B-Z DNA conformational transition in 1:1 electrolytes: dependence upon counterion size

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We have studied the B-Z transition of poly[d(G-C)] in the presence of alkali metal, tetramethylammonium and tetraethylammonium chlorides at room temperature. The measured critical salt concentrations increase in the order Na, K, Rb, TMA, Cs and are in good agreement with the theoretical values predicted from a statistical-mechanical treatment of the transition.

Left-handed DNA; Z-DNA; Alkali metal; Poly[d(G-C)]

1. INTRODUCTION

The right-to-left (B-Z) conformational transition of d[(G-C)] polymers and oligomers induced by increasing salt (NaCl) concentration is a classical example of an induced DNA structural transition in solution. It has been studied experimentally in great detail [1,2] and can be theoretically described in a quantitative manner using a recently proposed statistical-mechanical framework [3,4] for treating the contributions of polyion-diffuse ionic cloud interactions to the total free energy balance governing conformational equilibria. Information concerning the effects of other monovalent cations on this and other B-Z transitions is still fragmentary. It has been known for some time [5] that poly[d(G-C)] is also left-handed in 4 M CsCl, whereas neither LiCl [6] nor NH_4Cl [7] induces analogous structural transitions at room temperature. More recently, B-Z transi-

tions of poly[d(G-C)] in aqueous LiCl solutions have been observed [8,9], albeit at elevated temperatures. Transition data for other alkali metal halides and other 1:1 salts have not been reported. As recently discussed ([10] and original references therein) one expects on the basis of a large body of experimental evidence and a priori considerations that alkali metal halides (except Li salts which behave anomalously due to the small size of this cation) interact with DNA conformations according to the diffuse cloud model; i.e. they do not bind to specific DNA sites.

The statistical-mechanical theory mentioned above predicts that in this case the critical (midpoint) concentration $*c$ for the B-Z transition in aqueous solutions of alkali metal halides should increase in the same order as the effective distance of closest approach σ of a hydrated anion-cation pair decreases, i.e. in the order Na^+, K^+, Rb^+, Cs^+ (for a common anion, e.g. Cl^-). This theoretical prediction is quantitatively explored in this report.

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Abbreviations: TMA, tetramethylammonium; TEA, tetraethylammonium

2. MATERIALS AND METHODS

2.1. Chemicals and titrations

Poly[d(G-C)] was from P-L Biochemicals. Concentrations were determined using an ϵ of

7100 $M^{-1} \cdot cm^{-1}$ at 255 nm [1]. All alkali metal chlorides were analytical grade. The conformational state of poly[d(G-C)] was analysed quantitatively by measuring the A_{295}/A_{260} ratio with a thermoregulated Kontron Uvicon 820 spectrophotometer. The equilibrium properties of poly[d(G-C)] (40 μM) were determined after 6 h incubation at 20–25°C. The transition midpoints *c were obtained by determining the fractional B-Z transition θ from net absorbance ratios and plotting $\log[\theta/(1 - \theta)]$ vs $\log c$.

2.2. Theory

According to [3,4], the salt-dependent term $\Delta F_1(B,Z_I)$ in the free energy difference determining the all-none B-Z_I conversion of a DNA oligomer [d(C-G)$_6$ in our calculation] is given by

$$\Delta F_1(B,Z_I;c) = \sum_{i>j} \{ W_{11}[r_{ij}(Z_I);c] - W_{11}[r_{ij}(B);c] \} \quad (1)$$

where c is the bulk salt concentration and $r_{ij}(X)$ ($X = Z_I,B$) is the center-to-center distance between the i-th and j-th of the 24 negatively charged hard spheres of diameter σ modeling the phosphate charge distribution in conformation X as determined by X-ray analysis. $W_{11}(r)$ is the potential of mean force (effective interaction) of two anions at a distance r in an electrolyte characterized according to the restricted primitive model (RPM) by a bulk solvent dielectric constant ϵ ($\epsilon = 78.4$ here) and average distance of closest approach of an anion-cation pair σ. $W_{11}(r)$, which in addition depends on the temperature T and salt concentration c, has been obtained from solution of the hypernetted chain integral equation (HNC) [4] at room temperature for a wide range of c, σ and r values (this statistical-mechanical approximation is known to yield very accurate results for RPM electrolytes [11]). The published atomic coordinates of the B [12] and Z_I [13] DNA conformations have been used in conjunction with the calculated HNC potential of mean force (pmf) to estimate ΔF_1 via eqn 1.

