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BINDING OF CATIONIC PORPHYRINS TO BLOOD PROTEINS

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It is shown, that various porphyrins bind in different ways to the proteins depending on a structure of lateral functional groups. It is found, that the porphyrin binding ability of bovine serum albumin (BSA) is considerably (1,5 times) greater than that of hu-man serum albumin (HSA). Also it turned out that the porphyrin binding ability of hemoglobin is 2 times greater than the ability of HSA.

Blood proteins – cationic porphyrins – binding constants

Показано, что в зависимости от строения боковых функциональных групп разные порфирины по-разному связываются с белками. Определено, что порфирин-связывающая способность у бычьего сывороточного альбумина (БСА) значительно выше (в 1,5 раза), чем у человеческого СА (ЧСА). Оказалось также, что порфирин связывающая способность гемоглобина в 2 раза выше, чем у ЧСА.

Белки крови – катионные порфирины – константы связывания

Ցույց է տրված, որ կողմնային ֆունկցիոնալ խմբերի կառուցվածքից կախված զանազան պորֆիրինները տարբեր ձևով են կապվում սպիտակուցների հետ: Հայտնաբերվել է, որ ցուլի շիճուկային ալբումինի մոտ կապման ունակությունը զգալիորեն ավելի բարձր է (1,5 անգամ) մարդու շիճուկային ալբումինից: Պարզվել է նաև, որ հեմոգլոբինի հետ պորֆիրինի կապման ունակությունը մոտ 2 անգամ բարձր է մարդու շիճուկային ալբումինի համեմատ:

Արյան սպիտակուցներ – կատիոնային պորֆիրիններ– կապման հաստատուններ

Photosensitizers (PS), in particular porphyrins, are widely used in photodynamic therapy of tumours (PDT) [3, 6]. The photosensitizer, which selectively accumulate in a tumor, at PDT is injected into the patient and after excitation by light promotes generation of reactive oxygen species, which damage the components of cells and tissues, that finally adducts to the destruction of cells by necrosis and/or apoptosis [6, 10]. The high selectivity has been reached especially for cationic porphyrins which have been successfully used for destruction of tumors in vivo [9]. The proteins of blood can serve as carriers of medicines, which facilitate a selective delivery of porphyrins to areas of tumors. The affinity of serum albumin (SA), hemoglobin, lipoproteins of high and low density for porphyrins indicates the important role of these proteins as endogenous carriers of photosensitizers administered for PDT [5, 8, 11]. In connection with the above-stated, the investigation of binding and transfer of cationic porphyrins to proteins is of a great importance for PDT of tumors.

Materials and methods. BSA, BSA fatty acids free (BSA FAF), HSA fatty acids free (HSA FAF), human hemoglobin (HH) and cytochrome C were purchased from Sigma-Aldrich Chemical Co. (USA). Water-soluble cationic porphyrins and metallo-porphyrins have been synthesized at the Yerevan State Medical University according to the methods described in work [2]. All other reagents were of analytical grade.

The absorbance spectra were recorded on a Shimadzu UV-VISIBLE Recording Spectrophotometer UV-2100 (Japan) in 0,5 centimeter quartz cell. In analyzed cell the concentration of porphyrins was constant (5.10^{-6} M), and the concentration of proteins was changed (5.10^{-7} - $2{,}5.10^{-4}$ M) with their addition via Hamilton Co. Microliter Syringe (USA). The research of porphyrin-protein binding was carried out by defining the binding constant K via analyzing the reduction of absorption of a Soret band due to addition of a solution of protein to the solution of porphyrin. It is described by the following modified relation of Kapp et al. [7]:

$$A_o/A_o\text{-}A = 1/fK[C_{prot}] + 1 / f \qquad (1)$$

where A_o and A are the porphyrin solution absorbances in the absence and presence of the protein, respectively. In the plot of a adsorption isotherm A_o/A_o-A (ordinate) vs 1/[Cprot] (abscissa), 1/f is obtained from the intercept on the A_o/A_o-A axis, corresponding to 1 / [Cprot] = 0.

At definition of the values Ao and A of the absorption intensity we used the spectra processing software, namely "Spectra Manager for Spectra Analysis, Version 1.53.00, JASCO Corporation, 2000 ". This software corrects the value of absorption intensity A in view of spectra asymmetry and deviation from a base (zero) line. The value of K was defined according to relation of Kapp (1). The results of experiments have been processed statistically by using the criterion of Student.

