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Porphyrin and pyropheophorbide phosphorescence in synthetic molecules that mimic photosynthetic triplet energy transfer

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Abstract

Phosphorescence emission and excitation spectra and triplet lifetimes at 77 K have been obtained for a carotenoid–porphyrin–pyropheophorbide triad model for the photosynthetic triplet energy transfer relay and seven porphyrin and pyropheophorbide model compounds including methyl pyropheophorbide-*a*. The results confirm that triplet energy from the pyropheophorbide moiety of the triad is transferred to the carotenoid by a triplet energy relay involving endergonic transfer from the pyropheophorbide to the porphyrin, whose triplet is rapidly quenched by the carotenoid polyene.

Keywords: Photosynthesis; Phosphorescence; Porphyrin; Pyropheophorbide; Triplet energy transfer

1. Introduction

In order to survive in an oxygenous atmosphere, photosynthetic organisms must employ protection against reactive oxidizing species [1–5]. Carotenoid polyenes are part of this protective system, suppressing the formation of singlet oxygen. This carotenoid photoprotection involves quenching chlorophyll excited states so that they are not kinetically competent to sensitize singlet oxygen formation. The quenching is a triplet–triplet energy transfer process that produces the carotenoid triplet, whose energy is too low to permit singlet oxygen sensitization.

Efficient triplet–triplet energy transfer requires electronic overlap of the chlorophyll and carotenoid orbitals. However, photosynthetic reaction centers usually contain several chlorophyll or pheophytin molecules for each carotenoid, and the spatial organization of the chromophores is therefore a very important aspect of photoprotection. For example, in bacterial reaction centers, the carotenoid polyene is not located near the bacteriochlorophyll special pair (the chlorophyll triplet state of lowest energy), but rather adjacent to an accessory bacteriochlorophyll [6–8]. It has been suggested that carotenoid photoprotection in these reaction centers involves thermally activated energy transfer from the relatively low triplet level of the bacteriochlorophyll special pair to the more energetic triplet of the accessory bacteriochlorophyll, and then on to the carotenoid polyene [9–11]. A similar triplet energy transfer relay might be relevant to photoprotection of PSII reaction centers [12,13].

Synthetic model systems that mimic aspects of photosynthetic energy and electron transfer can be prepared [14–17]. For example, we recently reported the preparation and photophysical study of a carotenoid (C) porphyrin (P_{H2}) pyropheophorbide (Ppd) molecular triad (**1**) designed to help elucidate the mechanistic features of a thermally activated triplet energy transfer relay [18]. It was found that photoexcitation of the triad resulted in formation of the pyropheophorbide singlet state (C–P_{H2}–^{1}Ppd). Intersystem crossing generated the corresponding triplet, which was postulated to decay by thermally activated triplet energy transfer to yield ^{3}C–P_{H2}–Ppd via an intermediate C–$^3P_{H2}$–Ppd species. In aerobic solutions, this process efficiently reduced singlet oxygen production. Formation of ^{3}C–P_{H2}–Ppd was completely suppressed at liquid nitrogen temperatures. In the analogous triad containing a zinc porphyrin, triplet energy transfer was inefficient even at ambient temperatures, presumably due to the high energy of C–$^3P_{Zn}$–Ppd, which makes energy transfer from the pyropheophorbide thermodynamically unfavorable.

