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HYDRATION OF HUMAN AND BOVINE SERUM ALBUMIN COMPLEXES WITH CHLOROPHYLLIN INVESTIGATED BY DIFFERENTIAL EHF DIELECTROMETRY

D. A. Pesina, V. A. Kashpur, O. V. Khorunzhaya, A. V. Shestopalova
A. Ya. Usikov Institute for Radiophysics and Electronics of NAS of Ukraine,12 Akad. Proskury Str., Kharkiv, 61085, Ukraine
e-mail: darynapesina@gmail.com
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This study is designed to examine the hydration effects accompanying the interaction between chlorophyllin (CHLN), a potent inhibitor of experimental carcinogenesis, and human and bovine serum albumins (HSA and BSA) in aqueous solutions using differential dielectric method. This method allows studying both dielectric characteristics and hydration of the compounds under investigation. We show that for both complexes the process of complex formation is accompanied by changes of dielectric parameters. We also present calculations of the hydration of proteins and their complexes with ligand, which were carried out basing on the model concepts. We found that formation of CHLN complex with fatty acids free BSA is accompanied by a significant decrease of hydration while no notable hydration changes takes place upon formation of CHLN complex with HSA containing fatty acids. The former effect indicates that redistribution of free and bound water molecules takes place during the formation of the complex. The possible causes of effects observed are discussed.
KEY WORDS: serum albumin, chlorophyllin, serum albumin-chlorophyllin complex, hydration, EHF dielectrometry.

ГИДРАТАЦИЯ КОМПЛЕКСОВ СЫВОРОТОЧНОГО АЛЬБУМИНА ЧЕЛОВЕКА И БЫКА С ХЛОРОФИЛЛИНОМ: ИССЛЕДОВАНИЕ МЕТОДОМ ДИФФЕРЕНЦИАЛЬНОЙ КВЧ ДИЭЛЕКТРОМЕТРИИ

Д. А. Песина, В. А. Кашпур, О. В. Хорунжая, А. В. Шестопалова
Институт радиофизики и электроники имени А. Я. Усикова НАН Украины, Ул. Акад. Проскуры, 12, Харьков, 61085, Украина

Работа посвящена изучению гидратационных эффектов, сопровождающих взаимодействие между хлорофиллином (ХЛФН), потенциальным ингибитором экспериментального канцерогенеза, и сывороточного альбумина человека и быка (САЧ и САБ) в водных растворах с помощью дифференциального диэлектрического метода. Указанный метод позволяет изучать как диэлектрические параметры, так и гидратацию исследуемых веществ. Показано, что образование обоих комплексов сопровождается изменением диэлектрических характеристик растворов. Приведены основанные на модельных представлениях результаты расчета гидратации белков и их комплексов с лигандом. Обнаружено, что образование комплекса ХЛФН с САБ, свободным от жирных кислот, сопровождается значительным уменьшением гидратации, в то время как при образовании комплекса ХЛФН с САЧ, содержащим жирные кислоты, заметного уменьшения гидратации не происходит. Первое свидетельствует о перераспределении молекул свободной и связанной воды при формировании комплекса. Приводятся возможные объяснения наблюдаемых эффектов.
КЛЮЧЕВЫЕ СЛОВА: сывороточный альбумин, хлорофиллин, комплекс сывороточный альбумин - хлорофиллин, гидратация, КВЧ диэлектрометрия.

ГІДРАТАЦІЯ КОМПЛЕКСІВ СИРОВАТКОВОГО АЛЬБУМИНА ЛЮДИНИ ТА БИКА З ХЛОРОФІЛІНОМ: ДОСЛІДЖЕННЯ МЕТОДОМ ДИФЕРЕНЦІНОЇ НВЧ ДІЕЛЕКТРОМЕТРІЇ

Д. О. Песіна, В. А. Кашпур, О. В. Хорунжа, А. В. Шестопалова
Інститут радіофізики та електроніки імені О. Я. Усикова НАН України,Вул. Акад. Проскури, 12, Харків, 61085, Україна

Робота присвячена вивченню гідратаційних ефектів, що супроводжують взаємодію між хлорофіліном (ХЛФН), потенційним інгібітором експериментального канцерогенезу, та сироватковим альбуміном людини і бика (САЛ та САБ) в водних розчинах за допомогою

