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Effects of heavy central metal on the ground and excited states of chlorophyll

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Abstract Chlorophylls, owing to their adjustable π -electron system and intense, well-separated electronic transitions, can serve as convenient intrinsic spectroscopic probes of ligand–metal center interactions. They are also interesting for their photosensitizing properties. In order to examine the heavy-atom effects on the chlorophyll triplet state, a key intermediate in chlorophyll–photosensitized reactions, the synthesis of a novel Pt(II)-substituted chlorophyll a was carried out, and the effects of the substitution on steady-state and transient photophysical properties of chlorophyll were studied by absorption and fluorescence spectroscopies, and by laser flash photolysis. The presence of highly electronegative platinum as the central ion increases the energies of the chlorophyll main absorption transitions. As laser flash photolysis experiments show, in air-equilibrated solutions, chlorophyll triplets are efficiently quenched by molecular oxygen. Interestingly, this quenching by oxygen is more effective with metal-containing pigments, in spite of the increased spin–orbit coupling, introduced with the central metals. This points to occurrence of nonspecific interactions of molecular oxygen with metallochlorophylls. The differences in the effects exerted on the pigment triplet by the central metal become distinct after the removal of oxygen. The lifetime of a Pt-chlorophyll triplet remains very short, in the range of only a few microseconds, unlike in the free-base and

Mg- and Zn-substituted chlorophylls. Such drastic shortening of the triplet lifetime can be attributed to a large heavy-atom effect, implying that strong interactions must occur between the central Pt(II) ion and the chlorophyll macrocycle, which lead to a more efficient spin–orbit coupling in Pt-chlorophyll than in Pt-porphyrins.

Keywords Metallochlorophylls · Triplet state · Heavy-atom effect · Central metal effect · Platinum

Abbreviations BChl: Bacteriochlorophyll · BChla: Bacteriochlorophyll a · Chl: Chlorophyll · Chla: Chlorophyll a · DEAE: (Diethylamino)ethyl · F₂₈TPP: Perfluorinated tetraphenylporphyrin · HP: Hematoporphyrin · HPLC: High-performance liquid chromatography · ISC: Intersystem crossing · Mg-Chla: Magnesium-substituted chlorophyll a · PDT: Photodynamic therapy · Pheo: Pheophytin a · Pt-Chla: Platinum-substituted chlorophyll a · TPP: Tetraphenylporphyrin · Zn-Chla: Zinc-substituted chlorophyll a

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Introduction

The presence of metal ions is crucial to the functioning of many biological systems. For instance, metal ions directly participate in catalytic processes and have regulatory functions or act as signal transporters. Natural and synthetic metalloporphyrins are perhaps the most evident example of a chelating system, in which the type of (central) metal ion and its interactions with the environment (e.g., protein) determine the direction of processes taking place. If excited states of the complex are relevant to the functioning, the light elements are coordinated by the tetrapyrrole ligand. They do not affect the rates of intersystem crossing (ISC) from the ligand excited singlet state and, hence, triplet-state formation does not compete with singlet–singlet inter-

actions or its photochemistry. In consequence, the key photosynthetic pigments, chlorophylls (Chls; the structure of chlorophyll a, Chla, is shown in Fig. 1), bind Mg^{2+} (in rare cases also Zn^{2+}) as the central ion, light and moderately heavy elements with a simple frontier ns^2 electronic configuration. This permits the complex to retain both photochemical and redox activity. Therefore, owing to such a versatile design, a single type of pigment has the capacity to participate either in energy absorption and transfer (light harvesting) or in electron transfer (charge separation) [1].

The situation becomes less simple when tetrapyrroles chelate transition metal ions, which contribute their d electrons and interfere with the ligand π -electron system. When ions such as Fe^{2+} , Cu^{2+} , Ni^{2+} and Co^{2+} (d^6 , d^7 , d^8 and d^9 , respectively) are bound in the central pocket, the photochemical activity pathways of the complex can be out-competed. One extreme case of such metal-ion-controlled photoactivity is represented by porphyrins chelating Ni^{2+} . For instance, in the Ni-substituted bacteriochlorophyll (BChl), the excited-state lifetime is shortened to less than 40 fs, owing to an extreme enhancement of internal conversion [2, 3]. Nevertheless, the photochemically deactivated metalloporphyrins often retain high redox activity and/or the central ions are also able to participate in various (bio)chemical processes. In the heme, the affinity of the central Fe to small molecules (O_2 , CO and NO) is greatly enhanced; the heme binding centers serve in vivo as signal transporters and as the most important oxygen carriers; the hemoproteins also play a key role in the biological electron transfer [4, 5].

The nuclei of heavy central ions introduce another type of interaction, the so-called heavy-atom effect. This interaction, via the enhancement of spin-orbit coupling,

causes a mixing of states of differing spin multiplicity, and thus promotes otherwise formally spin-forbidden transitions. In such complexes, it affects the rates of ISC from an excited singlet state to a triplet state, and relaxation of the latter to the ground singlet state, leading to changes in the dynamics and yields of the formation and decay of triplet states [6, 7]. Here, Pt(II)-porphyrins are a representative example, which remain highly phosphorescent even at ambient temperature (triplet population approaches 100%) and hence they have found applications as organic light-emitting devices and optical sensors of in vivo oxygen [8–10].

Triplet formation, rather unwanted in biological systems, is of key relevance for singlet oxygen generation in applications for therapeutic purposes, such as photodynamic therapy (PDT) [11–13]. In that context, metal complexes of porphyrins and Chls have become increasingly attractive as second- and third-generation photosensitizers [14–22]. They are characterized by an exceptional electronic structure, which is prone to modulations and provides a means of controlling the relevant parameters, e.g., the absorption spectrum, to suit better the so-called therapeutic window (tissue penetration by light), triplet-state formation and singlet oxygen yields, and phototoxicity [23]. According to these criteria, chemical modifications of naturally occurring Chls via central metal substitution (Fig. 1) provide an interesting option, as recently shown for a water-soluble Pd-substituted derivative of BChl (TOOKAD) [20, 24–26].

