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Thermodynamics of interaction and structure of DNA complexes with phenacylimidazo[5,1-a]isoquinoline derivatives

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Abstract. Interaction of native calf thymus DNA (ctDNA) with phenacylimidazo[5,1-a]isoquinoline derivatives was studied by the methods of spectrophotometry, viscometry, isothermal titration calorimetry (ITC) and dynamic birefringence. It was found that both of investigated compounds form complexes with the DNA molecule, the structure of compounds affects the mode of binding these ligands to DNA. The primary binding mode can not be described by the classical models of groove binding or intercalation. It has been suggested that the primary mode of binding is "partial intercalation".

1. Introduction

Nowadays, the problem of the synthesis of new drugs, including drugs based on known compounds, whose effectiveness has been proved, is relevant. Alkaloids are a broad class of biological active compounds. Their chemical structure and mechanisms of action are various. One of well-known antispasmodic drug, isoquinoline derivative, is papaverine. The mechanism of its biological activity is to inhibit the phosphodiesterase [1].

The DNA molecule is a target for many biological active compounds possessing a flat heterocyclic chromophore such as anthracycline antibiotics, isoquinoline alkaloids and many others [2]. The interaction of these compounds with DNA may occur in various ways. The most common ones are groove binding and intercalation.

In the first case the molecule of the compound is located in the minor groove of the DNA double helix [3]. In the case of intercalation, flat hydrophobic chromophore of the molecule integrates into the DNA double helix between adjacent pairs of nitrogenous bases, causing local changes in the structure of the double helix [4].

Investigated in present work compounds - phenacylimidazo[5,1-a]isoquinoline derivatives, were synthesized as analogues of papaverine. They possess a flat heterocyclic chromophore that allows both intercalation and DNA groove binding [5].

Thermodynamic parameters of the interaction of these compounds with DNA, as well as mode of their binding with DNA double helix and structure of the complexes formed, were determined with calorimetric, spectroscopic and hydrodynamic methods.

2. Materials and methods

2.1. Materials

The interaction of DNA with phenacylimidazo[5,1-a]isoquinoline derivatives (figure 1) was studied by the methods of spectrophotometry, viscometry, ITC and dynamic birefringence.

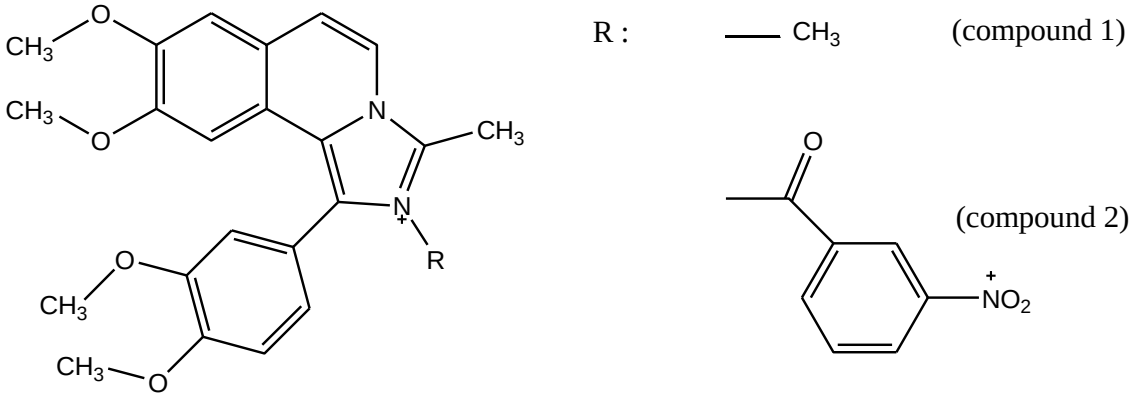

Figure 1. The structure of investigated compounds

The compounds were synthesized in the Research Institute of Hygiene, Occupational Pathology and Human Ecology FMBA of Russia by Krivorotov D. V. [6].

High-molecular-weight calf thymus DNA of firm "Sigma" (USA) has been used. Extinction coefficient ε_{260} = 6400–6700 $M^{-1}cm^{-1}$. The complexes were prepared by mixing the DNA and ligand solutions of the necessary concentrations. The ionic strength of the solution (μ) was 0.001 M and subsequently remained unchanged.

