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Thermodynamic extent of counterion release upon binding oligolysines to single-stranded nucleic acids

(polyelectrolytes/protein–nucleic acid interactions/salt effects)

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ABSTRACT **A major contribution to the binding free energy associated with most protein–nucleic acid complexes is the increase in entropy due to counterion release from the nucleic acid that results from electrostatic interactions. To examine this quantitatively, we have measured the thermodynamic extent of counterion release that results from the interaction between single-stranded homopolynucleotides and a series of oligolysines, possessing net charges z = 2–6, 8, and 10. This was accomplished by measuring the salt dependence of the intrinsic equilibrium binding constants—i.e., ($\partial \log K_{obs}/\partial \log[K^+]$)—over the range from 6 mM to 0.5 M potassium acetate. These data provide a rigorous test of linear polyelectrolyte theories that have been used to interpret the effects of changes in bulk salt concentration on protein–DNA binding equilibria, since single-stranded nucleic acids have a lower axial charge density than duplex DNA. Upon binding to poly(U), the thermodynamic extent of counterion release per oligolysine charge, z, is 0.71 ± 0.03, which is significantly less than unity and less than that measured upon binding duplex DNA. These results are most simply interpreted using the limiting law predictions of counterion condensation and cylindrical Poisson–Boltzmann theories, even at the high salt concentrations used in our experiments. Accurate estimates of the thermodynamic extent of counterion binding and release for model systems such as these facilitate our understanding of the energetics of protein–nucleic acid interactions. These data indicate that for simple oligovalent cations, the number of ionic interactions formed in a complex with a linear nucleic acid can be accurately estimated from a measure of the salt dependence of the equilibrium binding constant, if the thermodynamic extent of ion release is known.**

The interactions of proteins with nucleic acids are central to the control of gene expression and nucleic acid metabolism. A detailed understanding of how these processes are regulated requires information about the equilibrium affinity and pathways of association and dissociation of the protein–nucleic acid complexes involved. Structural data can provide information concerning the contacts made within protein–nucleic acid complexes; however, thermodynamic information is necessary to understand the stability of these complexes. One general feature of protein–nucleic acid interactions is that they are highly salt-dependent *in vitro*, such that the observed affinity decreases dramatically with increasing salt concentration. This phenomenon is observed for the interaction of any positively charged ligand with a linear nucleic acid and results from the polyelectrolyte nature of a linear nucleic acid (1–5). It has been demonstrated that the high electrostatic potential from the negatively charged backbone of a linear nucleic acid results in the accumulation of counterions (e.g., K^+) in the immediate vicinity of the nucleic acid to partially neutralize the closely spaced backbone phosphates (for a review, see ref. 2). The interaction of a positively charged ligand with the nucleic acid causes a perturbation of the electrostatic potential surrounding the nucleic acid with the result that some fraction of counterions are released into the bulk solution. The release of these counterions into a solution of low salt concentration causes a net increase in the entropy of the system, thus providing a major favorable component to the interaction free energy (1). The importance of this entropic contribution cannot be gleaned from purely structural studies. The thermodynamic extent of ion release upon formation of a ligand–nucleic acid complex can be estimated from the monovalent salt (MX) dependence of the equilibrium constant, K_{obs}, for formation of the complex, i.e., ($\partial \log K_{obs}/\partial \log[MX]$) (1).

A number of theoretical studies have sought to obtain a quantitative molecular interpretation of these dramatic salt effects (1, 3–7). Two of these were based on counterion condensation (CC) models that describe the electrostatic interaction of counterions with linear nucleic acids (1, 5). The CC models (5, 8) treat the nucleic acid as a uniform line charge and predict that a constant fraction of the phosphate charges is neutralized by the delocalized binding (condensation) of counterions. The extent of counterion condensation per phosphate, which is predicted by CC theory, is a function only of the linear charge density of the nucleic acid, the counterion charge, and the dielectric constant of the solvent and is independent of the bulk salt concentration (8). Manning's original CC theory (8) is strictly valid only as a limiting law (i.e., in the limit of zero salt concentration); however, a number of experimental studies suggest (1, 9) that many of the predictions are valid at much higher salt concentrations.