диференційного діелектричного методу. Зазначений метод дозволяє вивчати як діелектричні параметри, так і гідратацію речовин, що досліджуються. Показано, что утворення обох комплексів супроводжується зміною діелектричних характеристик розчинів. Представлено засновані на модельних уявленнях результати розрахунків гідратації білків і їх комплексів з лігандом. Встановлено, що утворення комплексу ХЛФН з САБ, вільним від жирних кислот, супроводжується значним зменшенням гідратації, в той час як при утворенні комплексу ХЛФН з САЛ, що містить жирні кислоти, помітного зменшення гідратації не відбувається. Перший з ефектів, що спостерігається, свідчить про перерозподіл молекул вільної та зв'язаної води при утворенні комплексу. Наведено можливі пояснення ефектів, що спостерігаються.
КЛЮЧОВІ СЛОВА: сироватковий альбумін, хлорофілін, комплекс альбумін-хлорофілін, гідратація, НВЧ діелектрометрія.

Protein–drug interactions play an important role in the pharmacological activity of many drugs. Drug binding to plasma proteins modulates the absorption, distribution, metabolism of drugs and affects its efficacy and toxicity, independent of the ultimate mechanism of the drug itself [1].

Interactions between ligand and protein take place in an aqueous environment and often involve conformational changes in the receptor [2]. Analysis of ligand-bound water molecules in high-resolution crystal structures of protein-ligand complexes [3] show that water molecules play an important role in protein-ligand binding not only by mediating the recognition process but also by maintaining the structure of the complex [4]. Water molecules can modify the shape and the flexibility of a ligand binding site in a protein, improving the complementarity between the protein and the ligand [3, 5]. Hydrogen (H-) bonds involving water are also shown to be ubiquitous in the active sites of proteins [6]. In some protein-ligand complexes water molecules can act as hydrogen-bonding bridges, shielding charges on the buried ligands and establishing a network of H-bonds which stabilize the protein-ligand interaction [3, 7, 8]. Recently attempts have been made to incorporate water molecules at the binding sites into ligands with a goal to improve binding affinities of ligands [2, 8, 9], and by computational docking it was shown that the inclusion of certain water molecules can lead to significant improvement in docking results [9].

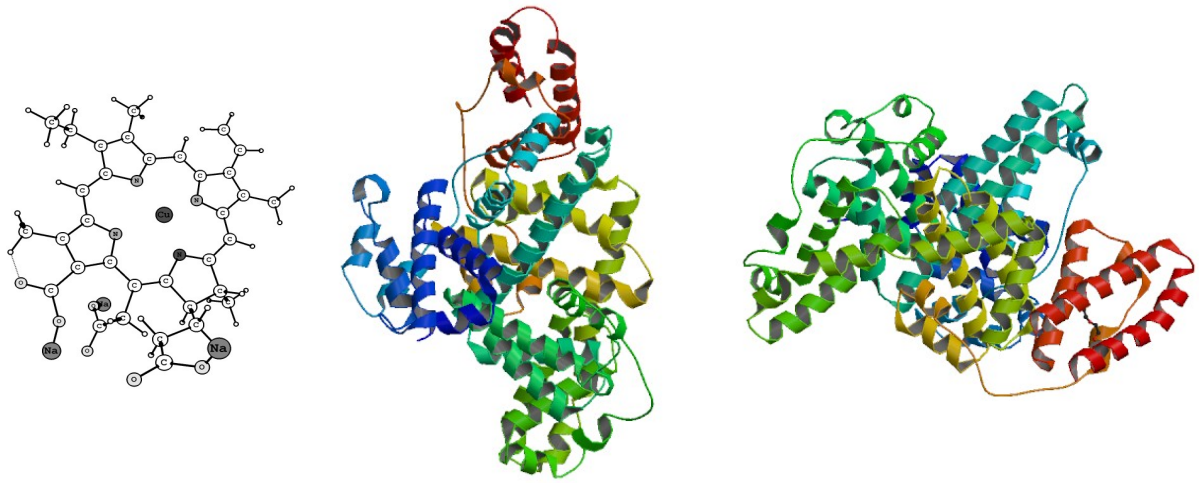
Taking together all roles of water in ligand-protein binding described above, the relevance of quantitative estimation of hydration of protein-ligand complexes and its changes that take place during formation of the complex becomes clear. It should be noted though that main sources of information on role of water molecules in protein-ligand binding are x-ray crystallography and computer simulation methods. The first method does not deal with solutions and information on hydration of substances obtained for crystals might differ from their hydration in solution, i.e. in the case which is closer to natural conditions. It does not give information on dynamics of restrained water either. The second method requires experimental verification.

