The Importance of Coulombic End Effects: Experimental Characterization of the Effects of Oligonucleotide Flanking Charges on the Strength and Salt Dependence of Oligocation (L^{8+}) Binding to Single-Stranded DNA Oligomers

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ABSTRACT Binding constants K_{obs}, expressed per site and evaluated in the limit of zero binding density, are quantified as functions of salt (sodium acetate) concentration for the interactions of the oligopeptide ligand KWK_6NH_2 (designated L^{8+}, with $Z_L = 8$ charges) with three single-stranded DNA oligomers (ss dT-mers, with $|Z_D| = 15$, 39, and 69 charges). These results provide the first systematic experimental information about the effect of changing $|Z_D|$ on the strength and salt dependence of oligocation-oligonucleotide binding interactions. In a comparative study of L^{8+} binding to poly dT and to a short dT oligomer ($|Z_D| = 10$), Zhang et al. (1996. *Proc. Natl. Acad. Sci. USA.* 93:2511–2516) demonstrated the profound thermodynamic effects of phosphate charges that flank isolated nonspecific L^{8+} binding sites on DNA. Here we find that both K_{obs} and the magnitude of its power dependence on salt activity ($|S_aK_{obs}|$) increase monotonically with increasing $|Z_D|$. The dependences of K_{obs} and S_aK_{obs} on $|Z_D|$ are interpreted by introducing a simple two-state thermodynamic model for Coulombic end effects, which accounts for our finding that when L^{8+} binds to sufficiently long dT-mers, both $\Delta G^o_{obs} = -RT \ln K_{obs}$ and S_aK_{obs} approach the values characteristic of binding to poly-dT as linear functions of the reciprocal of the number of potential oligocation binding sites on the DNA lattice. Analysis of our L^{8+}-dT-mer binding data in terms of this model indicates that the axial range of the Coulombic end effect for ss DNA extends over ~10 phosphate charges. We conclude that Coulombic interactions cause an oligocation (with $Z_L < |Z_D|$) to bind preferentially to interior rather than terminal binding sites on oligoanionic or polyanionic DNA, and we quantify the strong increase of this preference with decreasing salt concentration. Coulombic end effects must be considered when oligonucleotides are used as models for polyanionic DNA in thermodynamic studies of the binding of charged ligands, including proteins.

INTRODUCTION

Equilibrium constants (K_{obs}) for both specific and nonspecific binding of proteins or oligocations to polyanionic nucleic acids generally exhibit large negative power dependences on salt concentration or activity. We (Record et al., 1976, 1978, 1998) proposed that both the functional forms and the substantial magnitudes of these salt effects are the thermodynamic signatures of a polyelectrolyte effect. The local reduction in DNA axial density of negative (phosphate) charge that occurs upon binding an oligocation or protein greatly reduces the extent to which salt cations are accumulated in the vicinity of the ligand-DNA complex (Olmsted et al., 1995). We also proposed that the extent of cation release from DNA significantly exceeds the extent of anion release from the DNA-binding surfaces of a protein or other nonpolyanionic ligand, provided that non-Coulombic anion site-binding effects are absent (Record et al., 1976, 1978, 1998).

To test our proposal that the polyelectrolyte effect of DNA is the primary determinant of the power dependence of K_{obs} on salt mean ionic activity ($a_\pm$) for binding an oligocation to polyanionic DNA, Zhang et al. (1996) compared the binding of the C-amidated peptide KWK_6-NH_2 (designated L^{8+}) to polyanionic single-stranded (ss) poly dT and to the short oligoanion $dT(pdT)_{10}$ over a range of salt (NaAc) concentrations. Binding constants K_{obs}, expressed per site and extrapolated to zero binding density, are similar for poly- and oligo-dT at 0.3 M salt, but at 0.1 M salt the value of K_{obs} for poly dT exceeds that for $dT(pdT)_{10}$ by a factor of ~75. Values of the negative power-law exponent $S_aK_{obs} = d \ln K_{obs}/d \ln a_\pm$ are approximately twofold larger in magnitude for poly dT than for $dT(pdT)_{10}$. These data provide a quantitative demonstration of the polyelectrolyte effect: the dominant contribution ($\gtrsim$70%) to S_aK_{obs} of binding L^{8+} to poly dT arises from release of accumulated cations from poly dT, whereas the release of anions from L^{8+} makes a significantly smaller ($\lesssim$30%) contribution to S_aK_{obs}. Per L^{8+} bound, cation release from poly dT exceeds that from $dT(pdT)_{10}$ by ~2.6-fold. These findings demonstrated experimentally for the first time the importance of Coulombic end effects as the determinant of the large thermodynamic asymmetry between contributions to S_aK_{obs} of polyanionic DNA and an oligocationic ligand, and as the physical origin of the profound differences in the thermo-

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dynamics of binding of oligocationic ligands to polynucleotides and oligonucleotides, especially at lower salt concentrations. These experimental results are in accord with expectations based on Monte Carlo (MC) simulations (Olmsted et al., 1995; Olmsted, 1991; Shkel et al., unpublished observations).

In the present work we report binding data acquired for three ss oligonucleotides with lengths intermediate between those we investigated previously and thereby examine the dependences on $|Z_D|$ of $\Delta G^o_{obs} = -RT \ln K_{obs}$ and of $S_a K_{obs}$ for L^{8+} binding. To interpret these results we propose a novel thermodynamic two-state model of the Coulombic end effect, which is analogous to that introduced by Record and Lohman (1978) to analyze the effect of oligonucleotide length on the [salt] dependence of oligonucleotide helix-coil transitions. For binding L^{8+} to dT-mers, the two-state model predicts the functional forms of the approach of ΔG^o_{obs} and of $S_a K_{obs}$ to the values observed for binding L^{8+} to poly dT. Fitting the dT-mer data to equations based on this model yields semiquantitative predictions of the range of the Coulombic end effect and of the Coulombic preference exhibited by an otherwise nonspecific oligocationic ligand for interior sites as compared to terminal sites on DNA. We conclude that Coulombic end effects produce important differences in the binding to oligoanionic versus polyanionic DNA of any charged ligand (including a protein with a charged binding surface) and produce important differences in the thermodynamic affinities of terminal versus interior sites at which a positively (or, indeed, negatively) charged ligand binds to DNA, especially at low salt concentrations.

