Study of the DNA Packing Caused by Charged Compounds of Different Nature in Solution

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SUMMARY: A comparative study of DNA interaction with different counterions in solution has been conducted by the following methods: flow birefringence (FB), low-gradient viscometry, circular dichroism (CD), and UV-spectroscopy. The influence of counterions on the persistence length and polyelectrolyte swelling of DNA has been investigated. The process of DNA packing during the interaction with trivalent ions in solution has been studied, as well as the influence of Mn^{2+} on the binding of trivalent ions with DNA.

Introduction

It is known that in biological systems the DNA molecule is in very compact form. The process of its packing in solution is very unclear, because the DNA molecule has a high negative charge and the great thermodynamic rigidity. On the other hand, now it is known that DNA remains its secondary structure in compact form. The investigation of the initially process of DNA packing caused by charged compounds in model systems (the water solution of DNA with the definite concentration of supporting electrolyte) is useful for the understanding of the formation of supramolecular structures in biological systems.

Results

Electrostatic interactions occurring in a DNA molecule largely define its conformation in solutions: the long-range interactions are responsible for the polyelectrolyte swelling while the short-range interactions provide variation in the thermodynamic rigidity (persistence length).

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Consequently the DNA conformation is sensible determined by the efficiency of phosphate group screening varies with the concentration and valence of the solution counterions. By convention there are three regions of ionic strengths *I*. In the wide range of *I* the DNA volume change is proportional to R_d (Debye radius). This is evident from the viscometric data. The persistence length of DNA *a* changes only at low *I*. In that region of *I* the DNA volume changes not only due to the polyelectrolyte swelling but in consequence of increase of *a* as well. Polyelectrolyte swelling of DNA is extinct at high ionic strength. The swelling of DNA in these conditions results from the thermodynamical properties of solution.[1)]

The energy of electrostatic interaction of univalent counterions with a DNA molecule is comparable with that of thermal motion. For this reason, this kind of interaction is usually described by a diffuse sphere model. This type of ionic interaction with a macromolecule is commonly known as non-specific interaction. It is known that the charged phosphate groups guarantee the hydrofility of macromolecule. DNA retains its solubility until the concentration of univalent counterions in the solution reaches its maximum (5.45 M NaCl). One exception is LiCl: high concentrations of this salt dehydrate DNA, making it aggregate and precipitate at concentrations exceeding 9 M LiCl)[1)].

In contrast to univalent cations, bivalent ones can specifically bind to DNA electronegative groups to form relatively long-living complexes. The binding may proceed through two mechanisms: by losing a water molecule from the ion hydrosphere and though water. Eventually, there is an equilibrium between specific and non-specific bivalent ions involved in the interaction (equilibrium binding). Bivalent ions screen the DNA charge more effectively than univalent ions but can make DNA precipitate from the solution mainly only if the secondary molecular structure is damaged locally or if alcohol is added. In contrast, trivalent counterions make DNA precipitate at a concentration of 10^{-4} - 10^{-3} M (the concentration value varies with the solution ionic strength and the kind of counterion). Trivalent ions can form the energetically stronger binding.

It was shown that trivalent metal ions, in contrast to di- and monovalent, cause the shrinkage of DNA molecule even at high ionic strength solution (1 M NaCl)[2)] when the polyelectrolyte swelling disappears . We can see the initial process of the DNA packing, because the volume of macromolecule is below than at the total suppressing of the electrostatic repulsion in macromolecule: the linear swelling coefficient α for DNA ($M=9\times10^{6}$) is approximately equal 0.6÷0.7. Under these conditions the persistence length of DNA does not change. The

shrinkage is accompanied by the increasing in DNA optical anisotropy. However, for B-DNA conformation the optical anisotropy has a maximum value. We suppose that this is a result of an appearance of the mutually oriented parts of DNA chain due to the trivalent ions link distant along the chain positions on macromolecule. We suggested that such kind of DNA shrinkage my be the initial process of DNA packing caused by trivalent ions in solution. It seems quite likely that it is this restructuring of a molecule which provides its condensation later on. In order to check this assumption, we have undertaken similar experiments with Co(III) compounds: $[Co(NH_3)_6]Cl_3$ and $[Co(NO_2)_6]Na_3$. It is known that $[Co(NH_3)_6]Cl_3$ initiates DNA condensation from very dilute solutions to produce packing structures[3),4)]. It is a coordination compound. Experiments have shown that the formation of coordination bonds with a DNA molecule requires the presence of substitution acidoligands in the inner coordination sphere of the complex ion. It was sown for Platinum (II) compounds.[5)] In this work, we have studied also the DNA conformation in solutions with varying $[Co(NO_2)_6]Na_3$ contents. The dissociation of this compound produces a complex ion, $[Co(NO_2)_6]^3$; nevertheless, it interacts with DNA, inducing considerable changes in its conformation parameters. For the binding to occur, the complex ion must undergo a transition to its aqueous form: $[Co(NO_2)_2\ (H_2O)_4]^+$, $[Co(NO_2)(H_2O)_5]^{2+}$, or $[Co(H_2O)_6]^{3+}$. The methods of dynamic birefringence, low gradient viscometry and circular dichroism were used to investigate the DNA conformation in solution during the interaction with coordination compounds of Co(III). A comparison of the effects of $[Co(NH_3)_6]Cl_3$ and $[Co(NO_2)_6]Na_3$ can give an insight into the role of ligands comprising the inner coordination sphere of cobalt ions. As it was exhibited during DNA interaction with K_2PtCl_4 and $[Pt(NH_3)_4]Cl_2$[5)], the chlorine ions in the coordination sphere of platinum can be replaced by DNA groups. In contrast, $[Pt(NH_3)]^{2+}$ can not changes the ligands in the inner coordination sphere. It interacts with DNA without the formation of coordination binding.

