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REGULAR PAPER

Phosphorescence study of chlorophyll *d* photophysics. Determination of the energy and lifetime of the photo-excited triplet state. Evidence of singlet oxygen photosensitization

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Abstract Chlorophyll *d* (Chl *d*) is the major pigment in both photosystems (PSI and II) of the cyanobacterium *Acaryochloris marina*, whose pigment composition represents an interesting alternative in oxygenic photosynthesis. While abundant information is available relative to photophysical properties of Chl *a*, the understanding of Chl *d* photophysics is still incomplete. In this paper, we present for the first time a characterization of Chl *d* phosphorescence, which accompanies radiative deactivation of the photoexcited triplet state of this pigment. Reliable information was obtained on the energy and lifetime of the Chl *d* triplet state in frozen solutions at 77 K using diethyl ether and aqueous dispersions of Triton X100 as solvents. It is shown that triplet Chl *d* is effectively populated upon photoexcitation of pigment molecules and efficiently sensitizes singlet oxygen phosphorescence in aerobic solutions under ambient conditions. The data obtained are compared with the previous results of the phosphorescence studies of Chl *a* and Pheo *a,* and their possible biological implications are discussed.

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Introduction

Chlorophyll (Chl) *d* is the most abundant pigment present in the photosynthetic apparatus of the cyanobacterium *Acaryochloris marina*. Although the chemical nature of this chlorophyll has been known for over five decades (Manning and Strain 1943; Holt and Morley 1959), its involvement in biologically relevant processes has not been recognized until the discovery of the novel class of photosynthetic phototrophic organisms of which *A. marina* is the best characterized (e.g., Miyashita et al. 1996; Kühl et al. 2005). It has been demonstrated that in this organism, Chl *d* not only plays a major role in light harvesting, but it is part of the photochemical reaction centers (RC) of both Photosystem I and Photosystem II (PS I and PS II) (e.g., Hu et al. 1998; Tomo et al. 2007; Santabarbara et al. 2007; Schenderlein et al. 2008). Though, chemically, Chl *d* is distinct from Chl a only for a formyl substitution at C_3 position of ring I of the tetra-pyrrol ring (Holt and Morley 1959), the energy of the lowest singlet excited state is red-shifted by more than 30 nm in pigment solutions (Nieuwenburg et al. 2003) and by 30–40 nm in the photosynthetic apparatus of *A. marina* (e.g. Tomo et al. 2007; Schenderlein et al. 2008). This reflects in a loss of between 75 and 95 meV (3–4 k_bT) in energy available for the photochemical reactions, which is intriguing from the evolutionary point of view (Hu et al. 1998; Tomo et al. 2007; Santabarbara et al. 2007; Schlodder et al. 2007; Schenderlein et al. 2008).

For these reasons, there has been a surge of interest into investigating the photophysical properties of Chl *d*, which

have lead to a gathering of information relating to its redox properties (Kobayashi et al. 2007) as well as the vibrational (Telfer et al. 2010; Chen et al. 2004) and electronic energy levels (Schenderlein et al. 2008; Nieuwenburg et al. 2003). Yet, the characterization of the photo-excited triplet state, which is typically populated with high quantum yields in porphyrin-related molecules, is still incomplete. While the triplet states of Chls do not play a central role in productive photochemistry, they are one of the principal sensitizers of the reactive oxygen species active in light-induced damage of the photosynthetic apparatus, a process known as photoinhibition (Aro et al. 1993; Krasnovsky 1994). The triplet state of Chl *d* has been studied using Optically Detected Magnetic Resonance (Di Valentin et al. 2007) and Time-resolved ESR spectroscopy (Di Valentin et al. 2007; Schenderlein et al. 2008), from which the resonance frequencies and the zero-field splitting parameters (|D| and |E|) characterizing the photo-excited triplet state manifold have been estimated. More recently, the Chl *d* triplet state was also characterized by transient absorption spectroscopy in organic solvents (Niedzwiedzki and Blankenship 2010), in detergent micelles, and in reaction centers of PS I (Schenderlein et al. 2008). Still, the energy level of the triplet state, which is the primary factor determining the sensitization of $^1O_2$ ($^1\Delta_g$), and hence is of biological relevance, was not determined.

