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Mendeleev Commun., 2015, **25**, 307–309
Mendeleev Communications

Interaction between albumin and zinc tetra-4-[(4′-carboxy)phenylamino]phthalocyanine

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DOI: 10.1016/j.mencom.2015.07.027

The complex formation of bovine serum albumin with zinc tetra-4-[(4′-carboxy)phenylamino]phthalocyanine and its J-dimer in aqueous solutions was characterized.

Interactions between biopolymers and zinc(II) phthalocyanines containing different hydrophobic and hydrophilic groups are of great interest. Phthalocyanines are used for the diagnostics and therapy of cancer.[1] The studies of interactions of phthalocyanines and synthetic polyelectrolytes with proteins are promising for the fine regulation of protein properties such as transport ability and thermal stability.[2] It is well known that the location of macroheterocyclic compounds in binding sites, between subdomains or on the surface of protein globules depends on the hydrophobic/hydrophilic properties of phthalocyanines and the acid–base properties of a medium. The concentration of a macroheterocyclic compound in solution is a significant factor, especially, when metal phthalocyanines form stable dimer or oligomer species in solution.[3] The aim of this work was to study complex formation between bovine serum albumin (BSA) and zinc tetra-4-[(4′-carboxy)-phenylamino]phthalocyanine, which occurs as stable J-dimers in a concentration range from 1.8×10^{-5} to 3×10^{-5} mol dm^{-3}.†

The complex formation was valuated according to the Scatchard method[6] from fluorescence spectroscopic data. Figure 1 shows the fluorescence spectra of BSA in an aqueous solution of NaCl containing the phthalocyanine.

We constructed a plot in the coordinates $\lg[(F_0 - F)/(F - F_\infty)] = f(C_{Pc})$, where F_0 is the fluorescence intensity of BSA solution without phthalocyanine, F is the fluorescence intensity of BSA after each dose of phthalocyanine, F_∞ is the BSA fluorescence intensity after the addition of the last dose of phthalocyanine, and C_{Pc} is the phthalocyanine equilibrium concentration in solution after each dose (Figure 2). The slope of this straight line is equal to the number of protein binding sites to the macrocyclic molecule, the cross point of the plot with the abscissa is equal to the logarithm of the dissociation constant of the complex.

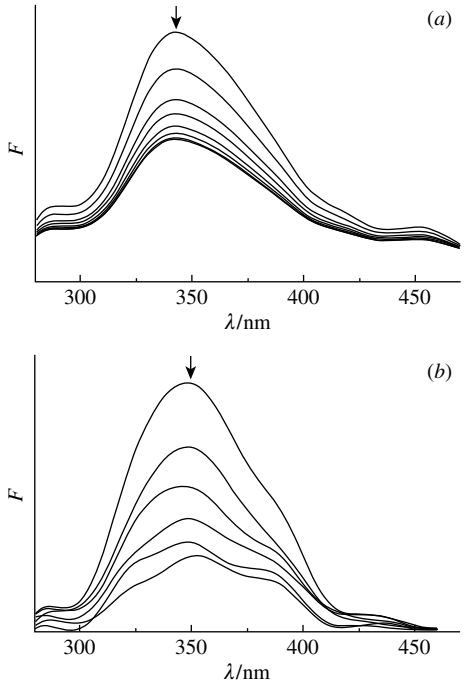

Figure 1 Fluorescence spectra (λ_{ex} = 295 nm) of BSA in an aqueous solution of NaCl (0.05 M) with the addition of ZnPc(4-NHC$_6$H$_4$COONa)$_4$ solution of concentration (*a*) from 0 to 4.5 μmol dm^{-3} and (*b*) from 10 to 30 μmol dm^{-3}.