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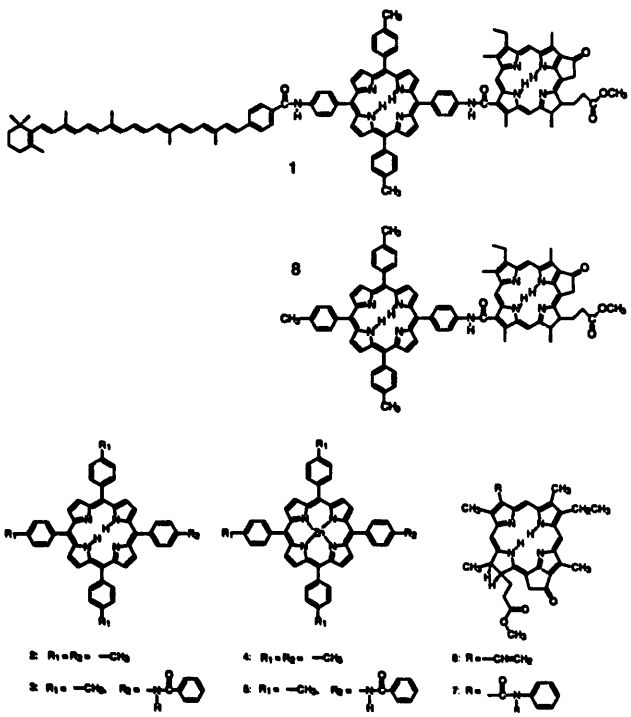
A knowledge of the triplet energies of the porphyrin and pyropheophorbide moieties in these triads and similar molecules is crucial for interpretation of photophysical studies. In the previous work [18], these energies were not available for **1** or closely related model compounds, and literature data for the phosphorescence of structurally related molecules were employed instead. In particular, the phosphorescence properties of pyropheophorbides were unknown. In the present paper, we report the phosphorescence properties of **1** and model compounds **2–7**, including methyl pyropheophorbide-*a* (**6**). The results confirm the conclusions of the earlier study and provide information concerning phosphorescence properties and triplet state energies and lifetimes of molecules that are useful components of a variety of photosynthetic model systems.



2. Experimental section

2.1. Synthesis

The preparation of molecules **1–8** has been described elsewhere. [18]

2.2. Phosphorescence spectra

Phosphorescence studies were performed on 1–20 μM solutions of **1–8** in acetone at 77 K. Phosphorescence was measured using a phosphorimeter based on a mechanical phosphoroscope [19,20]. The phosphoroscope consisted of three coaxial metal cylinders. The outer and inner were immobile while the third rotated between them on a shaft connected to an electric motor. Each immobile cylinder had one narrow rectangular window for excitation and a second for measuring phosphorescence. The rotating cylinder had two rectangular windows located 180° apart. As the cylinder rotated, these windows allowed light to pass through the excitation and measuring windows in sequence. The interval between complete closing of the excitation window and opening of the measuring window was about 200 μs. As a result, the phosphoroscope allowed detection of delayed luminescence with the lifetimes exceeding about 200 μs.

Excitation was provided by a 1 kW xenon lamp through a high intensity quartz condenser, an aqueous thermal filter and glass color filters. The xenon lamp radiation was focused through the phosphoroscope window onto a 3×3 mm spot on the sample, which was located in a Dewar inside the inner cylinder. The phosphorescence light was collected at a 105° angle with respect to the exciting beam and passed through the measuring window to the entrance slit of a monochromator. The monochromatic light was detected by an S-1 photomultiplier cooled to −60 °C, and the resulting response was amplified and analyzed by computer.

Phosphorescence excitation spectra were detected with a similar setup, but in this case the xenon lamp radiation was focused on the entrance slit of a grating monochromator. Monochromatic exciting light was focused on the sample as described above, and phosphorescence was detected through appropriate filters. For a single chromophore, excitation spectra generally correspond to absorption spectra of the phosphorescing compound. This follows from the equation: $L = I\varphi(1-10^{-A})$, where L is the luminescence intensity, I is the intensity of excitation, φ is the luminescence quantum yield and A is light absorbance. For an individual dye φ is usually independent of light wavelength, and the wavelength dependence of the L/I ratio coincides with the spectrum of $(1-10^{-A})$. When A is very low, ≤ 0.01, the shape of the $(1-10^{-A})$ spectrum coincides with the absorption spectrum. In the present case, because of very low phosphorescence intensity we used samples having A values in the Soret bands of about 0.3 in 5 mm cells at room temperature. After freezing in liquid nitrogen, the absorbance increased by a factor of about 2. Hence, the peak intensity ratios in the excitation spectra differ from those in the absorption spectra; the contribution of areas of low absorbance is accentuated.