Since the first reliable data on low-temperature (77 K) phosphorescence of chlorophylls and pheophytins were reported (Krasnovsky et al. 1973, 1974, 1975), phosphorescence measurements have proved a valuable and reliable technique to investigate the triplet states of the photosynthetic pigments in solutions, isolated fragments of the photosynthetic apparatus, etiolated and green leaves as well as whole algal and bacterial cells. In particular, phosphorescence of Chls (Chl *a*, *b*, protochlorophyll, bacteriochlorophylls *a*, *b*, *c* and *d*) and metal free analogs of these chromophores has been characterized in a series of previous investigations (see for refs Krasnovsky et al. 1977; Kleibeuker et al. 1978; Dvornikov et al. 1979; Hoff 1986; Krasnovsky 1982, 1994; Takiff and Boxer 1988; Neverov et al. 1996; Neverov and Krasnovsky 2004). Unlike fluorescence, which accompanies radiative deactivation of the excited singlet states of pigments, phosphorescence corresponds to radiative deactivation of the triplet states. Therefore, phosphorescence measurements provide direct information on the energies and the radiative and real lifetimes of the pigment triplet states. In this study we present, for the first time, a characterization of Chl *d* phosphorescence, in terms of its spectrum, energy, and lifetime at 77 K and show that the triplet state of Chl *d* can efficiently sensitize singlet oxygen phosphorescence in air-saturated solutions, under ambient conditions.

Materials and methods

Phosphorescence and fluorescence measurements

Phosphorescence was measured using two set-ups with mechanical phosphoroscopes, which allowed for detection of delayed light emission with the lifetime ≥500 µs (Krasnovsky 1979, 2004 and refs therein). In both set-ups, phosphorescence was excited by the focused light of a 1 kW Xenon lamp and registered with a S-1 photomultipliers (FEU-83) cooled to −50°C by flowing liquid nitrogen vapor. The photomultiplier signal was detected by a direct current amplifier, digitized, and stored in a personal computer.

One set-up allowed for measurement of the phosphorescence and fluorescence emission spectra using a high throughput grating monochromator placed between the samples and PMT. Phosphorescence emission spectra were recorded in the rotating phosphoroscope under excitation by 1 kW lamp via the water heat filter and sharp edge cut-off red glasses, covering the required spectral interval. Fluorescence was excited by the monochromatic light through the additional phosphoroscope window designed specially for fluorescence measurements. For fluorescence excitation, the 300 W halogen lamp and an additional grating monochromator were employed.

The second set-up was used for measurement of the phosphorescence excitation spectra. In this case, the monochromator was placed between the xenon lamp and the phosphoroscope as described by Krasnovsky 1979, 2004. Phosphorescence was excited by monochromatic light and registered via the IR cut-off absorption filter IKS-7 ($\lambda > 880$ nm).

Phosphorescence lifetime

Due to the asymmetric arrangement of the phosphoroscope windows, both set-ups allowed for the determination of the phosphorescence lifetimes based on comparison of the phosphorescence intensities upon forward (L_{dir}) and reverse (L_{rev}) rotation of the phosphoroscope cylinders, in the rotation frequency range of 1,500–5,000 rpm. The dependence of the phosphorescence intensity ratios on the rotation frequencies (*f*) was shown to obey the following expression (Krasnovsky 1979):

$$\ln\left(\frac{L_{dir}}{L_{rev}}\right) = (12 \cdot f \cdot \tau)^{-1} \quad (1)$$

where τ is the phosphorescence lifetime. The coefficient 12 is determined by the arrangement of the phosphoroscope windows and reflects the difference in duration of “dark” periods between the end of excitation phase and the

beginning of the measurement phase during the direct and the reverse phosphoroscope rotation.

Experimental conditions

Pigment phosphorescence was measured at 77 K using Chl *d* solutions frozen in the presence of air in special metal holders with optical path 5 mm. Under these conditions, the solutions formed white rigid snow. As shown previously, presence of air in frozen solvents does not influence the lifetime of the pigment triplet states because the viscosity of the frozen solvents is so high that quenching of triplets by dissolved oxygen does not occur (Krasnovsky et al. 1973, 1974, 1975). The absorbance of the samples in the sample holders at the maximum of the Chl *d* absorption spectra was about 0.6 at 77 K as estimated using a spectrophotometer equipped with the integrating sphere.

