

## Mutual Interactions of the Phosphate Groups in Locally Deformed Backbones of Various DNA Double Helices at High Salt Concentrations

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**Abstract.** Changes in the free energy of mutual phosphate group interactions are calculated that accompany bending of the A-, B- and Z-DNA backbones in 0.7, 2.1 and 4.2 mol/l NaCl aqueous solutions. The bending is often found to be favoured in the direction of the double helix grooves; B-DNA prefers bending into the major groove while minor groove is the preferred bending direction of A-DNA in the presence of 0.7 mol/l NaCl. Interestingly, the preferences are reversed in 4.2 mol/l NaCl. Further stabilization of A-DNA and B-DNA backbones is achieved in some cases if bending is combined with suitable local double helix twist alterations. Bending tendencies of Z-DNA backbone are generally weaker and they decrease, in contrast to B-DNA and A-DNA, with the increasing ionic strength.

**Key words:** DNA conformation — High salt — Double helix bending — Unwinding and winding

### Introduction

The DNA molecule is extremely compacted in bacteria, cell nuclei, sperm heads and virus capsids (Kelleberger 1988). The compaction is mainly driven by basic proteins which cause the same changes in natural DNA circular dichroism spectra and thus conformation as high-salt concentrations (Adler and Fasman 1971; de Murcia et al. 1978). This many times demonstrated fact makes studies of the high-salt concentration effects on DNA especially appealing because their conformational origin is not yet properly understood. This ignorance originated from the long time lasting lack of theory describing DNA properties in high-salt solutions. The theory is now available (Soumpasis 1984). In spite of an approximative nature, it has gained credibility in recent years by being capable not only to reproduce the B-to-Z isomerization in DNA (Soumpasis et al. 1987b;

Soumpasis 1988) but also its counterion size dependence (Soumpasis et al. 1987a). These encouraging results stimulated us to employ the theory to study tendencies of various types of DNA double helix to be bent in the presence of medium and high salt concentrations. Studies of DNA bending have now gained further impulse because it appears indisputable that bent DNA is an important molecular signal regulating gene expression (Travers 1989).

### Model and Method

Twenty four negative charges are placed in phosphate group positions of the backbones of B-, A- and Z-DNA. The phosphate group coordinates were taken from the literature (Arnott and Hukins 1972; Wang et al. 1981). Two Z-DNA molecules are considered, having GpC or CpG steps in the centre, because properties of these two steps are different in Z-DNA (Wang et al. 1981). All the model double helices are deformed in the centre and free energies (see below) are calculated which accompany the deformations. The deformations consist of bending in all directions and helical twist variations. Bending magnitude is the angle between the helical axes of the bottom and upper halves of the model molecules. Bending directions  $d$  of  $90^\circ$ ,  $108^\circ$ ,  $0^\circ$  and  $30^\circ$  correspond to bending into the double helix major grooves of A-DNA, B-DNA, Z-DNA (GpC) and Z-DNA (CpG), respectively. The corresponding values for the minor groove are  $270^\circ$ ,  $288^\circ$ ,  $180^\circ$  and  $210^\circ$ . We also consider the phosphate group configurations which are sterically not acceptable. These are indicated by light lines in the figures. Attached are numbers of the colliding phosphate group pairs. For each configuration of the system of 24 charges, a set of 276 interphosphate distances  $r_i$  is calculated (due to the double helix symmetry the distances are not all different). The distances are included in the expression for the free energy calculation. Free energy of a configuration is a sum of free energy contributions of each pair of charged spheres in the system. Free energy  $F_i$  (in kT units,  $k$  is the Boltzmann constant,  $T$  absolute temperature) of the  $i$ -th pair of charges is given by the expression (Soumpasis 1984)

$$F_i = -\ln g_o(r_i) + \frac{P \exp(C)}{1+C} \frac{\exp(-Cr_i)}{r_i}$$

where  $-\ln g_o(r_i)$  is the pair correlation function of a hard sphere fluid (Troop and Bearman 1965),  $P = e^2/ESkT$ , a dimensionless constant, where  $e$  is the electron charge,  $E = 78$  is the dielectric constant of water,  $S$  is the ionic diameter,  $r_i = r_i/S$ ,  $C = KS$ , where