ANALYSIS OF BINDING DATA FOR INTERACTIONS OF A PEPTIDE OLIGOCATION WITH SINGLE-STRANDED DNA OLIGOANIONS

Binding isotherms

We model the oligocationic ligand as a linear array of Z_L charges and the ss dT-mer lattice as a linear array of $|Z_D|$ phosphates. We assume that any group of Z_L consecutive phosphates constitutes a potential binding site, so that the binding site size is $n = Z_L$. The site-averaged binding constant K_{obs}, which characterizes the interaction of L^{8+} with any site on the dT-mer in the limit of zero binding density, is evaluated by fitting an isotherm whose functional form is derived from a simple combinatorial analysis (Latt and Sober, 1967; Epstein, 1978). This derivation, like that of the infinite linear lattice noncooperative McGhee–von Hippel (1974) isotherm, incorporates the assumption that all potential binding sites are thermodynamically equivalent, with an affinity unaffected by the number of bound ligands or the pattern of their locations on the lattice. Moreover, the number (n) of consecutive monomer lattice units occupied by binding one ligand is assumed to be independent of ligand binding density (and of any other property of the solution).

The noncooperative McGhee–von Hippel isotherm has been used to obtain values of K_{obs} and n for binding of cationic oligopeptides and other oligocations to polyanionic DNA, even though such binding may not be to discrete sites and may be expected to exhibit anticooperativity due to Coulombic repulsions between charged ligands that bind at nearby sites on the lattice. McGhee–von Hippel analyses of experimental data on the binding to polyanionic DNA of either compact multivalent ions, like Mg^{2+}, or oligocationic ligands with spatially separated univalent charges, such as putrescine^{2+}, yield values of n that are approximately the same as the number of charges on the ligand (i.e., $n \cong Z_L$; see, for example, McGhee and von Hippel, 1974; Record et al., 1976; Mascotti and Lohman, 1990, 1992, 1993; Zhang et al., 1996; Padmanabhan et al., 1997). The use of binding constants and the applicability of the McGhee–von Hippel isotherm to the analysis of competitive interactions of oligocations and univalent cations with a cylindrical polyion have occasionally been questioned on theoretical grounds (Ray and Manning, 1992; Rouzina and Bloomfield, 1997; Chen and Honig, 1997). However, theoretical calculations based on the Poisson-Boltzmann (PB) equation (Fogolari et al., 1992; Stigter and Dill, 1996) and PB and MC methods (Ni et al., unpublished observations) demonstrate that competitive Coulombic interactions of oligocations with DNA can be effectively treated in terms of salt-dependent binding constants K_{obs}. In addition, MC and PB calculations account for the empirical applicability of the functional form of the McGhee–von Hippel noncooperative binding isotherm as a means of evaluating a binding constant that characterizes the thermodynamic accumulation of a divalent small cation in the vicinity of polyanionic DNA in excess 1:1 salt (Ni et al., unpublished observations).

For an oligonucleotide lattice consisting of $|Z_D|$ phosphate charges and a ligand having Z_L ($< |Z_D|$) positive charges, if $n = Z_L$ for every binding site, then the maximum number of ligands that can bind to the lattice is the largest integer (designated J_{max}) less than $|Z_D|/Z_L$. Comparisons of our experimental binding curves with isotherms generated with the model assumptions specified above indicate that agreement is reasonable under the conditions investigated here, especially when the number of unoccupied DNA sites substantially exceeds the number of bound ligands (cf. Figs. 2 and 4). Even when $|Z_D|/Z_L$ is only slightly less than an integer, as for $dT(pdT)_{15}$, and when the (fixed) concentration of KWK_6-NH_2 is similar to the (variable) phosphate concentration, as in the initial stages of "reverse" titrations, the fraction of ligand-DNA complexes that involve fewer than Z_L phosphates appears to be insignificant (see Methods).

If each bound ligand occupies (and therefore effectively neutralizes) exactly Z_L consecutive phosphates on a dT-mer lattice consisting of $|Z_D|$ phosphates, the number of distinct arrangements, Ω_j, of j bound ligands on the lattice is

$$\Omega_j = \frac{(|Z_D| - (Z_L - 1)j)!}{j!(|Z_D| - Z_L j)!} \quad (1)$$

For this situation, the number of potential binding sites is $|Z_D| - Z_L + 1$, and the partition function, Σ, which is the summation of probabilities of all distinct configurations of all possible numbers of ligands bound to a finite oligonucleotide lattice, can be expressed (Epstein, 1978) as

$$\Sigma = \sum_{j=0}^{J_{max}} (K_{obs}[L_F])^j \Omega_j \quad (2)$$

where $[L_F]$ is the free ligand concentration. The average binding density, ν (the fraction of a ligand bound per phosphate), is calculated as

$$\nu = \sum_{j=0}^{J_{max}} jP_j/|Z_D| = \sum_{j=0}^{J_{max}} j(K_{obs}[L_F])^j \Omega_j/|Z_D|\Sigma \quad (3)$$

Here P_j is the probability that the DNA lattice has j ligands bound (irrespective of position) to Z_L consecutive phosphates. Epstein (1978) showed that as the number of sites ($|Z_D|$) becomes sufficiently large, Eq. 3 becomes identical to the noncooperative McGhee–von Hippel isotherm for an (effectively) infinite lattice. In our previous analysis of L^{8+} binding to the three potential binding sites on $dT(pdT)_{10}$ ($|Z_D| = 10$), an expression equivalent to Eq. 3 was used (Zhang et al., 1996). The model descriptions employed here for finite lattices are not intended to imply or test any unique molecular picture, but rather to provide a parameterization of the isotherm that suffices to obtain reliable values of K_{obs} from data at finite binding densities. The equivalent site-lattice approximation implies that each value of K_{obs} determined by application of the Epstein isotherm at a given salt concentration must be a site-weighted average over all accessible terminal and interior binding sites, as further considered in the following subsection.

Novel two-state model for Coulombic end effects on binding an oligocation (L^{Z_L}) to a DNA oligoanion $dT(pdT)_{|Z_D|}$

On a rodlike oligonucleotide, Coulombic end effects arise because the electric field diminishes in magnitude near either end of the linear lattice of nucleic acid phosphates. The physical manifestations of Coulombic end effects and theoretical calculations and experimental determinations thereof have recently been reviewed (Anderson and Record, 1995; Record et al., 1998; for recent theoretical work in this area, see Olmsted et al., 1989, 1991, 1995; Allison 1994; Manning and Mohanty, 1997). MC simulations demonstrate that the characteristic extents of accumulation of salt cations and exclusion of salt anions in the annular volume surrounding the polyion-like interior region of a sufficiently long ss DNA molecule decrease dramatically around the two terminal regions that extend in the axial direction ~10 phosphates from either end of DNA (Olmsted, 1991). At the salt activities for which these calculations were performed (expressed in concentration units as ~2–12 mM), the axial profile of the cation concentration at the DNA surface decreases approximately linearly from ~0.5 M (the value characteristic of the polyion-like interior) to <0.05 M at the ends. Single-stranded DNA oligomers shorter than ~20 phosphates are predicted to lack a polyion-like interior region, and the extent of cation accumulation everywhere on their surface is expected to be significantly lower than that characteristic of polyanionic DNA. For double stranded (ds) DNA at similar salt concentrations, the corresponding terminal regions are each predicted to span 15–20 phosphates by MC simulations (Olmsted et al., 1989) and by PB calculations (Allison, 1994).

