

The interaction of DNA with piperazine derivatives of benzoimidazophthalazine

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Abstract. The interaction of DNA molecule with new synthetic compounds, piperazine derivatives of benzo[4,5]imidazo[1,2-a]phthalazine, was investigated by the spectral, hydrodynamic and optical methods. The thermodynamic parameters of the interaction and the stoichiometry of the complexes were determined by spectrophotometric titration. A mode of binding and structure of the complexes were determined by analyzing the changes in the intrinsic viscosity and the optical anisotropy of the macromolecule upon complexation. It is shown that an increase in the intrinsic viscosity and the optical anisotropy with a small content of the ligand in the complex is caused by the increase in the thermodynamic rigidity of macromolecules upon the formation of the complex. Increase of the contour length of the macromolecule in the complex does not occur, that indicates nonintercalative mode of binding the piperazine derivatives of benzoimidazophthalazine with DNA.

1. Introduction

The mechanism of biological activity of various heterocyclic compounds: antibiotics, alkaloids, synthetic compounds, involves the formation of the complex with the DNA molecule [1]. This fact determines the relevance of studies of the interaction of new synthetic compounds with planar polycyclic chromophore with DNA *in vitro*.

There are several modes of binding of these compounds with DNA as monomers or as aggregates on the surface of the double helix [2, 3]. The most biologically significant binding is a monomeric ligand binding with DNA. In this case the chromophore intercalates into double helix, or it locates in the minor groove of the DNA double helix. Previously, it was shown on the interaction of DNA with derivatives of actinocine [4], xanthone [3], phenazine [5] that the localization of molecule of the ligand upon binding is determined by the ionic conditions [3] and its structural features [3-5].

Spectral and thermodynamic methods of research do not allow to distinguish between both types of monomeric ligand binding. It is necessary to study the changes in the macromolecular structure of DNA upon binding with the ligand to determine the structure of the complex. The local untwisting of the double helix and its elongation are typical changes upon the intercalation [6, 7]. The viscometry is commonly used method to determine the increase of the contour length of the double helix. The increase in reduced viscosity of DNA solution: $(\eta_r - 1)/C_{DNA}$, where η_r – relative viscosity, C_{DNA} – DNA concentration, upon formation of the complex is considered as a sign of intercalation [8, 9]. However, the change of the reduced viscosity of the solution of the macromolecule in the presence of low molecular weight ligand does not allow to make a conclusion about the presence of certain changes in



macromolecular parameters. This requires the determination of the value of the intrinsic viscosity $[\eta] = \lim (\eta_r - 1)/C_{DNA}$, when $C_{DNA} \rightarrow 0$, which is directly related to the size of the macromolecule.

In the case of high molecular DNA changes in intrinsic viscosity can occur not only as a result of changes in the contour length, but also due to changes in thermodynamic rigidity of the macromolecule [10]. Therefore, to detect changes in the contour length of the macromolecule more research is needed.

In this work the binding of new synthetic compounds, derivatives benzoimidazophthalazine (figure 1), with DNA was studied by the spectral, hydrodynamic and optical methods. Thermodynamic parameters of binding of compounds with DNA and the stoichiometry of the investigated complexes were determined by spectrophotometric titration (SPT). Changes of the macromolecular structure of DNA in the formation of DNA-ligand complex were determined by using a parallel measurement of the intrinsic viscosity and the optical anisotropy of the complexes [11]. The use of these two methods allows us to make unambiguous conclusions about the presence or absence of extension of the DNA double helix upon binding.