$$K = \left( \frac{4\pi e^2}{EkT} \sum_{a=1}^2 R_a Z_a^2 \right)^{1/2}$$

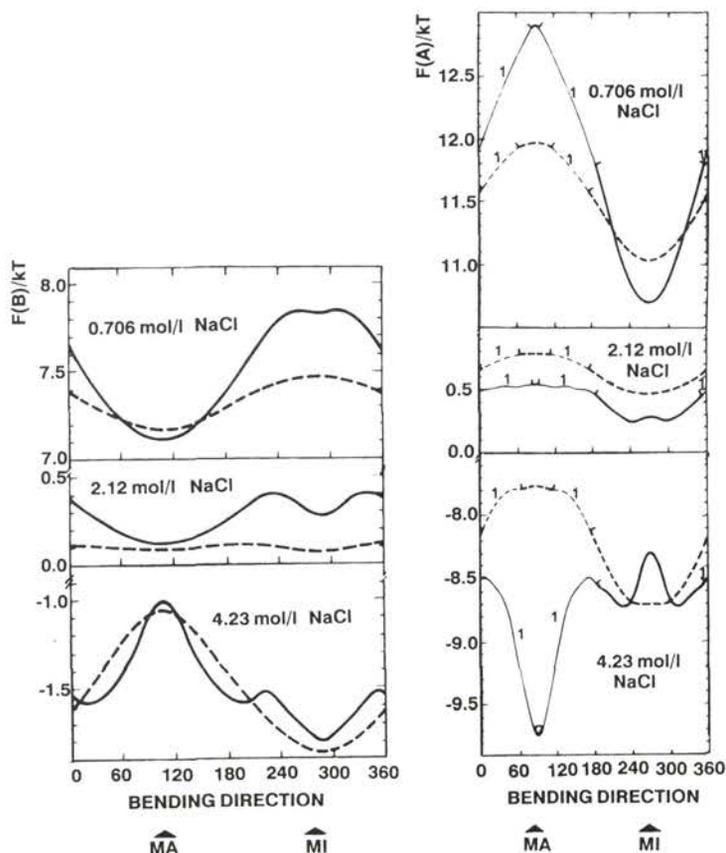


Fig. 1. Dependences on the bending direction of Helmholtz free energy  $F$  of the system of 24 phosphates in the B form (left) and the A form (right) for bending magnitudes  $10.0^\circ$  (-----) and  $22.5^\circ$  (—). The following notation is used in all figures: MI — minor groove, MA — major groove,  $m$  — bending magnitude,  $d$  — bending direction. Light lines indicate local deformations at which contacts between phosphates arise. The attached numbers indicate how many phosphate pairs collide.

is the Debye-Hückel screening parameter,  $a = 1$  ( $a = 2$ ) for anions (cations),  $Z_a$  is valency,  $R_a$  the ion number density. In this work  $S = 0.49$  nm, which corresponds to NaCl (Soumpasis 1984),  $T = 298.15$  K.

## Results

The first set of calculations performed concerned bending by  $10^\circ$  or  $22.5^\circ$  of the upper halves of our model double helices with respect to the fixed bottom halves

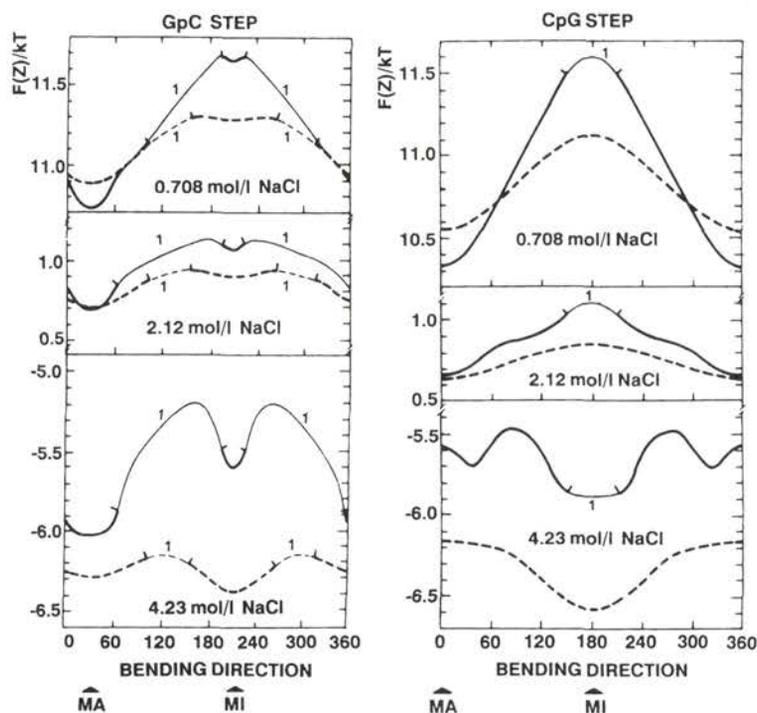


Fig. 2. The same as Figure 1 but for Z-DNA with a GpC (left) and CpG (right) dimer in the central deformed step.