The efficient formation of a photosensitizer triplet state of appropriate energy is a prerequisite for promoting the photodynamic effect according to the type II mechanism (via singlet oxygen) [13]. Therefore, when attempting to develop new photosensitizers, knowledge of factors determining triplet-state properties becomes critical. The triplet state of Chls has long been a subject of numerous studies, first of all, in relation to its possible participation in primary photosynthesis [27]; also the effects of moderately heavy central metals (Zn and Cd) on the Chl triplets have been investigated [28, 29]. More recently, triplet formation in water-soluble Chl derivatives has been investigated in relation to PDT [30]. However, the effects of still heavier atoms on the triplet state of Chl have not been investigated in detail. Therefore, we set out to obtain a Pt-substituted Chla (Pt-Chla), addressing the question of how the introduction of such a heavy element (atomic number $Z = 78$) and a drastic increase in the value of the spin-orbit ($L-S$) coupling parameter ξ (from 40 cm^{-1} in Mg to $4,481 \text{ cm}^{-1}$ in Pt) affect Chl, in particular the properties of its triplet state. The properties of the ground state of Pt-Chla were determined using steady-state absorption and fluorescence techniques. In order to determine the triplet state absorption spectra and lifetimes of magnesium-substituted Chla (Mg-Chla), zinc-substituted Chla (Zn-Chla), platinum-substituted Chla (Pt-Chla), and metal-depleted Chla (pheophytin a, Pheo) in solution, the laser flash photolysis technique was applied. The

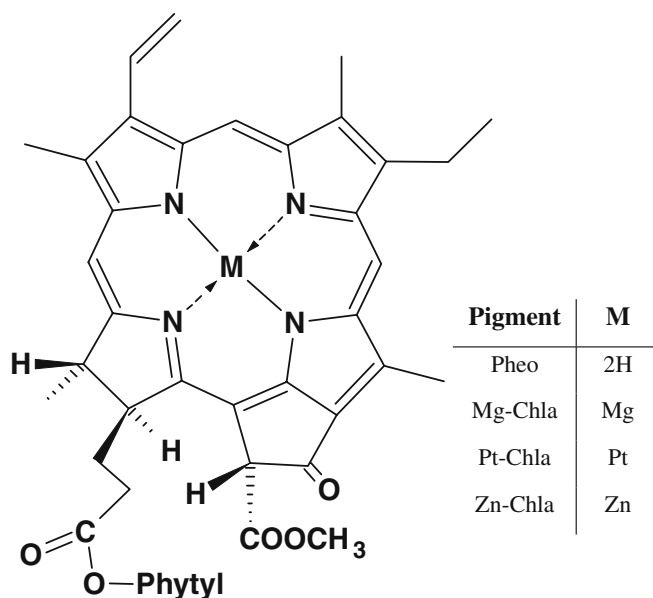


Fig. 1 Structural formulae of chlorophyll a (Chla) and its derivatives used in the present studies

effects of the Pt central ion are compared with those exerted by this ion on other porphyrins.

Materials and methods

Pigment preparation

Chla was isolated from the cells of cyanobacterium *Spirulina laporte*, obtained from the Culture Collection of Autotrophic Organisms in Trebon (Czech Republic), using selective precipitation, according to Iriyama et al. [31]. The pigment was purified first by column chromatography on (diethylamino)ethyl (DEAE)-Sephadex, following the method of Omata and Murata [32], and then by high-performance liquid chromatography (HPLC) on silica gel [33].

Pheo was prepared from pure Chla by demetalation in glacial acetic acid. The product was purified by column chromatography on DEAE-Sephadex as described before.

The synthesis of Zn-Chla was done via a direct metalation of Pheo with zinc acetate (analytical grade; POCh, Gliwice, Poland) in methanol at 40°C, under an N₂ atmosphere. The crude product was separated by flash chromatography on silica gel (elution with 20% acetone in chloroform). The final purification was done by column chromatography on DEAE-Sephadex [32].

Pt-Chla was synthesized by a direct insertion of Pt²⁺ into Pheo and the product was purified as described by Fiedor et al. [34]. The metalation was achieved by refluxing Pheo in glacial acetic acid with tenfold excess of K₂[PtCl₄] for 20 min at 80°C. After removing the solvent in a stream of N₂, the product was isolated by column chromatography on DEAE-Sephadex CL-6B (Pharmacia), using 5% MeOH in acetone (v/v) as the eluent. The identity of the product, Pt-Chla, was confirmed by mass spectroscopy (electrospray ionization technique, Finnigan 95S). The estimated molecular mass was 1,065 (*m/z*); the predicted molecular mass was 1,064.3.

Electronic absorption/fluorescence spectroscopy

The electronic absorption spectra were recorded with a Cary 50 Bio spectrophotometer (Varian, Mulgrave, USA). The measurements were done in 10-mm-pathlength quartz cuvettes at room temperature. The steady-state fluorescence measurements were done at room temperature, in 10-mm-pathlength quartz cuvettes, with an LS 50 spectrofluorimeter (PerkinElmer, Beaconsfield, UK). The samples were excited at 405 nm.

Laser flash photolysis

The flash photolysis experiments were carried out with a system comprising an LKS60 spectrometer (Applied Photophysics, Leatherhead, UK) and a SURELITE I-10

Nd:YAG laser (Continuum, USA), as described previously [35]. Spectral changes were monitored in the range between 250 and 750 nm, applying 10-nm increments. The measurements were done in 10-mm-pathlength quartz cuvettes at 20°C. At least six kinetics runs were recorded under each set of conditions and the data presented comprise their mean values. The fitting of the experimental data was performed using the software supplied by the instrument manufacturer.

