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Photodynamic effect of metallo 5-(4-carboxyphenyl)-10,15,20-tris(4-methylphenyl) porphyrins in biomimetic AOT reverse micelles containing urease

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Abstract

The photodynamic effect of 5-(4-carboxyphenyl)-10,15,20-tris(4-methylphenyl) porphyrin (H_2P) and its metal complex with Zn(II), Pd(II), Cu(II) and Ni(II) has been compared in homogeneous medium and in reverse micelles of *n*-heptane/sodium bis(2-ethylhexyl)sulfosuccinate (AOT)/water bearing urease as biological substrate model. The formation of porphyrin metal complex produces changes mainly in the free-base porphyrin characteristic absorption *Q*-bands and in the fluorescence quantum yields (ϕ_F). The singlet molecular oxygen, $O_2(^1\Delta_g)$, production was evaluated using 9,10-dimethylanthracene (DMA) in tetrahydrofuran (THF) yielding values of Φ_Δ ~0.48, 0.77 and 0.88 for H_2P, ZnP and PdP, respectively. DMA decomposition was not observed using CuP and NiP as sensitizer. The addition of β-carotene (Car) suppresses the $O_2(^1\Delta_g)$-mediated photooxidation of DMA. The photodynamic effect of these porphyrins on the urease activity was evaluated in water and in AOT reverse micelles. In both media, the enzyme photoinactivation increases with the sensitizer $O_2(^1\Delta_g)$ production. Also, the addition of azide ion photoprotects the urease activity. Therefore, the $O_2(^1\Delta_g)$ mediation appears to be mainly responsible for the enzyme inactivation in these media. The behavior of amphiphilic porphyrins in biomimetic microenvironments provides evidences about the promissory activity of these agents in photodynamic therapy (PDT).
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Keywords: Amphiphilic metalloporphyrins; Photodynamic effect; Urease; AOT reverse micelles

1. Introduction

One of the more recent and promising applications of porphyrins in medicine is in the detection and cure of tumors [1,2]. Photodynamic therapy (PDT) is an innovative and attractive modality for the treatment of cancer [3]. After administration of a photosensitizer, which is selectively retained by tumor cells, the subsequent irradiation with visible light in the presence of oxygen specifically inactivates neoplastic cells [4,5]. Basically two types of reactions can occur after photoactivation of the photosensitizer. One involves the generation of free radicals (type I photochemical reaction) and in the other, the production of singlet molecular oxygen, $O_2(^1\Delta_g)$, (type II) is the main species responsible for cell inactivation [3,6,7]. Both reactions can occur simultaneously and the ratio between two processes depends of the sensitizer, substrate and the nature of the medium [1].

Adequate photosensitizers are deemed to have specific chemical and biological properties [1]. Two of the photochemical requisites are a high absorption coefficient in the visible region of the spectrum and a long lifetime of triplet excited state to produce efficiently $O_2(^1\Delta_g)$. The spectroscopic properties and quantum yield of $O_2(^1\Delta_g)$ production (Φ_Δ) of porphyrins can be significantly changed by forming complexes with metals [8–11].

In several cases, amphiphilic porphyrin derivatives have shown potential use in the treatment of tumors [12]. The combination of hydrophobic and hydrophilic substituents in the sensitizer structure results in an intramolecular polarity axis. This property can produce a better accumulation in subcellular compartments, which is a prerequisite for an effective photosensitization [5]. One approach to this sensitizer architecture requires the formation of asymmetrically *meso*-substituted porphyrins. A convenient procedure for the synthesis of porphyrins bearing three identical molecular structures B and one different A (AB_3-porphyrins) was developed from *meso*-(substituted) dipyrromethanes [13–15]. In these cases, the structure A can bear a hydrophilic group, while B are substituted by groups that allow changing the

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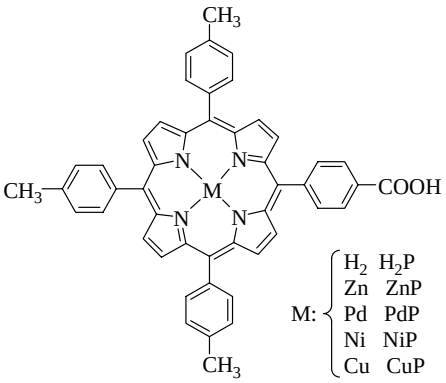

Scheme 1.

physical chemistry properties of the tetrapyrrolic macrocycle.

In previous studies, we have investigate the photodynamic activity of 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin in different biomimetic and biological media [16–18]. This synthetic porphyrin and their complex with metals are effective photosensitizers, which can be used as model compounds to investigate the theoretical and instrumental aspects of PDT. Recently, a novel asymmetrically substituted porphyrin bearing a trifluoromethyl group has been studied on Hep-2 and Hela cell lines as interesting photosensitizer [19,20].

