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Influence of Molecular Oxygen on the Charge Transfer Properties of a Co(II)Porphyrin-Al(III)Phthalocyanine Aggregate. Excited States Dynamics and Photobiological Activities

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We report the novel cooperative properties of a complex obtained by self-assembling in solution a tetracationic cobalt porphyrin, $Co^{II}P^{4+}$, and a tetraanionic sulfoaluminum phthalocyanine, $Al^{III}Pc^{4-}$. In the ground state, the complex displays a charge transfer character, $Co^{II-\delta}\ P^{4+}/(Al^{III}Pc^{4-})^{+\delta}$, and the oxidation of the phthalocyanine moiety is catalyzed in the presence of its partner. In aerated media, the neutral complex strongly binds molecular oxygen to form a stable zwitterionic species, $^{-}O_2Co^{II}P^{4+}/(Al^{III}Pc^{4-})^{+^{\circ}}$. The zwitterion can revert to the neutral form $Co^{II-\delta}P^{4+}/(Al^{III}Pc^{4-})^{+\delta}$ upon vigourous bubbling of the solution with an inert gas (N_2, Ar) or under light stimuli. In the excited state, the complex photoejects the oxygen and relaxes to the ground state via a triplet excited state. The latter species can transfer its excess energy to nearby oxygen molecules. The mechanism of photoproduction of $^1O_2$ was studied via steady-state and time-resolved absorption and fluorescence techniques over a wide time window, from nanoseconds to hundreds of femtoseconds. We also investigated the potentiality of the neutral and zwitterionic complexes as a sensitizer for the photodynamic inactivation of cancer cells.

I. Introduction

In our continuous effort to understand the properties of mixed aggregates of porphyrins and phthalocyanines, and in particular the influence of the nature of their interaction on their ground and excited state properties, we varied for each chromophore different parameters such as the central metallic ion,[1−3] the ionic peripheral substituents on the macrocycle,[2] and the solvent.[1] We showed that aggregation of the mixed complexes is governed by (i) the ability of the metal ion to coordinate one or two axial ligands, (ii) the donor or acceptor character of the solvent, and (iii) the relative position of the substituents on the macrocycles.[1] Although the 1/1 aggregates display the same optical properties in the ground state, independently of the metal ion, we discovered a particularly rich variety of behaviors in the excited states. Four categories emerge from our previous studies: (i) diamagnetic mixed complexes resulting from the pairing of two metallated and diamagnetic monomers or metalled diamagnetic monomer with a free base partner, (ii) paramagnetic mixed complexes, issued from coupling a paramagnetic porphyrin (or phthalocyanine) with a diamagnetic phthalocyanine (or porphyrin), (iii) mixed complexes with one oxidizable or reducible metal ion; and (iv) mixed complexes with one oxidizable and one reducible metal ion.[3] In the present study, we report on a new type of complex, the ground and excited state properties of which widely differ from the earlier reported mixed complexes.

This mixed aggregate is obtained by self-assembling in solution of two salts, the tetrachloride salt of the meso-tetrakis-(4-(*N*-methylpyridinio))cobalt(II) porphyrinato ($Co^{II}P^{4+}$) and the tetrasodium salt of the 4,4′,4″,4‴-tetrasulfonated aluminum-(III) phthalocyanine ($Al^{III}Pc^{4-}$). The ground state redox and optical properties are explored using cyclic voltammetry and spectroelectrochemistry. The excited state properties are studied via steady-state fluorimetry and transient absorption spectroscopy over a wide range of wavelengths (250−1500 nm) and time windows from milliseconds to hundreds of femtoseconds.

We also evaluated the photodynamic properties of the aggregate against tumor cells in vitro. Such activity was anticipated in view of the potential of the complex to generate, under light stimulus, singlet oxygen, which is the known cytotoxic intermediate in photodynamic therapy (PDT) of cancer. For comparative purpose, the photobiological activity of the $Al^{III}Pc^{4-}$ monomer was also included in the study.

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II. Experimental Section

Materials. The tetrachloride salt of $Co^{II}P^{4+}$ was purchased from Midcentury Chemicals and the tetrasodium salts of the 4,4′,4″,4‴-tetrasulfonated aluminum(III) phthalocyanine ($Al^{III}Pc^{4-}$) and 4,4′,4″,4‴-tetrasulfonated zinc phthalocyanine were synthesized according to Weber's method.[4] Dimethylsulfoxide (DMSO) of spectroscopic grade (Merck) was used for steady-state and transient spectroscopies. For electrochemistry experiments, we used ultrapure, water-free DMSO from Burdick and Jackson-Fluka, which was constantly kept under argon. The tetra-butylammonium hexafluorophosphate salt (Nbu_4PF_6) from Fluka (purum), used as supporting electrolyte, was recrystallized three times in an ethanol/ethylacetate mixture and dried under vacuum at 50 °C. Water was purified by Milli-ro and Milli-Q systems of Millipore to a resistivity >18 MΩ·cm^{-1}.

Methods. *Electrochemistry.* Cyclic voltammetry was used for the determination of the oxidation potential of the cobalt porphyrin and aluminum phthalocyanine, in their monomeric and aggregated forms. All of the solutions were saturated with argon and contained 10^{-2} M of NBu_4PF_6. This latter concentration was kept low to avoid the destruction of the electrostatically linked aggregate. The concentrations of $Co^{II}P^{4+}$ and $Al^{III}Pc^{4-}$ were varied between 10^{-4} and 2×10^{-4} M and 1/1 mixtures were used to study the aggregate. A glassy carbon electrode ($\Phi = 3$ mm) from Tokai and a gold electrode were the working and counter electrodes, respectively. The saturated calomel electrode (SCE) served as the reference electrode. Cyclic voltammograms were obtained using a home-built potentiostat and current measurer, a function generator (Parr 175), and an *X-Y* chart recorder (IFELEC 2502). The design of the spectro-electrochemistry cell was detailed elsewhere.[5] Electrolysis was monitored on a Pt grid electrode with a home-built potentiostat (50 V,1 A) and the spectra were recorded with a Varian 2300 spectrophotometer.

Steady-State Absorption and Fluorimetry. The UV−vis spectra of monomers and mixed aggregate of $Co^{II}P^{4+}$ and $Al^{III}Pc^{4-}$ were recorded with a Varian Cary 3E spectrophotometer. For emission studies, when the concentrations of the solutions were high, to collect the fluorescence we used the front face mode and thin cell to avoid spectral distortions resulting from the inner-filter effect and emission reabsorption. The fluorescence and excitation spectra were recorded with a SPEX fluorolog over the UV and visible domain. To collect luminescence in the visible−near IR, from 600 to 1500 nm, we used a SPEX Fluorolog equipped with a photomultiplier R928 and a cooled germanium photodiode (North Coast EO 817L).

Time-Resolved Techniques. Nanosecond Absorption Experiments. Detection of long-lived transient absorbing species was performed using a nanosecond absorption setup comprising, as the excitation source, a Quantel 580 Q-switch Nd-YAG operating at 1 Hz (full width at half-maximum = 15 ns) and equipped with frequency doubling, tripling, and quadrupling crystals. The excitation wavelength was 532 nm. The probing light, perpendicular to the excitation beam, comes from a pulsed Xenon lamp (XBO 500 W). The detection system is based on a photomultiplier, a digital oscilloscope, and a computer.

