

Formation of intermolecular crosslinks by the actinocin derivatives with DNA in interaction under conditions of semidilute solution

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Abstract. Interaction of native calf thymus DNA (ctDNA) with the actinocin derivatives containing protonated diethylamino groups, dimethylamino groups and unsubstituted amino groups and having different length of the alkyl chain have been studied by the method of viscometry. An anomalous hydrodynamic behavior of solutions of DNA with very low amount of ligands prepared under conditions of semidilute solution was revealed. We assumed that such an anomalous behavior of solutions of DNA complexes with actinocin derivatives associated with the formation of intermolecular crosslinks while the preparation of the complex was in terms of overlapping of macromolecular coils in solution. Comparative study of the hydrodynamic behavior of the DNA complexes with various actinocin structures lead us to the conclusion of the formation of crosslinks by the compounds containing protonated diethylamino groups.

1. Introduction

Currently, the problem of research of anticancer drugs is very relevant. The mechanisms of their action are various, one of them is disturbance of the DNA-dependent RNA synthesis. One of the most well-known antitumor antibiotics with a similar mechanism of action is actinomycin D. Actinomycin D was the first antitumor antibiotic that has been applied in the chemotherapeutic treatment of human tumors.

The most popular model of the interaction of actinomycin D with DNA is the intercalation binding model [1]. It is based on data from X-ray crystals diffraction of complex actinomycin D with deoxyguanosine [2]. Antibiotic chromophore is located between two GC pairs having the opposite orientation. The complex is formed by hydrogen bonds with the guanine residues on both sides of the chromophore. Intercalation occurs exclusively in the small groove.

In connection with the search of new effective antitumor antibiotics a large number of analogues of already known drugs has been synthesized. For example, actinocin derivatives containing dialkylaminoalkyl groups in the amide groups were synthesized. Previously it was shown that the actinocin derivatives of this group bind to DNA like actinomycin D by the way of intercalation of actinocin chromophore into DNA double helix [3].

These compounds possess three binding sites: a chromophore that can intercalate into DNA double helix and two protonated amino groups (figure 1).

Compounds with several binding sites, including the positively charged groups, can form intra- and intermolecular crosslinks. Intramolecular crosslinks are formed during a random approach of distant



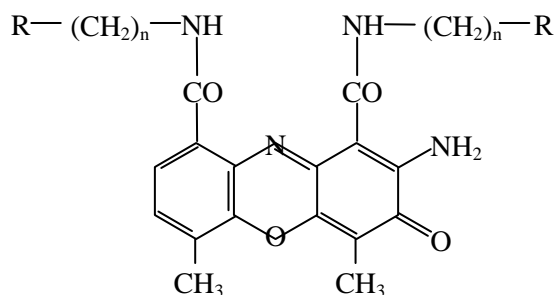
regions of DNA's chain, which are binding sites for specific groups of the ligand [4]. The molecules of histone H1 can form such crosslinks by interaction with a high-molecular DNA [5].

Intermolecular crosslinks can be formed under conditions of convergence of the elements of different molecules in solution. In the work [6] a method of molecular design, based on the "crosslinking" double-stranded nucleic acid molecules by polymeric chelate bridges, was proposed. Anthracyclines antibiotics which can form flat polymeric complexes were used to create the crosslinks. Favorable conditions for the formation of intermolecular crosslinks appear in the overlapping of macromolecular coils in semidilute polymer solutions [7][8].

In the present work we investigated complexes DNA with actinocin derivatives containing dialkylaminoalkyl groups in amide groups prepared in dilute and semidilute solutions.

2. Materials and methods

The interaction of DNA with actinocin derivatives containing various dialkylaminoalkyl groups in amide groups and having different length of the alkyl chain (figure 1) have been studied by the method of viscometry.



- Compound 1 $R=N(C_2H_5)_2$, $n=2$
- Compound 2 $R=N(C_2H_5)_2$, $n=3$
- Compound 3 $R=N(CH_3)_2$, $n=2$
- Compound 4 $R=N(CH_3)_2$, $n=3$
- Compound 5 $R=NH_3$, $n=2$
- Compound 6 $R=NH_3$, $n=3$

Figure 1. The structure of investigated compounds

The compounds were synthesized at St. Petersburg State Institute of Technology [9].

High-molecular-weight calf thymus DNA of firm "Sigma" (USA) has been used. Extinction coefficient $\varepsilon_{260} = 6400\text{--}6700 \text{ M}^{-1}\text{cm}^{-1}$. The complexes were prepared by mixing the DNA and ligand solutions of the necessary concentrations. The number of the bound ligand molecules per pair of DNA bases (r) was 0,1 or 0,01.