In this paper, the photodynamic activity of an amphiphilic free-base, 5-(4-carboxyphenyl)-10,15,20-tris(4-methylphenyl) porphyrin (H_2P), and its metal complex with Zn(II), Pd(II), Cu(II) and Ni(II) (Scheme 1) has been compared in homogeneous medium and in a simple biomimetic model formed by reverse micelles of *n*-heptane/sodium bis(2-ethylhexyl)sulfosuccinate (AOT)/water containing urease as biological substrate. Microheterogeneous systems such as reverse micelles are frequently used as an interesting model to mimic the water pockets that are often found in various bioaggregates such as proteins, enzymes and membranes [11,18–23]. Also, AOT reverse micelles form suitable and variable reaction media depending on water to surfactant ratio for the study of different types of organic and enzymatic reactions [21]. Thus, water-soluble and water-insoluble compounds can be dissolved simultaneously in reverse micelles. The results contribute to understand the photodynamic process induced by these amphiphilic porphyrins and their potential application as PDT agents.

2. Materials and methods

2.1. General

UV-Vis and fluorescence spectra were recorded on a Shimadzu UV-2401PC spectrometer and on a Spex FluoroMax fluorometer, respectively. Mass Spectra were taken with a Varian Matt 312 operating in EI mode at 70 eV. Silica gel thin-layer chromatography (TLC) plates 250 microns from Aldrich (Milwaukee, WI, USA) were used. All the chemicals from Aldrich were used without further purification. Urease (Wiener lab., Rosario, Argentina) and β-carotene (Car) (Sigma, St. Louis, MO, USA) was used as received. Sodium bis(2-ethylhexyl)sulfosuccinate, AOT (Sigma) was dried under vacuum. Solvents (GR grade) from Merck were distilled. Ultrapure water was obtained from Labonco equipment model 90900-01.

2.2. Porphyrins

5-(4-Carboxyphenyl)-10,15,20-tris(4-methylphenyl) porphyrin (H_2P) and its complex with zinc (ZnP) were synthesized as previously described [24]. H_2P absorption spectrum λ_{max} (THF) (nm) (ε, $dm^3\,mol^{-1}\,cm^{-1}$) 418 (455 000), 515 (19 000), 550 (10 000), 594 (5800), 649 (5300). ZnP absorption spectrum λ_{max} (THF) (nm) (ε, $dm^3\,mol^{-1}\,cm^{-1}$) 424 (405 000), 557 (16 500), 597 (6900).

Palladium 5-(4-carboxyphenyl)-10,15,20-tris(4-methylphenyl) porphyrin (PdP): To a solution of H_2P (30 mg, 0.043 mmol) in 15 ml of *N*,*N*-dimethylformamide (DMF) was added palladium(II) chloride (75 mg, 0.42 mmol). The mixture was stirred for 5 h at reflux in atmosphere of argon. The solvent was removed under reduced pressure and flash chromatography (silica gel, dichloromethane/methanol 8%) yielded 32 mg (94%) of PdP. TLC (silica gel) R_f (dichloromethane/methanol 10%) = 0.72. MS *m*/*z* 804 (M^+) (804.1717 calculated for $C_{48}H_{34}N_4O_2Pd$). Anal. Calcd. C 71.60, H 4.26, N 6.96; found C 71.52, H 4.29, N 6.88. Absorption spectrum λ_{max} (THF) (nm) (ε, $dm^3\,mol^{-1}\,cm^{-1}$) 416 (198 000), 523 (18 000), 555 (1800).

Copper 5-(4-carboxyphenyl)-10,15,20-tris(4-methylphenyl) porphyrin (CuP): To a solution of H_2P (32 mg, 0.046 mmol) in 4 ml of chloroform and 8 ml of acetic acid was added copper(II) acetate monohydrate (70 mg, 0.35 mmol). The mixture was stirred for 2 h at reflux in atmosphere of argon. Solvents were removed under vacuum and flash chromatography (silica gel, dichloromethane/methanol 6%) yielded 33 mg (94%) of CuP. TLC (silica gel) R_f (dichloromethane/methanol 10%) = 0.56. MS *m*/*z* 761 (M^+) (761.2078 calculated for $C_{48}H_{34}N_4O_2Cu$). Anal. Calcd. C 75.62, H 4.50, N 7.35; found C 75.67, H 4.43, N 7.38. Absorption spectrum λ_{max} (THF) (nm) (ε, $dm^3\,mol^{-1}\,cm^{-1}$) 416 (380 000), 540 (18 000), 577 (3100).