The McGhee–von Hippel (1974) and Epstein (1978) isotherms were derived for model lattices that are homogeneous with respect to ligand-binding affinity. If each potential binding site for L^{Z+} consists of exactly Z_L contiguous DNA phosphates, the number of such binding sites on a vacant DNA lattice is

$$N_B = |Z_D| - Z_L + 1 \quad (4)$$

The results reported in the present study of binding of L^{8+} to dT-mers indicate that the portion of the N_B sites located at or near the ends of an otherwise homogeneous DNA lattice becomes increasingly inhomogeneous (because of Coulombic end effects) with decreasing salt concentration. The fraction of affected sites of course decreases with increasing $|Z_D|$ and becomes negligible for poly dT. To analyze Coulombic end effects on the observed $|Z_D|$ dependences of K_{obs} and SK_{obs} on binding of L^{Z+}, we apply a two-state model analogous to that introduced by Record and Lohman (1978).

For model oligonucleotides long enough to have well-defined interior (I) and terminal or "end" (E) regions, N_B is represented as

$$N_B = N_E + N_I \quad (5)$$

For a homologous series of sufficiently long DNA oligomers, the two-state model assumes that N_E, the total number of binding sites in the two "end" regions of the linear DNA lattice, is constant (i.e., independent of N_B) at a given salt concentration, and that N_I, the number of interior binding sites, increases linearly with increasing N_B (Record and Lohman, 1978). This assumption is consistent with the $|Z_D|$ dependences of the axial profiles of surface cation concentrations calculated by MC simulations (Olmsted et al., 1989, 1995). The distinction between E and I sites is expected to decrease with increasing salt concentration, but not to disappear until the salt concentration is sufficiently high that the extents of cation accumulation (and anion exclusion) become indistinguishable in the I and E regions. This two-state model does not differentiate binding affinities within the end regions or recognize any transition region between end and interior sites. A more realistic multistate distribution of ligand binding affinities would introduce substantial computational complexity that does not appear to be needed for the present application.

The observed binding free energy $\Delta G^{o}_{obs} = -RT \ln K_{obs}$ is represented as a site-weighted average of contributions from terminal (ΔG^{o}_{E}) and interior (ΔG^{o}_{I}) sites:

$$\Delta G^{o}_{obs} = (N_I/N_B)\Delta G^{o}_{I} + (N_E/N_B)\Delta G^{o}_{E} \quad (6)$$

From Eqs. 4–6,

$$\Delta G^{o}_{obs} = \Delta G^{o}_{I} + N_E(\Delta G^{o}_{E} - \Delta G^{o}_{I})(|Z_D| - Z_L + 1)^{-1} \quad (7)$$

and

$$K_{obs} = K_I(K_E/K_I)^{N_E(|Z_D|-Z_L+1)^{-1}} \quad (8)$$

so

$$\begin{aligned} S_aK_{obs} &\equiv d \ln K_{obs}/d \ln a_{\pm} \\ &= S_aK_I + N_E(S_aK_E - S_aK_I)(|Z_D| - Z_L + 1)^{-1} \end{aligned} \quad (9)$$

From Eqs. 7 and 9, ΔG^{o}_{obs} and S_aK_{obs} are predicted to approach their respective polyion limits (that is, values characteristic of binding of L^{Z_L} to polyanionic DNA) as functions of the reciprocal of the total number of binding sites for L^{Z_L} on a vacant lattice: $(|Z_D| - Z_L + 1)^{-1}$. Eqs. 7–9 are used to analyze the effects of changes in $|Z_D|$ on ΔG^{o}_{obs}, K_{obs}, and S_aK_{obs} for the binding of L^{8+} to sufficiently long DNA oligomers. To implement the analysis, values of ΔG^{o}_{I}, K_I, and S_aK_I are obtained from our published experimental results for binding of L^{8+} to poly dT, and values of ΔG^{o}_{E}, K_E, and S_aK_E are approximated from experimental results for the short DNA oligomer $dT(pdT)_{10}$ (Zhang et al., 1996).

EXPERIMENTAL MATERIALS AND METHODS

Materials

All chemicals were reagent grade, and solutions were prepared with distilled deionized Milli-Q H_2O (Millipore). All experiments were performed in 5 mM sodium cacodylate, 0.2 mM Na_3EDTA buffer, at pH 6.0 at 25°C with variable amounts of sodium acetate (NaAc). This salt was chosen because Ac^-, unlike Cl^-, gives no indication of any specific interaction with oligopeptides (Mascotti and Lohman, 1990, 1992). KWK_6-NH_2 (K: L-lysine; W: L-tryptophan), a tryptophan-containing oligolysine amide, was synthesized and purified as described previously (Zhang et al., 1996). Oligopeptide identity was confirmed by mass spectrometry. Tryptophan was incorporated near the N-terminus to permit fluorescence measurements of binding and determinations of the oligopeptide concentration in 6 M guanidinium hydrochloride from the absorbance at 280 nm, using $\epsilon_{280} = 5690\ M^{-1}\ cm^{-1}$, where M refers to the molarity of the oligopeptide (Edelhoch, 1967).

The oligonucleotides $dT(pdT)_{15}$, $dT(pdT)_{39}$, and $dT(pdT)_{69}$ were synthesized using an Applied Biosystems 391 PCR-mate automated synthesizer with phosphoramidite chemistry and purified to >98% homogeneity by electrophoresis, using polyacrylamide gels as described elsewhere (Ferrari and Lohman, 1994). Stock solutions of dT-mers (~10 mM in DNA phosphate) were extensively dialyzed against a series of NaAc solutions of decreasing salt concentration, using dialysis tubing with appropriate molar mass cutoffs (in the range 10^3-10^4 g/mol) at 4°C. For this dialysis protocol, the final ratio of DNA phosphate concentration to $[Na^+]$ is ~1:1 (Stein et al., 1995). Concentrations of oligonucleotides were determined by UV absorbance at 260 nm on a Cary 210 spectrophotometer, using $\epsilon_{260} = 8100\ M^{-1}\ cm^{-1}$, where M refers to the molarity of the oligonucleotide monomer (Fasman, 1975).

