Cation radius effects on the helix-coil transition of DNA

Cryptates and other large cations

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ABSTRACT Most polyelectrolyte theories of the effect of ions on the thermal melting of DNA assume that the predominant influence of the cations comes through their charge. Ion size and structure are treated, for analytic convenience, as negligible variables. We have examined the validity of this assumption by measuring the melting temperature of calf thymus DNA as a function of salt concentration with four univalent cations of different hydrated radii. These are K^+ (3.3 Å), $(n\text{-}Pr)_4N^+$ (4.5 Å), $(EtOH)_4N^+$ (4.5 Å), and C222-K^+ (5 Å). C222-K^+ is a complex of cryptand C222 with K^+. With K^+ as the sole cation, T_m varies linearly with the log of ionic strength over the range 0.001–0.1 M. With all the K^+ sequestered by an equimolar amount of C222, T_m is depressed by 10–20°C and the slope of T_m vs. ionic strength is lower. At low ionic strength, an even greater reduction in T_m is achieved with $(n\text{-}Pr)_4N^+$; but the similar-sized $(EtOH)_4N^+$ gives a curve more similar to K^+. Theoretical modeling, taking into account cation size through the Poisson-Boltzmann equation for cylindrical polyelectrolytes, predicts that larger cations should be less effective in stabilizing the double helix; but the calculated effect is less than observed experimentally. These results show that valence, cation size, and specific solvation effects are all important in determining the stability of the double-helical form of DNA.

INTRODUCTION

Modern polyelectrolyte theory has been remarkably successful in explaining many of the effects of salts on the properties of DNA. The most widely used theory, counterion condensation theory (Manning, 1978), succeeds to a large extent despite ignoring all geometrical and chemical properties of the ionic species. The DNA is modeled as a line charge, and the small ions as point charges. Only the linear charge density of the DNA and the valences of the small ions enter the theory. More realistic theories can take DNA and ion size into account, but are relatively cumbersome for calculation of thermodynamic quantities. Theories based on the Poisson-Boltzmann equation (Klein et al., 1981; Le Bret and Zimm, 1984*a*; Stigter, 1975), the hypernetted chain approximation (Bacquet and Rossky, 1984; Soumpasis, 1984; Soumpasis, et al., 1987), and Monte Carlo simulations (Conrad et al., 1988; Le Bret and Zimm, 1984*b*; Mills et al., 1985) have been compared by several groups (Mills et al., 1985; Murthy et al., 1985). Further complications arise when the chemical nature of the cation must also be taken into account, as is often the case (e.g., Tam and Williams, 1985).

To recognize conditions requiring theories that incorporate ion structural features, it is important to understand the range of validity of counterion condensation theory. The thermal melting behavior of DNA is a much-studied and conveniently measured property. It therefore provides a convenient test of ideas that take cation size into account. One might hypothesize that the larger the cation, the less effective would be close-in screening of the negative phosphate charge. Then the helix stabilization contributed by counterion screening by condensed counterions might be less effective. Thus one might predict that T_m for DNA would be decreased as the cation radius increases, and the salt dependence of T_m would be lessened.

We measured the melting temperature of calf thymus DNA in the presence of four different monovalent cations; K^+, the tetrapropylammonium and tetraethanolammonium cations, and the complex of cryptand C222 with K^+. Cryptands are macrocyclic polyethers which are able to form a caged complex with various metal cations with high affinity. The resulting cryptate complex has the same charge as the metal ion, but a radius 1.7 Å larger. We find that two of the larger ions, tetrapropylammonium and C222 · K^+, significantly lower T_m compared with hydrated K^+ over the entire range of ionic strength. On the other hand, the tetraethanolammonium ion does so only at low ionic strength.

We have compared these experimental results with theoretical predictions based on the Poisson-Boltzmann theory for cylindrical polyelectrolytes, both the full nonlinear theory and the linear theory supplemented with a counterion condensation correction. This provides a first-

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order way to take account of finite ion size, though it neglects excluded volume and ion correlation effects considered in more complex theories. The calculations show that larger cations are less effective in stabilizing double-strand DNA against thermal melting; but the predicted magnitude of the change in T_m with cation radius is less than observed experimentally.