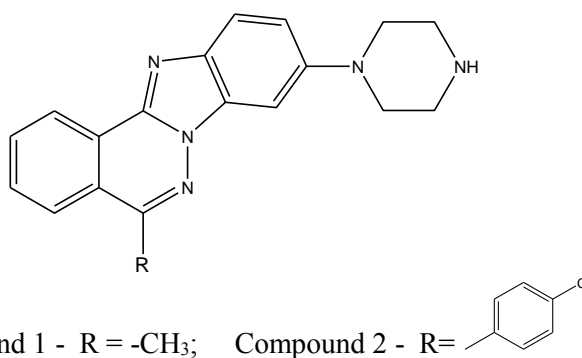


Figure 1. The structure of investigated compounds

2. Materials and methods

Calf thymus DNA “Sigma” with molecular weight $M=22 \cdot 10^6$ Daltons, defined by the value of the intrinsic viscosity $[\eta]$ in water solution of 0.15 M NaCl [12]. The DNA concentration was determined spectrophotometrically by the absorption of DNA hydrolyzate at $\lambda = 290$ and 270 nm [13]. Benzoimidazophthalazine derivatives were synthesized at the Research Institute of Hygiene, Occupational Pathology and Human Ecology FMBA of Russia. Synthesis and properties of these compounds have been described previously [14]. Compounds were dissolved in ethanol. Complexes were prepared by adding an alcoholic solution of the compound in water-salt solution of DNA. The concentration of ethanol in the final solution did not exceed 1%. The ionic strength of the solution $\mu = 0.001$. The stoichiometry of the DNA-ligand complex (r) was determined from the binding curve resulting from the spectrophotometric titration. The absorption spectra of the solutions were recorded with a spectrophotometer Shimadzu UV-1800.

The mode of binding of compounds with DNA was determined by analysing of changes in intrinsic viscosity and optical anisotropy of the macromolecule during the formation of the complex by the method described previously [11]. According to the model of free-jointed chain, the intrinsic viscosity of the macromolecule is related to the contour length L and the length of the Kuhn segment A by the Flory formula [10]:

$$[\eta] = F \frac{(LA)^{3/2}}{M} \gamma^3, \quad (1)$$

where F - Flory constant for a given polymer-solvent system, M – molecular weight of the macromolecule, γ – the coefficient of linear swelling. Then

$$\frac{L_r}{L_0} = \left(\frac{[\eta]_r}{[\eta]_0} \right)^{\frac{2}{3}} \cdot \frac{A_0}{A_r}, \quad (2)$$

here and below the codes "r" and "0" denote characteristics of the complex and free DNA, respectively.

To determine the change in contour length of the macromolecule during formation of the complex it is necessary to independently determine the change in the length of the Kuhn segment A . To this end, we determined the value of the Peterlin ratio [15] for the free DNA and its complexes with compounds 1 and 2 using dynamic birefringence. It is proportional to the optical anisotropy of the statistical segment of the macromolecule (α_1 - α_2):

$$\frac{\Delta n}{g(\eta - \eta_0)} \sim (\alpha_1 - \alpha_2) = (a_{\parallel} - a_{\perp}) \frac{A}{d}, \quad (3)$$

where Δn – the value of birefringence, g – the gradient of flow velocity, η и η_0 – viscosity of the solution and solvent, $(a_{\parallel}$ - $a_{\perp})$ – optical anisotropy of the monomer unit, d – thickness of the monomer unit.

Intrinsic viscosity was determined by using graphical extrapolation of the reduced viscosity to zero concentration. To preserve the stoichiometry of the complex in the process of the concentration measurement, the values of the ionic strength of the solution and equilibrium concentration of free ligand (C_{free}) is maintained equal to the corresponding values in the initial solution.

The value of birefringence was determined using experimental optical system [16]. Solution viscosity was determined using magnetic rotational viscometer [17].

3. Results and discussion

3.1. Spectrophotometric titration

The changes in spectral properties of ligand in the presence of DNA in solution are the main features of the interaction of compound with DNA. Both compounds have long-wavelength absorption band in the 280-450 nm region. In the presence of DNA decrease in intensity and a bathochromic shift of this absorption band were observed. The bathochromic shift increases with raising concentration of DNA in solution (figure 2).

