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Effect of cesium on the volume of the helix–coil transition of dA·dT polymers and their ligand complexes

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Abstract

The pressure dependence of the helix–coil transition of poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] in aqueous solutions of NaCl and CsCl at concentrations between 10 and 200 mM is reported and used to calculate the accompanying volume change. We also investigated the binding parameters and volume change of ethidium bromide binding with poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] in aqueous solutions of these two salts. The volume change of helix–coil transition of poly(dA)·poly(dT) in Cs^+-containing solutions differs by less than 1 cm^3 mol^{-1} from the value measured when Na^+ is the counter-ion. We propose that this insensitivity towards salt type arises if the counter-ions are essentially fully hydrated around DNA and the DNA conformation is not significantly altered by salt types. Circular dichroism spectroscopy showed that the previously observed large volumetric disparity for the helix–coil transition of poly[d(A-T)]·poly[d(A-T)] in solutions containing Na^+ and Cs^+ is likely result of a Cs^+-induced conformation change that is specific for poly[d(A-T)]·poly[d(A-T)]. This cation-specific conformation difference is mostly absent for poly(dA)·poly(dT) and EB bound poly[d(A-T)]·poly[d(A-T)].

Keywords: Poly[d(A-T)]·poly[d(A-T)]; Poly(dA)·poly(dT); Ethidium bromide; Counterions; Pressure

1. Introduction

As a highly charged polyanion, DNA is surrounded by counter-ions in aqueous solution which are essential for stabilizing its secondary structure. Processes such as the helix–coil transition of DNA and DNA binding with positively charged ligands are associated with a decrease of the charge density of DNA and a release of counter-ions. In turn, these processes are also sensitive to changes in the concentration of counter-ions in solution. An increase in bulk salt concentration is associated with increase of helix–coil transition temperature and a decrease in the affinity of ligands [1]. Although the influence of counter-ion concentration on conformational stability and ligand binding has been extensively studied very little effort has been invested in the study of the role the type of cation plays in these properties.

We have previously reported that the different alkali metal cations have a significant impact on the volume of helix–coil transition of poly[d(A-T)]·poly[d(A-T)] [2]. The monovalent alkali cations, Na^+, K^+, Rb^+, and Cs^+, differ greatly in their ionic radius and consequently in their charge density and the extent to which they are hydrated. For example, the Pauling ionic radius of Na^+ and Cs^+ are 0.95 and 1.69 Å, respectively [3]. A thermodynamic study [4] showed that by defining a hydration number, n, as the number of waters that binds sufficiently strong to become part of the ion, $n = 3.9 \pm 0.5$ for Na^+ compared to 0.6 ± 0.8 for Cs^+. The more extensive hydration of the sodium ion leads to it having a larger radius than the hydrated cesium ion, this, in turn results in weaker Coulomb electrostatic interactions and an increased distance between the DNA surface and sodium ions [5]. This size effect has been used to explain the weak selectivity observed for DNA counter-ion interactions [6–10]. Because of the relatively weak selectivity, there are few studies of the effect of different monovalent cations on DNA helix–coil transitions and DNA–ligand interactions [11–13].

With volumetric differences of the monovalent cations being an important factor of their DNA association selectivity, volumetric studies of the effect of salt type on biological process of DNA are especially intriguing. We have previously shown that there is a significant difference in the volume of the

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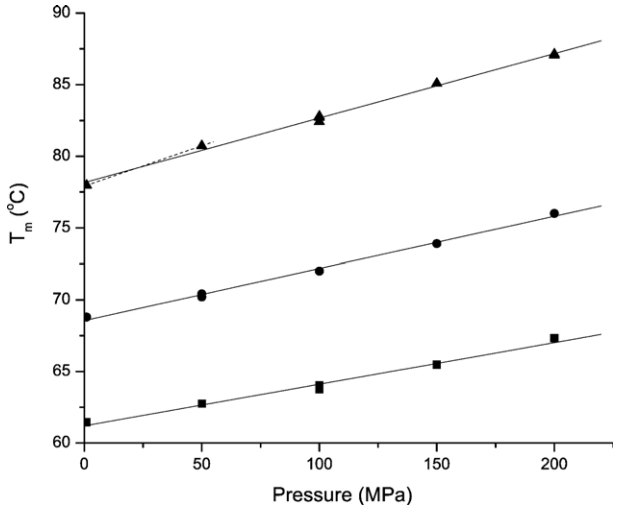

Fig. 1. Pressure dependence of helix–coil transition temperature of poly (dA)·poly(dT) in buffer solutions with various CsCl concentrations: 10 mM CsCl: ■, 50 mM CsCl: ●, 200 mM CsCl: ▲. The typical error for each data point is about 0.2 °C.

helix–coil transition of poly[d(A-T)]·poly[d(A-T)] in aqueous solutions containing Na^+ or Cs^+ [2]. In the present study, we further investigate the thermodynamic and volumetric differences in the effect of Na^+ and Cs^+ on helix–coil transition of DNA with different sequences, poly[d(A-T)]·poly[d(A-T)] and poly(dA)·poly(dT), and on the intercalation of ethidium bromide (EB) with these two polymers.

2. Materials and methods

2.1. Materials

Ethidium bromide (EB) and cesium chloride were obtained from Sigma-Aldrich Co. and used without further purification. All other low molecular weight chemicals were reagent grade or better. Poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] were purchased from Amersham Biosciences Corporation. The DNA polymers were dissolved in and then dialyzed against solutions containing 20 mM Tris–HCl, pH 7.2, 0.1 mM EDTA and the desired amount of NaCl or CsCl. The concentrations of the resulting DNA solutions were determined spectrophotometrically using molar extinction coefficients: $\varepsilon_{259}=12{,}000\ M^{-1}\ cm^{-1}$ for poly(dA)·poly(dT) [14,15], $\varepsilon_{262}=13{,}200\ M^{-1}\ cm^{-1}$ for poly[d(A-T)]·poly[d(A-T)] [15]. The concentrations are in moles of base pairs. The concentration of EB was also determined spectrophotometrically using $\varepsilon_{480}=5{,}850\ M^{-1}\ cm^{-1}$ [16].

