

Immobilized phthalocyanines as red-light photosensitizers

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Abstract

Phthalocyanines are well known red-light photosensitizers particularly relevant in applications such as cancer therapy and water and blood sterilization. Their homogeneous photochemistry is dominated by their ability to produce singlet molecular oxygen. Triplet quantum yields and lifetimes are the key parameters if type II processes are considered to be responsible for photodynamic action. However, heterogeneity plays a major role in the cellular environment and the proximity of dyes and molecular targets may activate charge transfer processes. Factors controlling the photophysical activity of phthalocyanines forced to interact strongly with themselves and with other molecules are considered.

Keywords: Phthalocyanines; Red-light photosensitizers; Singlet molecular oxygen

1. Introduction

Phthalocyanines (Pcs) have been extensively considered as sensitizers in photodynamic therapy (PDT) [1], destruction of bacteria [2] and inactivation of viruses [3]. Their strong absorption in the red (Q-band) overlaps the region of maximum light penetration in tissues, allows microbiological decontamination to be driven under irradiation in the visible region of the spectrum, and permits blood sterilization. Cellular damage in PDT is implicitly related to the ability of dyes to yield $^1\text{O}_2$. Aside from the nature of the excited species directly responsible for photobiological activity, a strong influence has been assigned to thermodynamic factors, such as partition coefficients [4] and specific binding to cellular components [5]. Comparatively less relevance has been given in the literature to the possibility that type I, i.e. charge transfer mechanisms could be involved.

Selection of the macrocycle substituents and the complexed metal allows tuning of the photophysical behavior and thermodynamic properties of Pcs. Aggregation is strongly influenced by peripheral substituents or axial ligands, which have, in turn, a strong effect on the hydrophilic–hydrophobic character of the dye and, therefore, on the localization of dyes in particular tissues and on selective binding. Excited state lifetimes and redox potentials of Pcs are a function of the complexed metal and are almost independent of substituents. In spite of this fact, substituents have an indirect influence on the photophysical behavior, as dimers and higher oligomers are normally inactive. This is particularly important if high local dye concentrations are desired.

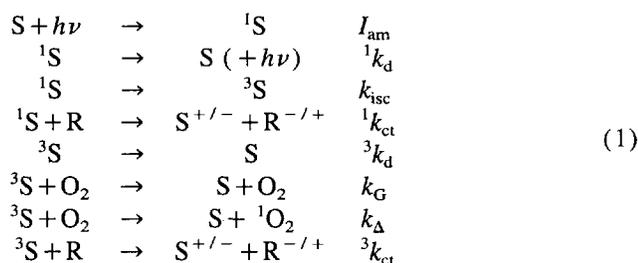
An implicit rule in the selection of Pcs for their application in PDT seems to be the requirement that their triplet states are long lived. Metallophthalocyanines containing diamagnetic metals such as aluminum or zinc are characterized by a long triplet lifetime and a relatively high triplet quantum yield and are, therefore, common candidates for this kind of applications. In aerated solutions, the red-light photoactivity of these Pcs is strongly dominated by their ability to yield singlet molecular oxygen ($^1\text{O}_2$, $^1\Delta_g$) [6,7]. Their singlet states are short-lived and their triplet states are readily quenched by molecular oxygen, so charge transfer reactions are quite improbable.

In the cellular environment, heterogeneity plays an important role and a proximity of the photosensitizer to molecular targets at distances lower than the excited state diffusion length is needed [8]. On relating photobiological activity with photophysics, properties in heterogeneous systems rather than in solution should be taken into account. An interesting point concerning Pcs, and in general red-light absorbing photosensitizers with low triplet excitation energy, is the reduced ability of triplet states to induce charge transfer reactions owing to their unfavorable redox potentials. A way to avoid this restriction is to take advantage of the larger singlet excitation energy, increasing the proximity of dyes and targets to overcome the substantially lower singlet lifetime.

In what follows, factors controlling the photophysical activity of dyes forced to interact strongly with themselves and with other molecules will be briefly considered, giving special attention to results on the photophysics of tetracarboxyphthalocyanines obtained in our laboratory.

2. Phthalocyanine photophysics in solution and micellar systems

The following simplified mechanism will be considered:



where S is the photosensitizer and R is a substrate capable of undergoing charge transfer (molecular oxygen may be included). I_{am} is the photon flux absorbed by monomers, provided that dimers and higher oligomers are cofacial and decay through radiationless channels [6] without any further effect. Energy transfer to substrates other than molecular oxygen is not taken into account and will be considered separately.

