Study of DNA interaction with cobalt disulfophthalocyanine

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Abstract. DNA interaction with disulfophthalocyanine of cobalt (CodSPC) was studied by UV-VIS spectrophotometry and low gradient viscometry. The thermal denaturation of DNA in complexes with CodSPC was compared with free DNA denaturation. The small increase in melting temperature indicates that the secondary structure of DNA in complexes is stable. As a result of experimental research the external binding of CodSPC to DNA was determined. The only one type of binding is accompanied by stacking of heterocycles at high concentrations of CodSPC. An increase in viscosity of DNA solutions with the growth of CodSPC concentration can be explained by intermolecular DNA contacts via CodSPC.

Most of the antitumor drugs are aimed at binding to the DNA molecule in the cell. This helps to prevent cell division or leads cancer cells to apoptosis. Different types of interaction between drugs and double-stranded DNA can be distinguished as major or minor groove binding, external binding with phosphates and intercalation. DNA interaction with phthalocyanine, porphyrine and their compounds with metals has a significant interest because of their spectral features, which have medical application, such as photodynamic therapy [1]. Metal phthalocyanines can be used also as pigments and catalysts. The absorption in infrared area of spectrum, high quantum yield of fluorescence and an ability to cleave DNA promote their application in medical and biological practice. An aggregation of phthalocyanines in water solutions is a well-known fact. For this reason the usage of phthalocyanines in chemotherapy is limited. Nevertheless, an addition of sulfonic, carboxylic or amino groups to the peripheral sites can reduce aggregation between molecules [2, 3]. The type of the coordination metal also influences on aggregation. Although study of cationic complexes of phthalocyanines are in the focus of investigators, the anionic complexes are much less studied [3]. In this paper the possibility of negative DNA interaction with anionic CodSPC was regarded. We used UV-VIS spectrophotometry, low gradient viscomery and thermal denaturation of DNA (DNA melting). Also binding constant was calculated with Wolf-Shimer equation [4].
1. Experimental section

1.1. Materials
High molecular calf thymus DNA (Sigma Aldrich, CAS 73049-39-5; D1501-1G Type I “Highly Polymerized”, fibrous preparation) with molecular mass $9\times10^6$ that was determined from the intrinsic viscosity of DNA in 0,15 M NaCl was used. Water solution of NaCl (chemically pure) in concentration 0.005 M was used as solvent for the preparation of DNA solution. DNA concentrations was determined by spectral method. For that the difference in absorption at 270 and 290 nm for DNA denaturated in 6% HClO$_4$ (in 15 min at 100$^\circ$C) was measured. This procedure allows one to independently determine the molar extinction coefficient for a given DNA sample by an absorbance at 260 nm for native molecule (without thermal denaturation). The sodium salt of anionic complex of cobalt disulfophthalocyanine was used. The structure of the compound is shown in Fig.1. Sulfonic groups make CodSPC water soluble. The Tris buffer was used in all our experiments. Consequently in this work all solutions of CodSPC (with or without DNA) contain Tris buffer.

![Fig.1 Structure of cobalt disulfophthalocyanine (CodSPC).](image)

1.2 Methods.
1.2.1 UV-VIS absorption spectrophotometry
All experiments were conducted in 0,005 M NaCl and Tris buffer solutions. Spectrophotometer SF-56 (Russia) with operating range from 190 nm — 1100 nm was used. Quartz cuvettes were used with optical pans 1 cm. Spectrophotometric titration allows to determine binding constant according to Wolfe-Shimer equation:

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\frac{[\text{DNA}]}{(\varepsilon_a-\varepsilon_f)} = \frac{[\text{DNA}]}{(\varepsilon_b-\varepsilon_f)} + \frac{1}{K_b(\varepsilon_b-\varepsilon_f)}
$$

where $[\text{DNA}]$ is the DNA concentration in moles of base pairs, $\varepsilon_a$ corresponds to the $A_{\text{compound}}$, where $A$ is the absorption of the compound at the selected wavelength within the analyzed band, $[\text{compound}]$ is the concentration of compound, $\varepsilon_b$ and $\varepsilon_f$ are molar extinction coefficient of free compound and compound fully bonded to DNA respectively. The plot of $\frac{[\text{DNA}]}{(\varepsilon_a-\varepsilon_f)}$ value on $[\text{DNA}]$ called Wolfe-Shimer plot can give the binding constant value [4].

1.2.2 Low-gradient viscometry
In order to determine the specific viscosity $\eta_r$-1 of DNA solutions at constant concentration of DNA the low-gradient rotation Zimm-Crothers type viscometer was used. All solutions were prepared with constant DNA concentration $C_{DNA}$=0.005% (determined from spectral data). All systems contain NaCl salt (0.005M) and Tris buffer in small concentrations.

1.2.3 Thermal denaturation study (DNA melting)
Two systems were prepared in 0.005 M NaCl with Tris buffer. Specord 200 Plus spectrophotometer was used.