Results and Discussion. Investigations of interactions of a proteins with ligands at simulative conditions, starting from simple two-componential systems, such as monomers of porphyrins and proteins are of of special importance for definition of porphyrin-protein binding constants. However even such seemingly simple systems can be transformed during experiment into the multicomponential systems by dimerization and polymerizations (aggregation) of proteins (especially at their higher concentrations), thus corrupting the results of constants and quantity of places of ligand binding to protein. In spectrophotometric analysis one of the basic criteria for presence of two-componential system in a solution is the formation of isosbestic points (the general point of spectral curves crossing) [1]. To obtain correct values of binding constants of protein-ligand pair, the titration of a porphyrin solution with increasing concentration of proteins, by tracing the formation of isosbestic points for spectral curves was investigated.

From Fig. 1A one can see, that results of the titration process of a porphyrin solution can be divided on two parts: the first group of the spectral curves of solutions, obtained via titration of porphyrin by solution of BSA up to a ratio protein : porphyrin = 2,5:1 and, the second group of spectral curves having significant shift of absorption peak of a Soret band (on 6-8 nanometers) and obtained via titration of porphyrin by solution of BSA up to a ratio protein : porphyrin = 40:1 ($[C_{prot}] = 2.10^{-4}$ M). From Fig. 1A it follows, that in the first group of spectral curves the isosbestic point is observed (the top five curves) whereas spectral curves of the second group do not pass through the isosbestic point. Hence, for the correct definition of a binding constant (K) it is necessary to titrate up to that concentration of a protein at which curves of spectra still pass through the isosbestic point.

The interaction of 8 porphyrins and metalloporphyrins with 4 different blood proteins, and also with cytochrome C has been investigated according to

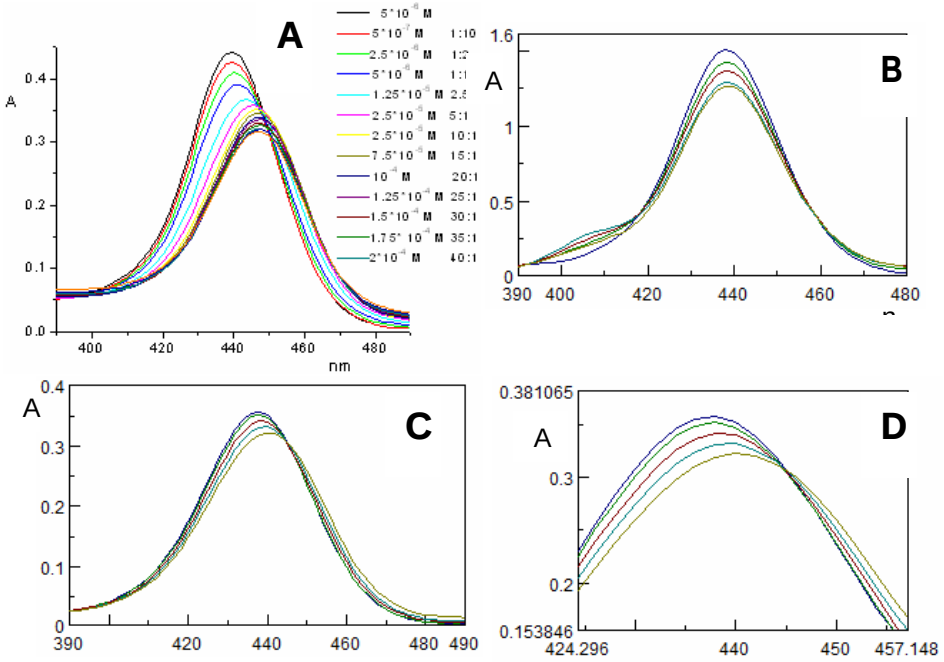

Fig.1. Absorption spectra of porphyrins solutions (5.10^{-6} M in 0,01 M phosphate buffer pH 7,2) at their titration by proteins solutions. A – titration of Zn-TOE4PyP by BSA solution; B – titration of Zn-TBut4PyP by HH solution; C - titration of Zn-TBut4PyP by BSA FAF solution; D – the same like in C with magnification of isosbestic point spectral region.

this criterion. In Fig. 1B and 1C the absorption spectra of some porphyrins at their titration rather low concentration of various proteins (a ratio of protein : porphyrin = 0,5:1; 0,75:1; 1:1; 1,75:1; 2,5:1) are presented. The observed spectra on the presence of isosbestic points for all porphyrins and proteins were investigated.

