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NANOSCALE CYCLODEXTRIN SYSTEMS FOR DELIVERY OF TETRAPYRROLE PHOTOSENSITIZERS

Kablov I.V.[1], Kaskex V.[1,2], Kravchenko I.E.[1], Zorina T.E.[1], Zorin V.P. [1,2]

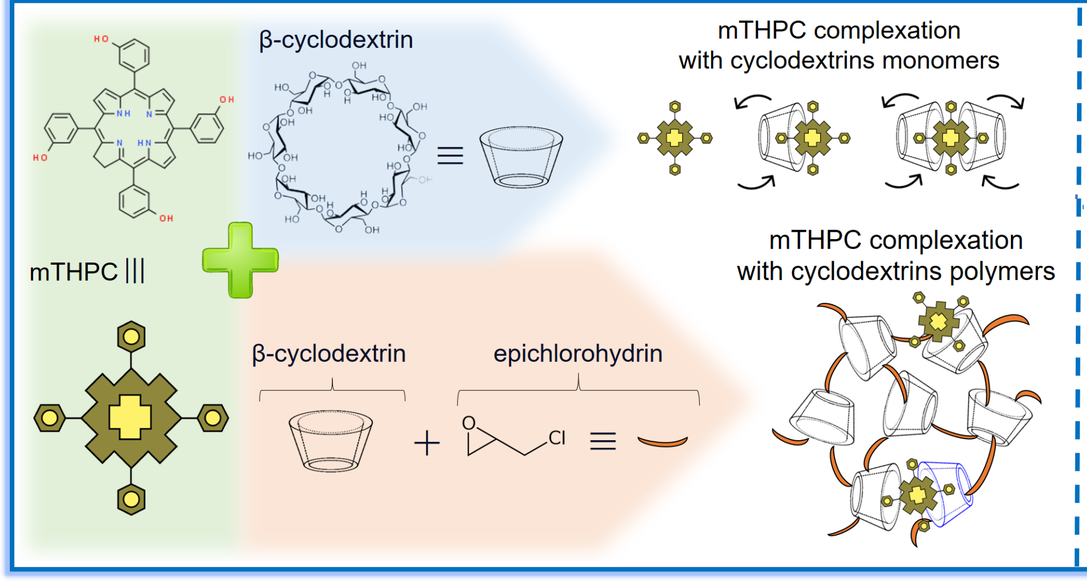
[1] *Department of Biophysics, Belarussian State University, Minsk, Belarus*
[2] *Department of General and Medical Physics, International Sakharov Environmental Institute, Minsk, Belarus.*

INTRODUCTION & AIM

Cyclodextrins (CDs) are widely applied in medicine and pharmaceutical industry for the development of new pharmacological forms of drugs because of their ability to non-covalently bind in a host-guest manner and increase the bioavailability of drugs. The formation of CDs complexes with drug compounds provides a reduction in the compounds toxicity, increasing their stability in the body, as well as improving pharmacokinetic and pharmacodynamic properties. One of the promising directions for the development of drug delivery systems using CDs is the use of polymer systems based on them.

The aim of this work was a comparative study of the peculiarities of the formation of inclusion complexes of monomeric and polymeric derivatives of β-cyclodextrin (β-CD) with the known photosensitizer (PS) meta-tetra(hydroxyphenyl)chlorine, used in photodynamic therapy, and evaluation of the accumulation and localization of such complexes in cellular systems.