The solutions of the pigments were prepared freshly before the measurements; the optical density of the samples was kept between 0.25 and 0.40 at the excitation wavelength (355 nm). To confirm the samples' integrity during measurement, their absorption spectra were compared before and after each flash photolysis experiment. The samples were stable to about 1,000 laser flashes, showing less than 10% degradation, with only Pt-Chla being more labile, showing 15% degradation.

For the photolysis experiments in the presence of oxygen, solvents in equilibrium with air at 20°C were used. In order to remove oxygen from the samples, the solutions were purged with argon, until no change in the signal decay rate was observed. The solutions were kept under argon also during the measurements.

Solvents

All solvents used during the preparations of pigments were of analytical grade (POCh); *n*-hexane and 2-propanol were degassed and filtered prior to use in HPLC. Acetone, methanol and toluene applied in the laser flash photolysis measurements were of spectroscopic grade (Uvasol Merck, Darmstadt, Germany), and pyridine was of analytical grade (Merck).

Results and discussion

The effects and functioning of biologically important metal ions have been studied with an extensive use of metalloporphyrins, owing to the advantageous properties of their π -electron system and the ease of applying spectroscopic techniques. In terms of studying the ligand-metal center interactions, chlorins, and in particular bacteriochlorins, provide a very convenient model system. Their intense optical transitions, in the region between 350 and 800 nm, are well resolved and show a high sensitivity to the centrally chelated metal ion, including its coordination sphere [36–38]. Recent studies on metallosubstituted BChls have already shown the usefulness of these pigments as probes for monitoring interactions of metal ions with ligands of biological importance [39, 40].

A similar approach was applied in the present studies, aimed at probing the heavy-atom effects on the π -electron system of Chl in a newly synthesized Pt-derivative of Chla. The insertion of Pt(II) ion into the Chla macrocycle was confirmed by the molecular mass

measurement (experimental m/z value 1,065 versus calculated 1,064.3) and through specific changes induced in the spectroscopic properties of this ligand, which are discussed in detail later. The structural formulae of Chla and its three derivatives Pheo, Zn-Chla and Pt-Chla are shown in Fig. 1. The four pigments differ with respect to the ligands of the central nitrogens; in Pheo (demetallated Chla is equivalent to free base) two protons are present; in the metallosubstituted pigments the following ions are ligated: Mg^{2+} (24.3 au), Zn^{2+} (65.4 au) and Pt^{2+} (195.1 au). The three metals differ greatly in terms of the spin-orbit coupling parameter ξ , whose value spans over 2 orders of magnitude: from 40 cm^{-1} for Mg and 390 cm^{-1} for Zn, to as much as 4,481 cm^{-1} for Pt [7]. The electronegativities of these central metals also differ considerably: 1.3 for Mg, 1.7 for Zn and 2.3 for Pt (according to Pauling's scale [41]).

Steady-state absorption and fluorescence spectra

The ground-state absorption spectra of the four pigments, recorded in methanol at room temperature, are presented in Fig. 2. The spectra show two intense transitions, characteristic of chlorin absorption: a broad Soret band near 400 nm and a narrower Q_y band at the red edge of the visible region. In between the two transitions are located several weaker and somewhat overlapping bands. The relative intensities of the two major transitions vary, depending on the species bound into the central pocket; the Q_y band becomes weaker in Zn-Chla and in particular in Pheo, while its intensity in Pt-Chla is comparable to that in Mg-Chla. In other solvents (acetone, pyridine and toluene), except for some shifts in the positions of the main absorption bands (listed in

Table 1), no major changes occur in the spectra of the four pigments (not shown).

There is a clear solvent effect on the absorption spectrum of Pt-Chla, as in pyridine, the Soret transition is redshifted by 1,000 cm^{-1} , relative to its position in acetone; the corresponding shift (pyridine versus acetone) of the Q_y band amounts to 111 cm^{-1} (Table 1). However, in spite of the fact that pyridine is a strongly coordinating agent [42], these solvent effects on the spectrum of Pt-Chla are not necessarily indicative of changes in the metal coordination state. Quite similar solvent shifts also occur in Pheo (Table 1) [43], and, moreover, the Pt^{2+} ion in square-planar complexes is quite unlikely to accept axial ligands [44].

In porphyrins, the energy levels of the electronic transitions are determined to a large extent by electrostatic interactions between the N-centered π -electron densities and the metal center, and hence the effect of the central metal is related to its electronegativity [45, 46]. Electronic transitions of metallosubstituted BChls and Chls are also affected by central metal substitution [39, 47]. For instance, the presence of the central Zn^{2+} ion in Chla causes 5–6-nm blueshifts of the Soret and Q_y transitions (Fig. 2, Table 1), in agreement with previous studies [47, 48]. The insertion of highly electronegative Pt into the Chla macrocycle has a more substantial effect on the absorption spectrum: both major electronic transitions are blueshifted by approximately 30 nm (Fig. 2, Table 1). In similar complexes of Pd with Chla and bacteriochlorophyll a (BChla) [39, 48], the absorption bands also undergo comparable blueshifts, which (in BChla) has been explained as originating from the electrostatic attraction of the π electrons to the metal center [49]. The electronegativity of Pt is as high as that of Pd (2.2) and, therefore, a similar origin of the spectral shifts in Pt-Chla can be assumed [47].

The relationship between the central metal electronegativity and the band energies in Chla is confirmed by linearity of the plot of the Soret band energy versus the Q_y energy in several metallosubstituted Chls (Mg, Zn, Cu, Ni and Pt complexes, Fig. 3). The plot has a slope of nearly unity, indicating not only that various central metal ions affect the two electronic transitions in Chla in a very similar way (constant degree of configuration interaction) but also that the substitution does not lead to major structural changes [50]. Also in porphyrins and metallosubstituted (unreduced) chlorins, the planarity of the tetrapyrrole macrocycle is not significantly perturbed by the insertion of heavy-metal ions [50].