Zinc tetra-4-[(4′-carboxy)phenylamino]phthalocyanine in aqueous solutions is inclined to association. Unlike ZnPc-(4-OC$_6$H$_4$COONa)$_4$ and ZnPc(4-SC$_6$H$_4$COONa)$_4$, which form π–π associates, ZnPc(4-NHC$_6$H$_4$COONa)$_4$ forms J-aggregates [(ZnPc(4-NHC$_6$H$_4$COONa)$_4$]$_2$.[7] Analysis of the electronic absorption spectra of ZnPc(4-NHC$_6$H$_4$COONa)$_4$ showed that, in a concentration range from 10^{-7} to 10^{-6} mol dm^{-3}, the phthalocyanine mainly occurs as monomer species. However, as the phthalocyanine concentration was increased to $(1–6)\times10^{-5}$ mol dm^{-3}, the association equilibrium was shifted towards dimer species. The linear plot of absorbance *vs.* phthalocyanine concentration in a concentration range of $(1–6)\times10^{-5}$ mol dm^{-3} indicates that higher order associates were not formed. It may be explained by coordination interaction between the central metal atom of one phthalocyanine molecule and the nitrogen atom of the peripheral substituent of another phthalocyanine molecule. According to published data,[8,9] the coordination of an electron-donating ligand

† Zinc tetra-4-[(4′-carboxy)phenylamino]phthalocyanine was synthesized and purified according to a published procedure.[4] Bovine albumin, fraction V, for biochemistry, pH 7.0 (Acros Organic) was used without further purification. For BSA as a polyelectrolyte, an effect of polyelectrolyte swelling (*i.e.*, conformational alterations of the protein in solution) takes place. As found earlier,[5] an aqueous NaCl (0.05 M) solution was added to an aqueous BSA solution (0.08 wt%) to eliminate this effect.

The spectroscopic investigation was performed using a Unico 2800 spectrophotometer (United Products and Instruments) in a range of 200–800 nm, in 10 mm quartz cuvettes. The fluorescence spectra were measured using an Avantes AvaSpec-2048 spectrophotometer, an Ivolga OMS-1 light source (Lumix) and a LM-4 monochromator.

The IR spectra of crystal samples (KBr disks) were recorded in a range of 4000–400 cm^{-1} using a VERTEX 80v spectrometer (Germany).

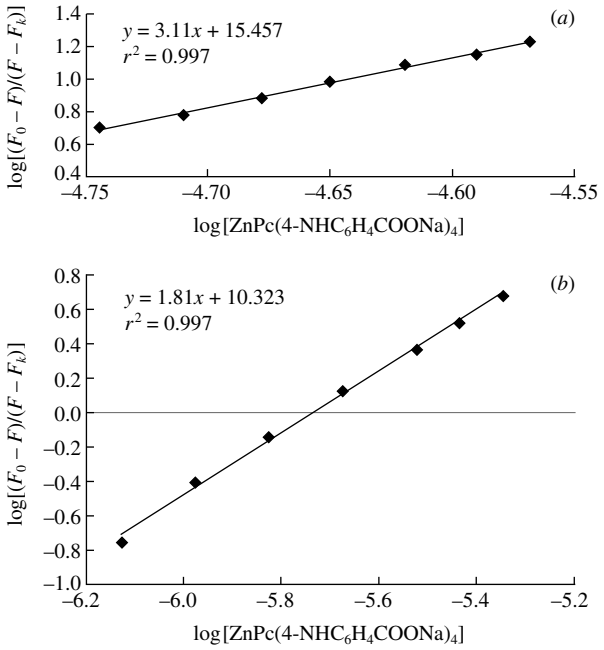

Figure 2 Plot in Scatchard coordinates bond fluorescence spectra of BSA upon titration by $ZnPc(4\text{-}NHC_6H_4COONa)_4$ with concentrations: (*a*) 0–4.5 μmol dm^{-3} and (*b*) 10–30 μmol dm^{-3}.

by metal phthalocyanines results in an out-of-plane shift of the metal towards the ligand that, in turn, complicates coordination of the second electron-donating ligand. It is likely that the distortion of a planar structure and noncompensated additional energy consumption due to the drawing of the metal into the macrocycle plane to coordinate the second ligand prevent from formation of the $ZnPc(4\text{-}NHC_6H_4COONa)_4$ J-associates of a higher order.