Nickel 5-(4-carboxyphenyl)-10,15,20-tris(4-methylphenyl) porphyrin (NiP): A solution of H_2P (30 mg, 0.043 mmol) in 10 ml of chloroform was treated with nickel(II) acetate tetrahydrate (80 mg, 0.32 mmol) in 4 ml of methanol. The mixture was stirred for 3 h at reflux in atmosphere of argon. Solvents were evaporated under reduced pressure and flash chromatography (silica gel, dichloromethane/methanol 10%) yielded 31 mg (95%) of NiP. TLC (silica gel) R_f (dichloromethane/methanol 10%) = 0.48. MS *m*/*z* 756 (M^+) (756.2035 calculated for $C_{48}H_{34}N_4O_2Ni$). Anal. Calcd. C

76.11, H 4.52, N 7.40; found C 76.03, H 4.58, N 7.36. Absorption spectrum λ_{max} (THF) (nm) (ε, $dm^3\,mol^{-1}\,cm^{-1}$) 416 (260 000), 528 (17 100).

2.3. Spectroscopic studies

Absorption spectra were recorded at 25.0 ± 0.5 °C using 1 cm optical path quartz cuvette. The fluorescence quantum yield (ϕ_F) of porphyrins were calculated by comparison of the area below the corrected emission spectrum in tetrahydrofuran (THF) with that of tetraphenylporphyrin (TPP) as a fluorescence standard, exciting at $\lambda_{exc} = 550$ nm. A value of $\phi_F = 0.10$ for TPP in THF was calculated by comparison with the fluorescence spectrum in toluene using $\phi_F = 0.11$ and taking into account the refractive index of the solvents [25].

2.4. Partition coefficient measurements

1-Octanol/water partition coeffiecients (P) were determined at 25 °C using equal volumes of water (2 ml) and 1-octanol (2 ml). Typically, a solution of each porphyrin (∼10 μM) was stirred in the thermostat after the equilibrium was reached (8 h). An aliquot (100 μl) of aqueous and organic phases were dissolved in 2 ml of THF and the final porphyrin concentration determined by absorption spectroscopy [26].

2.5. Steady-state photolysis

Solutions of 9,10-dimethylanthracene (DMA, 35 μM) and photosensitizer ($\lambda = 550$ nm, absorbance 0.1) in THF (2 ml) were irradiated in quartz cuvettes with monochromatic light at $\lambda = 550$ nm from a 75 W high-pressure Xe lamp trough a high intensity grating monochromator (Photon Technology Instrument). The light intensity was determined as 1.2 mW/cm^2 (Radiometer Laser Mate-Q, Coherent). The kinetics of DMA photooxidation were studied by following the decrease of the absorbance at $\lambda_{max} = 378$ nm. The observed rate constants (k_{obs}) were obtained by a linear least-squares fit of the semilogarithmic plot of $\ln A_0/A$ versus time. Photooxidation of DMA was used to determine singlet molecular oxygen, $O_2(^1\Delta_g)$, production by the photosensitizers [19,27]. TPP was used as the standard ($\Phi_\Delta = 0.62$) [28]. Measurements of the sample and reference under the same conditions afforded Φ_Δ for the porphyrins by direct comparison of the slopes in the linear region of the plots. The studies in presence of β-carotene were performed using a [Car] = 14 μM. All the experiment were performed at 25.0 ± 0.5 °C. The pooled standard deviation of the kinetic data, using different prepared samples, was less than 5%.

2.6. Urease activity essays

A stock solution of urease (0.63 μmol NH_3/min) was prepared dissolving 50 μl of commercial urease in 950 μl of water. The appropriated amounts of urease stock solution (20–100 μl) were transferred into a 5 ml glass vials and diluted to 0.4 ml with phosphate-buffered saline (PBS) solution. After that, 100 μl of urea (0.5 M) was added and the solutions were incubated at 37 °C for 10 min. The enzyme activity was determined using the indophenol spectrophotometric method [29]. Thus, the samples were treated with 1 ml of reactant 1 (532 mM of phenol, 0.85 mM of sodium nitroferricianure and 0.3 mM of manganese(II) diethyldithiocarbamate) and 1 ml of reactant 2 (36.6 mM of sodium hipochloride, 0.12 mM of *p*-toluensulfoncloramide and 0.625 mM of sodium hydroxide). The mixture was incubated for 20 min at 37 °C. Then, an aliquot of 100 μl was dissolved in 2 ml of water and the absorbance was determined at 630 nm using a quartz cuvette. A linear repose was obtained (Fig. 1) using enzymatic activities between 0.030 and 0.16 μmol NH_3/min. Therefore, urease of 0.11 μmol NH_3/min was used in all sensitized experiments.

