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Computer simulation of the thermodynamics of the B → Z-DNA transition: effect of the ionic size and charge

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The B- to Z-DNA transition free energy as a function of the size and charge of added ions is investigated by Monte Carlo simulation for simple grooved B- and Z-DNA models. It is shown that the electrostatic contribution to the free energy depends almost linearly on the logarithm of the salt concentration for all the systems. The effect of increasing the size of the ions is to make the curves steeper, although its influence on the transition midpoint is more complex. Divalent cations markedly reduce both the slope and the transition midpoint with respect to monovalent ions. These conclusions are in agreement with the experimental findings. The effect of increasing the charge of the anions—not yet experimentally studied—is less pronounced.

1. Introduction

Interest in the physicochemical behaviour of DNA is primarily determined by the relationship between DNA structure and function [1, 2]. The biological role of the different conformers was not very clear but, recently, there is increasing evidence of its presence *in vivo*. In particular, the discovery that certain classes of proteins bound to Z-DNA with high affinity and great specificity is indicative of a biological role [3]. As well as this, recent studies show a topological change of DNA from B-DNA to Z-DNA conformation in the hippocampus of a brain with Alzheimer's disease [4]. With this new information, it is clear that the investigation of the relative stabilities of the conformers becomes more relevant.

The conformational equilibrium between the B and Z forms of DNA [5] involves dramatic structural changes: the double-helix twist has opposite senses in both forms—right-handed in B-DNA and left-handed in Z-DNA—and the base pairs are flipped over in one form with respect to the other relative to the sugar-phosphate backbone [6]. The tracking of the conversion of one DNA form into another is out of reach with current computing power, but the same does not hold for the thermodynamics of the transition. An important aspect of the B → Z transition is its dependence on the ionic concentration. The free energy difference has been experimentally determined for the transition induced by the addition of NaCl to short dGdC oligomers [7]. By examining the dependence on the number of nucleotides and on salt concentration, Pohl fitted the experimental data to a simple expression. For an infinite polyion and concentrations in the 2–5 M range, it reads

$$\Delta G^{\mathrm{B}\to\mathrm{Z}} = m^{\mathrm{B}\to\mathrm{Z}} \ln \frac{C}{C^{\mathrm{B}\to\mathrm{Z}}}. \qquad (1)$$

The relation indicates that the free energy difference is essentially—an additional point at 1.5 M is slightly below the estimation of equation (1)—linear in the logarithm of salt concentration. For $C = C^{\mathrm{B}\to\mathrm{Z}}$ the free energy difference vanishes and marks the point at which the populations of both forms are the same. For this reason, $C^{\mathrm{B}\to\mathrm{Z}}$ is usually referred to as the transition midpoint.

The theoretical interpretation of these experiments is not trivial. In solution, DNA is a negatively charged polyelectrolyte due to the complete ionization of the acidic phosphate groups [1]. As Z-DNA is thinner than B-DNA, its charged phosphates are closer to each other giving stronger repulsions among them so that B is, in principle, a more stable DNA form. Thus, the stabilization of the Z form produced by a change in the solution ionic strength is a theoretical challenge. Several theoretical treatments have been proposed [8–13] with different degrees of success. One important drawback of some of these studies is that, given the complexity of the system studied, further simplification is required and the final underlying model is somewhat unphysical. Computer simulation has the advantage of allowing one to check the quality of the theoretical approximations and,

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sometimes more importantly, the validity of the models used. All-atom models have already been used in the simulation of the structural DNA changes [14–16]. Nevertheless, these studies dealt only with punctual aspects of the conformational change since the underlying all-atom model is far too complex to evaluate the free energy difference in a number of thermodynamic conditions. Recently, we have carried out computer simulation studies on simple DNA models to investigate the thermodynamics of the $B \rightarrow Z$ transition. The simulation was used [17] to check several theoretical approaches whose underlying DNA model is extremely simple. We analysed the assumptions of the Soumpasis potential of mean force theory [8] and, to a lesser extent, the counterion condensation theory of Manning and co-workers [11] and the polymer reference interaction site model theory of Hirata and Levy [10]. Later, we have used the same simulation procedure to investigate the ability of different DNA models to account for the essential experimental trends. We have shown that, when the salt concentration increases, the coupling of the DNA shape with its high charge induces important effects in the ionic cloud around the polyion [18] (see also [19]) which eventually brings about the transition [20]. These studies confirmed the usefulness of a relatively simple DNA model [21] reproducing the DNA grooved geometry. In particular, the so-termed grooved 'primitive' model (GP) was able to properly account for the thermodynamics of the $B \rightarrow Z$ transition and its dependence on the concentration of added monovalent salt [17].