Weak structural effects in metallosubstituted Chls would be in line with the increased rigidity of the Chl macrocycle, caused by the presence of the isocyclic ring. Unfortunately, in the absence of crystal structural data on Chla and metallosubstituted Chla, the contribution of conformationally induced spectral shifts cannot be conclusively estimated at present.

It is interesting to compare the effects of the Pt central ion on the ligand ground-state properties in Chl with those in less reduced porphyrins, e.g., by comparing the

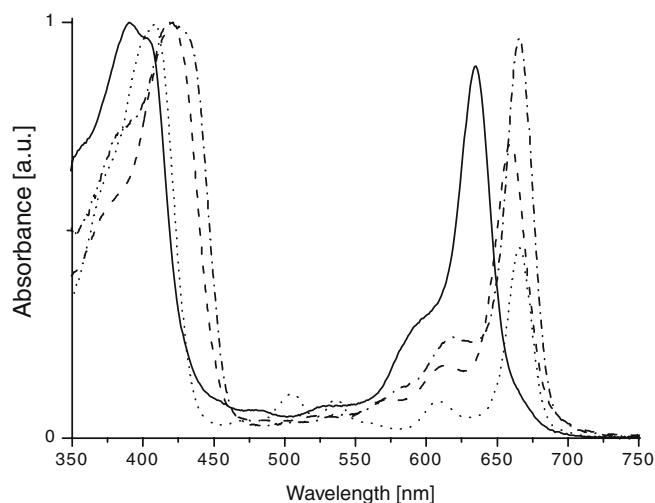


Fig. 2 Steady-state electronic absorption spectra of Chla and its derivatives in methanol; pheophytin a (*Pheo*) (dotted line), magnesium-substituted Chla (*Mg-Chla*) (dash-dotted line), zinc-substituted Chla (*Zn-Chla*) (dashed line) and platinum-substituted Chla (*Pt-Chla*) (solid line)

Table 1 Energies of the main absorption bands of free-base chlorophyll a (pheophytin) and its metalloderivatives in organic solvents

Solvent	Free base		Mg-Chla		Zn-Chla		Pt-Chla	
	Soret	Q _y	Soret	Q _y	Soret	Q _y	Soret	Q _y
Acetone	24,450* (409)	15,015 (666)	24,272 (412)	15,129 (661)	23,753 (421)	15,244 (656)	25,510 (392)	15,760 (634)
Methanol	24,510 (408)	15,015 (666)	23,697 (422)	15,037 (665)	23,866 (419)	15,220 (657)	25,445 (393)	15,736 (636)
Pyridine	24,096 (415)	14,925 (669)	22,936 (436)	14,914 (671)	23,256 (430)	15,128 (661)	24,510 (408)	15,649 (639)
Toluene	24,154 (414)	14,903 (671)	23,529 (425)	15,060 (664)	23,474 (426)	15,198 (658)	24,480 (409)	15,662 (638)

*Energy values in reciprocal centimeters. Values in parentheses are given in nanometers

shifts in their absorption maxima, associated with the insertion of the Pt ion. The energies of the main absorption bands of several free-base tetrapyrroles and their Pt(II) complexes, found in the literature [50–52] and obtained in this work, are compiled in Table 2. Among tetraphenylporphyrin (TPP), hematoporphyrin (HP), perfluorinated TPP (F₂₈TPP), chlorin and Chla, the largest differences between the free and the complexed ligand are induced in the ground-state spectra of Chla and TPP, with the Soret transition being most affected (blueshifted by 1,485 and 1,072 cm⁻¹, respectively). In HP and a heavily substituted F₂₈TPP, the corresponding shifts are somewhat smaller (700–800 cm⁻¹), while only a small opposite (red) shift is induced by Pt in chlorin. Equally significant differences are seen in the effects of the central Pt on the position of the Q transition. It amounts to 733 and 338 cm⁻¹ of a blueshift in Chla and TPP, respectively, an opposite (red) 240 cm⁻¹ shift in HP, and practically to no change of the band position for chlorin.

The previous analysis shows that the effects exerted by the central Pt ion on the energies of ligand frontier

orbitals widely vary, even in structurally closely related ligands (tetrapyrroles). Depending on the details of the tetrapyrrole structure, the central metal induces opposite effects (redshift or blueshift) on electronic transitions of the same origin. Apparently, in the previously described series of tetrapyrrolic ligands, the π -electron system of Chla seems to be more easily influenced by the central metal. A similar sensitivity to the central metal is observed in metallosubstituted BChls [39].

Most probably, it is the a_{2u} molecular orbital which is affected by these interactions more in BChla and Chla than in less reduced porphyrins. It is thought to be localized on all four central nitrogens and its energy is the main factor which determines the energy of both the Soret (B_y) and the Q_y transitions [39]. In porphyrins, the highest occupied molecular orbital is degenerate, being composed of two orbitals, the a_{2u} and the a_{1u} (with no electron density on N), and therefore the contribution of the former to the energies of the electronic transitions is diminished. This degeneracy is removed in reduced porphyrins and the energies of the a_{1u} and $e_{g,y}$ orbitals rise, leading to a better separation of optical transitions, as in BChls and Chls [53]. It seems that the differences in the contributions from the a_{1u}/a_{2u} orbitals are amplified when atoms with higher charge densities (Pd, Pt, etc.) interact with the four nitrogens forming the central pocket. In that respect, somewhat surprising is a relatively weak heavy-atom effect on the electronic transitions in metallochlorins. A partial loss of the molecular D_{4h} symmetry renders them an intermediate case between porphyrins and Chls, but apparently, here the central atom interacts strongly also with the $e_{g,x}$ and $e_{g,y}$ orbitals (which reside on two pyrrolic nitrogens and partly contribute to the B/Q transitions) and thus the shifts of the a_{2u} orbital energy are compensated.

