Spectral Properties of Cooper (II) and Zinc (II) Complexes with Mesoporphyrinic Ligands in Micellar Media

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Several Cu(II) and Zn(II) complexes with mesoporphyrinic ligands were studied for their molecular absorption and fluorescence properties in different solvents and in Triton X-100 direct and reverse micelles in order to better understand the possible localization of the porphyrinic compound at the molecular level on the living cell. The experiments intended to mimic the chemical–physical relationship between microenvironments in organism and the amphiphilic properties of porphyrins for photodynamic therapy drugs design. The results obtained from the spectral studies indicate for all compounds a localisation at the interface between the polyethylene oxide chains and the tert-octyl-phenyl etheric residue of the Triton X-100 molecules. The Zn (II) complexes seems to be situated in a more hydrophobic area of the polyethyleneoxidic chains as compared to the corresponding Cu(II) complexes.

Keywords: mesoporphyrinic complexes, micelles, Triton X-100, spectroscopy

The continuous interest lately associated to the properties exhibited by porphyrins and metalloporphyrins in micellar media comes from the possible use of such compounds in photodynamic therapy (PDT) and as contrast agents in magnetic resonance imaging of solid tumors [1].

The use of PDT as in the therapy of cancer is mostly attractive because of its fundamental specificity and selectivity [2]. Accordingly, PDT is based on the administration of a pharmaceutical form of a given photosensitizer having a high affinity to malignant tissues and other non-normal cells; the subsequent exposure to visible light of the photosensitizer in the presence of oxygen specifically inactivates neoplastic cells. Despite significant advantages, the porphyrinic compounds as photosensitizers have a few limitations. Due to the large π conjugate systems, they easily form aggregates, which have a significantly lower ability to form reactive oxygen species and consequently decrease the photodynamic activity. In recent years, nanostructured materials such as liposomes, nanoparticles and micelles have been considered as potential carriers for porphyrinic compounds that may resolve the aforementioned problems [3-7]. Therefore, before the study of porphyrin compounds in pharmaceutic formulation, it is necessary to study the spectroscopic and aggregation properties of these compounds in membrane mimetic media, such as micelles, in order to determine the factors that modulate porphyrin - membrane interactions.

Micelles are simplified models of the biological membranes and they are characterized by an approximate spherical or spheroidal shape possessing significant flexibility. The presence of polar headgroups and hydrophobic chains in micellar structures allows one to study the potential affinity of a porphyrinic structure to cell-membrane type systems [8–14].

Newly developed complexes with Cu(II) and Zn(II) of unsymmetrically and symmetrically substituted porphyrins were studied in micellar solutions of *tert*-octylphenoxy-polyethoxyethanol (Triton X-100) [15-17]. The abilities of the porphyrinic compounds to localize in these micelles were determined by UV-Vis and fluorescence spectroscopy.

The following compounds (fig.1) were studied: 5,10,15,20–meso-*tetrakis*-(4-carboxymethylphenyl)-21,23-Zn(II) porphine, (Zn(II)TCMP), 5-(3-hydroxyphenyl)-10,15,20-*tris*-(4-carboxymethylphenyl)-21,23-Zn(II) porphine, (Zn(II)TCMPOH$_m$), 5,10,15,20–meso-*tetrakis*-(4-carboxymethylphenyl)-21,23-Cu(II)porphine, (Cu(II)TCMP), 5-(3-hydroxyphenyl)-10,15,20-*tris*-(4-carboxymethylphenyl)-21,23-Cu(II)porphine, (Cu(II)TCMPOH$_m$).

The spectral properties of the four porphyrinic complexes compounds in Triton X-100 micellar systems were studied and the results compared to reference homogeneous systems, which mimics the local micellar micropolarity. For this purpose comparative spectra of metalloporphyrins in polyethyleneglycol (PEG 300) as well as the spectra of the same compounds dissolved in different organic solvents (cyclohexane, methanol, dimethylsulfoxide and dichloromethane) were also registered.

Experimental part

Materials and methods

Porphyrinic ligands and their complexes with Cu(II) and Zn(II) were synthesized as previously described [15-17]. The organic solvents (methanol, dimethylsulfoxide, dichloromethane, cyclohexane) were purchased from Sigma-Aldrich and used without any further treatment. The water used was previously doubly distilled.