The cobalt and platinum compounds used in this work were synthesised and studied at the Chemical Pharmaceutical Institute (St.-Petersburg, Russia). Calf thymus DNA (Serva) with $M=9x10^6$ and $20x10^6$ were used. The temperature during the experiments was 21°C. The DNA concentration was determined from the difference in absorption at two wavelengths for DNA hydrolysed at 100°C with $HClO_4$. The hyperhcromism was about 40%. Circular dichroism spectra were recorded in a Jobin-Ivon Mark-IV dichrograph.

The results received with circular dichroism method (Fig.1) can suggest to some extent that the DNA phosphate groups are the most probable binding sites for $[Co(NH_3)_6]^{3+}$ ions.

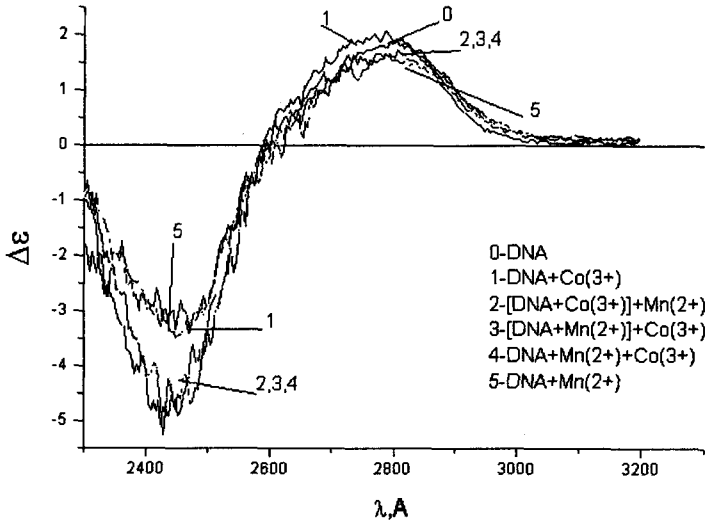

Fig. 1. CD spectra of DNA and its complexes with Co(3+) and Mn(2+)

The DNA interaction with $[Co(NH_3)_6]^{3+}$ and Mn^{2+} ions in solution was studied. It is known that different divalent ions prefer different binding position on DNA. For example, Mn^{2+} ions interact with DNA not only at phosphate groups but at base pairs as well. If the latter is the case, the change in DNA CD-spectrum is exhibited. The $MnCl_2$ was added to solution contains 0.005 M NaCl and DNA-Co(3+) complexes formed during one day at 4°C after the mixing of requisite Co(3+) and DNA concentrations. Here Co(3+) used arbitrarily and corresponded $[Co(NH_3)_6]Cl_3$ or $[Co(NH_3)_6]^{3+}$, in contrast to Co(3-) for $[Co(NO_2)_6]Na_3$. After the addition of the second compound to the solution systems again held for 12 hours. The measurements was carried out in one day with the control solutions involving DNA-Mn(2+), DNA-Co(3+) or [DNA +Co(3+) +Mn(2+)] complexes. Last was formed when both compounds added simultaneously. The control solutions held at the same conditions as the systems under study. It was shown that CD spectrum of DNA in solution does not depend on the procedure of preparation the complex systems consist of DNA, $[Co(NH_3)_6]Cl_3$ and $MnCl_2$ (Fig.1, curves 2,3,4). Although from Fig.1 it follows that the CD- spectra of DNA-$[Co(NH_3)_6]^{3+}$ and DNA-Mn^{2+} complexes defer sufficiently. We can suggest that DNA-$[Co(NH_3)_6]^{3+}$ and DNA-Mn^{2+} bonds realise independently of one another. The similar result was obtained for DNA-Fe^{3+}-Mn^{2+} complexes (Fig.2).

