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SUPRAMOLECULAR AGGREGATES PORPHYRINS-PHTHALOCYANINES AND THEIR IMPLICATIONS IN PHOTOMEDICINE

Simona-Florentina Pop[1,2] and Rodica-Mariana Ion[1,2]

[1] National Research & Development Institute for Chemistry and Petrochemistry – ICECHIM, Spl. Independentei, 202, sect. 6, Bucharest, Romania

[2] Valahia University, 2 Carol I Bd, Targoviste, Romania

E-mail: popsimfc@yahoo.com

Abstract

The aggregates of porphyrins and phtalocyanines can provide promising properties in the search of new molecular materials for medicine, and could be readily monitored spectroscopically, since it causes strong perturbations of their absorption spectra. The formation of water-soluble heteroaggregates from Ni,Co,Pd,Rh complexes of 5,10,15,20-tetra-methyl-pyrydil porphyrin (Me-TMPyP) and Zn complex of 2,9,16,23-tetra-carboxylate-phthalocyanine (ZnTCPc) has been investigated in this paper by using UV-Vis and IR spectra. The formation of such adducts involves intensive interactions between two differently charged macrocycles. The singlet oxygen generation is also stimulated by this heteroaggregation. Some data about the application of such heteroaggregates in photodynamic therapy of Saccharomices Cerevisiae cells were evaluated.

Key words: *porphyrins, phthalocyanine, heteroaggregates, photodynamic therapy.*

1. INTRODUCTION

The photodynamic therapy of tumour is an innovative method for cancer diagnosis and treatment of various types of tumours by the combined action by oxygen, light and sensitizer (r.m. ion 1997, 1998).

PDT is an indirect treatment; firstly the photosensitizer dyes are applied to the sample. following illumination these usually produce singlet oxygen that acts efficiently on the cells. the uptake and the retention time of the dyes should be different for the normal and cancer tissue (i. rosenthal 1991).

The sensitizer should have some special conditions:

- be incorporated into malignant cells at a much great efficiency than its incorporation into normal cells;
- be efficiently fluorescent and produce the singlet oxygen, or the other species which can destroy malignant cells;
- be non toxic for the healthly cells and, if possible, it should be quickly expelled from the organisms.

Porphyrins and phtalocyanines are promising candidates for sensitizing PDT action, because:

- are typical NIR dyes with red absorption band located in a region of low absorption of tissues;
- are non-toxic for different healtly cells;
- are much better incorporated into different kind of human cells than other dyes;

- are much soluble in different polar or aprotic solvents;
- due to their aggregation capacity, they could penetrate much better the membrane cells, after this being recovered into their monomeric forms;

The molecular mechanisms involved in photodynamic therapy are:

- radicalic sensitized photooxidation (type I reaction);
- singlet oxygen sensitized photooxidation (type II reaction);
- photosensitized reactions without oxygen(type III reaction).

In spite of the contribution of each such mechanisms, the main active specie causing damage of cancer cells is singlet oxygen (r.m.ion 1996, p. gregory 1991).

The generation of $^1O_2$ molecules occurs through the following mechanism:

$$P(S_o) + h\upsilon \longrightarrow P(S_1) \xrightarrow{ISC} P(T_1)$$

$$P(T_1) + 3O_2 \longrightarrow P(S_0) + {}^1O_2$$

In spite of their good properties, the porphyrins easily support a photodegradation reaction (called “photobleaching”) which occur under irradiation in oxygen atmosphere, leadind in a short time to some toxic products and lower efficiency (r.m.ion 1994, j. goc, d. wrobel, r.m.ion 1998).

Porphyrins and phthalocyanines are the most used compounds in the photodynamic therapy of cancer, due to their nir absorbing wavelenghts, non-toxicity and high singlet efficiency generation (d. frackowiak 1997, r.f.pastarnack, l.francesconi, r.raff 1973, m. ochsner 1996).

Phthalocyanines, which are structurally related to the porphyrins, are more stable than porphyrins and with adequate properties for photodynamic therapy (absorb visible light at a substantially longer wavelenghts than porphyrins).

By coupling porphyrins with phthalocyanines, could be obtained heterocomplexes useful for photodynamic applications. surprisingly, in the last decade the association of the two compounds has not received much attention in the literature (t.h.tran-thi 1988, 1989, 1992, j.-f. lipskier 1993, t.h.tran-thi, j.-f. lipskier 1992). their association is based on "electrostatic" mixed dimers by pairing porphyrin and phtalocyanine bearing oppositelly charge substituents, and have higher efficiency and stability (t.h.tran-thi 1997).