Record *et al.* (1) proposed that the binding of a ligand of charge $+z$ to a linear nucleic acid would neutralize z phosphates, resulting in the release of the counterions that had been thermodynamically associated with those z phosphates. These thermodynamically bound ions include those counterions that are physically associated with the nucleic acid, although in rapid exchange, as well as those ions involved in "screening" the z phosphates (1). The "screening" ions are those that are perturbed electrostatically by the charge that remains on the z phosphates after counterion binding (1, 8). In the absence of preferential ion effects associated with the ligand and hydration effects, Record *et al.* (1) predict that the salt dependence of the intrinsic equilibrium binding constant, K_{obs}, for a ligand with charge $+z$, can be described by Eq. **1**, over a wide range of salt concentrations.

$$\partial \log K_{obs}/\partial \log[M^+] = -z\psi. \qquad [1]$$

That is, $z\psi$ counterions should be thermodynamically "released" into solution, where ψ is the fraction of a counterion

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Abbreviations: ss, single stranded; CC, counterion condensation; KWK_p-NH_2, L-Lys-L-Trp-(L-Lys)$_p$-NH_2.

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thermodynamically associated per phosphate in the absence of the ligand. Theoretical predictions of the thermodynamic extent of counterion association, ψ, were first made by Record *et al.* (1), using the limiting-law CC model (8). However, identical analytical expressions for ψ have been obtained based on the cylindrical Poisson–Boltzmann cell model (7, 10). Since the Poisson–Boltzmann approach does not invoke the hypothesis of counterion condensation, Eq. **1** is a general limiting-law expression. In aqueous solution containing only monovalent cations, ψ is predicted to equal 0.88 counterions per phosphate for duplex B-form DNA (1).

Alternatively, Manning (5), using a subsequent formulation of the CC model that is based on a molecular thermodynamic two-phase model (11), predicts that z counterions should be released upon binding a ligand of charge $+z$ to a linear nucleic acid, as stated in Eq. **2**.

$$\partial \log K_{obs}/\partial \log[M^+] = -z. \quad [2]$$

As formulated by Manning (5), the release of these z counterions is independent of the conformation of the nucleic acid, i.e., duplex or single stranded (ss). Daune (3), using a different approach, also obtained a result equivalent to Eq. **2**. We emphasize that K_{obs} in both Eqs. **1** and **2** represents the intrinsic equilibrium binding constant obtained upon extrapolation to zero binding density.

Most experimental studies of the salt dependence of the binding of simple ligands of known positive charge to linear nucleic acids have been performed with duplex DNA (1, 12–15), so that the predictions of Eqs. **1** and **2** differ by only 12%, which is usually within the experimental limits of the measurements. Although most of these studies indicate that $(\partial \log K_{obs}/\partial \log[M^+]) < z$, an unequivocal experimental test of the predictions of the two approaches requires the use of a linear nucleic acid with a lower axial charge density. The ss homopolynucleotides possess average axial charge spacings that are significantly lower than for duplex DNA, with values of ψ ranging from 0.68 to 0.78 monovalent cations per phosphate (16). Therefore, we have carried out quantitative measurements of the monovalent salt dependence of K_{obs} for the interaction of ss homopolynucleotides with a series of oligolysines containing a single tryptophan, with sequences, L-Lys-L-Trp-(L-Lys)$_p$-NH$_2$ (KWK$_p$-NH$_2$), where $z = p + 2$, when fully protonated. The predictions of Eqs. **1** and **2** differ by 22–32% for the binding of these peptides to ss homopolynucleotides, which should be easily measurable. These data will also facilitate the interpretation of salt effects on protein–ss nucleic acid interactions.