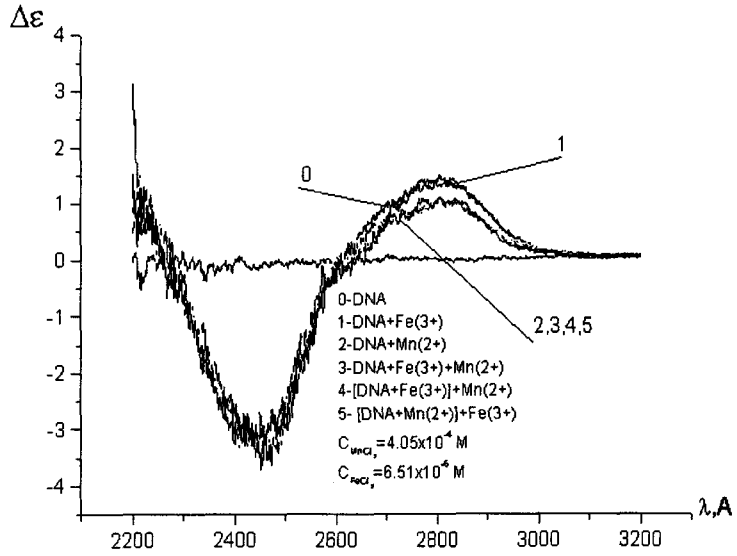

Fig. 2. CD-spectra of DNA and its complexes with Fe(3+) and Mn(2+)

It should be noted that Fe^{3+}-DNA binding does not influence the CD-spectrum of DNA in the present conditions (curves 0 and 1). We can see that DNA-Fe^{3+} binding is realised from the FB method data[2]. It is clear from the experiments that the optical anisotropy of DNA change variously during its interaction with Mn^{2+} and trivalent ions[2]. The interaction of Mn^{2+} with DNA was studied in detail at numerous experiments. There is much apparent from publications that Mn^{2+} interacts with DNA at N7 Guanine position and causes change in CD-spectrum of DNA. The data obtained showed that the addition of Mn^{2+} to DNA-Fe^{3+} complexes and the addition of Fe^{3+} to DNA-Mn^{2+} complexes lead to the same result as DNA+Mn^{2+}-Fe^{3+} simultaneously binding. The binding of Mn^{2+} with DNA realises independently on the Fe^{3+} binding. We can conclude that Fe^{3+} ions under study do not interact with base pairs. By this means we can suggest that our results demonstrate that used trivalent ions prefer the binding with DNA phosphate groups.

At Fig.3 the dependence of relative change in the optical anisotropy of DNA $(\gamma_1 - \gamma_2)/(\gamma_1 - \gamma_2)_0$ on the concentration of trivalent ions in solution C_{Me} presents.

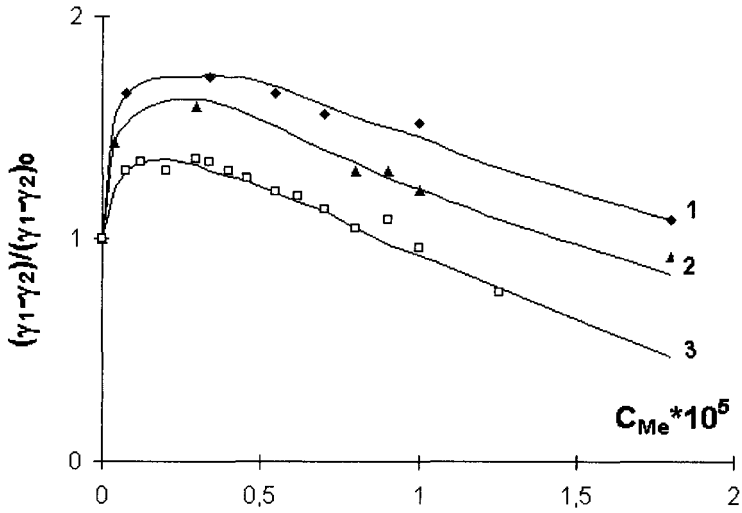

Fig. 3. Dependence of $(\gamma_1 - \gamma_2)/ (\gamma_1 - \gamma_2)_0$ on C_{Me} for DNA-Co(3+) (1), DNA-Co(3-) (2) and DNA-Fe^{3+} complexes in 0.005 M NaCl

We can see that the type of the considered dependence is common to all trivalent ions used in the present work. Even a low cobalt content is shown to produce DNA shrinkage, as is indicated by an intrinsic viscosity. It is accompanied by the increased DNA optical anisotropy. This effect was observed for both Co(III) compounds used. The comparison of these data with earlier results on DNA-Fe^{3+} complexation has revealed some features common to the DNA binding to trivalent metal ions: the shrinkage of macromolecule is accompanied by the increase in DNA optical anisotropy during its interaction with trivalent ions. The concentration of trivalent ions in solution was near 10^{-6}. The DNA concentration during the formation of complex was 0.006%. We have suggested that the mutual orientation of DNA statistical segments is changed due to the formation of intramolecular linkage between trivalent ions and remote DNA chain segments.

Conclusion

It has been shown that DNA coordination compounds of Co(III) as well as Fe^{3+} ions cause the DNA shrinkage in solution. It is accompanied by the increased DNA optical anisotropy. The analysis of these data has revealed some features common to the DNA binding to trivalent

metal ions. We have suggested that the mutual orientation of DNA statistical segments is changed due to the formation of intramolecular linkages between trivalent ions and remote DNA chain segments.

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