The phosphorimeter also allowed measurement of phosphorescence lifetimes. This was done by comparing the steady-state phosphorescence intensities obtained with direct and reverse rotation of the electric motor [19]. Absorption spectra of the frozen, light-scattering solutions were measured using an SF-18 double-beam spectrophotometer with an integrating sphere.

3. Results

3.1. Free-base porphyrins 2 and 3

Porphyrins **2** and **3** have very similar phosphorescence emission spectra and triplet lifetimes (Table 1). The results

Table 1
Principal phosphorescence maxima and phosphorescence lifetimes in acetone at 77 K

Compound	Maxima [a] (nm)	τ (ms)	Compound	Maxima [a] (nm)	τ (ms)
1	962, 1075	1.0±0.2	5	792, 878	21±5
2	868, 984	3.8±0.5	6	940, 1065	1.0±0.2
3	872, 992	3.8±0.5	7	975, 1085	1.0±0.2
4	798, 894	21±3	8	962, 1075	1.0±0.2

[a] The short-wavelength maxima were determined with an accuracy of ±2 nm, and the long-wavelength maxima with an accuracy of ±5 nm.

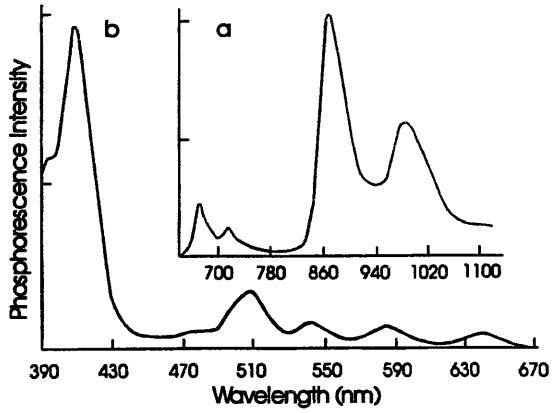


Fig. 1. (a) Phosphorescence emission spectrum of an approximately 1×10^{-5} M solution of free base porphyrin 2 in acetone at 77 K. Excitation was with red light (>600 nm) and the detection monochromator bandpass was 12 nm. (b) Phosphorescence excitation spectrum of the same solution for emission at >900 nm. The excitation monochromator bandpass was 5 nm.

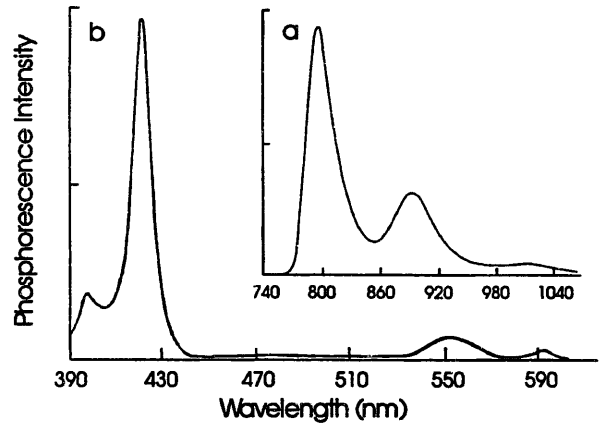


Fig. 2. (a) Phosphorescence emission spectrum of an approximately 1×10^{-5} M solution of zinc porphyrin 4 in acetone at 77 K. Excitation was with yellow light (>500 nm) and the detection monochromator bandpass was 12 nm. (b) Phosphorescence excitation spectrum of the same solution for emission at 790 nm. The excitation monochromator bandpass was 5 nm.