In this paper, the existence of such porphyrin-phtalocyanine heteroaggregates will be demonstrated using uv-vis absorption spectroscopy. also, the singlet oxygen generation capacity for all the studied compounds are evaluated by means of 1,3-diphenylisobenzophuran test (r.m.ion 1999).

2. EXPERIMENTAL

2.1 Materials

Ni(II)TMPyP, Rh(III)TMPyP, Pd(II)TMPyP and Co(II)TMPyP were synthesized following the procedures in the references (A.D.Adler 1967). The metal complexes of TMPyP have been converted into their chloride salts by passing them through the VIONIT AS-14 ion-exchange column in the chloride form. First of all, the phthalocyanine was transformed into its sodium salt. A 0.088 g sample of ZnTCPc was dissolved on a stoichiometric amount (1:4) of NaOH (3.9 ml , 0.1 M).

The solution was filtrated and the filtrate was dried under a stream of air overnight. A 0.05 g amount of this product was dissolved in 30 ml of water. In another beaker, an equimolar amount of Me-TMPyP (0.05 g) was dissolved in the same amount of water and the two solutions were mixed. The final solutions were left for three weeks to ensure complete precipitation and then filtered by gravity.

In order to evaluate the photochemical efficiency was used a special trap for singlet oxygen test -1,3-diphenylisobenzofuran(DPBF) prepared and purrified in the laboratory after the literature data (R.M.Ion 1994).

2.2 Apparatus

UV-Vis spectra (for freshly solutions) were recorded on a SPECORD M400, Carl Zeiss Jena spectrophotometer with a microprocessor. IR spectra (for KBr pellets) were run on a SPECORD M80 Carl Zeiss Jena spectrometer with a computer command.

2.3.Singlet oxygen test

Measurements were carried out in a quartz cell (1cm x 1cm) at 20 ºC. A DMSO solution (2.3 ml) containing sensitizer (4.8 x 10^{-5} M) and DPBF (2.7 x 10^{-5} M) was irradiated with light beam from a UV-Vis spectrophotometer. Solutions of sensitizers were freshly prepared and kept in the dark before measurements. The decreasing of the DPBF concentration was followed by a special programm ruled on a computer at the absorbance from 415 nm (the molar coefficient of absorption for DPBF is 23300 $M^{-1}cm^{-1}$) as function of the irradiation time (irradiation cycles 50 x 25 s). The reaction showed a zero order-kinetics in the first 100 s. The incident photon flow was 4.65 x 10^{-9} M.s-1. Using the absorption spectra of the photosensitizer, the absorbed photon flow (I_{abs}) was evaluated. The quantum yield of the photooxidation of DPBF was calculated from the eq.l

$$\Phi_{DPBF} = \left[\frac{[DBPF]}{I_{abs}} \cdot V \right]; \text{ V= 3 cm}^3$$

The quantum yield for singlet oxygen generation was calculated from eq.2.

$$\frac{1}{\Phi_{DPBF}} = \frac{1}{\Phi^1 O_2} + \left(\frac{1}{\Phi^1 O_2} \cdot \frac{K_d}{K_a} \right) \cdot \left(\frac{1}{[DBPF]} \right) \quad (2)$$

From the intercept of the Stern-Volmer plots, we obtained the quantum yield for singlet oxygen generation.

2.4.The Job method

The control of the complex formation and determination of their stoichiometry were carried out according to Job's method (P.Job 1928). This method is based on the spectrophotometric analysis of mixtures of equimolar solutions of all the reagents from the studied system. It was made for example, by adding the measured volume of TSPP 10^{-4} M concentration to the solution of the phthalocyanine (Pc56) 10^{-5} M. Were obtained the absorbance changes of the series of solutions in which the ratio of the three differently charged compounds varied in increments from 10:0 up to 0:10.

By coupling porphyrins with phthalocyanines, it could be obtained heterocomplexes usefull for photodynamic applications. Their association is based on pairing porphyrin and phthalocyanine bearing oppsotelly charge substituents, the obtained heterodimers having higher efficiency and stability.

In this study we report about the synthesis, spectral characterization and application in photodynamic applications of some water-soluble heterodimers MP/MPc by pairing MTMPyP with ZnTCPc .