Our report aims to fill in the gap of experimental information on hydration of protein-ligand complexes in solutions. We present experimental results on hydration changes, that accompany interaction between chlorophyllin (CHLN) and human and bovine serum albumins (see fig. 1, a-c), obtained using the differential dielectric method. This method was effectively applied earlier for investigation of the hydration effects accompanying interaction between different types of ligands with DNA [10].

Serum albumin was selected as a model protein since it is one of the well studied and most abundant plasma proteins. This protein acts as a transporter for a wide variety of drugs and endogenous compounds in human body including porphyrins like chlorophyllin [11-13]. Serum albumin has three homologous domains (I-III), each containing two subdomains (A and B) [11, 12]. Most drugs bind with high affinity to one of two sites of the protein, called

site I and site II. These sites are formed as hydrophobic pockets in subdomains IIA and IIIA respectively [12, 13].

Chlorophyllin is a water-soluble semi-synthetic derivative of the green pigment chlorophyll and is shown to have an ability to inhibit the mutagenic activity of a variety of chemicals and complex mixtures [14] (for example, CHLN is a potent inhibitor of aflatoxin B1 in hepatocarcinogenesis in rainbow trout [15]). CHLN is shown to be an antioxidant. Scavenging of radicals and/or interaction with the active group of mutagenic compounds may be responsible for its antimutagenic activity. CHLN has been also used for radiation sensitization therapy of cancer [16] and is targeted to interact with cellular DNA and protein [13].



(a) (b) (c)

Fig. 1. Structure of (a) CHLN ($C_{34}H_{31}CuN_4Na_3O_6$) and crystal structures of (b) HSA and (c) BSA (PDB ID 1e78 and 4f5s respectively)

As far as we know, this paper is the first one concerning hydration effects that take place upon CHLN-serum albumin complex formation.

MATERIALS AND METHODS

Chlorophyllin sodium salt and bovine serum albumin (fatty acids free, protein content >98%) were obtained from Sigma Aldrich, human serum albumin (protein content >85%) was purchased from Fluka, all the samples were used without further purification. All measurements were performed in water solutions. The ligand solution was added dropwise to the solutions of proteins with constant stirring to ensure the formation of homogeneous solutions. When preparing the solution of BSA-CHLN complex, we used the stock solutions with concentrations of 1.74% for CHLN and 4.40% for BSA. For preparation of HSA-CHLN complex, stock solutions with concentrations of 2.07% for CHLN and 4.15% for HSA were used. For each protein its concentration both in the solutions of proteins and in the solutions of complexes was the same. Concentrations of solutions of CHLN, BSA, HSA, BSA-CHLN and HSA-CHLN complexes were as follows: 1.9%, 2.89%, 2.91%, 3.5% and 3.56% respectively. All concentrations are expressed as weight percents. In tables 1-3 these concentrations are presented taking into account concentrations of sodium ions or/and admixture salts. The latter concentrations were determined by conductivity measurements. Protein-ligand weight ratios were 4.9 and 4.7 for BSA-CHLN and HSA-CHLN complexes respectively.

EHF (extremely high frequencies) range correspond to millimeter waves and are close to the frequencies of relaxation vibrations of water molecules, so that the permittivity of

biomolecule solution is a complex value: $\varepsilon^* = \varepsilon' - i\varepsilon''$. The decrease of mobility of hydration (bound) water results in decrease of general permittivity of the solution containing both free and bound water. Determining complex permittivity of solution and using some known models, we can estimate the amount of bound water molecules and its change under various external influences [10, 17].