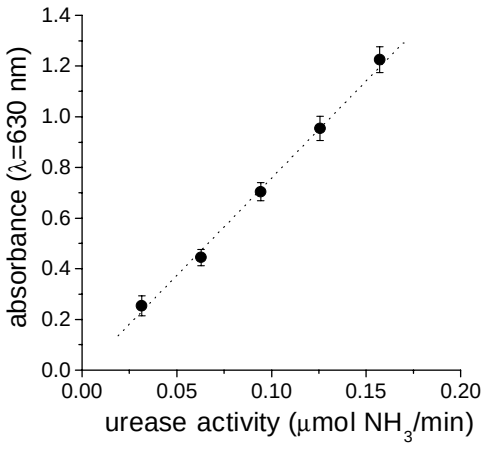

Fig. 1. Linear repose of urease activity in aqueous solution incubated at 37 °C with 0.5 M of urea. Values represent mean ± S.D. of three separated experiment.

2.7. Urease photoinactivation studies

The glass vials containing 0.4 ml of urease (0.11 μmol NH_3/min) and an appropriate amount of the corresponding porphyrin (∼1 μl from a concentrate porphyrin solution in DMF, spectral area 300 cm^{-1}) were exposed to visible light for 1 h at room temperature. The light source used was a Novamat 130 AF slide projector equipped with a 150 W lamp. The light was filtered through a 3 cm glass cuvette filled with water to absorb heat. A wavelength range between 350 and 800 nm was selected by optical filters [30]. The light intensity at the treatment site was 70 mW/cm^2 (Radiometer Laser Mate-Q, Coherent). After irradiation, the samples were treated as previously described to determine the enzymatic activity. Every experiment was compared with a control in dark and they were performed by triplicate.

2.8. Studies in AOT reverse micelles

A stock solution of AOT 0.1 M was prepared by weighing and dilution in *n*-heptane [11]. An appropriate amount of urease in PBS was added to obtain the same concentration than that used in aqueous solutions. The amount of water present in the system was expressed as the molar ratio between water and the AOT present in the reverse micelle ($W_0 = [H_2O]/[AOT]$) [31,32]. The addition of PBS saline solution to obtain the corresponding W_0 was performed using a calibrated microsyringe. The glass vials with 0.4 ml of AOT micelles bearing urease were treated as described above for aqueous solutions.

3. Results and discussion

3.1. Spectroscopic studies and porphyrin properties

The absorption spectra of porphyrins in THF show the typical Soret and *Q*-bands, characteristic of a free-base H_2P and the corresponding matalloporphyrins [33,34]. Fig. 2 shows porphyrin *Q*-bands. The spectra were also analyzed in different media. The Soret band absorption maxima of the porphyrin are summarized in Table 1. Sharp absorption bands were obtained in organic solvents indicating that the porphyrin is mainly no aggregated in these media. The solvatochromic effect on the location of absorption bands showed a slight blue shift upon solubilization in water with respect to organic solvents. Also, a broadening of Soret band infers that aggregation occurs in aqueous solution, as it is typical for many porphyrin derivatives [23,35,36].

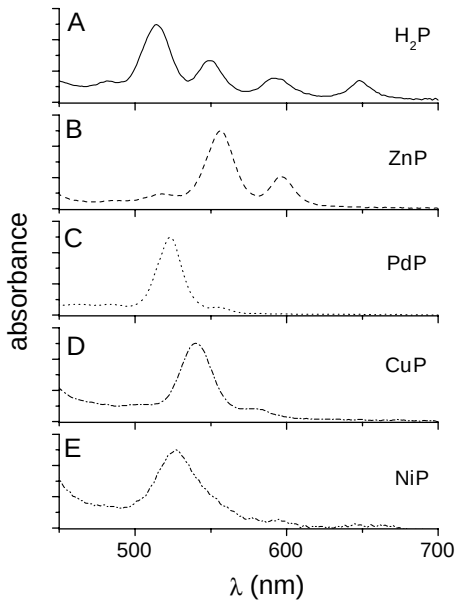

Fig. 2. Absorption spectra (*Q*-bands) of porphyrins in THF: (A) H_2P, (B) ZnP, (C) PdP, (D) CuP, (E) NiP.

Table 1
The Soret band absorbance peak of porphyrins in different media

Medium	H_2P	ZnP	PdP	CuP	NiP
THF	417.5	424.2	416.1	415.9	415.8
DMF	419.3	427.0	418.2	417.3	417.2
n-Heptane	417.3	423.1	416.1	414.4	414.4
Octanol	418.9	426.8	417.6	415.9	415.9
Water	412.5	422.3	415.9	410.3	413.4

The steady-state fluorescence emission spectra of these porphyrins were analyzed in THF. No detectable fluorescence bands were observed for CuP and NiP. The emission spectra of H_2P, ZnP and PdP are shown in Fig. 3A. The same values of λ_{em} were obtained exciting the sample at the wavelength of maximum absorption of the Soret and the *Q*-bands. The fluorescence quantum yields (ϕ_F) of the porphyrins were calculated by steady-state comparative method using TPP as a reference (Table 2). Taking in account the porphyrin energy of the 0–0 electronic transitions, the energy levels of the singlet excited stated were calculated giving values of 1.90, 2.07 and 2.12 eV for H_2P, ZnP and PdP, respectively.

