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# Investigation of DNA complexes with iron ions in solution

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**Abstract**

The optical anisotropy and intrinsic viscosity of DNA–$Fe^{3+}$ complexes have been investigated. It was shown that the binding of iron ions to DNA causes the shrinkage of the macromolecule. The formation of such complexes is accompanied by increasing DNA optical anisotropy. We suggest that the binding of iron ions to widely spaced along the chain DNA groups creates the conditions for initiation of mutually oriented DNA fragments, thus, ensuring a higher molecular optical anisotropy. © 1998 Elsevier Science B.V.

*Keywords:* DNA–$Fe^{3+}$ complexes; Optical anisotropy; Intrinsic viscosity

## 1. Introduction

The polyelectrolytic nature of the DNA molecule favours the study of DNA interaction with different charged ligands, including metal ions, in solution. The biological role of metal ions is significant and varies with their nature and valence. The content of some metals in biological systems is negligible, nevertheless, they perform unique functions in the human body. It is known that their deficiency can lead to distressing consequences. On the other hand, excessive metal ions are harmful for the health. Most metals are in the cells as positively charged ions. Biological systems are very complicated, and the mechanism of metal ion action is poorly understood. In this connection, it would be useful to investigate a model system, for example DNA aqueous solution with metal ions. Experiments of this kind are also cognitive for the understanding of polyelectrolytic properties of DNA.

At present, we know fairly much about the behaviour of DNA molecular parameters with changing monovalent ion concentration. Variation in the solution ionic strength is known to influence the long- and short-range electrostatic interactions in the polymer chain. Flow birefringence and viscometric data show that the DNA swelling in a solution observed over a wide range of ionic strengths from 1 M to 2 mM (NaCl) is due only to a change in the long-range electrostatic interactions in the molecule. However, when the ionic strength decreases to less than 2 mM, the persistence length of the DNA molecule increases because of the change in the short-range electrostatic interactions. In this case, the higher equilibrium rigidity of the macromolecule makes the major contribution to further increase of the molecular effective volume [1]. At sufficiently high counte-

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rion concentrations, the polyelectrolytic properties of DNA are suppressed and its conformation is determined by non-electrostatic long- and short-range interactions [2].

There have been a large number of publications on the interaction between divalent ions and the DNA molecule. It was shown that the alkaline earth metal ions $Mg^{2+}$, $Ba^{2+}$, and $Ca^{2+}$ interact with the phosphate groups of the macromolecule, whereas the transition metal ions $Mn^{2+}$, $Ni^{2+}$, and $Cu^{2+}$ also bind to DNA bases. This difference was detected, for example, in the study of the optical anisotropy of DNA complexes with these ions. It is interesting to note that the decrease in the DNA polyelectrolytic swelling is not very sensitive to the nature of divalent cations and their binding positions [3,4]. Experiments have shown that, at the same ionic strength, the divalent ions shield the charge of the macromolecule more effectively than monovalent ions [4]. This means that the change in the DNA persistence length in the presence of divalent ions starts at a lower ionic strength.

The interaction of trivalent metal ions with the DNA molecule and the influence of the binding on the DNA conformation has not been studied in detail. Most such experiments deal with coordination compounds of Co(III) [5–8]. It is known that Cr(III) and Co(III) ions can form coordination bonds with the phosphate groups of nucleotides [9,10]. There is an opinion that Co(III) can induce DNA condensation. The phenomenon of DNA condensation in aqueous solutions of polyamines has been studied experimentally and theoretically [11–16]. The DNA collapse was observed earlier by Lerman [17,18]. Compact DNA structures of various shapes were observed in water–ethanol mixtures of different ionic strengths [19]. It was suggested that cations can induce DNA condensation in 89–90% coverage of the phosphate groups [13]. Monovalent ions cannot provide an effective screening of these groups because they interact with DNA mostly without loss of hydration water. In this case, the counterions belong to the solvent. On the contrary, divalent ions bind to DNA at definite positions. In some cases, DNA–$Me^{2+}$ complexes are formed with the loss of hydration water of the ion [20]. Distinct divalent ions prefer different vacant positions on the double helix during the binding process.