The emission properties of Chla, as expected, are drastically affected by the heavy-metal effect in the presence of the Pt²⁺ ion in the central pocket. As seen in Fig. 4, it causes a complete quenching of the fluorescence from Chl (the low-intensity signal, on the basis of its position at 672 nm, can be attributed to residual Pheo). Taking into account the high value of the spin-orbit coupling parameter ζ and in analogy to the effects of other heavy central metals [7], also in the case of Pt one would expect an efficient ISC, resulting, e.g., in a high yield of the triplet state in Pt-Chla. The complete loss of fluorescence might be such an indication of efficient ISC in Pt-Chla, which should be reflected as well in

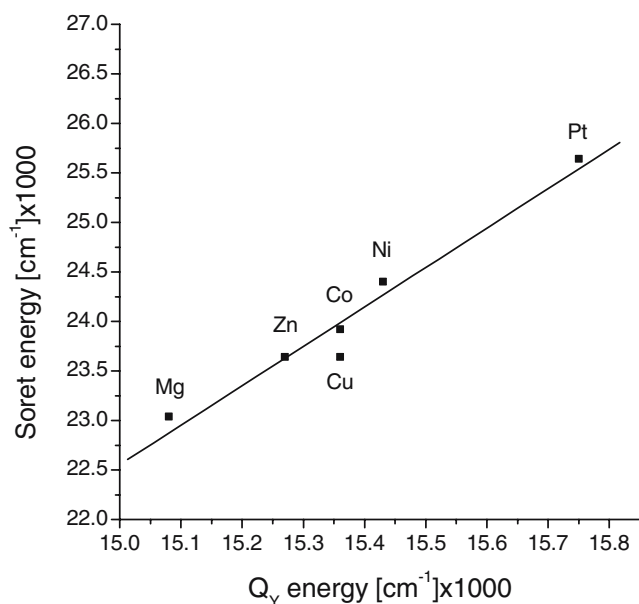


Fig. 3 Changes of the Soret transition energy as a function of the Q_y transition energy in a series of metallosubstituted chlorophylls; the data for the Co-substituted, Cu-substituted and Ni-substituted derivatives were taken from Ref. [48]

Table 2 Comparison of energies of the main absorption bands of various tetrapyrrolic ligands and their complexes with Pt(II) ion

Ligand	Free base		Pt-coordinated		Δ_{Soret} (cm ⁻¹)	Δ_{Q} (cm ⁻¹)	Reference
	Soret band	Q band	Soret band	Q band			
TPP	23,866* (419)	18,215 (549)	24,938 (401)	18,553 (539)	1,072	338	[66]
F ₂₈ TPP	25,510 (392)	—	26,178 (382)	18,762 (533)	668	—	[52]
HP	24,938 (401)	18,692 (535)	25,773 (388)	18,450 (542)	835	-242	[67]
Chlorin	25,000 (400)	16,474 (607)	24,814 (403)	16,556 (604)	-186	82	[50]
Pheo	24,156 (414)	15,015 (666)	25,641 (390)	15,748 (635)	1,485	733	This work

*Energy values in reciprocal centimeters. Values in parentheses are given in nanometers.

TPP tetraphenylporphyrin, F₂₈TPP perfluorinated tetraphenylporphyrin, HP hematoporphyrin

the increase of phosphorescence intensity in that complex, expected to be seen in the near IR [54–56], which extends, unfortunately, beyond our detection range.

Transient absorption spectra

The kinetics of the transient absorption changes (ΔA) of the four metallochlorophylls in solution were determined by laser flash photolysis. Laser flashes (6 ns, $\lambda = 355$ nm) were applied to produce excited states of pigments by excitation to the Soret band and the transients were recorded in a time span from several hundreds of nanoseconds to several hundreds of microseconds. The spectra were constructed point by point from the sets of the decay curves, collected at individual wavelengths between 270 and 750 nm with 10-nm intervals, covering major absorptions of Chla.

A representative example of the evolution in time of the transient spectra of the four Chla derivatives in Ar-saturated methanol is shown in Fig. 5. In all cases, the

transient absorption profiles show similar features; strong negative signals are seen in the regions coinciding with the maxima of ground-state absorption (i.e., the Soret and Q_y transitions). In the remaining parts of the spectral range, a broad positive absorption is seen, peaking near 440 nm. In each case, the transient absorption profiles cross at a zero value of ΔA , forming four distinct isosbestic points (Fig. 5). Analogous laser flash photolysis experiments, performed with the same four pigments in other solvents, acetone, pyridine and toluene, showed very similar spectral characteristics (not presented).

In the case of Pheo, Chla and Zn-Chla, the transient features, both negative and positive, decayed within 200 μ s after the excitation, regardless of the solvent used, while the transients observed for Pt-Chla showed much shorter lifetimes and decayed completely after less than 20 μ s, again showing no significant solvent dependence.

Similar transient spectral features were observed in the laser photolysis experiments performed with Chla derivatives in air-equilibrated solutions (not shown), but in that case much faster kinetics, by at least 2 orders of magnitude, was determined (Table 3). Among the four pigments, no significant differences in the transients' lifetimes were found, apparently owing to quenching by oxygen. This strong dependence of the kinetics on the presence of oxygen allows us to attribute the transient spectra to triplet–triplet absorption. This is further confirmed by the good agreement of the transiently recorded spectra with the T₁ state absorption, obtained for Chla in organic solvents in earlier flash photolysis experiments [30, 57, 58].