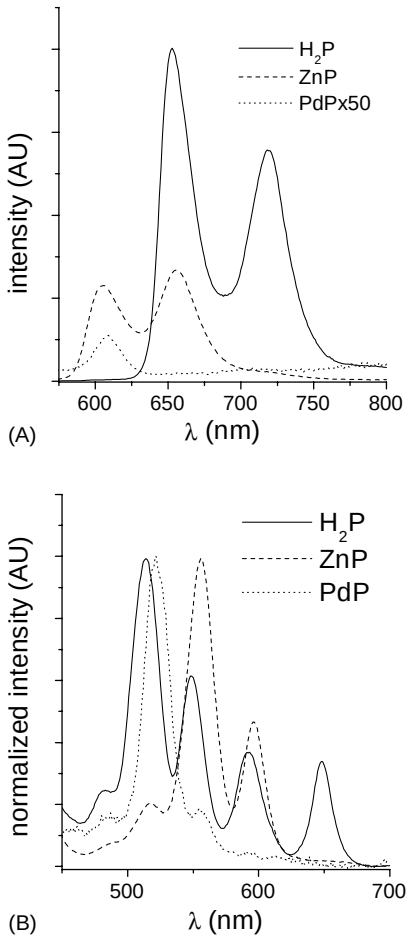

Fig. 3. Fluorescence spectra of porphyrins in THF: (A) emission ($\lambda_{exc} = 550$ nm); (B) excitation ($\lambda_{em} = 720$, 660, 610 nm, respectively for H_2P, ZnP and PdP).

Table 2
Fluorescence quantum yields (ϕ_F) and partition coefficients (P) of porphyrins

Porphyrin	ϕ_F	P	Log P
H_2P	0.11 ± 0.01	4.48	0.65
ZnP	0.054 ± 0.002	50.2	1.70
PdP	(1.3 ± 0.2) × 10^{-4}	85.6	1.93
CuP	–	31.3	1.50
NiP	–	49.1	1.69

The corrected fluorescence excitation spectra of porphyrins were recorded in THF (Fig. 3B) monitoring the emission at 725, 655 and 610 nm for H_2P, ZnP and PdP, respectively. In all cases, the spectra resemble the absorption spectra (Fig. 2), indicating that these porphyrins are mainly not aggregated in this medium.

The *n*-octanol/water partition coefficients of porphyrins (P) were evaluated at 25 °C ($P = [\text{porphyrin}]_o/[\text{porphyrin}]_w$) [1,26]. As can be observed in Table 2, the lipophilic character increases in these metalloporphyrin with respect to free-base H_2P, being PdP the less soluble porphyrin in water.

3.2. Photooxidation of 9,10-dimethylanthracene (DMA)

The aerobic irradiations with monochromatic light of photosensitizers in THF were performed in the presence of 9,10-dimethylanthracene. This substrate quenches $O_2(^1\Delta_g)$ by exclusively chemical reaction [37]. A time-dependent decrease in the DMA concentration was observed by following a decrease in its absorbance (Fig. 4). From these plots, the values of the observed rate constant (k_{obs}^{DMA}) were calculate for DMA (Table 3). The quantum yield of $O_2(^1\Delta_g)$ production (Φ_Δ) was calculated from the slopes of the plots for the porphyrins compared with the corresponding slope obtained for the reference, TPP, $k_{obs}^{DMA} = (6.2 \pm 0.1) \times 10^{-4}\,s^{-1}$. The values of Φ_Δ were estimated in 0.48, 0.77 and 0.88 for H_2P, ZnP and PdP, respectively. A considerable increase in Φ_Δ was obtained by forming a metal complex of porphyrin with Zn and Pd, while DMA photodecomposition was negligible using either CuP or NiP as sensitizer. These results are quite reasonable values for free-base porphyrin and metal complexes in this solvent [27].