2.2. High-pressure DNA melting

We measured the helix–coil transition temperature, T_m, of poly(dA)·poly(dT) at different pressures, ranging from 1 to 200 MPa (0.1 MPa=1 bar=0.9678 atm) by monitoring the change of UV absorbance with increasing temperature. The pressure dependence of the T_m was used to deduce the volume changes of these denaturation processes at atmosphere pressure using the Clapeyron equation: $dT_m/dP=T_{m,\ 1atm}\Delta V°/\Delta H$. A more detailed description of the instrumentation and experimental methods can be found in previous publications [17].

2.3. Fluorometric titrations

Fluorescence titrations were performed on a Spex FluoroMax 3 spectrofluorometer (Jobin Yvon, Inc., Edison, NJ) at room temperature to determine the equilibrium binding parameters of EB binding with poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)]. The excitation and emission wavelengths were 512 nm and 600 nm, respectively. Binding parameters, such as the binding constant K, binding site size, n, and binding cooperativity, ω, were obtained through fitting spectroscopic data with site-exclusion model [18]. More detailed information was reported earlier [17].

3.3. High-pressure fluorescence measurements

The molar volume change of DNA–ligand binding was determined by measuring the pressure dependence of the binding constants and employing the standard thermodynamic relationship: $(\partial \ln K_a/\partial P)_T=-\Delta V°/RT$, where R is the gas constant. The change of binding affinity with pressure was monitored by Fluoromax 3 spectrofluorimeter. A more detailed description can be found in our previous publications [17]. The data were fitted with second order polynomial using Origin software (OriginLab Corporation, Northampton, MA) to obtain $\Delta V°$ and its pressure derivative, the isothermal compressibility.

4. Results

4.1. Helix–coil transition of poly(dA)·poly(dT) in aqueous solutions of CsCl

The pressure dependence of helix–coil transition temperature (T_m) of aqueous solutions of poly(dA)·poly(dT) at various CsCl concentrations are summarized in Fig. 1. The increase in T_m with increasing pressure implies that pressure stabilizes the DNA duplex and that, under these conditions, the helix–coil transition is associated with a positive volume change.

The molar volume change of the helix–coil transition at different salt concentrations was calculated using Clapeyron equation; the results are given in Table 1. The values of ΔH at different transition temperatures were calculated using calorimetrically measured temperature dependent enthalpies of

Table 1
Volume change of the helix–coil transition of poly(dA)·poly(dT) at different salt concentrations*

[CsCl] (mM)	T_m (°C)	100 ($\Delta T_m/\Delta P$) (°C MPa^{-1})	ΔH^a (kJ mol^{-1})	ΔV^a (cm^3 mol^{-1})
10	61.2±0.3	2.90±0.16	39.9±1.2	3.46±0.21
50	68.6±0.2	3.63±0.12	41.6±1.3	4.41±0.20
200	78.2±0.2	4.49±0.11	43.8±1.5	5.60±0.23

*All measurements in 20 mM Tris–HCl, pH 7.2.
[a] ΔH and ΔV are per mole of base pairs.

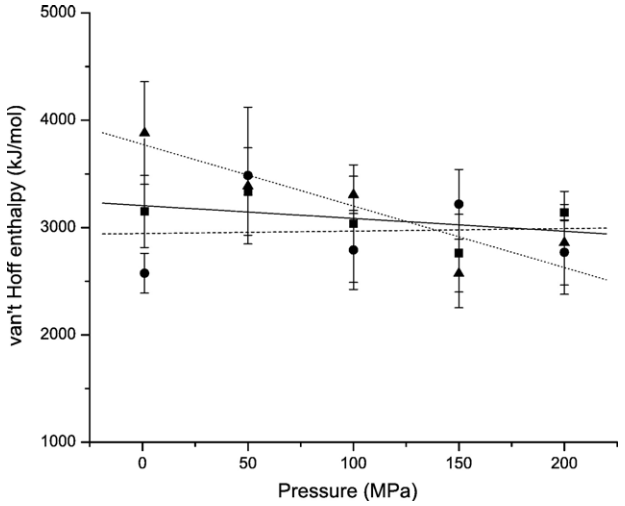

Fig. 2. van't Hoff enthalpy of the helix–coil transition of poly(dA)·poly(dT) at different CsCl concentrations (10 mM: ■, solid line; 50 mM ●, dashed line; 200 mM ▲, dotted line) at different pressures.

helix–coil transition. Thus, ΔH_{ref}=39.2 kJ mol^{-1} at 58.2 °C with ΔC_p=228 J mol^{-1} K^{-1} [19]. This calorimetric study [19] was carried out in aqueous NaCl solutions at atmospheric pressure, and there are no other similar literature calorimetric studies using cations other than sodium or obtained at an elevated pressure. In our analysis of data acquired at high pressure we have assumed that ΔH has no significant pressure dependence. This assumption is supported by the absence of a pressure dependence of the van't Hoff enthalpies for helix–coil transitions [2,20] as well as the data presented in Fig. 2. We have also assumed that the transition enthalpy is not strongly dependent on the nature of the counter-ion (Na^+, Cs^+). This has been suggested in the literature [2]. The value of the T_m in given in Table 1 is its value at atmosphere pressure (0.1 MPa). The pressure dependence of the T_m, dT_m/dP, was calculated over the full pressure range (1–200 MPa) for three salt concentrations. From Table 1, T_m, dT_m/dP and ΔV all increase with increasing salt concentration.