Dielectric measurements are carried out at 7.6 mm (39.5 GHz) wavelength using an original differential dielectrometer [10, 17]. For solutions of CHLN and proteins the differences in attenuation coefficients $\Delta\alpha$ and the phase constants $\Delta\beta$ are measured directly between the solvent and solution while the same differences for solutions of complexes are measured directly between protein and complex solutions. The dielectric data in tables 1-3 are given as differences between dielectric parameters of water and corresponding solutions.

Solutions of CHLN and proteins are measured against water while solutions of complexes are measured against solutions of proteins. Measurements are carried out multiple times, and the data are averaged.

Basing on the electrodynamic relationships real ε' and imaginary ε'' parts of the dielectric constant of the solution are calculated:

$$\begin{aligned} \varepsilon' &= (\frac{\lambda}{\lambda_{cr}})^2 + \left(\frac{\lambda}{2\pi}\right)^2 \left[(\beta - \Delta\beta)^2 - (\alpha - \Delta\alpha)^2\right], \\ \varepsilon'' &= 2(\frac{\lambda}{2\pi})^2 (\alpha - \Delta\alpha)(\beta - \Delta\beta) \ , \end{aligned} \tag{1}$$

where λ is the wavelength in free space, λ_{cr} is a critical wavelength in the waveguide, α and β are the attenuation coefficient and the phase constant of the solvent. The method registers $\Delta\varepsilon'$ and $\Delta\varepsilon''$ with an accuracy of up to 0.1% of the values of ε', ε''. A contribution to ε'' resulting from presence of ions in samples is determined by the electroconductivity measurements with 1% accuracy using an AC bridge and calibration solutions of NaCl [18, 19]. Values of $\Delta\varepsilon''$ are presented in tables 1-3 taking into account this contribution.

Since in EHF range ε' and ε'' are frequency-dependent, it is efficient to describe the dielectric properties of the solutions under investigation using frequency-independent parameters of Debye theory of polar liquids: ε_s (low-frequency limit of ε') and ε_∞ (high limit of ε') [20]. In Debye theory which is valid with high accuracy for both water and aqueous solutions of biomolecules, ε_s is related to ε' and ε'' as:

$$\varepsilon_s = \varepsilon' + \frac{\varepsilon''^2}{(\varepsilon' - \varepsilon_\infty)} \tag{2}$$

Quantitative estimates of the hydration extent can be done using formula for dielectric permittivity of mixtures [17, 21]. Because of the low concentrations c that we use and the absence of absorption by both proteins and ligand in EHF range, difference $\Delta\varepsilon_s$ is a linear function of biomolecules volume υ and bound water volume ω:

$$\Delta\varepsilon_s = \varepsilon_s^w - \varepsilon_s^s = pc[(\varepsilon_s^w - \varepsilon_\infty^b)\upsilon + (\varepsilon_s^w - \varepsilon_\infty^w)\omega], \tag{3}$$

where ε_s^w and ε_s^s are ε_s of solvent and solution respectively; p is a form factor (1.58 for all cases); c is the concentration (in g of solute per cm^3 of solution); ε_∞^b and ε_∞^w are the high frequency limits of ε' of biomolecules (~4 [22]) and water (~5.7 [20]) respectively; υ is a partial volume of solute (0.733 cm^3/g for both proteins [23, 24] and 0.899 cm^3/g for CHLN, the latter value we obtained using densitometry; 0.761 cm^3/g and 0.762 cm^3/g and for BSA-CHLN and HSA-CHLN complexes respectively, these values were obtained using corresponding values for proteins and CHLN and taking into account relative concentrations of complex components).

The obtained value of hydration corresponds to the overall amount of water bound with all types of dissolved biomolecules in the aqueous system.

Ions that are present in solutions of biomolecules not only cause the conductivity of the solutions but also lower their ε_s. In order to get the value of dielectric decrement $\Delta\varepsilon_s$ which corresponds to biomolecules and their hydration only we subtract $\Delta\varepsilon_s$ corresponding to ions from experimentally obtained value of the dielectric decrement.