The shapes of the transient spectra and the presence of well-defined isosbestic points, together with the identity of the decay rates (Table 3), as determined individually for the negative and positive signals, all indicate that following sample excitation by laser flashes only two spectrally distinct species appear, which interchange with one another. Apparently, the spectral changes correspond to a relaxation of the pigment T₁ state, leading to a gradual recovery of the ground state. The observed transient spectra result from the overlap of ground-state bleaching and T₁ absorption.

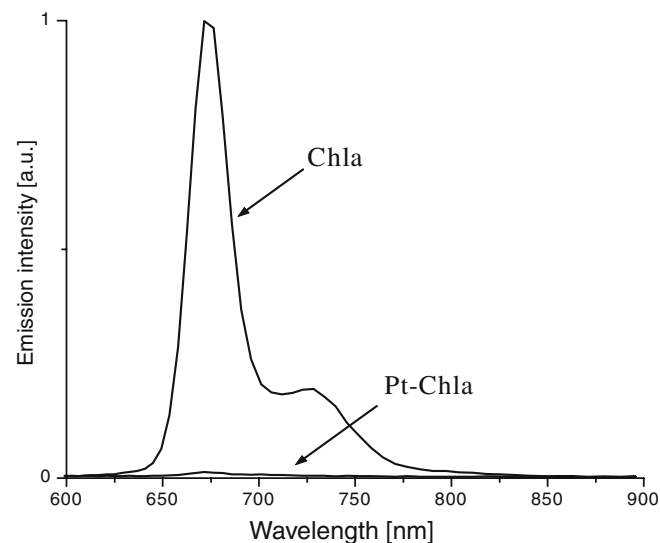
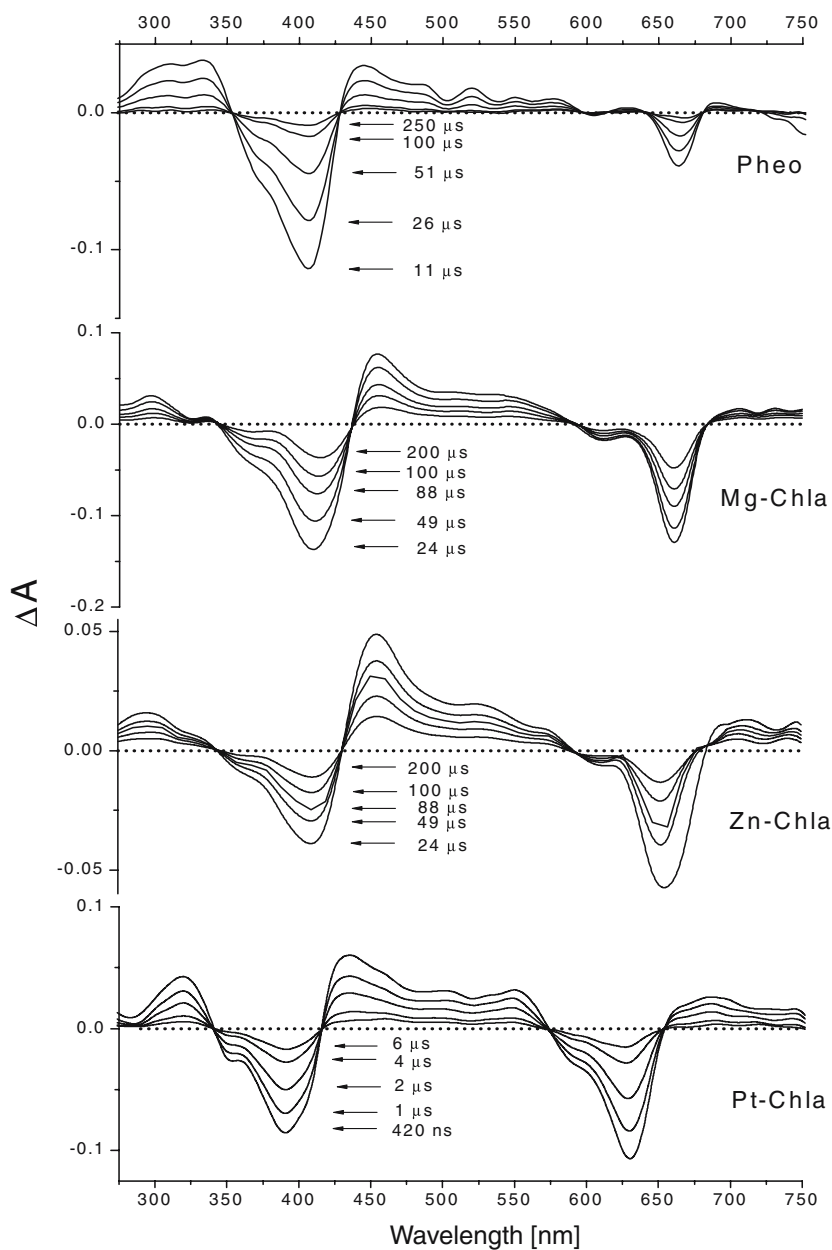


Fig. 4 Fluorescence spectra of Chla and Pt-Chla in acetone, normalized to match the absorbances at the excitation wavelength (405 nm)

Fig. 5 Transient absorption spectra (differential) of Chla and its derivatives in Ar-saturated methanol, recorded at room temperature in laser flash photolysis experiment (see text for details)



Triplet-state dynamics

The decay rates of T_1 states of Chla and its derivatives, determined in the present studies, are listed in Table 3. Their values, estimated independently at several wavelengths near the maximal signals in the transient spectra, are uniform across the monitored spectral range, as a narrow distribution of their values was obtained ($\pm 7\%$). In the presence of oxygen, the triplets of all pigments are very short lived, owing to efficient quenching. They decay within 150–270 ns, showing only weak solvent dependence. The longer values (355–549 ns) of the triplet lifetimes in pyridine are in line with the diffusion-controlled quenching [59], slowed down in more viscous solvents ($\eta = 0.925 \times 10^{-3}$ Pa·s for pyridine versus 0.303×10^{-3} Pa·s for acetone,

0.593×10^{-3} Pa·s for methanol, 0.586×10^{-3} Pa·s for toluene, all at 20°C) [7].