for an acetone solution of **2** appear in Fig. 1. The emission spectrum features phosphorescence maxima at 870 and 980 nm and some delayed fluorescence in the 700 nm region. The phosphorescence lifetime is 3.8 ms. The intensity of the delayed fluorescence was proportional to the square of the excitation intensity, and is likely due to triplet–triplet annihilation or other intermolecular phenomena. Similar delayed fluorescence has been observed in solutions of chlorophyll-*a* [21,22]. The phosphorescence excitation spectrum (Fig. 1(b)) has maxima at 413, 510, 544, 586 and 641 nm, which correspond to maxima in the low-temperature absorption spectra. The peak intensities are distorted because of the high absorbance of the solution, as discussed above. In general, the phosphorescence properties of **2** and **3** are similar to those of 5,10,15,20-tetraphenylporphyrin, which has the major phosphorescence maximum at 853–865 nm and a triplet lifetime of 4.6–6.6 ms. [23–26]

3.2. Zinc porphyrins 4 and 5

Zinc porphyrin **4** has phosphorescence maxima at 798 and 894 nm (Fig. 2). The triplet lifetime is 21 ms (Table 1). The peak maxima of the excitation spectrum, at 423, 554 and 593 nm, corresponded with those of the low-temperature absorption spectrum. The quantum yield of phosphorescence was 400 times higher than that for **2**. The results for **5** were similar (Table 1). The phosphorescence properties of these two molecules are similar to those reported previously for zinc 5,10,15,20-tetraphenylporphyrin, whose phosphorescence maximum is observed at about 780 nm [24–27].

3.3. Pyropheophorbides 6 and 7

The phosphorescence maxima of **6** occurred at 940 and 1065 nm (Fig. 3). The phosphorescence lifetime was about 1 ms, and the phosphorescence intensity was less than that of **2** by a factor of 5. The excitation spectrum is also shown in Fig. 3. The maxima are generally in accord with those of the

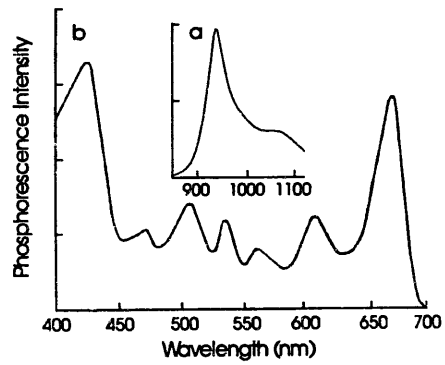


Fig. 3. (a) Phosphorescence emission spectrum of an approximately 1×10^{-5} M solution of methyl pyropheophorbide (**6**) in acetone at 77 K. Excitation was with red light (>650 nm) and the detection monochromator bandpass was 16 nm. (b) Phosphorescence excitation spectrum of the same solution for emission at >900 nm. The excitation monochromator bandpass was 6 nm.

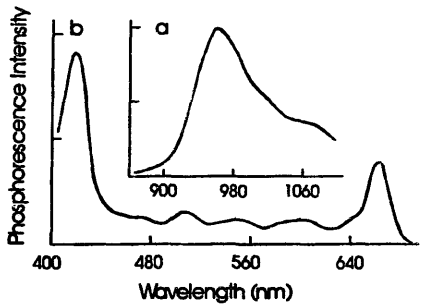


Fig. 4. (a) Phosphorescence emission spectrum of an approximately 1×10^{-5} M solution of triad **1** in acetone at 77 K. Excitation was with red light (>650 nm) and the detection monochromator bandpass was 16 nm. (b) Phosphorescence excitation spectrum of the same solution for emission at >900 nm. The excitation monochromator bandpass was 8 nm.

absorption spectrum at 298 K. Compound **7** has its phosphorescence maxima at slightly longer wavelengths than those of **6** (Table 1). The triplet lifetime is also about 1 ms. Pheophorbide-*a*, which has a similar conjugated ring system, has its main phosphorescence maximum at 930 nm [28]. Thus, the triplet energies of **6** and **7** are slightly lower than that of pheophorbide-*a*.

