrometer (Bruker ER 200D), equipped with a standard TE_{102} rectangular cavity and a helium flow cryostat (Oxford ESR 900) adapted for fast response in conjunction with a pulsed laser system. An excimer laser (Lambda Physik LPX 100, pulse width 20 ns, 150–200 mJ per pulse, 308 nm, repetition rate 22 Hz) pumping a dye laser (Lambda Physik FL 2000, rhodamine 6G dye, 582 nm, 20–25 mJ) was used as the light source. The transient signals were detected with either a boxcar averager (EG&G mod. 162) or a transient recorder (Lecroy mod. 9361). No field modulation and phase sensitive detection was used.

Electronic absorption spectra were recorded using an UV–VIS–NIR spectrophotometer (Varian Cary-5).

The synthesis of the model pyropheophorbide and of the covalently-linked carotenopyropheophorbides has already been described [13]. Samples (10^{-2}–10^{-3} M) were prepared in 2-methyltetrahydrofuran (Fluka), which had been purified by distillation from sodium metal. They were prepared on a high-vacuum line, degassed by numerous freeze–pump–thaw cycles, and then sealed.

Home-made programs, working on a Digital VAX 4000 computer, were used for the simulation of the spectra.

3. Results

3.1. Electronic absorption and fluorescence excitation spectra

The corrected fluorescence excitation spectra of the free-base carotenopyropheophorbide and of the zinc caroteno-

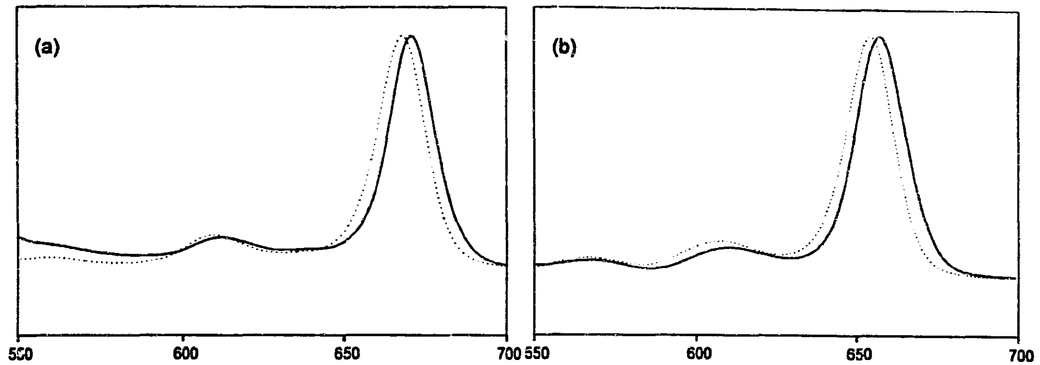

Fig. 2. Room-temperature electronic absorption spectra of (a) free-base pyropheophorbide (····) and free-base carotenopyropheophorbide (—), (b) zinc pyropheophoride (····) and zinc carotenopyropheophorbide (—) in 2-methyltetrahydrofuran. The spectra have been normalized on the maximum of the Q_y band.

pyropheophorbide (not shown) have carotenoid features, indicative of singlet–singlet energy transfer. The singlet–singlet energy transfer efficiency from the carotenoid to the pheophorbide in the free-base compound is comparable to published data [4].

The differences in the absorption spectra between the pyropheophorbide and the corresponding carotenopyropheophorbide, in the region 550–700 nm, are shown in Fig. 2a for the free-base compounds and in Fig. 2b for the zinc compounds. Both carotenopyropheophorbide absorption spectra are slightly red-shifted compared to those of the corresponding model compound, which is indicative of π-interaction between the carotenoid and pheophorbide moieties [4].