Experimental determinations of binding isotherms by fluorescence measurements

To quantify the binding of KWK_6-NH_2 to dT-mers, tryptophan fluorescence quenching was monitored at an excitation wavelength of 296 nm and an emission wavelength of 350 nm, using an SLM-Aminco 8000C spectrofluorometer (Spectronics Instruments, Rochester, NY), as described previously (Zhang et al., 1996). "Reverse" titrations (Lohman and Mascotti, 1992) were performed at a fixed concentration of (excess) salt by adding oligonucleotide salt solution to an oligopeptide salt solution. At 25°C, reverse titrations were performed at three different oligopeptide concentrations to obtain binding isotherms. Fluorescence quenching data were analyzed as described previously (Bujalowski and Lohman, 1987; Zhang et al., 1996) to evaluate: the binding density (ν), defined as the average fractional number of ligands bound per DNA phosphate; the free ligand concentration ($[L_F]$); and the maximum fluorescence quenching (Q_{max}). To determine best fitted values of the site-averaged binding constant K_{obs}, nonlinear least-squares software NONLIN (Johnson and Frasier, 1985) was used to fit the finite lattice binding isotherm (Eq. 3) to experimental values of ν as a function of the total ligand and DNA concentrations.

Experimental determination of the salt concentration dependence of K_{obs}

"Salt-back" titrations (Lohman and Mascotti, 1992), in which preformed oligopeptide-DNA complexes are dissociated by titrating with a concentrated (2 M) NaAc solution, were used to evaluate K_{obs} at low binding densities over a range of NaAc activities. For each set of conditions, at least three independent salt-back titrations were performed by monitoring the recovery of tryptophan fluorescence of the oligopeptide upon dissociation from each of the dT-mers. Salt-back titrations are suitable for the present application, because the fluorescence intensities of KWK_6-NH_2 and the parameters Q_{max} and n do not vary significantly over the salt concentration range investigated (0.1–0.4 M) (data not shown; cf. Mascotti and Lohman, 1990, 1992), and because analyses of titrations at 0.1 M or 0.2 M salt show that Q_{corr}/Q_{max} is proportional to $[L_B]/[L_T]$ (as shown in Fig. 1).

To promote the applicability of Eq. 3 to determination of values of K_{obs}, initial binding densities in salt-back titrations were selected to be less than 30% of the saturation value ($\nu = 0.125$). Equation 3 was found to yield adequate fittings, even for $dT(pdT)_{15}$, apparently because complexes involving fewer than eight phosphates are uncommon. The possibility that some ligands occupy fewer than Z_L phosphates under our experimental conditions has been investigated. For binding of KWK_6-NH_2 to $dT(pdT)_{15}$, small but apparently systematic deviations were observed when Eq. 3, which posits a single binding mode ($n = 8$), was fitted to the fluorescence quenching data of Fig. 2 *C* as a function of DNA concentration. These deviations may indicate that some of the bound ligands occupy fewer than eight consecutive DNA phosphates (i.e., $n < 8$) at high ligand:DNA ratios, where the ligand binding density is relatively high. Equation 3 was therefore modified (Zhang, 1996) to allow for the possibility of two site sizes ($n = 8$ and $n = 7$). The modified equation provides a slightly better fitting of the data for $dT(pdT)_{15}$ (not shown) but does not significantly affect the reported value of the binding constant (corresponding to the $n = 8$ binding mode). Even at the highest ligand:DNA ratio examined, we conclude that no more than 10% of all bound L^{8+} involve the $n = 7$ mode (Zhang, 1996).

At each fixed salt concentration, each value of K_{obs} for ligand binding to an oligonucleotide was obtained as the only positive root of a polynomial equation based on Eq. 6 (the highest power of the polynomial is J_{max}), by using Laguerre's root-finding method, as incorporated in the IMSL algorithm DZPLRC (IMSL, Houston, TX). Values of K_{obs} obtained by analyzing fluorescence quenching data were averaged for at least three salt-back titrations, and standard errors were calculated.

Effects of changing the activity of the excess salt on the binding equilibrium were analyzed by plotting log K_{obs} as a function of log $a_{\pm}$. Values of $a_{\pm}$ for NaAc were interpolated from literature data on mean ionic activity coefficients and solution density, as described previously (Zhang et

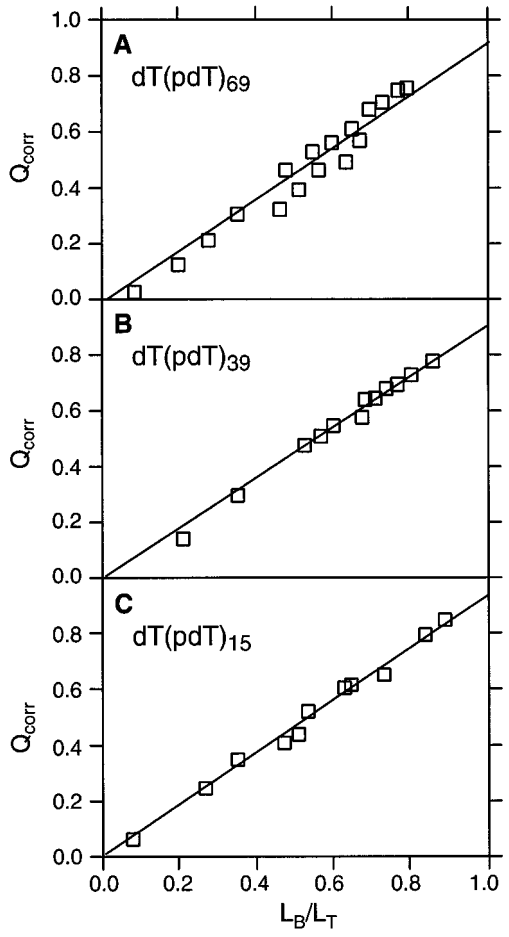

FIGURE 1 Demonstration of proportionality between corrected fluorescence quenching Q_{corr} (Lohman and Mascotti, 1992) and the ratio ($[L_B]/[L_T]$) of concentrations of bound oligopeptide and total oligopeptide for binding KWK_6-NH_2 to (*A*) $dT(pdT)_{69}$ at 0.2 M NaAc, and to (*B*) $dT(pdT)_{39}$ and (*C*) $dT(pdT)_{15}$, both at 0.1 M NaAc. Values of the maximum fluorescence quenching obtained from the extrapolation to $[L_B]/[L_T] = 1$ are listed in Table 1. Oligopeptide concentrations ranged from 1 to 3 μM, and DNA phosphate concentrations ranged from 1 to 100 μM in these experiments (pH 6.0, 25°C).

al., 1996). Under the experimental conditions of interest here, the concentrations of DNA phosphate and of the ligand are much smaller (by a factor of at least 10^3) than the salt concentration. Consequently, the magnitude and salt concentration dependence of $a_{\pm}$ are assumed to be indistinguishable from the corresponding properties in a solution that contains only NaAc.

