Ytterbium-porphyrins as a new class of the luminescent labels

M Tsvirko¹, Yu Korovin² and N Rusakova²
¹ Institute of Chemistry and Environmental Protection, Jan Dlugosz University, 13/15 Armii Krajowej Av., Częstochowa, Poland
² A.V.Bogatsky Physico-Chemical Institute of the National Academy of Sciences of Ukraine, 86 Lustdorfskaya doroga, 65080 Odessa, Ukraine

m.tsvirko@ajd.czest.pl

Abstract. New complexes of ytterbium with asymmetric porphyrins containing substituents in β-positions and hydrophobic meso-(monophenyl-p-oxypropyl)triphenylporphyrin (OPP) were obtained and characterized by elemental analysis, IR, UV-Vis absorption and luminescence spectroscopy. Electronic absorption, luminescence and luminescence excitation spectra of these complexes were studied at 295 K in DMF solutions and in the water-lecithin medium. The 4f-luminescence of ytterbium-porphyrins in the near infrared (IR) spectral region (λmax = 980 nm) is observed under excitation in Soret band (400-430 nm). The effect of substituent in porphyrin macroring on the 4f-luminescent properties was also investigated. The conjugates of these compounds with protein molecules - bovine serum albumin (BSA) were investigated as well. These compounds are interesting at the initial stage of diagnostics of tumor tissues as IR-luminescent probes due to their spectral-luminescent characteristics and some biochemical properties.

1. Introduction

The near-IR luminescence of lanthanide ions, appears to be especially promising (in particular, at the biomedical investigations due to the relative transparency of tissue at these wavelengths). In recent years the potential of near IR luminescence has increased enormously due to the introduction of new methods of investigations and applications [1].

We would like to outline perspectives first of all porphyrins as luminescent labels in near IR - region because they can form stable complexes with lanthanide ions and have high absorbance [2]. Moreover, it is known that porphyrins plays the important role in different biological systems and their properties can be adjusted by corresponding modifications of the electronic distribution on the heteroaromatic ring via substitution in meso- and/or β-positions.

Nevertheless, till now only few attempts [3, 4] were undertaken to find out how the nature of porphyrins (type of substituents and extra-ligands, symmetry, distribution of charges in a macroring) influences on intensity of 4f-luminescence. Besides, most investigations are dedicated to porphyrins with limited structural variation (with symmetrical tetrasubstitution, as a rule) [5, 6].

In this paper we report study the complexes of ytterbium with some porphyrins containing the asymmetric substituents. Synthesis of ytterbium complexes with porphyrins is caused by that from all of
lanthanide series only Yb$^{3+}$ ions are capable to show the strong 4f-luminescence in these objects [7]. Additionally, if ytterbium-porphyrins will be used as tags in bio-medical investigations, then asymmetric ligands will allow a proper linkage via covalent bonding between the label and the target biomolecule.

2. Experimental
All starting reagents and solvents were obtained from commercial sources and used without additional purification. The porphyrins P$_1$-P$_7$ (figure 1), meso-(monophenyl-p-oxypropyl)triphenylporphyrin (OPP) and Yb-OPP complex (figure 2) were selected after preliminary screening in the series of similar compounds with the account of spectral-luminescent characteristics.

**Figure 1.** Structures of the porphyrins P$_1$-P$_7$.

**Figure 2.** Structure of the Yb-OPP complex.

The ytterbium complexes were prepared by a modified method described earlier [8] through the interaction of a 5 to 10-fold excess of Yb(acac)$_3$ (acac - is acetylacetone as an extra-ligand) and free porphyrin (P$_1$-P$_7$) in 1,2,4-trichlorobenzene on boiling under nitrogen atmosphere for 4-7 h depending on porphyrin. The solid residues were vacuum dried overnight, dissolved in methanol-chloroform and applied into column with neutral Al$_2$O$_3$. Then the complexes (the following ratio Yb:P$_n$ = 1:1) were eluted with DMF (concentration 5×10$^{-5}$ mol/L were used for the spectral-luminescent measurements). The purity of the compounds was controlled by TLC chromatography, UV-Vis and IR spectroscopy. The complexation was confirmed by the characteristic absorption spectra and elemental analysis data (the difference between calculated and measured values does not exceed 0.2% for C and H, 0.3% for N and Yb).