For determination of a binding constants K according to relation of Kapp (1) the adsorption isotherms in coordinates A_o/A_o-A vs $1/[C_{prot}]$ have been plotted (Fig. 2).

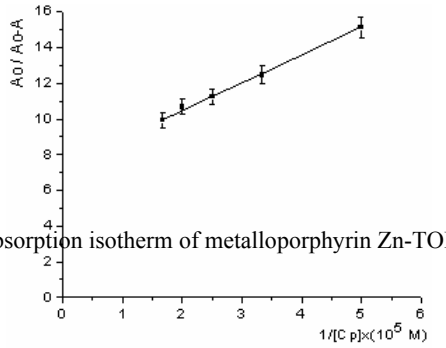

Fig.2. Absorption isotherm of metalloporphyrin Zn-TOE4PyP to BSA

The results of definition of binding constants (K) for 8 porphyrins and metalloporphyrins with 5 various proteins are presented in the table, where we also present the values $K_{aver} = \sum K/8$ for each protein, which reflects the average connecting ability of the given protein to cationic porphyrins.

From the table follows, that the porphyrin binding ability of BSA and fatty acids free BSA are practically identical and are considerably higher (1,5 times) than that of HSA. Also it turned out that the binding ability of hemoglobin in 2 times higher, than the ability of HSA. High affinity to cationic porphyrins was displayed by cytochrome C (2-3 times higher than BSA). Because of a positive charge and lipophilic properties the cationic porphyrins can be localized with high probability in mitochondrion owing to a negative charge of an internal part of mitochondrion membranes; such arrangement of porphyrins can testify that mitochondrion are the primary place of cells destruction at PDT [10].

Table. The binding constants (K) of porphyrins and metalloporphyrins with various proteins

	Porphyrins and metallo-porphyrins	BSA (K) $\cdot 10^{-5}$ M	BSA Fatty Acids Free (K) $\cdot 10^{-5}$ M	HSA Fatty Acids Free (K) $\cdot 10^{-5}$ M	Human Hemoglobin (K) $\cdot 10^{-5}$ M	Cytochrome C (K) $\cdot 10^{-5}$ M
1.	TOE4PyP	4,10 ± 0,29	2,60 ± 0,19	1,55 ± 0,11	3,36 ± 0,23	8,18 ± 0,49
2.	Zn-TOE4PyP	2,60 ± 0,16	2,65 ± 0,15	3,00 ± 0,21	4,82 ± 0,31	7,47 ± 0,39
3.	TBut4PyP	3,13 ± 0,20	4,71 ± 0,29	3,10 ± 0,21	2,99 ± 0,19	7,84 ± 0,43
4.	Zn-TBut4PyP	3,80 ± 0,22	4,10 ± 0,25	0,40 ± 0,02	2,07 ± 0,12	1,58 ± 0,09
5.	TAll4PyP	0,70 ± 0,04	1,07 ± 0,06	1,60 ± 0,09	2,02 ± 0,11	3,56 ± 0,21
6.	Zn-TAll4PyP	1,42 ± 0,08	1,52 ± 0,08	1,50 ± 0,08	1,75 ± 0,1	0,60 ± 0,03
7.	TMetAll4PyP	1,98 ± 0,11	0,37 ± 0,02	0,78 ± 0,04	1,89 ± 0,11	5,52 ± 0,33
8.	Zn-TMetAll4PyP	0,76±0,04	1,80 ± 0,11	0,19 ± 0,01	5,29±0,29	2,87 ± 0,16
	$K_{aver} \cdot 10^{-5}$ M	2,31	2,26	1,52	3,02	4,70

From the table it follows, that the porphyrin binding ability of BSA and fatty acids free BSA are practically identical and are considerably higher (1,5 times) than that of HSA. Also it turned out that the binding ability of hemoglobin is 2 times higher, than the ability of HSA. High affinity to cationic porphyrins was displayed by cytochrome C (2-3 times higher than BSA). Because of a positive charge and lipophilic properties the cationic porphyrins can be localized with high probability in mitochondrion owing to a negative charge of an internal part of mitochondrion membranes; such arrangement of porphyrins can testify that mitochondrion are the primary place of cells destruction at PDT [10].

The knowledge of binding constants of cationic porphyrins with the various proteins can promote the study of mechanisms of photosensitizers transport at PDT of tumors and facilitate the synthesis of porphyrins which will be more efficient in therapy of tumours.

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