Initial solutions were prepared in two ways: 1 - in a semidilute solution, when $[\eta] \cdot C_{DNA} > 1$, where $[\eta]$ - intrinsic viscosity of DNA ($C_{DNA} = 8 \cdot 10^{-3}\%$); 2 - in a dilute solution, when $[\eta] \cdot C_{DNA} < 1$ ($C_{DNA} = 4 \cdot 10^{-3}\%$). The ionic strength of the solution (μ) was 0.1 or 0.001 M and subsequently remained unchanged.

We measured the viscosity of solutions of pure DNA and DNA-ligand complexes. Viscosity of solutions 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 in a wide range of velocity gradient. Velocity gradient of viscometer $g = (0.1 - 0.5) \text{ s}^{-1}$. Viscometer is a device with two coaxially arranged cylinders. In the gap between them investigated solution is placed. The inner cylinder (rotor) is rotated by a rotated magnetic field. Rotor velocity depends on the frequency of magnetic field and viscosity of solution.

3. Results and discussion

The results of measurements of the dependence of the viscosity from concentration of DNA in solution at $r = 0.1$ are shown in figure 2, $\mu = 0.001$.

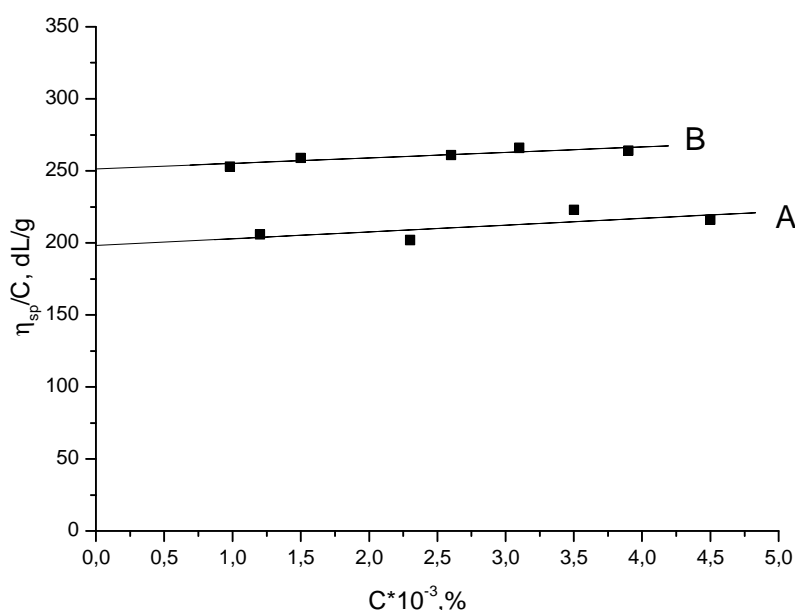


Figure 2. The dependence of the η_{sp}/C_{DNA} from concentration of DNA, $\mu=0.001$, A - free DNA, B - complexes of compounds 1-6 with DNA at $r=0.1$

The concentration dependence of the viscosity of solutions of DNA and its complexes with ligands is rectilinear in dilute solutions regardless of the preparation conditions. This allows determine the intrinsic viscosity of DNA and its change in the process of interaction with the actinocin derivatives.

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

$$[\eta] = F \frac{(\overline{h_0^2})^{3/2}}{M} \alpha^3, \quad (1)$$

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 - molecular weight, α - the coefficient of linear swelling.

For freely jointed chain:

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

where L - contour length, A - length of statistical segment. According to a Flory formula [11], it is possible to write

$$\frac{[\eta]_r}{[\eta]_{DNA}} = \left(\frac{L_r A_r}{L_0 A_0} \right)^{3/2}. \quad (3)$$

According to the intercalation model [1] when ligand bind with DNA $L_r = L_0(1+r)$.

For all investigated compounds at $r=0.1$ the relative change of $[\eta]$: $[\eta]_{0.1}/[\eta]_{DNA}=1.25$, which corresponds to intercalation mode of binding of ligand in these conditions [3].

The results of measurements of the viscosity dependence from concentration of DNA in solution at $r = 0.01$ are shown in figure 3.