2.2. Isothermal titration calorimetry (ITC)

ITC experiments were performed using a TA Instruments Nano ITC 2G Microcalorimeter at Thermogravimetric and Calorimetric Research Centre St.Petersburg State University. Reaction cell volume was 1.4 mL. Aliquots of investigated compounds (7 μL) were injected from a 250-μL rotating syringe (300 rpm) into the isothermal sample cell containing 1.4 mL of DNA solution. All the experiments were carried out at 21°C. Delay time between each injection was 500 s. Each injection generated a heat burst curve (μJ per second), the area under which was determined by integration using Origin software version 8.6 that gave the measure of the heat of reaction associated with the injection. The heat associated with dilution of each ligand injection was subtracted from the corresponding heat associated with the ligand-DNA injection to give the heat of ligand binding for that injection. To determine the thermodynamic parameters of binding - binding constant, enthalpy of complex formation and the number of binding sites, the equation 1 was used. It corresponds to the model - 1 ligand molecule for 1 binding site [7].

$$Q = \frac{nM\Delta HV_0}{2}\left[1+\frac{X}{nM}+\frac{1}{nKM}-\sqrt{\left(1+\frac{X}{nM}+\frac{1}{nKM}\right)^2-\frac{4X}{nM}}\right], \quad (1)$$

where Q – the heat released or absorbed during the titration, ΔH – binding enthalpy, V_0 – cell volume, X – ligand concentration, M – DNA concentration in mol base pares (bp), K – intrinsic binding constant, n – number of binding sites.

2.3. Spectrophotometric titration (SPT)

Absorption spectra were measured by Shimadzu UV-1800. The titration was carried out in a wide range of ratios of concentrations ligand/DNA when concentration of the ligand was constant. The obtained data were analyzed in two ways: the neighbor exclusion model of McGhee and von Hippel (equation 2) [8] and Scatchard model with 1 ligand molecule for 1 binding site (equation 3) [9]:

$$\frac{r}{C_f} = k(1-lr)\left[\frac{(1-lr)}{[1-(l-1)r]}\right]^{l-1}, \quad (2)$$

where r – molar ratio of binding ligand to DNA (bp), C_f – concentration of free ligand, k – binding constant, l – the binding site size in bp;

$$\frac{r}{C_f} = K(n-r), \quad (3)$$

where r – molar ratio of binding ligand to DNA (bp), C_f – concentration of free ligand, K – binding constant, n – the number of binding sites.

2.4. Viscometry

The viscosity of solutions of pure DNA and DNA-ligand complexes has been measured with the magnetic rotational viscometer [10]. Measurements were carried out at the experimental setup to determine the viscosity of non-Newtonian fluids. Velocity gradient of viscometer g= (0.1 – 0.5) s^{-1}.

According to Flory formula [11], the intrinsic viscosity of the macromolecule is related to its parameters by the ratio:

$$[\eta] = F\frac{\left(\overline{h_0^2}\right)^{3/2}}{M_w}\alpha^3, \quad (4)$$

where $[\eta]$ - the intrinsic viscosity, F - Flory constant for a given polymer-solvent system, $\overline{h_0^2}$ – the mean-square distance between the ends of the chain, M_w – molecular weight, α – the coefficient of linear swelling.

For freely jointed chain:

$$h_0^2 = LA, \quad (5)$$

where L – contour length, A - length of statistical segment.

2.5. Dynamic birefringence

In parallel with the viscometry studies the dynamic birefringence of the same solutions was studied. The value of dynamic birefringence Δn was measured in the titanium dynamo-optimeter with internal rotor using the optical device with half-shadow elliptical compensator [12]. The Peterlin ratio [13] was calculated to determine the changes in thermodynamic rigidity of macromolecule upon complex formation (A_r/A_0). The Peterlin ratio $\Delta n/g(\eta - \eta_0)$ proportional $(a_1-a_2)S$, where Δn – value of the dynamic birefringence, g – gradient flow rate, η и η_0 – viscosity of the solution and solvent respectively, (a_1-a_2) – polarizability difference of monomer residues along and perpendicular to the axis of the statistical segment of the macromolecule, S – the number of monomers in the segment. Analysis of the results was carried out according to the formula:

$$\frac{A_r}{A_0} = \frac{S_r}{S_0} = \left(\frac{\Delta n}{g(\eta-\eta_0)}\right)_r \Big/ \left(\frac{\Delta n}{g(\eta-\eta_0)}\right)_0 \bullet \frac{(a_1-a_2)_0}{(a_1-a_2)_r}, \quad (6)$$

3. Results and discussion

The thermodynamic parameters of the interaction and the stoichiometry of the complex were determined by ITC and SPT.