3.3. Photoprotector effect of β-carotene (Car)

The photooxidation reaction of DMA sensitized by these porphyrins in THF, was studied in the presence of Car

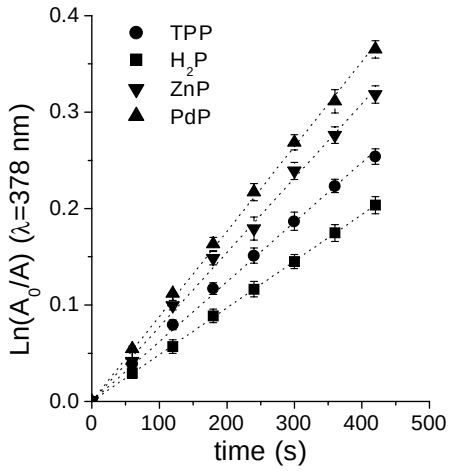

Fig. 4. First-order plots for the photooxidation of DMA (35 μM) photosensitized by TPP (●), H_2P (■), ZnP (▼) and PdP (▲) in THF. Values represent mean ± S.D. of three separated experiment.

(14 μM). Under these conditions, Car can quench $O_2(^1\Delta_g)$ through energy transfer or chemical reaction [38–41]. From the semilogarithmic plots of Fig. 5A, the values of the observed rate constant in presence of Car ($k_{obs}^{DMA+Car}$) were calculated. The results are gathered in Table 3. Under this conditions, the reaction of DMA was quenched with an efficiency ($\eta_q = 1 - k_{obs}^{DMA+Car}/k_{obs}^{DMA}$) of ∼0.85 for the three porphyrins used as sensitizers.

On the other hand, the photochemical reaction of DMA was studied in the presence of different Car concentrations (0–14 μM) using TPP as sensitizer (Fig. 6). From the Stern–Volmer plot ($k_{obs}^{DMA}/k_{obs}^{DMA+Car} = 1 + K_{SV}[Car]$, $K_{SV} = \tau^0 k_q$ where k_q represents the quenching constant of Car and τ^0 the excited state lifetime of $O_2(^1\Delta_g)$ in the absence of Car), a value of $K_{SV} = 3.9 \pm 0.2 \times 10^5\,M^{-1}$ was obtained. This result was used to calculate the ratio of $k_{obs}^{DMA+Car}/k_{obs}^{DMA} = 0.15$ for [Car] = 14 μM, which suggest that $O_2(^1\Delta_g)$ is the main species responsible for DMA photooxidation in THF.

Under these conditions Car also quenches $O_2(^1\Delta_g)$ by chemical reaction. The observed rate constants of Car (k_{obs}^{Car}) were calculated from the first-order plot at λ = 490 nm (Fig. 5B) [41]. The values of k_{obs}^{Car} are shown in Table 3. The reaction rate constant of Car photooxidation (k_r^{Car}) was determined using DMA as actinometer [16,42], giving a value of $\sim 1.7 \times 10^7\,s^{-1}\,M^{-1}$ using H_2P, ZnP or PdP as sensitizers. On the other hand, considering $\tau^0 = 23$ μs in THF [42], a value of $k_q = 1.7 \times 10^{10}\,s^{-1}\,M^{-1}$ was calculated.

Table 3
Kinetic parameters for the photooxidation of DMA and Car sensitized by porphyrins in THF

Porphyrin	k_{obs}^{DMA} ($\times 10^{-4}$, s^{-1})	$k_{obs}^{DMA+Car}$ ($\times 10^{-4}$, s^{-1})	k_{obs}^{Car} ($\times 10^{-4}$, s^{-1})	k_r^{Car} ($\times 10^7$, $s^{-1}\,M^{-1}$)	η_q
H_2P	4.8 ± 0.1	0.71 ± 0.03	1.8 ± 0.1	1.8 ± 0.2	0.85
ZnP	7.7 ± 0.2	1.10 ± 0.04	2.3 ± 0.1	1.5 ± 0.2	0.86
PdP	8.8 ± 0.2	1.19 ± 0.05	3.1 ± 0.1	1.7 ± 0.2	0.86

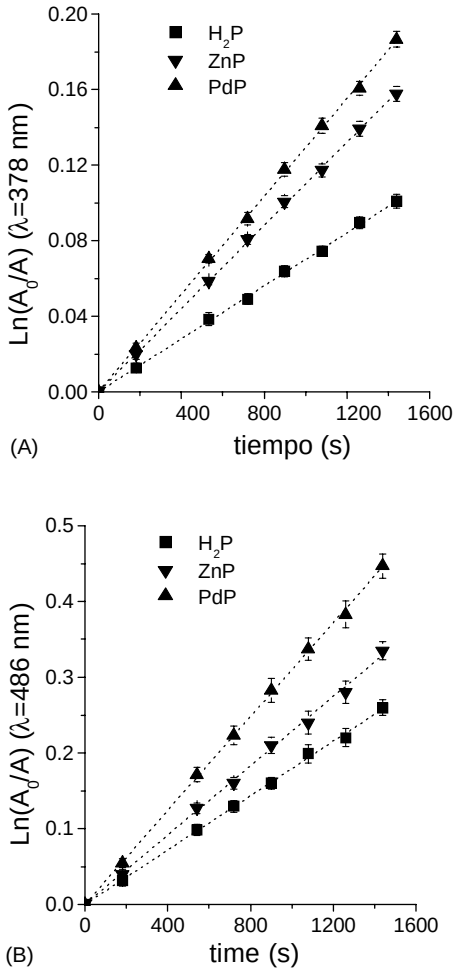

Fig. 5. First-order plots for the photooxidation of: (A) DMA (35 μM) in the presence of Car (14 μM); (B) Car (14 μM) sensitized by H_2P (■), ZnP (▼), and PdP (▲) in THF. Values represent mean ± S.D. of three separated experiment.