So it would be useful to study the DNA interaction with trivalent metal ions and to compare the experimental data with those for the DNA–Me(II) binding.

The present work deals with the effect of $Fe^{3+}$ ions on the conformation of the DNA molecule at different concentrations of the supporting electrolyte (NaCl).

## 2. Materials and methods

The calf thymus DNA (Serva) used had a molecular mass $M = 9 \times 10^6$, as evaluated from the intrinsic viscosity $[\eta]$ at 0.15 M NaCl. The DNA concentration in the solution was determined from its absorption at $\lambda = 270$ and 290 nm following hydrolysis with $HClO_4$ [21]. $C_{DNA}$ is the monomer concentration (bp). The complex formation usually occurred at the same DNA concentration $C_{DNA} = 10^{-4}$ M (bp). In the concentration experiments, the condition $C_{Fe}/C_{DNA}$ = constant was preserved. However, with other dilution procedures, the complex also remained stable. For this reason, the $C_{Fe}/C_{DNA}$ ratio is valid mainly during the complex formation. The DNA native state was tested before and after the investigations by different methods, using the molar extinction coefficient $E_{260}$ (P). The measurements were made at 21°C.

## 3. Viscometry

The relative viscosity $\eta_r$ of the solution at different flow rate gradients $g$ was measured for different DNA concentrations $C$ in the Zimm–Crothers type magnetic rotation viscometer [22] with subsequent determination of the DNA intrinsic viscosity for all the systems under investigation:

$$[\eta] = \lim_{\substack{C \to 0 \\ g \to 0}} \frac{\eta_{r-1}}{C}$$

The value of $[\eta]$ is related to the parameters of the macromolecule by the Flory equation:

$$[\eta] = \Phi(\varepsilon) \frac{(LA)^{3/2}}{M} \alpha^3 \qquad (1)$$

where $L$ is a hydrodynamic length of a macromolecule, $A$ is a length of the statistical chain segment, $\Phi(\varepsilon)$ is the Flory coefficient depending on swelling and equilibrium rigidity of a macromolecule, and $\alpha$ is its linear swelling coefficient. For random coils, $A$ is equal to $2a$, where $a$ is a chain persistence length. It follows from Eq. (1) that the value of $[\eta]$ depends on both long- and short-range interactions in the macromolecule. Therefore, it is not possible to establish unequivocally the contribution from each of these factors to the observed change of $[\eta]$ using viscometry only. This problem, however, can be solved by the flow birefringence method.

## 4. Flow birefringence

The dependence of the birefringence value $\Delta n$ for DNA solutions of different concentrations on the flow rate gradient can yield the dynamooptical constant:

$$[\eta] = \lim_{\substack{g\to 0 \\ C\to 0}} \frac{\Delta n}{gC\eta_0}$$

where $\eta_0$ is the solvent viscosity. The ratio $[n]/[\eta]$ is proportional to the optical anisotropy of the macromolecule, regardless of the model concept. In absence of the macroform effect, the value $\frac{(\Delta n/g)_{g\to 0}}{(\eta_r - 1)\eta_0}$, which is determined at any finite solution concentration, coincides with the $[n]/[\eta]$ ratio related to the optical anisotropy of the statistical segment of the macromolecule $(\alpha_1 - \alpha_2)$ by the Kuhn equation [23–25]:

$$\frac{(\Delta n/g)_{g\to 0}}{(\eta_r - 1)\eta_0} = \frac{[n]}{[\eta]} = \frac{4\pi}{45\,kT}\frac{\left(n_s^2 + 2\right)^2}{n_s} \times (\alpha_1 - \alpha_2) \qquad (2)$$

