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ABSORPTION AND FLUORESCENCE SPECTRA PARAMETERS OF CATIONIC PORPHYRINS FOR PHOTODYNAMIC THERAPY OF TUMORS

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Determination of a number of spectral parameters of cationic porphyrins for their identification in absorption and fluorescence spectra, as well as in complexes with proteins, is an urgent task in connection with their use in photodynamic therapy of tumors. We have carried out studies and these parameters have been determined for a number of cationic porphyrins and metalloporphyrins.

Cationic porphyrins and metalloporphyrins – photodynamic therapy of tumors – molar extinction coefficient – fluorescence emission wavelength

Կատիոնային պորֆիրինների մի շարք սպեկտրալ պարամետրերի որոշումը դրանց նույնականացման համար կլանման և ֆլուորեսցենտային սպեկտրներում, ինչպես նաև սպիտակուցների կոմպլեքսներում, հրատապ խնդիր է կապված դրանց օգտագործման հետ ուռուցքների ֆոտոդինամիկ թերապիայում: Մեր կողմից իրականացրել են ուսումնասիրություններ և այդ պարամետրերը որոշվել են մի շարք կատիոնային պորֆիրինների և մետաղապորֆիրինների համար:

Կատիոնային պորֆիրիններ և մետաղապորֆիրիններ – ուռուցքների ֆոտոդինամիկ թերապիա – մոլյար էքստինքցիայի գործակից – ֆլյուորեսցենցիայի էմիսիայի ալիքի երկարության

Определение ряда спектральных параметров катионных порфиринов для их идентификации в спектрах поглощения и флуоресценции, а также в комплексах с белками является актуальной задачей в связи с их применением в фотодинамической терапии опухолей. Нами были проведены исследования и эти параметры были определены для ряда катионных порфиринов и металлопорфиринов.

Катионные порфирины и металлопорфирины – фотодинамическая терапия опухолей – коэффициент молярной экстинкции – длина волны эмиссии флуоресценции

Photodynamic therapy (PDT) of tumors is one of the most promising and rapidly developing areas in the treatment of oncological diseases [2, 4]. In PDT, photosensitizers

(PS) are used as anticancer drugs, mainly cationic porphyrins [8], which selectively accumulate in tumors [1, 8] and, under the influence of light, cause the formation of singlet oxygen and free cytotoxic radicals, leading to cell death [1, 4, 8]. The binding and delivery of porphyrins (photosensitizers) to the tumor is one of the important tasks of the PDT method [8]. Delivery is carried out through such blood proteins as serum albumin, hemoglobin and lipoproteins, which play a big role in this problem [3, 9, 10, 12]. Among photosensitizers for PDT, cationic porphyrins are currently most actively studied and used [8, 14]. Earlier in Armenia, a number of cationic porphyrins with various peripheral groups (oxyethyl-, butyl-, allyl-, metallil-) and central metal atoms (Zn, Ag, Co, Cu, etc.) were synthesized [11, 13].

Materials and methods. The absorption and fluorescence spectra of porphyrins/metalloporphyrins and their complexes with proteins by absorption and fluorescence spectroscopy were studied. The absorption spectra of porphyrins and their complexes with proteins on a Shimadzu UV-VISIBLE Recording Spectrophotometer UV-2100 (Japan) in a quartz cuvette (0.1 or 1 cm) were recorded. Changes in the absorption spectra of cationic porphyrins and metalloporphyrins were recorded for the Soret band (420-440 nm) with an accuracy of 0.1 nm. Fluorescence spectra on an MPF 44 spectrofluorimeter (Perkin-Elmer, USA) in a quartz cuvette (0.1 or 1 cm) were recorded. All measurements at room temperature were carried out.

Statistical analysis. Statistical parameters (mean values, standard deviation) were calculated using Microsoft Excel and Origin 7.0 software (Origin Lab Corporation). Tab. 1 shows the average values of five independent experiments (n = 5). The standard deviation of the values did not exceed 5%. $p \leq 0.05$.

Results and Discussion. Currently, cationic porphyrins are also synthesized in a number of international companies in America (USA), Europe (UK) and Asia (Japan). The possible use of cationic porphyrins in PDT has also been demonstrated in our recent works, where their complexation with such important blood proteins as transferrin and ceruloplasmin was shown [5-7]. In fig. 1 shows the spectra of one of the cationic porphyrins and its binding to the ceruloplasmin as an example.