RESULTS AND DISCUSSION

Binding isotherms and binding constants

Binding isotherms for interactions of the cationic ligand (KWK_6-NH_2) with three ss DNA oligomers ($dT(pdT)_{15}$, $dT(pdT)_{39}$, and $dT(pdT)_{69}$, collectively designated dT-mers) were determined by analysis of tryptophan fluorescence quenching in reverse and salt-back titrations (as described in Materials and Methods) performed over the same range of solution conditions examined in our previous study of the binding of KWK_6-NH_2 to poly dT and $dT(pdT)_{10}$ (Zhang et al., 1996). Fig. 1, constructed using the model-independent procedure described by Bujalowski and Lohman (1987), shows for all three dT-mers that the corrected tryptophan fluorescence quenching (Q_{corr}) is proportional to $[L_B]/[L_T]$, where $[L_B]$ and $[L_T]$ are the concentrations of bound and total oligopeptide, respectively. Confirmation of this proportionality allows free and bound ligand concentrations to be quantified directly from corrected quenching data. The intercepts (extrapolated) at $[L_B]/[L_T] = 1$, where all oligopeptide is bound to DNA, correspond to the maximum fluorescence quenching (Q_{max}) for these dT-mers. For the binding of KWK_6-NH_2, values of Q_{max} obtained for the intermediate-length dT-mers (cf. Table 1) are indistinguishable (outside experimental uncertainty) from one another and from the values of Q_{max} for poly dT and $dT(pdT)_{10}$ reported by Zhang et al. (1996).

Fig. 2, *A–C*, presents the results of reverse (DNA) titrations that characterize the binding of KWK_6-NH_2 to $dT(pdT)_{69}$, $dT(pdT)_{39}$, and $dT(pdT)_{15}$, respectively, at 0.1 M NaAc, pH 6.0, 25°C. A nonlinear least-squares program was used to determine each value of K_{obs} by fitting Eq. 3 to the quenching data for L^{8+} binding to each dT-mer as a function of DNA phosphate concentration. The excellent agreement between the solid smooth curves constructed with the best-fitted parameters listed in Table 1 and the quenching data obtained at different oligopeptide concentrations indicates that Eq. 3 (and hence the noncooperative binding model with site-averaged binding constants) is satisfactory for analyzing the systems and experimental conditions investigated in the present study. Values of K_{obs} at 0.1 M NaAc, obtained from the resulting nonlinear fittings, increase from $(2.1 \pm 0.2) \times 10^5$ M^{-1} for $dT(pdT)_{15}$ to $(1.6 \pm 0.1) \times 10^6$ M^{-1} for $dT(pdT)_{69}$ (cf. Table 1). These values are intermediate between the values of K_{obs} that we

TABLE 1 Experimentally determined parameters for the [salt]dependences of the binding interactions of KWK_6-NH_2 with dT-mers

dT-mer	Q_{max}	K_{obs} (M^{-1}) ($[Na^+] = 0.1$ M)	$-S_aK_{obs}$	log K_o (1 M Na^+)
$dT(pdT)_{10}$	0.90 ± 0.02	$(4.6 \pm 0.2) \times 10^4$	3.5 ± 0.1	+0.8 ± 0.3
$dT(pdT)_{15}$	0.93 ± 0.02	$(2.1 \pm 0.2) \times 10^5$	4.5 ± 0.2	+0.3 ± 0.1
$dT(pdT)_{39}$	0.94 ± 0.02	$(9.5 \pm 0.5) \times 10^5$	6.0 ± 0.1	−0.4 ± 0.1
$dT(pdT)_{69}$	0.93 ± 0.02	$(1.6 \pm 0.1) \times 10^6$	6.1 ± 0.1	−0.6 ± 0.1
poly dT	0.90 ± 0.02	$(3.5 \pm 0.3) \times 10^6$	6.4 ± 0.1	−0.5 ± 0.2