It was observed that by using ionizing radiation (electron or photons beam) was possible to short the synthesis time and to increase the synthesis yield .

2.5. Photodynamic test on Saccharomices Cerevisiae

In all the experiments described in this paper, subcultures of the diploid SC-6 starin of the yeast SC were grown on malt agar slants at 30 ^{0}C for 48 h to 72 h. Immediatelly prior to irradiation or other experimental procedures, these resting cells were harvested, washed, and resuspended in double glass distilled water, to a final concentration of 10^6 cells/ml.

The survival fraction of irradiated cells was determined by measuring the coloni-forming ability of the cells. The cells were inoculated in 25 cm^3 plastic flasks and incubated for 5 hours for proper attachement to the substratum. The cells were then exposed to 10 mM of each sensitizer for 18 h and subsequently to light from 650 nm monochromatic light provided from a medium-pressure Hg lamp. After light exposure the cells were incubated in a sensitizer free medium for 6 days at 37 ^{0}C and then the colonies counted.

2.6. PDT-induced tumour regression

Was used a brain tumor provided from mice (50 mm^3). It was then treated with a PDT protocol that has been described previously (Pascu 1998).

In brief, the tumour were exposed to 650 nm wavelenght light emitted from an argon-pumped dye laser 24 h after innoculation with 2 ml from the solution of the best singlet oxygen generator Rh(III)TMPyP/ZnTCPc - 0.93).

The fluence was 135 W/cm^2 and was delivered at a fluence rate of 0.084 W/cm^2. The treatment was 1 cm in diameter, which included at least 1 mm margin of the non-neoplastic tissue.

3. RESULTS AND DISSCUSION

The porphyrins and phthalocyanines represent two classes of very chemically versatile compounds. Depending of the nature of the central metals and the peripheral substituents, a wide range of photophysical, photochemical properties and also interesting applications can be obtained, the photodynamic therapy being the most important between them .

Phtalocyanines are azaporphyrins consisting of four benzoindole nuclei connected by nitrogen bridges in a 16-membered ring of alternating carbon and nitrogen atoms arround a central metal atoms which form stable chelates with metal cations (dia or paramagnetic ion), Figure 1.

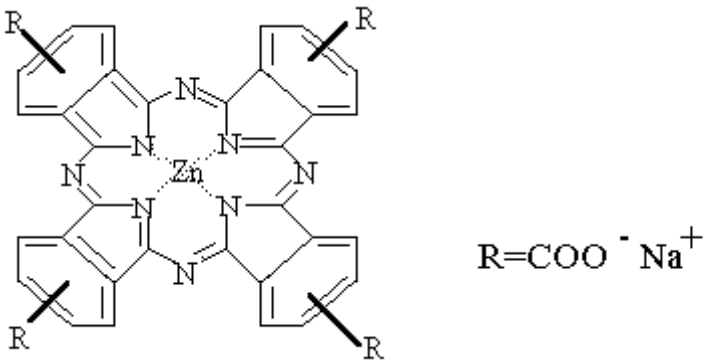


Figure 1 The structure of the phthalocyanine molecule

Porphyrins are tetrapyrrolic pigments based on porphine, Figure 2, which made up of four pyrrole subunits joined together by four methine bridges to give a cyclic molecule.