Femtosecond Absorption Experiments. For the short-lived transients, we use the femtosecond time-resolved pump-and-probe technique. The generation of high-power femtosecond pulses, which are based on a colliding mode-locked dye laser and further amplification, was detailed elsewhere.[6] The excitation wavelength is 620 nm (150 fs, 100 μJ, 10 Hz repetition rate) and the generation of a continuum of white light through a 10 mm water cell allows the transient absorbing species to be probed over the 380−900 nm domain. The probing and reference (no excitation) beams, passing through a 2 mm sample cell, are dispersed into a monochromator and collected with a cooled diode array. The signals were analyzed with a computer at various delays between the pump and the probe, and transient differential spectra were established over the 380−900 nm domain.

Photobiological Studies. Dye Solutions. The stock solution of $Co^{II}P^{4+}/Al^{III}Pc^{4-}$ (8.8×10^{-4} M) was prepared in dimethylsulfoxide (DMSO) and $Al^{III}Pc^{4-}$ (5.5×10^{-4} M) in a phosphate-buffered saline solution (PBS). The concentration of the latter was determined spectroscopically after dilution in dimethylformamide (ϵ: 2.0×10^5 M^{-1} cm^{-1}, λ_{max} 680 nm).

Cell Photoinactivation. EMT-6 mouse mammary tumor cells were maintained in a Waymouth's medium supplemented with 15% fetal bovine serum (FBS) and 1% L-glutamine (Gibco, Canada), according to an established protocol,[7] as previously described.[8] The photocytotoxicity test was conducted by means of the colorimetric 3-(4-5-dimethylthiazol-2-yl)-diphenyl-tetrazolium bromide (MTT) assay.[9,10] Briefly, 15×10^3 EMT-6 cells per well were inoculated in 100 μL Waymouth's growth medium in 96 multiwell plates and incubated overnight at 37 °C and under a partial pressure of CO_2 (5%). Six plates were used, three for incubation with $Co^{II}P^{4+}/Al^{III}Pc^{4-}$ and three for incubation with $Al^{III}Pc^{4-}$. The cells were rinsed twice with PBS and incubated for 24 h at 37 °C with 100 μL of the dye prepared at $1-20 \times 10^{-3}$ M in Waymouth 1% FBS. After incubation, the cells were rinsed twice with PBS and refed with 25 μL Waymouth 15% FBS to avoid desiccation. The plates were then placed without lid in a translucent air-tight chamber. Two plates (one with $Co^{II}P^{4+}/Al^{III}Pc^{4-}$, one with $Al^{III}Pc^{4-}$) were deaerated with nitrogen at 1.3 L/min for 30 min before and during the 10 min of irradiation. Two other plates (one with $Co^{II}P^{4+}/Al^{III}Pc^{4-}$, one with $Al^{III}Pc^{4-}$) were left for 30 min in the chamber before and during irradiation, while the two last plates (one with $Co^{II}P^{4+}/Al^{III}Pc^{4-}$, one with $Al^{III}Pc^{4-}$) were left for 40 min in the chamber without irradiation. The red light source consisted of two 500 W tungsten/halogen lamps (GTE Sylvania, Canada) fitted with a circulating, refrigerated, aqueous Rhodamine filter. The fluence rate calculated over the absorbance peaks of the dyes (660−700 nm) was 10 mW cm^{-2} for a total fluence of 6 J cm^{-2}. The cells were then refed with 75 μL Waymouth 15% FBS and incubated at 37 °C overnight before cell viability was assessed. Fifty microliters of a fivefold diluted MTT stock solution (5 mg/mL PBS) in Waymouth 15% FBS was added to each well. After 3 h, 100 μL of sodium dodecyl sulfate (SDS 10% in 0.01 N HCl) was added in the wells. Plates were incubated overnight at 37 °C whereafter the absorbance was read at 595 nm by means of a microplate reader (BioRad, Mississauga, Ontario, Canada). The average absorbance of the blank wells in which cells were omitted was subtracted from each other well. The average absorbance of the control cells, which were incubated with dye-free Waymouth 1% FBS, represents 100% cell survival. Also, control cells which were incubated with the maximum amount of DMSO (2.3%) present in the $Co^{II}P^{4+}/Al^{III}Pc^{4-}$ solutions showed no significant toxicity. The drug dose required to inactivate 90% of the cells (LD_{90}) was extrapolated from the survival curves. Eightfold replicates were run per drug dose and the experiment was repeated three times.

III. Results

III.1. Spectrophotometric Measurements. We recorded the evolution of the UV−vis absorption spectrum during the titration

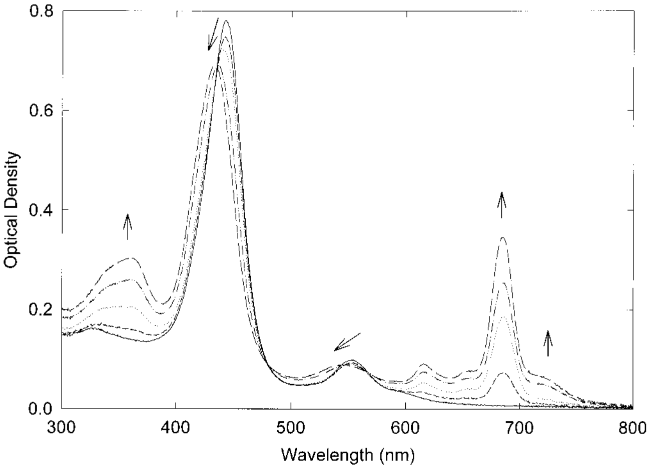


Figure 1. Spectral evolution during the titration a solution of $Co^{II}P^{4+}$ (1.54×10^{-5} M) with a concentrated solution of $Al^{III}Pc^{4-}$ (1.47×10^{-3} M). Solvent: DMSO. Optical pathlength: 0.5 cm. (—) Starting solution of $Co^{II}P^{4+}$; (— — —) addition of 0.26 equivalent of $Al^{III}Pc^{4-}$ (0.38×10^{-5} M); (···) addition of 0.52 equivalent of $Al^{III}Pc^{4-}$ (0.76×10^{-5} M) (—··—··—) addition of 0.77 equivalent of $Al^{III}Pc^{4-}$ (1.13×10^{-5} M); (— —) addition of 1.03 equivalent of $Al^{III}Pc^{4-}$ (1.51×10^{-5} M).