MATERIALS AND METHODS

Reagents and Buffers. All chemicals were reagent grade and buffers were prepared with doubly distilled-deionized (Milli-Q, Millipore) H_2O. Buffer CK contained 10 mM cacodylic acid and 0.2 mM Na_3EDTA, titrated with KOH to pH 6.0 [5.2 mM ($Na^+ + K^+$)]. Buffer CN contained 10 mM sodium cacodylate and 0.2 mM Na_3EDTA, titrated with HCl or CH_3CO_2H to pH 6.0 (10.6 mM Na^+).

Polynucleotides. Poly(dT) ($s_{20,W}$ = 10.1 S) and poly(dU) (10 S) were from Midland Certified Reagent (Midland, TX); poly(U) (9.5 S) was from Boehringer Mannheim; poly(A) (7.8 S) and poly(C) (7.8 S) were from Pharmacia. All polynucleotides were dialyzed extensively against the desired buffer and concentrations were determined spectrophotometrically (17, 18).

Oligopeptides. Peptides were synthesized as C-terminal amides [KWK$_p$-NH$_2$ (p = 1, 2, 4, 6, and 8)] or with free carboxyl groups [KWK$_p$-CO$_2$ (p = 1, 4)] on a Biosearch 9500 synthesizer using solid-phase t-Boc methods (Texas Agricultural Experiment Station Biotechnology Laboratory, Texas A&M) and purified by HPLC on a semi-preparative C_{18} μBondapak column (Waters), using water/acetonitrile gradients in the presence of 10 mM heptafluorobutyrate. Peptide purity was determined by HPLC and composition was verified by fast atom bombardment mass spectrometry (University of Texas Medical Center, Houston). Peptide stock concentrations were determined by measuring tryptophan absorbance in 6.0 M guanidine hydrochloride/20 mM Tris, pH 6.8 at 25°C, by using ε_{280} = 5690 $M^{-1} \cdot cm^{-1}$ (19).

Fluorescence Measurements and Construction of Binding Isotherms. Tryptophan fluorescence quenching was used to monitor peptide binding to ss nucleic acids (20, 21), using an SLM-Aminco 8000 (Urbana, IL) fluorometer (λ_{ex} = 292 nm; λ_{em} = 350 nm). Titrations of peptide with concentrated polynucleotide were performed with corrections for dilution and inner-filter effects as described (18). The method of Bujalowski and Lohman (22) was used to obtain model-independent estimates of the peptide binding density, ν (peptides bound per nucleotide), and the free peptide concentration (L_F) at each point in a titration. By using this method (22), Q_{obs} (observed fluorescence quenching) was found to be directly proportional to L_B/L_T (where L_B is the concentration of bound peptide and L_T is the concentration of total peptide) over the range of binding densities that was covered and Q_{max} (the fluorescence quenching when all of the peptide is bound) was determined from a linear extrapolation of Q_{obs} to L_B/L_T = 1. Since $Q_{obs}/Q_{max} = L_B/L_T$ for each peptide–homopolynucleotide interaction studied here, values of ν and L_F at any point in a titration can be calculated, using Eq. **3** (22);

$$\nu = (Q_{obs}/Q_{max})\,(L_T/D_T) \quad [3a]$$

and

$$L_F = (1 - Q_{obs}/Q_{max})L_T, \quad [3b]$$

from which binding isotherms can be constructed from a single titration, where D_T is the total nucleotide concentration. Aggregation of the free peptides was not observed as judged by fluorescence polarization measurements.

Equilibrium Binding Constants. The intrinsic equilibrium binding constants are defined as K_{obs} = [LD]/[L][D], where LD, L, and D are the peptide–polynucleotide complex, free peptide, and free peptide binding sites (nucleotides) on the polynucleotide, respectively. Values of K_{obs} were obtained in the zero-binding density limit, based on the noncooperative model of McGhee and von Hippel (23). The site sizes n (i.e., number of nucleotides occluded by the bound peptide) for each peptide were estimated from a linear extrapolation of the low binding density values of ν/L on a Scatchard plot (see Fig. 1) to ν/L = 0; the point of intersection on the ν axis is equal to $(2n - 1)^{-1}$ (23). For each peptide, n was equal to the number of amino acids in the peptide. K_{obs} was then obtained by comparing the experimental binding isotherm to theoretical isotherms (23),