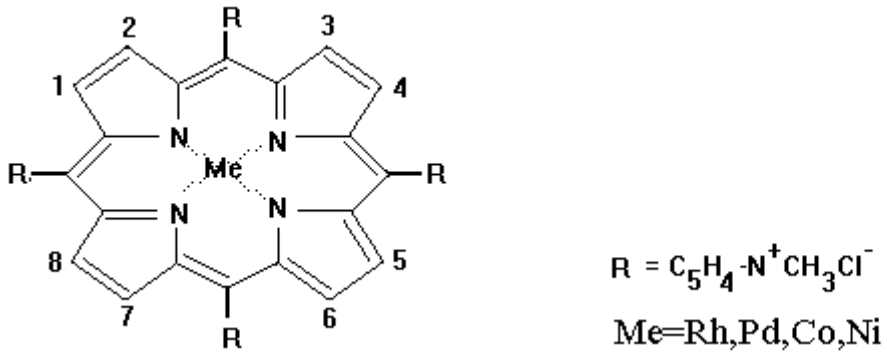


Figure 2. The structure of the porphyrin molecule

The hetero-aggregates of porphyrins and phthalocyanines grafted with ionic substituents of opposite charges have been subjected to extensive investigations in the last two decades, because such assemblies can provide promising properties in the search for new molecular materials for electronics or photodynamic therapy (Radzki 1987,1986). The hetero-aggregation will occur only between N^+ substituents from the porphyrin and the sulfonated ones in the 4,4',4',4''' positions of the phthalocyanine ring. The electronic spectra of porphyrins differ drastically from those of the phthalocyanines. The porphyrins display an intense Soret band and four Q bands in the 500-700 nm spectral region (T.H.Tran Thi 1992). During the contact between the porphyrin and the phthalocyenine a hypsochromism and a broadening of the Soret band of the porphyrin were observed (Figure 3) this observation being as an evidence for the aggregation between both components (Table 1).

Table 1. UV-Vis wavelengths of the heterodimers Me-P/Me-Pc

Compound	Soret band (nm)			Q band (nm)			
NiTMPyP	420	440	553		600		
ZnTCPc		435			635	686	
NiTMPyP/ZnTCPc	415	470	535	560			
CoTMPyP	430	455	545				
CoTMPyP/ZnTCPc	431		546		626	696	
RhTMPyP	430		500	550	590		
RhTMPyP/ZnTCPc	428			556	596	648	
PdTMPyP	438		520	565			
PdTMPyP/ZnTCPc	431			545		646	

Knowing that at a constant wavelength, when two equimolar solutions are mixed in equal volumes, the absorbance of the mixture should be equal with the sum of the absorbances of the individual solutions (if there has been no interaction between the two solutions), in the studied case were observed relevant differences between the expected and found absorbances, Table 2.

Table 2. UV-Vis absorption intensities (A) for the hetetodimers MeP/MePc

Compound	Soret band (nm)	A found	A calculate
NiTMPyP/ZnTCPc	415	2.02	2.67
CoTMPyP/ZnTCPc	431	1.23	1.67
RhTMPyP/ZnTCPc	428	1.94	2.07
PdTMPyP/ZnTCPc	431	1.67	2.03

$A_{calculated} = A_{MeP} + A_{MePc}$

This observation reprezent another proof for the heterodimerization between MeTMPyP and ZnTCPc. The IR spectra recorded for the studied systems, showed some strong differences between the heteroaggregates and the monomeric forms, especially for the band around 1500 cm^{-1}. For ZnTCPc upon dimerization, the band from 1390 cm^{-1} (attributed to symmetric strech of carboxylate ion) shifts to 1372 cm^{-1} and the band from 1585 cm^{-1} (attributed to asimmetric frecquency of the same carboxylate ion) shifts to higher frequency, Table 3.

Table 3. IR data for carboxylated stretch in cm^{-1}

Compound	Sym. stretch (cm^{-1})	Asym.strech (cm^{-1})
NiTMPyP/ZnTCPc	1377	1590
CoTMPyP/ZnTCPc	1375	1588
RhTMPyP/ZnTCPc	1374	1590
PdTMPyP/ZnTCPc	1379	1589
NiTMPyP	1382	1582
CoTMPyP	1390	1586
RhTMPyP	1392	1585
PdTMPyP	1389	1584
ZnTCPc	1390	1583

The observed data could argue the symetry distorsion of phthalocyanine upon dimerization. Before testing these photosensitizer systems into some photodynamic applications, was necessary to evaluate their capacity for singlet oxygen generation. All the studied systems are good sensitizers for photo-oxidation of DPBF with high quantum yields (Table 4).

Table 4. Singlet oxygen generation by the studied compounds

Compound	$\Phi(^1O_2)$
NiTMPyP/ZnTCPc	0.7
CoTMPyP/ZnTCPc	0.801
RhTMPyP/ZnTCPc	0.85
PdTMPyP/ZnTCPc	0.93
NiTMPyP	0.07
CoTMPyP	0.13
RhTMPyP	0.23
PdTMPyP	0.3
ZnTCPc	0.7

From this table could be observed that the best photosensitizer is the system RhTMPy/ZnTCPc with a very high value for the quantum yield of singlet oxygen-0.93.