2. Results and discussion

2.1 Absorption spectroscopy
The absorption spectrum of CodSPC has bands in UV and visible areas (Fig. 2A). Shortwave UV band in intersects with DNA absorption spectrum. It is difficult to use for the analysis of binding. Near UV band has maximum at 345 nm (so-called Soret or B band). This band associates with the nature of complexing metal. Visible red split band with main maximum at 693 nm and unresolved maximum around 595 nm (Q-bands) associate with electronic structure of heterocycles. It was shown that the addition of DNA into CodSPC solution causes change in spectrum of compound. Indeed, all bands undergo a change: one can see hypsochromic shift with hyperchromism in Soret and minor Q bands and hypochromism with blue shift in main Q band (Fig. 2). In this way, spectral data show that DNA interacts with CodSPC in a solution. Spectrophotometric titration demonstrates the presence of isosbestic points in Soret and Q bands, which indicates only one type of CodSPC binding to DNA.

Titrations are the most convenient way to determine the binding constants [5]. Aggregation of phthalocyanines in solutions without DNA was not recognized. But according to the Fig.2 C the hyperchromism in Q band can indicate some association of chromophores. We could not see this effect for Soret band. It is known that B-band is common for all phthalocyanines and related to the $\pi$-$\pi$ electron transitions [6]. One type of binding, according to isosbestic point [7] associates with decrease in absorption of phthalocyanines. Although there is no significant changes in the DNA absorption (we calculate this value believing that absorption of CodSPC in this region does not change).

According to the literature such spectral changes support the idea about external binding of CodSPC to DNA [8]. To estimate the binding constant, the Wolfe-Shimmer equation was applied (Fig.3). According to the data obtained, the value of $K_b$ is quite small for the intercalation and huge for an electrostatic binding [2, 5]. However, the calculated binding constant is correct for an external interaction. The values of hypochromism and hypsochromism as well as $K_b$ are presented in the Table 1.

Table 1 Hypochromic (%) effect, hypsochromic ($\Delta \lambda$) shift for $C_{DNA}$= 0.007% binding constant and shift in DNA melting temperature

| Complex | Hypochromic effect (%) | Hypsochromic Effect (nm) | $K_b$, M$^{-1}$ | $\Delta T_m$ |
|---------|------------------------|--------------------------|----------------|------------|
| Co(C8H4)$_2$(C8H4SO3Na)$_2$N$_8$ | 4.38 | 1.6 | $3.6*10^{-4}$ | 3.5 |
Fig. 2 CodSPC absorption spectrum with (solid lines) and without DNA (segment line) in 0.005 M NaCl solution with Tris buffer (a), the results of titration in B-(b) and Q-(c) bands. [CodSPC] = 2.5*10^{-5} M a) and two bands of compound absorption (b and c). DNA concentration was changed from 0 to 0.015%

Fig. 3 Wolfe-Shimmer plot in order to determine the binding constant (see in text)

2.2 DNA melting study
Another method which can give an information about stability of DNA secondary structure during binding is DNA thermal denaturation studies. According to the experimental data, the intercalation is
accompanied by a large change in DNA melting temperature, while groove binding or an electrostatic interaction cause only small shift in DNA melting temperature respectively [9]. In our experiment free DNA in 0.005 M NaCl (C = 0.005%) and complex of DNA with CodSPC (C_{CoPc} = 5.0*10^{-5}M) were heated from 25 °C to 98 °C. The dependences of absorption at 260 nm (maximum of DNA absorption band) on T are shown in Fig. 4. Analysis of the melting curves provides us information about melting temperature (middle point of transition). The small increase in the melting temperature of DNA was served in complexes (see Table 1). In Fig. 4 the curve with open circles indicates free DNA melting, and the curve with the black one shows melting of DNA in complexes. The data do not contradict with an idea about external binding of CodSPC to DNA.

![Fig.4 Melting curves of free DNA (pen circles) and DNA in complexes with CodSPC (black circles)](image)

**2.3 Low-gradient viscometry**

The binding of small molecules to DNA can effect on DNA volume in a solution and on DNA rigidity [10]. To investigate the influence of CodSPC on DNA tertiary structure we use low gradient viscosity. The measurements were carried out at room temperature (21°C). DNA concentration in all systems was constant, C_{DNA}=0.005%. The compound CodSPC was added into DNA solution by the mixing of DNA and CodSPC solutions in same solvents. Different concentrations of CodSPC cause increase in solution viscosity. The dependence of reduced viscosity of DNA solutions on CodSPC concentration is presented in Fig. 5. The data show an increase (28%) in the reduced viscosity of DNA solutions with CodSPC respect to DNA solution without compound.

![Fig.5 Dependence of reduced viscosity of DNA solutions with CodSPC on concentration of the compound](image)
The intercalation of heterocycles of CodSPC can induce the observed increase in viscosity, but spectral data and melting experiment indicate the external binding. We can assume that the only one type of binding - the external binding of CodSPC to DNA, is accompanied by stacking of heterocycles outside of DNA helix. An increase in viscosity of DNA solutions with the growth of CodSPC concentration can be explained by intermolecular DNA contacts via CodSPC. For better understanding further investigation is necessary.

3. Conclusions
Our study show that CodSPC in experimental condition interacts with DNA. Only one type of binding is realized. The binding is accompanied by changes in CodSPC spectrum and by increase in viscosity of DNA solutions. The binding does not cause destabilization of DNA secondary structure. For better understanding of processes of binding further experiments should be carried out. The support of this work was partially provided by Saint-Petersburg State University Research Park Center of Diagnostics of Functional Materials for Medicine, Pharmacology and Nanoelectronics.

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