It is well known that the condensation of ions around DNA is strongly influenced by the charge of the surrounding ions. Theoretical treatments and simulation studies [19, 22–27] predict a significant accumulation of the multivalent counterions around polyions more or less representative of DNA. Interesting phenomena, such as the competition between different ions or the overneutralization of the DNA charge, have been revealed by these studies. Unfortunately, to our knowledge, no simulations have been carried out to investigate the effect of the ionic charge on the thermodynamics of the $B \rightarrow Z$ transition. The situation is similar for the influence of the ionic size. Simulation has been used to investigate the competitive binding of different monovalent cations to A- and B-DNA [28] but similar studies are non-existent in the context of the the $B \rightarrow Z$ conformational change. The objective of the present work is to investigate by computer simulation the variation of the transition free energy in the presence of added salt with different ionic sizes (monovalent electrolytes) and charges (1:1, 2:1, and 2:2 salts). Section 2 describes the DNA model used and the methodology of the simulations, section 3 presents the free energy results using the GP model and section 4 is devoted to a final discussion and summary of the more significant conclusions.

2. Model and methods

The DNA model used in this work has been described elsewhere [21], we give here a brief description for completeness. As most of the DNA charge lies at the phosphate groups, unit negative charges are placed at the phosphorus positions. The double-helical array of charged spheres is held fixed in a continuum solvent of relative dielectric constant equal to that of water at 25°C. In addition to the phosphates, a big sphere mimics the central DNA core while each nucleotide is completed by another uncharged sphere of the same size as the phosphate one (see figure 1). For B-DNA, the ensemble of the big central spheres makes them almost indistinguishable from a repulsive cylinder with its axis coincident with that of DNA. In this way, the model reproduces very accurately the grooved DNA shape while retaining a considerable simplicity.

The ion–ion and ion–DNA sites' potential includes, apart from the Coulombic term, a repulsive interaction which, for simulation convenience, is of the r^{-n} type [21, 29–31], r being the interionic distance and $n = 9$ the steepness parameter of the repulsive interaction. It is well known that the structure of this type of repulsive potential is very close to that of an 'equivalent' hard model. In practice, the equivalence is given by the coincidence of the distance at at which the first peak of the radial distribution appears [31]. For this reason, and for the sake of simplicity, we will refer throughout this paper to the 'equivalent' hard ionic radius. The radius of the cations is identical to that of the anions for all the salts considered in this work.

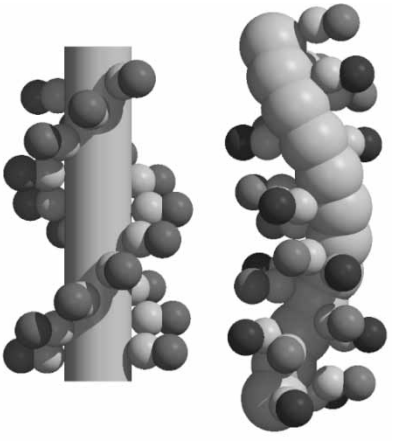

Figure 1. Grooved primitive models: B-DNA (left) and Z-DNA (right). Dark spheres represent the charged phosphate groups. The two distinct types of phosphates in Z-DNA are depicted with a different grey scale.