After exposure of the yest Saccharomices Cerevisiae to certain agents (light and porphyrinis drug) a great decrease in viability is observed if the treated cells are incubated in the dark for 6 hours or more in distilled water at 30 ^{0}C. It seems reasonable to assume that the light inactivation of Saccharomices Cerevisiae is due to the enzymatic destruction of DNA (K.Gunaydin 1999). Different applications of

PDT have been, in the last 4-5 years, effectively developed. The selective degradation of cancerous cells is obtained, for example, by the intravenous injection of photosensitizer followed by illumination of the pacient with red light. Such procedure has already been applied on more than 10,000 occasions. It is primarily applied in clinical treatment, because only one photosensitizer (Photofrin II) has been approved for wider applications in hospitals. In clinics and, of course, in model investigations, various group of dyes are being investigated to find the most effective photosensitizers for a given type of therapy having the highest selectivity of incorporation into malignant and healthy cells.

Up to now, several dyes, e.g., merocyanines, phthalocyanines, and several types of porphyrins have been most widely investigated (Frackowiak 1998).

Microorganisms are proposed to be an attractive simplified experimental model for studying the photosensitization mechanisms (T.H.Tran-Thi 1988). The yeast Saccharomices Cerevisiae was used as a model eukaryotic organism to study the uptake of porphyrins and their phototoxicity. The eukariotic yeast cells provide a bridge between the simple prokariotic systems and the more complex mammalian cells (T.H.Tran-Thi 1989). In aerobic eukaryotic cells porphyrin-mediated mitochondrial photodamage appears to be the primary photobiological effect (T.H.Tran-Thi 1992).

Yeast cells (SC-132) used in the present experiments, has a sufficiently large dimension (diameter 5 μm) (3-14 μm). Yeast cells can tolerate suspension in distilled water for several days without detectable damage grow under these conditions. Under photodynamic treatment, these cells are inactivated as it is shown in Table 1. Photoinactivation of the respiratory activity and photolethality of whole cells caused by porphyrin accumulation in mitochondrial membranes are well known (J.-F.Lipskier 1993), Figure.3. Mitochondria play a central role in oxidative metabolism in eukaryiotes

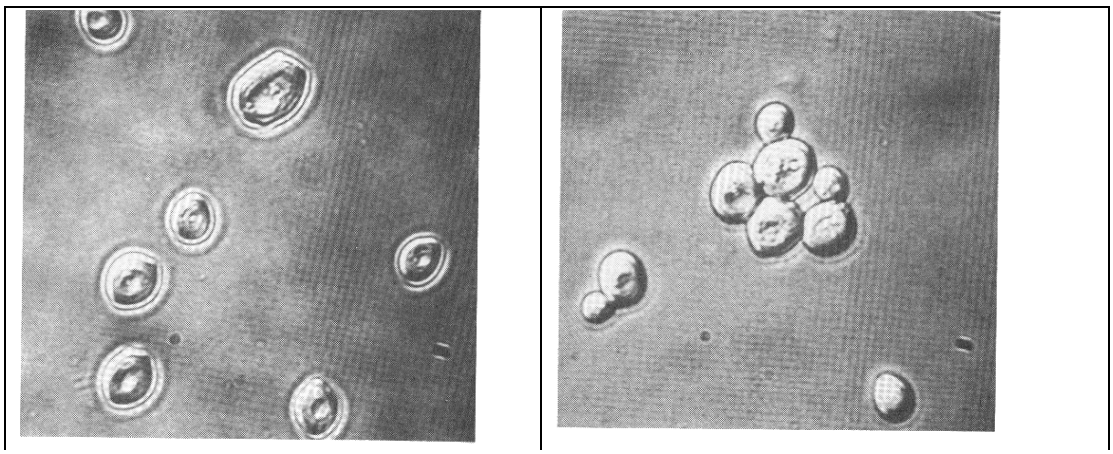


Figure.3. The aspects of SC cells before (left) and after (right) PDT treatment

(T.H.Tran-Thi 1992, P. Job 1928).

4. CONCLUSION

The heterodimerization based on pairing porphyrins and phthalocyanines bearing appositelly charge substituents, yields to the final heterodimers having higher efficiency and stability.

The hetero-aggregation will occur only between Cl- substituents of the porphyrin and the sodium ion from the carboxilated substituents of the phthalocyanine ring.

The heterodimerization was investigated by spectral methods (UV-Vis,IR spectroscopy) and the final heterodimer was tested as photosensitizer by DPBF test, the best being the system RhTMPyP/ZnTCPc.

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