of a $Co^{II}P^{4+}$ solution in DMSO ($c \approx 10^{-5}$ M) with $Al^{III}Pc^{4-}$ ($c \approx 10^{-3}$ M). The spectral evolution is very dependent on the freshness of the $Co^{II}P^{4+}$ solution. For a freshly prepared solution of the cobalt porphyrin monomer, the spectrum is characterized by a Soret band at 434 nm ($\epsilon = 1.04 \times 10^5$ $M^{-1}\cdot cm^{-1}$) and a smaller Q band at 548 nm. For a few-day-old solution kept in the dark under aerated conditions, both Soret and the Q bands are shifted to the red to 442 and 557 nm, respectively. Figure 1 displays the titration of an old solution of $Co^{II}P^{4+}$ with $Al^{III}Pc^{4-}$. Up until we reach the molar equivalence, the addition of the phthalocyanine induces a slight blue shift in the porphyrin bands from 442 to 436 and 557 to 550 nm, accompanied by a small diminution of the absorption intensity; at the same time, we observe the growth of a wide absorption band at ca. 680 nm, reminiscent of the phthalocyanine main Q band concomitant with a new absorption peaking around 720 nM and extending to 800 nm. After the equivalence is reached, we observe only the addition to the spectrum of the absorption of the nonaggregated phthalocyanine. Using a method derived from that of Job,[1] we find that the formation constant is equal to $5 \pm 4 \times 10^6$ M^{-1}. If we then do the same titration with a freshly prepared solution of the cobalt porphyrin, we observe a slight red shift of both Soret and Q bands of the porphyrin from 434 to 435 and 548 to 549 nm. Note that the final maxima are practically the same than that found in the previous titration (Figure 1 and Table 1). The spectral evolution on the phthalocyanine side is identical to the one observed in the previous titration, with the peak at 680 and a shoulder at 720 nm. When we kept an equimolar solution exposed to the air for several weeks or if we kept it saturated with dioxygen for several days (in both cases in the dark), we noted that, while the absorption bands from the porphyrin are no more displaced (436 and 550 nm) but slightly decrease in intensity, a drastic change occurred in the phthalocyanine band, which was then split into a band peaking at 712 nm and a shoulder at 688 nm. By intensively bubbling the solution of the 1/1 aggregate with argon, we observed a complete blue shift of the absorption band at 712 nm which then peaked at 690 nm. On the porphyrin side, both Soret and Q bands are also blue-shifted from 436 to 425 and 550 to 534 nm. All of these spectral properties are reported in Table 1.

III.2. Electrochemical Measurements. The voltammetric measurements, performed on an argon degassed solution of $Co^{II}P^{4+}$ in DMSO, reveal four reduction waves at −0.52, −0.73, −0.95, and −1.04 V vs SCE (Figure 2). All four reactions are quasi-reversible and respectively are one-, one-, two- and two-electron reductions. In the same conditions, no clear peak could be obtained when positive potentials up to +0.7 V/SCE were applied, although an oxidation was occurring. The process was thus nonelectrochemically reversible but was found to be chemically reversible. The first reduction wave of $Al^{III}Pc^{4-}$ was observed at potentials between that of the first two reductions of the porphyrin, but no oxidation was detected below 0.7 V/SCE. Similar measurements were performed on an equimolar solution of $Co^{II}P^{4+}$ and $Al^{III}Pc^{4-}$. The reduction waves of the two compounds were observed and were superimposed. More interestingly, an anodic peak at +0.45 V/SCE was detected. A similar peak was observed with a solution of porphyrin alone that was not perfectly deaerated (i.e., in which dioxygen was dissolved). To identify the various oxidation and reduction products, we performed some spectroelectrochemical experiments and recorded the UV−vis absorption spectra of solutions of $Co^{II}P^{4+}$, $Al^{III}Pc^{4-}$, and $Co^{II}P^{4+}/Al^{III}Pc^{4-}$ (1/1) when the potential applied varied between −0.6 and +0.6 V/SCE (Figure 3 and Table 1).

For the porphyrin alone (Figure 3a), at positive potentials (>−0.1 V), bathochromic displacements of all the bands were observed (from 435 to 441 nm for the Soret band and from 545 to 552 nm for the Q band); the initial spectrum was recovered by applying a −0.3 V/SCE potential. At lower negative potentials (−0.6 to −0.45 V/SCE), hypsochromic displacements of the bands (to 427 and 538 nm) were observed. Once again, the initial spectrum was recovered with a −0.3 V potential.

The phthalocyanine solution (Figure 3b) was stable for potentials between −0.4 and +0.6 V/SCE, which was consistent with the voltammetric measurements. The spectrum of the reduction product of the phthalocyanine, obtained for potential lower than −0.5 V/SCE, is characterized by the disappearance of the Soret and Q bands of the molecule and the growth of a broad and less intense band peaking at 565 nm. The original compound was recovered when a null potential was applied.

We note that the mixture prepared in DMSO containing the electrolyte has an absorption spectrum slightly different from that shown in Figure 1: the Soret and Q bands maxima of the cobalt porphyrin in the complex correspond to the absorption maxima observed in solutions bubbled with argon (425 and 534 nm); the Q band of the phthalocyanine moiety is sharper and peaks at 680 nm are due to the excess of phthalocyanine. If we apply a potential below −0.5 V/SCE, we observe the concomitant reduction of both the porphyrin and the phthalocyanine (spectrum not shown), as was expected. When a null potential is imposed (0 V), we first observe a small change in the band at ca. 425 nm (small decrease in intensity and slight red shift (2 nm) of the porphyrin Soret band) and an important diminution of the band at 680 nm, as well as the growth of a shoulder at ca. 710 nm (Figure 3c, dashed line). At positive potential, +0.2 V, we observe a bathochromic shift of the 427 and 534 nm bands to 435 and 550 nm, respectively. Concomitant decrease of the 680 nm band and increase of the 712 nm band are also observed. This phenomenon is amplified when the potential is increased to +0.4 V: the final maxima for the porphyrin Soret and Q bands are 440 and 556 nm, which are very close to the maxima of the porphyrin monomer displayed in Figure 3a (dotted line). Once again, the initial spectrum is recovered by applying a −0.3 V/SCE potential for a sufficient length of time.

III.3. Steady-State Irradiation. Samples containing solutions of $Co^{II}P^{4+}$ and $Co^{II}P^{4+}/Al^{III}Pc^{4-}$ and bubbled with argon or

TABLE 1: Spectral Properties of the Solutions of Cobalt Porphyrin and of the 1/1 Phthalocyanine/Porphyrin Aggregate in DMSO Under Various Experimental Conditions

	exp. conditions	$\lambda_{\mathrm{Soret}}(\mathrm{CoP}^{4+})$ nm	λ_{Q} (CoP^{4+}) nm	$\lambda_{\mathrm{Q}}(\mathrm{AlPc}^{4-})$ nm
$\mathrm{Co}^{\mathrm{II}}\ \mathrm{P}^{4+}$	argon saturated	434	548	
$\mathrm{Co}^{\mathrm{III}}\ \mathrm{P}^{4+}{-}\mathrm{O}_2^{-}$	air or O_2 saturated	442	557	
$(\mathrm{Co}^{\mathrm{III}}\ \mathrm{P}^{4+})^{\circ+}$	argon saturated, electrochemistry	441	552	
$(\mathrm{Co}^{\mathrm{I}}\ \mathrm{P}^{4+})^{\circ-}$	argon saturated, electrochemistry	427	536	
$\mathrm{Co}^{\mathrm{II}-\delta}\ \mathrm{P}^{4+}/(\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-})^{+\delta}$	argon saturated	425	534	690
$^{-}\mathrm{O}_2{-}\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}/(\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-})^{\circ+}$	air or O_2 saturated	436	550	712
$\mathrm{Co}^{\mathrm{II}}\ \mathrm{P}^{4+}/(\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-})^{\circ+}$	argon saturated, electrochemistry $E = 0$ to $+0.2$ V	435	550	712
$(\mathrm{Co}^{\mathrm{III}}\ \mathrm{P}^{4+})^{\circ+}/(\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-})^{\circ+}$	argon saturated, electrochemistry $E = +0.4$ V	440	556	730