The transition free energy can be formally split into several contributions [32]

$$\Delta G^{B\to Z} = \Delta G^{B\to Z}_{intra} + \Delta G^{B\to Z}_{hydr} + \Delta G^{B\to Z}_{electr}\,, \qquad (2)$$

where the subscript 'intra' represents the intramolecular contributions except the electrostatic ones which are included in the electrostatic term. $\Delta G^{B\to Z}_{electr}$ is then essentially independent of the molecular sequence so simple models are suitable for its calculation. $\Delta G^{B\to Z}_{intra}$ has been estimated for the alternating guanine–cytosine hexamer to be about $-0.1\,k_BT$ per phosphate and almost independent of salt concentration [32]. Water is included in our model as a dielectric continuum and, thus, the hydration term $\Delta G^{B\to Z}_{hydr}$ cancels as long as both conformers are made with the same elements. This is the main limitation of our model because it is known that the hydration state of each conformer is different [33]. Nevertheless, despite the importance of DNA hydration, its influence on the B–Z transition is controversial [34, 35]. Besides, it seems reasonable that the specific hydration contribution remains substantially invariant with the salt concentration. Notice that if a neglected contribution is independent of the salt concentration, the calculated free energies should differ from experiment by a constant factor so the *slope* would be the same while the transition midpoint would be shifted to a different concentration. This is why we paid more attention to the dependence of the free energies on the ionic strength than to the precise determination of the transition midpoint.

We split the computation of the electrostatic term for each conformer into two steps [17, 36]. The first one consists of the *set-up* of the *uncharged* conformer structure from uncharged particles within a solution at the ionic concentration of interest. Notice that, as long as the final systems are conformers, the starting solution with uncharged particles is common for both states. Besides, even though the conformers are structurally dissimilar, the set-up term gives a small contribution. The other step corresponds to a *charging process*, in which the polyion sites are charged up while a number of counterions exactly compensate its charge (see [17] for details). In that paper, we also show that, for a simple model, the charging term accounts for about 90% of the total change in the electrostatic free energy difference. Moreover, the *set-up* term cannot be easily calculated for a more elaborated model such as the one used in this paper so we will omit such a contribution. In summary, our expression for the transition free energy is then

$$\Delta G^{B\to Z} = \Delta G^{B\to Z}_{intra} + \Delta G^{B\to Z}_{charging}. \qquad (3)$$

For the calculation of the latter term we use a charging process; the charging free energy is then given by the usual integral of the electrostatic potentials φ^{Ω} over the charging parameter λ [17]. As in our model only the phosphate groups are charged, the summation of the electrostatic potentials involves simply a sum over p, the number of structurally distinct phosphates in each DNA form (1 for B-DNA and 2 for Z-DNA). The charging free energy—in kT units per phosphate—is then given by

$$\beta\Delta G^{\Omega}_{charging} = \int_0^1 \frac{-1}{p}\sum_p \phi^{\Omega}_p(\lambda)\,d\lambda\,, \qquad (4)$$

where $\phi = \beta\varphi e$ is the reduced electrostatic potential and Ω represents either the B or Z-DNA forms. For the intramolecular term, the estimation of García and Soumpasis [32] will be used.

Tables 1 to 2 present the parameters used in the simulations and the resulting bulk concentrations for the systems when the charging parameter is 1. The parameters used in the corresponding simulations for other values of the charging parameter are the same as those for $\lambda = 1$. The simulations have been done for an infinitely long DNA molecule at infinite dilution. To this end, we have used the modulated bulk as a fuzzy boundary (MBFB) technique described in [37]. The simulations with the asymmetrical salt 2:1 require an additional parameter γ_{AB} (see [38]) which has been estimated by means of the HNC integral equation. The values of γ_{AB} have been 0.28, 0.24, 0.19 and 0.162 for the concentrations 0.5, 1.25, 2.7 and 4.8 M, respectively. Notice that in the simulations with added salt it is not possible to know in advance the exact final bulk concentration although, with some experience, it is possible to anticipate approximately the final concentration (the relevant input data are the number of counterions and the excess of co- and counterions together with the simulation box size). As a consequence, the concentrations in the bulk region are not round numbers. In order to make the reading of the text more comfortable we will use nominal concentrations to refer to the simulation bulk values. Both the nominal concentrations and the actual values are given in tables 1 and 2. Obviously, the calculations in which the concentration is relevant have been done with the actual values.