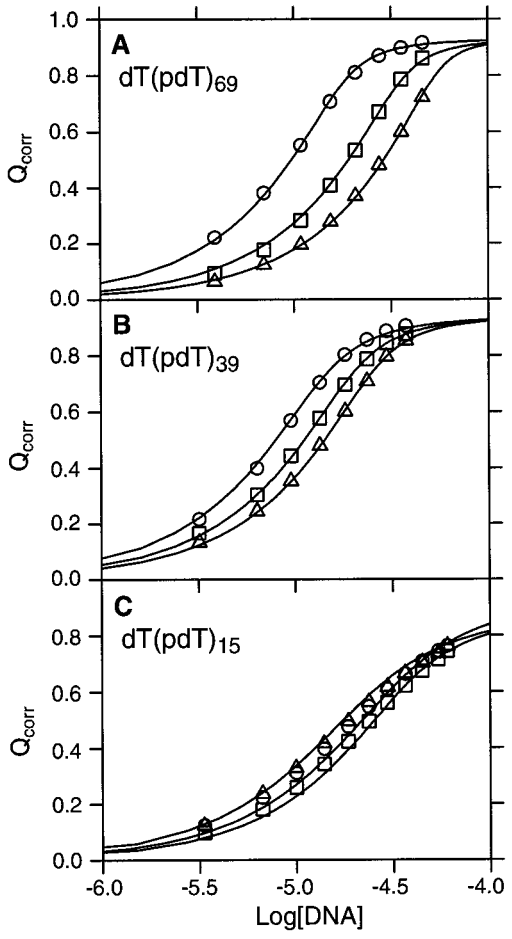

FIGURE 2 Binding isotherms obtained at constant ligand concentrations by monitoring the fluorescence quenching as a function of the DNA phosphate concentration at 25°C, 0.1 M Na^+, pH 6.0. (*A*) KWK_6-NH_2 binding to $dT(pdT)_{69}$ at initial oligopeptide concentrations of 0.99 μM (○), 1.98 μM (□), and 2.96 μM (△), respectively. The solid smooth curves are theoretical fittings of Eq. 3 with $K_{obs} = 1.6 \times 10^6\ M^{-1}$, $Q_{max} = 0.93$, and $n = 8$. (*B*) KWK_6-NH_2 binding to $dT(pdT)_{39}$ at initial oligopeptide concentrations of 0.99 μM (○), 1.49 μM (□), and 1.98 μM (△), respectively. The solid smooth curves are theoretical fittings of Eq. 3 with $K_{obs} = 9.5 \times 10^5\ M^{-1}$, $Q_{max} = 0.94$, and $n = 8$. (*C*) KWK_6-NH_2 binding to $dT(pdT)_{15}$ at initial oligopeptide concentrations of 0.99 μM (△), 1.49 μM (○), and 1.98 μM (□), respectively. The solid smooth curves are theoretical fittings of Eq. 3 with $K = 2.1 \times 10^5\ M^{-1}$ and $Q_{max} = 0.93$.

(Zhang et al., 1996) reported for binding of this L^{8+} to $dT(pdT)_{10}$ ($(4.6 \pm 0.2) \times 10^4\ M^{-1}$) and to poly dT ($(3.5 \pm 0.3) \times 10^6\ M^{-1}$) at the same salt concentration. Fig. 3 presents plots of K_{obs} versus the reciprocal of the number of potential binding sites per lattice to illustrate the large difference between the value of K_{obs} for poly dT and that of each intermediate-length dT oligomer at 0.1 M NaAc. Even for the oligomer with $|Z_D| = 69$ phosphates, K_{obs} is only one-half that for poly dT.

Effects of salt concentration on binding constants (K_{obs}) for different lengths of DNA

Binding constants for the dT-mers at different salt concentrations were determined by applying Eq. 3 to analysis of

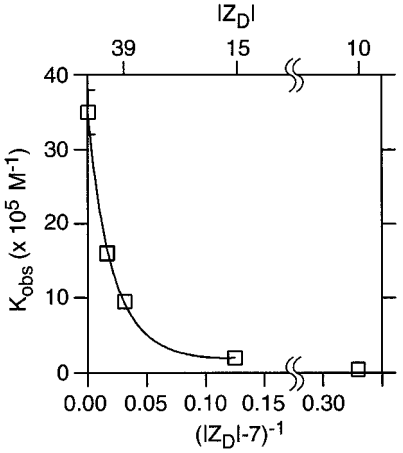

FIGURE 3 Values of K_{obs} (data points from Table 1) for binding L^{8+} to dT oligomers at 0.1 M NaAc plotted versus the reciprocal of the number of ligand binding sites per dT-mer $(|Z_D| - 7)^{-1}$. The solid curve is a plot of values of K at 0.1 M NaAc calculated from $\log K_{obs} = S_a K_{obs} \log [NaAc] + \log K_o$ (1 M Na^+) with the best linear fitted parameters specified in Table 1. Selected values of the number of dT-mer phosphates $|Z_D|$ are plotted on the top nonlinear scale.

fluorescence quenching data obtained from salt-back titrations. Typical salt-back titrations of the complexes of KWK_6-NH_2 with $dT(pdT)_{69}$, $dT(pdT)_{39}$, and $dT(pdT)_{15}$ are shown in Fig. 4. The strong decreases in fluorescence quenching with increasing salt concentration indicate the strong dependences of L^{8+} binding constants (K_{obs}) on salt concentration for all three oligonucleotide lattices. In every case titration to high ($\gtrsim$0.4 M) salt concentrations causes a reduction in the extent of the fluorescence quenching to less than 2%, which demonstrates essentially complete dissociation of the complexes. This behavior is consistent with that reported in salt-back titrations of other nonspecific oligopeptide-DNA complexes (e.g., Mascotti and Lohman, 1990, 1992; Zhang et al., 1996). Fig. 5 presents plots of $\log K_{obs}$ as a function of $\log a_\pm$ for binding of KWK_6-NH_2 to all of the dT-mers. Best-fitted linear plots were analyzed to determine values of the slope, $S_a K_{obs}$, and values of K_o, the binding constant extrapolated to the 1 M salt reference state. These are tabulated in Table 1. Nonlinearity of the $\log K_{obs}$ vs. $\log a_\pm$ plots at high salt concentration, noted previously by Zhang et al. (1996) but not analyzed here, is expected to cause convergence of values of K_{obs} for the various dT-mers at high salt concentration. Extrapolated values of K_o (1 M) therefore do not represent predictions of the actual L^{8+} binding constant for these lattices at 1 M salt.

The negative slopes ($S_a K_{obs}$) of the plots, which quantify the net release of thermodynamically accumulated counterions that accompanies ligand binding to the various dT-mers, can be interpreted with reference to the thermodynamic expression for $S_a K_{obs}$ derived by Anderson and Record (1993). The decrease in magnitude of $S_a K_{obs}$ with decreasing $|Z_D|$ can be attributed entirely to a decrease in the extent of release of thermodynamically accumulated cations from the dT-mer when a complex is formed, because the

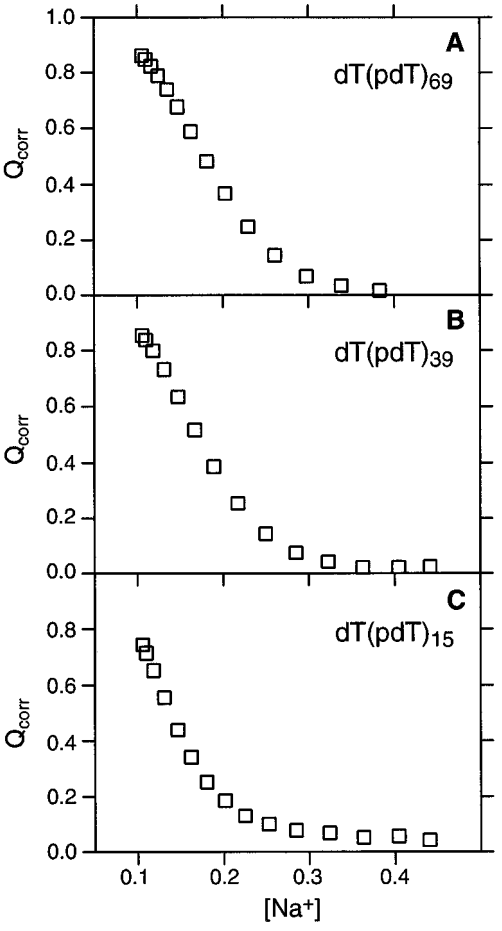

FIGURE 4 Representative salt-back titrations at 25°C, pH 6.0, exhibiting decreases in fluorescence quenching with increasing salt concentration. (*A*) $dT(pdT)_{69}$. (*B*) $dT(pdT)_{39}$. (*C*) $dT(pdT)_{15}$. The initial concentrations of DNA phosphate and oligopeptide were 46.2 μM and 2.89 μM (*A*), 37.5 μM and 1.94 μM (*B*), and 60.4 μM and 1.90 μM (*C*), respectively.