After the removal of oxygen, the triplet lifetimes, with the remarkable exception of the Pt derivative, extend by approximately 3 orders of magnitude, reaching almost 0.1 ms in pyridine (Table 3). These values compare reasonably well with the ones previously obtained for the Chla T_1 state under cryogenic conditions [28, 30]. This can be taken as a good indication that, at ambient temperatures, only residual oxygen is present in the Ar-saturated solutions.

As seen from the kinetics of transient absorption decay (Fig. 6, Table 3), the oxygen effect is much less pronounced in the case of Pt-Chla. After saturation with Ar, the lifetime of the Pt-Chla T_1 state is extended merely by tenfold, regardless of the solvent applied.

Table 3 The values (with their mean deviation) of triplet lifetimes of Chla and its three derivatives, determined from the fitting to the transient signal decay curves, obtained by laser flash photolysis at room temperature in organic solvents

Pigment	Acetone		Methanol		Pyridine		Toluene	
	Air τ_T (ns)	Ar τ_T (μ s)	Air τ_T (ns)	Ar τ_T (μ s)	Air τ_T (ns)	Ar τ_T (μ s)	Air τ_T (ns)	Ar τ_T (μ s)
Pheo	161 \pm 2	34.26 \pm 0.49	150 \pm 7	40.56 \pm 0.48	372 \pm 7	70.15 \pm 1.67	200 \pm 1	42.52 \pm 0.42
Mg-Chla	208 \pm 4	39.76 \pm 0.49	174 \pm 7	70.10 \pm 1.51	355 \pm 5	69.57 \pm 1.87	268 \pm 1	66.12 \pm 1.27
Zn-Chla	180 \pm 5	43.48 \pm 0.44	180 \pm 3	66.69 \pm 4.96	366 \pm 2	86.39 \pm 0.40	273 \pm 2	35.37 \pm 0.48
Pt-Chla	202 \pm 6	3.55 \pm 0.03	198 \pm 5	2.04 \pm 0.24	549 \pm 3	4.08 \pm 0.10	192 \pm 3	4.33 \pm 0.16

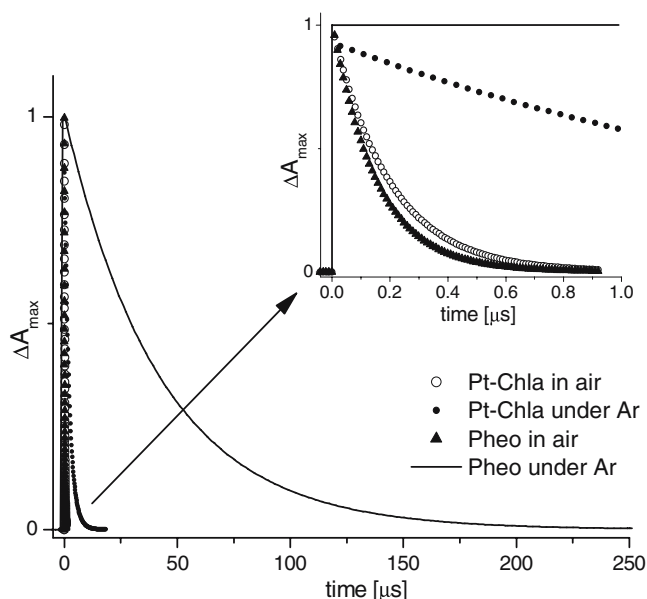
The kinetics of the transient signals were monitored at the Q_y absorption maximum of each pigment

Effects of Mg and Zn

In a simplified view, the mixing of the singlet and triplet states, induced by magnetic interactions of electrons with heavy nuclei, is described by the following interaction operator:

$$H_{SO} = \xi \mathbf{L} \cdot \mathbf{S}, \quad (1)$$

where ξ is the spin–orbit coupling constant, and \mathbf{L} and \mathbf{S} are, respectively, the orbital and spin angular momenta. In isolated atoms, the spin–orbit coupling constant is expected to be proportional to the atomic number Z to the power of 4 ($\xi \approx Z^4$). However, even in simple organic molecules this relationship depends on several factors and hence becomes difficult to estimate. Moreover, in complexes of metal ions with larger ligands, such as porphyrins, in estimating the rates of radiationless transitions, tunneling effects have to be taken into account and, in addition, the contributions of heavy-atom effects to total ISC rates show dependence on the heavy-atom position in the complex [60].

**Fig. 6** The effects of oxygen on the kinetics of the decay of the T_1 state of Pheo and Pt-Chla in methanol, determined by laser flash photolysis at room temperature (see text for details). In the inset the expansion of 1- μ s region is shown

The values of the spin–orbit coupling constants in Mg and Zn are low, 40 and 390 cm^{-1} , respectively, but in any case are nonnegligible and therefore one would expect some heavy-atom effect to occur, i.e., an increased state mixing, resulting not only in a more efficient triplet formation but also in triplet quenching, especially in Zn-Chl. Intriguingly, the trends in the present set of data somewhat contradict this line of reasoning. The comparison of the triplet lifetimes reveals a remarkable dependence, that in three solvents, acetone, methanol and toluene, the values for the metal complexes are always longer than those for the free ligand (Table 3). The relative differences consistently amount to 15–25%, irrespective of the central metal, solvent and for Mg and Zn even of oxygen concentration (except for Zn-Chl in toluene). In pyridine, in equilibrium with air, the triplet lifetimes of the three pigments are nearly identical (approximately 360 ns), while after oxygen removal, the Zn-Chl triplet lifetime is again longer than in the other two pigments. Seemingly, the pigment triplets are quenched by oxygen somewhat slower in the presence of a central metal in Chl.