Taken together, these results show that cation structure and solvation, as well as simple size and charge, need to be taken into account for a full understanding of ion effects on double helix stability.

MATERIALS AND METHODS

KOH as a volumetric standard, C222 (Kryptofix 222) and KCl at 99.99+% were obtained from Aldrich Chemical Co. (Milwaukee, WI). Analytical grade boric acid was obtained from Malinckrodt Inc. (St. Louis, MO). Tetraethanolammonium bromide [$(EtOH)_4NBr$] was synthesized and purified as described previously (Evans et al., 1988). Tetrapropylammonium bromide (Pr_4NBr) was purchased from Kodak and recrystallized from acetone-ether. Chemicals were dissolved in glass-distilled water which had been passed through an ion exchange resin and had a conductivity of <10 Mohm^{-1}-cm^{-1}.

High-molecular weight calf thymus DNA (Sigma type I) was dissolved in 0.1 M NaCl, 0.1 M Tris, 0.01 M EDTA, pH 7.6 buffer and mildly sheared by passage through a 20-gauge needle. The DNA was then deproteinized by proteinase K digestion followed by repeated phenol/chloroform extractions according to standard procedures (Maniatis et al., 1982). After removal of phenol by extraction with ether, the DNA was twice precipitated with isopropanol and dialyzed against 10 mM sodium cacodylate and 0.1 mM EDTA, pH 6.5 for storage at ~1.4 mg/ml. Before use in melting temperature experiments, the DNA was further dialyzed against a low salt (1 mM KOH, 0.9 mM boric acid) pH 10 buffer.

For the T_m experiments with K^+ as the cation, potassium was supplied from a stock solution of 150 mM KOH adjusted to pH 10 with boric acid (final concentration of boric acid was 195 mM). When C222 · K^+ was the cation, a 300-mM stock solution equimolar in KCl and C222, pH 10.68, was the source.

For the melting temperature experiments, DNA to give a final concentration of 50 μg/ml and appropriate amounts of KOH/borate buffer, C222 · KCl, or quaternary ammonium salts were mixed with water to give a final volume of 0.750 ml. The pH of the solutions was measured using glass microelectrode (model 6030-OZ; Ingold Electrodes Inc., Andover, MA) on a Solution Analyzer (model 4603; Markson Science Inc., Phoenix, AZ).

The samples were placed in water-jacketed quartz cells (Helma type 160) in a spectrophotometer (DU-8; Beckman Instruments Inc., Fullerton, CA). A teflon-covered thermistor (model 44104; Omega Engineering, Inc., Stamford, CT) was inserted into each sample through a tight-fitting teflon cap. The optical density and temperature of each of four samples was monitored continuously by an Apple IIe computer as the temperature was increased ~1° per min from 20 to 95°C by means of a circulating water bath. The optical density measurements from concurrently run air blanks or C222 · KCl with no DNA were subtracted from the experimental curves. The net absorbance curves were smoothed with several passes of a smoothing routine (Bevington, 1969), and the T_m was determined from the maximum of the first derivative of the smoothed data.

TABLE 1 Hydrated radii R_i of cations (Å).

K^+	3.3
$(n\text{-}Pr)_4N^+$	4.5
$(EtOH)_4N^+$	4.5
C222-K^+	5.0

RESULTS

The melting temperature was determined for DNA in the presence of 1–100 mM concentrations of various cations, whose ionic radii are shown in Table 1 (Nightingale, 1959). When C222 · K^+ was the cation, the pH of the DNA-cation solution was kept above 9.0 to ensure nearly total complexation of the potassium with negligible amounts of free K^+ and diprotonated C222 (Fig. 1). The K^+ · DNA samples were run at pH 9.0 or slightly higher. Although the helix is most stable at neutral pH, we found that up to pH 10.2 there was no significant decrease in T_m (results not shown). The pH of the $EtOH_4NBr$ and Pr_4NBr DNA solutions varied from 6.90 to 7.74.