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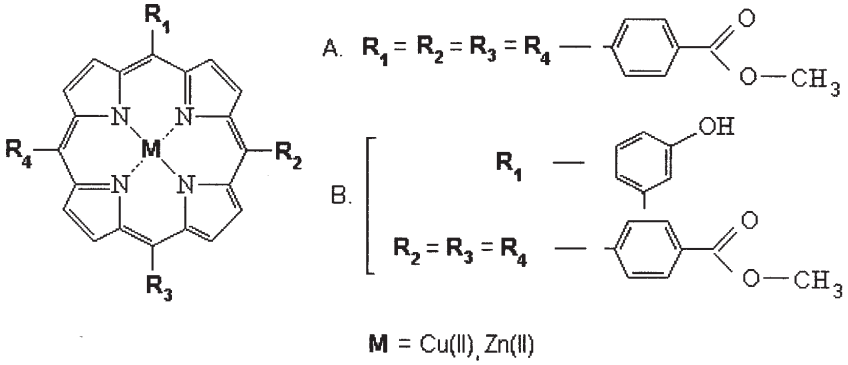

Fig. 1. Structures of the metalloporphyrins used in this study

A = 5,10,15,20–meso-*tetrakis*-(4-carboxymethylphenyl)-21,23-M(II) porphine (TCMP)

B =5- (3-hydroxyphenyl)-10,15,20-*tris*-(4-carboxymethylphenyl)-21,23-M(II) porphine ($TCMPOH_m$)

For the micellar solutions the surfactant *tert*-octylphenoxypolyethoxyethanol (Triton X-100, Sigma, >99% purity) was used. For this purpose 0.24 mM Triton X-100 in water direct micelles (DM) and 0.66 M Triton X-100 in cyclohexane reverse micelles (RM) solutions were prepared [18]. As reference, polyethylene glycol having an average molecular weight of 300 was used.

Appropriate volumes of metalloporphyrins in dichloromethane solutions were evaporated to dryness at room temperature on the bottom of a test tube. Aliquots of 3mL of Triton X in water and cyclohexane were added, and then the tubes were mildly vortex mixed for 5 minutes, capped and then left still overnight to ensure the solubilisation and diffusion of the metalloporphyrins into the micelles. The final concentration of each porphyrinic compound in micellar media was set at 2.5×10^{-6} M; the solutions were kept in dark to prevent photodegradation before the measurements, which were performed 24 h after preparation.

Results and discussions

Microheterogeneous systems such as micelles are frequently used as interesting models to mimic the water pockets that are often found in various bioaggregates such as proteins, enzymes and membranes [10, 11, 19-26]. Also, water-soluble and water-insoluble porphyrinic compounds can be inserted simultaneously in micellar systems. The results are expected to lead to a better understanding of the photodynamic process induced by amphiphilic porphyrins and their potential application as PDT agents.

UV-Vis spectra

In all media, the tested metalloporphyrins exhibits a characteristic, strong Soret band in the spectral range of 412-420 nm. Furthermore, they present one (for copper complexes) or two (for zinc complexes) Q bands in the region from 538 to 600 nm (table 1). In all the studied systems (organic solvents and Triton-X 100 micelles) and for the concentrations used, the metalloporphyrin was not in a dimer aggregation state form, which can be observed clearly from the spectral profile of Q and Soret bands (not shown) in the micellar media [27].

The batochromic shifts observed for the UV absorption bands are due to the porphyrins incorporation in the micellar media [28].

The analysis of the spectal data obtained for the Cu (II) and Zn (II) porphyrinic compounds in micellar media and in solvents with different polarities (table 1) lead to the following observations performed on the basis of the Soret and Q bands position.

The absorption spectra of Cu(II) mesoporphyrinic compounds exhibit a slight hypsocromic shifts in Triton X-100 DM as compared to the reference PEG 300 media (table 1); also, their Soret bands are bathochromically shifted in methanol (the most polar solvent used). That can be due to the localisation of Cu(II) complexes in the area of the polyethyleneoxidic chains of Triton X-100.

The Zn(II) complexes Soret band exhibit a slight batochromic shift in Triton X-100 DM as compared to their corresponding spectra in PEG 300; they also are bathochromically shifted as compared to the methanolic solutions.

The above observations indicate a localisation of the Zn (II) complexes in Triton X-100 DM in a more hydrophobic area of the polyethyleneoxidic chains as compared to the corresponding Cu(II) complexes.

The same spectral parameters (table 1), being very close to those obtained in PEG 300, sugesting a localisation of all compounds rather at the interface between the polyethylene oxide chains and the *tert*-octylphenyletheric residue of the Triton X-100 molecule, and not to the interface between the oxyethylene chains and water.

In Triton X-100 RM, the Soret bands are situated at the same wavelengths as those in PEG 300 (table 1), indicating a localisation of the complexes inside the micelles in the area of oxyethylenic chains.