The molar volume changes for the helix–coil transition of poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] in Na^+- and Cs^+-containing solutions are plotted against log([salt]) in Fig. 3. To achieve a more accurate comparison with our measurements, the values taken from the literature in Fig. 3 were recalculated from original data using the enthalpy calculation methods applied in this work. Thus, ΔH_{ref}=39.2 kJ mol^{-1} at 58.2 °C with ΔC_p=228 J mol^{-1}K^{-1} for poly(dA)·poly(dT) and ΔH_{ref}=33.7 kJ mol^{-1} at 50.9 °C with ΔC_p=178 J mol^{-1}K^{-1} for poly[d(A-T)]·poly[d(A-T)] [19]. From Fig. 3, the molar volume change becomes more positive with increasing salt concentrations for all four systems. For three of the four systems, the two sodium-based systems and poly(dA)·poly(dT) in Cs^+, the changes are linear with respect to log([salt]) and the slopes are similar. In contrast, exchanging Na^+ with Cs^+ results in a nonlinear salt dependence for poly[d(A-T)]·poly[d(A-T)] [2], For both polymers, the molar volume is more positive in Cs^+-containing solutions than in those containing sodium ions at the same salt concentration. The difference is about 0.7 cm^3 mol^{-1} for poly(dA)·poly(dT) regardless of salt concentration. Exchanging sodium with cesium appears to stabilize poly (dA)·poly(dT) with no significant effect on molar volume change. On the other hand, exchanging sodium with cesium at concentrations greater than 50 mM results in a more positive molar volume change (Fig. 3).

The number of counter-ions released during a helix–coil transition is proportional to salt concentration dependence of T_m [1] and can be calculated using the equation:

$$\Delta T_m/\Delta\log([M^+]) = \left(2.303RT_m^2/\Delta H\right)\Delta n$$

where Δn is the number of released counter-ion per phosphate, ΔH is the helix–coil transition enthalpy of a base pair, and

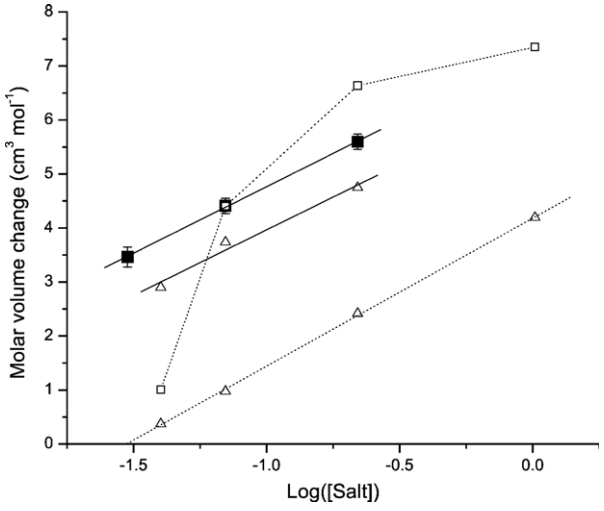

Fig. 3. Molar volume change of helix–coil transition of poly(dA)·poly(dT) (solid line, Na^+: △, Cs^+: ■) and poly[d(A-T)]·poly[d(A-T)] (dashed line, Na^+: △, Cs^+: □) as a function of salt concentration in aqueous NaCl and CsCl. The values for poly (dA)·poly(dT) in aqueous NaCl solution and for poly[d(A-T)]·poly[d(A-T)] in aqueous NaCl and CsCl solutions are from [2].

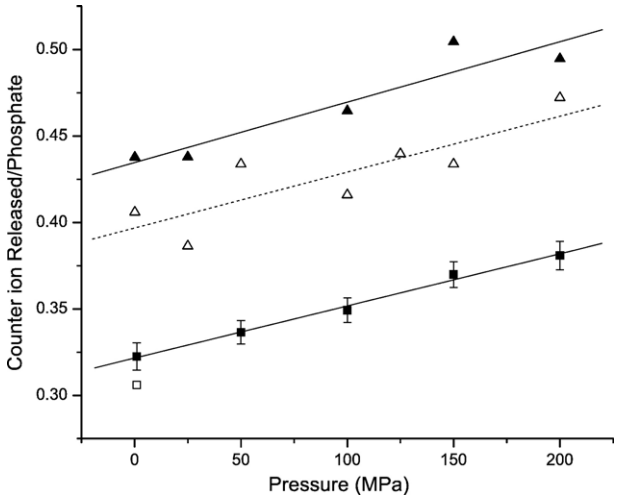

Fig. 4. Number of counter-ions released upon the helix–coil transition of poly (dA)·poly(dT) (Na^+: ▲, Cs^+: ■) and poly[d(A-T)]·poly[d(A-T)] (Na^+: △, Cs^+: □) in aqueous NaCl and CsCl solutions. Data points without error bars are calculated from the literature values [2,20].

$R = 8.31$ J K^{-1} mol^{-1}. The calculated Δn of the helix–coil transition of poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] based on data from this study and the literature [2,20] are plotted in Fig. 4. Due to the unusual nonlinear pressure dependence of T_m, only the lowest pressure point of poly[d(A-T)]·poly[d(A-T)] in Cs^+-containing solutions are plotted. A few observations can be made from Fig. 4. First of all, the data show similar pressure dependence regardless of type of DNA or cation, with Δn increasing by ~0.06 over the pressure range we investigated. Secondly, the value of Δn of poly(dA)·poly(dT) is slightly larger than that of poly[d(A-T)]·poly[d(A-T)] for the same type of salt. Finally, the value of Δn observed for solutions containing Na^+ is much larger than that observed when cesium is the counter-ion, with $\Delta\Delta n = \Delta n_{Cs} - \Delta n_{Na} = 0.10 \pm 0.01$ for poly(dA)·poly(dT). According to [1], Δn is the difference between the number of counter-ions associated with the helix and coil per phosphate, and for B-DNA Δn is about 0.37, which is within the range of our results. Note that the value of Δn obtained here is the stoichiometric term, which is different from Δn reported in [20] where it is a thermodynamic quantity and is half of the stoichiometric term.