3.2. EPR spectra

The transient triplet state EPR spectra of the free-base pyropheophorbide and of the zinc pyropheophorbide are shown in Fig. 3a and b, respectively. The spectra have been detected at 0.6 µs delay after the laser flash. They are spin-polarized, in a EAEAEA and AAEAEE fashion, respectively. Their simulation was achieved using a computer program for polarized powder triplet EPR spectra. For the free-base pyropheophorbide the following parameters have been derived from the simulation of the spectrum: the zero-field parameters $|D| = 336 \times 10^{-4}$ cm^{-1} and $|E| = 41 \times 10^{-4}$ cm^{-1}, the relative populations of the three triplet sublevels P_x:P_y:P_z = 1.0: 0.5:0.28. For the zinc pyropheophorbide, the parameters are the following: $|D| = 301 \times 10^{-4}$ cm^{-1} and $|E| = 41 \times 10^{-4}$ cm^{-1}, P_x:P_y:P_z = 0.62:0.0:1.0. They are comparable with published data for similar compounds [14,15].

The transient triplet state EPR spectra of the free-base carotenopyropheophorbide and of the zinc carotenopyropheophorbide are shown in Fig. 4a, c and Fig. 4b, d, respectively. The spectra have been detected at different delays after the laser flash: at 0.6 µs delay (Fig. 4a and b) and at 9 µs delay (Fig. 4c and d). The zero-field splitting parameters $|D| = 358 \times 10^{-4}$ cm^{-1} and $|E| = 32 \times 10^{-4}$ cm^{-1}, derived from the simulation of the spectra of both compounds at two different delay times, are consistent with those for a triplet state of a 9 double-bond carotenoid polyene covalently-linked to a porphyrin moiety [16]. The spectra at 0.6 µs delay are spin-polarized and the relative triplet sublevel populations, derived from the simulation of the spectra, are P_x:P_y:P_z = 1:0.34:0.66 for the free-base cartenopyropheophorbide and P_x:P_y:P_z = 1:0.65:1 for the zinc compound. The spectra at 10 µs are still spin-polarized but with a different polarization pattern, in a EAEAEA fashion, as obtained previously for all carotenoid triplets using the CW–EPR technique [16,17].

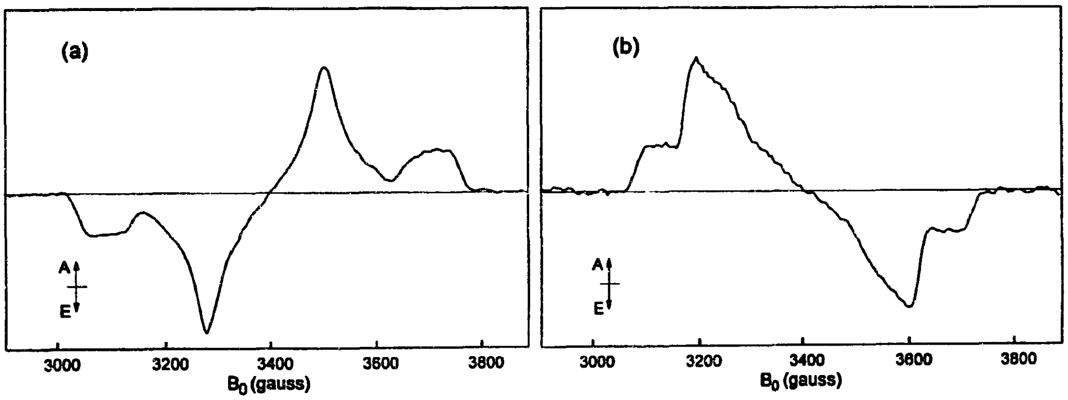

Fig. 3. Time-resolved triplet-state EPR spectra by direct detection of (a) free-base pyropheophorbide, (b) Zinc pyropheophorbide in 2-methyltetrahydrofuran. $T = 20$ K, $P_{MW} = 14.5$ mW, delay time after the laser pulse = 0.6 µs, integration gate = 0.3 µs. The ordering of the zero-field triplet sublevels is $Z^2 > Y^2 > X^2$. A and E stand for absorption and emission, respectively.