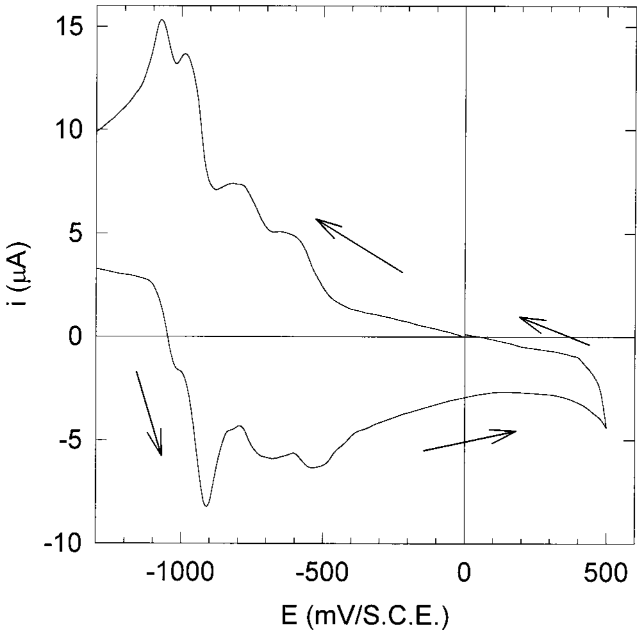


Figure 2. Voltammogram of a $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}$ solution in DMSO, $v = 100$ mV/s.

oxygen were submitted to 40−45 min continuous irradiation from a xenon lamp (200 W). UV radiation below 300 nm was cut off using filters. No change was observed in the absorption spectra of the Ar-degassed solutions. On the other hand, when the solutions were bubbled with oxygen, a clear evolution of the spectra was detected (Figure 4): for $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}$, the initial absorption bands at ca. 442 and 557 nm shift to ca. 435 and 548 nm, whereas for $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}/\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-}$, the band at 712 nm almost totally disappears while the intensity of the 690 nm band increases. The irradiated solutions recover their initial spectra if we keep the solution in contact with air long enough.

III.4. Radiative Pathway of the Complex. Luminescence spectra were recorded for the three solutions of $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}$, $\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-}$, and $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}/\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-}$ in DMSO, under air-free and oxygenated conditions. No luminescence was detected over the 600−1500 nm domain for the solutions of $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}$. On the contrary, we observed for $\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-}$ monomer an intense luminescent band peaking at 684 nm with a long tail in the near IR extending to 1400 nm, the intensity of which decreased in the presence of oxygen. For the complex, an excess of the cobalt porphyrin is added to displace the equilibrium toward the complex, thus avoiding any residual fluorescence coming from the highly fluorescent $\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-}$ monomer. In deaerated solutions, no luminescence was detected over the same domain of exploration, indicating that the excited state of the complex relaxes mainly through nonradiative pathways. When oxygen is present and kept long enough in the solution after bubbling, we observe no new luminescence in the near IR domain which could be attributed to singlet oxygen emission. When the solvent is water, $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}/\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-}$ ground state absorption spectrum displays the same but much wider bands than the bands observed in DMSO. Similar to the complex in DMSO, the nonradiative decay pathways predominate because no emission was detected over the whole explored domain from visible to near IR.

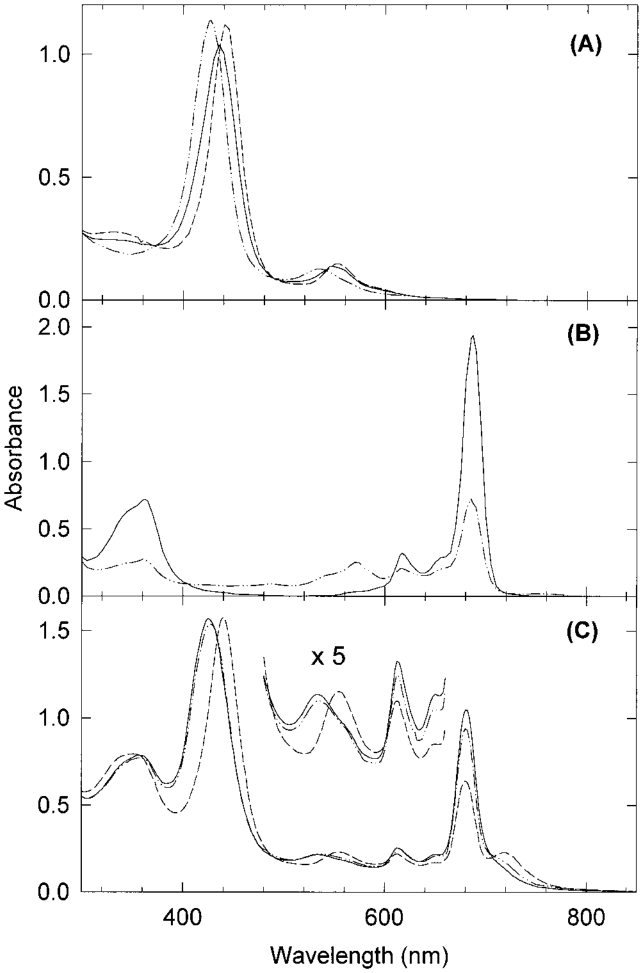


Figure 3. Spectroelectrochemistry: UV−vis absorption spectrum of DMSO solutions of (A) $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}$ (2×10^{-4} M): (—) no potential, (— —) +0.4 V/SCE, (—••—) −0.6 V/SCE. (B) $\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-}$ (2×10^{-4} M): (—) no potential, (—••—) −0.6 V/SCE. (C) $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}/\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-}$ (2×10^{-4}/2×10^{-4} M): (—) no potential, (—••—) 0 V/SCE, (— —) +0.65 V/SCE. In all cases, the solutions were saturated with argon and contained 10^{-2} M of $\mathrm{NBu_4PF_6}$.

III.5. Transient Absorption. With the nanosecond time-resolved pump and probe experiments on deaerated solutions of $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}$ and $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}/\mathrm{Al}^{\mathrm{III}}\mathrm{Pc}^{4-}$, no transient absorbances were detected, thus indicating that the transient absorbing species must be short-lived. We report the femtosecond time-resolved experiments on a deaerated $\mathrm{Co}^{\mathrm{II}}\mathrm{P}^{4+}$ solution in DMSO ($c \approx 10^{-4}$ M^{-1})

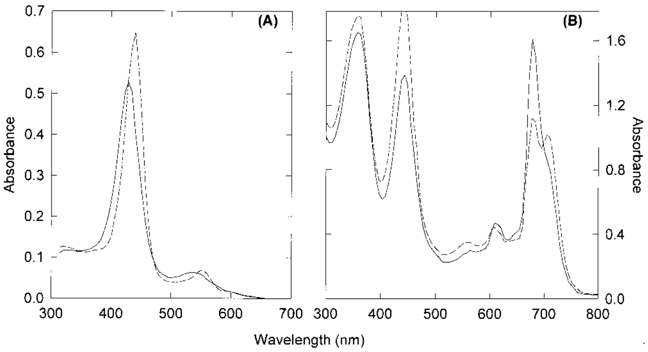


Figure 4. UV−vis absorption spectra of **(A)** $Co^{II}P^{4+}$ and **(B)** $Co^{II}P^{4+}$/$Al^{III}Pc^{4-}$ solutions in DMSO continuously irradiated for 45 min with a 450 W Xe lamp (λ_{cutoff} = 300 nm) and previously bubbled with O_2. (− − −) before and (—) after irradiation.