in the presence of 0.7, 2.1 or 4.2 mol/l NaCl. A general conclusion following from these calculations is that the double helix grooves play prominent roles in the bending (Figs. 1,2). A closer inspection of the results reveals that the grooves play opposite roles in some conformations and that the roles are reversed upon changing from medium (0.7 mol/l NaCl) to high (4.2 mol/l NaCl) ionic strength. Specifically, bending is favoured into the major groove but unfavoured into the minor groove with B-DNA at 0.7 mol/l NaCl while at 4.2 mol/l NaCl the tendencies are opposite. Interestingly, just a reverse behaviour in all respects is displayed by A-DNA which prefers bending into the minor groove at 0.7 mol/l NaCl while major groove is the preferred direction for bending at 4.2 mol/l NaCl (Fig. 1). The situation is different with Z-DNA in both GpC and CpG steps. In particular, steric clashes occur on many occasions when Z-DNA is bent and the bending of Z-DNA at high ionic strength has a destabilizing effect (Fig. 2), in contrast to B-DNA and A-DNA.

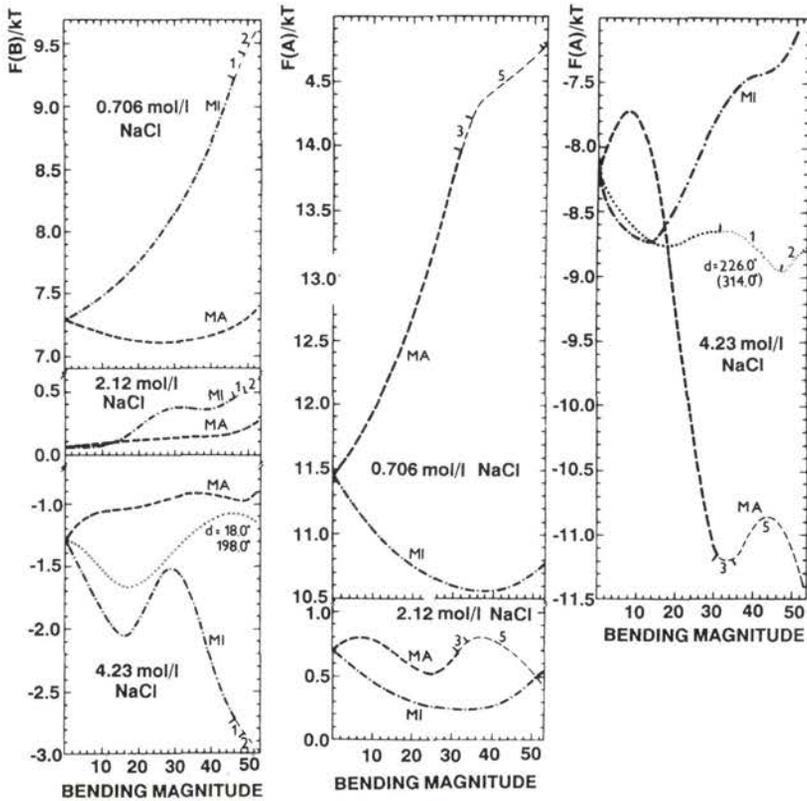


Fig. 3. Dependence of Helmholtz free energy  $F$  of the system of 24 phosphates in the B form (left) and the A form (middle and right) on the bending magnitude in the directions of double helix grooves. The curves (.....) correspond to non-groove directions in which additional energy minima occurred in Fig. 1.

It follows from the above analysis that the double helix grooves play crucial roles in DNA bending. Consequently, we dealt in more detail with the energy dependences on bending in the groove directions. B-DNA evidently resists bending into the minor groove at 0.7 mol/l NaCl while showing an extensive tolerance to bending into the major groove up to a  $50^\circ$  magnitude (Fig. 3). This tolerance is not substantially changed up to 4.2 mol/l NaCl while the resistance to bending into the minor groove direction weakens with the increasing ionic strength. In fact, at high-salt B-DNA bending is more favourable into the minor groove than into the major one. A-DNA exhibits remarkably opposite properties as compared to B-DNA (Fig. 3), which not only concerns the opposite roles