$$\nu/L_F = K_{obs}\,(1 - n\nu)\,\{(1 - n\nu)/[1 - (n-1)\nu]\}^{(n-1)}, \quad [4]$$

generated using Eq. **4** with K_{obs} as the sole parameter. The dependences of K_{obs} on salt concentration were obtained by performing separate titrations at different salt concentrations or by performing "salt-back" titrations as described (18). The latter approach was only possible since $Q_{obs}/Q_{max} = L_B/L_T$ and Q_{max} and n are independent of salt concentration. The value of Q_{obs} at each point in the salt-back titration was used to calculate K_{obs} at that [K^+], using Eqs. **3** and **4**.

RESULTS

Characterization of Peptide–ss Homopolynucleotide Binding. An equilibrium binding isotherm for the interaction of the peptide, KWK_2-NH_2 with poly(U) in buffer CN+27 mM NaCl is shown in Fig. 1. This isotherm was constructed from a binding density function analysis (22) of seven titrations at peptide concentrations ranging from 1.4 to 6.0 μM. A theoretical isotherm generated using Eq. 4 with $n = 4$ and $K_{obs} = 5.4 \times 10^4$ M^{-1} is also shown in Fig. 1, indicating that a noncooperative binding isotherm describes the data well, in agreement with previous studies of oligopeptide–duplex DNA interactions (13). All values of K_{obs} reported in this study were determined from an extrapolation to zero binding density from isotherms that cover a low range of binding densities (<30% saturation of the polynucleotide). This was necessary to avoid compaction and eventual precipitation of the peptide–polynucleotide complex that occurs at higher binding densities (D.P.M., unpublished results).

Preferential Anion Effects Are Negligible in Acetate Salts for Oligolysine–ss Polynucleotide Binding. In general, (∂log K_{obs}/∂log[MX]) is a measure of the difference in the thermodynamic degree of association (preferential interaction) of cations, anions, and water between the product and reactant species (4, 24). Therefore, this quantity is a measure of the thermodynamic extent of counterion release from the polynucleotide only when preferential anion interactions and preferential hydration are negligible. A previous study (12) suggested that preferential anion interactions are not significant for pentalysine binding to duplex DNA; however, we examined this further by measuring the salt dependence of K_{obs} for the poly(U)–KWK_4-NH_2 ($z = +6$) interaction for a series of monovalent salts, differing in the anion. The results, plotted in Fig. 2, indicate that preferential anion interactions do not differ significantly among Cl^-, acetate, Br^-, and F^-. However, experiments with KWK_8-NH_2 ($z = +10$), which were obtained at higher salt concentrations (0.25–0.5 M), show that K_{obs} is slightly lower in the presence of Cl^- than in acetate or F^- and the values of (∂log K_{obs}/∂log[MX]) are slightly more negative (by less than 10%) in the chloride salts. From this, we conclude that preferential interactions of acetate and F^- with the oligopeptides studied here are negligible. As a result, we have used KCH_3CO_2 to vary the monovalent counterion concentration, since in KCH_3CO_2, (∂log K_{obs}/∂log[K^+]) should only reflect counterion (K^+) release from the polynucleotide. We note that the data in Fig. 2 for NaCl and KCl are superimposable indicating that for this system, preferential binding to poly(U) of Na^+ and K^+ does not differ.