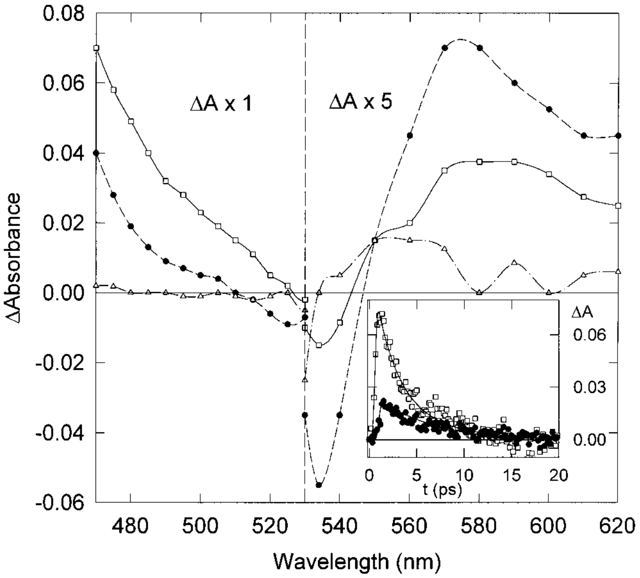


Figure 5. Differential absorption spectra of $Co^{II}P^{4+}$ (10^{-4}M) in DMSO at various time delays: (−□−) 1 ps, (−●−) 2.5 ps, and (−·△−·) 10 ps·λ_{exc}: 400 nm. Inset: kinetics decays at (□) 472 and (●) 580 nm.

and aerated $Co^{II}P^{4+}$/$Al^{III}Pc^{4-}$ equimolar solution in DMSO ($c \approx 10^{-4}$ M^{-1}) excited with 150 fs laser pulses at 400 and 620 nm, respectively.

The differential absorption spectra for $Co^{II}P^{4+}$ are presented on Figure 5. The first excited state detected is characterized by an absorption between 470 and 510 nm, the peak of which is below 470 nm. At the same time, we observe a small bleaching between 510 and 550 nm as well as a small absorption over 550 nm peaking at ca. 585 nm. This state then disappears over the next few picoseconds and we then observe a new excited state characterized by a smaller intensity for the band at higher energy, a more severe bleaching at 530 nm, and an increase in the low energy band, the peak of which shifts to ca. 575 nm. This new spectrum totally disappeared after 20 ps. The kinetic data can be fitted with a double exponential decay with the following lifetimes: $\tau_1 = 2.0 \pm 0.5$ ps and $\tau_2 = 5.2 \pm 1.0$ ps.

Figure 6 shows the transient differential spectra obtained at various time delays after the 620 nm laser pulse exciting the $Co^{II}P^{4+}$/$Al^{III}Pc^{4-}$ solution. The spectrum obtained just after the excitation pulse is characterized by bleachings at 435 and 680 nm, and absorptions at 405, 460, and 715 nm. This spectrum evolves very quickly, and within 2 ps, we observe a small reduction of intensity of the bleachings and of the band at 405 nm, together with an important increase of the absorption at 460 nm. The absorption band at 460 nm shifts toward a band at 475 nm with a shoulder at 500 nm and we also observe a shift of the red band from 715 to 730 nm with a slight intensity decay for the last band. Then, we detect a continuous decay over all the spectra during the next 70−100 ps, after which the spectra is stable and similar to that observed previously[2,3,6] for the first singlet excited state of the phthalocyanine. No consistent first- or second-order scheme could be fitted to the kinetic traces over the explored domain from 380 to 900 nm.

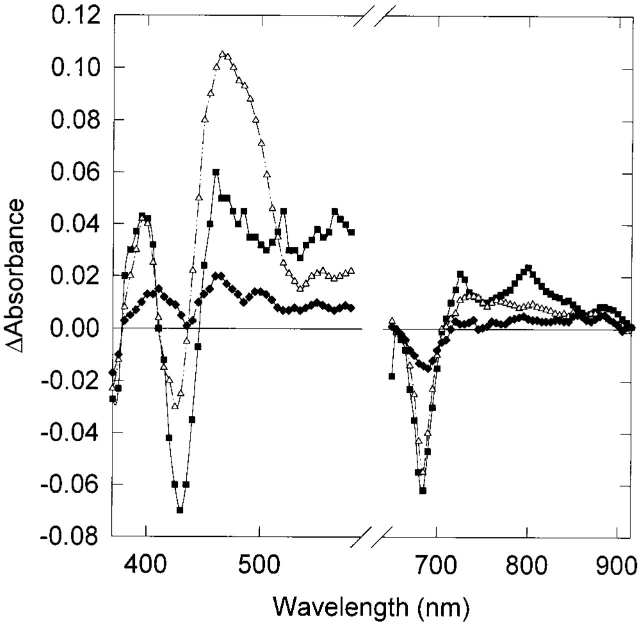


Figure 6. Differential absorption spectra of $Co^{II}P^{4+}$/$Al^{III}Pc^{4-}$ (10^{-4}/10^{-4}M) in DMSO at various time delays: (■) 0.6 ps, (△) 2.1 ps, and (◆) 70 ps. λ_{exc}: 620 nm.

III.6. Biological Studies. The effect of red light on the survival of EMT-6 cells incubated with increasing concentrations of CoP^{4+}/$Al^{III}Pc^{4-}$ and $Al^{III}Pc^{4-}$ in aerated and hypoxic conditions is shown in Figure 7. Under both conditions, no dark cytotoxicity was observed following the 24 h incubation with the dyes.

In deaerated conditions, $Al^{III}Pc^{4-}$ was slightly photoactive indicating that anoxia was not complete, whereas $Co^{II}P^{4+}$/$Al^{III}Pc^{4-}$ showed no photoactivity. In aerated conditions, $Co^{II}P^{4+}$/$Al^{III}Pc^{4-}$ was shown to be about two times less active than $Al^{III}Pc^{4-}$ (LD_{90}: 15 and 7 μM, respectively).