OBJECTS OF STUDY & METHODS



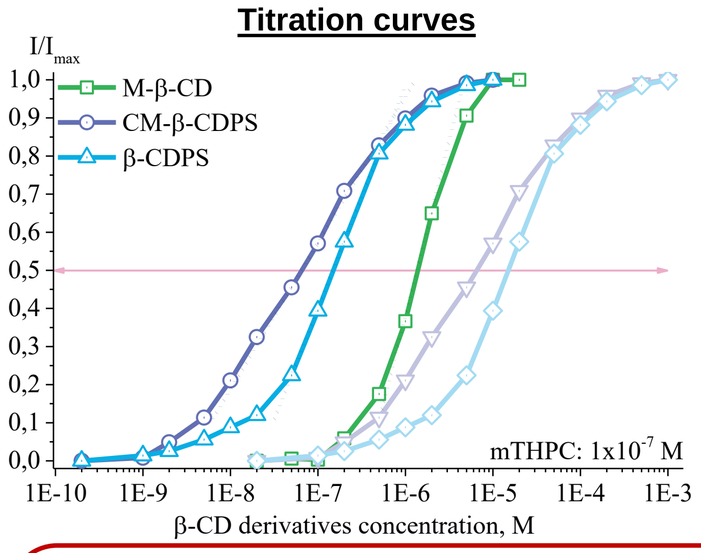
- ❑ **meta-tetra(hydroxyphenyl)chlorine (mTHPC), Biolitec (Germany)**
- ❑ **methyl-β-cyclodextrin (M-β-CD), AraChem (Netherlands)**
- ❑ **carboxymethyl-β-cyclodextrin polymer (CM-β-CDPS), CycloLab (Hungary)**
- ❑ **β-cyclodextrin polymer (β-CDPS), CycloLab (Hungary)**
- ❑ **dipalmitoylphosphatidylcholine (DPPC), SIGMA (USA)**

- ❖ Binding constants of PS with β-CD derivatives and comparison of their extrication rates from β-CD inclusion complexes were evaluated using spectral analysis methods // spectrofluorimeter Solar CM-2203, Belarus.
- ❖ The level of PS accumulation was assessed on myeloid leukemia cells line K562 (cell culture collection of Belarusian Research Center of Oncology, Hematology and Immunology) // cytofluorimeter FC 500, Beckman Coulter, USA.
- ❖ To analyse the processes of intracellular localization of mTHPC, we compared the distribution of PS fluorescence and cell organelle colocalization in cells by fluorescence microscopy // LeicaTCSSPE fluorescence microscope, Germany.

RESULTS & DISCUSSION

mTHPC complexation with β-cyclodextrin derivatives

Titration curves



Binding constants (*K*)

CD type	*K*, M^{-1}
M-β-CD	6,79x10^5
CM-β-CDPS	1,44x10^7
β-CDPS	6,31x10^6

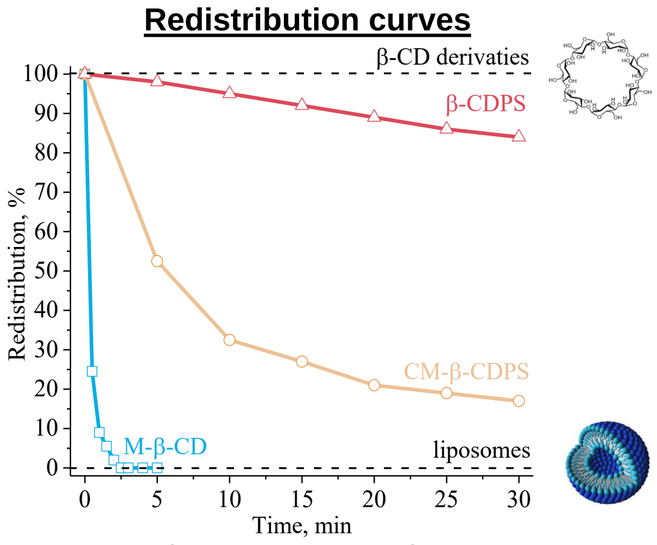
CD type	*K**, M^{-1}
CM-β-CDPS	1,31-1,44 x10^5
β-CDPS	5,74-6,31 x10^4

* values of *K* calculated per CD-unit in polymeric CDs

Relative affinity of mTHPC to monomeric CD is significantly higher compared to the affinity to a structural units of polymeric β-CD derivatives. Taking into account the degree of polymerization, the affinity of mTHPC to CM-β-CDPS and β-CDPS polymers is higher than to M-β-CD. The differences in the shape of the binding isotherms between mTHPC and β-CDs are probably determined by the influence of polymer structure on the formation of inclusion complexes.