According to mechanism (1) and in the absence of charge transfer processes:

$$\phi({}^1O_2) = \frac{\phi_T}{\frac{1}{S_{\Delta}} + \frac{1}{\tau_T \cdot k_{\Delta} \cdot [O_2]}} \quad (2)$$

where ϕ_T is the triplet quantum yield, $S_{\Delta} = k_{\Delta} / (k_{\Delta} + k_G)$, and $\tau_T = ({}^3k_d)^{-1}$ is the triplet lifetime. For zinc or aluminum Pcs $\tau_T \approx 400 \mu\text{s}$ [9] and, assuming $k_{\Delta} = 10^9 \text{ M}^{-1} \text{ s}^{-1}$ and $S_{\Delta} \approx 1$, the 1O_2 quantum yield will coincide with the triplet quantum yield for $[O_2] > 2.5 \times 10^{-6} \text{ M}$, well below the molecular oxygen concentration in air saturated solutions. For dyes having very short triplet lifetimes, such as copper Pcs ($\tau_T \approx 50 \text{ ns}$ [9]), $\phi({}^1O_2)$ is normally lower than ϕ_T and depends strongly on the concentration of molecular oxygen. In spite of that, monomeric copper(II)-tetrapentylloxycarbonylphthalocyanine in toluene ($\phi({}^1O_2) = 0.36$) is as good as aluminum Pcs as 1O_2 sensitizer [6].

The singlet state lifetime of monomeric Pcs is not larger than a few nanoseconds [9], so diffusion-controlled charge transfer from the singlet state is not expected to make any noticeable contribution in solution for $[R] < 0.1 \text{ M}$. Diffusion-controlled charge transfer from the triplet state will reduce the 1O_2 quantum yield when $[R] > [O_2] / 10$. For zinc Pcs, reductive or oxidative charge transfer from the triplet state is thermodynamically favorable if $E_{R/R}^{\circ} > -0.45 \text{ V}$ or $E_{R^{+}/R}^{\circ} < 0.24 \text{ V}$ vs. SCE respectively, while aluminum Pcs require $E_{R/R}^{\circ} > -0.26 \text{ V}$ or $E_{R^{+}/R}^{\circ} < 0.54 \text{ V}$ vs. SCE respectively [9]. Thus aluminum Pcs are more oxidizing and less reducing than zinc Pcs. In homogeneous solution, however, the back reaction will normally prevent the formation of stable oxidation or reduction products. In a very few cases of oxidative quenching where back reaction is not possible [10], Pc radical cations are produced

which disproportionate leading to dye bleaching in most solvents, as is found in the case of zinc dicarboxydiamide-phthalocyanine (I) [11].

On passing to micellar systems the situation is quite different. Hydrophobic Pcs such as compound I are soluble in cetyltrimethylammonium chloride (CTAC) and bromide (CTAB) and sodium dodecylsulphate (SDS) micelles [12]. Solutes such as quinones [13] and to a lesser extent amines [14] may enter the micellar pseudophase, reaching extremely high effective concentrations. In this case simultaneous singlet and triplet charge transfer quenching is observed, which leads to fluorescence quenching and to a reduction of the singlet oxygen quantum yield. Molecular oxygen does not have any effect on the singlet quenching. Using amines as quenchers in CTAC micelles, stabilization of the Pc radical anion is provided by the micellar charge, hindering back charge transfer. Radical anions arise simultaneously from the singlet and triplet Pc excited states.

Two points are relevant considering reactivity in micelles: (1) the lower reactivity of the triplet state caused by its lower oxidizing and reducing power is compensated by its longer lifetime, and (2) singlet reactions are enhanced by the attainment of very high substrate local concentrations. The last point deserves further attention. The oxidative quenching of compound I by quinones [13] occurs with a reactive singlet rate constant of 2 to $5 \times 10^7 \text{ s}^{-1}$ depending on the quinone, when one quinone molecule is present in the micelle. This number should be compared with the natural singlet lifetime ($\approx 3 \text{ ns}$), leading to a singlet quenching efficiency of 6–15%. As the number of substrate molecules in the micelles increases, this efficiency also increases and it is possible to quench most excited Pcs at relatively low overall quinone concentration. The 1O_2 quantum yield is further reduced in this case because the triplet quantum yield is lowered by singlet quenching.

3. Phthalocyanines attached to non-reactive and reactive surfaces

Adsorption or chemical binding of dyes to insoluble organic or inorganic supports may be used to allow recovery of the photosensitizer after usage [15], as a means to improve extracorporeal PDT or water sterilization. Fluorescence and 1O_2 quantum yields were determined for different Pcs chemically attached to hydrophobic [16] and hydrophilic [17] polymers. Very high surface concentrations were attained, while maintaining the overall mass of sensitizer per gram of polymer at low values (less than 1%). Binding of hydroxaluminum tricarboxymonoamidophthalocyanine (II) and copper(II) tetracarboxyphthalocyanine (III) to the hydrophobic matrix Amberlite IRA-93 yields quite different results owing to the different aggregation tendencies of both Pcs [16].