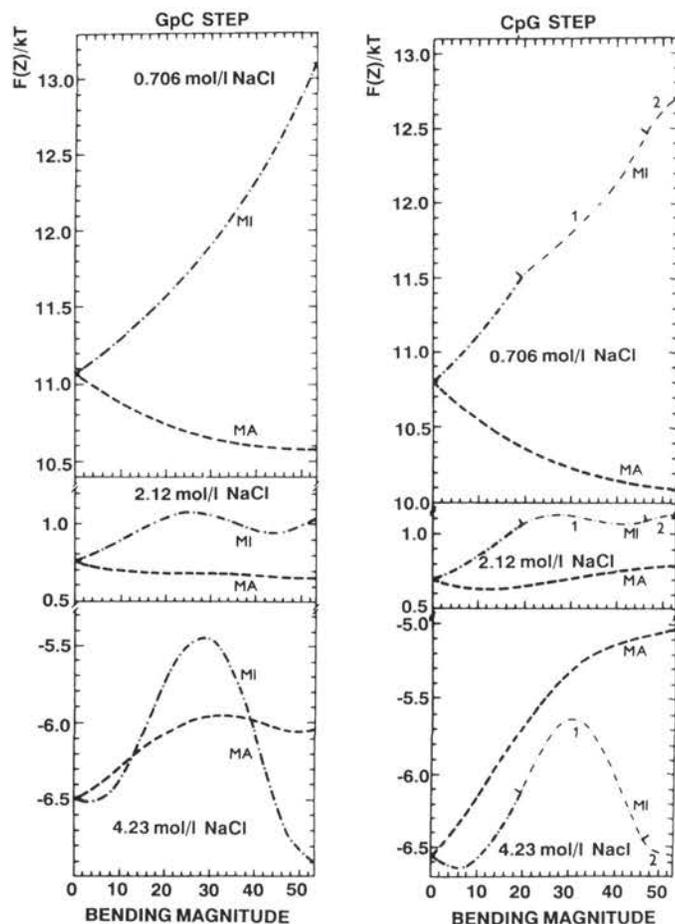


Fig. 4. The same as Figure 3 but for Z-DNA with the central GpC (left) and CpG (right) step.

of the grooves but also the dependences on ionic strength. The minor groove is an unfavourable direction for bending in both steps of Z-DNA almost regardless of ionic strength (Fig. 4).

Bending is not the only imaginable local deformation of DNA. In fact, it is often, if not always, combined with local changes in the double helix twist (Frederick et al. 1984). That is why we calculated free energy dependences on the helical twist changes in the central flexible hinge of our model double helices for straight and bent molecules. The calculations show that at 0.7 mol/l NaCl the