4.2. The effect of Na^+ and Cs^+ on the intercalation of ethidium bromide (EB) with poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)]

Table 2 summarizes the thermodynamic binding parameters, K_a, n and ω, $dK_a/d(\log([salt]))$, the molar volume change, and the compressibility change for EB binding with poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] in aqueous solutions of NaCl and CsCl (note that this Table includes values from this study and from [21]). The binding parameters, K_a, n, and ω, for binding in the presence of sodium and cesium ions are almost identical for poly(dA)·poly(dT) and exhibit small differences in case of poly[d(A-T)]·poly[d(A-T)]. The binding of EB to poly[d(A-T)]·poly[d(A-T)] in Cs^+-containing solutions has the same binding site size, $n = 2$, has a small positive cooperativity, and is somewhat weaker compared to what is observed when sodium is the counter-ion. The value of the binding constants as a function of counter-ion concentration is shown in Fig. 5. The slope, $-d\ln K/d\ln[salt]$, is related to the number of counter-ions released upon binding according to polyelectrolyte theory [22]. From Fig. 5 it is evident that the number of counter-ions released is approximately independent of the nature of the counter-ion for these two polymers.

Table 2
The effect of NaCl and CsCl on EB–DNA binding

	poly[d(A-T)]·poly[d(A-T)]		Poly(dA)·poly(dT)	
	CsCl	NaCl[a]	CsCl	NaCl[a]
n	2	2	3	3
ω	2.6±0.2	0.8±0.1	2.4±0.5	1.9±0.6
K_a ($\times 10^6$ M^{-1})[b]	0.43	0.89	0.037	0.037
ΔG (kcal mol^{-1})[b]	−7.7	−8.1	−6.2	−6.2
$-d\ln K_a/d\ln[salt]$	−1.29± 0.01	1.26± 0.02	−1.26± 0.02	1.29± 0.05
ΔV (cm^3 mol^{-1})[c]	−13.0± 1.0	−13.9± 0.2	4.4±2.1	5.4±0.3
Compressibility change (10^{-3} cm^3 mol^{-1} MPa^{-1})[c]	−12±5	−17±1	7±11	−5±3

[a] Data from Ref. [21].
[b] Corresponding to solution with 50 mM added NaCl.
[c] Average of values from different salt concentrations.

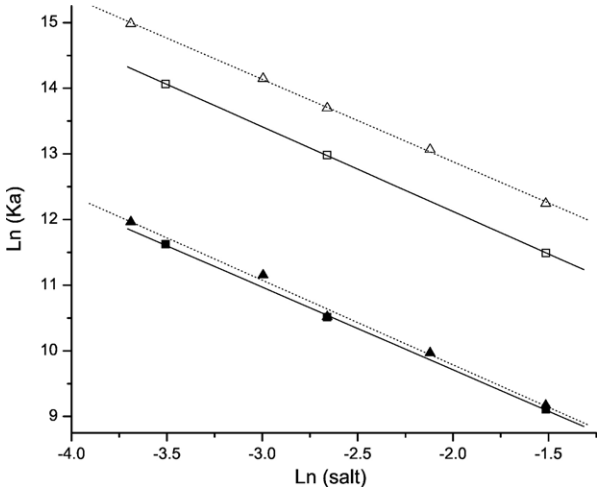

Fig. 5. Salt dependence of the binding constant, K_a, of EB intercalation with poly(dA)·poly(dT) (Na^+: ▲, Cs^+: ■) and poly[d(A-T)]·poly[d(A-T)] (Na^+: △, Cs^+: □) in aqueous NaCl and CsCl solutions. The typical error for each data point is about 0.1.

The values for the molar volume change of EB–DNA binding at different salt concentrations are summarized in Fig. 6. The molar volume changes showed no significant dependence on the type or concentration of salt. Averaged over the different salt concentrations, the molar volume changes exhibit a difference of about 1 cm^3 mol^{-1} in sodium- and cesium-containing solutions, the values are slightly more positive for cesium in the case of poly[d(A-T)]·poly[d(A-T)] and slightly more negative for cesium in the case of poly(dA)·poly(dT). However, the differences are small and within range of uncertainty. The differences in

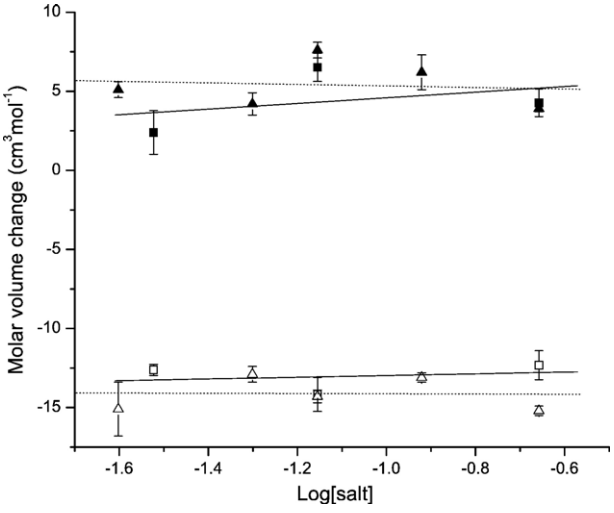

Fig. 6. Molar volume change of EB binding with poly(dA)·poly(dT) (Na^+: ▲, Cs^+:■) and poly[d(A-T)]·poly[d(A-T)] (Na^+: △, Cs^+: □) in aqueous NaCl (dotted line) and CsCl (solid line) solutions.

compressibility changes of binding with the same DNA are also within range of experimental errors.