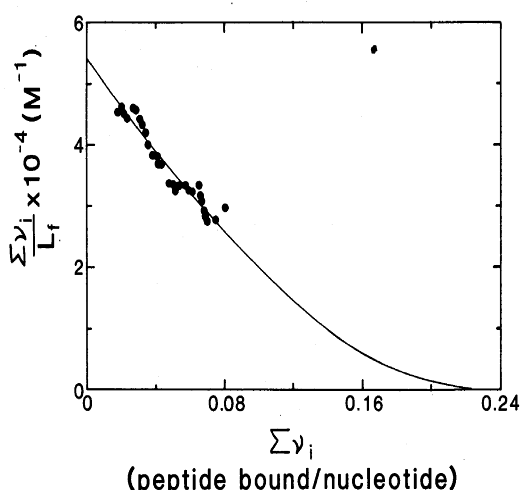

FIG. 1. Equilibrium binding isotherm constructed from a general method (22) for the binding of KWK_2-NH_2 to poly(U) [buffer CN+27 mM NaCl (37 mM Na^+), 25.0°C, pH 6.0]. The smooth curve was generated using the noncooperative model of McGhee and von Hippel (23) with $n = 4$ and $K_{obs} = 5.4 \times 10^4$ M^{-1}.

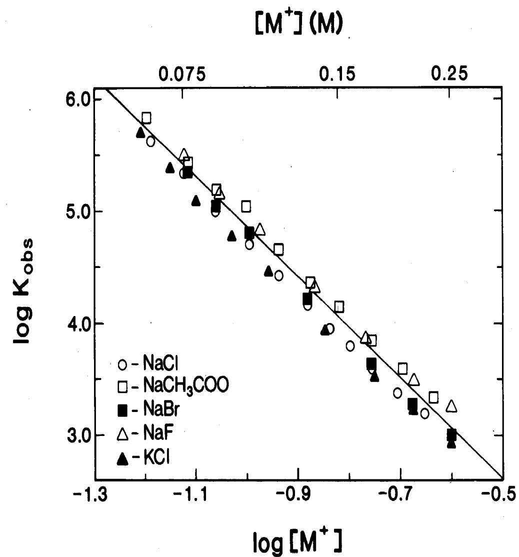

FIG. 2. Dependence of K_{obs} on total monovalent counterion concentration, M^+, for the interaction of KWK_4-NH_2 with poly(U) (25.0°C, pH 6.0) in the presence of monovalent salts differing in the anion. A linear least squares line {log K_{obs} = 0.4(± 0.4) − 4.5(± 0.4)log[M^+]} is shown.

The Thermodynamic Extent of Counterion Release Is Less Than One Per Oligolysine Net Charge. For the binding to poly(U), we have measured K_{obs} as a function of [K^+] for the series of peptides, KWK_p-NH_2, with p = 1, 2, 4, 6, and 8, at 25.0°C and pH 6.0 by using KCH_3CO_2 to vary the salt concentration. These peptides possess only formal positive charges with z = +3, +4, +6, +8, and +10, respectively, when fully protonated at pH 6.0 (25). We have also measured K_{obs} for the zwitterionic peptides, KWK_p-CO_2, with p = 1 and 4 (z = +2 and +5, respectively, at pH 6.0). These binding constants are plotted as a function of [K^+] in Fig. 3. Within experimental error, log K_{obs} is a linear function of log[K^+] for each peptide, and the value of −(∂log K_{obs}/∂log[K^+]) is significantly lower than the net peptide charge z, as summarized in Table 1.

In Fig. 4, we have plotted {−(∂log K_{obs}/∂log[K^+])} as a function of the net charge on the peptide. Within experimental error, the thermodynamic extent of counterion release