3. Results

Figure 2 shows the integrand in the charging term integral—the negative of the reduced electrostatic energy at the phosphates, $-\phi^{\Omega}$—as a function of the ionic radius for several concentrations of monovalent

Table 1. Parameters of the simulations in the presence of added 1:1 salt. N_{n+} is the number of DNA charged sites, N_+ indicates the excess number of counterions (additional to those needed to compensate the DNA charge) and N_- the number of co-ions. L and *Ap* refer to the geometry of the hexagonal simulation box (the axial length and the hexagon apotheme, respectively). R^{I} and r_c are parameters used in the MBFB method (the cylindrical cell radius of the inhomogeneous region and the cut-off in the homogeneous region, respectively).

		Number of ions			Geometry				
DNA Form	$C_{\mathrm{bulk}}^{\mathrm{nominal}}$/M	N_{n+}	N_+	N_-	L	R^{I}	Ap	r_c	C_{bulk}/M
1:1 salt, equivalent ionic radius 1.5 Å									
B	1.0	40	205	205	67.6	32.5	40.4	13.0	0.980
”	2.6	”	194	194	33.8	22.0	34.8	9.0	2.48
”	4.5	20	200	200	”	22.5	26.9	8.0	4.42
”	7.8	20	280	280	”	20.9	24.1	6.4	7.75
Z	1.0	48	256	256	89.2	32.5	39.1	13.0	0.975
”	2.6	”	160	160	44.6	22.0	27.4	9.0	2.65
”	4.5	24	265	265	”	22.5	26.7	8.0	4.46
”	7.8	24	380	380	”	20.9	24.2	6.4	7.85
1:1 salt, equivalent ionic radius 2.1 Å									
B	1.0	40	205	205	67.6	32.5	40.4	13.0	0.989
”	2.6	”	194	194	33.8	22.0	34.8	9.0	2.52
”	4.5	20	200	200	”	22.5	26.9	8.0	4.45
”	7.8	20	280	280	”	20.9	24.1	6.4	7.72
Z	1.0	48	256	256	89.2	32.5	39.1	13.0	0.986
”	2.6	”	160	160	44.6	22.0	27.4	9.0	2.64
”	4.5	24	265	265	”	22.5	26.7	8.0	4.50
”	7.8	24	380	380	”	20.9	24.2	6.4	7.70
1:1 salt, equivalent ionic radius 2.45 Å									
B	1.0	40	205	205	67.6	32.5	40.4	13.0	0.994
”	2.6	”	194	194	33.8	22.0	34.8	9.0	2.50
”	4.5	20	200	200	”	22.5	26.9	8.0	4.42
Z	1.0	48	256	256	89.2	32.5	39.1	13.0	0.995
”	2.6	”	160	160	44.6	22.0	27.4	9.0	2.63
”	4.5	24	265	265	”	22.5	26.7	8.0	4.44

Table 2. Parameters of the simulations in the presence of divalent counterions. Symbols as in table 1.

		Number of ions			Geometry				
DNA Form	$C_{\mathrm{bulk}}^{\mathrm{nominal}}$/M	N_{n+}	N_+	N_-	L	R^{I}	Ap	r_c	C_{bulk}/M
2:1 salt, ionic radius 2.1 Å									
B	0.5	20	102	204	67.6	32.0	39.2	14.0	0.504
”	1.25	20	190	380	”	29.5	34.4	9.0	1.24
”	2.7	”	160	320	33.8	25.5	30.4	”	2.71
”	4.8	10	250	500	33.8	24.0	28.3	8.0	4.77
Z	0.5	24	140	280	89.2	28.0	39.8	14.0	0.505
”	1.25	24	250	500	”	29.5	34.2	9.0	1.24
”	2.7	”	210	420	44.6	25.5	30.1	”	2.73
”	4.8	12	327	654	”	23.9	28.0	8.0	4.78
2:2 salt, ionic radius 2.1 Å									
B	0.2	20	100	100	67.6	48.0	59.7	22.0	0.204
”	0.4	20	125	125	”	38.0	47.2	17.0	0.415
”	1.0	20	205	205	”	32.5	40.4	13.0	0.950
”	1.9	20	250	250	”	27.0	32.5	11.0	1.85
Z	0.2	24	113	113	89.2	44.0	55.1	22.0	0.204
”	0.4	24	162	162	”	38.0	46.7	17.0	0.415
”	1.0	24	256	256	”	32.5	39.1	13.0	0.958
”	1.9	24	334	334	”	27.0	32.5	11.0	1.86