Fluorescence spectra

The results obtained via the fluorescence spectra obtained for the Zn (II) porphyrinic complexes in micellar media and in different polarities organic solvents are presented in figure 2-3 and table 2.

Refered at the first band, for the symetrical tetrapyrollic structure with Zn(II) ion, a batochromic shift in Triton X-100/water micellar systems was registered as compared to PEG 300 systems and methanolic solution, suggesting a localisation of the complex Zn(II)TCMP in a less polar area of the micelle compared to the localisation of $Zn(II)TCMPOH_m$.

For $Zn(II)TCMPOH_m$ in Triton X-100/water micellar media, the emission maxima are situated between PEG 300 and methanol, indicating a higher local polarity, thus a localisation in a domain of the oxiethylenic chains having a greater hydration degree. That is due to the more hydrophile character of $Zn(II)TCMPOH_m$ granted by the presence of the *meta*-hydroxyphenylic substituent.

In Triton X-100/cyclohexane micellar systems, the fluorescence spectra of Zn(II)TCMP and $Zn(II)TCMPOH_m$ confirm their localisation in the area of the polyethyleneoxidic chains, as the maximum wavelength is situated very close to that one recorded in the reference system of PEG 300. $Zn(II)TCMPOH_m$ exhibits the fluorescence peak at a wavelength very close to that one

Table 1

ABSORPTION MAXIMA WAVELENGTHS (λ_{max} AND EXTINCTION MOLAR COEFFICIENTS (logε) OF THE PORPHYRINIC COMPLEXES IN DIFFERENT SOLVENTS AND MICELLAR MEDIA (c=2.5 x 10^{-6} M)

Solvent	$\lambda_{max(nm)}$/[logε] (L mol^{-1} cm^{-1})		
	Soret band B(0,0)	Qy(1,0)	Qy(0,0)
	Cu(II)TCMP		
MeOH	412 [5.637]	538 [4.024]	
CH_2Cl_2	416 [5.697]	539 [4.387]	
DMSO	422 [5.582]	544 [4.334]	
Chx	414 [5.773]	538 [4.350]	
PEG300	417 [5.709]	540 [4.423]	
TX/ H_2O	416 [5.704]	539 [4.447]	
TX/Chx	417 [5.675]	538 [4.505]	
	Zn(II)TCMP		
MeOH	424 [5.591]	557 [4.146]	597 [3.806]
CH_2Cl_2	422 [5.618]	549 [4.274]	588 [3.778]
DMSO	431 [5.634]	560 [4.428]	601 [4.000]
Chx	425 [5.616]	558 [4.170]	598 [3.778]
PEG300	427 [5.596]	559 [4.236]	598 [4.017]
TX/ H_2O	428 [5.595]	560 [4.146]	601 [3.857]
TX/Chx	427 [5.598]	560 [4.146]	600 [3.857]
	Cu(II)TCMPOH$_m$		
MeOH	413 [5.486]	538 [4.255]	
CH_2Cl_2	416 [5.598]	539 [4.310]	
DMSO	421 [5.468]	544 [4.265]	
Chx	413 [5.570]	538 [4.134]	
PEG300	416 [5.464]	538 [4.049]	
TX/ H_2O	415 [5.580]	538 [4.236]	
TX/Chx	415 [5.571]	538 [4.182]	
	Zn(II)TCMPOH$_m$		
MeOH	424 [5.725]	557 [4.326]	598 [3.924]
CH_2Cl_2	421 [5.677]	548 [4.365]	586 [3.806]
DMSO	430 [5.688]	561 [4.318]	602 [4.017]
Chx	425 [5.743]	557 [4.255]	596 [3.748]
PEG300	426 [5.740]	558 [4.274]	598 [3.852]
TX/H_2O	427 [5.695]	559 [4.318]	599 [3.982]
TX/Chx	426 [5.711]	559 [4.265]	598 [3.857]

MeOH = methanol, CH_2Cl_2 = dichloromethane, DMSO = dimethylsulfoxide, Chx =cyclohexane, PEG300 = polyethylenglycol300, TX=Triton X-100

of the fluorescence spectrum in methanol, the most polar solvent used, thus supporting its localisation in the more hydrophilic part of the ethyleneoxydic chain.

The analysis of the fluorescence spectra of Zn (II) porphyrinic complexes in different solvents reveal smaller shifts of the peaks due to the solvent polarity for Zn(II)TCMPOH$_m$ compared to Zn(II)TCMP.