5. Discussion

5.1. The difference in the amount of Cs^+ and Na^+ counter-ions released during helix–coil transition of DNA

The helix–coil transition of a DNA duplex can be written as:

$$\text{Helix}\cdot 2n_h M_h^+ \leftrightarrow 2\left(\text{Coil}\cdot n_c M_c^+\right) + 2(n_h - n_c)M_{bulk}^+$$

where n_h and n_c are the number of counter-ions associated with each nucleotide of the helix and coil forms of DNA, respectively, thus, $\Delta n = n_h - n_c$ is the number of counter-ions released per phosphate. According to the polyelectrolyte theory [1,23,24],

$$\Delta n = n_h - n_c = (1 - b_h/c) - (1 - b_c/c) = (b_c - b_h)/c$$

where b is the axial charge density of DNA in Å, and the parameter c is proportional to temperature and the dielectric constant of the solvent, ε. The pressure dependence of Δn observed in Fig. 4 could originate partly from a pressure dependence of $T\varepsilon_{water}$, which increases about 10% from 0.1 to 200 MPa [25]. Since the pressure dependences of Δn are similar among the different systems we studied (Fig. 4), the differences in Δn at the same pressure are likely due to differences in $\Delta b = b_c - b_h$. The much smaller value of Δn observed for systems with Cs^+ relative to those with Na^+ could be the result of smaller b_c or larger b_h. Because duplex DNA is much more rigid than single stranded DNA, it seems likely that b_c is more prone to change than b_h. Between a typical A and a typical B form DNA, b_h differs by about 0.2 Å, which could account for a change in $\Delta\Delta n$ of ~0.03. Within the salt concentration range used in this study, we expect the DNA duplexes involved to have much smaller variances in conformation than an A–B transition rendering the contribution of b_h to $\Delta\Delta n$ insignificant. Therefore, the difference we observed, about 0.10 in $\Delta\Delta n = \Delta n_{Na+} - \Delta n_{Cs+}$, is likely a result of a smaller b_c for Cs^+-containing solutions than for Na^+-containing solutions. The cesium ion has been reported to have a higher association affinity towards DNA than Na^+ [6–9] due to its smaller hydration sphere [9,10]. That, for Cs^+, the parameter, b_c would be smaller than for Na^+ is likely the result of a reduced phosphate–phosphate repulsion in the presence of Cs^+ due to the fact that the smaller hydrated Cs^+ might be closer to phosphate of DNA than hydrated Na^+. Finally, if our assumption that the helix–coil transition enthalpy is the same in solutions containing Na^+ or Cs^+ is not true, then the enthalpy differences may also contribute to the observed differences in Δn.

5.2. Effect of Cs^+ on volumetric properties of helix–coil transition of DNA

The partial molar volume of a solute in an aqueous solution can be decomposed into an intrinsic volume, V_I, and a hydration volume, V_H. The intrinsic volume is the sum of internal volume, V_{int}, which is the volume enclosed by the van der Waals surface of the solute, and the thermal volume, V_T, the void between solute and solvent generated by thermal vibration. The hydration volume, V_H, is the volume of the water molecules which are interacting with the solute. The molar volume change of helix–coil transition of DNA, ΔV_{hc}, can be considered as a sum of contributions from DNA, ΔV_{DNA} and the release of counter-ions, ΔV_{M+}. The parameters ΔV_{DNA} and ΔV_{M+} also have intrinsic and hydration factors. When comparing the values of ΔV_{hc} observed in the presence of the two cations used in this study, Na^+ and Cs^+, we found the value of ΔV_{hc} for poly(dA)·poly(dT) to be insensitive to the nature of the cation. This behaviour was in sharp contrast to that observed for poly[d(A-T)]·poly[d(A-T)] in the presence of these two ions [2]. We interpret this behaviour in terms of ΔV_{M+} and ΔV_{DNA}.

Although monovalent cations, such as Na^+ and Cs^+, have been considered to be fully hydrated and delocalized around DNA [26], recent X-ray crystallography [27–30] and NMR studies [8] have suggested that they can partly replace bound water to bind in the minor groove of AT-rich sequences, especially A-tract sequences. However, it has been suggested that this localized binding is temperature dependent [8] with significantly higher occupancy observed in crystal data under cryogenic temperature (120–160 K) and much lower occupancy in NMR data obtained at 277 K. Under our experimental conditions, i.e. room temperature and above, specific minor groove binding of Na^+ and Cs^+ ought to be negligible. Therefore, to a first approximation, we assume that Na^+ and Cs^+ are fully hydrated when condensed onto DNA under our experimental conditions.

5.2.1. ΔV_{M+}

With Na^+ and Cs^+ being fully hydrated, V_I and V_H of the cations are not greatly influenced by DNA. The value of ΔV_M is presumably simply the volume change of transferring a counter-ion from a high salt concentration area around DNA to the bulk solution of a lower salt concentration. The apparent molar volume, V_Φ, of NaCl and CsCl in condensed and bulk solution can be roughly estimated based on the concentration and temperature dependence of densities of NaCl and CsCl solutions [31]. To estimate the difference between Na^+ and Cs^+ for our systems at atmospheric pressure, we use 20 mM, 200 mM and 1 M as typical salt concentrations in bulk solution, in the vicinity of DNA coil and in the vicinity of DNA helix, respectively [32]. We can then write,

$$\Delta V_M + 2\Delta n^* V_\Phi(20\text{mM}) + 2n_c{}^* V_\Phi(200\text{mM}) - 2n_h{}^* V_\Phi(1M)$$

where the factor 2 makes the term ΔV_{M+} per base pair instead of per nucleotide. To an approximation, we set n_h equal to 0.76 for all cases. For poly(dA)·poly(dT), $\Delta V_{M+}(Cs) \approx 2*0.32*V_{\Phi,Cs,61°C}(20\ \text{mM}) + 2(0.76-0.32)*V_{\Phi,Cs,61°C}(200\ \text{mM}) - 2*0.76*V_{\Phi,Cs,61°C}\ (1\ \text{M}) \approx -1.89\ \text{cm}^3\ \text{mol}^{-1}$, similarly $\Delta V_{M+}(Na) \approx -2.43\ \text{cm}^3\ \text{mol}^{-1}$ and $\Delta\Delta V_{M+} = \Delta V_{M+}(Cs) - \Delta V_{M+}(Na) \approx 0.54\ \text{cm}^3\ \text{mol}^{-1}$. Similarly, for poly[d(A-T)]·poly[d(A-T)], $\Delta\Delta V_{M+} = \Delta V_{M+}(Cs) - \Delta V_{M+}(Na) \approx -1.93 - (-2.44) \approx 0.51\ \text{cm}^3\ \text{mol}^{-1}$. These calculations show that