RESULTS AND DISCUSSION

Chlorophyllin hydration

Table 1 presents the dielectric parameters of aqueous solution of CHLN at 20°C. Dielectric parameters of water are obtained using interpolation scheme that allows determining such characteristics in a wide range of frequencies $0< f <100$ GHz and temperatures $0<T<60$ °C [20].

Table 1.
Dielectric parameters and hydration of chlorophyllin

Substance / Parameter	CHLN	Substance / Parameter	Water [20]
c, %	1.76	c, %	–
T, °C	20	T, °C	20
$\Delta\alpha$ (± 0.0006), Np/mm	0.0503	α, Np/mm	2.7
$\Delta\beta$ (± 0.0004), radian/mm	0.0667	β, radian/mm	4.9
$\Delta\varepsilon'$ (± 0.005)	0.385	ε'	17.2
$\Delta\varepsilon''$ (± 0.01)	0.97	ε''	26.9
$\Delta\varepsilon_s$ (± 0.06)	2.84	ε_s	80.5
$\Delta\varepsilon_s^{ion}$ (± 0.1)	2.34		
ω (± 0.03), *g/g*	0.21		
n±1, water molecules	7-8		

Conductivity measurements showed that solution has high electroconductivity (2.9×10^{-3} S·cm^{-1}) which is caused by presence of sodium ions. These measurements indicate that: a) the contribution of the ionic conductivity of the ligand to ε'' of solution is 0.12; b) the concentration of sodium ions calculated from the conductivity value of the solution is 0.064 M. Considering that 1 M of Na^+ lowers ε_s by 8 units [25], the contribution of sodium ions in the dielectric decrement ($\Delta\varepsilon_s$-$\Delta\varepsilon_s^{ion}$) is 0.5.

The calculation based on the relationship (3) indicates that 0.21 g of water is bound to 1 g of the ligand. This value corresponds to 7-8 bound water molecules per one molecule of the ligand. Since CH and CH_2 groups of the ligand are not hydrated (they do not form strong H-bonds and are not charged) they do not influence the water structure. The found hydration

extent correlates with number of H-bonds that chlorophyllin (Fig.1a) is able to form with water molecules: 3 H-bonds formed by CO groups and 4 H-bonds formed by nitrogen atoms.

Hydration of BSA-CHLN complex

BSA sample used was fatty acids free and thus with all binding sites available for binding by CHLN. Table 2 shows dielectric parameters of BSA and BSA–CHLN complex at 22 °C.

Presence of sodium ions from CHLN sample in BSA-CHLN complex is taken into account both in its $\Delta\varepsilon''$ and $\Delta\varepsilon_s$ values. The contributions of the ionic conductivity to ε'' of BSA and BSA-CHLN complex solutions caused by admixture ions are 0.05 and 0.09 respectively.

Table 2.
Dielectric properties and hydration of BSA and BSA-CHLN complex

Substance / Parameter	BSA	BSA–CHLN	Substance / Parameter	Water [20]
c, %	2.83	3.40	c, %	–
T, °C	22		T, °C	22
$\Delta\alpha$ (± 0.0006), Np/mm	0.0832	0.0955	α, Np/mm	2.7
$\Delta\beta$ (± 0.0004), radian/mm	0.1180	0.1367	β, radian/mm	5.1
$\Delta\varepsilon'$ (± 0.005)	0.731	0.851	ε'	18.3
$\Delta\varepsilon''$ (± 0.01)	1.52	1.78	ε''	27.8
$\Delta\varepsilon_s$ (± 0.06)	3.88	4.57	ε_s	79.8
$\Delta\varepsilon_s^{ion}$(± 0.07)	3.75	4.28		
ω (± 0.02), *g/g*	0.38	0.30		

Our results on hydration of BSA (0.38 g/g) are consistent with the value of hydration of an average globular protein – 0.35 g water per gram of protein [26]. They are also in an agreement with results of another dielectric study on albumin hydration [27]. The weight of total restrained water per protein weight in the abovementioned study was estimated of 0.34 g/g.

