Interactions of human serum albumin with acridine and phenazine

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Interactions of human serum albumin with acridine and phenazine have been studied by means of spectrophotometry. The equilibrium constant of albumin—acridine interaction was found to be $1.54 \pm 0.20 \times 10^5$ l mol^{-1} at pH 7.4 and 20°C. Equilibrium constant of the phenazine—albumin interaction was $3.88 \pm 0.19 \times 10^5$ l mol^{-1} under identical conditions.

При помощи спектрофотометрического метода подвергалось исследованию взаимодействие альбумина человеческой сыворотки крови с акридином и феназином. Установлено, что константа равновесия взаимодействия альбумин—акридин является $1,54 \pm 0,20 \times 10^5$ л моль$^{-1}$ при pH 7,4 и 20°C. В тех же самых условиях константа равновесия взаимодействия феназина с альбумином является $3,88 \pm 0,19 \times 10^5$ л моль$^{-1}$.

Blood serum albumin (HSA) forms complexes with a number of different compounds. These nonspecific interactions of albumin are considered to be related to structural properties of HSA molecules in solutions. The nucleus of HSA globule preserves its highly organized structure. The loosely organized HSA molecule surface consists of subunits linked by a polypeptidic bridge and can respond to changes in environmental conditions by configurational changes [1—4]. This "configurational adaptability" of the albumin surface enables HSA molecules to interact with several compounds [5—7].

In this paper the results of spectrophotometric study of HSA—acridine and HSA—phenazine interactions are presented.

Experimental

HSA used was a product of Imuna, Šarišské Michaľany (Czechoslovakia), prepared by a modified Kohn method. It has a 96% electrophoretic purity grade (we have considered 68 000 as average molecular weight of HSA).

Acridine — (Lachema, Brno), purified by crystallization from diluted ethanol.

Phenazine — product of BDH Chemicals Ltd., purified by crystallization from diluted ethanol.

Solutions of HSA and the ligands investigated were prepared in 0.1 M phosphate buffer of pH 7.4; solutions of phenazine also in buffer solution as previously mentioned but adjusted with 0.1 M-NaOH to pH 10, and with 0.1 M phosphoric acid to pH 3, respectively. Absorption spectra of the complexes and the pure compounds were registered in a double-beam Specord UV VIS spectrophotometer (Zeiss, Jena). All solutions were held at 20 ± 0.2°C, including sample holders during measuring.

Results and discussion

Equilibrium constants of HSA—ligand interactions were evaluated based on the equation

$$1/R = 1/n\ K\ C_{\mathrm{f}} + 1/n$$

where R is number of ligand moles bound per HSA mole, C_{f} is concentration of the free ligand form, n is number of bond sites in the HSA molecule, K is equilibrium constant. The $1/R$ *vs.* $1/C_{\mathrm{f}}$ was found to be linear at interactions following Langmuir's adsorption isotherm.

Spectrophotometric study of molecular interactions does not allow to determine directly concentration of the free ligand in ligand—HSA systems. This concentration was evaluated by use of molar absorptivities of the free (ε_{f}) and bound (ε_{b}) ligand [8]. Molar absorptivity of the bound ligand form can be calculated from the linear part of the plot expressing the $A/C\ d$ *vs.* C/C_{p} dependence by using *Westphal*'s method [9], where A is absorbance of the system at the wavelength given, C is total concentration of the ligand, C_{p} is albumin concentration, and d is length of the light path through the solution measured. Calculations of molar absorptivities of bound ligand forms, equilibrium constants, and bond site number were made by the least square method based upon linear dependences.

Due to HSA—ligand interactions, the electronic absorption spectra of both compounds investigated showed shifting of the absorption maxima towards higher wavelengths in the u.v. region. The series of curves of the absorption spectra of ligand—HSA systems showing different degrees of interaction developed well defined isosbestic points: these were, for the HSA—acridine systems, observed at 251.5 nm, for HSA—phenazine systems, at 252 nm. Isosbestic points indicate formation of one complex only.

Evaluations of equilibrium constants were carried out at 254 nm for acridine and at 255 nm for phenazine. Table 1 shows the molar absorptivities of free and bound ligands at given wavelengths, whereas interaction characteristics are summarized in Table 2.

Table 1

Molar absorptivities of free (ε_f) and bound (ε_b) forms of acridine and phenazine with their mean deviations

Ligand	λ, nm	ε_f cm^2 mmol^{-1}	ε_b cm^2 mmol^{-1}
Acridine	254	31 060 ± 600	143 630 ± 670
Phenazine	255	27 730 ± 450	80 800 ± 443

Table 2

Equilibrium constants of acridine—HSA and phenazine—HSA complexes (t = 20°C), number of bond sites and correlation coefficient of the $1/R$ *vs.* $1/C_f$ function

Ligand	pH	$K \cdot 10^{-5}$ l mol^{-1}	n	r
Acridine	7.4	1.54 ± 0.20	1.11 ± 0.08	0.982
Phenazine	7.4	3.88 ± 0.19	0.35 ± 0.02	0.975
Phenazine	10.0	2.40 ± 0.28	0.65 ± 0.04	0.979

HSA—heterocyclic compound interactions are affected by n—π interactions localized on the heteroatoms; however, the importance of hydrophobic forces between the aromatic nucleus of the ligand and hydrophobic areas of the HSA molecule must be emphasized [10]. We have assumed that, with acridine—HSA and phenazine—HSA complexes, hydrogen bonds have been created between the nitrogen atom in the heterocyclic molecule and the donor site of the protonized hydrogen atom of the HSA molecule. The higher number of bond sites of HSA molecules, in case of HSA—acridine interactions, could point to a higher basicity level of the acridine nitrogen atom compared to phenazine (higher pK_a value). The significance of basicity of the bond site in the heterocycle during the interactions described, has been confirmed also by following of pH dependences; while at higher pH values of the medium an increase in bond sites of phenazine on the HSA molecule (Table 2) was observed, in more acidic (pH 3) conditions interactions were depressed to an extent as to render impossible, by the method used, to determine their parameters.

The equilibrium constant increase shown at phenazine—HSA interactions as compared to the same constant at acridine—HSA interactions, is in accordance with the assumed phenazine—HSA complex stabilization by means of interaction taking place on two nitrogen atoms in the phenazine molecule. The lower number of bond sites in the albumin molecule than 1 (Table 2) indicates the possibility of interaction of two nitrogen atoms in the phenazine molecule taking place with several albumin molecules.

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