Complexing the K^+ ion with the larger C222 moiety decreased the T_m 10–20 degrees relative to the K^+ · DNA samples (Fig. 2). Increasing C222 · K^+ above 0.01 M did not further increase T_m. The ions $(EtOH)_4N^+$ and its alkyl analogue Pr_4N^+ (Table 1) affected T_m in different ways. The $(EtOH)_4N^+$ ion was nearly as effective as K^+ in stabilizing double stranded DNA, whereas the Pr_4N^+ ion depressed T_m by 20° relative to K^+. The slope of the T_m vs. log ionic strength plot is also lower for C222 · K^+ than for K^+ alone, though this is not true for the other two cations.

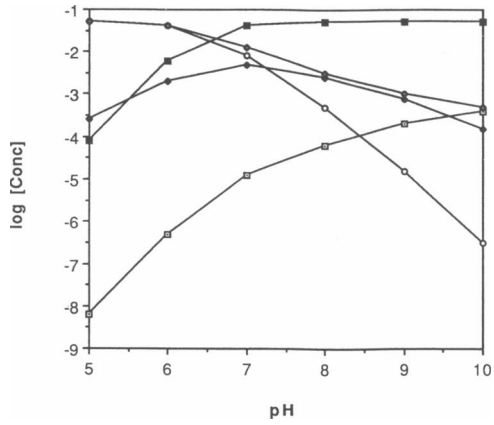

FIGURE 1 The concentration of species in 50 mM C222, 50 mM KCl solution, calculated according to the equilibrium constants tabulated by Lehn and Sauvage (Lehn and Sauvage, 1975). This is a revision of Fig. 9 in Evans et al. (1988). The symbols correspond to C222 (□); C222 H^+ (♦); C222 H^{2+} (○); K^+ (◊); C222 · K^+ (■).

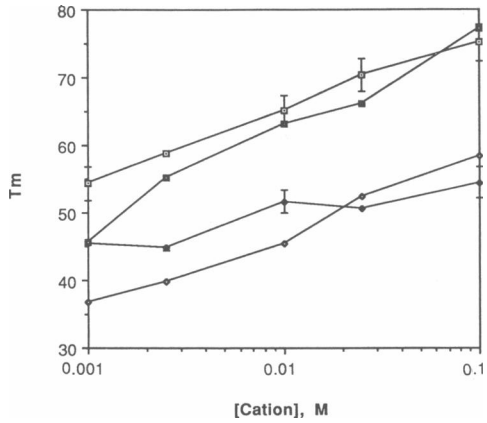

FIGURE 2 T_m of calf thymus DNA, as a function of cation concentration and type. The symbols correspond to K^+ (□); C222 · K^+ (♦); $(EtOH)_4N^+$ (■); $(n - Pr)_4N^+$ (◊). The error bars for K^+ and C222 · K^+ are standard deviations from two to four measurements.

The absolute values of T_m are somewhat lower, and the slope of the T_m vs. log $[K^+]$ plot in Fig. 2 is about half, what is normally observed at neutral pH (Record, 1975). Part of this discrepancy is due to the elevated pH in our experiments with K^+ and C222 · K^+. Titration of the bases becomes appreciable above pH 9, and our experiments were carried out slightly above pH 9 to assure complete complexation of the K^+ by crytand (see Fig. 1). Our results fall between those reported by Record (1975) for NaCl at pH 9.0 and 10.8, but are a bit lower than would be expected from his data. Whatever the source of this modest discrepancy, our experiments should at least yield internally consistent results.

DISCUSSION

To understand the effect of cation size on the helix-coil transition in DNA, one needs a theory of the effect of ion size on the apparent equilibrium constant K_{obs} for the reaction $H = C$. A general expression is

$$\ln K_{obs} = \ln K_{\dagger}^{\circ} - N_u \Delta W/kT, \qquad (1)$$

where $K_{\dagger}^{\circ}$ is the standard state equilibrium constant, N_u is the number of nucleotides in the cooperative melting unit, and $\Delta W = W_c - W_h$ is the salt-dependent difference in free energy per nucleotide between coil and helix.