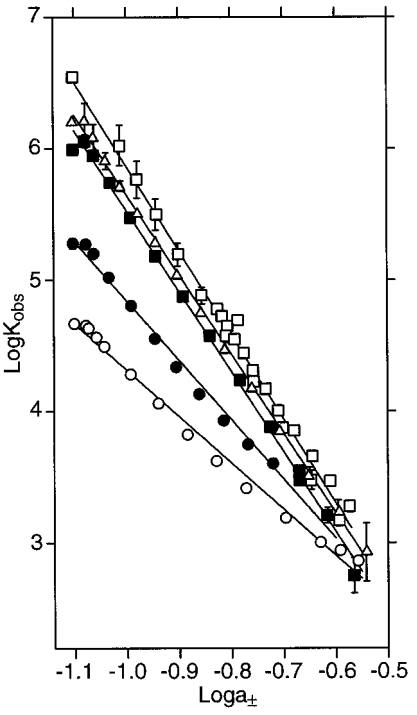

FIGURE 5 Log-log plot of K versus ionic activity ($a_\pm$) of sodium acetate for KWK_6-NH_2 binding to poly dT (□), $dT(pdT)_{69}$ (△), $dT(pdT)_{39}$ (■), $dT(pdT)_{15}$ (●), and $dT(pdT)_{10}$ (○). Parameters for the best linear fittings are summarized in Table 1. For comparison, linear fittings to the corresponding range of K versus $a_\pm$ for poly dT (□) and $dT(pdT)_{10}$ (○) from Zhang et al. (1996) are also shown.

release of thermodynamically accumulated anions from L^{8+} should be the same for binding of this ligand to any of the dT-mers. The large negative values of S_aK_{obs} for all dT-mers and the small value of K_o for polydT indicate that a reduction in salt concentration drives binding of KWK_6NH_2 to all dT-mers by the release of counterions accumulated in the vicinity of the reactants as a result of Coulombic interactions (Record et al., 1976, 1978; Lohman et al., 1980; Mascotti and Lohman, 1992).

Two-state interpretation of the Coulombic end effect on S_aK_{obs} and ΔG^o_{obs} of binding L^{8+} to oligo- and polyelectrolyte DNA

According to the two-state interpretation of Coulombic end effects on the thermodynamics of L^{Z_L} binding to an oligonucleotide (specified by Eqs. 4, 7, 9), both the standard binding free energy ΔG^o_{obs} and the exponent S_aK_{obs} describing the power dependence of K_{obs} on salt activity should approach the corresponding values for L^{Z_L} binding to a polyanionic lattice (poly dT) as a linear function of N_B^{-1}, the reciprocal of the number of L^{Z_L} binding sites per DNA oligoelectrolyte. Analyses of the binding data shown in Figs. 3 and 5 using this two-state model are shown in Fig. 6, *A* (S_aK_{obs}) and *B* (ΔG^o_{obs} at four salt concentrations between 0.1 and 0.3 M). Fig. 6, *A* and *B*, shows that values of S_aK_{obs} and ΔG^o_{obs} for binding L^{8+} to sufficiently long ss DNA oligoanions ($|Z_D| \geq 15$) and to polyanionic DNA are linear functions of $N_B^{-1} = (|Z_D| - 7)^{-1}$. Slopes and intercepts of these plots are summarized in Table 2. In each case, the thermodynamic data for the shortest oligonucleotide ($dT(pdT)_{10}$; $|Z_D| = 10$) deviate from the trend observed for the longer oligonucleotides. In the context of the two-state model for Coulombic end effects, we infer that $dT(pdT)_{10}$ is too short to have a polyion-like interior region.

The parameter N_E (as defined in Eq. 6) is evaluated from the data of Table 2 (cf. Fig. 6) by assuming that ΔG^o_E and S_aK_E may be approximated by the values determined by Zhang et al. (1996) for L^{8+} binding to $dT(pdT)_{10}$, and that ΔG^o_I and S_aK_I are determined by the intercepts (Table 2) at $N_B \rightarrow \infty$ of Fig. 6, which are the same within error as the values determined for poly dT. Application of Eqs. 7 and 9 to the data of Table 2 indicates that $N_E \cong 5$ sites, independent of salt concentration over the range 0.1–0.3 M. If there are $N_E/2 = 2$–3 such sites at each end of a sufficiently long oligonucleotide, and each site for L^{8+} consists of eight phosphates ($n = 8$), then in the two-state description the length of each terminal region is ~10 phosphates, which is

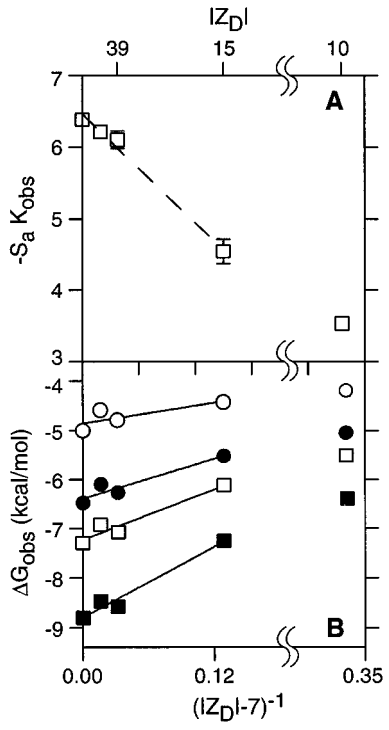

FIGURE 6 Variation with the reciprocal of the number of oligocation (L^{8+}) binding sites $N_B^{-1} = (|Z_D| - 7)^{-1}$ of (*A*) the exponent S_aK_{obs} describing the power dependence of the binding constant K on mean ionic activity of salt ($a_\pm$) from Fig. 5 and (*B*) standard binding free energies ΔG^o_{obs} (20°C) for salt concentrations of 0.1 M (■), 0.15 M (□), 0.2 M (●), and 0.3 M (○), respectively. These values of ΔG^o_{obs} were obtained by interpolation of the log K_{obs} shown in Fig. 5, using the best linear fitting parameters given in Table 1. Selected values of the number of dT-mer phosphates are plotted on the top nonlinear scale. Solid lines in *A* and *B* are generated by linear regression of the data ($|Z_D| \geq 15$). Slopes and intercepts are given in Table 2.

consistent with the range of the Coulombic end effect predicted by Olmsted (1991) on the basis of MC simulations on (uncomplexed) ss DNA at lower salt concentrations ($a_\pm$ = 2 mM, 12 mM). (These calculations were used to analyze salt effects on conformational transitions of oligonucleotides (Olmsted et al., 1991) and therefore pertain to a model of ss DNA with no bound ligands.)