Both Pcs are soluble in DMF but not in water and in most organic solvents. Compound **II** does not aggregate in DMF solution up to 10^{-4} M due to a probably bent structure, while compound **III** shows dimerization in far less concentrated solutions building up face-to-face dimers [6]. Fluorescence quantum yields of compound **II** bound to the polymer in DMF suspension are quite similar to those of the free Pc in the same solvent but are somewhat reduced in toluene suspension, where the free dye is not soluble. The last solvent enhances interaction among dye moieties through solvophobic forces and singlet self-quenching occurs. However, aggregation is not evidenced through UV-vis spectra. Compound **III** is not fluorescent and UV-vis extinction spectra performed on the polymer-bound dye show a high degree of aggregation in DMF suspension.

Polymer-bound compound **II** in DMF suspension shows $^1\text{O}_2$ quantum yields of nearly 10% of those in solution of the same solvent, which are further reduced by a factor of 10 on passing to toluene suspension. Though aggregation is not evidenced in this case, triplet self-quenching is seen to operate effectively in both solvents, particularly in toluene. $^1\text{O}_2$ photogeneration is severely restricted in these polymers due to the reduced lifetime of the triplet state. In contrast, energy transfer from the singlet state to adsorbed substrates is verified, as almost quantitative fluorescence quenching is observed when a cyanine dye is adsorbed on the polymer deposited on glass plates [16].

When the same Pcs are bound to hydrophilic polymers such as Sephadex and suspensions are formed in water or DMF, aggregation is verified for both Pcs [17], at a larger extent for compound **III**. This fact reduces further fluorescence and $^1\text{O}_2$ quantum yields of compound **II** and inhibits $^1\text{O}_2$ generation for compound **III**. The aggregation degree of the last compound decreases when CTAC is added to the water suspension. In these cases, it is very difficult to separate the effect of the solvent on the dye and on the polymer matrix, as enhanced interaction among dyes may arise either from solvophobic forces or from changes in the polymer conformation.

Reactive surfaces offer the possibility to produce charge transfer intermediates, as it occurs when dyes adsorbed or linked to semiconductors are irradiated [18]. On red-light excitation of compound **II** adsorbed on TiO_2 in water suspension [19], electrons are injected in the semiconductor conduction band and Pc radical cations are formed. Fluorescence is inhibited completely, showing that charge transfer involves the Pc singlet state. UV-vis extinction spectra show that the dye is moderately aggregated, though almost complete surface coverage is achieved. Interaction of Pc carboxyl groups with TiO_2 hydroxyl groups may act in reducing the dye aggregation tendency on the surface.

According to its redox potential (0.94 V vs. SCE), the radical cation of compound **II** is more oxidizing than its triplet state (0.54 V vs. SCE), but less than its singlet state (1.15 V vs. SCE [9]). Substrates as phenols and thiophenols with $E_{\text{R}^{\cdot+}/\text{R}}^{\circ} < 0.94$ V vs. SCE may be irreversibly photo-oxidized

by red-light irradiation of TiO_2/Pc suspensions in water [19].

4. Conclusions

Photodynamic action is commonly related to a Type II mechanism, i.e. to the ability of photosensitizers to yield $^1\text{O}_2$. Therefore, an implicit requirement for the design of drugs useful in PDT is a large triplet quantum yield and lifetime. However, type I mechanisms may also be involved and these rely on the availability of excited states or charged intermediates with appropriate lifetime and redox potentials.

Charge transfer processes from Pcs excited in their Q-band may take place either from the singlet or the triplet states and involve metal or ligand centered processes. The last processes prevail in Pcs of non-transition or closed-shell metals, such as aluminum or zinc. These compounds are characterized by long-lived and low-lying triplet states. Charge transfer from the triplet state may occur for quencher concentrations large enough to compete with quenching by molecular oxygen. Quencher redox potentials are limited to a narrow window. Charge transfer from the singlet state requires close vicinity of quenchers but is possible within a broader range of redox potentials. Rapid back reaction occurs unless some potential barrier exists, as in the case of charged micelles.

As a general rule, a concentration of Pcs on non-reactive surfaces enhances aggregation and promotes excited state self-quenching, thus lowering photoactivity, while concentration of substrates in microheterogeneous media or by adsorption on surfaces may enhance activity of singlet Pc. As a result, Pcs chemically bound to the surface of organic polymers may transfer energy efficiently to adsorbed substrates from the singlet state. Interaction of Pcs with the matrix may be also decisive in determining reactivity, as is particularly true for semiconducting supports such as TiO_2 .

The ability of dyes bound or adsorbed to solid supports to induce photodynamic action is an open problem and much work remains to be done in this field. One of the main advantages of binding dyes to insoluble supports is their easy recovery from the reaction medium. This advantage may be levelled off by deactivation of excited states by concentration quenching. Concentration effects will be larger when Pcs are not soluble in the suspension medium and when no specific interactions between dyes and support exist.

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