According to counterion condensation theory (Manning, 1978; Record et al., 1978), the free energy can be divided into a sum of contributions from counterion condensation (W_{cc}) and residual electrostatic interaction (W_{el}) between polyelectrolyte and ion atmosphere. Using the notation of Record et al. (1978),

$$W_{i,cc}/kT = -N_u^{-1} \ln \Sigma_i^{\circ} = \xi_i^{-1} \ln a_M^+ \qquad (2)$$

and

$$W_{i,el}/kT = N_u^{-1} \ln \gamma_i^{\circ} = -\xi_i^{-1} \ln \kappa b_i, \qquad (3)$$

where $i = h$ or c. In these equations, Σ_i° is a binding polynomial, γ° is the polyion activity coefficient, a_M^+ is the activity of monovalent cation (taken equal to the salt molarity C in our calculations), κ is the inverse Debye length ($\approx 0.33\ C^{1/2}\ Å^{-1}$ in aqueous solution), and ξ is the ratio of the Bjerrum length $b_j = e^2/DkT$ to the charge spacing b along the polyion backbone. For water at 20°C, $b_j = 7.14$ Å. For B-form double helical DNA, $b = 1.7$ Å and $\xi_h = 4.2$. For single-stranded DNA, we take $b = 4.2$ Å (Record et al., 1978), giving $\xi_c = 1.7$. Thus the charge densities of helix and coil DNA are reduced by factors of 0.24 and 0.41. Combining Eqs. 2 and 3 with these definitions yields

$$\begin{aligned} W_i/kT &= (W_{i,cc} + W_{i,el})/kT \\ &= \xi_i^{-1}[0.5 \ln C - \ln 0.33 b_i] \\ &= \xi_i^{-1}[\ln \kappa - \ln 0.109 b_i]. \end{aligned} \qquad (4)$$

Because counterion condensation theory idealizes polyions as line charges, these equations do not have any dependence on polyion or counterion radius. Such dependence is provided by theories based on the Poisson-Boltzmann (P-B) equation. The linearized P-B equation was solved by Hill (1955) to give

$$W = \frac{Z^2 e^2}{DL}\left[\frac{K_0(\kappa a)}{\kappa a K_1(\kappa a)} + \ln \frac{a}{R}\right] \qquad (5)$$

for the electrostatic work required to bring a cylindrical rod of length L and radius R to the charge Ze. The sum of polyion radius (R_h for helix, R_c for coil) and ionic radius R_i is the exclusion radius a. K_0 and K_1 are the modified Bessel functions of orders 0 and 1. W_{el} is related to the polyion activity coefficient by $RT \ln \gamma = W_{el}(\kappa) - W_{el}(\kappa = 0)$. Because the charge spacing $b = L/Z$, Eq. 5 can be written per nucleotide as

$$W/kT = \xi\left[\frac{K_0(\kappa a)}{\kappa a K_1(\kappa a)} + \ln \frac{a}{R}\right], \qquad (6)$$

where a and R, hence W, will have different values for helix and coil.

It is well known that the linearized P-B equation does not give accurate results for highly charged polyelectrolytes such as nucleic acids. Eq. 6 can be modified in two ways to obtain better results. The first is an ad hoc hybrid with counterion condensation theory. That is, we assume that the effective charge is reduced from Z to Z/ξ, and that the free energy of counterion condensation, Eq. 2,

must be added to the electrostatic free energy. This yields

$$W/kT = \xi^{-1}\left[\frac{K_0(\kappa a)}{\kappa a K_1(\kappa a)} + \ln\frac{a}{R}\right] + \xi^{-1}\ln C. \qquad (7)$$

The other, more firmly based, approach is to use the numerical solution to the full, nonlinear P-B equation. We use the convenient formulation of Stigter (Stigter, 1975, 1982) who expresses his results in terms of a table of correction factors δ to the linear P-B free energy (without the small ln $[a/R]$ term). This model thus yields

$$W/kT = \frac{\xi}{\delta}\left[\frac{K_0(\kappa a)}{\kappa a K_1(\kappa a)}\right]. \qquad (8)$$

To obtain δ, we fit the correction factor β for the reduced surface potential y_0 to a double polynomial in reduced distance x_0 and (ξ/x_0) using Table 2 in Stigter (1982). This enabled determination of y_0, and thereby δ from Table 2 in Stigter (1975) (using a double polynomial in x_0 and y_0).