Our tentative studies allow us to suppose that the composition of the phthalocyanine–BSA complexes and the location of $ZnPc(4\text{-}NHC_6H_4COONa)_4$ in the protein globule depend on the concentration of phthalocyanine in solution.

The Scatchard parameters for the complexes are given in Table 1. The complex of BSA with $ZnPc(4\text{-}NHC_6H_4COONa)_4$ monomer species is more stable than that with the J-dimers. A comparison of the stability constant of BSA with $ZnPc(4\text{-}NHC_6H_4COONa)_4$ with published data[10,11] showed that the same stability constant is typical of albumin with macroheterocyclic compounds located in IB and IIA subdomaines. Both of the sites have a hydrophobic pocket enclosed by positively charged and polar groups and contain tryptophan. Therefore, anion phthalocyanines are located in IB and IIA subdomains. The isoelectric point of BSA is 4.6,[12] *i.e.*, the protein globule surface in the test solutions is negatively charged. This fact and the high constants exclude the interaction of $[ZnPc(4\text{-}NHC_6H_4COONa)_4]_2$ with the protein surface. Thus, based on the fluorescence data, it can be concluded that $[ZnPc(4\text{-}NHC_6H_4COONa)_4]_2$ is located either in subdomains IB and IIA or near to them, for instance, between IB–IIIB and IIA–IIIA/IIB. It is obvious that the location of $[ZnPc(4\text{-}NH\text{-}C_6H_4COONa)_4]_2$ in the protein globule (inside of the subdomains or between them) would affect the conformational state of the protein and its thermal stability.

Table 1 Stability constants ($K \pm 3\%$) and the number of binding sites (n) of $ZnPc(4\text{-}NHC_6H_4COONa)_4$ and $[ZnPc(4\text{-}NHC_6H_4COONa)_4]_2$ with BSA, calculated by the Scatchard method.

Complex	K	n
BSA–$ZnPc(4\text{-}NHC_6H_4COONa)_4$	5.4×10^5	2
BSA–$[ZnPc(4\text{-}NHC_6H_4COONa)_4]_2$	9.4×10^4	3

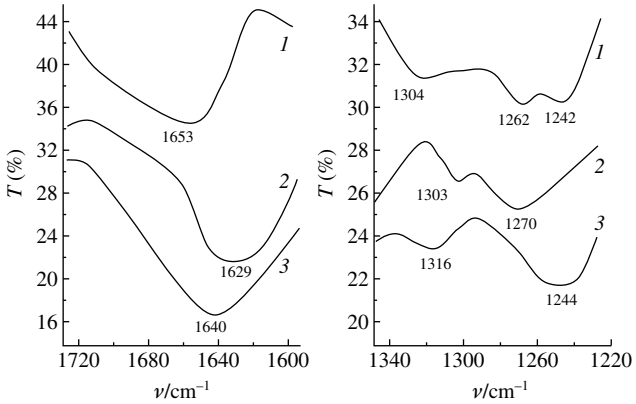

Figure 3 IR spectra of (*1*) BSA, (*2*) BSA complex with $ZnPc(4\text{-}NHC_6H_4COONa)_4$ and (*3*) BSA complex with $[ZnPc(4\text{-}NHC_6H_4COONa)_4]_2$ in amide I (left) and amide III (right) regions.

The initial IR spectrum of BSA (Figure 3) is typical of a native conformation.[13] In the amide I region, an intense broad band at 1653 cm^{-1} is detected. In the amide III region, three bands are observed at 1242, 1262 and 1304 cm^{-1}. The α-helix content of the secondary structure of BSA is no less than 67%.[14–16] Other 23% include unordered and strand structures. The protein is thought to possess little or no β-sheet structure. The band at 1653 cm^{-1} is assigned to α-helical structure.[17–19] In the amide III region, the α-helical (1330–1290 cm^{-1}), β-sheet (1290–1260 cm^{-1}) and unordered (1250–1220 cm^{-1}) structures of BSA are observed.[20,21]

The IR spectra of the complexes show that the complex formation of BSA with both $ZnPc(4\text{-}NHC_6H_4COONa)_4$ and its J-dimers leads to the alteration of the secondary structure of the protein (Figure 3).

