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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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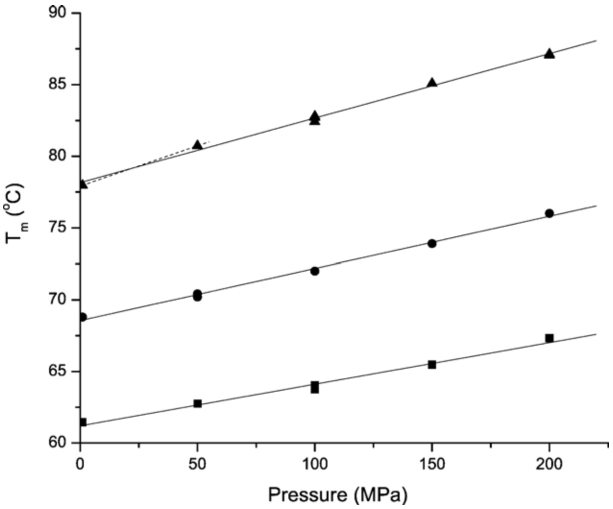

Fig. 1. Pressure dependence of helix–coil transition temperature of poly(dA)·poly(dT) in buffer solutions with various CsCl concentrations: 10 mM CsCl: ■, 50 mM CsCl: ●, 200 mM CsCl: ▲. The typical error for each data point is about 0.2 °C.

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.