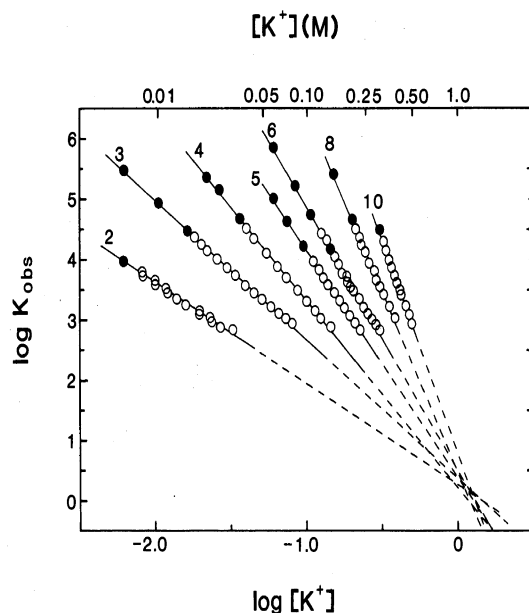

FIG. 3. Dependence of log K_{obs} on log[K^+] for a series of positively charged oligopeptides binding to poly(U) (25.0°C, buffer CK, pH 6.0). The data are plotted as a function of the total potassium concentration. KCH_3CO_2 was used to vary the K^+ concentration. The peptides were KWK_p-NH_2, with p = 1, 2, 4, 6, and 8 (z = +3, 4, 6, 8, and 10), and KWK_p-CO_2, with p = 1, 4 (z = +2 and +5). The net positive charge of the peptide is indicated for each line. ●, Data from titrations at a constant salt concentration; ○, data from salt-back titrations. Linear least square lines are shown (see Table 1 for the equations). Dashed lines are extrapolations of the least square (solid) lines.

Table 1. Effect of oligopeptide charge on the salt dependence for binding to poly(U)

Peptide	z	$(\partial \log K_{obs}/\partial \log[K^+])$	$\log K_{obs}$(1 M KCH_3CO_2)
KWK-CO_2	2	−1.68 (± 0.20)	0.26 (± 0.24)
KWK-NH_2	3	−2.30 (± 0.19)	0.36 (± 0.24)
KWK_2-NH_2	4	−3.10 (± 0.21)	0.20 (± 0.22)
KWK_4-CO_2	5	−3.76 (± 0.22)	0.37 (± 0.22)
KWK_4-NH_2	6	−4.36 (± 0.22)	0.49 (± 0.22)
KWK_6-NH_2	8	−5.95 (± 0.25)	0.46 (± 0.24)
KWK_8-NH_2	10	−7.02 (± 0.34)	0.77 (± 0.27)

Buffer CK (pH 6.0, 25.0°C) using KCH_3CO_2 to vary the counterion concentration. Numbers in parentheses are SEM.

from poly(U) is directly proportional to z, even for the zwitterions, with the average value of $\{-(1/z)(\partial \log K_{obs}/\partial \log[K^+])\} = 0.74 \pm 0.04$. This indicates that there is less than one counterion thermodynamically released per phosphate from poly(U) upon binding these z-valent peptides. The solid line describing the data in Fig. 4 has been constrained to intersect at the origin, whereas the linear least-squares (dashed line) has a slope of 0.68, with a nonzero intercept of +0.33. A nonzero intercept might be expected if the tryptophan in each peptide can intercalate between two consecutive bases, thereby changing the axial charge spacing and resulting in an additional small extent of counterion release (26). However, within the uncertainty of the data, this intercept is not significantly different from zero. As a result, however, we can only state that the thermodynamic extent of counterion release per oligopeptide net charge falls within the range from 0.68 to 0.74.

Similar measurements with other ss homopolynucleotides indicate that the thermodynamic extent of counterion release upon binding KWK_4-NH_2 is also significantly less than z (data not shown). The experimental values of $(-1/z)(\partial \log K_{obs}/\partial \log[K^+])$ for poly(dU) and poly(U) are similar (≈0.74 ± 0.04), whereas the value for poly(dT) is slightly smaller (≈0.68 ± 0.04) and the values for poly(A) and poly(C) are slightly larger (≈0.77 ± 0.04). Although the differences among these values are within our experimental error, each is clearly less than unity.