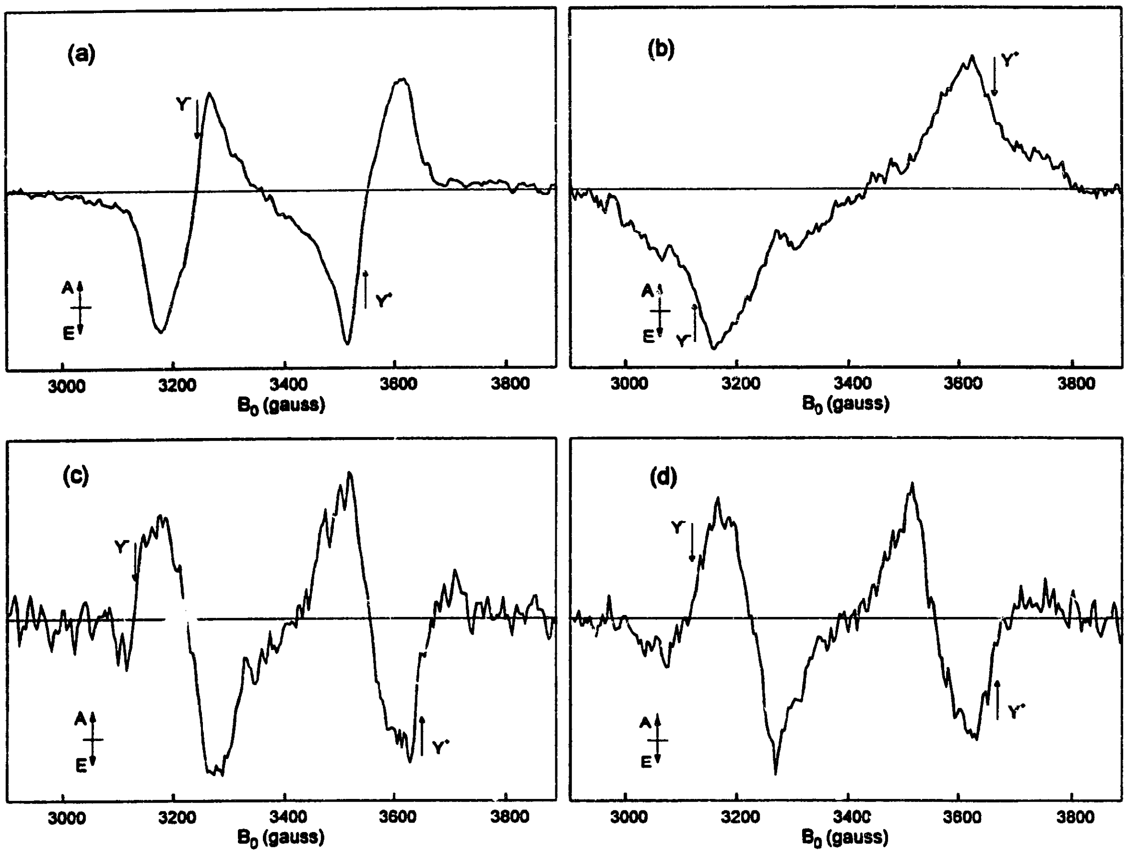

Fig. 4. Time-resolved triplet-state EPR spectra by direct detection of (a), (c) free-base carotenopyropheophorbide and (b), (d) Zinc carotenopyropheophorbide in 2-methyltetrahydrofuran. (a), (b) Delay time after the laser pulse = 0.6 μs. (c), (d) Delay time after the laser pulse = 9 μs. $T=20$ K, $P_{MW}=14.5$ mW, integration gate = 0.3 μs. The ordering of the zero-field triplet sublevels is $Z^2>Y^2>X^2$; y^- and y^+ are the two EPR transitions where the y-axis of the zero-field splitting tensor is parallel to the magnetic field. The triplet state EPR decay curves in Fig. 5 were obtained at these magnetic field positions. A and E stand for absorption and emission, respectively.

The triplet state EPR decay curves of the low field y transition of the triplet EPR spectra of the free-base carotenopyropheophorbide and of the zinc compound are shown in Fig. 5a and b, respectively. The decay curves of the high field y transition display the same kinetics but are opposite in sign. The decay curves have been simulated using a computer program describing the dynamics of the excited triplet state as described previously [12]. The analysis of the triplet state dynamics is accomplished neglecting spin-lattice relaxation, since no temperature dependence of the decay curves is observed in the low temperature range (10–40 K), and neglecting any influence of the induced EPR transitions on the kinetics. The kinetic traces of the z and x peaks have not been analyzed because of their even poorer signal-to-noise ratio. The parameters derived from the decay kinetics of the y transition, together with the EPR parameters used for the