This apparent paradox can be explained by considering the ability of oxygen to interact with metal centers, perfectly known, e.g., for Fe and Cu porphyrins. By analogy, molecular oxygen may have some affinity to central metals chelated to other tetrapyrroles and hence is able to transiently (weakly) interact also with central metal ions in Chls. Such interactions between molecular oxygen (either in the triplet or the singlet state) and metallochlorophylls have been indicated by increased photooxidation rates of metallobacteriochlorophylls (versus the free base), observed earlier in our model photodynamic system [61, 62] and, on the other hand, it would also account for Mg-containing Chls being reportedly much better physical quenchers of singlet oxygen than the corresponding free bases [63].

The occurrence of weak interactions between oxygen and metal-center-containing pigments is very consistent with the trends in our present data, simply by considering a sort of competition between oxygen diffusion and its interactions with metal centers. Consequently, the process of quenching of the triplets by molecular oxygen is entirely diffusion-controlled only in the case of free ligand, Pheo, but slows down in the presence of metallocomplexes.

Obviously, the interaction of oxygen molecules with metallochlorophylls is only transient, since in a solvent of higher viscosity (pyridine) the process becomes again diffusion-controlled in the presence of oxygen. Moreover, these interactions with oxygen must be rather nonspecific, as the three metal complexes show similar behavior; the differences between central metal ions become meaningful after the removal of oxygen.

Effects of Pt

According to earlier studies [29, 64], the mixing of the singlet and triplet states in Chls becomes more efficient owing to the heavy-atom effect only when heavier metal ions, such as Cd and Pd, are coordinated to the pigment central pocket. Therefore, it is expected to be very efficient also in the complex with Pt. Indeed, as mentioned before, the complete quenching of fluorescence in Pt-Chla is very likely to result from state mixing. Second, as shown in the flash photolysis experiment, the central Pt(II) ion causes a drastic shortening of pigment triplet-state lifetime, which becomes as short as 2–4 μ s. This value is very different from the triplet lifetimes normally found in nonhalogenated Pt-porphyrins (approximately 100 μ s), e.g., in Pt-octaethylporphyrin [65]. This significant difference in the triplet lifetimes of more than 1 order of magnitude, is another indication, in addition to the Pt-induced shifts of the electronic transitions (see earlier, Table 2), that the host π -electron system in Pt-Chla is strongly affected by interactions with the central Pt ion, and hence also the state mixing in this molecule might be more efficient than in Pt-porphyrins.

The shortening of the triplet lifetime in Pt-Chla could also result, e.g., from the lowering of the triplet energy in Chl and an increase of nonradiative decay rates. However, taking into account the increase of the S_1 state (Q_y) energy in Pt-Chla (Table 1), this alternative mechanism might be rather ineffective (unless the heavy atom severely affects also the singlet–triplet splitting). Still another possibility would be a contribution of charge transfer states to the excited-state decay in Pt-Chla, but that mechanism has been found operative only for Pt(IV) complexes [51].

A few mechanisms can be suggested to account for the increase in the singlet–triplet mixing in Pt-Chla. First, it may originate from the direct influence of the heavy nucleus with a large spin–orbit coupling ($\xi = 4,481 \text{ cm}^{-1}$) on the π orbitals, affecting both x and y spin sublevels in Chla [29]. The strong interactions of the central Pt(II) ion with the nitrogen centers in the Chl molecule are evidenced by shifts of absorption transitions in Pt-Chla, which are larger than the shifts induced by Pt in porphyrins (see earlier, Table 2). Second, state-mixing enhancement may invoke interactions of the metal d electrons with the z (out-of-plane) spin sublevel in Chla, as a consequence of the redirection of the spin sublevel activity [29]. Actually, both mechanisms are expected to be involved, since, as discussed before

(Fig. 3), the Pt^{2+} ion almost certainly assumes an in-plane position in the complex, where it is in a position to generate the maximum effect on the macrocycle. The very effective spin–orbit coupling then promotes triplet relaxation via ISC in Pt-Chla, which competes with the energy transfer to molecular oxygen. However, a question arises as to what degree the photosensitizing properties of Chla are affected by effective spin–orbit coupling in Pt-Chla. This is a subject for further investigation.

Conclusions

The introduction of a heavy-metal Pt center into a Chla molecule is very specifically manifested in the spectral properties of the Pt-Chla complex. Owing to the high electronegativity of the metal center, the electronic transitions of the complex are shifted to higher energies. Similar shifts are observed in the spectral characteristics of triplet-state pigments. A comparison of the Pt-induced spectral shifts shows that the effects of heavy-metal insertion on the π -electron system are greater in Chla than in other tetrapyrroles (unreduced porphyrins).

The central metals affect the interactions of Chl with oxygen, causing an increase in its triplet lifetime, as compared with that of the free base. Most likely, molecular oxygen and metallochlorophylls transiently nonspecifically interact, which slows down the diffusion of oxygen.

In Pt-Chla, the internal heavy atom drastically changes the properties of the pigment excited state, as it causes a complete quenching of fluorescence and strongly affects the dynamics of the triplet state. The mixing of the triplet and singlet states in Pt-Chla is much more efficient than in simpler Pt-porphyrins, probably owing to stronger interactions of the macrocycle π -electron system with the in-plane-positioned central Pt(II) ion. As a consequence, the Pt-Chla triplet is quenched less effectively by molecular oxygen than in the case of the unsubstituted Chls but the question remains open as to whether (and how) this influences the photosensitizing properties.

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