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SPECTRAL TECHNIQUES FOR PHOTOSENSITIZER REDISTRIBUTION IN BLOOD SERUM

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Предложен новый метод флуоресцентного анализа процессов распределения неполярного порфиринового фотосенсибилизатора между основными белками сыворотки крови. Метод включает в себя использование циклических олигосахаридов (циклодекстринов) для предотвращения агрегации препарата и количественного определения основных характеристик его связывания с белками сыворотки крови на основании кривых конкурентного связывания.

It is well recognized that drug pharmacokinetics depends strongly on drug interactions with plasma proteins after i.v. injection. The drug distribution between serum proteins governs its penetration and localization into the tissues. Thus, the evaluation of drug distribution mechanisms in blood serum proteins is very important to understand their pharmacokinetics behavior. In spite of the fact, there are a large number of physical-chemical methods for direct determination of drug molecules affinity to serum proteins, most of them meet with difficulties, when dealing with non-polar compounds such as porphyrin photosensitizers. In this work, we describe a number of fluorescent techniques that can be useful when the distribution mechanisms of porphyrin photosensitizers in blood serum is studied.

Porphyrin derivatives, especially chlorins, are widely used in photodynamic diagnostic and therapy of oncological diseases [1]. Temoporfin (mTHPC), one of the most effective photosensitizer, in photodynamic therapy of solid tumors encounters several complications resulting from its insolubility in aqueous medium [2]. mTHPC molecules form aggregates in aqueous bulk that complicates PS biodistribution in organism after injection in blood. The aggregation leads also to the loss of mTHPC fluorescent ability and effects on their affinity to biological structures, such as plasma proteins and cell membranes. For this reason mTHPC aggregation restricts the applicability of fluorescent measurements for the quantitative characterization of mTHPC distribution processes in blood serum.

In our study we have used cyclic oligosaccharides (cyclodextrins) to prevent the photosensitizer aggregation and to calculate the binding constants of mTHPC to the main serum proteins. It is widely known that cyclodextrins 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. Interaction of mTHPC with β-CDs leads to the formation of inclusion complexes that completely abolish mTHPC aggregation in aqueous solutions. The processes of complex formation between mTHPC and β-CD derivatives have been studied by means of fluorescent techniques. Computational mathematical analysis allowed us to estimate the stoichiometry and the apparent binding constants of inclusion complexes. It has been shown that complexation between mTHPC and CDs is a two-stage process. The complexes between mTHPC and β-CDs have stoichiometry 1:1 or 1:2 (Figure 1) and are characterized by the high values of binding constants, i.e. for Me-β-CD the binding constants were calculated as $K_{11}=1.0\text{x}10^{-7}$ M^{-1} and $K_{12}=3.5\text{x}10^{-5}$ M^{-1}.

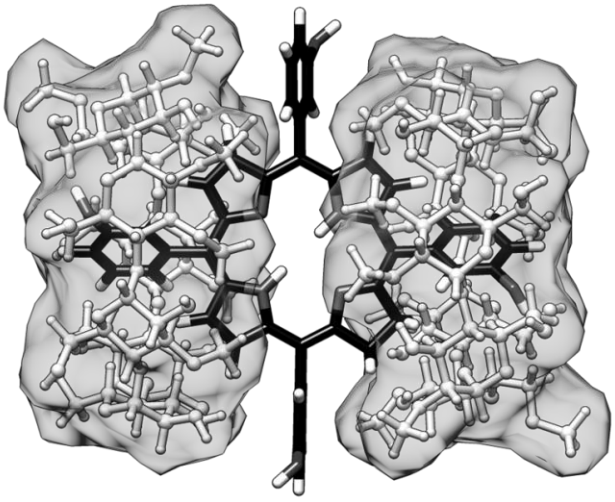
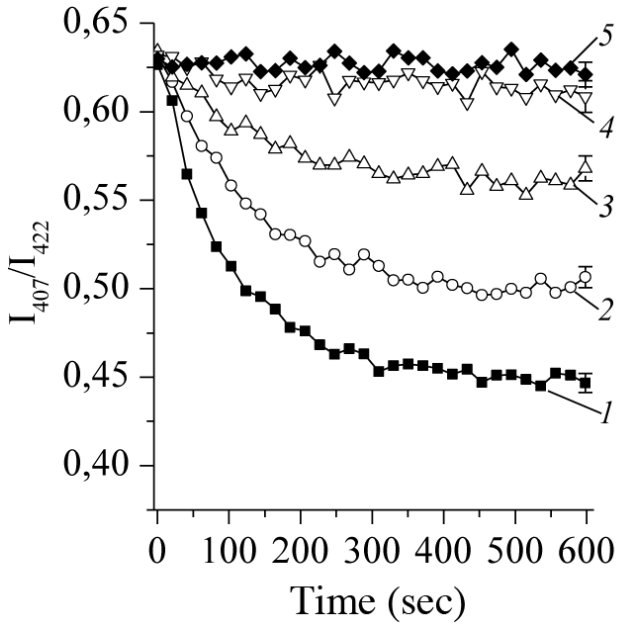