Singlet oxygen ($^1O_2$) sensitization by Chl *d* was measured at room temperature by monitoring $^1O_2$ phosphorescence at 1270 nm in the air-saturated pigment solutions using the same phosphoroscope set-ups, which were used for measurement of the Chl *d* phosphorescence. For these measurements, Chl *d* was dissolved in hexafluorobenzene, in which the singlet oxygen lifetime is known to be equal to 15–20 ms (see for refs Krasnovsky 1998, 2004).

Chl *d* was isolated from *A. marina* cells and purified according to the procedure described by Di Valentin et al. 2007. As solvents, diethyl ether (reagent grade, additionally purified by distillation), hexafluorobenzene (Piminvest, Moscow, 99.9%), and aqueous dispersions containing 2% detergent Triton X100 (Merck) were used. The aqueous systems were prepared by mixing water with the pigment solutions in ethanol. The final concentration of ethanol in aqueous detergent solutions was 5%.

Results

Figure 1 shows the fluorescence and phosphorescence emission spectra of Chl *d* dissolved in diethyl ether at 77 K. The maxima of fluorescence and phosphorescence spectra were observed at 699 $\pm$ 1 and 978 $\pm$ 2 nm, respectively. The half band width is $\sim$19 nm for the fluorescence and 67 $\pm$ 3 nm for the phosphorescence band ($\sim$750 cm^{-1}). The fluorescence spectrum shows also a weaker long-wavelength band at 765 nm, whereas the phosphorescence spectrum shows a weaker maximum at 1108 $\pm$ 3 nm, whose relative amplitude was about 1/3 compared to the main maximum at 978 nm. Similar fluorescence and phosphorescence emission spectra were obtained when Chl *d* was dissolved in aqueous dispersions of Triton X-100. The difference from the ether solutions resides in a moderate shift of the emission spectra (1–3 nm) toward longer wavelengths (Table 1).

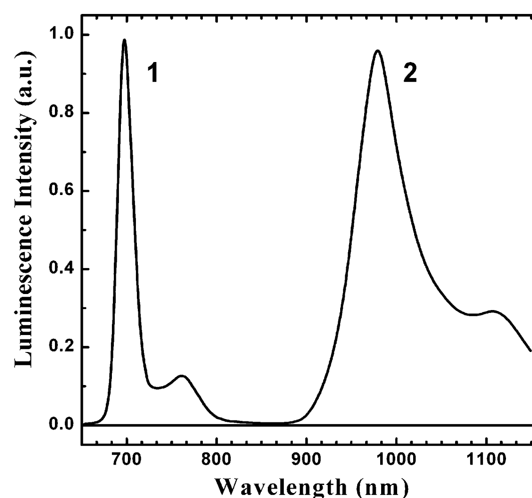

Fig. 1 Fluorescence (*1*) and phosphorescence (*2*) spectra of Chl *d* in diethyl ether at 77 K. Fluorescence was recorded with the monochromator slits corresponding to 1 nm upon excitation by monochromatic light 440 nm. Phosphorescence was excited by xenon lamp via the cut-off red glass transmitting at $\lambda > 670$ nm with the monochromator slits corresponding to 16 nm. The absorbance of the samples in the red band was <0.1 during fluorescence measurements and about 0.6 during phosphorescence measurements. The spectra are corrected for the spectral response of the detector

It should be noted that the intensity ratio of the main and long wavelength maxima in the phosphorescence spectra of Chl *d* coincides with that observed previously in solutions of Chl *a* and pheophytin (Pheo) *a* (Krasnovsky and Semenova 1981; Krasnovsky et al. 1990). The fluorescence spectra obtained in this study are similar to those reported at 77 K in methanol (Nieuwenburg et al. 2003) and at 1.8 K in tetrahydrofuran (Di Valentin et al. 2007).

Figure 2 shows the excitation spectrum of the Chl *d* phosphorescence. Because of the low intensity of the pigment phosphorescence, the excitation spectra were measured using relatively high Chl *d* absorbance and rather broad excitation band (12 nm) that caused an apparent smoothing of the excitation spectrum and a relative increase of the minor excitation bands. Nevertheless, the main maxima in the phosphorescence excitation spectra were found at 443 and 685 nm, in good agreement with the main absorption bands of the Chl *d* solutions at room temperature, which were observed at 445 and 686 nm. Figure 3 illustrates the dependence of $\ln(L_{dir}/L_{rev})$on the phosphoroscope rotation frequency *f*. Fitting of the experimental data to Eq. 1 results in the lifetime of Chl *d* phosphorescence close to 1 ms.