where $n_s$ is the refractive index of the solvent. The optical anisotropy of the segment is equal to $S\Delta\beta$, where $\Delta\beta$ is the difference between the polarizabilities of a nucleotide pair along the helix axis and the normal to it, and $S$ is the number of pairs in a statistical segment; $S = A/l$, where $l$ is the length of the monomer residue. It has been shown by calculations and numerous experiments that the macroform effect for DNA is negligible, as compared to the intrinsic anisotropy of the macromolecule [26,27]. This fact follows from $\frac{(\Delta n/g)_{g\to 0}}{(\eta_r - 1)\eta_0}$ being independent of the DNA concentration in the solution. This result has also been obtained for all the systems investigated here.

## 5. Results and discussion

DNA precipitates from a solution as loose flakes when the concentration of $FeCl_3$ is greater than $C_{Fe} \cong 1.5 \times 10^{-4}$ M. A detailed spectral analysis has shown that we deal with a true molecular solution only at $C_{Fe}$ less than $5 \times 10^{-5}$ M. This range of $FeCl_3$ concentrations was used in our experiments. We ignored the contribution of the $Fe^{3+}$ concentration to the ionic strength, because it is far less than that of monovalent ions.

It is known that the proportions of bound and free ligands in the solution during the concentration investigations of DNA complexes with small ligands should be maintained constant. The investigation of DNA complexes with divalent metal ions may be accompanied by the dialysis of the initial solution against the solvent with a given ionic concentration. This solvent subsequently serves as a diluent [3]. In contrast to divalent ions, $Fe^{3+}$ ions form, with the macromolecule, relatively stable complexes which are not disrupted by dilution, as is evident from experiments using different dilution methods. This fact may be due not only to the considerably higher electrostatic forces, than those in the case of mono and divalent ions, but also to the ability of iron to form coordination bonds [28]. In fact, experiments have shown that the formation of a $Fe^{3+}$–DNA complex takes some time (over 10 h) for the DNA molecular parameters to be changed. In this sense, $Fe^{3+}$ ions behave like platinum coordination compounds [29,30].

Fig. 1 shows the effect of $Fe^{3+}$ ions on the hydrodynamic behaviour of the DNA molecule. It can be seen that the DNA intrinsic viscosity decreases with increasing $FeCl_3$ concentration in the solution up to $C_{Fe} \cong 8 \times 10^{-6}$ M at 0.005 M NaCl. Further increase in the iron content has no effect on the $[\eta]$ value. This fact may indicate that the vacant sites on the DNA molecule for the binding of $Fe^{3+}$

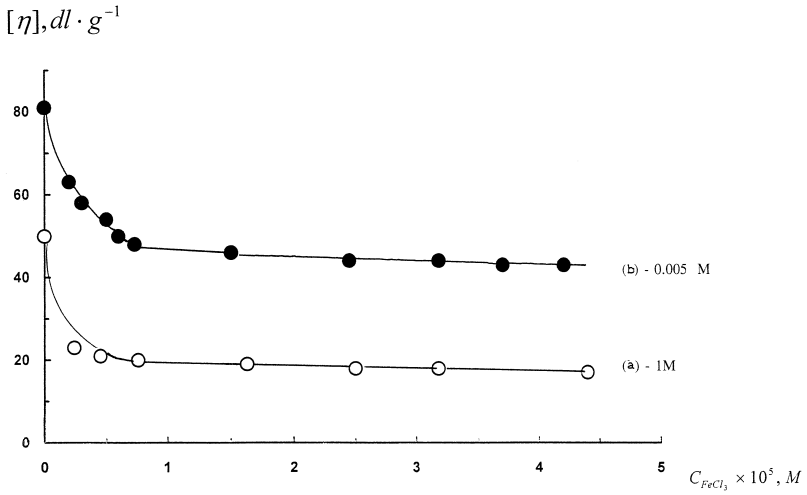


Fig. 1. Dependence of the intrinsic viscosity on iron concentration in solution at $I = 1$ M NaCl (a) and 0.005 M NaCl (b).