3.4. Dyad 8 and triad 1

The phosphorescence emission spectrum of triad **1**, observed with excitation by red light (>650 nm), has maxima at 962 and 1075 nm (Fig. 4), and generally resembles those of model compounds **6** and **7**. Dyad **7** has an identical spectrum. Thus, only the pyropheophorbide triplet state is observed; the porphyrin triplet states are not significantly populated. The excitation spectrum of the 962 nm phosphorescence band of **1** (Fig. 4(b)) features pyropheophorbide maxima at 508, 534, 610 and 655 nm and porphyrin maxima at 585 and 640 nm. The Soret region has contributions from both the porphyrin and pyropheophorbide moieties. The carotenoid absorption bands in the 400–530 nm region contribute little to the excitation spectrum. Under excitation with red light, the phosphorescence quantum yields and lifetimes of pyropheophorbide emission in **1**, **6**, **7**, and **8** are identical, within experimental error.

4. Discussion

4.1. Singlet–singlet energy transfer

The phosphorescence spectra of triad **1** and dyad **8**, obtained under excitation with red light which is absorbed by both the porphyrin and pyropheophorbide moieties, show only pyropheophorbide emission. The porphyrins are silent. The phosphorescence excitation spectra indicate that light absorbed by the porphyrin does give rise to pyropheophorbide emission. In principle, this could be due either to rapid singlet–singlet energy transfer from the porphyrin to the pyropheophorbide, or to rapid triplet–triplet energy transfer between the same moieties. In previous work, fluorescence studies at ambient temperatures showed that excitation of the porphyrin moieties of **1**, **8**, or their zinc analogs results in rapid singlet–singlet energy transfer to the pyropheophorbide, which is the only fluorescing species. The yield of this transfer is near unity [18]. In model carotenoporphyrin dyads having structures similar to the carotenoporphyrin portion of **1**, singlet–singlet energy transfer from the carotenoid to the porphyrin has a quantum yield of about 0.1 at ambient temperatures [29]. As mentioned above, the phosphorescence excitation spectrum of **1** has strong contributions from the porphyrin and the pyropheophorbide and only a weak contribution from the carotenoid moiety. Thus, these singlet energy transfer phenomena evidently occur at 77 K. These results show that the pyropheophorbide is the singlet-energy sink in the triad. Light absorbed by the carotenoid is either transferred to the attached porphyrin or degraded through internal conversion. (Intersystem crossing quantum yields in such carotenoids are essentially zero.) The porphyrin singlet state decays by singlet–singlet energy transfer to the pyropheophorbide, which undergoes normal intersystem crossing to yield the triplet state, which in turn gives rise to the observed phosphorescence.

4.2. Triplet–triplet energy transfer

In acetone solution at ambient temperatures, transient absorption studies showed that the pyropheophorbide triplet state of **1**, C–P_{H2}–^{3}Ppd, is quenched by triplet–triplet energy transfer to yield the carotenoid triplet state with a rate constant k_{ent} of 2.9×10^6 s^{-1} [18]. This transfer was postulated to occur not by direct quenching of the pyropheophorbide by the carotenoid, but rather by a triplet energy relay involving the porphyrin. Endergonic triplet energy transfer from C–P_{H2}–^{3}Ppd yields C–$^3P_{H2}$–Ppd, which is rapidly quenched by triplet energy transfer to the carotenoid, giving ^{3}C–P_{H2}–Ppd. The carotenoid triplet was not observed at low temperature, as expected from this mechanism. The phosphorescence results are in accord with this proposal. The only phosphorescence observed in **1** and **8** was due to the pyropheophorbide, which had an unperturbed lifetime of about 1 ms. Thus, triplet energy transfer did not occur.