Table 1 presents a comparison of the phosphorescence parameters characterizing Chl *d* in diethyl ether and water-detergent dispersions with those of Chl *a* and Pheo *a*, which have been most carefully studied by Krasnovsky and

Table 1 Comparison of the phosphorescence parameters of Chl *d*, Chl *a*, and Pheo *a* in diethyl ether and aqueous detergent dispersions at 77 K

Pigments	Diethyl ether		Water + 2% TX-100		E_t^a, eV
	Maxima, nm (±3 nm)	Lifetimes, ms (±0.05 ms)	Maxima, nm (±3 nm)	Lifetimes, ms (±0.05 ms)	
Chl *d*	978, 1108	1.05	981, 1110	1.1	1.26
Chl *a*[b]	929, 1060	2.7	931, 1060	2.6	1.33
Pheo *a*[c]	932, 1060	1.0	931, 1060	1.0	1.33

[a] E_t is the energy corresponding to the main maxima of the pigment phosphorescence spectra

[b] Krasnovsky and Semenova (1981)

[c] Krasnovsky et al. (1990)

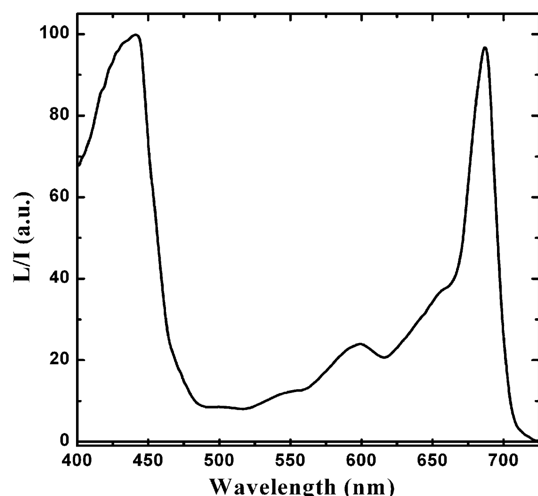

Fig. 2 Excitation spectrum of the Chl *d* phosphorescence in diethyl ether at 77 K. Phosphorescence was recorded through the cut-off infrared filter IKS-7 transmitting light at $\lambda > 880$ nm with the width of the monochromator slits corresponding to 12 nm. Absorbance of the sample was ~0.6 in the red absorption band. L/I is the ratio of the phosphorescence intensity to the excitation intensity

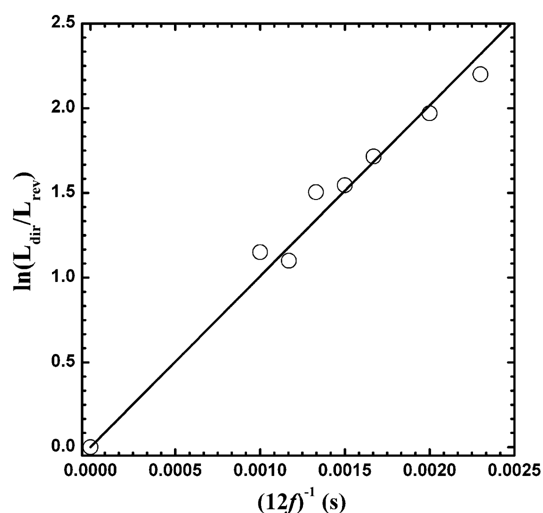

Fig. 3 Determination of the lifetime time of the Chl *d* phosphorescence at 77 K

Semenova (1981) and Krasnovsky et al. (1990). As shown previously, in diethyl ether and aqueous dispersions of Triton X100 frozen as snow, Chl *a* phosphorescence corresponds to the pigment molecules whose central magnesium atom is either penta- or tetra-coordinated, i.e., the central magnesium atom is axially coordinated by one solvent molecules, or not connected with solvent molecules at all (Krasnovsky 1982; Krasnovsky et al. 1975, 1977; Dvornikov et al. 1979; Losev et al. 1987). It is likely that Chl *d* coordination in these solvent systems (diethyl ether and Triton X-100 micelles) is analogous to that of Chl *a*, as also recently discussed based on the resonance Raman and fluorescence line narrowing experiments (Telfer et al. 2010) for the case diethyl ether solvation at low temperatures.