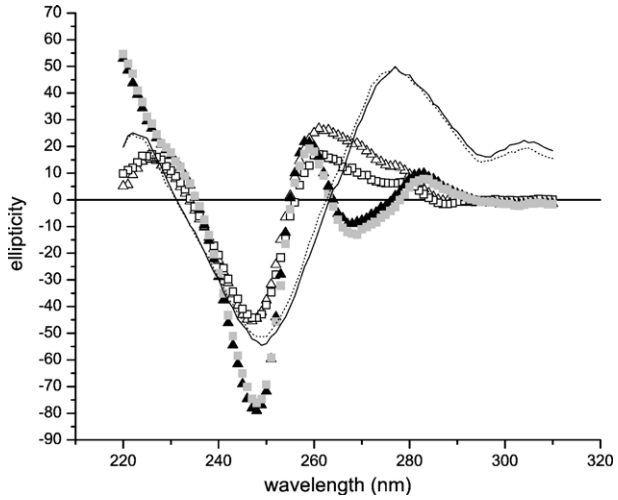

Fig. 7. Circular dichroism (CD) spectra of poly(dA)·poly(dT) (Na^+: ▲, Cs^+: ■) and poly[d(A-T)]·poly[d(A-T)] (Na^+: △, Cs^+: □) in aqueous buffer solution with 55 mM NaCl and 50 mM CsCl plus 5 mM NaCl solutions. And CD spectra of poly[d(A-T)]·poly[d(A-T)] with bound EB (EB:base pair=0.2) in aqueous buffer solution with 200 mM NaCl (dotted line) and CsCl (solid line).

counter-ion release resulting from a DNA helix–coil transition gives rise to a negative volume change of about −2 cm^3/mol with ΔV_{M+}(Cs) being about 0.5 cm^3/mol more positive than ΔV_{M+}(Na) for either polymer. This small difference accounts for the experimentally measured small positive $\Delta\Delta V = \Delta V(Cs) - \Delta V(Na)$ for poly(dA)·poly(dT), which in turn suggests that ΔV_{DNA} contribution is insignificant for poly(dA)·poly(dT). On the other hand, for poly[d(A-T)]·poly[d(A-T)], the value of ΔV(Cs) is more than 3 cm^3 mol^{-1} more positive than the value of ΔV(Na), suggesting a dominate contribution of ΔV_{DNA}.

In a similar manner we can calculate the compressibility change of counter-ion release by fitting compressibility data taken from the literature for CsCl and NaCl solutions [33]. Thus, $\Delta\kappa_{s\ M+} \approx -0.77\times10^{-2}$ cm^3 mol^{-1} MPa^{-1} for $M^+ = Cs^+$ and -0.79×10^{-2} cm^3 mol^{-1} MPa^{-1} for $M^+ = Na^+$, the difference between Cs^+ and Na^+ is only about 0.02×10^{-2} cm^3 mol^{-1} MPa^{-1}, or about 0.04 cm^3 mol^{-1} per 200 MPa, which is not detectable under our experimental conditions.

5.2.2. ΔV_{DNA}

Even with the sodium and cesium ions fully hydrated they could still have a differential effect on DNA, which would be reflected as differences in the value of ΔV_{DNA}. Based on the above analysis of ΔV_{M+}, the value of ΔV_{DNA} for poly(dA)·poly(dT) is not significantly different in the presence of sodium or cesium suggesting that the conformation and hydration of this polymer are probably independent of the nature of the monovalent cation. On the other hand, ΔV_{DNA} of poly[d(A-T)]·poly[d(A-T)] is significantly more positive in Cs^+-containing solutions than in those containing sodium ions. We propose that the difference in volumetric properties results from the conformation and hydration state of poly[d(A-T)]·poly[d(A-T)] being strongly influenced by the type of cation.

Poly[d(A-T)]·poly[d(A-T)] undergoes a gradual conformational shift towards an alternate B form with increasing salt concentration; the transition is associated with a positive-to-negative inversion of ellipticity at 275 nm [34,35]. The change is most effectively induced by high concentrations of CsF, and more effective by CsCl than NaCl [35,36]. At concentrations of CsF greater than 3 M, poly[d(A-T)]·poly[d(A-T)] eventually changes into a new conformation called X-DNA [36], which is similar to the D-form conformation poly[d(A-T)]·poly[d(A-T)] adopts as a dehydrated fiber [37,38]. It has been suggested that the alternate B form of poly[d(A-T)]·poly[d(A-T)] has a more gauche–gauche geometry with base overlapping being improved at one step and weakened at the next step [34,39].