Bilayer vesicular membranes with the encapsulated porphyrin (or Yb-porphyrin) were prepared by method [9] in a following way: solutions of lipid were mixed (lecithin was used as such one) and porphyrin in a weight ratio 9:1 in mixture chloroform : methanol = 5:1 and evaporated in vacuum to dry. After water addition the sample was shaken 10 min and obtained dispersion was treated at the temperature 278 K 15 min by ultrasound at frequency 22 kHz. Then lipid dispersion was centrifuged 10 min and used for the spectral research. Conjugation of OPP and Yb-OPP was realized by carbodiimide method according to [10]. The conjugate content was determined by the spectrophotometric method [10].

All absorption and corrected luminescence spectra were recorded at temperature of 295±1 K with the use of UV-Vis spectrophotometer Lambda 9 (Perkin-Elmer), SDL-2 and Fluorat-02-Panorama spectrofluorimeters (Lumex, St.-Petersburg, Russia) with a Nd:YAG laser and 150 W Xe lamp as the excitation sources. The relative quantum yields of 4f-luminescence ($\phi$) of Yb$^{3+}$ in the complexes (a methanolic solution of Zn-tetraphenylporphyrin as a standard, $\phi$ = 0.0315) were determined as described previously [7].

3. Results and Discussion
The characteristic changes in the electronic absorption spectra of free porphyrins and complexes were observed. Spectra of porphyrins in DMF solutions are characterized by the presence of strong Soret band and $Q$-bands (I-IV) in the visible region. Correlation of intensities of the absorption $Q$-bands allows the relation of these spectra to the “etio”-type (IV>III>II>I). Two $Q$-bands were observed in spectra of the
complexes instead of four (table 1). The lower-energy band has the electronic origin $Q(0,0)$ of the lowest singlet excited state $S_1$ and the second band is its vibrational overtone - $Q(1,0)$.

**Table 1.** Spectral-luminescent properties of the ytterbium complexes with porphyrins

| Ligand/Complex | Absorption $\lambda_{max}$, nm / $\lg e$ | Lum.$^a$ $\phi \times 10^3$ |
|----------------|----------------------------------------|----------------------------|
|                | IV                                      | III                                     | II                                      | I                                      |
| P1             | 429.4/5.35                             | 522.4/4.28                             | 561.1/3.94                             | 600.2/3.80                             | 658.7/3.62                             | 1.3                                      |
| Yb-P1          | 427.8/4.61                             | 554.2/3.80                             | 592.6/3.19                             | 602.9/3.20                             | 645.3/3.76                             | 4.7                                      |
| P2             | 432.3/5.42                             | 524.6/4.44                             | 566.2/4.13                             | 603.1/3.98                             | 640.4/3.76                             | 0.9                                      |
| Yb-P2          | 428.9/4.63                             | 561.2/3.89                             | 592.6/3.20                             | 602.9/3.20                             | 645.3/3.76                             | 0.9                                      |
| P3             | 421.4/5.26                             | 517.8/4.00                             | 553.3/3.58                             | 595.5/3.45                             | 651.3/3.38                             | 0.9                                      |
| Yb-P3          | 429.4/4.24                             | 561.8/3.29                             | 599.4/3.00                             | 651.8/3.75                             | 651.8/3.75                             | 0.9                                      |
| P4             | 420.7/5.46                             | 519.2/4.33                             | 553.6/3.92                             | 595.9/3.80                             | 651.8/3.75                             | 0.9                                      |
| Yb-P4          | 430.2/3.62                             | 559.0/2.79                             | 599.5/2.59                             | 651.8/3.75                             | 651.8/3.75                             | 0.9                                      |
| P5             | 428.2/5.37                             | 523.1/4.34                             | 563.2/4.05                             | 602.5/3.96                             | 658.0/3.85                             | 0.5                                      |
| Yb-P5          | 418.6/3.16                             | 498.1/2.56                             | 537.5/1.63                             | 621.4/2.38                             | 621.4/2.38                             | 0.4                                      |
| P6             | 407.2/4.16                             | 498.7/3.87                             | 535.0/3.95                             | 574.3/3.91                             | 621.4/2.38                             | 0.4                                      |
| Yb-P6          | 403.3/2.90                             | 459.6/2.68                             | 550.0/1.48                             | 621.0/3.94                             | 621.0/3.94                             | 0.4                                      |
| P7             | 405.1/5.27                             | 497.5/4.30                             | 530.2/4.15                             | 567.5/4.02                             | 621.0/3.94                             | 0.4                                      |
| Yb-P7          | 400.3/2.96                             | 470.0/2.70                             | 502.1/1.95                             | 621.0/3.94                             | 621.0/3.94                             | 0.4                                      |