IV. Discussion

IV.1. Ground State Reactions. We previously[1−3] demonstrated that the mixture in DMSO of porphyrins MP and phthalocyanines M′Pc, with various metal ions M and M′, resulted in the predominant formation of bimolecular aggregates at stoichiometric equivalence, according to the following equilibrium:

$$\mathrm{MP} + \mathrm{M'Pc} \leftrightarrow (\mathrm{MP/M'Pc}) \qquad \text{(IV.1-1)}$$

We can then determine the aggregates formation constants by applying a variant of Job's method[1] to the UV−vis absorption spectra obtained by titration of one of the molecules by the other:

$$K_{\mathrm{ass}} = \frac{[\mathrm{MP/M'Pc}]}{[\mathrm{MP}]\cdot[\mathrm{M'Pc}]} \qquad \text{(IV.1-2)}$$

For the couple $Co^{II}P^{4+}$/$Al^{III}Pc^{4-}$, we obtain K_{ass} between 10^6 and 10^7 M^{-1}. Although this value is small compared to that obtained for other aggregates (for the bimolecular aggregates

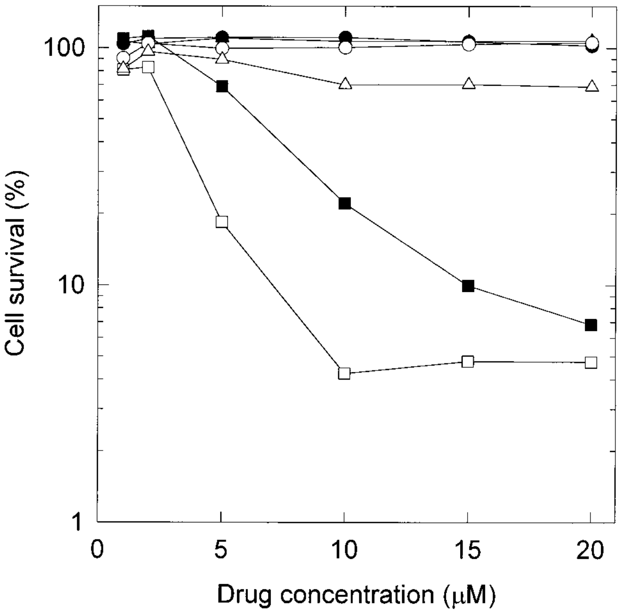


Figure 7. Survival of EMT-6 cells incubated for 24 h with increasing concentrations of $Co^{II}P^{4+}/Al^{III}Pc^{4-}$ or $Al^{III}Pc^{4-}$ and exposed to red light (10 mW/cm², 6 J/cm² between 660 and 700 nm) in aerated and hypoxic conditions: (●) $Co^{II}P^{4+}/Al^{III}Pc^{4-}$, flushed with air 40 min; (▲) $Co^{II}P^{4+}/Al^{III}Pc^{4-}$, flushed with N_2 30 min and irradiated during 10 min; (■) $Co^{II}P^{4+}/Al^{III}Pc^{4-}$, flushed with air 30 min and irradiated during 10 min; (○) $Al^{III}Pc^{4-}$, flushed with air 40 min; (△) $Al^{III}Pc^{4-}$, flushed with N_2 30 min and irradiated during 10 min; (□) $Al^{III}Pc^{4-}$, flushed with air 30 min and irradiated during 10 min.

formed with $Mg^{II}P^{4+}$, $Zn^{II}P^{4+}$, H_2P^{4+} and H_2Pc^{4-}, $Cu^{II}Pc^{4-}$ and $Al^{III}Pc^{4-}$, (5×10^8 M^{-1} < K_{ass} < 5×10^{10} M^{-1}),[1,3,11] it is still quite large and similar to that determined for $Mn^{III}P^{4+}/Al^{III}Pc^{4-}$ (4×10^6 M^{-1}) and $Co^{II}P^{4+}/Cu^{II}Pc^{4-}$ (1.7×10^7 M^{-1}).[3,11] We note that the uncertainty on the K_{ass} value is quite high for the $Co^{II}P^{4+}/Al^{III}Pc^{4-}$ complex compared to the other bimolecular aggregates. This is due to the existence of two equilibria in solution, which involves molecular oxygen.

The binding of molecular oxygen or nitrogen oxide to the Co^{II} ion is a well-established phenomenon.[12,13] In this case, the presence of air in the solution of $Co^{II}P^{4+}$ induces, with time, a shift of the Soret band from 434 to 442 nm and of the main Q band from 548 to 557 nm (Figure 4a). The spectrum then observed is very similar to that obtained by Araullo-McAdams and Kadish[14] by oxidation of Co^{II} to Co^{III} in the porphyrin. Indeed we too observe this spectrum when the cobalt porphyrin is electrochemically oxidized (Figure 3a). Hence, we propose that by binding O_2, $Co^{II}P^{4+}$ forms a charge transfer aggregate $Co^{III}P^{4+}{-}O_2^{-}$ and the following equilibrium is observed:

$$\mathrm{Co^{II}P^{4+} + O_2 \Leftrightarrow Co^{III}P^{4+}{-}O_2^{-}} \tag{IV.1-3}$$

It should be noted, however, that this process is neither instantaneous nor quick: even in oxygen-saturated solution it takes a few days before it is complete, which may explain why Kadish's group did not observe a similar aggregate in dimethylformamide while studying the catalytic reduction of dioxygen by $Co^{II}P^{4+}$. The cobalt porphyrin spectrum initially observed is also obtained if we irradiate the air-exposed solution continuously with UV−vis light ($\lambda > 300$ nm). This proves that the complex is destroyed by an intense enough exposition to light: back electron transfer and dioxygen ejection occur, and we need to wait again if we wish to recover the oxygenated complex.

When $Co^{II}P^{4+}$ and $Al^{III}Pc^{4-}$ are already aggregated in oxygen-free solutions, the position of the maxima of the absorption bands, 425 , 534, and 690 nm, very close to those of the $Co^{I}P^{4+}$ radical anion (427 and 536 nm) and of the radical cation, $(Al^{III}Pc^{4-})^{\circ+}$ (712 nm), indicates the existence of a charge transfer (CT) character, $Co^{II-\delta}$ $P^{4+}/(Al^{III}Pc^{4-})^{+\delta}$. This partial charge transfer favors the binding of molecular oxygen on the cobalt ion. The main effect of the binding of oxygen on the preexisting complex is to enhance the charge transfer from the phthalocyanine moiety to the oxygen through the porphyrin macrocycle. As a consequence, the Soret band of the porphyrin moiety shifts from 425 ($Co^{I}P^{4+}$) to 436 (neutral $Co^{II}P^{4+}$) while the phthalocyanine Q band shifts from 690 to 712 nm (fully oxidized $(Al^{III}Pc^{4-})^{\circ+}$). The 712 nm band is generally characteristic of the cation of metallated phthalocyanines[15]. A zwitterion, ${}^{-}O_2{-}Co^{II}P^{4+}/(Al^{III}Pc^{4-})^{+\circ}$, is formed and the following equilibrium is established:

$$\mathrm{Co^{II}P^{4+}/Al^{III}Pc^{4-} + O_2 \Leftrightarrow {}^{-}O_2{-}Co^{II}P^{4+}/(Al^{III}Pc^{4-})^{+\circ}} \tag{IV.1-4}$$

Such a zwitterion can also be obtained when a phthalocyanine solution is mixed with an oxygen-saturated solution of cobalt porphyrin. We showed that the binding of oxygen induced a charge transfer from the $Co^{II}P^{4+}$ ion to form ${}^{-}O_2{-}Co^{III}P^{4+}$; the evolution of the porphyrin/phthalocyanine aggregate suggests then that Co^{II} can still ligand O_2, and that an electron is transferred from the cobalt ion to the oxygen, and simultaneously an electron is transferred from the phthalocyanine to the cobalt, which can be depicted by the following reaction:

$$\mathrm{{}^{-}O_2{-}Co^{III}P^{4+} + Al^{III}Pc^{4-} \Leftrightarrow {}^{-}O_2{-}Co^{II}P^{4+}/(Al^{III}Pc^{4-})^{+\circ}} \tag{IV.1-5}$$