The CD spectra of poly(dA)·poly(dT) display relatively minor salt-induced changes even at high concentrations of CsF [35]. Thus, the conformation of poly[d(A-T)]·poly[d(A-T)] is more sensitive to the salt condition than poly(dA)·poly(dT). The experiments we report here were carried out at salt concentrations much lower than those reported in the literature. In order to verify the importance of salt type at low salt concentration, we recorded CD spectra of these two polymers under our low salt concentration range; the results are summarized in Figs. 7 and 8. Fig. 7 shows that poly[d(A-T)]·poly[d(A-T)] in CsCl and NaCl exhibits distinctly different conformations at salt concentrations as low as 50 mM, while the difference for poly(dA)·poly(dT) in aqueous CsCl or NaCl solutions is observable but much smaller. Furthermore, as shown in Fig. 8A, the conformation of poly[d(A-T)]·poly[d(A-T)] continues to change with increasing CsCl concentration while it stays roughly the same as the concentration of NaCl increases. The subtle differences in the CD spectra of poly(dA)·poly(dT) caused by NaCl and CsCl do not change at salt concentrations above 50 mM although we only investigated concentrations up to 200 mM.

The CD spectrum of calf thymus DNA also shows a significant difference at low concentrations of NaCl and CsCl [40], although the difference is smaller than what we observed for poly[d(A-T)]·poly[d(A-T)]. Hanlon et al. [40] proposed that calf thymus DNA undergoes a partial transformation to C-DNA (20% at 10 mM CsCl) in the presence of Cs^+ and that the resulting C-DNA is partially dehydrated and more compact relative to B-DNA [41]. The effect of Cs^+ at low concentrations appears to be sequence dependent with the alternating AT sequences being favoured. We propose that this effect originates from the higher affinity of this cation for DNA and a stronger neutralizing effect on DNA phosphate, which causes dehydration of the phosphate, a change in conformation of the backbone, and compaction of DNA. We interpret the lack of an effect with poly(dA)·poly(dT) in terms of the fact that the first layer hydration water in the minor groove of poly(dA)·poly(dT) only has hydrogen bonds with the bases, while the hydrogen bonding with backbone sugars are also involved in case of poly[d(A-T)]·poly[d(A-T)] [42]. Thus, hydration of poly(dA)·poly(dT) might be less dependent on changes of phosphate back bone than poly[d(A-T)]·poly[d(A-T)]. Secondly, the structure of poly(dA)·poly(dT), being similar to C and X form DNA, is already more over-wound [43] than a typical B-form DNA; the conformation of poly(dA)·poly(dT) does not have sufficient flexibility to undergo further changes.

The more positive value of ΔV_{DNA} for the helix–coil transition of poly[d(A-T)]·poly[d(A-T)] observed in the presence of

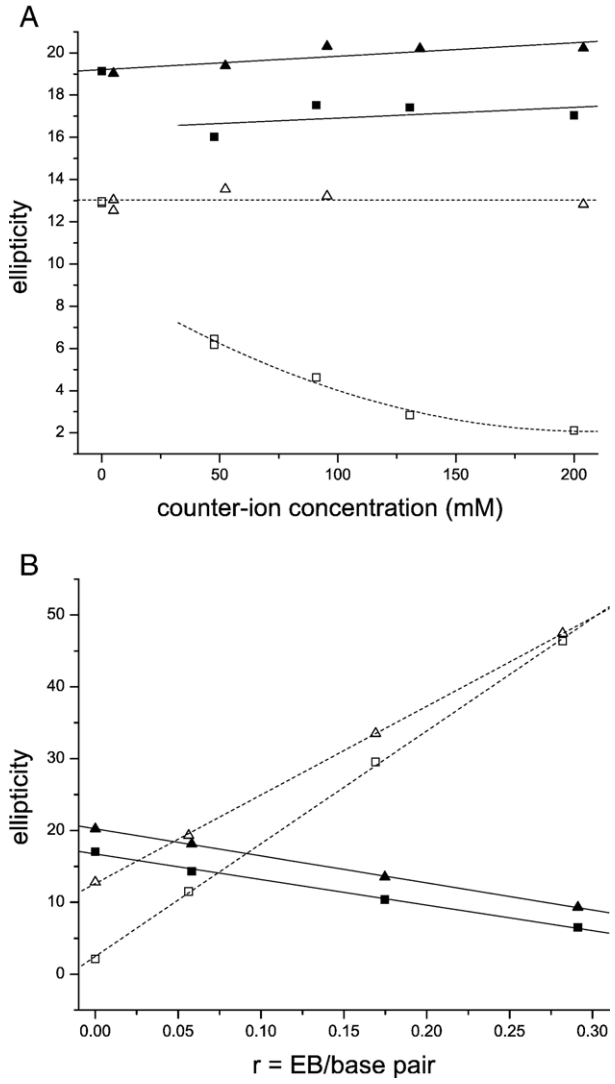

Fig. 8. Plots of ellipticity of poly(dA)·poly(dT) (259 nm; Na^+: ▲, Cs^+: ■) and poly[d(A-T)]·poly[d(A-T)] (275 nm; Na^+: △, Cs^+: □) changing with A) counter-ion concentration and B) ratio of EB/base pairs.

cesium [2] implies a smaller partial molar volume of the cesium form, B_c form, than the normal B form found in the presence of sodium ions. The observed compressibility [2] may originate from a less compressible B_c form than B form. This proposal agrees well with volumetric data for poly[d(A-T)]·poly[d(A-T)] in CsCl and NaCl, both at 50 mM, 25 °C [44]. The partial molar volume of DNA-Cs (183.0 $cm^3\ mol^{-1}$) is reported to be about 19.6±2.5 $cm^3\ mol^{-1}$ larger than DNA-Na (163.4 $cm^3\ mol^{-1}$) [44]. Since the partial molar volume of Cs^+ is about 22.8 $cm^3\ mol^{-1}$ larger than Na^+ [45], V_{DNA} of the Cs^+ form is about 3.2±2.5 $cm^3\ mol^{-1}{}_{nt}$ or 6.4±5.0 $cm^3\ mol^{-1}{}_{bp}$ smaller than the Na^+ form. Similarly, the adiabatic compressibility of DNA-Cs is about $5.2\pm4.0\times10^{-3}$ $cm^3\ mol^{-1}\ MPa^{-1}$ more positive than DNA-Na [44] with the difference between Cs^+ and Na^+ being about $12.8\pm0.7\times10^{-3}$ $cm^3\ mol^{-1}\ MPa^{-1}$ [33,46]. Thus, the cesium form is about $7.6\pm4.0\times10^{-3}$ $cm^3\ mol^{-1}{}_{nt}\ MPa^{-1}$ or $15.2\pm8.0\times10^{-3}$ $cm^3\ mol^{-1}{}_{bp}\ MPa^{-1}$ less compressible than the sodium form. The results in volume and compressibility both agree with the helix–coil transition observation assuming the dependence of the volumetric properties on salt type arises mainly from the helix form.