Stability of mTHPC/β-CD derivatives inclusion complexes

Redistribution curves



The mean squared error measurement is less than 3%.

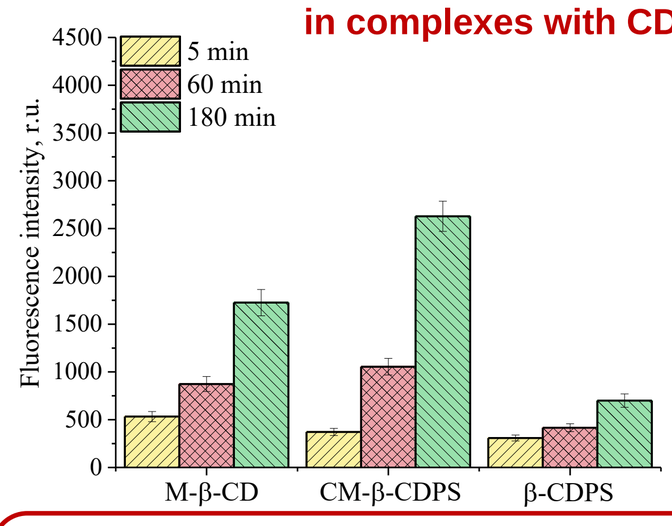
The mTHPC dissociation rates from nanocarriers were compared by analyzing the redistribution rates of PS molecules from inclusion complexes onto DPPC liposomes (Bengem's method).

mTHPC ($1\cdot10^{-7}$ M)
M-β-CD ($1\cdot10^{-5}$ M)
CM-β-CDPS ($1\cdot10^{-7}$ M)
β-CDPS ($1\cdot10^{-7}$ M)
DPPC ($2\cdot10^{-4}$ M)

The incubation temperature is 45 °C.

The rate of mTHPC dissociation from inclusion complexes with polymeric derivatives of β-CD is significantly lower compared to monomeric M-β-CD. It should be noted that there is no correlation between the PS release rates and its binding constants.

Accumulation of mTHPC administered in complexes with CD in K562 cells



T = 37 °C
mTHPC ($5\cdot10^{-7}$ M)
serum proteins (2%)
K562 (1x10^6 cells/ml)
M-β-CD ($2,5\cdot10^{-5}$ M)
β-CDPS ($2,5\cdot10^{-7}$ M)
CM-β-CDPS ($2,5\cdot10^{-7}$ M)

When a photosensitizer is introduced as a part of complexes with various cyclodextrins, the rates of its accumulation in K562 cells differ significantly. The highest level of accumulation in cells was observed for mTHPC introduced with CM-β-CDPS. It is assumed that the main factor controlling the rate of intracellular accumulation is the rate of PS release from the complexes with CD.

Intracellular localization of mTHPC

PC, complexes	Pearson correlation coefficients (PCC) for different cell compartments		
	MitoView™ Green *mitochondria*	$DiOC_6$ (3) Iodide *endoplasmic reticulum*	LysoView™ 488 *lysosomes*
mTHPC	0,915±0,081	0,931±0,084	0,111±0,051
mTHPC-M-β-CD	0,761±0,058	0,950±0,069	0,450±0,072
mTHPC-CM-β-CDPS	0,871±0,079	0,983±0,074	0,650±0,061
mTHPC-β-CDPS	0,867±0,079	0,972±0,071	0,510±0,076

PCC from 1.00 to 0.70 — relatively strong correlation, from 0.69 to 0.36 — moderate correlation, less than 0.20 — non-correlation.

According to the data obtained, the introduction of mTHPC in complexes with CD leads to changes in the characteristics of accumulation and localization of PS in cells and, as a consequence, the vectorization of photodynamic action of PS.

CONCLUSIONS:

PS effectively binds to monomeric and polymeric cyclodextrins. The use of cyclodextrins ensures the introduction of mTHPC in monomeric form. Differences in the rates of mTHPC release from complexes with monomeric and polymeric cyclodextrins suggest their different influence on the processes of PS biodistribution in the organism.