As it is shown in the table 2, for the solution of BSA the value of $\Delta\varepsilon_s$ is 3.88. The expected contribution of CHLN to the dielectric decrement of complex basing on the experimental value of 2.84 for $\Delta\varepsilon_s$ of CHLN solution in concentration 1.9% is 0.91 (the concentration of CHLN in the complex is 0.61%). When adding this value to 3.88, we obtain the expected value for $\Delta\varepsilon_s$ of complex – 4.79, which is larger than the experimental value of the dielectric decrement of complex (4.57). The decrease of the dielectric decrement, or the difference between these two values, is 0.22. It is approximately 3 times greater than the experimental error, and thus the observed effect can be considered as reliable. The effect can be explained by a decrease of amount of bound water molecules in the system. The calculated complex hydration (0.30 g/g) is less than the weighted sum of hydration of the complex components (0.35 g/g) indicating the release of bound water molecules into bulk solvent.

As far as we know, interaction between BSA and sodium copper chlorophyllin has been studied earlier by Gao and coauthors only [28]. They observed the strong interaction between BSA and CHLN with apparent association constant $K_a = 2.17\times10^5$ M^{-1} and binding site $n = 1.12$ which indicates that BSA has only one binding site for CHLN. It was shown that CHLN can bind to both Tyr and Trp residues but the interaction between the latter one and the ligand is stronger. Since Trp residue is located in a hydrophobic pocket of subdomain IIA, CHLN is likely to bind with binding site I, which is formed there.

Our experimental results consisting in the dehydration of the complex also serve as an evidence of BSA–CHLN interaction. The decrease of amount of bound water molecules can be explained by a number of factors including the dehydration of pigment, the displacement of water molecules from the ligand binding site and a general change in the protein hydration caused by ligand-induced conformational changes.

Hydration of HSA-CHLN complex

HSA sample that was used in the experiment contained fatty acids (FA). It represents normal physiological conditions when HSA binds with approximately 0.1-2 mol of FA per 1 mol of protein [29]. It is more correct then to consider the system under investigation as a triple one: HSA–FA–CHLN.

The dielectric parameters of HSA and its complex with CHLN at 23°C are presented in the table 3. The contributions to ε'' resulting from presence of ions in solutions of HSA and HSA-CHLN complex are 0.04 and 0.08 respectively.

Table 3.
Dielectric properties and hydration of HSA and HSA-CHLN complex

Substance / Parameter	HSA	HSA–CHLN	Substance / Parameter	Water [20]
c, %	2.86	3.46	c, %	–
T, °C	23		T, °C	23
$\Delta\alpha$ (± 0.0006), Np/mm	0.0806	0.0967	α, Np/mm	2.8
$\Delta\beta$ (± 0.0004), radian/mm	0.1306	0.1526	β, radian/mm	5.1
$\Delta\varepsilon'$ (± 0.005)	0.885	1.018	ε'	18.8
$\Delta\varepsilon''$ (± 0.01)	1.56	1.89	ε''	28.2
$\Delta\varepsilon_s$ (±0.06)	3.52	4.41	ε_s	79.4
$\Delta\varepsilon_s^{ion}$(± 0.07)	3.42	4.14		
ω (±0.02), *g/g*	0.27	0.25		

The calculation shows that investigated HSA sample is less hydrated than BSA: its extent of hydration is 0.27 g of water per 1 g of the protein. The difference in hydration of two studied proteins can be caused by two major factors: 1) FA binding sites of BSA might be available for interaction with water (hydration active sites are more exposed to water environment) and 2) conformational changes of HSA caused by FAs can lead to decrease of solvent accessible area of the protein.

Unlike BSA-CHLN complexation, the formation of HSA–CHLN complex is accompanied only by a slight decrease of the dielectric decrement of complex in comparison with the sum of $\Delta\varepsilon_s$ of the complex components taking into consideration their concentrations in the complex. As shown in the table 3, for the solution of HSA the value of $\Delta\varepsilon_s$ is 3.52. The expected contribution of CHLN to $\Delta\varepsilon_s$ of complex is 0.96 (the concentration of CHLN in the complex is 0.65%). The expected value for $\Delta\varepsilon_s$ of complex obtained as the sum of this value and 3.52 is 4.48, while the experimentally obtained value is 4.41. The difference of these two values is 0.07, which is of the order of the experimental error. As calculations show, hydration of HSA–CHLN complex is 0.25 g/g. Taking into account relative concentrations of HSA and CHLN in the complex and the calculated CHLN hydration, one should expect 1 g of the complex to bind 0.26 g of water. Thus our results show only the tendency towards the decrease of hydration under the formation of HSA-CHLN complex.