$^a$Errors in measurement of luminescence quantum yield are $\pm 15\%$.

In complexes with porphyrin-chalkones (P1 and P2) the Soret band is shifted (blue shift), decreasing at the same time on intensity due to significant disturbance of the planarity of complexes. The similar effect was observed for meso-$\beta$-substituted ytterbium-porphyrins (Yb-P5-Yb-P7) as well. At the same time, the absorption bands of complexes with pyrrole derivative porphyrin (Yb-P3) have the red shift in comparison with oxygen-containing complexes (Yb-P6 and Yb-P7).

The 4f-luminescence of Yb$^{3+}$ ions in the complexes is observed at 960-1040 nm (main maximum at 980 nm, electronic transition $^2F_{5/2} \rightarrow ^2F_{7/2}$) on the excitation in a wide spectral range (300-600 nm). However, the greatest luminescence efficiency is achieved at excitation in the maximum of Soret band (400-430 nm). The coincidence between the absorption and excitation spectra of the complexes clearly shows that the ligand-to-lanthanide ion energy transfer from the triplet levels of porphyrins (“antenna-chromophore”) to the resonance $^2F_{5/2}$-level of Yb$^{3+}$ (10200 cm$^{-1}$) takes place. It should be noted that absorption maxima of ytterbium-porphyrins Yb-P1,7 have extinction coefficients $10^4 - 10^5$ M$^{-1}$cm$^{-1}$ that almost four order higher than the extinction coefficients for direct excitation of the near IR transition of the Yb$^{3+}$ themselves. Hence, porphyrin-excitation provides a much more efficient pathway to obtain strong 4f-luminescence of Yb$^{3+}$ ions than direct excitation. All free porphyrins display molecular luminescence (two broad bands with maxima c.a. at 650 and 720 nm). This luminescence is practically supressed in complexes confirming effective energy transfer from the organic (i.e. porphyrin) moiety of the complex to the lanthanide ion.

The highest 4-f luminescence quantum yield $\phi$ is observed for complexes Yb-P2 (with “secondary” phenyl substituent), and Yb-P5 (with pyrrole substituent) (table 1). For the complexes with P1-P4 these data confirm the action of aromatic chromophores as the “photoantennae” accumulating of excitation energy [11]. Absence of similar effect in complexes with porphyrin-pyrazoles Yb-P1 and Yb-P4 most likely due to

[3]
substituents in P3 and P4 can shield a cavity of a macrocycle, complicating the complexation process. The correctness of the given assumption has been proved by essentially greater surplus of Yb(acac)3 used at synthesis of these complexes.

The hydrophilicity of substance administrated in organism is not obligatory requirement for the medicinal practice. The overwhelming majority of synthetic porphyrins and their metal complexes are slightly soluble in water. But this factor is eliminated when liposomal forms of metal porphyrins are used for the administration in organism (or at the stage of preliminary research). It is known [12] that hem molecules (i.e. porphyrins) in natural enzymes are located in hydrophobic cavities which are organized in specific manner. Therefore research of different properties of porphyrin molecules is important task at the modeling of membrane bioprocess.