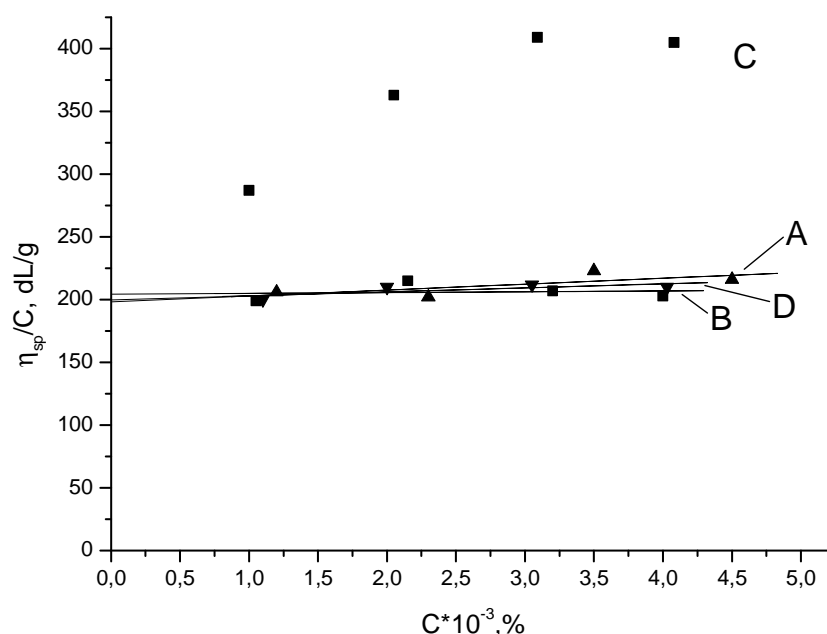


Figure 3. The dependence of the η_{sp}/C_{DNA} from concentration of DNA, $\mu=0.001$, A - free DNA; B- complexes of compounds 1-6 with DNA, prepared under conditions of dilute solution, $r=0.01$; C- complexes of compounds 1,2 with DNA, prepared under conditions of semidilute solution, $r=0.01$; D- complexes of compounds 3-6 with DNA, prepared under conditions of semidilute solution, $r=0.01$

As figure 3 shows, there is no change between the viscosity of free DNA and viscosity of ligand-DNA complex while preparation of solutions of complexes of all compounds with DNA was under conditions of the dilute solution. However, while complexes were prepared under conditions of overlapping of macromolecular coils and then were diluted in 2 times an anomalous increase of the viscosity of the compounds 1, 2 was observed. In the case of compounds 3-6, the influence of the preparation conditions on the viscosity dependence of complexes from DNA concentration wasn't observed. Thus, we can see an anomalous increase in the viscosity of compounds containing diethylamino groups. Alkyl chain length doesn't affect the hydrodynamic behavior of the complex.

Similar studies of complexes at $r = 0.01$ were performed at ionic strength $\mu = 0.1$. In this case we didn't observe any differences in viscosity of complexes compared to viscosity of free DNA, regardless of the preparation method of initial solutions of complexes.

Studies of viscosity dependence of complexes according to the velocity gradient showed that increasing gradient leads to disappearing of anomalous increase in viscosity.

We assumed that the observed anomalous behavior of solutions of DNA with actinocin derivatives related to the formation of intermolecular crosslinks in the process of preparation of the complex in terms of overlapping of macromolecular coils in solution. Saving these crosslinks upon dilution of the solution results in an increasing of specific viscosity due to the formation of large coiled aggregates composed of several DNA molecules. Their preservation in the measurement of viscosity due to low values of velocity gradients used in the gradient viscometer. Increasing of gradient to 0.5 s^{-1} leads to destruction of crosslinks and decreasing of viscosity.

Effect of ionic strength on the behavior of complex DNA with compounds 1, 2 shows that electrostatic interactions play an important role in the formation of crosslinks. We assumed that these

interactions can exist between the protonated diethylamino groups of compound and phosphate groups of DNA.

At the same time, it was found that such crosslinks are formed in the case of compounds containing unsubstituted amino and dimethylamino groups. This points to the important role of hydrophobic interactions in the formation of intermolecular ligand-DNA crosslinks.

Difference in one CH₂ group (2 or 3) in the alkyl chain of the compound did not affect its ability to form a crosslink.

It was unexpected that complexes containing only very low amount of bound ligand ($r = 0.01$) have anomalous hydrodynamic behavior. While increasing this parameter to a value of 0.1 only intercalation complexes are formed. This indicates that the amount of ligand binding sites able to form crosslinks is very low, and the binding constant is high.

4. Conclusions

In this work an anomalous hydrodynamic behavior of DNA solutions with very low amount of actinocin derivatives, containing amide diethylamino groups and prepared in semidilute solution, was found. It has been suggested that this behavior is caused by the formation of intermolecular crosslinks. Study of the dependence of the hydrodynamic behavior from the ionic strength, structure of the compounds and velocity gradient has shown that this kind of interaction is electrostatic in nature, occurs with the participation of hydrophobic interactions and destroyed by increasing the velocity gradient.

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