ions have been filled up. The limiting number of bound $Fe^{3+}$ ions is about 1 per 10 base pairs (bp). Fig. 1 also shows that the concentration of the supporting electrolyte does not affect the character of the dependence of $[\eta]$ on the $Fe^{3+}$ concentration. Nevertheless, the independence of the $[\eta]$ value on $C_{Fe}$ begins in 1 M NaCl at lower iron concentration than in 0.005 M. This means that the vacant positions on DNA in 1 M are completed with iron ions at a small $C_{Fe}/C_{DNA}$ ratio. Again, the $[\eta]$ values at $C_{Fe} > 8 \times 10^{-6}$ M differ in solutions of low and high ionic strengths, indicating that the degree of shrinkage varies with the initial size of the macromolecule. In other words, the negative charges on the DNA molecule are not totally shielded by the bound iron ions, and the swelling of the iron-bonded macromolecule is still observed at a low ionic strength, although its size diminishes after the binding. One should note that no polyelectrolytic swelling of the DNA molecule is actually observed in 1 M NaCl and its volume is determined from the thermodynamic properties of the solution [2]. Hence, a decrease in the $[\eta]$ value at a high ionic strength cannot be caused by a change in the DNA polyelectrolytic swelling. On the other hand, the persistence length cannot change with additional screening of the DNA negative charge by the bound iron ions. It seems likely that the binding of iron ions initiates the formation of intramolecular links, which decrease the volume of the macromolecule. The intermolecular cross-linking is unlikely at the DNA concentration used. Moreover, the formation of intermolecular complexes would contribute to the $[\eta]$ value. It is pertinent to note that the addition of $Fe^{3+}$ to the DNA solution can cause a change in the DNA effective charge. This effect, indeed, can partially account for decreasing intrinsic viscosity at 0.005 M NaCl. But at 1 M NaCl, that explanation would be inadequate. At high ionic strength, the polyelectrolytic swelling is absent. The binding of iron ions cannot decrease the electrostatic repulsion because it is negligible. In these conditions, the decrease in the $[\eta]$ value can be explained only in terms of the reorganisation of the tertiary structure of the DNA molecule in solution.

It is significant that the viscometric data cannot provide unambiguous information about the nature of conformational changes in the molecule during the binding. We can only state its shrinkage in the solution as a result of interaction with $Fe^{3+}$ ions. The essential decrease in the DNA size at a high ionic strength demonstrates a non-electrostatic nature of the shrinkage.

Let us analyze the dynamic birefringence results. Fig. 2 shows the dependence of the dynamooptical constant $[n]$ on the iron concentration in the solution. At constant molecular optical anisotropy, the $[n]$ value is known to vary with that of $[\eta]$. A compari-

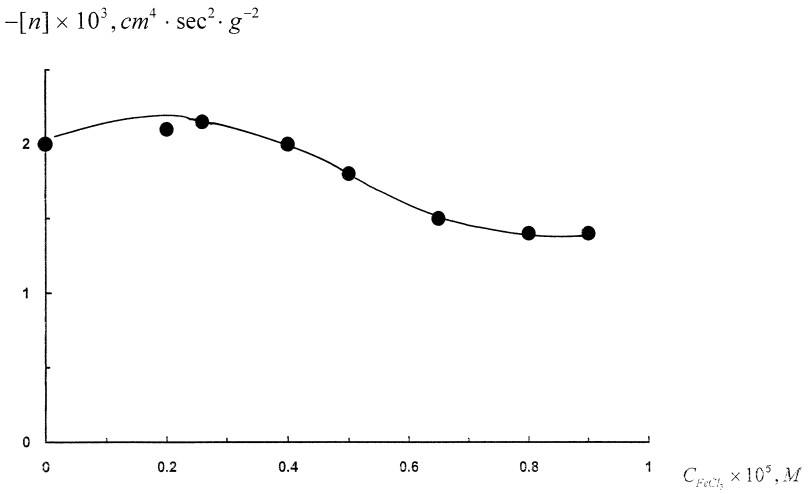


Fig. 2. Dependence of the dynamooptical constant of DNA on iron concentration in solution at $I = 0.005$ M NaCl.

son of the curves in Figs. 1 and 2 shows no synchronism; hence, the optical anisotropy of the DNA molecule changes during its binding to $Fe^{3+}$ ions.