We have studied spectral-luminescent properties of hydrophobic OPP and complex Yb-OPP which with the help of water-lecithin dispersion was possible to transfer into water phase as well as conjugates of these compounds with protein molecules - bovine serum albumin (BSA). The choice of albumin was caused by possibility of its covalent bounding with complex Yb-OPP. Latest have the high value of 4f-luminescence quantum yield ($\phi = 4.0 \times 10^{-3}$). The $\phi$ is practically equal to such one for the complex of ytterbium-tetraphenylporphyrin ($\phi = 4.2 \times 10^{-3}$), but it is 7.6 - 8.4 times higher that $\phi$ for the natural Yb-porphyrins reported before [13, 14].

To evaluate degree of sorption (non-covalent) binding of BSA with Yb-OPP the control model experiment was carried out. The conjugation of BSA was realized with analogous complex where carboxylic group was non-activated. It was established that for such non-covalent conjugate the number of porphyrin molecules related to one protein molecule is lesser than 0.1. Thus, the share of nonspecifically bounded porphyrin is small in conjugate Yb-OPP-BSA.

Comparison of absorption spectra of water-lecithin dispersions OPP and Yb-OPP (pH=6-7) with their spectra in chloroform-methanol mixture demonstrated that firsts ones have insignificantly been shifted into short-wave region relatively the second ones. Moreover, broadened Soret band in chloroform-methanol solution can indicates aggregation of porphyrin molecules.

It has been established that 4f-luminescence quantum yield of Yb-OPP complex in water-lecithin dispersion is lower as compared to such one for the methanol solvent of complex - $2.1 \times 10^{-3}$ and $4.0 \times 10^{-3}$, respectively. At the comparison of spectral-luminescent characteristics of Yb-OPP complexes (nonbounded and conjugated with BSA) it is noteworthy that their absorption spectra are similar. The difference is that a band with a maximum at 225 nm is appeared in conjugate spectrum that proves modifications only in linker chain ($p$-oxy-propyl) of porphyrin cycle.

Coincidence of excitation spectra of 4f-luminescence with the absorption spectra of Yb-OPP and Yb-OPP-BSA complexes and complete absence of its low temperature phosphorescence proves the efficient energy transfer to the level $^3F_{5/2}$ of Yb$^{3+}$ ions. In these complexes the molecular luminescence is observed, although its intensity is not high. As to 4f-luminescence, then in conjugate it keeps (figure 3) that proves that there is no destruction of complex in result of joining of Yb-porphyrin to protein. Secondly, lesser intensity of conjugate signal indicates that due to the large molecular mass of BSA and insignificant quantity of graft-molecules of Yb-complex (no more than 14) the concentration of the later in conjugate solution is not high.

Afterwards it was established that considered porphyrins and their Yb-complexes kept their luminescent characteristics in protein media, it seemed reasonable to realize some of toxicological research, because namely since this stage the appropriateness of substance as a medicinal agent must be determined. In particular, we have realized assessment of sharp toxicity of free base OPP and complex Yb-OPP. LD$_{50}$ was 250 and 160 mg/kg, respectively (15 mice were used in experiments).

Investigation of phototoxicity of above listed compounds was realized as well. With this aim the substances in doze 200 mg/kg were intraperitoneally administrated into experimental mice. The surface of skin was enlightened with the help of lamp 100 W from 50 cm distance. The animals’ state and behavior were fixed through the certain time period.
Figure 3. 4f-Luminescence spectra of Yb-OPP (1) and Yb-OPP-BSA (2); \( \lambda_{\text{exc}} = 420 \text{ nm} \).

From the comparison of data on the intact and experimental animals it could be concluded that OPP influences phototoxically, however, it is substantially lower as compared to hematoporphyrin which is used in clinical practice [9]. As to Yb-OPP, then its phototoxicity was not founded practically.

Distribution of Yb-OPP in organism was investigated at the final stage of research. Complex was administrated intraperitoneally (75 mg/kg doze) to 36 mice of the BALB/c series with the induced by methylcholanthrene tumor. On the base of dissection results it was concluded that Yb-porphyrin is mainly accumulated in tumor tissues, namely in integument tissues of both skin and the lungs.