This hypothesis is corroborated by the results obtained when the porphyrin/phthalocyanine aggregate is electrochemically submitted to potentials such that the cobalt ion should be oxidized but not the phthalocyanine. We first observe (Figure 3c) the formation of oxidized phthalocyanine, once again characterized by its absorption band at ca. 712 nm, followed by the oxidation of Co^{II} in the porphyrin, while some phthalocyanine monomers retain their neutral form. Hence, only the aggregated phthalocyanine is oxidized during the first step, and the excess phthalocyanine remains in the solution while the cobalt ion is in turn affected:

$$\text{first step: } \mathrm{Co^{II}P^{4+}/Al^{III}Pc^{4-} \rightarrow Co^{II}P^{4+}/(Al^{III}Pc^{4-})^{+\circ}} \tag{IV.1-6}$$

$$\text{second step: } \mathrm{Co^{II}P^{4+}/(Al^{III}Pc^{4-})^{+\circ} \rightarrow Co^{III}P^{4+}(Al^{III}Pc^{4-})^{+\circ}} \tag{IV.1-7}$$

Such a phenomenon is only possible if the interactions between the phthalocyanine π orbital and the cobalt d orbital are significant, possibly through an interaction with the porphyrin π cloud.

The data reported in the literature concerning various other cobalt porphyrins solubilized in nonaqueous solvents with various ligands[16] or peripheral substituents[17] and similar porphyrins with different metal ions[18] (Cu, Zn, VO) enable us to assign the four reduction waves detected during the cyclic voltammetry experiment performed on $Co^{II}P^{4+}$. The first two reduction products are $Co^{I}P^{4+}$ and the radical dianion $(Co^{II}P^{4+})^{2-}$, where two electrons are transferred to the porphyrin π system. This radical is very reactive, and because of the highly hygroscopic character of DMSO, could react by adding protons to the macrocycle to form a phlorin, despite all experimental care. The last two waves observed correspond to the reductions of the methylpyridinum groups. The electrochemical irreversibility of the oxidation is not a surprise, as such a behavior was observed for other cobalt porphyrins;[16,17] the coordination change (from 5 to 6 ligands when going from Co^{II} to Co^{III}) is the explanation generally accepted. However, this observation differs from the data published by Araullo-McAdams and Kadish,[14] who report a reversible oxidation at +0.26 V/SCE for the same molecule in solution in DMSO, but is comparable to what these authors observed for the dimethylformamide solution.

The 1/1 $Co^{II}P^{4+}/Al^{III}Pc^{4-}$ complex displays a very unusual property: the oxidation of the phthalocyanine moiety is catalyzed in the presence of the porphyrin, which in turn becomes less oxidizable, because its oxidation only occurs when the electron withdrawal from the phtalocyanine moiety is complete.

IV.2. Excited States Relaxation. In the Co^{II} ion, seven electrons occupy the 3d orbitals, and one unpaired electron is present in a d_{z^2} orbital. According to Ake and Gouterman,[19] interaction of the latter electrons with the singlet and triplet states of the porphyrin macrocycle induces the formation of doublet and quadruplet states; the ground state is a doublet, whereas the lower ($\pi-\pi^*$) excited states are a singdoublet, $^2Q(\pi,\pi^*)$, a tripdoublet, $^2T(\pi,\pi^*)$, and a tripquadruplet, $^4T(\pi,\pi^*)$, from the highest to the lowest. Moreover, theoretical calculations[20] predict the presence of a (π,d) charge transfer state, obtained by transfer of an electron from the porphyrin $a_{2u}(\pi)$ orbital to the cobalt d_{z^2} orbital. To explain the lack of luminescence of cobalt porphyrins, which contrasts with the observations reported for other paramagnetic (copper or vanadyl) porphyrins, these authors suggested the following deactivation pathway in two steps:

$$^2Q(\pi,\pi^*) \rightarrow {}^2T(\pi,\pi^*) \rightarrow (\pi,\mathrm{d})\ \mathrm{CT\ state} \quad \text{(IV.2-1)}$$

This hypothesis was supported by the results of laser flash photolysis studies on cobalt(II) octaethylporphyrin ($Co^{II}OEP$) and cobalt(II) tetraphenylporphyrin ($Co^{II}TPP$) reported by Holten's group.[21] Using 35 ps laser pulses to excite the solutions, these authors observed two excited states, the lifetimes of which were estimated to be less than 20 ps; they assigned these states to the tripdoublet and the CT states predicted by Antipas and Gouterman.[20] The evolution of the differential absorption spectrum that we observe for $Co^{II}P^{4+}$ (Figure 5) is almost identical to that reported by Tait et al.[21] for $Co^{II}OEP$ and $Co^{II}TPP$, and we detect two transient states; the analysis of the kinetic traces reveals a two-exponential decay with lifetimes of 2 ps and 5.2 ps. A comparison with the transient spectra reported for other paramagnetic porphyrins[22,23] enables us to conclusively assign the first transient to the tripdoublet excited state. The spectrum of the second transient is similar to those reported for porphyrin radical cations, the neutral form fundamental of which is a doublet.[24,25] On the basis of these similarities and the theoretical predictions mentioned previously, we assign the second transient to the (π,d) CT state.

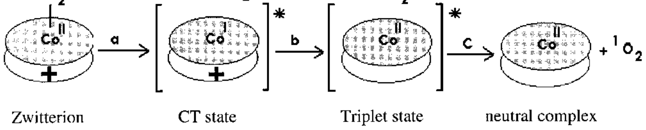
For the porphyrin/phthalocyanine aggregate, we must note first that the solution is not deaerated. However, neither is it oxygen-saturated nor has it been kept exposed to the atmosphere for very long, so that as discussed earlier, not all $Co^{II}P^{4+}$ molecules have bound a molecular oxygen. Second, the aggregate formation reaction is an equilibrium, and for a formation constant of $5 \pm 4 \times 10^6\ M^{-1}$ and a total concentration of 10^{-4} M in porphyrin and phthalocyanine, the free porphyrin and free phthalocyanine molecules represent 3−10% of the total concentration. The solution we are studying thus comprises: $Co^{II}P^{4+}$, $^-O_2-Co^{III}P^{4+}$, $Al^{III}Pc^{4-}$, $Co^{II-\delta}P^{4+}/(Al^{III}Pc^{4-})^{+\delta}$, and $^-O_2-Co^{II}P^{4+}/(Al^{III}Pc^{4-})^{+\circ}$. We use a laser excitation pulse at 620 nm, and the free porphyrin absorption, with or without oxygen ligand, is very weak at this wavelength, and its concentration is at least one order of magnitude less than that of the aggregate. No effect concerning these species will be detectable on the transient absorption spectra. On the other hand, the three last species have similar absorption bands at 620 nm and their excited states relaxation will be detected.