One can see that in the solvents investigated, the phosphorescence of Chl *d* is shifted ~50 nm to longer wavelengths compared to that of Chl *a* and Pheo *a*. The lifetime of the Chl *d* triplet is about three times less than the triplet lifetime of Chl *a* and it is equal to the lifetime of the Pheo *a* triplet state. The quantum yield of Chl *d* phosphorescence (Φ_P) was estimated by comparing with that of Pheo *a*, whose phosphorescence quantum yield is known to be $\Phi_P = (3 - 5) \times 10^{-5}$ (Krasnovsky et al. 1977; Dvornikov et al. 1979). The quantum yield of the Chl *d* phosphorescence in 2% Triton X100 aqueous dispersions was found to be 0.6 ± 0.1 of the Pheo phosphorescence yield in the same medium. Hence, for Chl *d*, $\Phi_P = (1.5 - 3.5) \times 10^{-5}$.

As listed in Table 1, the energy of the Chl *d* triplet state, 1.26 eV, is markedly higher than the energy of the singlet $^1\Delta_g$ state of molecular oxygen, which is known to correspond to 0.98 eV, i.e., ~1270 nm (see for refs Krasnovsky 1979; Krasnovsky 2004). Based on the energy and lifetime of the Chl *d* triplet state, one would expect efficient energy transfer from triplet Chl *d* to oxygen in air-saturated systems.

In accord with this assumption, millisecond phosphorescence of singlet oxygen at ~1270 nm was observed at room temperature upon irradiation of the air-saturated Chl *d* solutions in hexafluorobenzene (Fig. 4). The Chl *d* phosphorescence was not detected under these conditions because the pigment triplet states are strongly quenched by

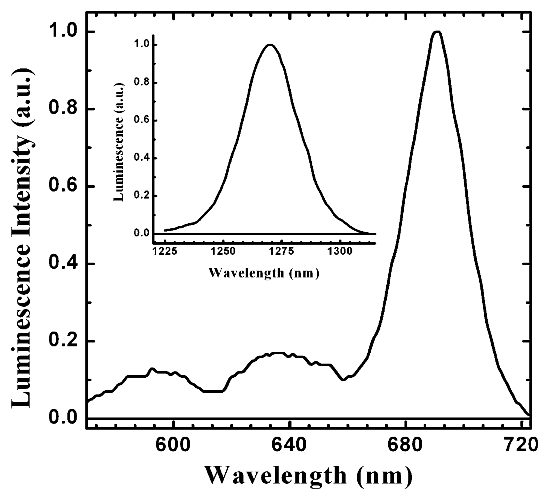

Fig. 4 Excitation and emission spectra (insert) of singlet oxygen phosphorescence in air-saturated solutions of Chl *d* in hexafluorobenzene at room temperature recorded using the mechanical phosphoroscopes. Absorbance of the Chl *d* solution was ~0.15 in the *red* absorption band. The spectra were recorded with the monochromator slits corresponding to 4 and 16 nm, respectively

dissolved oxygen that usually causes the reduction of the triplet lifetime to a few hundreds nanoseconds what is much below the phosphoroscope time resolution (Krasnovsky 1998, 2004 and refs therein). As Fig. 4 shows, the red maximum of the excitation spectrum (689 nm) of the singlet oxygen phosphorescence corresponds to the red absorption maximum of the Chl d solutions. The signal of singlet oxygen phosphorescence disappeared after addition of 15% acetone, which is known (Krasnovsky 1998) to decrease the singlet oxygen lifetime below time resolution of the phosphoroscope (500 μs). The quantum yield of singlet oxygen production (Φ_Δ) was estimated as described by Krasnovsky (1979). Tetraphenylporphyrin (TPP) was used as a reference dye, for which the most probable Φ_Δ value is 0.7 ± 0.05 (see for refs Krasnovsky et al. 1990; Krasnovsky 1998, 2004). The quantum yield of singlet oxygen generation by Chl *d* was found to be 0.93 ± 0.03 of the TPP singlet oxygen yield. Hence, $\Phi_\Delta = 0.65 \pm 0.06$ in the Chl *d* solution. This value is slightly higher than that obtained for Chl *a*, $\Phi_\Delta = 0.55 \pm 0.05$ (Krasnovsky et al. 1990; Krasnovsky 1979, 1994). Recently, we learned that photosensitized phosphorescence of singlet oxygen was also detected in Chl *d* solution in acetone (Okazaki et al. 2010). Thus, these data indicate that Chl *d* efficiently populates the triplet state upon photoexcitation and also efficiently photosensitizes singlet oxygen formation.