4. Conclusions

The new ytterbium-porphyrins were synthesized. It was shown that the efficiency of the 4f-luminescence in these compounds depends on the nature of asymmetric substituents in porphyrin macroring. The IR-luminescence of BSA conjugates with \textit{meso}-(monophenyl-p-oxypropyl)triphenyl-porphyrin and its Yb-complex have been investigated. We have demonstrated that these conjugates possess enough high luminescence quantum yields. Therefore we hope that investigated ytterbium-porphyrins shold to attract a wide attention a luminescent labels.

5. Acknowledgement

The presented work was supported by the Scientific Grant from “Fundamental Researches Joint Projects Competition, BRFFI - UFFI - Program”.

References

[1] Bünzli J-C G and Piguet C 2005 Taking advantage of luminescent lanthanide ions \textit{Chem. Soc. Rev.} \textbf{34} 1048
[2] Gouterman M 1978 The Porphyrins, vol 3 (Academic Press)
[3] Korovin Yu, Zhilina Z, Rusakova N, Kuzmin V, Vodzinsky S and Ishkov Yu 2001 Spectral-luminescent effects in heterometallic complexes of crown-porphyrins \textit{J. Porphyrins Phthalocyanines} \textbf{5} 481
[4] He H S, Wong W K, Li K F and Cheah K W 2004 Synthesis, characterization and photoluminescence properties of monoporphyrinate lanthanide complex \textit{Synth. Met.} \textbf{143} 81
[5] Foley T J, Harrison B S, Knefely A S, Abboud K A, Reynolds J R, Schanze K S and Boncella J M 2003 Facile preparation and photophysics of near-infrared luminescent lanthanide(III) monoporphyrinate complexes \textit{Inorg. Chem.} \textbf{42} 5023
[6] Dargiewicz-Nowicka J, Makarska M, Villegas M A, Legendziewicz J and Radzki S 2004 Photophysics of the porphyrins; unusual fluorescence of europium porphyrin complex entrapped in sol-gel silica matrix \textit{J. Alloys Compd.} \textbf{380} 380
[7] Tsvirko M P, Stermaik G F, Pyatosin V E, Solovyov K N, Kachura T F, Piskarskas A S and Gadonas R A 1986 Fast electronic relaxation in lanthanide porphyrins \textit{Chem. Phys.} \textbf{106} 467
[8] Wong C P, Venteicher R F and Horrocks W de W, Jr 1974 Lanthanide porphyrin complexes. A
potential new class of nuclear magnetic dipolar probe J. Am. Chem. Soc. 96 7149

[9] Joshi K and Joshi P 1992 Binding of hematoporphyrin derivative to brain tumor cells - a fluorescence spectroscopic study Photochem. Photobiol. 55 113

[10] Morliere P, Momenteau M, Candide C, Simonin V, Santus R, Maziere J C, Dubertret L, Goldstein S and Hüppe G 1990 Synthesis, cellular uptake and cell photosensitization by a porphyrin bearing a quinoline group J. Photochem. Photobiol. B. 5 49

[11] Alpha B, Lehn J M and Mathis G 1987 Energy transfer luminescence of europium(III) and terbium(III) cryptates of macrobicyclic polypyridine Angew. Chem., Int. Ed. 26 266

[12] He H, Zhao Z, Wong W, Li K, Meng J and Chean K 2003 Synthesis, characterization and near-infrared photoluminescent studies of diethyl malonate appended mono-porphyrinate lanthanide complexes J. Chem. Soc. Dalton Trans. 980

[13] Gaiduk M, Grigoryants V, Mironov A, Rumyantseva V, Chissov V and Sukhin G 1990 Fibre-laser IR luminescence diagnostics of malignant tumours using rare earth porphyrins J. Photochem. Photobiol. B. 7 15

[14] Gaiduk M, Grigoryants V, Mironov A and Rumyantseva V 1991 Spectro-luminescent characteristics and kinetics of Yb\(^{3+}\) complexes of porphyrins Proc. Estonian Acad. Sci. Phys. Math. 40 198