We have also measured the dependence of K_{obs} on $[Na^+]$ for the binding of KWK_4-NH_2 (z = +6) to duplex plasmid DNA (pUC8) at 25°C and pH 6.0 by using NaCl to vary the salt concentration. The value of $(\partial \log K_{obs}/\partial \log[Na^+]) = -5.7 \pm 0.5$ indicates that the thermodynamic extent of counterion release is greater than from any of the ss homopolynucleotides. This is expected if the higher linear

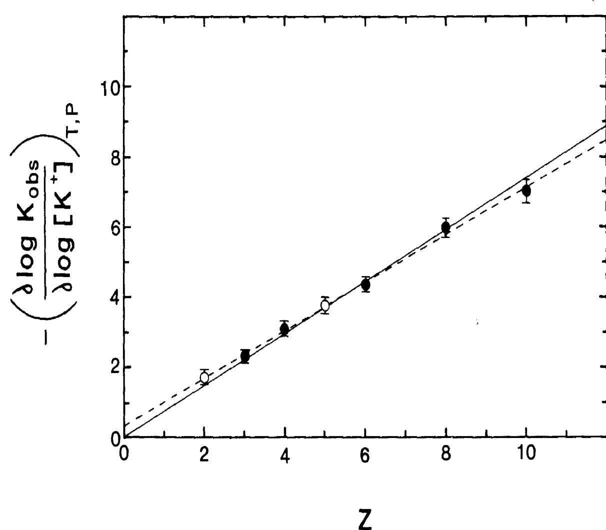

FIG. 4. Thermodynamic extent of ion release $\{-(\partial \log K_{obs}/\partial \log[K^+])\}$ is proportional to the net positive charge z on each peptide (data from Fig. 3). ●, C-terminal amidated peptides, KWK_p-NH_2; ○, zwitterions, KWK_p-CO_2. The solid line was constrained to intersect at the origin (slope = 0.74 ± 0.04), whereas the linear least squares dashed line has a slope and intercept of 0.68 and 0.33, respectively.

charge density that exists for duplex B-form DNA (ψ = 0.88) influences the extent of thermodynamic counterion release.

DISCUSSION

The Thermodynamic Extent of Counterion Release Is Determined by the Oligopeptide Charge and the Linear Charge Density of the Nucleic Acid. Most previous studies of the salt dependences of the binding of well-defined oligovalent cations to nucleic acids have been performed with duplex DNA (1, 12–15). These studies indicate that the thermodynamic extent of counterion release is proportional to the charge on the oligopeptide, z. However, the high charge density of duplex DNA made it difficult to determine whether the proportionality constant differs from unity. We have used ss homopolynucleotides in the studies reported here, since these possess a lower axial charge density than duplex DNA, thus making it easier to answer this question. The results show definitively that upon formation of a complex between a positively charged oligopeptide and a ss polynucleotide, in the zero binding density limit, the thermodynamic extent of counterion release, as measured by $|(\partial \log K_{obs}/\partial \log[K^+])|$, is less than, although proportional to, the net charge on the oligopeptide. Furthermore, the salt dependence is significantly larger, although still less than z, for the binding of these oligopeptides to duplex DNA, consistent with its higher charge density (16, 27, 28).

Comparison with Theoretical Models. The results presented here can be used to test various predictions of the extent of counterion release upon binding of a charged ligand to a linear nucleic acid. The data in Figs. 3 and 4 are in quantitative agreement with the interpretation of cation effects on charged ligand–nucleic acid equilibria given by Record *et al.* (1) (see Eq. **1**). On the other hand, these data are qualitatively but not quantitatively consistent with the predictions of Manning (5), Friedman and Manning (6), and Daune (3) (see Eq. **2**). Although these data for the interactions of ss homopolynucleotides with oligopeptides possessing net charges of $2 \leq z \leq 10$ are clearly most consistent with the model proposed by Record *et al.* (1), it is possible that z-valent ligands that bind in different modes may yield different quantitative salt dependences.

The dramatic decrease in K_{obs} with increasing salt concentration observed for these oligolysine–ss nucleic acid interactions results from the increase in entropy upon release of counterions from the nucleic acid into a solution of low salt concentration (1). These salt effects, as well as those observed for protein–nucleic acid interactions, result from the direct binding of cations to the polynucleotide and cannot be explained by models that treat the effects of salt as a purely electrostatic "screening" or ionic strength phenomenon. This has been clearly demonstrated by a number of studies of the effects of mixtures of mono- and divalent cations on the binding of charged ligands to linear nucleic acids (12, 29, 30) and is also true for the oligopeptides studied here (D.P.M., unpublished experiments). Therefore, any theoretical explanation of these salt effects must account for the direct binding of ions to the macromolecules.