Conclusion

In this study, we described for the first time the phosphorescence, energy, and lifetime of the Chl *d* triplet state and demonstrate that Chl *d* is an efficient photosensitizer of singlet oxygen production in air-saturated solutions. The data indicate that the Chl *d* triplet state, like the triplet states of Chl *a* and Pheo *a*, might transfer energy to oxygen with singlet oxygen formation also under aerobic physiological conditions. This process can cause photooxidative stress and photoinhibition of the photosynthetic apparatus. More detailed studies to elucidate these issues are in progress.

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References

Aro E-M, Virgin I, Andersson B (1993) Photoinhibition of photosystem II. Inactivation protein damage and turnover. Biochim Biophys Acta 1143:113–134

Chen M, Zeng H, Larkum AW, Cai ZL (2004) Raman properties of chlorophyll *d*, the major pigment of *Acaryochloris marina*: studies using both Raman spectroscopy and density functional theory. Spectrochim Acta A 60:527–534

Di Valentin M, Ceola S, Agostini G, Telfer A, Barber J, Böhles F, Santabarbara S, Carbonera D (2007) The photo-excited triplet state of Chlorophyll *d* in methyl-tetrahydrofuran studied by optically detected magnetic resonance and time-resolved EPR. Mol Phys 105:2109–2117

Dvornikov SS, Knyukshto VN, Solovyov KN, Tsvirko MP (1979) Phosphorescence of chlorophylls and their pheophytins. Opt Spektr (URSS) 46:689–695

Hoff AJ (1986) Triplets: phosphorescence and magnetic resonance. In: Govindjee AmetzJ, Fork DC (eds) Light emission of plant and bacteria. Academic Press Inc, New York, pp 225–266

Holt AS, Morley HV (1959) A proposed structure for chlorophyll *d*. Can J Chem 37:507–514

Hu Q, Miyashita H, Iwasaki I, Kurano N, Miyachi S, Iwaki M, Itoh S (1998) A photosystem I reaction center driven by chlorophyll *d* in oxygenic photosynthesis. Proc Natl Acad Sci USA 95:13319–13323

Kleibeuker JF, Platenkamp RJ, Schaafsma TJ (1978) The triplet state of photosynthetic pigments. Chem Phys 27:51–64

Kobayashi M, Ohashi S, Iwamoto K, Shiraiwa Y, Kato Y, Watanabe T (2007) Redox potential of chlorophyll *d* in vitro. Biochim Biophys Acta 1767:596–602

Krasnovsky AA Jr (1979) Photoluminescence of singlet oxygen in pigment solutions. Photochem Photobiol 29:29–36

Krasnovsky AA Jr (1982) Delayed fluorescence and phosphorescence of plant pigments. Photochem Photobiol 36:733–741

Krasnovsky AA Jr (1994) Singlet oxygen and primary mechanisms of photooxidative damage of chloroplasts. Studies based on detection of oxygen and pigment phosphorescence. Proc Roy Soc Edinburgh 102B:219–235

Krasnovsky AA Jr (1998) Phosphorescence studies of singlet oxygen in photobiochemical systems. Membr Cell Biol 12:665–690

Krasnovsky AA Jr (2004) Singlet oxygen and photodynamic action. Biofizika 49:289–306

Krasnovsky AA Jr, Semenova AN (1981) Parameters of the triplet state and spectral properties of the monomeric chlorophyll in liposomes at −196°C. Photobiochem Photobiophys 3:11–18

Krasnovsky AA Jr, Romaniuk VA, Litvin FF (1973) On the phosphorescence and delayed fluorescence of chlorophylls and pheophytins *a* and *b*. Dokl AN SSSR (Biophysics) 209:965–968