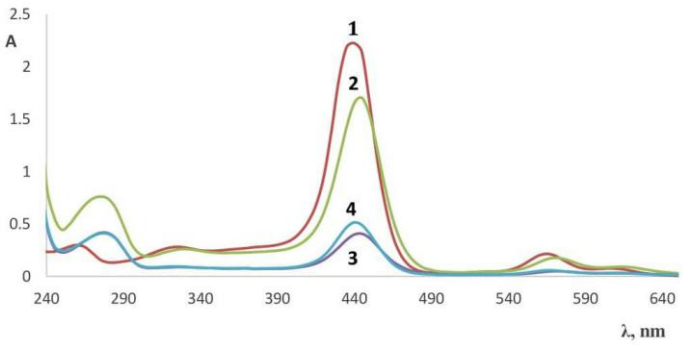

Fig. 1. Absorption spectra of cationic metalloporphyrin Zn-TOEt4PyP and its change upon binding to ceruloplasmin (CP). 1 - 1.6×10^{-4} M Zn-TOEt4PyP in 0.01 M phosphate buffer (PBS), pH 7.2; 2 - spectrum of the complex [CP + Zn-TOEt4PyP] 3 min after binding of the protein with porphyrin (in 0.01 M PBS pH 7.2).

From the fig. 1 it can be seen that, upon binding of metalloporphyrin Zn-TOEt4PyP (curve 1) with ceruloplasmin, the absorption decreases and the absorption of the Soret peak (439 nm) shifts to longer wavelengths (curve 2), indicating the complexation of porphyrin with the protein. Such changes in the protein-porphyrin

binding spectra require precise determination of the position of the spectral peaks and their changes.

In connection with the important aspects of the application of cationic porphyrins in PDT and their wide range of production, the determination of a number of spectral parameters of cationic porphyrins for their identification in absorption and fluorescence spectra, as well as in complexes with proteins, is an urgent task. As such spectral parameters, can serve with high accuracy defined the absorption values of the maxima of the Soret absorption band (420-440 nm), the values of the molar extinction coefficient, as well as the emission wavelengths of such compounds in a fluorescence spectra. We have carried out studies and these parameters have been determined for a number of cationic porphyrins and metalloporphyrins produced in Armenia and the UK (tab. 1).

Table 1*. Spectral parameters of cationic porphyrins and metalloporphyrins

NN	Porphyrins, metalloporphyrins	Maximum absorption of the Soret peak, λ_{max}, nm	Molar extinction coefficient, ε, $M^{-1}\cdot cm^{-1}$	Fluorescence emission wavelength, λ_{em}, nm
1	TOEt4PyP (Arm)	423.8	$1.823 \cdot 10^5$	710.0 (λ_{ex}=424 nm)
2	TOEt4PyP (UK)	423.5	$1.681 \cdot 10^5$	712.0 (λ_{ex}=424 nm)
3	TBut4PyP (Arm)	421.5	$3.182 \cdot 10^5$	710.0 (λ_{ex}=424 nm)
4	TBut3PyP (Arm)	417.0	$3.084 \cdot 10^5$	662.0 and 708.0 (λ_{ex}=424 nm)
5	Zn-TOEt4PyP (Arm)	439.4	$1.469 \cdot 10^5$	637.0 (λ_{ex}=440 nm)
6	Zn-TOEt4PyP (UK)	439.0	$1.433 \cdot 10^5$	637.0 (λ_{ex}=440 nm)
7	Zn-TBut4PyP (Arm)	436.8	$1.754 \cdot 10^5$	637.0 (λ_{ex}=440 nm)
8	Zn-TBut4PyP (UK)	438.0	$1.928 \cdot 10^5$	637.0 (λ_{ex}=440 nm)
9	Zn-TBut3PyP (Arm)	429.5	$1.958 \cdot 10^5$	610.0 and 662.0 (λ_{ex}=424 nm)
10	Ag-TOEt4PyP (Arm)	432,0	$1.679 \cdot 10^5$	712.0 (λ_{ex}=424 nm)
11	Ag-TOEt4PyP (UK)	433.5	$1.931 \cdot 10^5$	685.0 (λ_{ex}=424 nm)
12	Ag-TBut4PyP (UK)	432.0	$1.607 \cdot 10^5$	685.0 (λ_{ex}=440 nm)
13	Ag-TAll4PyP (UK)	433.5	$1.544 \cdot 10^5$	685.0 (λ_{ex}=424 nm)
14	Ag-TAll3PyP (UK)	427.0	$1.920 \cdot 10^5$	670.0 and 720.0 (λ_{ex}=424 nm)
15	Co-TAll4PyP (Arm)	435.5	$1.048 \cdot 10^5$	712.0 (λ_{ex}=424 nm)
16	Cu-TAll4PyP (Arm)	425.5	$1.186 \cdot 10^5$	695.0 (λ_{ex}=424 nm) Absolutely negligible peak of fluorescence
17	Cu-TOEt3PyP (Arm)	420.0	$2.101 \cdot 10^5$	No emission (λ_{ex}=424 nm)

*In Table shows the average values of five independent experiments (n = 5). The standard deviation of the values did not exceed 5%. $p \leq 0.05$.

Thus, spectral parameters of a number of cationic porphyrins and metalloporphyrins, determined with high accuracy, will make it possible to carry out their identification during complexation with proteins.

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