The results of calorimetric titration of DNA by compound 1 are shown in Figure 2. For compound 2 the results are similar.

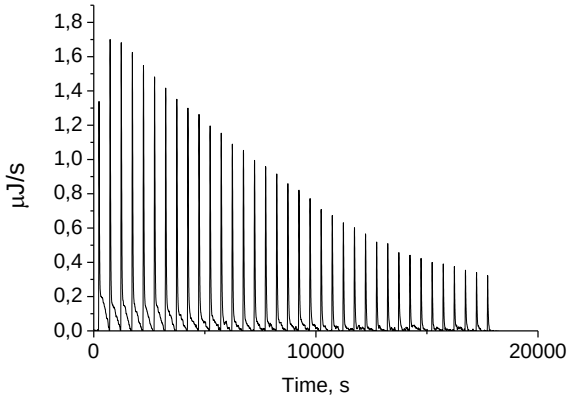

Figure 2. The raw ITC data of compound 1 (640 μM) binding to ctDNA (200μM)

The resulting thermograms are shown in Figure 3.

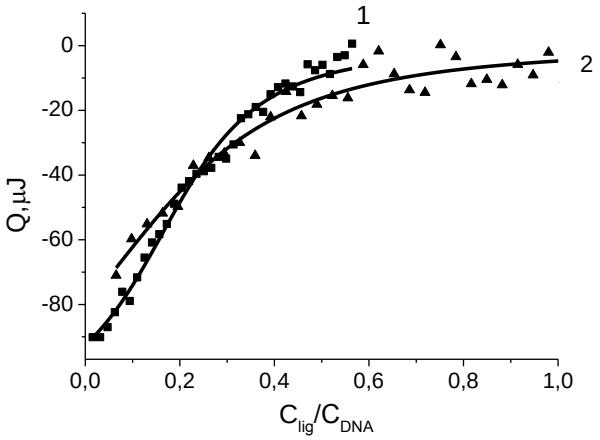

Figure 3. The thermogram of investigated compounds binding to ctDNA (1-compound 1 (640 μM) ctDNA (200μM (bp)); 2 –compound 2 (600 μM), ctDNA (100 μM (bp)). The solid lines correspond to the best fit to the data

The solid lines in figure 3 correspond to the best fit to the data using equation 1 with the following parameters: for compound 1: K= (9.3±0.6)*$10^4$$M^{-1}$, n= (0.22±0.01), H= (-6.2±0.2) kcal/mol; for compound 2: K= (7.6±0.6)*10^4 M^{-1}, n= (0.24±0.02), H= (-6.4±0.4) kcal/mol.

The results of the spectrophotometric titration of the compounds 1 and 2 are shown in Figure 4. C_{lig1} = 24μM, C_{lig1}/C_{DNA} was varied from 0.4 to 6, C_{lig2} =28 μM, C_{lig2}/C_{DNA} was varied from 0.2 to 4.7. The presence of an isobestic point allows us to plot the binding curves and determine the binding parameters. Binding isotherms for both compounds are shown in figure 5. Thermodynamic parameters of binding for compound 1 were calculated using the McGhee and von Hippel model (equation 2) k= (1.88±0.07)*$10^5$$M^{-1}$, l= (2.24±0.06), and using Scatchard model: K= (4.7±0.3)*$10^5$$M^{-1}$, n= (0.36±0.03). For compound 2 the character of binding is more complex, when r> 0.5 there is a second way of binding, therefore determination of binding parameters from the absorption spectra is not possible.