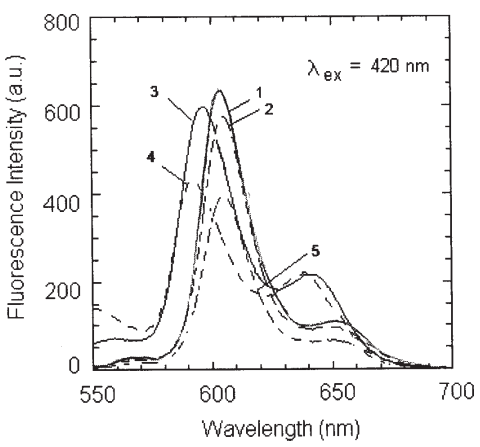

Fig. 2. Fluorescence spectra of Zn(II)TCMP (2.5 x 10^{-6} M) in different media (1-methanol, 2-Triton X-100 DM, 3-dichloromethane, 4-cyclohexane, 5-Triton X-100 RM)

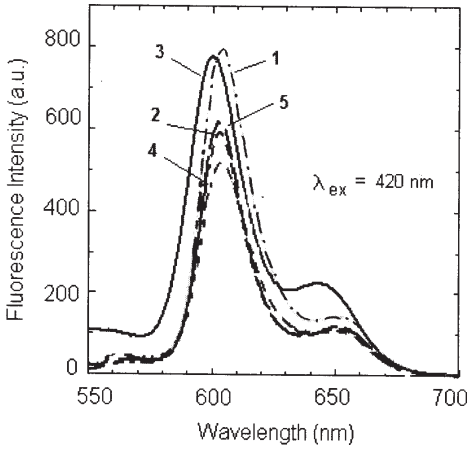

Fig. 3. Fluorescence spectra of Zn(II)TCMPOH$_m$ (2.5 x 10^{-6} M) in different media (1-methanol, 2-Triton X-100 DM, 3-dichloromethane, 4-cyclohexane, 5-Triton X-100 RM)

Table 2
THE FLUORESCENCE SPECTRAL PARAMETERS (λ_{max} aND FLUORESCENCE INTENSITY I_f) OF ZN(II)TCMP AND ZN(II)TCMPOH$_M$ IN SOLVENTS WITH DIFFERENT POLARITIES AND MICELLAR MEDIA (c=2.5x10^{-6} M, λ_{ex}=420 nm)

Solvent	λ_{max} (nm)/[I_f] (a.u.)	
	Zn(II)TCMP	
MeOH	603 [637.2]	652 [111.0]
CH_2Cl_2	608 [505.7]	653 [73.6]
DMSO	597 [598.0]	641 [216.0]
Chx	592 [430.5]	638 [222.3]
PEG300	604 [547.0]	653 [79.7]
TX/H_2O	605 [577.0]	650 [96.3]
TX/Chx	605 [395.0]	651 [66.9]
	Zn(II)TCMPOH$_m$	
MeOH	604 [801.4]	652 [147.9]
CH_2Cl_2	608 [470.5]	657 [72.2]
DMSO	600 [770.5]	651 [109.2]
Chx	604 [517.8]	651 [104.2]
PEG300	602 [517.0]	650 [90.5]
TX/ H_2O	603 [593.4]	650 [114.2]
TX/Chx	603 [613.3]	651 [120.0]

MeOH = methanol, CH_2Cl_2 = dichloromethane, DMSO = dimethylsulfoxide, Chx =cyclohexane, PEG300 = polyethylenglycol300, TX=Triton X-100

Conclusions

In this paper was monitored the ability of the metalloporphyrinic complexes with Cu(II) and Zn(II) to localize in different micellar media, using UV-Vis and fluorescence spectroscopy methods. Micelles as nanostructured materials prove to be interesting host systems for the metalated porphyrinic forms. This will set the basis for the future choice of porphyrin-carrier dyade for the potential pharmaceutical formulation.

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References

1. KADISH, K.M., MAYA G,.B., ARAULLO, C., GUILARD, R., Inorg. Chem., **28**, 1989, p. 2725
2. ALEXIADES-ARMENAKAS, M., Clinics in Dermatol., **24**, 2006, p. 16
3. LAI, PS, LOU, PJ, PENG, CL, PAI, CL, YEN WN, HUANG, MY, et al. J Control Release, **122**,. 1, 2007, p. 39

