Interaction of indole-papaverine with DNA in solutions of various ionic strength

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Abstract. Interaction of synthetic alkaloid of isoquinoline series, which is an analogue of the biologically active compound papaverine, was studied by spectral, microcalorimetric, optical and hydrodynamic methods at different ionic strengths of medium. It was found that the investigated compound may interact with DNA in various ways depending on the ratio of ligand - DNA concentrations and ionic strength of solution (µ). When µ = 0.001, indole-papaverine intercalates into the double helix of DNA. The increase of µ resulted in a decrease of the affinity of the compound to DNA and a change its binding method.

1. Introduction
The study of the formation mechanism of non-covalent reversible complexes of biologically active substances with DNA is an actual task, since the understanding of these processes plays an important role in the improvement of existing drugs, and may serve as the first step in the design of new ones.

Intercalation and groove binding are mainly caused by van der Waals interactions and hydrogen bonds that are non-covalent interactions. Forasmuch as DNA in solution at neutral pH is a polyanion with negatively charged phosphate groups of the sugar phosphate backbone, electrostatic interactions also play a significant role in the formation of such complexes [1]. At intercalation there is embedding of planar aromatic molecules of ligand between DNA base pairs [2]. At external interactions there is a fitting of ligand molecules into the grooves or a formation of aggregates on the surface [3].

This paper presents the results of a study of interaction of DNA with the indole derivatives of isoquinoline - indole-papaverine, which has a positive charge on the isoquinoline chromophore, at various ionic strengths of medium.

2. Materials and methods
There were used calf thymus DNA from the company «Sigma» (USA) with molecular weight \( M = 10^7 \) Da. DNA concentration was determined by the spectrophotometrical method described in [4]. Indole-papaverine was synthesized at the Research Institute of Hygiene, Occupational Pathology and Human Ecology. [5] Aqueous solutions of NaCl of different ionic strength, \( \mu \), and pH 6.0 - 6.5 were used as a solvent. Complexes were prepared by direct mixing of DNA and the substance at a certain concentrations of the latter.

The stoichiometry of complexes, defined as the amount of bound ligand per pair of nitrogenous bases of DNA \( r \), was obtained from the spectrophotometric titration data (SFT). The absorption spectra of the solutions were recorded using «Specord UV-Vis» and «Shimadzu UV-1800" spectrophotometers. Circular dichroism spectra were recorded at dichrographs MarkV.

The enthalpy of interaction and thermodynamic parameters of the indole-papaverine binding were determined by isothermal titration calorimetry (ICT) using microcalorimeter TA Instruments Nano ITC 2G at the Resource Center of St. Petersburg State University "Thermogravimetric and calorimetric methods of investigation". [6] The calculation of the thermodynamic binding parameters in this case was carried out on the bases of a model with two independent binding sites [7].
The structure of complex and the way by which the compound and DNA interact during the formation of complex were established by means of viscosimetry and birefringence. Analysis of changes in the intrinsic viscosity, \([\eta]\), and the optical anisotropy of statistical segments of the macromolecule, \((\alpha_1 - \alpha_2)\), was performed according to methods described previously [1,8]. We measured the dependence of the relative viscosity of solution, \(\eta_r\), on DNA concentrations in the solution, \(C_{DNA}\), using a magnetic rotational viscometer [9]. Thus, \([\eta]\) was determined as

\[
[\eta] = \lim \left( \eta_r - 1 \right) / C_{DNA}, \text{ at } g \to 0 \text{ and } C_{DNA} \to 0,
\]

where \(g\) is the velocity gradient.

To determine the changes in the magnitude of \((\alpha_1 - \alpha_2)\), resulted from the formation of the complex, Peterlin’s relationship was used [10], which is proportional to the optical anisotropy of the statistical segment of the macromolecule:

\[
\Delta n / \left( g \left( \eta - \eta_0 \right) \right) \sim (\alpha_1 - \alpha_2),
\]

where \(\Delta n\) - the magnitude of the birefringence, \(\eta_0\) - viscosity of the solvent.

\(\Delta n\) was measured in the titanium dynamo-optimeter using the experimental optical setup with a mica half- shadow compensator [11].