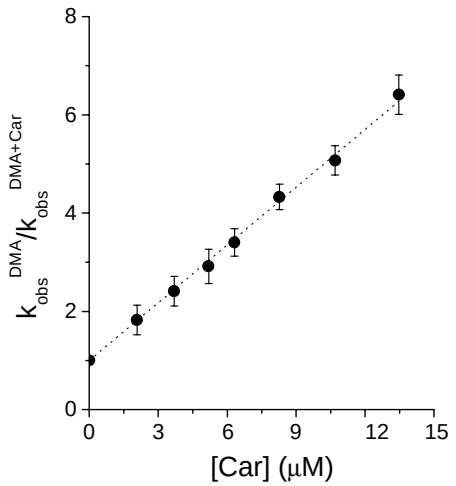

Fig. 6. Stern–Volmer plot of DMA (35 μM) photooxidation in the presence of Car sensitized by TPP. Values represent mean ± S.D. of three separated experiment.

Therefore, the protective function of Car is highly efficient because the chemical rate constant (k_r^{Car}) is several orders of magnitude lower than the physical quenching [39,40].

3.4. Photosensitized inactivation of urease

The aqueous solutions of urease containing the porphyrins were irradiated with visible light for 1 h. After that, the enzyme activity was determined as showed in Fig. 7A. As can be observed, the photoinactivation of urease sensitized by these porphyrins followed the order H_2P < ZnP < PdP. In contrast, enzymatic activity remained high (>95%) for solutions containing either CuP or NiP.

On the other hand, the enzymatic photoinactivation was analyzed in *n*-heptane/AOT (0.1 M)/water (W_0 = 20) reverse micelles. In this system, urease is exclusively solubilized in the polar side of the interface, while the amphiphilic porphyrins localize assumedly at the interface with the carboxy group in polar part of the reverse micelle and the

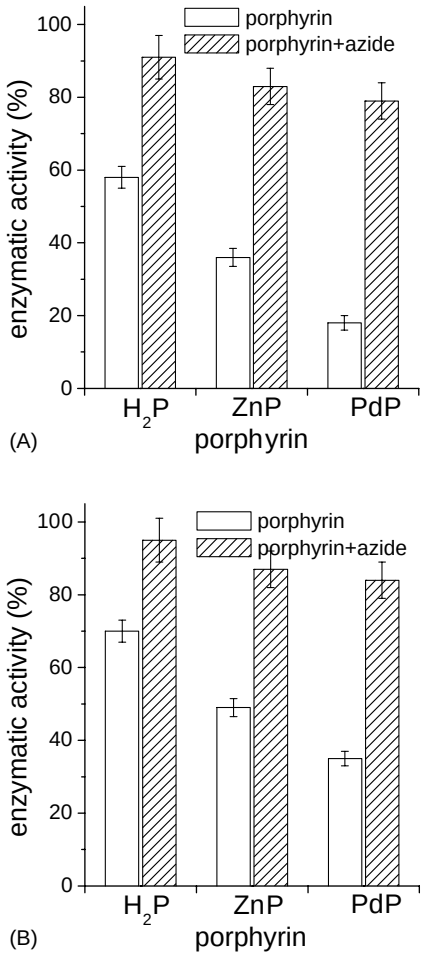

Fig. 7. Photoinactivation of urease by porphyrins in absence of azide and in the presence of 0.01 M azide: (A) aqueous solution, (B) AOT reverse micelles, irradiated with visible light for 1 h. Porphyrin spectral irradiated area 300 cm^{-1}. Values represent mean ± S.D. of three separate experiments.

porphyrin skeleton near the apolar phase [23]. When the absorption spectra of H_2P was studied varying AOT concentration, an increase in the intensity of the Soret band was observed as the [AOT] increases. This effect can be attributed to the interaction between the porphyrin and the micelle [31,32]. The strength of the association between H_2P and AOT was determined through the binding constant, $K_b = [H_2P_b]/[H_2P_f][AOT]$ (where the terms $[H_2P_b]$ and $[H_2P_f]$ refer to the concentration of bound and free porphyrin, respectively, and [AOT] is the total surfactant concentration). Thus, the spectral changes were analyzed using the Ketelaar Eq. (1) [32]:

$$\frac{1}{A - A_{Hp}} = \frac{1}{(\varepsilon_b - \varepsilon_{Hp})[P]_0} + \frac{1}{(\varepsilon_b - \varepsilon_{Hp})[P]_0 K_b[AOT]} \quad (1)$$

where $[P]_0$ is the total concentration of the porphyrin, A the absorbance at different [AOT], A_{Hp} the absorbance in *n*-heptane, and ε_b and ε_{Hp} are the molar absorptivity for the porphyrin bound to the interface and in the organic medium, respectively.