Using Eq. 4, 7, or 8 to obtain the dependence of W/kT on salt concentration, we combine Eq. 1 with the thermodynamic identity (Record et al., 1978)

$$\frac{dT_m}{d\ln a_\pm} = -\frac{RT_m^2}{N_u\Delta H^\circ_{obs}}\left(\frac{\partial \ln K_{obs}}{\partial \ln a_\pm}\right)_T = \alpha\beta\left(\frac{\partial \ln K_{obs}}{\partial \ln a_\pm}\right)_T, \qquad (9)$$

where ΔH°_{obs} is the observed enthalpy of the transition per mole of nucleotides, α is a correction for ionic activity, and $\beta = RT_m^2/N_u\Delta H^\circ_{obs}$. In our calculations, we took $a_\pm = C$, $\alpha = 1$, and $\beta = 50°$, independent of C and T (Privalov et al., 1969). We then numerically integrated Eq. 9 to find the depression ΔT_m of T_m at C relative to its value at $C = 1$ M. Thus all ΔT_m's are zero at $C = 1$ M.

The results are shown in Figs. 3 and 4, using cation radius $R_i = 3$ or 5 Å and several choices for R_h and R_c. In all cases, T_m is lower for the larger cation than for the smaller, in accord with our experimental observations comparing K^+ with Pr_4N^+ and C222 · K^+. However, the effect of ion radius is small, only a few degrees compared with the 10–20 degrees observed experimentally. Moreover, the difference in T_m is calculated to decrease with increasing salt concentration, in contrast to the experimental trend. In Fig. 3, the counterion condensation theory curve has a slope equal to the experimental value in NaCl solution (Record, 1975), because b_c was adjusted to reproduce the experimental result. The nonlinear P-B theory agrees with this quite well, and the results are relatively insensitive to choice of helix or coil radii (Fig. 4). The linearized P-B theory with condensation correction gives significantly poorer agreement, but is qualitatively reasonable. In contrast, the linearized P-B theory without condensation correction, Eq. 6, gives results that

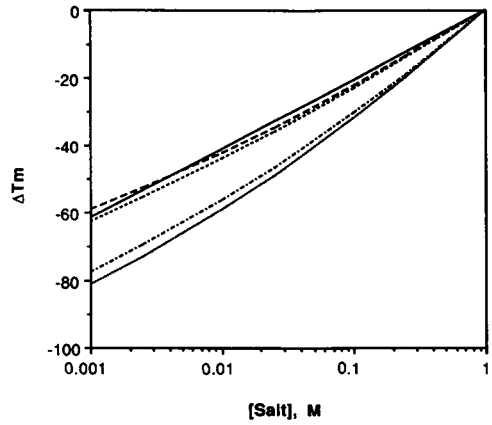

FIGURE 3 Theoretical salt dependence of DNA helix-coil transition temperature, relative to its value at 1 M salt, for two values of cation radius R_i, with helix and coil radii R_h = 10 Å, R_c = 5 Å. Line types correspond to counterion condensation theory, Eq. 4: (——); nonlinear Poisson-Boltzmann theory, Eq. 8: R_i = 3 Å (— — —), R_i = 5 Å (– – –); linear P-B theory with condensation, Eq. 7: R_i = 3 Å (· – · – · –), R_i = 5 Å (· · · · ·).

are grossly inaccurate (results not shown). It is possible that theories that take more detailed account of DNA helix geometry and dielectric discontinuities (Troll et al., 1986; Conrad et al., 1988; Jayaram et al., 1989) would produce a larger ion radius effect, but it seems unlikely that they could reproduce the large effects seen in Fig. 2. Another possibility is that different cations bind to single stranded DNA in such a way as to produce fairly large