Figure 1 – Schematic structure of CD/mTHPC inclusion complex

The alteration mechanisms of mTHPC biodistribution between the main serum proteins by methyl-β-CD have been studied. Me-β-CD had a concentration-dependent effect on the process of mTHPC distribution in blood serum. It was showed that the using of low concentrations of Me-β-CD (less than $5x10^{-6}$ M) leads to the acceleration of mTHPC redistribution between biological structures [3,4]. In addition, the binding constants of mTHPC to the main transport serum proteins were estimated by means of developed fluorescence polarization technique.

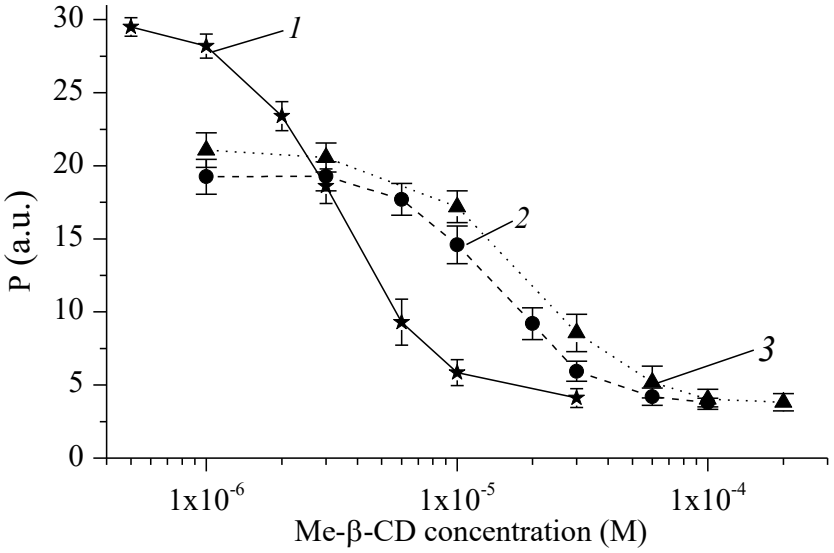


mTHPC concentration – 0.5 μM. CD concentration: 1 – 5; 2 - 10; 3 - 20; 4 - 50; 5 – 200 μM. Serum concentration – 1%.

Figure 2 – mTHPC redistribution kinetics from CD inclusion complexes in blood serum

It was shown that CDs can be also used in indirect techniques of binding constants determination. To determine the mTHPC affinity to biological structures we estimated mTHPC relative affinity to biological structures. 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].



1 – human serum albumin (2 mg/ml); 2 – low density lipoproteins (0,1 mg/ml);
3 – high density lipoproteins (0,1 mg/ml). mTHPC concentration – 0,5 μM.

Figure 3 - Titration curves of mTHPC in serum proteins solutions by Me-β-CD by means of fluorescence polarization technique

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