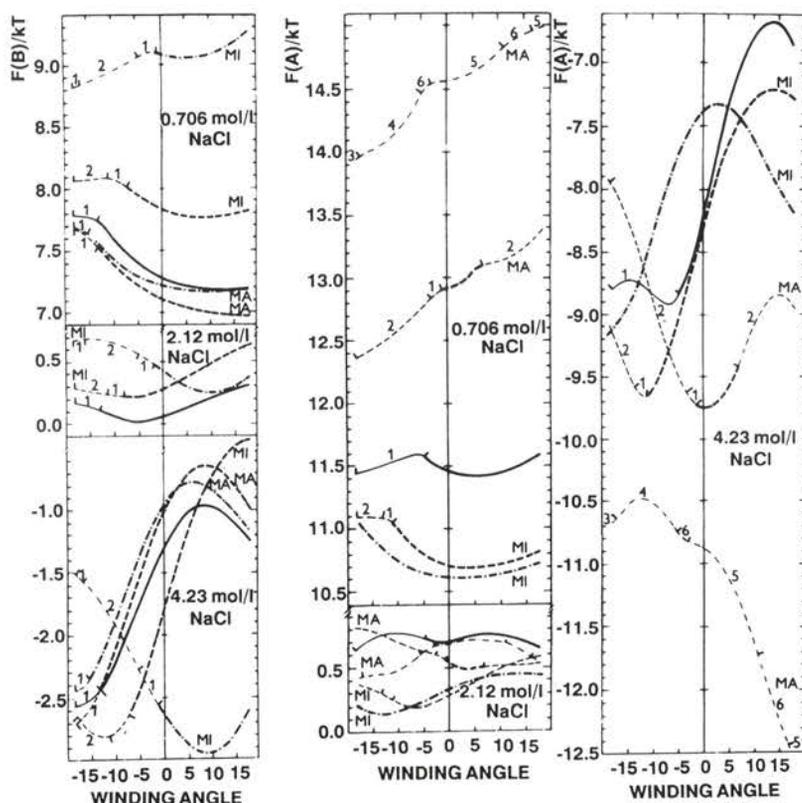


Fig. 5. Dependences of the Helmholtz free energy  $F$  of the system of 24 phosphates in the B form (left) and the A form (middle and right) on local helix twist alterations for the straight molecules (—) and molecules bent [ $m = 22.5^\circ$  (---) and  $45.0^\circ$  (-·-·-)] in the major (MA) and minor (MI) groove directions. The curves in the case of the B-DNA bent into the major groove at 2.12 mol/l NaCl (not shown) have a shape similar (their energy difference is less than 0.1 kT) to that of the curve obtained for the straight molecule (solid line). (The numbers of phosphate group collisions in A-DNA at 2.12 mol/l NaCl are the same as indicated at the corresponding curves obtained at 0.7 mol/l and 4.2 mol/l NaCl.) Zero winding angles correspond to  $36.0^\circ$  with B-DNA and  $32.7^\circ$  with A-DNA.

phosphate group interactions tend to increase the helical twist of straight B- and A-DNAs (Fig. 5). On the other hand, base stacking — another important force operating in the double helix — shows an opposite tendency (Šponer and Kypr 1989). Thus it appears that DNA duplex folding into the B and A forms is the consequence of a delicate balance between these two forces. In contrast, the phosphate group interactions tend to unwind B- and A-DNA at 4.2 mol/l NaCl, and this tendency is likely to drive the known high-salt induced DNA isomeriza-

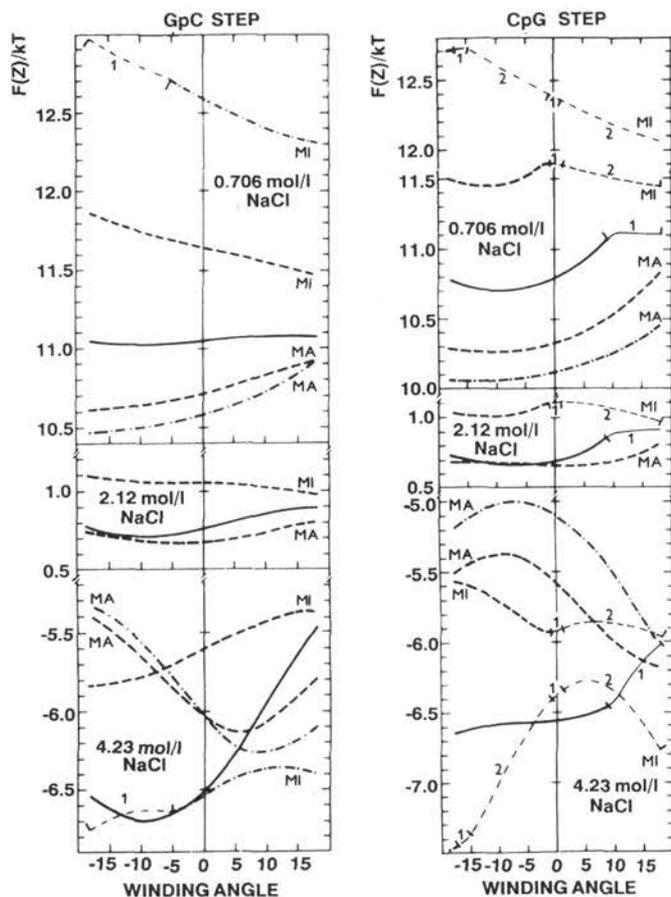


Fig. 6. The same as Figure 5 but for Z-DNA with the central GpC (left) or CpG (right) step.

tions into unwound non-B double helices (Jovin et al. 1983; Vorlíčková and Kypr 1985; Kypr and Vorlíčková 1988). Z-DNA backbone tolerates local helix winding changes at 0.7 mol/l NaCl at GpC step and 4.2 mol/l NaCl at the CpG step while its unwinding is favoured by the phosphate group interactions at the CpG steps at 0.7 mol/l NaCl and especially at the GpC ones at 4.2 mol/l NaCl (Fig. 6).