The formation of complex between BSA and $ZnPc(4\text{-}NHC_6H_4COONa)_4$ results in a shift of the band in the amide I region towards low frequencies and the appearance of a band at 1630–1620 cm^{-1} due to a transition from α- to β-structure.[16] In the amide III region, the shift of the band at 1262 cm^{-1} towards high frequencies associated with the β ordering of the protein structure and the disappearance of the bands associated with unordered fragments of the protein molecules are observed. Thus, the spectral changes indicate the promotion of local conformational transitions in a BSA globule from the unordered to β-structure induced by $ZnPc(4\text{-}NHC_6H_4COONa)_4$. However, α-helical structure remains dominant.

In the amide I region, the complex formation between the protein and $[ZnPc(4\text{-}NHC_6H_4COONa)_4]_2$ is accompanied by an intense absorption at 1640 cm^{-1} associated with short α-helical structures.[13] In the amide III region, the shift of the band at 1262 cm^{-1} towards low frequencies is due to transition from α-helix to short segment α-sheet structures.

Information on the interaction between protein and macroheterocyclic compound can be obtained from studies of thermal effect on the protein stability (Table 2, Figure 4). The thermal stability of protein was estimated from a decrease of the fluorescence spectra of BSA, which are sensitive to lose of the protein native conformation.[22] The denaturation temperature of BSA obtained from the fluorescence spectra is 61 °C. Note that the denaturation temperature depends on different factors, such as the presence of electrolyte, the rate of heating and the phase state. According to published data,[23] the denaturation temperatures of BSA in a crystal and in solution at pH 6.7 are 68 and 64 °C, respectively. The strong stabilizing effect of NaCl solutions (0–1.0 M) on the native BSA structure was reported[24,25] (the denaturation temperature increases from 56 to 72 °C). No significant change was observed in the denaturation temperature of

Table 2 Thermal stability of BSA and its complexes with ZnPc(4-NH-$C_6H_4COONa)_4$ and $[ZnPc(4\text{-}NHC_6H_4COONa)_4]_2$.

System	$T/\pm5$ °C
BSA	61
BSA–$ZnPc(4\text{-}NHC_6H_4COONa)_4$	56
BSA–$[ZnPc(4\text{-}NHC_6H_4COONa)_4]_2$	90

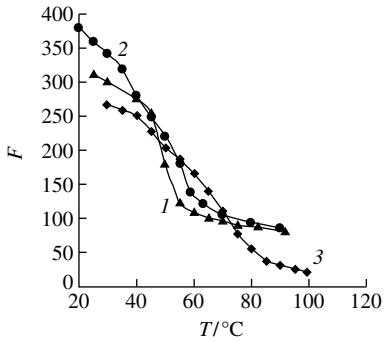

Figure 4 The temperature dependence of the fluorescence intensity of (*1*) free BSA and complexes of BSA with $ZnPc(4\text{-}NHC_6H_4COONa)_4$ at phthalocyanine concentration of (*2*) 0.2 and (*3*) 20 μmol dm^{-3}.

BSA upon its interaction with $ZnPc(4\text{-}NHC_6H_4COONa)_4$. On the contrary, the interaction of the protein with $[ZnPc(4\text{-}NHC_6H_4COONa)_4]_2$ increased it by 30 °C. The same difference confirms the assumption about the localization of J-dimers between subdomaines IB–IIIB and IIA–IIIA/IIB.

Thus, the stability of phthalocyanine complexes with BSA and the localization of the phthalocyanine protein globule depend on the association state of phthalocyanine in solution. The monomer $ZnPc(4\text{-}NHC_6H_4COONa)_4$ forms stable complexes with albumin. The formation of complex between phthalocyanine J-dimer and BSA changed the secondary structure of the protein.

The results obtained can contribute to the studies of interactions between phthalocyanines and proteins, which are perspective for fine regulation of the protein properties such as transport ability and thermal stability.

This work was supported by the Russian Science Foundation (grant no. 14-23-00204).

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Received: 29th December 2014; Com. 14/4539