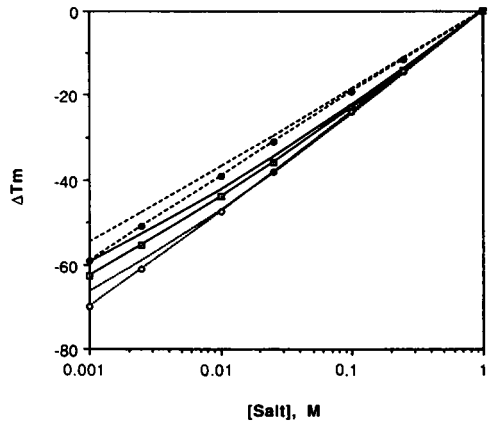

FIGURE 4 Theoretical salt dependence of DNA helix-coil transition temperature, relative to its value at 1 M salt, according to nonlinear Poisson-Boltzmann theory. Calculations were made for various choices of helix and coil radii R_h and R_c, and cation radius R_i = 3 Å (without symbol) or 5 Å (with symbol). Line types correspond to R_h = 12 Å, R_c = 5 Å (— — —); R_h = 10 Å, R_c = 5 Å (——); R_h = 12 Å, R_c = 8 Å (· · · · ·).

differences in b_c. However, there is no independent evidence for this ad hoc hypothesis.

Physically, the reduced T_m in the presence of larger cations may be explained by the decreased ability of large cations to screen phosphate–phosphate repulsions close to the DNA decreased ability of large cations to screen phosphate–phosphate repulsions close to the DNA backbone, an effect which is magnified in double-stranded DNA compared with the thinner single-stranded DNA. These results parallel those observed when cryptands interact with amphiphiles (Miller et al., 1987). Normally, adding salt to a micellar solution increases the aggregation number because more effective screening of coulombic repulsion allows headgroups to move closer together. This in turn leads to more efficient packing of micellar bound surfactants. Complexing of counterions by cryptands results in decreased aggregation numbers which are independent of added salt (C222:NaCl) up to 0.4 M. When cryptands are added to anionic double chain amphiphiles, liquid crystals are transformed to unilamellar vesicles or spherical micelles depending on the amount of cryptand added.

It is clear from Fig. 2 that factors other than cation charge and radius affect the melting of DNA. Pr_4N^+ has essentially the same ionic radius as C222 · K^+, but it is even more effective in lowering T_m at low salt, whereas the dependence of T_m on log (salt) is greater. Even more striking is the effect of $(EtOH)_4N^+$, which behaves similarly to K^+, though its ionic radius is 50% larger. In this case we may speculate that the hydroxyl groups of the cation allow greater penetration of the hydration shell of the DNA, allowing $(EtOH)_4N^+$ to approach more closely and screen phosphate charge more effectively than do the less hydrophilic C222 · K^+ and Pr_4N^+. Another plausible speculation, advanced by an anonymous referee, is that the cations with hydrophobic surfaces, Pr_4N^+ and C222 · K^+, bind to and stabilize the exposed bases of single-stranded DNA, whereas the more hydrophilic $(EtOH)_4N^+$ and K^+ do not. When complexed with metal ions, all the oxygen and nitrogen atoms of C222 are directed toward the inside of the cage (Metz et al., 1971), leaving a hydrocarbon exterior.

Early studies of the effects of various monovalent cations on DNA melting (Dix and Straus, 1972; Hamaguchi and Geiduschek, 1962) did not detect major differences, but these studies did not use the large range of cation sizes employed here. Significant dependence of DNA melting on structure of isovalent cations was noted in a study of the effects of methylene spacer length in the series of trivalent spermidine homologues $NH_3^+(CH_2)_3NH_2^+(CH_2)_nNH_3^+$, where n varies from 2 to 8 (Thomas and Bloomfield, 1984). In this case, a less effective stabilization of the helix was observed for $n = 2$ and 3, perhaps as the result of a too short amino-imino spacing to interact with neighboring phosphates. Taken together, these results show that simple electrostatic theories using a point charge model explain a good deal of the polyelectrolyte behavior of DNA; but full agreement with experiment requires consideration of counterion size, structure, and solvation.

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