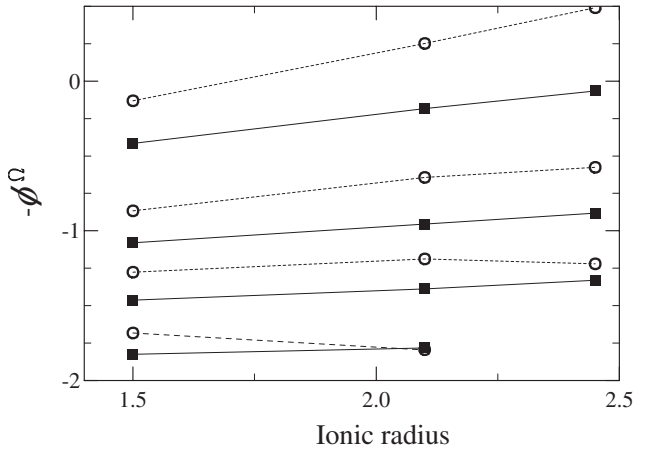

Figure 2. Reduced electrostatic energy at the phosphates for DNA with added monovalent salt as a function of the ionic radius. Solid lines and squares, B-DNA; dashed lines and circles, Z-DNA. Each pair of curves are the results for a given salt concentration; from top to bottom 1, 2.6, 4.4 and 7.8 M, respectively. Lines are drawn as a guide to the eye.

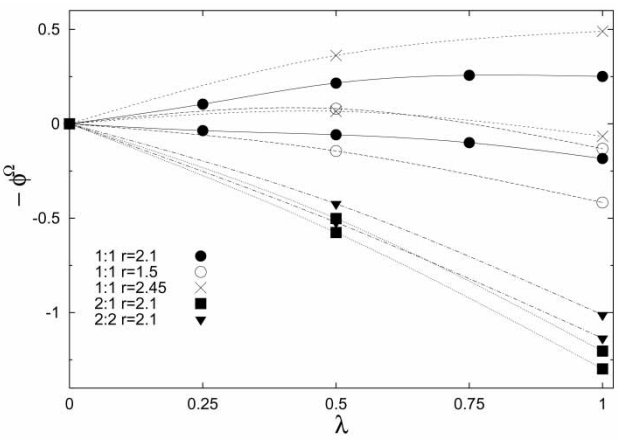

Figure 3. Integrand of the charge integral for the systems at (approximately) 1 M salt concentration as a function of the coupling parameter λ. Filled circles, 1:1 salt with ionic 2.1 Å radius; open circles, 1:1 salt with 1.5 Å ionic radius; crosses, 1:1 salt with 2.45 Å ionic radius; squares, 2:1 salt (the resulting bulk salt concentration for this system is somewhat higher, 1.25 M); inverted triangles, 2:2 salt; For each system, the upper curve correspond to Z-DNA and the lower to B-DNA. Lines are drawn as a guide to the eye.

salt. For Z-DNA, the mean value for the two distinct types of phosphates is given. In general, both DNA forms have lower electrostatic energies for small ions than for large ones. This is strictly true for B-DNA where the dependence of $-\phi_p^{\Omega}$ with the ionic radius is linear for all the salt concentrations studied. Nevertheless, for Z-DNA, the variation is more complex. The slope of the curves is positive at low salt concentrations but it tends to flatten out as the concentration increases and becomes negative for the higher salt concentration. This preliminary result is in accordance with the tendency of the experimental data which indicate that the ability to induce the B→Z-DNA transition is higher for larger cations [39].

Figure 3 shows the dependence of the integrand of equation (4) with the coupling parameter, λ, for the systems at (approximately) 1 M salt concentration. The integrands for the divalent systems are smaller than those for the monovalent salts as expected for a system with larger ionic strength. The results for the system with a 2:2 salt are quite similar—but slightly larger—than those for the 2:1 salt, although it should be taken into account that the actual bulk salt concentrations differ noticeably in this case, namely 0.954 and 1.24 M respectively (see table 2). The area between the curves for the B- and Z-DNA forms in figure 3 is just the transition free energy difference. It is lower for the system with 2:1 added salt than for the system in the presence of monovalent ions. In other words, the asymmetric salt has a higher ability to induce the B→Z transition, in accordance with the experimental tendency [5]. It seems that the effect of the anions is minor since the the curves for the 2:2 salt are close to those for the system in a 2:1 electrolyte solution.