3. RESULTS AND DISCUSSION

In accordance with earlier studies [6,8,9] it is found that LiCl does not induce a B-Z transition at room temperature. Similarly, addition of TEA

chloride up to a concentration of 3 M does not lead to a B-Z transition either. All other salts give rise to cooperative B-Z transitions (fig.1) and the critical concentrations *c measured are compiled in table 1. To compare with theory it is necessary to assign values for σ to every salt. In principle, this average distance of closest approach can be directly determined from anion-cation pair correlation functions measured via differential neutron or X-ray scattering [14]; unfortunately, due to technical problems this has not yet been achieved. The more common practice is to obtain numerical values for σ from fits of RPM theoretical expressions to experimental activity and osmotic coefficient data. In this case the values obtained depend somewhat on the theoretical expressions used (e.g. finite ion size Debye-Hückel theory with corrections for hard corde contributions [15,16]; mean spherical approximation [17], etc.) but σ is invariably found to be larger than the sum of the corresponding crystal ionic radii and smaller than the sum of the hydrated ionic radii. In the case of salts with a common anion, σ decreases with increasing cationic crystal radius, i.e. in the order Li$^+$, Na$^+$, K$^+$, Rb$^+$, Cs$^+$. A typical set of σ values known to yield good fits to colligative data over a wide range of salt concentrations is included in table 1 along with the sum of bare ionic radii for each salt.

The theoretical dependence of the critical concentration *c on σ (in the range 3.5–6.0 Å) has been calculated using the method outlined above and the condition

$$\Delta F_1(B,Z_I;*c) = 0 \quad (2)$$

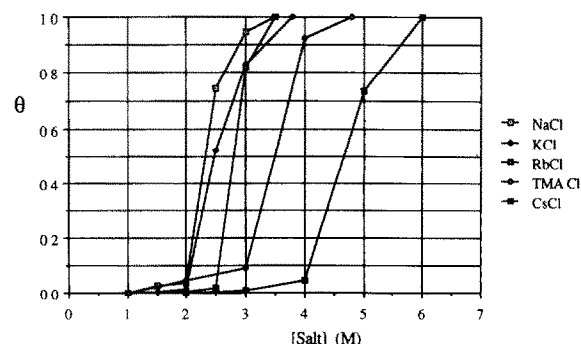which is valid at the midpoint of the transition

Fig.1. Cooperative B-Z transition of poly[d(G-C)] in different 1:1 electrolytes. See text for details.

Table 1

Parameters for the B-Z transition of poly [d(G-C)] in aqueous 1:1 electrolytes

Salt	*c (M)	σ_c (Å)	σ (Å)
NaCl	2.3	2.76	4.0
KCl	2.6	3.14	3.8
RbCl	2.9	3.29	3.6
CsCl	4.8	3.50	3.0

All data at room temperature (20–25°C); *c is the salt concentration at the transition midpoint; σ is the distance of closest approach: the data in the second column are obtained from the sum of the Pauling crystal radii [18] and in the third column from [15]

Fig.2. Correlation of transition midpoint *c with effective distance of closest approach σ. Theoretical calculations are shown by the solid line. See text for details.

where the two conformations are equally probable for oligonucleotides and (in the present case) polynucleotides. The results of this calculation are shown in fig.2 (solid drawn curve) along with experimental *c values and corresponding σ values adjusted as follows. It has been found [3,4] that in the case of NaCl the value of σ required to reproduce accurately the whole experimental transition curve [2] is not the one included in table 1 (i.e. 4.0 Å) but the higher value of 4.9 Å. We have applied the same correction factor (4.9/4.0 = 1.23) to the entries in the last column of table 1 in order to generate the σ values used in fig.2.

The present results provide good evidence that Na, K, Rb and Cs salts affect the B-Z equilibrium in qualitatively the same way, i.e. via diffuse ionic cloud interactions. Furthermore, it is seen that the theory invoked is capable of rationalizing the differential effects observed in a quantitative way. In the case of TMA, the measured *c value of 3.5 M also indicates predominance of the diffuse cloud mode of interaction with DNA but, unfortunately, we could not find a reliable σ value for this salt in the literature. The failure of TEA to induce the transition may be simply due to its lower solubility which is below the necessary critical concentration. If LiCl were to behave similarly to the other alkali metal chlorides, theory would predict that it should induce the B-Z transition at 1.88 M, whereas the measured value [9] at a temperature (30°C) closest to room temperature is approx. 5.0 M. Considering this finding and the fact that the transition in the presence of LiCl is strongly
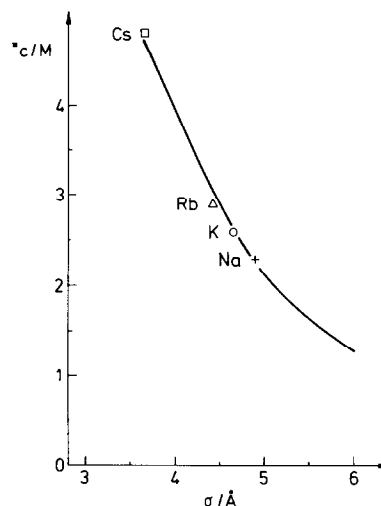
temperature-dependent (higher temperatures favor the Z conformation in the presence of high salt concentration [8,9]), it is likely that this salt stabilizes the B conformation via preferential site binding. The behavior of Li^+ has been found to be anomalous in other cases of biological interest as well. For example, its interactions with charged membrane components give rise to alterations in the shape and size of human erythrocytes completely different from those obtained in the presence of the other alkali metal halides [19].

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