Fig. 3 shows the dependence of the ratio $[n]/[\eta]$ (which is proportional to the difference in the polarizabilities of the molecule) on the $FeCl_3$ concentration at low and high ionic strengths of the solution. One can see that the points corresponding to the values of $\frac{(\Delta n/g)_{g\to 0}}{(\eta_r - 1)\eta_0}$ also fall on this curve. This fact indicates that the macroform effect in the systems under investigation is negligible. It is also clear that the binding of iron ions to the DNA molecule causes a relatively drastic increase in the absolute value of the $[n]/[\eta]$ ratio even at low $FeCl_3$ concentrations. The concentration of monovalent ions in the solution does not affect much the process of $Fe^{3+}$ binding. The change in the optical anisotropy is smaller at 0.005 M NaCl than at a high ionic strength. The

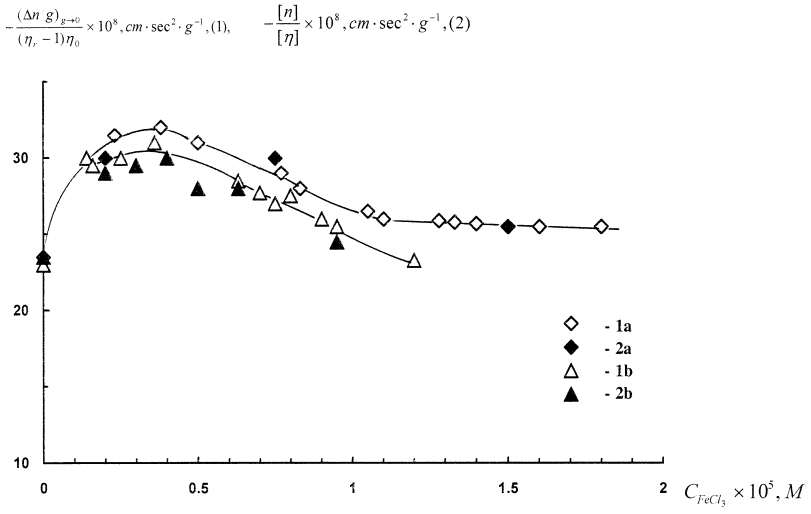


Fig. 3. Dependence of $\frac{(\Delta n/g)_{g\to 0}}{(\eta_r - 1)\eta_0}$ (1) and $[n]/[\eta]$ (2) on iron concentration in solution at $I = 1$ M NaCl (a) and 0.005 M NaCl (b).

increase in the optical anisotropy of the DNA segment may be due to its larger length or to a higher $\Delta\beta$ parameter. However, for the B-form conformation, $\Delta\beta$ has the maximum value. Alternatively, the suggestion that the equilibrium rigidity of the DNA molecule increases during its binding to $Fe^{3+}$ ions contradicts the viscometric data. The only assumption that remains is that the observed changes in the DNA optical anisotropy results from certain structural rearrangements in the polymer chain, caused by its interaction with the iron ions. It may be suggested that regions of mutually oriented helical chain fragments, held side by side by common iron ions, are formed during the shrinkage of the DNA molecule. It is known that in a random coil there is a preferential orientation of statistical segments along the major axis of the molecular ellipse. The orientation of the segments gives the direction for the arrangement of the DNA fragments linked to one another by iron ions. This packaging can lead to a higher DNA optical anisotropy and does not contradict the viscometric data obtained. The suggested formation of more or less parallel elements of the DNA molecule can result from the iron ions binding to the phosphate groups widely spaced along the chain. Indeed, for cross-links to be formed, the ions must be located on the helix surface. Therefore, we suppose that they are bound to the phosphate groups. Actually, the binding of small ligands to the DNA bases causes usually the decreasing of the molecular optical anisotropy. The increasing of this parameter is very difficult to interpret in any other way except by the structural rearrangement of molecular coil. The binding with the DNA bases also causes the change in absorption spectra. In our experiments, we did not detect it.