3. Results and discussions

3.1. Spectrophotometric titration

At \(\mu = 0.001\) there are two isosbestic points (Fig.2), which reflect not only two methods of binding, but also the high affinity of the ligand to DNA \((k_{bind} > 10^7 \text{ M}^{-1})\). The limiting spectrum is observed at \(C_{ligand} / C_{DNA} \leq 0.1\), which confirms the possibility of a primary monomer binding. The availability of isosbestic point at a wavelength \(\lambda = 377\) nm and at \(C_{ligand} / C_{DNA} \leq 2.0\) indicates that there is no considerable amount of free ligand in the system. Changes in the intensity and spectrum shift occur due to the transition of monomeric binding type to dimeric one [12, 13]. Isosbestic point at \(\lambda = 413\) nm corresponds to the band of spectra which comprises spectrum of free ligand and complexes at \(C_{ligand} / C_{DNA} \leq 2.0\), and characterizes the equilibrium between free ligand and dimeric bound.

Two isosbestic points blurred with the increase of ionic strength to \(\mu = 0.003\), (Fig.3), which may indicate both the decrease of the affinity of the compound to DNA and the lack of a balance between the two types of monomer-dimer binding methods.
3.2. Isothermal microcalorimetric titration

The results of ICT at $\mu = 0.001$ (Fig. 4) are consistent with the SFT data. The monomer binding occurs at $C_{\text{ligand}} / C_{\text{DNA}} \leq 0.2$, while dimer binding takes place at $0.2 \leq C_{\text{ligand}} / C_{\text{DNA}} \leq 1.5$. If we have $C_{\text{ligand}} / C_{\text{DNA}} \geq 1.5$, it is possible to talk about the external aggregative nonspecific binding. When the ionic strength of the medium is increased, the general form of thermal image changes significantly (Fig. 5).

The thermodynamic parameters obtained from the ICT data by the processing program of microcalorimeter assuming the model of two independent types of binding sites are shown in Table 1.

![Figure 2](image1.png)

**Figure 2.** The absorption spectra of the compound during the titration with a constant concentration $C_{\text{ligand}} = 4.20 \times 10^{-5}$ M, $\mu = 0.001$, $0.12 \times 10^{-3} \text{ M} \leq C_{\text{DNA}} \leq 4.63 \times 10^{-4}$ M.

![Figure 3](image2.png)

**Figure 3.** The absorption spectra of the compound during the titration with a constant concentration $C_{\text{ligand}} = 4.00 \times 10^{-5}$ M, $\mu = 0.003$, $0.57 \times 10^{-3} \text{ M} \leq C_{\text{DNA}} \leq 4.70 \times 10^{-4}$ M.

![Figure 4](image3.png)

**Figure 4.** Thermogram of titration of DNA by ligand (upper part) and dependence of the amount of heat per one mole of the ligand from $C_{\text{ligand}} / C_{\text{DNA}}$ at $\mu = 0.001$ (lower part). $C_{\text{DNA}} = 2 \times 10^{-5}$ M. The black solid line reveals the approximation by the model of two types of independent binding sites.

![Figure 5](image4.png)

**Figure 5.** Thermogram of titration of DNA by ligand (upper part) and dependence of the amount of heat per one mole of the ligand from $C_{\text{ligand}} / C_{\text{DNA}}$ at $\mu = 0.003$ (lower part). $C_{\text{DNA}} = 2 \times 10^{-5}$ M. The black solid line reveals the approximation by the model of two types of independent binding sites.

| Complex | $\Delta H_1, \text{ kcal/mol}$ | $\Delta H_2, \text{ kcal/mol}$ | $\Delta S_1, \text{ kcal/mol K}$ | $\Delta S_2, \text{ kcal/mol K}$ | $k_{\text{bind1}}, M^{-1}$ | $k_{\text{bind2}}, M^{-1}$ | $n_1$ | $n_2$ |
|---------|-------------------------------|-------------------------------|-----------------------------|-----------------------------|---------------------|---------------------|-------|-------|
| $\mu=0.001$ | -1.50 | -9.60 | 35.3 | 4.5 | $1.3 \times 10^8$ | $1.3 \times 10^6$ | 0.4 | 1.0 |
| $\mu=0.003$ | -1.56 | -5.24 | 28.4 | 64.6 | $2.3 \times 10^7$ | $2.0 \times 10^7$ | 1.7 | 3.1 |

The comparison of colorimetric titration curves at ionic strengths, equal to 0.001 and 0.003, indicates that the secondary binding at $\mu = 0.003$ occurs when it is a large excess of the ligand and its thermal
characteristics very similar to the non-specific aggregative binding of the ligand at $\mu = 0.001$ and
$C_{\text{ligand}} / C_{\text{DNA}} \geq 1.5$. At the same time the binding of ligand dimers observed at $\mu = 0.001$, while at $\mu = 0.003$ does not occur.