Interaction between human serum albumin (fatty acids free) and CHLN, to the best of our knowledge, has been studied only by Ouameur and coauthors by absorption, CD and FTIR

spectroscopy methods [13]. They showed that CHLN is located along the polypeptide chains of protein with no specific interaction. The spectral changes observed using FTIR spectroscopy in [13], in authors' opinion, are due to the participation of the pigment polar group in H-bonding network in the CHLN-HSA complex that contributes to structural stabilization of metalloporphyrin-protein complex. The association constant calculated for pigment-HSA complex ($K=7\times10^3$ M^{-1}[13]) showed weaker interaction in respect to complexes of HSA with other ligands [30] and fatty acids ($K_{FA}\sim10^6$ M^{-1} [31]). Although association constant for HSA-CHLN complex found in [13] is rather small, there is an evidence of interaction between HSA and the pigment. CD data obtained for aqueous solution showed the reduction of the α-helix (from 66 to 55%) in favor of the β-sheet and turn structures upon pigment complexation. This, as shown by Ouameur and coauthors [13], is the indication of a partial unfolding of protein in the presence of pigment.

As shown in [29, 32], fatty acids may affect the interactions between HSA and drugs because some FA binding sites overlap with drug binding sites I and II. The HSA-FA complexes are also shown to display conformational changes from the defatted HSA structure [33]. The binding of FA molecules to HSA can cause some rearrangements at the I–II and II–III domain interfaces and conformational changes of the side chains of drug binding site I [29].

Absence of reliable hydration changes upon complex formation observed in our experiment might indicate that under experimental conditions the HSA–CHLN complexation is unfavorable. Since information on interaction between HSA (both free and bound with fatty acids) and CHLN lacks, we can only suggest that due to 3-order higher association constant fatty acids might occupy binding sites that CHLN is able to bind with. Another possible explanation, which is based on data in [29, 33, 34], is the ability of fatty acids molecules to cause such conformational changes of protein molecule that complementarity between CHLN and its binding site on protein becomes broken. The latter suggestion is contrary to the absence of specific interaction as observed in [13]. On the other hand, studies of interaction between HSA and hemin, another porphyrin that has similar to CHLN structure, indicate a single binding site for ligand within hydrophobic cavity of subdomain IB which corresponds to a binding site for fatty acids [35]. In [28] CHLN is shown to interact with BSA, which is structurally close to HSA, specifically via its hydrophobic aromatic part at the binding site located in subdomain IIA of BSA. In order to get more complete description of processes accompanying formation of HSA–FA–CHLN complex one need to carry out some additional computer simulations (including molecular docking) and experiments on hydration changes upon complex formation of fatty acids free HSA with CHLN.

Quantitative dielectrometry-based estimation of hydration and its changes upon formation of HSA–CHLN and BSA–CHLN complexes is just the first step in solution of the problem of the water role in the functioning of complex. To obtain more complete description of the systems one should involve direct methods of energy and structure estimation such as calorimetry and computer modeling (docking, molecular dynamics simulations). The combination of these methods allows obtaining more detailed structures of complexes with the direct regard for water surroundings [36].

CONCLUSIONS

The dielectric characteristics of solutions of chlorophyllin, human and bovine serum albumins and complexes of the proteins with CHLN were studied in the EHF range using an original high-sensitivity differential dielectrometer. Basing on some model concepts we have made the quantitative estimation of hydration extent of the investigated substances and its changes upon the formation of the HSA–CHLN and BSA–CHLN complexes. Significant decrease of hydration observed for BSA–CHLN complex formation can be contributed to the

redistribution of free and bound water molecules in system and serve as an evidence of BSA–CHLN interaction. It was also found that within experimental error no hydration changes accompany formation of HSA–CHLN complex (there is only a slight tendency towards some decrease of hydration). This result might indicate that such complexation is unfavorable due to the presence of fatty acids that bind with HSA with higher affinity than CHLN.

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