These links should lead to a decrease in the DNA volume and, hence, to its lower intrinsic viscosity. The appearance of the first link greatly facilitates that of the subsequent links. This probably account for the relatively narrow range of $FeCl_3$ concentrations, in which the decrease in the $[\eta]$ value has been observed.

It is noteworthy that during the DNA interaction with iron ions, the changes in $[\eta]$ and $\frac{(\Delta n/g)_{g\to 0}}{(\eta_r-1)\eta_0}$ depend weakly on the ionic strength of the solution. This may be due to the presence of a large number of $Na^+$ ions, and, consequently, the suppression of polyelectrolytic swelling favours the mutual orientation of the DNA molecular pieces.

A comparison of conformational changes in the DNA molecule occurring during its interaction with di- and trivalent metal ions shows that they differ greatly. This fact was established in the study of DNA hydrodynamic and optical parameters. For divalent metal ions, a decrease in the $[\eta]$ value was observed only at a low ionic strength, regardless of the position of the ion binding to the molecule,

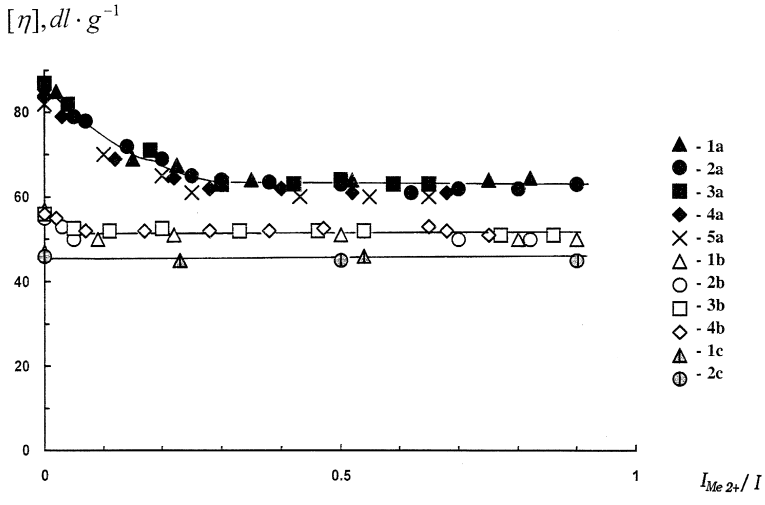


Fig. 4. Dependence of the intrinsic viscosity of DNA on $I_{Me^{2+}}/I$ ($I$ is the ionic strength supplied by divalent ions) at total ionic strength $I = 0.005$ (a), 0.1 (b) and $I = 1$ (c) for $Mg^{2+}$ (1), $Mn^{2+}$ (2), $Ba^{2+}$ (3), $Ni^{2+}$ (4) and $Cu^{2+}$ (5).

which indicates that its polyelectrolytic swelling has decreased. At a high ionic strength, the $[\eta]$ value did not change appreciably after the divalent ion binding to DNA (Fig. 4). In these conditions, the polyelectrolytic swelling of the DNA molecule was negligible.

A different situation was observed in the study of DNA complexes with trivalent iron ions. Lower $[\eta]$ values were found in a narrow iron concentration range independent of the ionic strength. Moreover, at high ionic strengths, the decrease in $[\eta]$ even slightly exceeded that observed at low ionic strengths, and the evaluation of the linear swelling coefficient from Eq. (1) gave $\alpha \cong 0.7$. This value indicates that the size of the DNA molecule in a complex is far smaller than that obtained in absence of polyelectrolytic swelling. In other words, the $Fe^{3+}$ binding causes the DNA shrinkage. This has nothing to do with the screening of DNA negative charges, as differentiated from the action of divalent ions.