3.3. Circular dichroism
Parallel to SFT were made spectropolarimetric studies at $\mu = 0.001$ and $\mu = 0.003$ in regeon of $C_{\text{ligand}} / C_{\text{DNA}} \leq 1.0$ (Fig. 6, 7). At $\mu = 0.001$ ICD appears when $C_{\text{ligand}} / C_{\text{DNA}} > 0.4$ and has the exciton form of spectrum corresponding to the interaction of the bound ligand molecules, which confirms SFT and ICT data about the emergence of dimer binding under these conditions. At $\mu = 0.003$ ICDs exciton nature is not observed, indicating the absence of dimers in the system.

![Figure 6. Circular dichroism spectra at $\mu = 0.001$. 0.88·10^{-6} \text{M} \leq C_{\text{ligand}} \leq 1.85 \cdot 10^{-5} \text{M, } C_{\text{DNA}} = 2.00 \cdot 10^{-5} \text{M.}]

![Figure 7. Circular dichroism spectra at $\mu = 0.003$. 0.10·10^{-6} \text{M} \leq C_{\text{ligand}} \leq 1.78 \cdot 10^{-5} \text{M, } C_{\text{DNA}} = 2.00 \cdot 10^{-5} \text{M.}]

3.4. Viscometry and dynamic birefringence
To determine way of the compound binding there have been identified the characteristic viscosity and the Peterlin’s ratio for free DNA and its complex with a ligand at $r = 0.2$ (tab.2).

At $\mu = 0.001$, there is the increase of intrinsic viscosity during the formation of the DNA complex with indole-papaverine. In the case of high molecular weight of DNA, such changes can be caused by an increase in contour length ($L$), and an increase in the thermodynamic rigidity of the macromolecule which determined by the length of the statistical segment ($A$) [14].

| Complex | [\eta], m^3/kg | $\Delta n / (n - n_{\text{DNA}})$, m s/kg |
|---------|---------------|-------------------------------------|
| DNA     | 15±1          | 26 ± 2                              |
| r=0.2   | 20±1          | 27 ± 2                              |
| $\mu=0.003$ |                 |                                     |
| DNA     | 7.0±0.5       | 22±2                                |
| r=0.2   | 5.5±0.5       | 22±2                                |

The value of Peterlin’s ratio, which is proportional to the thermodynamic rigidity of macromolecules, remains constant within the error. Therefore, the intrinsic viscosity increase upon complexation DNA with ligand at $\mu = 0.001$ occurs as a result of change its contour length. The calculation of this change was performed using the Flory’s formula for coiled macromolecules [14]

$$[\eta] = \Phi \frac{(h_0^2)^{1/2}}{M} \gamma^3,$$

where $\Phi$ – Flory constant for a given polymer-solvent system, $h_0$ - the distance between the ends of the macromolecule, $\gamma$ - linear swelling ratio. As a result, it was received $L_{\text{ligand}} / L_{\text{DNA}} = 1.2 \pm 0.1$, which
corresponds to the relative change in the contour length of the macromolecule, expected at the ligand intercalation into the double helix of DNA at \( r = 0.2 \).

At \( \mu = 0.003 \) the intrinsic viscosity of the complex with the ligand content is even slightly smaller than the intrinsic viscosity of pure DNA which eliminates intercalative binding.

4. Conclusions
The investigated compound has a positive charge on the isoquinoline chromophore, so the ionic strength of medium plays a significant role in the complexation process with DNA.

On the basis of obtained experimental data we can propose the model of ligand binding to DNA depending on the ionic strength of medium and amount of ligand molecules in the complex. When \( \mu = 0.001 \), the interactions with DNA at \( 0.1 \leq r \leq 2.0 \) come in two ways: monomeric and dimeric. These ways of binding are interdependent: associated monomer is a place of secondary ligand binding and, as a result, there forms dimer. Formation of the latter is accompanied by the release of heat. Free ligand and the third non-specific binding mode as external units appeared at \( r \geq 1.5 \). Intercalative way of binding of monomers to DNA is shown using birefringence and viscosimetry tests.

Binding way of monomers become non-intercalative with the increase of ionic strength of medium. This binding is characterized by the negative ICD, the absence of enthalpy change and the reduction of intrinsic viscosity of macromolecules during the formation of complex. For both \( r \geq 1.2 \) and low ionic strength of medium, there is the nonspecific aggregation binding way that result in an excess of the ligand in the system.

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