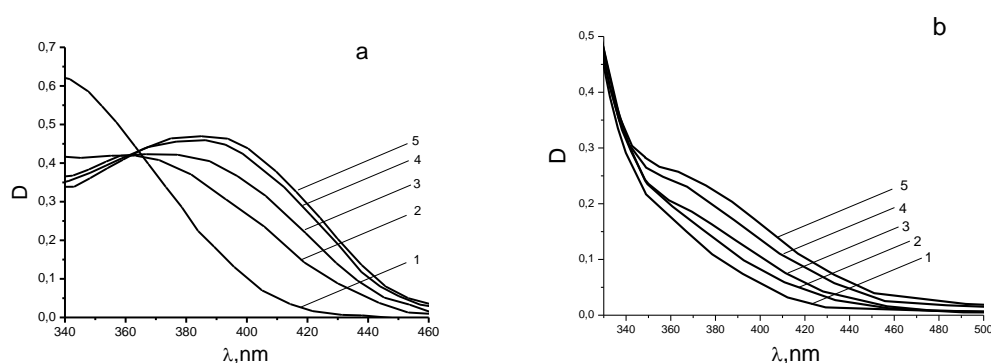


Figure 2. The absorption spectra of compounds 1 (a) and 2 (b) when $C_{lig} = 2.5 \cdot 10^{-5} M$ in the presence of DNA at different ratios of molar concentrations of ligand and DNA (C_{lig}/C_{DNA}) in 0.001M NaCl: a) $C_{DNA} = 0$ (1), $C_{lig}/C_{DNA} = 1.1$ (2), 0.6 (3), 0.3 (4), 0.1 (5); б) $C_{DNA} = 0$ (1), $C_{lig}/C_{DNA} = 5.0$ (2), 0.7 (3), 0.3 (4), 0.2 (5)

Based on the SPT data the binding curves (dependencies of r on C_{free}) were constructed (figure 3).

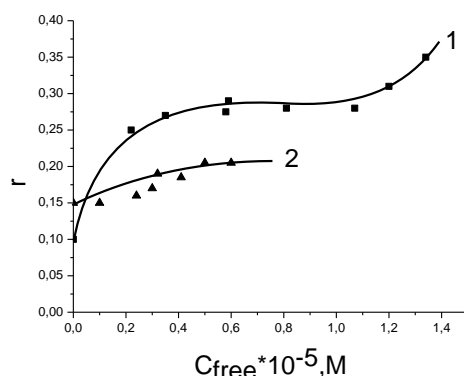


Figure 3. The binding curves: compound 1 (1) and compound 2 (2) in 0.001 M NaCl

The second, weaker type of binding occurs only with the large excess of free ligand in solution. By using the model with excluded places of binding [18] the binding constants (k) and the number of places of binding per pair of DNA bases (n) of first, the energetically stronger binding, were determined: $k_1 = (19 \pm 2) \cdot 10^5 \text{ M}^{-1}$, $n_1 = 0.32 \pm 0.05$, $k_2 = (5.5 \pm 0.5) \cdot 10^5 \text{ M}^{-1}$, $n_2 = 0.20 \pm 0.5$ for compounds 1 and 2, respectively.

3.2. Viscometry and dynamic birefringence

To determine the mode of binding, intrinsic viscosity and an optical anisotropy of DNA-ligand complexes were measured with different concentrations of the ligand in the complex under existence of only a primary binding mode. Figure 4 shows the dependence of the reduced viscosity of the solution from DNA concentration for the free DNA and for DNA-ligand complexes. These dependencies were used to determine the corresponding intrinsic viscosity. Their linearity indicates isoionic dilution and preservation of the stoichiometry of the complexes upon dilution of initial solutions. Obtained in this way values of intrinsic viscosity of free DNA and its complexes with ligands are shown in table 1.

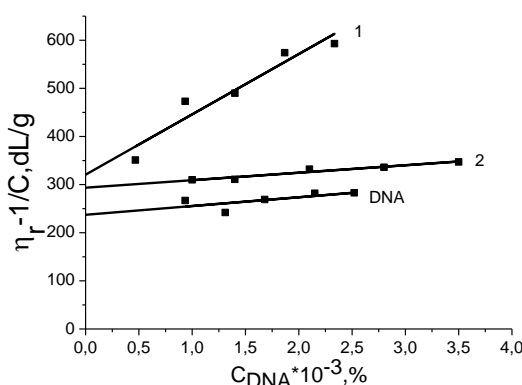


Figure 4. Dependence of $\eta_r - 1/C$ on C_{DNA} (1) and its complexes with compound 1 at $r = 0.1$ (2), $r = 0.2$ (3) and the compound 2 at $r = 0.1$ (4) in 0.001 M NaCl

The value of birefringence, Δn , was determined for the same solutions and Peterlin ratio (equation 3) was calculated. The results are shown in table 1.