A fundamental difference is also observed in the behaviour of optical anisotropy of DNA complexes with di- and trivalent metal ions. In case of the divalent ions $Mg^{2+}$, $Ba^{2+}$, $Ca^{2+}$, which are bound only to phosphate groups, the optical anisotropy of the DNA molecule does not change appreciably. Its decrease is observed only in the presence of $Mn^{2+}$, $Ni^{2+}$, $Cu^{2+}$ ions capable of binding to nitrogen bases of the molecule (Fig. 5). In this case, the behaviour of the optical anisotropy of the complexes is independent of the solution ionic strength. On the contrary, on addition of iron ions to a DNA solution, the optical anisotropy increases. As pointed out above, this result can be explained only by the appearance of mutually oriented pieces of the DNA molecule. These data show that the diminishing of the size of the DNA molecule caused by its interaction with di- and trivalent ions differs essentially.

The binding of divalent ions decreases the DNA molecular size at $C_{Me^{2+}} > 10^{-5}$ M due to the suppression of polyelectrolytic swelling. In contrast, when trivalent iron ions are bound to DNA at the concentration of about $10^{-6}$ M, the DNA shrinkage is observed even at high ionic strengths, and the estimate of the linear swelling coefficient ($\alpha = 0.7$) is unrelated to a change in the intramolecular electrostatic repulsion. Indeed, the polyelectrolytic swelling is suppressed in 1 M NaCl. The decrease of the DNA volume at 1 M NaCl with $\alpha = 0.7$ suggests a shrinkage of the macromolecule. The reasons for the formation of a compact structure may be the intramolecular cross-links, formed by iron ions bound to phosphate groups.

We hope that further investigations will make it possible to offer a more specific interpretation of the DNA shrinkage by trivalent metal ions.

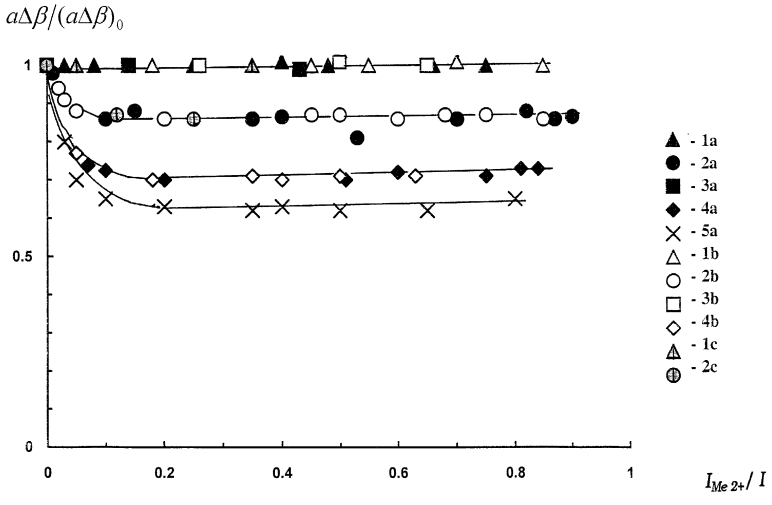


Fig. 5. Dependence of $a\Delta\beta/(a\Delta\beta)_0$ on $I_{Me^{2+}}/I$ at $I = 0.005$ (a), and 0.1 (b), where $a$ is the persistence length of the macromolecule, $\Delta\beta$ is the optical anisotropy of base pair, $I$ is the total ionic strength, $(a\Delta\beta)_0$ are the DNA molecular parameters in NaCl only. The $a$ value does not change during interaction [4]. The symbols are the same as on Fig. 4.

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