Table 3 presents the numerical values of the electrostatic free energies of each conformer and their differences at the concentrations investigated. The free energy differences are depicted in figures 4 and 5. The analysis of the effect of the ionic size on the B→Z transition free energy is not simple. Experimentally, the critical concentration of alkaline chlorides which induce the transition of poli(dGdC) increases with the atomic number [5, 39, 40]. But the monovalent ions associate to DNA without loss of their hydration sphere. Thus, the relevant ionic size is that of the hydrated ion and this is lower when going to larger atomic number. With this interpretation, experimental data indicate that smaller ions induce the transition at higher salt concentrations [39]. The dependence of the computed electrostatic contribution on the ionic size is shown in figure 4. The curves are almost linear and have a negative slope which is in agreement with the experimental data for NaCl. The steepness increases as the ionic size increases. But the value of $\Delta G^{B\to Z}_{charging}$ at low salt is larger for the bigger ions and, as a consequence, all the curves cross. In this situation, the addition of a constant term to the free energy difference not only changes the numerical values of the transition midpoint but also the relative stability of both DNA forms for the different salts. Our results indicate that the above mentioned experimental findings are fulfilled if the contribution to the free energy

Table 3. Electrostatic free energies (in $k_B T$ units per phosphate).

Salt	Ionic radius/Å	$C^{bulk}_{nominal}$/M	$G^{\Omega}_{charging}$ B	$G^{\Omega}_{charging}$ Z	$\Delta G^{B\to Z}_{charging}$
2:1	2.1	0.5	−0.393	−0.283	0.110
”	”	1.25	−0.600	−0.535	0.065
”	”	2.7	−0.781	−0.756	0.025
”	”	4.8	−1.019	−1.017	0.002
2:2	”	0.2	−0.116	0.013	0.129
”	”	0.4	−0.274	−0.164	0.110
”	”	1.0	−0.537	−0.451	0.086
”	”	1.9	−0.782	−0.698	0.084
1:1	1.5	1.0	−0.117	0.005	0.122
”	”	2.6	−0.373	−0.280	0.093
”	”	4.4	−0.504	−0.438	0.066
”	”	7.8	−0.632	−0.590	0.042
1:1	2.45	1.0	0.033	0.323	0.290
”	”	2.6	−0.398	−0.277	0.121
”	”	4.4	−0.677	−0.660	0.017

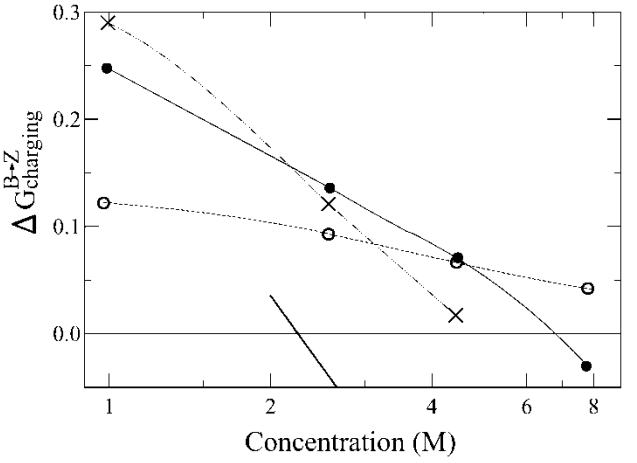

Figure 4. Electrostatic free energy difference of the B → Z transition for systems with added monovalent salt as a function of the ionic size. Dashed line and open circles, ionic radius 1.5 Å; solid lines and filled circles, 2.1 Å; dash-dotted lines and crosses, 2.45 Å. The thick solid line at the bottom is the experimental curve for added NaCl. Lines are drawn as a guide to the eye. Notice the logarithmic scale of the concentrations.