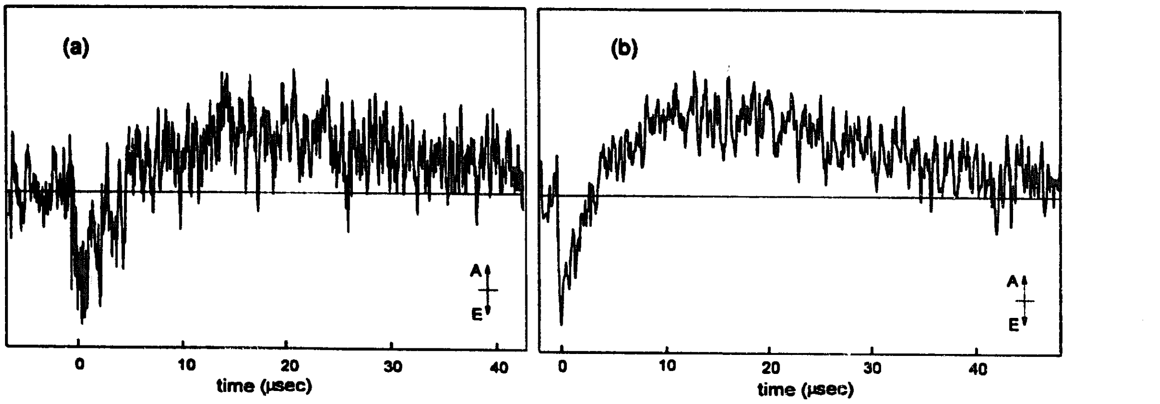

Fig. 5. Triplet state EPR decay curves of the low field y transition (y^-) of the triplet state EPR spectra (see Fig. 4) of (a) the free-base carotenopyropheophorbide and of (b) the zinc carotenopyropheophorbide in 2-methyltetrahydrofuran. $T=20$ K, $P_{MW}=140$ μW, average of 1000 traces, sample interval 3 ns. The kinetic traces were obtained by subtracting on- and off-resonance signals to eliminate the back-ground signal caused by the laser pulse. The ordering of the triplet sublevels is $Z^2>Y^2>X^2$. A and E stand for absorption and emission, respectively.

Table 1
Carotenoid triplet state kinetic parameters resulting from the simulation of the triplet state EPR decay curves and the triplet state EPR spectra of free-base carotenopyropheophorbide and of zinc carotenopyropheophorbide (see Fig. 3 and Fig. 4). K_x, K_y, K_z are the triplet spin sublevel decay rates (in s^{-1}). P_x, P_y, P_z are the relative population probabilities of the three triplet sublevels (extrapolated to 0 μs delay from the laser flash). The ordering of the zero-field triplet sublevels is $Z^2 > Y^2 > X^2$

	Free-base carotenopyro pheophorbide	Zinc carotenopyro pheophorbide
K_x	2.2×10^5	1.5×10^5
K_y	3×10^4	6×10^4
K_z	1.5×10^5	1.3×10^5
P_x	1.0	1.0
P_y	0.3	0.6
P_z	0.6	1.0

simulation of the transient EPR spectra at 0.6 and 9 μs delays, have been used to extrapolate the initial EPR spectrum (at 0 μs). The carotenoid triplet state decay rates and the relative initial populations of the three triplet sublevels (extrapolated to 0 μs delay from the laser flash) obtained from this computer simulation are shown in Table 1 for both compounds. The triplet state lifetime, evaluated from the average of the zero field rate constants, is in the 10 μs range typical of carotenoid triplets.