The triplet energies for the various molecules, calculated from the short-wavelength phosphorescence maxima, appear in Table 2. The energy of C–$^3P_{H2}$–Ppd is higher than that of C–P_{H2}–^{3}Ppd by 0.13 eV, as calculated based on model porphyrin **3** (ΔE in Table 2), and triplet energy transfer from the pyropheophorbide is endergonic, as expected. Triplet–triplet energy transfer rates are properly treated by the Dexter approach. [30] However, the rate constant for endergonic energy transfer from C–P_{H2}–^{3}Ppd to yield C–$^3P_{H2}$–Ppd at various temperatures, k_{ent}, may be estimated according to Eq. (1), where k_0 is a temperature-independent pre-exponential factor, R is the gas constant, and ΔE is the energy difference between the two states (Table 2). The measured [18] k_{ent} in **1** at about 293 K, 2.9×10^6 s^{-1}, and the ΔE value for model porphyrin **3** may be used to estimate

Table 2
Triplet energies of the triad and model compounds (E_t) and energy gaps (ΔE) between the porphyrin triplet states of the listed compounds and the triplet state of the pyropheophorbide moiety of **1**

Compound	Phosphorescence maximum (nm)	E_t (eV)	ΔE (eV)	$\exp(-\Delta E/RT)$	
				293 K	77 K
2	868	1.43	0.14	3.9×10^{-3}	6.9×10^{-10}
3	872	1.42	0.13	5.8×10^{-3}	3.10×10^{-9}
4	798	1.55	0.26	3.4×10^{-5}	9.59×10^{-18}
5	792	1.57	0.28	1.5×10^{-5}	4.7×10^{-19}
6	940	1.32	–	–	–
7	975	1.27	–	–	–
1, 8	962	1.29 [a]	–	–	–

[a] For the pyropheophorbide moiety.

$$k_{ent} = k_0 \exp\left(\frac{-\Delta E}{RT}\right) \quad (1)$$

the value of k_0 as $5.0\times10^8\ s^{-1}$. Thus, at 77 K, k_{ent} for **1** is about $1.6\ s^{-1}$. As the triplet lifetime of C–P_{H2}–^{3}Ppd at 77 K is about 1 ms, triplet transfer should not be, and is not, observed.

If we assume that k_0 in Eq. (1) for the zinc porphyrin analog of **1** is also approximately $5\times10^8\ s^{-1}$, then we can use Eq. (1) and the ΔE value from Table 2 for model porphyrin **4** to estimate a rate constant for energy transfer at 293 K from C–P_{Zn}–^{3}Ppd to yield C–$^3P_{Zn}$–Ppd of about $2\times10^4\ s^{-1}$. This compares relatively well with the experimental value of $3.7\times10^4\ s^{-1}$. [18] At 77 K, the corresponding rate constant would be infinitesimal, as expected from the lack of triplet energy transfer at that temperature.

The data presented herein confirm the proposed triplet relay exhibited by **1** and reported earlier [18]. In addition, they provide experimentally determined phosphorescence spectra, phosphorescence lifetimes and triplet energies for triad **1**, methyl pyropheophorbide-*a*, and model compounds **2–4**, **7** and **8**. These will be useful for evaluating the triplet properties of a large number of existing and proposed multicomponent molecular systems that employ chromophores of these types.