Plotting the left-hand side term of Eq. (1) versus 1/[AOT], the value of K_b can be calculated from the slope and the intercept as it is shown in Fig. 8. From this plot, a value of $K_b = 319\,M^{-1}$ was calculated for H_2P. Similar value was previously obtained for carotenoid bearing a carboxylic acid group in AOT reverse micelles [32]. In these systems, the strong hydrogen bond donors of the acid produces a similarly large value of K_b. The binding constants of ZnP and PdP were not possible to estimate using this approached because the porphyrins is completely bind to the polar heads of AOT.

The aerobic irradiation with visible light of AOT micelles containing urease and the sensitizer produce enzyme inactivation as showed in Fig. 7B. For the three cases, a smaller effect of photoinactivation was obtained in AOT micelles than in homogeneous medium.

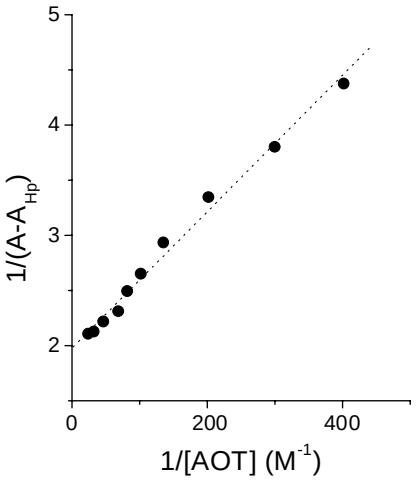

Fig. 8. Variation of $1/(A - A_{Hp})$ vs. AOT concentration for H_2P in *n*-heptane/AOT (0.1 M)/water ($W_0 = 20$). Dotted line: linear regression fit by Eq. (1). $\lambda_{max} = 417$ nm.

In these systems, the ion azide was used as a hydrophilic photoprotector against type II photoprocess [1,43]. Studies of the urease in the presence of different azide concentration (1×10^{-3} to 1×10^{-2} M) indicated that the urease activity remain unchanged under these conditions. The addition of 1×10^{-2} M azide produced a diminution in the urease photoinactivation and the enzyme is practically not affected by the photodynamic effect of these porphyrins in both media (Fig. 7). These results indicate that the $O_2(^1\Delta_g)$ mediation appears to be mainly responsible for the enzyme inactivation.

4. Conclusions

These studies provide information on the photodynamic activity of amphiphilic metalloporphyrin derivatives. The formation of metal complex produces changes mainly in the porphyrin absorption *Q*-bands and in the fluorescence quantum yields. No emission was detected for CuP and NiP, and it was very small for the complex with Pd. This effect can be disadvantage for detection and quantification of these agents in biological media. The photodynamic activity of these porphyrins in the presence of DMA increase in the order: NiP ~ CuP ≪ H_2P < ZnP < PdP. Similar tendency was observed when the photooxidative reaction was performed in the presence of Car as a lipophilic $O_2(^1\Delta_g)$ quencher. Also, the quenching effect of Car is in concordance with a type II photoreaction process in THF. In the presence of urease, the relative photosensitizing effectiveness of the porphyrins followed the tendency found for DMA decomposition. Thus, CuP and NiP produced a very small photoinactivation, which is consistent with the lack of type II process for these metal complexes [27]. In AOT micelles, the high amphiphilic capability of the sensitizers binds the porphyrin to the interfacial region of reverse micelles. Also, in this microheterogeneous system the enzyme activity remained higher than in homogeneous solution, probably due to the distribution of $O_2(^1\Delta_g)$ mainly in the organic phase than in the micellar pseudophase [11]. The presence of azide, as a water-soluble quencher, photoprotected the enzyme in both systems. Therefore, porphyrins with appreciable Φ_Δ appear to inactive urease mainly with the intermediacy of $O_2(^1\Delta_g)$. Although many other factor can contribute in biological systems, the results in this simple biomimetic medium indicate that an increase in the photodamage can be expected for porphyrins with high $O_2(^1\Delta_g)$ generation, such as ZnP and PdP.

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