4. Discussion

The analysis of the experimental results has been performed in terms of spin angular momentum conservation during triplet–triplet energy transfer, in an external magnetic field, from the pheophorbide triplet to the carotenoid triplet. A home-made computer program has been used for the analysis. The directions of the spin magnetic axes for the pheophorbide moiety and the ordering of the triplet sublevels have been chosen in accord with earlier studies [18,19]. The directions of the spin magnetic axes for the carotenoid moiety have been chosen in accord with previous results [20] (z is the molecular chain axis, x is the normal in chain-plane and y is the normal out of chain-plain). The ordering of the zero-field triplet sublevels is: $Z^2 > Y^2 > X^2$. The donor population parameters P_x, P_y, P_z, used in the analysis are those obtained from the simulation of the transient EPR spectrum of the pyropheophorbide model compound at 0.6 μs delay. No extrapolation at 0 μs delay was necessary since both the free-base and the zinc pheophorbide triplet decay kinetics are in the milliseconds range and they have no influence on the relative populations at 0.6 μs. Furthermore, the donor initial populations have no time to evolve during the carotenoid triplet formation period (≤ 50 ns according to [13]).

The following conformational hypotheses, based on literature data, have been used in the analysis: (1) both carotenopyropheophorbides have an extended conformation [21], (2) the amide group linking pyropheophorbide and carotenoid moieties is planar and in the Z conformation [22].

The variable parameter in the simulation analysis of the carotenoid triplet EPR spectrum is the rotation angle Φ around the bond joining the phenyl group to the first carotenoid double bond (see Fig. 6). The effect of this rotation on the relative configuration between the donor and the acceptor magnetic axes is the same as that produced by the rotation around the bond joining the phenyl and amide groups or an additive rotation around both bonds. The conformational analysis has been done varying the angle Φ every 15°. The carotenoid triplet EPR spectra have been computed by varying the angle Φ every 15° and calculating the different projections of the donor principal magnetic axes on the acceptor magnetic axes.

The angular dependence of the time-resolved EPR spectra at intervals of 60° is shown in Fig. 6 (the spectra are equivalent for opposite rotation angles).

We may now compare the carotenoid triplet transient EPR spectra, calculated as described above (assuming spin conservation during triplet–triplet energy transfer), to the carotenoid triplet EPR spectra extrapolated to a 0 μs laser flash delay, as described in Results. As shown in Fig. 7, the carotenoid triplet EPR spectrum at 0 μs is compatible with the $\Phi = 180°$ simulation in the case of the free-base carotenopyropheophorbide, and to the $\Phi = 60°$ simulation for the corresponding zinc compound. Simulations obtained adding the contributions for the angles $\Phi = 180°$ and $\Phi = 180 \pm 15°$ for the free-base compound, and similarly for $\Phi = 60°$ and $\Phi = 60 \pm 15°$ for the zinc compound, can still reproduce the P_x, P_y, P_z parameters of Table 1.

The main conclusion from this computer analysis is that different conformations seem to be active in the triplet–triplet transfer process for the free-base carotenopheophorbide and for the zinc compound. In the case of the free-base carotenopyropheophoride, the π-systems of the two moieties are in the same plane, while in the zinc compound there is a 60° dihedral angle between the π-systems.

According to Dexter [23], triplet–triplet energy transfer requires strict constraints on the interaction between the donor and the acceptor, as the electronic exchange terms are the dominant ones in the electronic coupling elements which mix the initial and final states. Therefore, an all planar extended conformation should be the most suitable arrangement for a high efficiency of triplet–triplet transfer in both dyads. This conformation allows the maximum degree of overlap between the carotenoid and pyropheophorbide π-systems. This condition is fulfilled for the free-base but not for the zinc compound. The only way to explain the EPR results for the zinc carotenopheophorbide is to admit an influence of the central metal on the dyad conformation, and this would also influence the triplet–triplet transfer efficiency. This is an unexpected result, especially if the carotenoid moiety is extended away from the pyropheophorbide macrocycle, as the NMR data suggest [21]. One cannot, however, exclude a central metal interaction with, for example, the amide bond, which could produce a mutual rotation of the carotenoid and pheophorbide π-systems. On the other hand, the similar red-