Although both the two-state model and the assumptions regarding use of the L^{8+} − $dT(pdT)_{10}$ binding data to evaluate ΔG^o_E and S_aK_E are highly simplified, the observations regarding the $|Z_D|$ independence, salt concentration insensitivity, and axial range of the Coulombic end effect are in qualitative accord with the available MC simulations on ds and ss DNA (Olmsted et al., 1989, 1991, 1995; Olmsted, 1991). Thus far, no calculations based on MC simulations or any other method have addressed the problem of calculating S_aK_{obs} for nonspecific oligocationic binding to the total ensemble of potential binding sites whose affinities vary with position on an oligoanion of length not much greater than that of the ligand. In particular, the pioneering computational study of Olmsted et al. (1995) presented MC predictions of S_aK_{obs} for only one example of a ligand-oligonucleotide complex, in which ligands bind specifically at the center of the DNA lattice. No such constraint on the positioning of the bound ligand exists for the nonspecific binding process that we now have studied experimentally by fluorescence quenching. In addition, MC calculations by Olmsted et al. (1995) were performed for duplex DNA and at salt concentrations (1–12 mM) that are lower than those where equilibrium binding experiments have proved to be feasible. Consequently, although those calculations correctly predicted the importance of Coulombic end effects for binding of an oligocation to DNA oligomers, they cannot be compared directly with the results of experiments reported here, which are analyzed using the two-state (end, interior) model. This analysis indicates that $dT(pdT)_{15}$ has no polyion-like interior binding sites (N_I = 0), so that any site occupied by L^{8+} is at least partly within a terminal region. Therefore, the slopes in Fig. 6, *A* and *B*, may yield only a minimum estimate of the magnitude of N_E. To refine these estimates, further experiments and detailed analyses by PB and MC calculations are in progress.

SUMMARY

In this study and a previous one (Zhang et al., 1996), we have quantified two large Colombic end effects on the DNA binding of an oligocation (hence, by implication, any oligocationic DNA binding site on a protein surface). Zhang et al. (1996) demonstrated the profound asymmetry between the two contributions to S_aK_{obs}: ~30% from anion release from an oligocationic ligand versus ~70% from cation release from polyanionic DNA. The substantial difference between these two contributions to S_aK_{obs} arises because the oligocationic ligand is short enough (i.e., has a small enough number of charges $|Z_D|$) so that it exhibits a relatively small extent of counterion (anion) accumulation per structural charge. By comparison, DNA is a polyanion with large $|Z_D|$ that therefore exhibits a larger extent of counterion (cation) accumulation per structural charge. This phenomenon is not a trivial consequence of the greater number of monomeric charges on a polyanion as compared with a relatively short oligoanion having the same average axial charge density. The small extent of counterion accumulation near an oligocationic ligand of the type studied here itself is a consequence of Coulombic end effects. When an oligocation is too short to exhibit a polyion-like interior region, the electric field due to the superposition of Coulombic interactions involving its structural charges does not drive extensive counterion (i.e., anion) accumulation.

Our application of a two-state model to quantify the range of the Coulombic end effect in single-stranded DNA indicates that sites within the first ~10 phosphates at each end of ss DNA of any length have an (average) oligocation binding affinity that is significantly lower than that of the interior phosphates. The ratio of affinities for interior compared with terminal sites increases strongly with decreasing salt concentration below ~0.3 M. Even for relatively long

TABLE 2 Two-state parameters for the dependences of S_aK_{obs} and ΔG^o_{obs} (20°C) on the reciprocal of the number of ligand binding sites on DNA oligomers ($|Z_D| \geq 15$)

S_aK_{obs} (Fig. 6 *A*)				
$[Na^+]$ (M)	S_aK_I (intercept)	$N_E(S_aK_E - S_aK_I)$ (slope)	$S_aK_E - S_aK_I$	N_E
0.1–0.3	−6.40 ± 0.04	15.1 ± 0.7	2.9 ± 0.1	5.2 ± 0.3
ΔG^o_{obs} (Fig. 6 *B*)				
	ΔG^o_I (intercept)	$N_E(\Delta G^o_E - \Delta G^o_I)$ (slope)	$\Delta G^o_E - \Delta G^o_I$	
0.10	−8.75 ± 0.08	12.0 ± 1.0	2.4 ± 0.1	5.0 ± 0.4
0.15	−7.18 ± 0.10	8.5 ± 1.2	1.7 ± 0.1	5.1 ± 1.1
0.20	−6.34 ± 0.11	6.6 ± 1.3	1.3 ± 0.2	5.1 ± 1.6
0.30	−4.81 ± 0.13	3.2 ± 1.6	0.6 ± 0.2	5.1 ± 3.9

ss oligonucleotides (e.g., $|Z_D| = 39$ to 69 phosphates), the Coulombic end effect significantly reduces the average binding strength (i.e., the magnitude of K_{obs}) at low salt concentrations and somewhat reduces the average extent of thermodynamic ion release (i.e., the magnitude of S_aK_{obs}) upon complexation. For binding of L^{8+} to ds oligonucleotides, the Coulombic end effect is expected to be detectable even for $|Z_D|$ larger than 69, because of the twofold larger axial range predicted for the diminution of the electric field in the terminal regions (20 phosphates versus 10 phosphates; Olmsted et al., 1989; Olmsted, 1991). Therefore, Coulombic end effects should be taken into account in the analysis and interpretation of [salt]-dependent effects on protein-DNA binding in systems where oligonucleotides are used as models for polyanionic DNA, especially in the typical experimental range of salt concentrations. In work in progress, the values of S_aK_{obs} determined here are being compared with corresponding theoretical predictions obtained by using Poisson-Boltzmann calculations and Monte Carlo simulations to implement the thermodynamic analysis of S_aK_{obs} of ligand-polyion binding presented by Anderson and Record (1993).

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