Interestingly, smaller volume and negative compressibility changes are normally associated with stronger hydration. Although it more closely resembles the C or X form of DNA, the cesium-induced form of DNA is expected to be more dehydrated than the sodium form. However, the dehydration in case of C, X, and other forms of DNA under conditions of high salt and low water activity is actually a decrease in quantity of hydration water combined with improved efficiency and quality of remaining hydration water. In the case of the low salt cesium form, to have a conformation that is capable of having higher quality hydration water under similar water activity as the sodium form might actually have volumetric effect of improved hydration rather than dehydration. On the other hand, the conformations of the cesium form might differ from that of the sodium form in tightness of stacking and solvent assessable surface, which will result in different values of V_I, V_T, and V_H. This would also contribute to the volumetric differences observed between sodium and cesium forms. Tikhomirova and Chalikian [44] interpreted their data as arising from the partial dehydration of counter-ion by poly[d(A-T)]·poly[d(A-T)], while we interpret our results in terms differential hydration of poly[d(A-T)]·poly[d(A-T)] by the two counter-ions.

5.3. Effect of cesium on the intercalation of EB with poly[d(A-T)]·poly[d(A-T)] and poly(dA)·poly(dT)

In case of poly(dA)·poly(dT), the observed similarity in binding parameters in solutions containing either Na^+ or Cs^+ ions indicates that both ions have similar impact on the ligand binding of this polymer. As measured by the helix–coil transition temperature, and the CD spectra, Na^+ and Cs^+ exert a similar affect on free poly(dA)·poly(dT). Furthermore, as can be seen in Fig. 8B, the change in DNA conformation after binding EB is also independent of the type of cation; Na^+ and Cs^+ cause the CD spectra to change in the same manner at similar concentrations. Thus, neither the poly(dA)·poly(dT)-EB complex nor free DNA depend on the nature of the cation. In addition, the counter-ion release volume for ligand binding, ΔV_{M+}, differs by only about 0.20 $cm^3\ mol^{-1}$ for the two counter-ions, which is smaller than experimental error. This result is consistent with the similarity of the other parameters we measured for this system.

For poly[d(A-T)]·poly[d(A-T)] binding with EB, the volumetric properties are also insensitive to the type of cation. The volume change of binding was determined by measuring the effect of pressure on partially bound poly[d(A-T)]·poly[d(A-T)]. We propose that the partially bound poly[d(A-T)]·poly[d(A-T)] has already lost most of the salt-type dependent conformational differences that existed in ligand-free poly[d(A-T)]·poly[d(A-T)]. CD spectra acquired at increasing EB:DNA ratios, depicted in Fig. 8B, and CD spectra in Fig. 7 show that the difference in the DNA conformation caused by Na^+ and Cs^+ disappears with intercalation of EB. There is no difference at $r=0.2$, which shows that the conformational transition from

the Cs^+-sensitive free DNA form, B_c form, to the salt-type insensitive bound form is complete at a low binding density. Our experiments were carried out at $r \sim 0.1$, we would expect the conformation of the DNA to be mostly in the salt-type insensitive bound form and pressure perturbation will only able to probe a small fraction of the cesium volume effect of poly[d(A-T)]·poly[d(A-T)]. With the cesium form, B_c, having a smaller partial molar volume than the sodium form, the transition to the bound state from the cesium form would have a more positive volume change. Although small the volume change observed is about 1 ± 1 cm^3 mol^{-1} more positive for cesium than sodium, in agreement with this idea. The smaller binding constant in the presence of Cs^+ might indicate a lower binding affinity with the cesium form than the sodium form, and the small positive cooperativity in the presence of cesium could be caused by an intercalation-induced conformational transition from the cesium form to a high affinity conformation at the neighbouring binding sites.

6. Conclusions

Focusing on volumetric properties, we have studied the effect of Na^+ and Cs^+ on helix–coil transition and ethidium binding of poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)]. If these cations are fully hydrated, the difference in volumetric properties of counter-ion release is small. The differences in the volumetric properties between the two cations are likely a result of salt-type dependent DNA conformation and hydration. The conformation of poly[d(A-T)]·poly[d(A-T)] is more easily disturbed by salt and the difference in the effect of Na^+ and Cs^+ is apparent at salt concentrations as low as 50 mM. On the other hand, conformation and hydration pattern of poly(dA)·poly(dT) are more independent of salt type; no differences were observed in the range of concentrations we investigated. Although partially dehydrated, the cesium form of poly[d(A-T)]·poly[d(A-T)] has a smaller partial molar volume than the sodium form, probably due to a stronger hydration and a more compact structure. The volumetric properties of DNA–ligand binding appear to be insensitive to both salt concentration and salt type. The intercalation of the positively charged EB reduces the effectiveness of the counter-ion as well as limiting the flexibility of the DNA conformation. The resulting DNA–ligand complexes exhibit conformation and hydration states in sodium- and cesium-containing solutions that are more similar than those of free poly[d(A-T)]·poly[d(A-T)]. The difference remains small for poly(dA)·poly(dT) in the bound and free states.

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