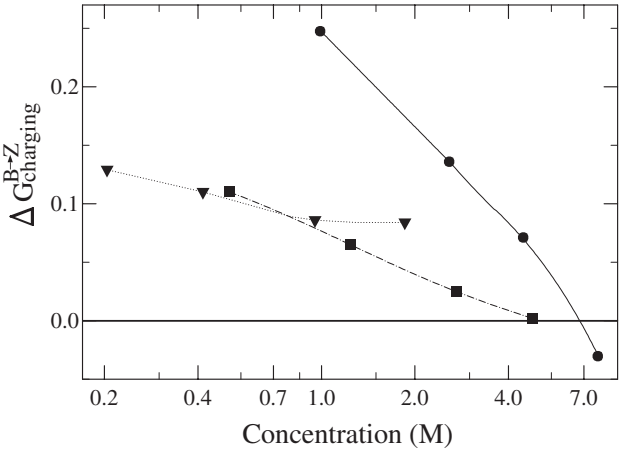

Figure 5. Electrostatic free energy difference of the B → Z transition in the presence of added salt as a function of the ionic charge. Solid line and filled circles, 1:1 salt; dash-dotted line and squares, 2:1 salt (the salt concentration of this system is 1.25 M); dotted line and inverted triangles, 2:2 salt. Lines are drawn as a guide to the eye. Notice the logarithmic scale of the concentrations.

of the terms neglected in figure 4—set-up, hydration and intramolecular—is negative and less than $0.065\,k_B T$ per phosphate (the point at which the curves corresponding to ionic sizes 1.5 and 2.1 Å cross). This quantity is of the order of the intramolecular term calculated by García and Soumpasis [32], $\Delta G^{B\to Z}_{intra} \approx -0.1\,k_B T$/P. Incorporating this contribution into the electrostatic term, the transition B → Z would take place at 2.8 M for the 2.45 Å salt, closer to the experimental data—2.5 M for NaCl—than the value 3.4 M obtained for the ions with a 2.1 Å radius. Notice that 2.45 Å is the size used by Soumpasis to reproduce the experimental data [8]. The radius 1.5 Å is probably too low for CsCl. In fact, Soumpasis *et al.* used the value 1.85 Å for this salt [39]. A rough interpolation on figure 4 for a 1.85 Å curve would lead to a prediction of the transition midpoint around 3.8 M which is also close to the experimental value for CsCl, 4 M [40]. Anyway, our interest is not to fit the experimental transition midpoints but to investigate the tendencies observed in the B → Z-DNA transition. Before proceeding to a fine tuning of the parameters, a

Tabel 4. Slope of the variation of the electrostatic contribution to the B → Z transition free energy with the logarithm of the salt concentration $m^{B\to Z}$ and its ratio with respect to the experimental value.

Salt	Ionic radius/Å	$m^{B\to Z}_{charging}$	$m^{B\to Z}_{charging}/m^{B\to Z}_{exp}$
1:1	1.5	−0.048	1/6.3
1:1	2.1	−0.122	1/2.5
1:1	2.45	−0.190	1/1.6
2:1	2.1	−0.050	–
2:2	,,	−0.029	–

refinement of other details of the model (especially the effect of the solvent) would be needed. Table 4 presents the slope of the curves shown in figure 4 and 5. The slope for 1:1 salts have been calculated between 2 and 5 M—as in Pohl's experiment—and around 0.7 M for 2:1 salts (the transition midpoint for poli(dGdC) in the presence of $MgCl_2$ [5]). The slope of the curves for the monovalent salt with radius 2.45 Å is 1.5 times lower than the experimental one. This result is clearly better than that obtained for ions with a 2.1 Å radius which is 2.5 times lower.