Krasnovsky AA Jr, Lebedev NN, Litvin FF (1974) Spectral characteristics of phosphorescence of chlorophylls and pheophytins, Dokl. AN SSSR (Biophysics) 216:1406–1409

Krasnovsky AA Jr, Lebedev NN, Litvin FF (1975) Detection of the triplet state of chlorophyll and chlorophyll precursors from measurement of their delayed fluorescence and phosphorescence in leaves and chloroplasts. Dokl AN SSSR (Biophysics) 225:207–210

Krasnovsky AA Jr, Lebedev NN, Litvin FF (1977) Phosphorescence and delayed fluorescence of chlorophyll and its precursors in solutions, leaves and chloroplasts at 77 K. Studia Biophys B65:81–89

Krasnovsky AA Jr, Neverov KV, Egorov SY, Roeder B, Levald T (1990) Photophysical studies of pheophorbide *a* and pheophytin *a*: phosphorescence and photosensitized singlet oxygen luminescence. J Photochem Photobiol B Biol 5:245–254

Kühl M, Chen M, Ralph PJ, Schreiber U, Larkum AW (2005) Ecology: a niche for cyanobacteria containing chlorophyll *d*. Nature 433:820

Losev AP, Nichiporovich IN, Sagun EI, Vasilenok GD (1987) Detection of nonliganded forms in solutions of chlorophyll and protochlorophyll. Dokl AN BSSR 31:131–134

Manning WM, Strain HH (1943) Clorophyll *d*, a green pigment of red algae. J Biol Chem 151:1–19

Miyashita H, Ikemoto H, Kurano N, Adachi K, Chihara M, Miyachi S (1996) Chlorophyll *d* as a major pigment. Nature 383:402

Neverov KV, Krasnovsky AA Jr (2004) Phosphorescence analysis of the chlorophyll triplet states in preparations of PS 2. Biofizika 49:493–498

Neverov KV, Shalygo NY, Averina NG, Krasnovsky AA Jr (1996) Formation of the pigment triplet states in green plant leaves treated with chelators of iron. Russ J Plant Physiol 43:89–99

Niedzwiedzki DM, Blankenship RE (2010) Singlet and triplet excited state properties of natural chlorophylls and bacteriochlorophylls. Photosynth Res 106:227–238

Nieuwenburg P, Clarke RJ, Cai ZL, Chen M, Larkum AW, Cabral NM, Ghiggino KP, Reimers JR (2003) Examination of the photophysical processes of chlorophyll *d* leading to a clarification of proposed uphill energy transfer processes in cells of *Acaryochloris marina*. Photochem Photobiol 77:628–637

Okazaki S, Tomo T, Mimuro M (2010) Direct measurement of singlet oxygen produced by four-ringed chlorophyll species in acetone solution. Chem Phys Lett 485:202–206

Santabarbara S, Chen M, Larkum AW, Evans MCW (2007) An electron paramagnetic resonance investigation of the electron transfer reactions in the chlorophyll *d* containing photosystem I of *Acaryochloris marina*. FEBS Lett 581:1567–1571

Schenderlein M, Çetin M, Barber J, Telfer A, Schlodder E (2008) Spectroscopic studies of the chlorophyll *d* containing photosystem I from the cyanobacterium, *Acaryochloris marina*. Biochim Biophys Acta 1777:1400–1408

Schlodder E, Çetin M, Eckertet H-J, Scmittt F-J, Barber J, Telfer A (2007) Both chlorophyll *a* and *d* are essential for the photochemistry in photosystem II of the cyanobacteria Acaryochloris marina. Biochim Biophys Acta Bioenerg 1767:589–595

Takiff L, Boxer SG (1988) Phosphorescence spectra of bacteriochlorophylls. J Am Chem Soc 110:4425–4426

Telfer A, Pascal AA, Bordes L, Barber J, Robert B (2010) Fluorescence line narrowing studies on isolated chlorophyll molecules. J Phys Chem B 114:2255–2260

Tomo T, Okubo T, Akimoto S, Yokono M, Miyashita H, Tsuchiya T, Noguchi T, Mimuro M (2007) Identification of the special pair of photosystem II in a chlorophyll *d*-dominated cyanobacterium. Proc Natl Acad Sci USA 104:7283–7288