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References

[1] M. Griffiths, W.R. Sistrom, G. Cohen-Bazire and R.Y. Stanier, *Nature (London), 176* (1955) 1211–1214.
[2] A.A. Krasnovsky, Jr., *Proc. R. Soc. Edinburgh, 102B* (1994) 219–235.
[3] P. Mathis and C.C. Schenck, in G. Britton and T.W. Goodwin (eds.), *Carotenoid Chemistry and Biochemistry*, Pergamon, Oxford, 1981.
[4] N.I. Krinsky, in W. Bors, M. Sarah and D. Tait (eds.), *Oxygen Radicals in Chemistry and Biology*, Walter de Gruyter, Berlin, 1984, pp. 45–51.
[5] K. Asada and M. Takashashi, *Top. Photosynthesis, 9* (1987) 227–287.
[6] J.P. Allen, G. Feher, T.O. Yeates, D.C. Rees, J. Deisenhofer, H. Michel and R. Huber, *Proc. Natl. Acad. Sci. USA, 83* (1986) 8589–8593.
[7] C.H. Chang, D. Tiede, J. Tang, U. Smith, J. Norris and M. Schiffer, *FEBS Lett., 205* (1986) 82–86.
[8] J. Deisenhofer, O. Epp, K. Miki, R. Huber and H. Michel, *Nature (London), 318* (1985) 618–624.
[9] C.C. Schenck, P. Mathis, M. Lutz, D. Gust and T.A. Moore, *Biophys. J., 41* (1983) 123a.
[10] C.C. Schenck, P. Mathis and M. Lutz, *Photochem. Photobiol., 39* (1984) 407–417.
[11] L. Takiff and S.G. Boxer, *J. Am. Chem. Soc., 110* (1988) 4425–4426.
[12] P. Mathis, K. Satoh and O. Hansson, *FEBS Lett., 251* (1989) 241–244.
[13] Y. Takahasi, O. Hansson, P. Mathis and K. Satoh, *Biochim. Bioiphys. Acta, 893* (1987) 49–59.
[14] D. Gust and T.A. Moore, *Adv. Photochem., 16* (1991) 1–65.
[15] D. Gust and T.A. Moore, *Top. Curr. Chem., 159* (1991) 103–151.
[16] D. Gust, T.A. Moore and A.L. Moore, *Acc. Chem. Res., 26* (1993) 198–205.
[17] M.R. Wasielewski, *Chem. Rev., 92* (1992) 435–461.
[18] D. Gust, T.A. Moore, A.L. Moore, A.A. Krasnovsky, Jr., P.A. Liddell, D. Nicodem, J.M. DeGraziano, P. Kerrigan, L.R. Makings and P.J. Pessiki, *J. Am. Chem. Soc., 115* (1993) 5684–5691.
[19] A.A. Krasnovsky, Jr., *Photochem. Photobiol., 29* (1979) 29.
[20] A.A. Krasnovsky, Jr., *SPIE Proc., 1887* (1993) 177–186.
[21] A.A. Krasnovsky, Jr., and A.N. Semenova, *Photobiochem. Photobiophys., 3* (1981) 11–18.
[22] A.A. Krasnovsky, Jr., *Photochem. Photobiol., 36* (1982) 733–741.
[23] M. Gouterman and G.-M. Khalil, *J. Mol. Spectrosc., 53* (1974) 88–100.
[24] A. Voelcker, H.J. Adick, R. Schmidt and H.D. Brauer, *Chem. Phys. Lett., 159* (1989) 103–108.
[25] A. Harriman, *J. Chem. Soc., Faraday Trans. 1, 76* (1980) 1978–1985.
[26] G. Egorova, V. Knyukshto, K. Solovyev and M. Tsvirko, *Opt. Spectrosc. (USSR), 48* (1980) 1101–1109.
[27] D.J. Quimby and F.R. Longo, *J. Am. Chem. Soc., 97* (1975) 5111–5117.
[28] A.A. Krasnovsky, Jr., K.V. Neverov, S.Y. Egorov, B. Roeder and T. Levald, *J. Photochem. Photobiol. B: Biology, 5* (1990) 245–254.
[29] D. Gust, T.A. Moore, A.L. Moore, C. Devadoss, P.A. Liddell, R. Hermant, R.A. Nieman, L.J. Demanche, J.M. DeGraziano and I. Gouni, *J. Am. Chem. Soc., 114* (1992) 3590–3603.
[30] D.L. Dexter, *J. Chem. Phys., 21* (1953) 836–850.