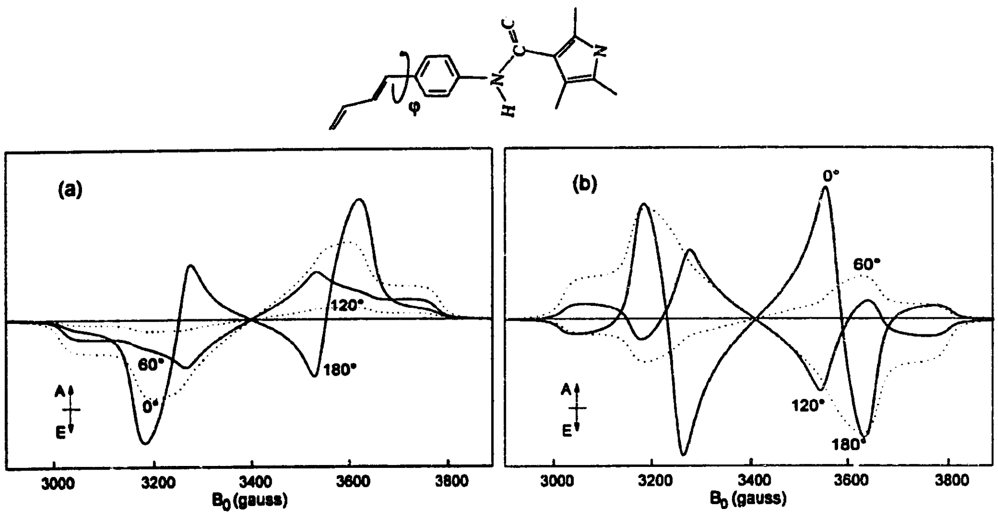

Fig. 6. Angular dependence of the computer calculations of the time-resolved EPR spectrum of the carotenoid triplet of (a) the free-base carotenopheophorbide and (b) the zinc carotenopyropheophorbide compound. The spectra were simulated using the hypothesis of spin angular momentum conservation during triplet–triplet energy transfer from the pyropheophorbide triplet. The angle of rotation Φ is the variable parameter in the simulations. A and E stand for absorption and emission, respectively.

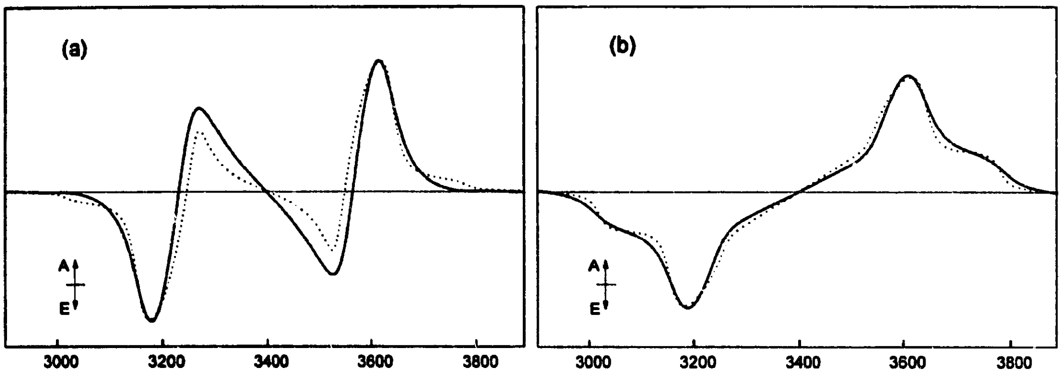

Fig. 7. (a) Comparison between the computer calculations of the time-resolved EPR spectrum of the carotenoid triplet using the hypothesis of triplet–triplet transfer for $\Phi = 180°$ (····), and the time resolved EPR spectrum at 0 μs (—), simulated using the P_x, P_y, P_z population values of Table 1, for the free-base carotenopyropheophorbide. (b) Comparison between the computer simulations of the time-resolved EPR spectrum of the carotenoid triplet for $\Phi = 60°$ (····), and the time resolved EPR spectrum at 0 μs (—), for the zinc carotenopyropheophorbide. The angle of rotation Φ is shown in Fig. 6. A and E stand for absorption and emission, respectively.

shift observed in the absorption spectra of the two dyads compared to those of the corresponding model compounds (see Fig. 2) is indicative of a similar π-interaction between the carotenoid and pyropheophorbide moieties in both compounds. This interaction should be reduced if the dihedral angle between the carotenoid and pyropheophorbide π-systems is 60°, but such a reduction could well be counteracted by a metal d-orbital interaction.