Table 1. Hydrodynamic and optical parameters of DNA and its complexes with compounds 1 and 2

	r	$[\eta]$ (m ³ /kg)	$\Delta n/g(\eta - \eta_0) \cdot 10^7$ (m·s ² /kg)
DNA	-	24.0±0.5	26±1
DNA-compound 1	0.1	32.0±0.5	33±1
	0.2	32.5±0.5	34±1
DNA-compound 2	0.1	29.0±0.5	31±1

The value of the Peterlin ratio does not depend on the contour length of the macromolecule. Its increase in the complex formation may be caused by the increase of the thermodynamic rigidity and/or the increase of the average optical anisotropy of the monomeric unit by the addition of the ligand molecule. In the case of intercalation binding, the average optical anisotropy of the monomeric unit can be calculated by the equation:

$$(a_{||} - a_{\perp})_r = \frac{(a_{||} - a_{\perp})_0 + r \cdot (a_{||} - a_{\perp})_l}{1 + r}, \quad (4)$$

where $(a_{||} - a_{\perp})_l$ – optical anisotropy of the ligand in the axes of its main polarizabilities.

In the case of an alternative method of binding, when the ligand molecule is located in the minor groove of the DNA double helix, the average optical anisotropy of the monomeric unit will be expressed by the equation:

$$(a_{||} - a_{\perp})_r = (a_{||} - a_{\perp})_0 + r(a_{||} - a_{\perp})_l \cdot \frac{3\cos^2 \beta - 1}{2}, \quad (5)$$

The orientation angle of the plane of the ligand chromophore relative to the axis of the macromolecule segment β in the case of intercalation is equal to zero, since the plane of intercalated chromophore in the double helix is perpendicular to the axis of the DNA double helix. In the case of binding in the minor groove this angle is 45° [19]. The optical anisotropy of the monomeric unit of free DNA and the ligand molecule in its own axes can be approximately calculated by using the scheme of tensor additivity of polarizabilities of valence bonds.

When $r = 0.1$ in both cases a change in average optical anisotropy of a monomeric unit $\frac{(a_{||} - a_{\perp})_r}{(a_{||} - a_{\perp})_0}$

is slightly: for intercalation model the ratio is 1.04, and for the model of groove binding - 1.035. The relative change in length of the Kuhn segment A_r/A_0 upon complex formation can be calculated by equation:

$$\frac{A_r}{A_0} = \left(\frac{\Delta n}{g(\eta - \eta_0)} \right)_r / \left(\frac{\Delta n}{g(\eta - \eta_0)} \right)_0 \cdot \frac{(a_{||} - a_{\perp})_0}{(a_{||} - a_{\perp})_r}, \quad (6)$$

The results of calculation of this value for the intercalation and groove binding models are presented in Table 2.

Using the obtained values of A_r/A_0 to determine the value of change of the macromolecule contour length (L_r/L_0) during the formation of the complex from the experimental values of the intrinsic viscosity (equation 1) shows that this value stays practically unchanged (table 2), which indicates the nonintercalative type of binding of investigated compounds with DNA. We assume that these compounds bind to the minor groove of DNA.

Table 2. Changes in the macromolecular parameters of the DNA in the formation of complexes, calculated from experiment and theory for different models

	r	$(A_r/A_0)_{\text{exp}}$		$(L_r/L_0)_{\text{exp}}$		$(L_r/L_0)_{\text{theor}}$	
		Intercalation	Groove binding	Intercalation	Groove binding	Intercalation	Groove binding
DNA-comp. 1	0.1	1.22±0.05	1.22±0.05	0.99±0.05	0.99±0.05	1.1	1
	0.2	1.22±0.05	1.22±0.05	1.08±0.05	0.99±0.05	1.2	1
DNA-comp. 2	0.1	1.15±0.05	1.15±0.05	0.99±0.05	0.99±0.05	1.1	1

4. Conclusions

The studies show that the benzoimidazophthalazine derivatives containing residue of the piperazine in 9th position interact with the DNA molecule by forming the equilibrium complexes in a water-salt solution at $\mu = 0.001$. In the formation of the complex, an increase of the intrinsic viscosity and the optical anisotropy of the macromolecule are observed. These changes are caused by an increase in the thermodynamic rigidity of macromolecules in the formation of the complex. Increasing the contour length of the macromolecule is not happening. Therefore, these compounds do not intercalate into the double helix of DNA upon binding. Probably, the ligand molecules are located in one of the grooves of DNA double helix, presumably in a small one.

Thus, an increase in the characteristic viscosity, and especially in the reduced viscosity, during the formation of complexes of DNA with a variety low molecular weight compounds is not unambiguous evidence of the intercalation binding of these compounds with a macromolecule.

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