The phthalocyanine monomer first singlet excited state is well known and its lifetime is 5.0 ± 0.1 ns;[6,26] it can be readily identified as the species responsible for the spectrum observed at "long" time delay (>100 ps). Moreover, the continuous irradiation experiments showed us that light irradiation of the aggregate $^-O_2-Co^{II}P^{4+}/(Al^{III}Pc^{4-})^{+\circ}$ resulted in the photoejection of O_2 and a relaxation toward $Co^{II}P^{4+}/Al^{III}Pc^{4-}$. Immediately after photoexcitation (Figure 6, Δt = 0.6 ps), we observe in the transient spectrum an absorption band at 715 nm, and we already know that the radical cation of phthalocyanine is characterized by such a band. The rest of this transient is not very different from what we observe in other MP/M′Pc aggregates.[2,3] We therefore suggest that the first observed transient is an excited state of the form $[Co^{I}P^{4+}/(Al^{III}Pc^{4-})^{+\circ}]^*$, and that the same excited state is obtained from both the oxygenated and nonoxygenated aggregates, through an extremely fast relaxation respectively after the dioxygen photoejection or directly after the photoexcitation:

$$^-O_2-Co^{II}P^{4+}/(Al^{III}Pc^{4-})^{+\circ} \xrightarrow{h\nu} [Co^{I}P^{4+}/(Al^{III}Pc^{4-})^{+\circ}]^* + O_2\ \text{(ejected in the vicinity)} \quad \text{(IV.2-2)}$$

$$Co^{II-\delta}P^{4+}/(Al^{III}Pc^{4-})^{+\delta} \xrightarrow{h\nu} [Co^{I}P^{4+}/(Al^{III}Pc^{4-})^{+\circ}]^* \quad \text{(IV.2-3)}$$

This first transient, $[Co^{I}P^{4+}/(Al^{III}Pc^{4-})^{+\circ}]^*$, decays in less than 2 ps to form a new excited species, the transient spectrum of which between 470 and 600 nm is close to that of the first transient observed after the excitation of $Co^{II}P^{4+}$ alone. However, when this latter species, assigned as a tripdoublet excited state, decays with a 2.0 ps lifetime, the decay of the aggregate new excited state is much longer lived (70 to 100 ps) and is nonexponential. We attribute this state to the "triplet" excited state of the complex: the relaxation of the aggregate could go through this pathway because of a complete reordering of the relative energies of the various excited states in the complex and the nature of the initial steps of the relaxation. The charge transfer state would now be higher than the triplet. The latter would be longer-lived than the similar state of the corresponding porphyrin monomer. This longer lifetime would enable the triplet to react with the molecular oxygen molecules ejected in the vicinity, which would explain the nonexponential decay. Hence, we would observe the following reaction scheme:



We tried to prove the existence of $^1O_2$ via the study of the luminescent properties of $Co^{II}P^{4+}$, $Al^{III}Pc^{4-}$, and $Co^{II}P^{4+}/Al^{III}Pc^{4-}$, exploring the well-established luminescence domain of $^1O_2$.[27] However, at room temperature, no luminescence of $^1O_2$ could be detected either for $(Co^{II}P^{4+})^*$, which is too short-lived to diffuse and react with O_2, or for the triplet excited state of $Al^{III}Pc^{4-}$ monomer, which is known to diffuse and react transiently with molecular oxygen.[28] Although the quantum yield of triplet excited states of $Al^{III}Pc^{4-}$ monomer is high and that of the 1/1 complex is expected to be close to unity, no luminescence of $^1O_2$ was observed at room temperature. On the other hand, it was not possible to lower the temperature of these solutions because the solvent, DMSO, crystallizes around 13 °C. The absence of $^1O_2$ luminescence, under our experimental conditions, does not imply that $^1O_2$ is not formed. To check our hypothesis of $^1O_2$ generation under light stimulus, we compared the photoinactivation of the cells, in vitro, by $Co^{II}P^{4+}/Al^{III}Pc^{4-}$ and $Al^{III}Pc^{4-}$, the latter being known to generate singlet oxygen.

IV.3. Biological Studies. The loss of biological activity of the complex in hypoxia can be explained in terms of the lifetime of the transient species formed upon excitation. Both the CT state, $Co^{I}P^{4+}/(AlPc^{4-})^{+\circ}$, and triplet state of the complex are too short-lived to be able to diffuse and react with any residual oxygen present in the cells. However, in aerated conditions, the complex was photoactive. This result corroborates our findings and supports the hypothesis that the zwitterionic species, which is formed in the presence of oxygen, undergoes a sequence of reactions under a light stimulus: photoejection of O_2, relaxation of the CT to triplet excited state, and energy transfer to the O_2 remaining in its vicinity.

These data also show that the zwitterion is about two times less active than the $Al^{III}Pc^{4-}$ at low concentration (compare c and f of Figure 7). Various possible explanations could account for this difference: (a) a displacement of the equilibria established in DMSO (eqs IV.1-4 and IV.1-5) in biological media. The loss of O_2 (eq IV.1-4) or the separation of the aggregate (eq IV.1-5) through interactions with the cells would both diminish the concentration of the active zwitterion; (b) the presence of water also favors the formation of high-order aggregates[1] and thus disfavors the binding of O_2 on the complex.

Conclusion

We showed that the mixing in DMSO solutions of $Co^{II}P^{4+}$ and $Al^{III}Pc^{4-}$ induces the formation of a bimolecular aggregate $Co^{II}P^{4+}/Al^{III}Pc^{4-}$ with a formation constant K_{ass} varying from 10^6 to 10^7 M^{-1}, depending on the concentration of oxygen. The complex displays new and unconventional properties. First, the oxidation potential of the phthalocyanine moiety in the aggregate is greatly lowered (at least 500 mV less) compared to the corresponding monomer. The spectroelectrochemical measurements showed that oxidation of the latter could not be detected in DMSO, whereas in the aggregate, the phthalocyanine moiety was oxidized before the Co^{II} ion. Second, both the cobalt porphyrin monomer and the aggregate can bind molecular oxygen, and then form charge transfer species, $^{-}O_2(Co^{III}P^{4+})^{+\circ}$ and $^{-}O_2Co^{III}P^{4+}/(Al^{III}Pc)^{\circ+}$. This process is slow and takes several days to a few weeks depending on the O_2 concentration. Both species are destroyed by visible light while the oxygen molecule is photoejected. Following the photoejection of O_2, singlet oxygen is generated exclusively in the case of the complex. Femtosecond laser flash photolysis experiments allowed us to identify all the steps preceeding the $^1O_2$ formation and to elucidate the nature of the transients involved. The oxygen is photoejected instantaneously within the pulse duration and the CT state, $Co^{I}P^{4+}/(AlPc^{4-})^{+\circ}$, is formed and relaxes very quickly to the triplet excited state, $^{T}(Co^{II}P^{4+}/AlPc^{4-})^{*}$. The triplet can then react with the oxygen which is still in the vicinity, or relax to the ground state via a different pathway.

We also tried to estimate the efficacy of the complex to generate $^1O_2$ by comparing its photoactivity with that of the corresponding monomer, $Al^{III}Pc^{4-}$. However, in vitro, the zwitterion is less active than the corresponding $Al^{III}Pc^{4-}$. This result is explained in terms of the displacement of the equilibrium and the loss of oxygen through interactions with the biological medium.

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