4. TAKEUCHI, Y, ICHIKAWA, K, YONEZAWA, S, KUROHANE, K, KOISHI T, NANGO M, et al., J Control Release, **97**, 2, 2004, p. 231
5. VARGAS, A, PEGAZ, B, DEBEFVE, E, KONAN-KOUAKOU, Y, LANGE, N, BALLINI, JP, et al., Int J Pharm, **286**, 1–2, 2004, p. 131
6. IDETA R, TASAKA F, JANG, WD, NISHIYAMA N, ZHANG, GD, HARADA, A, et al. Nano Lett, **5**, 12, 2005, p. 2426
7. JANG WD, NAKAGISHI Y, NISHIYAMA N, KAWAUCHI S, MORIMOTO Y, KIKUCHI M, et al., J Control Release, **113**, 1, 2006, p. 73
8. SANTIAGOA, P. S., NETOA, D. S., GANDINIA S,.C.M., TABAK, M., Colloids and Surfaces B: Biointerfaces, **65**, 2008, p. 247
9. BORISSEVITCH,. I.E. BORGES C.P.F., BORISSEVITC G.P., YUSHMANOV V.E., LOURO, S.R.W., TABAK, M., Z. Naturforsch J. Biosci. **51**, 1996, p. 578
10. CAETANO, W., TABAK, M., J. Colloid Interface Sci., **225**, 2000, p. 69
11. PERUSSI, J.R., YUSHMANOV, V.E., MONTE, S.C., IMASATO, H., TABAK, M., Physiol. Chem. Phys. Med., **27**, 1995, p. 1
12. GANDINI, S.C.M., YUSHMANOV, V.E., BORISSEVITCH, I.E., TABAK, M., Langmuir, **15**, 1999, p. 6233
13. GANDINI, S.C.M., YUSHMANOV, V.E., TABAK, M., J. Inorg. Biochem., **85**, 2001, p. 263
14. MAITI, N.C., MAZUMDAR, S., PERIASAMY, N, J. Phys. Chem. B, **102**, 1998, p. 1528
15. SOCOTEANU, R., BOSCENCU, R**.**, NACEA, V., SOUSA OLIVEIR,A A., VIEIRA, FERREIRA, L.F., Rev. Chim. (Bucuresti), **59**, no. 8, 2008, p. 498
16. BOSCENCU, R., SOCOTEANU, R., ILIE, M., SOUSA, OLIVEIRA A., CONSTANTIN C., VIEIRA FERREIRA L. F, Rev. Chim. (Bucuresti), **60,** no.. 10, 2009, p.1006
17. BOSCENCU, R., ILIE, M., SOCOTEANU, R., SOUSA OLIVEIR,A A., CONSTANTIN C., VIEIRA FERREIRA L. F. ,J. Serb. Chem. Soc., submitted Jully 2009
18. CARAGHEORGHEOPOL, A., BANDULA, R., CALDARARU, H., JOELA H., J.Molecular Liquids, **72**, 1997, p. 105
19. SCALISE, I., DURANTINI, E. N., Journal of Photochemistry and Photobiology, A: Chemistry**, 162,** 2004, p. 105
20. MILANESIO, M.E., ALVAREZ, M.G., YSLAS, E.I., BORSARELLI, C.D., SILBER J.J., RIVAROLA V., DURANTINI E.N., Photochem. Photobiol., **74**, 2001, p.14
21. LA PENNA, M., ALVAREZ, M.G., YSLAS, E.I., RIVAROLA, V., DURANTINI, E.N.,Bioorg. Chem., **29,** 2001, p. 130
22. MILANESIO, M.E., MORAN, M.S., YSLAS, E.I., ALVAREZ, M.G., RIVAROLA V., DURANTINI E,.N., Bioorg. Medicinal Chem., **9**, 2001, p. 1943
23. VILLANUEVA, A., DURANTINI, E.N., STOCKERT, J.C., RELLO, S., VIDANIA, R., CAÑETE, M., JUARRANZ, A., ARRANZ, R., RIVAROLA, V., Anti-Cancer Drug Design, **16**, 2002, p. 279
24. CHAUHAN, S.M.S., MOHAPATRA, P.P., KALRA, B., KOHLI, T.S., SATAPATHY, S., J. Mol. Catal. A: Chem., **113**, 1996, p. 239
25. CHAUHAN, S.M.S., SAHOO, B.B., Bioorg. Med. Chem., **7**, 1999, p. 2629
26. YU, J.H., WENG Y.X., WANG, X.S., ZHANG, L., ZHANG, B.W., CAO, Y., J. Photochem. Photobiol. A: Chem., **156**, 2003, p. 139
27. SIBATAA, M.N., TEDESCOB, A.C., MARCHETTIA, J.M., European Journal of Pharmaceutical Sciences, **23**, 2004, p. 131
28. BALASUBRAMANIAM, E., NATARAJAN, P., J. Photochem. Photobiol. A: Chem., **103**, 1997, p. 201

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