DETERMINATION OF ARYL-PORPHYRINS BINDING CONSTANTS TO BIOLOGICAL STRUCTURES BY INDIRECT SPECTRAL APPROACH

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The evaluation of drugs affinity to different biological structures (serum proteins, membranes and others) is very important to understand their pharmacokinetics features. In spite of the fact, there are a large number of physical-chemical methods for direct determination of the characteristics of affinity; most of them meet with difficulties, when dealing with non-polar compounds. In the current work we present a new indirect spectral fluorescent method, which allows to quantify relative affinity of aryl-porphyrins to biomolecules and biomembranes.

Aryl-porphyrins (APs) are widely used in photodynamic diagnostic and therapy of oncological diseases [1]. Most of them are hydrophobic molecules and form aggregates in aqueous surroundings. The formation of aggregates complicates APs biodistribution in organism after injection in blood. The aggregation leads also to the loss of APs fluorescent ability and affects on their affinity to biological structures, such as plasma proteins and cell membranes.

In our study we used cyclic oligosaccharides (cyclodextrins, CDs) to prevent the APs aggregation and to estimate the binding constants of aryl-porphyrins to biological membranes and serum proteins. It is widely known that CDs readily form inclusion complexes with many drugs by incorporating a drug molecule or more commonly a lipophilic moiety of the molecule into the central cavity. It has been shown, that CDs efficiently form an inclusion complexes with AP and can be photosensitizer vehicles via complexation [2].

As object to study we used following APs: 5,10,15,20-Tetra(m-hydroxyphenyl)chlorin (mTHPC), 5,10,15,20-Tetra(o-sulfophenyl)porphyrin (TSPP) and 5,10,15,20-Tetra(o-carboxyphenyl)porphyrin (TCPP). The complex formation processes between listed above APs and methyl-β-cyclodextrin (Me-β-CD) have been studied (Figure 1) and the spectral techniques for determination of complexation stoichiometry and quantitative parameters have

been developed [3]. According to the data obtained, the complexes between APs and Me-β-CD have a stoichiometry 1:2 and can be characterized by the binding constants values over 10^{12} M^{-2}.

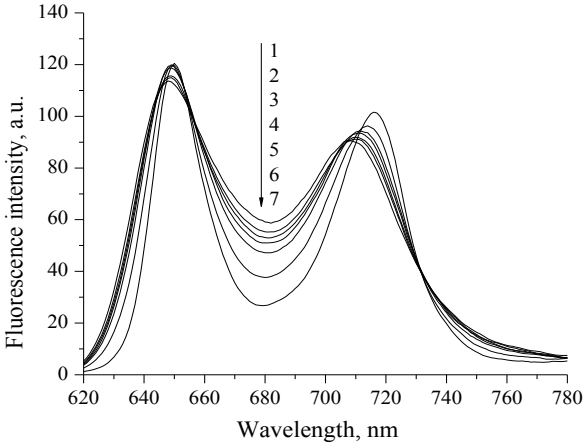

Figure 1 – TSPP fluorescence spectra in water solutions in presence of various Me-β-CD concentration: 1 – 0; 2 – 0.4 μM; 2 – 0.6 μM; 3 – 0.8 μM; 4 – 1 μM; 5 – 2 μM; 6 – 6 μM.

To determine the APs affinity to biological structures we have analyzed the processes of APs binding to Me-β-CD in the serum proteins solutions (human serum albumin, low and high density lipoproteins) and in the lipid vesicles suspensions (Figure 2). The obtained titration curves and previously determined binding constants values for the APs association with Me-β-CD process were used to estimate relative affinity of porphyrins to biological structures. In the case of mTHPC, the following values of the distribution coefficient were obtained: 2.6 $(mg/ml)^{-1}$ for human serum albumin, $4.8x10^{2}$ $(mg/ml)^{-1}$ for low density lipoproteins and $1.0x10^{3}$ $(mg/ml)^{-1}$ for high density lipoproteins. The ratios of mTHPC distribution coefficients in plasma compounds were in a good accordance to the data obtained by means of the gel-chromatography [4].

The data obtained confirm the interest of CDs in mTHPC-PDT.

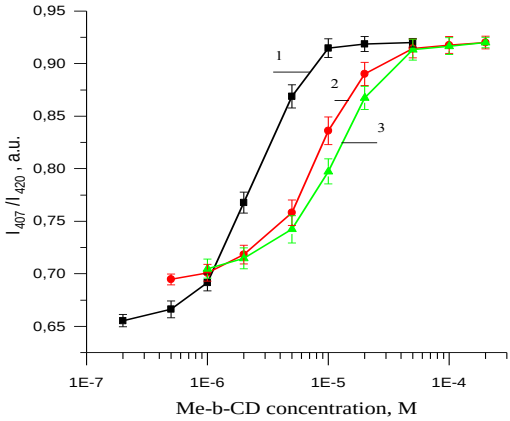

Figure 2 – The titration curves of mTHPC by Me-β-CD in solutions of serum albumin (1), low density lipoproteins (2), high density lipoproteins (3)

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