As for the effect of the ionic charge, figure 5 shows that divalent cations reduce the steepness of the free energy difference curves. Again, the free energy difference is almost linear on the logarithm of salt concentration. Conversely to the case for the ionic size, the curves do not cross within the range of concentrations investigated. Thus, an increase in the charge of the cations moves the transition midpoint to significantly lower concentrations. If we add the intramolecular term to the electrostatic contribution, the transition midpoint for the 2:1 salt occurs at 0.65 M. This tendency is coincident with the experimental trends. In the particular case of poli(dGdC), the transition midpoint in the presence of NaCl is 2.5 M, to be compared with a 0.7 M concentration with added $MgCl_2$ [5]. The slope of the 2:1 system is $\approx -0.05\,k_BT/P$, considerably lower than that corresponding to the monovalent salt with ions of the same size. In principle, this theoretical prediction cannot be contrasted since, to the best of our knowledge, there are no estimations of the experimental quantity. Nevertheless, a wide range of transition midpoints for different salts and DNA sequences is available and, from them, it is possible to give a rough estimation of the slope. For instance, the difference between the intramolecular free energy of the sequences poli(dGdC) and its 5-methylated variant is $-0.36\,k_BT/P$ [41, 42]. It seems reasonable that the dependence on salt concentration is unaffected by such a mutation and that equation (1) also holds for poli(dG^5dC). If we assume that the slopes for both systems are the same, we may write

$$\Delta G^{B\to Z}_{poli(dGm^5C)} - \Delta G^{B\to Z}_{poli(dGdC)} = m^{B\to Z}_{1:1} \ln\left(\frac{C^{B\to Z}_{poli(dGdC)}}{C^{B\to Z}_{poli(dGm^5dC)}}\right)_{1:1}, \tag{5}$$

which results in a value $-0.3\ln(2.25/0.7) = -0.35 k_BT/P$, almost coincident with the previous one. Using the same expression for the 2:1 salt, it is possible to estimate the slope

$$m^{B\to Z}_{2:1} = \frac{\Delta G^{B\to Z}_{poli(dGdC)} - \Delta G^{B\to Z}_{poli(dGm^5C)}}{\ln\left(C^{B\to Z}_{poli(dGdC)}/C^{B\to Z}_{poli(dGm^5dC)}\right)_{2:1}}, \tag{6}$$

whose value is $-0.36/\ln(0.7/0.006) = -0.05\,k_BT/P$, which is coincident with the slope calculated with our simulations of the GP model (see table 4).

Finally, the effect of the charge of the anions is slightly more pronounced than that anticipated from figure 3. A higher charge decreases the slope of the free energy results (table 4). The curves for the 2:1 and the 2:2 salts cross at approximately 0.7 M which explains the similarity observed in figure 3. As the electrostatic free energy difference at that concentration is about $0.1\,k_BT/P$, if one assumes that the intramolecular term is adequate for both salts, the predicted transition midpoints would be almost coincident. Notice that the coincidence is a consequence of the addition of two terms with opposite sign but similar absolute value. For other DNA sequences with different intramolecular contributions to the free energy difference, the predicted transition midpoints would differ for the 2:1 and the 2:2 salts.

4. Discussion

The results of this work together with those of [20] indicate that simple grooved DNA models are able to reproduce the essential features of the B- to Z-DNA transition. The main contribution to the free energy difference is electrostatic. In particular, the charging term accounts essentially for most of the dependence of $\Delta G^{B\to Z}$ on the ionic strength (i.e. the slope of the free energy difference curve). But other smaller contributions (such as the intramolecular term) are very important for the precise determination of the transition midpoint and cannot be neglected. A major question still unsolved is that of the role of the solvent. A number of studies on the various DNA forms indicate that water forms stable structures connecting parts of the molecule and that these structures are different for each DNA form. The success of the Soumpasis potential of mean force theory and our computer simulation studies (both based in

continuum solvent models that nullify the hydration contribution) seem to indicate that this term is not the determinant one. However, there is room for improvement: our results for the slope of the $\Delta G^{B\to Z}$ curve for monovalent ions is clearly lower than the experimental result and the location of the transition midpoint is surely affected by the distinct state of hydration of the B- and Z-DNA conformers. Unfortunately, there is no simple way to check this question. All-atom DNA models in an explicit water solution containing a sufficient number of ions so as to give accurate values of the electrostatic potentials would involve over 10 000 particles. A single run is possible with such a sample size but the same does not hold when the objective is to investigate the effect of the ion charge and the ionic size (notice that the present work required more than 100 simulation runs). Finally, it is worth noting that studies like the present one are also limited by the availability of experimental data. Although monovalent ion solutions have been thoroughly measured, there is a lack of systematic data for multivalent ions. This is important because the transitions between the various DNA forms can be an excellent test of the quality of a DNA force field.

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