We also calculated free energy dependences on the local helix winding angle for molecules bent in the groove directions. The bend magnitudes were chosen  $22.5^\circ$  and  $45^\circ$ . It follows from Fig. 3 that B-DNA does not bend into the minor groove at 0.7 mol/l NaCl, and this is only slightly changed upon helical

twist variations (Fig. 5). But at 4.2 mol/l NaCl a strongly overwound and substantially bent into the minor groove B-DNA structure is favoured with a local helix winding value of approx  $45^\circ$ . Interestingly, low bending magnitudes are not favourable for this strongly overwound structure to invoke an imagination that there is a local bending switch in B-DNA, provided a base sequence exists allowing the backbone phosphates to control the switch in dependence on their ionic screening.

In A-DNA at 0.7 or 2.1 mol/l NaCl, the local helix twist alterations more or less only modulate the much stronger dependence on bending if sterically acceptable spatial arrangements of the phosphate groups are only taken into account. On the other hand, at 4.2 mol/l NaCl the stability is strongly dependent on the helix winding angle (Fig. 5). Among the sterically acceptable configurations those are the most stable which are moderately bent into the grooves; bending into the minor groove requires a substantial double helix unwinding while bending into the major groove occurs at the helix twist values occurring in A-DNA. Z-DNA favours unwinding and bending into the major groove in both steps at 0.7 mol/l NaCl. At high-salt, straight molecules of Z-DNA are the most stable ones (Fig. 6).

## Discussion

An exhaustive description of forces which influence DNA conformation is so complex that its practical use is not tractable even using supercomputers. Thus the various forces influencing DNA conformation should be studied separately with the hope that this approach will finally yield the possibility to analyze the whole DNA conformation including the bound solvent molecules and ions. At least, this approach might provide reasonable starting conformations which can already be reasonably optimized including all forces which influence DNA conformation. In a previous work we dealt with base stacking forces operating in DNA (Šponer and Kypr 1989) while the effect of high salt concentrations on mutual interactions of the backbone phosphate groups has been studied here. The present study of a system of 24 negative charges placed in positions of backbone phosphates of unperturbed or locally deformed A-, B- and Z-DNA double helices brought several pieces of information.

It is immediately obvious from the Figures that the backbone of B-DNA is stabilized by about 10 kT units upon changing from 0.7 to 4.2 mol/l NaCl while the stabilization energy of A-DNA is twice as high. In addition, the B-DNA backbone is more stable than that of A-DNA at 0.7 and 2.1 mol/l NaCl while the opposite is true at 4.2 mol/l NaCl. There are experimental data indicating a higher stability of A-DNA in comparison with B-DNA at high-salt

(Borah et al. 1985; Nishimura et al. 1986; Sarma et al. 1986). It is also worth of being noticed that at 0.7 mol/l NaCl the backbones of both B- and A-DNA tend to overwind, presumably to counteract base stacking tendencies to unwind the double helices (Šponer and Kypr 1989). At high-salt, even the interphosphate interactions promote DNA unwinding and are thus the driving force of DNA isomerizations into the unwound non-B conformations (Jovin et al. 1983; Vorlíčková and Kypr 1985; Kypr and Vorlíčková 1988).

It follows from previous studies considering all intramolecular forces operating in DNA, but not solvent and ions, that grooves play crucial role in DNA bending (Zhurkin et al. 1979). We extend this result by two findings. First, the grooves play opposite roles in B- and A-DNA; bending is favoured into the major groove in the former structure while minor groove is the favoured bending direction in the latter structure. No doubt, the fact that phosphate groups in A-DNA are turned into the major groove plays a decisive role. Secondly, the bending tendencies are reversed at high-salt when B-DNA favours bending into the minor groove while A-DNA tends to bend into the major groove. It follows from our calculations that the interphosphate interactions promote deformations of the B- and A-DNA double helices and that these tendencies get stronger with the increasing ionic strength. Z-DNA deformability is substantially less in comparison with that of A- and B-DNA, it is less dependent on the ionic strength and there are no energy gains connected with high-salt Z-DNA deformation. Deformations of both B- and A-DNA backbones are connected with free energy gains of the order of 1 kT unit. They can naturally be opposed by the resistance of, first of all, base stacking forces. The resistance of stacking forces to bending certainly is a base sequence dependent property (Gartenberg and Crothers 1988) deserving a detailed theoretical study. However, the average energy required to bend real DNA can reliably be estimated from its persistence length. This approach shows (Jursa and Kypr, in preparation) that free energies of the mutual backbone phosphate interactions acquired upon DNA bending (this concerns A- and B-forms but not Z-form) in the favourable directions are sufficient for bending of real DNA by 8–10°. This result indicates that bending tendencies promoted by the interphosphate interactions are generally non-negligible while at low bending magnitudes they even dominate over other forces operating in DNA.

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