The thermodynamic predictions of the limiting-law CC (8) and limiting-law Poisson–Boltzmann cell models (7, 10) are theoretically valid only in the limit of zero salt concentration, as long as the counterion concentration remains in excess over the polyelectrolyte structural charges. Therefore, use of these limiting laws to interpret the results of experiments performed at higher salt concentrations, such as those reported here, is an approximation. In addition, the analysis of Record *et al.* (1), as well as that of Manning (5), also neglects end effects in the vicinity of the bound ligand and assumes that the activity coefficients of the ligand and counterions cancel (1, 4). However, it remains that the experimental

results reported here are described very well by these limiting laws (7, 8, 10). In fact, the data shown in Figs. 3 and 4, which were obtained over the range from 6 mM to 0.5 M K^+, show no change in the thermodynamic extent of counterion release per net peptide charge, within experimental error. The reasons for this excellent agreement between the limiting-law theories and experiment require further study; however, it may be due to salt-dependent terms that are not considered in the limiting-law theories but that are nearly compensating at higher salt concentrations (31).

In spite of these caveats, the data reported here indicate that the application of the limiting-law CC or Poisson–Boltzmann cell model, as described by Record *et al.* (1), provides a simple and accurate description of the quantitative effects of changes in counterion concentration on the equilibrium binding of charged ligands to linear nucleic acids. These measurements on simple z-valent peptides will enable rigorous tests of theoretical approaches to the interpretation of salt effects on charged ligand–nucleic acid interactions.

Preferential Anion Effects Are Minimized in Acetate and Fluoride Salts. As expected from previous studies of similar peptides (12), we have observed only minor anion effects on the oligolysine–ss homopolynucleotide interactions. The only significant effects are for the peptide KWK_8-NH_2 with $z = +10$, which displays a decrease in K_{obs} and a slightly larger salt dependence in chloride salts. However, the data in fluoride and acetate are identical within experimental error, from which we conclude that preferential anion effects are negligible in acetate and fluoride salts. The preferential binding of anions to several nucleic acid binding proteins also decreases in the order $Br^- > Cl^- >$ acetate $\approx F^- \approx$ glutamate (18, 32–35), although the effects are much larger than with the peptides. A similar ranking ($Br^- > Cl^- > F^-$) was observed for the interaction of these anions with polyacrylamide, which was used as a model for the peptide backbone (36). All of the above studies suggest that acetate, glutamate, or fluoride salts should minimize preferential anion binding in protein–nucleic acid interactions. Since glutamate is one of the major monovalent anions in *Escherichia coli*, it has been suggested that the use of glutamate or acetate salts *in vitro* might provide a more appropriate comparison with interactions *in vivo* (34, 37).

Our determinations of the thermodynamic extent of counterion release have been made using salt concentrations rather than activities. The rationale for this has been discussed (1, 2, 4) and is based on the fact that the values of the activity coefficients of the oligopeptides or their dependence on salt concentration are not known. Since this information is usually lacking, especially for proteins, it has been argued that $(\partial \log K_{obs}/\partial \log[M^+])$ is the more appropriate measure of the thermodynamic extent of ion release (1, 2, 4). However, upon plotting the data in Fig. 3 as a function of log $a_\pm$, rather than $\log[K^+]$, the slopes, normalized by z, are still significantly less than one, therefore, the main conclusion presented here is independent of this consideration.

Relationship to Protein–ss Nucleic Acid Binding. The model studies reported here will facilitate the quantitative interpretation of equivalent data for protein–nucleic acid equilibria, although clear differences exist. For instance, although the salt dependence of K_{obs} is determined by the net charge of the oligopeptides, this is not the case for protein–nucleic acid interactions, since many nucleic acid binding proteins have a net negative charge at pH ≥ 7 and yet still bind strongly to the negatively charged nucleic acid.

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