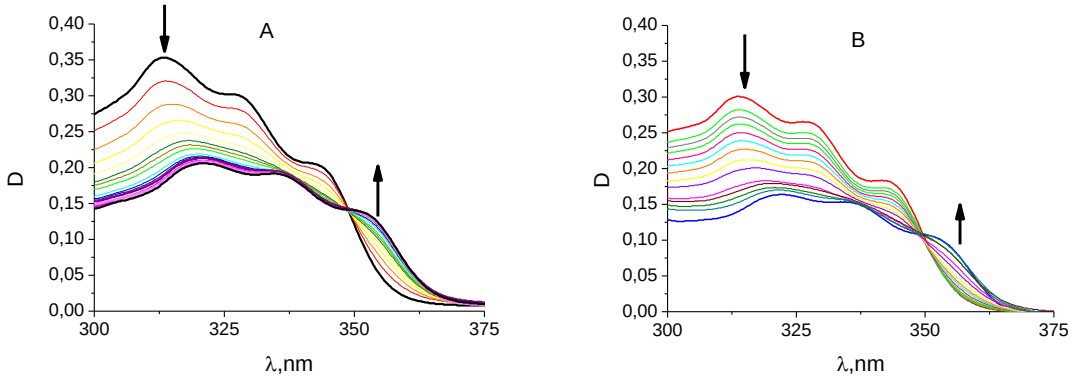

Figure 4. Absorption spectra of complexes DNA with compound 1(C_{lig}=24 μM) (A), with compound 2(C_{lig}=28 μM) (B)

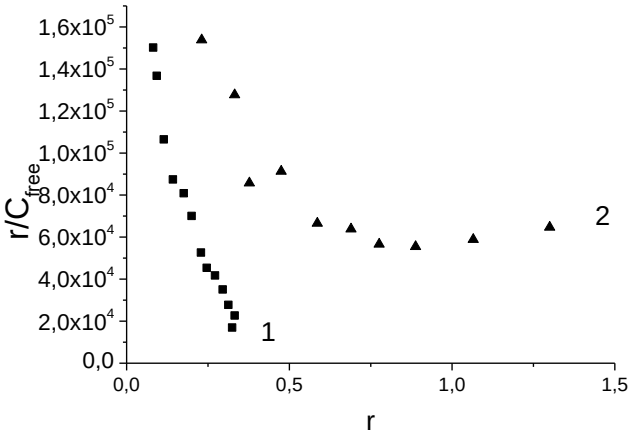

Figure 5. Binding isotherms 1- complexes DNA-compound 1; 2- complexes DNA-compound 2

Comparison of the results obtained from both methods shows that the results obtained from the spectrophotometric titration give significantly higher binding constant value regardless of the model used for the calculations. It can be assumed that the reason for the difference lies in the different titration procedure applied at ITC and SPT.

A mode of binding of investigated compounds with DNA was determined by using viscometry method and method of dynamic birefringence [14]. For this purpose, parallel measurement of the intrinsic viscosity and optical anisotropy (the Peterlin ratio [9]) of the DNA-ligand complexes was carried out (r = 0.2). The results of measurement are given in the table 1. The absence of significant changes of Peterlin ratio during the binding of ligands with DNA indicates constancy of the thermodynamic rigidity of macromolecule. Therefore, the relative change of the contour length of the macromolecule (L_r/L_0), calculated from the values of intrinsic viscosity for complex DNA-compound 1 is $(L_r/L_0)_1$= (1.13±0.05) and for complex DNA-compound 2 is $(L_r/L_0)_2$= (1.09±0.05). In the case of classical intercalation binding when r= 0.2, the change of the contour length (L_r/L_0)=1.2. We can assume that in this case there is a partial or incomplete intercalation [5, 15] of chromophores of investigated compounds into the DNA double helix.

Table 1. The results of measurements of viscosity and dynamic birefringence

	$[\eta]$ (dL/g)	$\Delta n/g(\eta - \eta_0) \cdot 10^8$ (cm·s^2/g)
DNA	150±10	25±1
DNA-compound 1	175±10	25±1
DNA-compound 2	174±10	26±1

4. Conclusions

Both of the investigated compounds form complexes with the DNA molecule. The presence of bulky substitute in the isoquinoline chromophore affects the nature of the binding to macromolecule. Thus, compound 2 besides the primary binding mode has secondary one. It was shown that both compounds have similar values of enthalpy, binding constants, and the number of primary binding sites determined by ITC. The binding constant and the number of primary binding sites determined by spectrophotometry have higher values regardless of the model used for the calculation. Changes of macromolecular parameters upon binding of both compounds with DNA are not consistent with the expected in the case of classical intercalation or groove binding. It can be assumed that the compounds partially intercalated into the DNA double helix.

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