We conclude in any case that if the two dyads maintain the same conformation, it is necessary to invoke an alternative mechanism of carotenoid triplet formation to explain the EPR results. Transient absorption spectroscopy with the appropriate time-resolution would be welcome in order to find a still lacking direct correlation between the kinetics of the carotenoid triplet formation and the pheophorbide triplet disappearance.

5. Conclusions

In this paper, we have shown that the widely different initial polarization of the carotenoid triplet formed in an illuminated carotenopyropheophorbide, observed in the presence and absence of a Zn atom in the center of the porphyrin ring, are certainly not only due to the well known differences in the population kinetics of the porphyrin moiety following metallation. Different conformations have to be invoked in order to account for the experimental data on the hypothesis that conventional triplet–triplet transfer is the mechanism giving rise to the carotenoid triplet. Should the conformational explanation not stand further required tests, then a different mechanism must be invoked for the birth of the triplet.

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References

[1] R.J. Cogdell and H.A. Frank, Biochim. Biophys. Acta 895 (1987) 63.

[2] Y. Koyama, J. Photochem. Photobiol. B: Biol. 9 (1991) 265.

[3] R. van Grondelle, J.P. Dekker, T. Gillbro and V. Sundstrom, Biochim. Biophys. Acta 1187 (1994) 1.

[4] D. Gust and T.A. Moore, Adv. Photochem. 16 (1991) 1.

[5] A.L. Moore, A. Joy, R. Tom, D. Gust, T.A. Moore, R.V. Bensasson and E.J. Land, Science 216 (1982) 982.

[6] D. Gust, T.A. Moore, R.V. Bensasson, P. Mathis, E.J. Land, C. Chachaty, A.L. Moore, P.A. Liddell and G.A. Nemeth, J. Am. Chem. Soc. 107 (1985) 3631.

[7] A. Angerhofer, F. Bornhauser, A. Gall and R.J. Cogdell, Chem. Phys. 194 (1995) 259.

[8] J.F. Shepanski, D.J. Williams and Y. Kalisky, Biochim. Biophys. Acta 766 (1984) 116.

[9] T. Imamura, O. Onitsuka, H. Murai and K. Obi, J. Phys. Chem. 88 (1984) 4028.

[10] H. Murai, Y. Mizunuma, K. Ashikawa, Y. Yamamoto and Y.J. I'Haya, Chem. Phys. Lett. 144 (1988) 417.

[11] K. Akiyama, S. Tero-Kubota, T. Ikoma and Y. Ikegami, J. Am. Chem. Soc. 116 (1994) 5324.

[12] W. McGann and H. Frank, Chem. Phys. Lett. 121 (1985) 253.

[13] P.A. Liddell, D. Barrett, L.R. Makings, P.J. Pessiki, D. Gust and T.A. Moore, J. Am. Chem. Soc. 108 (1996) 5350.

[14] A. Angerhofer, in: H. Scheer (Ed.), Chlorophylls (CRC Press, Boca Raton, 1990) pp. 945–991.

[15] J.F. Kleibeuker, R.J. Platenkamp and T.J. Schaafsma, Chem. Phys. 27 (1978) 51.

[16] H.A. Frank, B.W. Chadwick, J.J. Oh, D. Gust, T.A. Moore, P.A. Liddell, A.L. Moore, L.R. Makings and R.J. Cogdell, Biochim. Biophys. Acta 892 (1987) 253.

[17] D. Carbonera, G. Giacometti, G. Agostini and A. Toffoletti, Gazzetta Chim. It. 119 (1989) 225.

[18] S.J. van der Bent and T.J. Schaafsma, J. Chem. Phys. 68 (1978) 187.

[19] W.G. van Dorp, W.H. Schoemaker, M. Soma and J.H. van der Waals, Molec. Phys. 30 (1975) 1701.

[20] J. Frick, PhD Thesis, Stuttgart 1992.

[21] M.R. Wasielewski, P.A. Liddell, D. Barrett, T.A. Moore and D. Gust, Nature 322 (1986) 570.

[22] F.D. Lewis and E.L. Burch, J. Phys. Chem. 100 (1996) 4055.

[23] D.L. Dexter, J. Chem. Phys. 21 (1953) 836.