Thermodynamics of Oligoarginines Binding to RNA and DNA†

David P. Mascotti‡ and Timothy M. Lohman*

Department of Biochemistry and Molecular Biophysics, Box 8231, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110

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ABSTRACT: We have examined the equilibrium binding of a series of synthetic oligoarginines (net charge $z = +2$ to $+6$) containing tryptophan to poly(U), poly(A), poly(C), poly(I), and double-stranded (ds) DNA. Equilibrium association constants, K_{obs}, measured by monitoring tryptophan fluorescence quenching, were examined as functions of monovalent salt (MX) concentration and type, as well as temperature, from which $\Delta G°_{obs}$, ΔH_{obs}, and $\Delta S°_{obs}$ were determined. For each peptide, K_{obs} decreases with increasing $[K^+]$, and the magnitude of the dependence of K_{obs} on $[K^+]$, $\partial \log K_{obs}/\partial \log[K^+]$, increases with increasing net peptide charge. In fact, the values of $\partial \log K_{obs}/\partial \log[K^+]$ are equivalent for oligolysines and oligoarginines possessing the same net positive charge. However, the values of K_{obs} are systematically greater for oligoarginines binding to all polynucleotides, when compared to oligolysines with the same net charge. The origin of this difference is entirely enthalpic, with ΔH_{obs}, determined from van't Hoff analysis, being more exothermic for oligoarginine binding. The values of ΔH_{obs} are also independent of $[K^+]$; therefore, the salt concentration dependence of $\Delta G°_{obs}$ is entirely entropic in origin, reflecting the release of cations from the nucleic acid upon complex formation. These results suggest that hydrogen bonding of arginine to the phosphate backbone of the nucleic acids contributes to the increased stability of these complexes.

Protein−nucleic acid interactions are central to most aspects of gene regulation as well as DNA metabolism. In order to understand how these proteins function, it is necessary to know not only the nucleic acid sites to which they bind but also the affinities and the kinetics of these interactions. An understanding of the molecular basis for the stabilities of these complexes requires information on the thermodynamics of the interactions. The stabilities of most protein−nucleic acid complexes, both sequence specific and nonspecific, have large contributions from electrostatic interactions [for reviews, see Record et al. (1978, 1991) and Lohman and Mascotti (1992a)] stemming from the fact that DNA and RNA possess highly negatively charged phosphate backbones, and the nucleic acid binding sites of these proteins generally possess positively charged amino acids. Attempts to understand the origins and magnitudes of the electrostatic contributions to complex protein−nucleic acid systems have been greatly aided by studies of the energetics of binding (thermodynamics) of well-defined model peptides to nucleic acids [for a review, see Lohman and Mascotti (1992a)].

Previous thermodynamic studies of positively charged oligopeptides binding to both single-stranded (ss) and duplex DNA and RNA have demonstrated that the major driving force for forming such complexes results from the thermodynamic release of cations (e.g., Na^+, K^+, and Mg^{2+}) previously bound to the nucleic acid, which is primarily an entropic effect (Record et al., 1976; Mascotti & Lohman, 1990, 1992, 1993; Lohman et al., 1980). Such cation release also contributes to the stability of protein−nucleic acid interactions (de Haseth et al., 1977; Record et al., 1977, 1978), although ion binding and release from the protein can also play large roles (Overman et al., 1988; Overman & Lohman, 1994; Lohman et al., 1996). Nearly all thermodynamic studies of charged peptides binding to DNA and RNA have used oligolysines, yet many proteins also contain arginine in their nucleic acid binding sites. In addition to carrying a positive charge, arginine also possesses a guanidinium group capable of forming bifurcated hydrogen bonds (Cotton et al., 1973), which can potentially stabilize the protein−nucleic acid complex (Helene & Maurizot, 1981; Helene & Lancelot, 1982). In fact, it has been shown that arginine, within the dipeptide Arg-Glu, can form hydrogen bonds with the nucleic acid phosphates, as well as the cytosines within ss nucleic acids and guanosines in both ss and duplex nucleic acids (Lancelot et al., 1979). Arginine interactions also appear to play dominant roles in the binding of a number of proteins to RNA (Kenan et al., 1991; Mattaj, 1993) and DNA (Oliva & Dixon, 1991; Bradbury, 1977; Johns, 1977).

One example of the arginine-rich family of RNA-binding proteins is the HIV-1 trans-activating (Tat) protein which interacts with specific RNA sequences referred to as the trans-activating region (TAR) (Weeks et al., 1990; Calnan et al., 1991; Tao & Frankel, 1992). Studies of peptides containing arginine led to the proposal of an "arginine fork", whereby a single arginine residue could impart sequence specificity to the binding of a peptide by forming hydrogen bonds to two successive phosphates within a bulge contained within an RNA hairpin (Weeks et al., 1990). Although oligolysine peptides could also bind with high affinity, no specific interaction with the phosphates of the RNA bulge was observed (Weeks et al., 1990). It has also been shown

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* Address correspondence to this author: Department of Biochemistry and Molecular Biophysics, Box 8231, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110.

‡ Current address: Chemistry Program, NAMS Division, Richard Stockton College, Jim Leeds Rd., NAMS Division, Pomona, NJ 08240.

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that free arginine can exhibit similar binding specificity (Tao & Frankel, 1992).

These observations illustrate the need to better understand the thermodynamic differences between the binding of lysine and arginine to nucleic acids. Toward this end, we have undertaken a systematic thermodynamic study of the binding of a series of oligoarginines to ss polynucleotides and duplex DNA and compare their properties to those found for oligolysines. These model peptide studies will facilitate interpretations of the thermodynamics of more complex peptide− and protein−nucleic acid interactions.

MATERIALS AND METHODS

Buffers and Reagents. All solutions were made as described (Lohman & Mascotti, 1992b; Mascotti & Lohman, 1992, 1993). The standard buffers are CB6 and 1.0 mM KCH_3CO_2 and CB7 and 1.0 mM KCH_3CO_2. To prepare these buffers, a solution of 10 mM cacodylic acid, 0.2 mM Na_3EDTA, and 1.0 mM KCH_3CO_2 was titrated to pH 6.0 or 7.0 with 5 M KOH. Our standard high-salt buffers are identical to the low-salt buffers, except that [KCH_3CO_2] was 2.0 M.

Peptides. Oligopeptides containing L-arginine (R) and L-tryptophan (W) of the general form RWR_2-NH_2, RWR_4-NH_2, and RWR-CO_2 were synthesized by the TAES support laboratory (Texas A&M University, College Station, TX). RWR-NH_2 was synthesized by the Protein Chemistry Laboratory (Washington University School of Medicine). All peptides were purified and characterized as described (Mascotti & Lohman, 1990). Stock peptide concentrations were determined spectrophotometrically, as described (Mascotti & Lohman, 1990; Lohman & Mascotti, 1992b).

Polynucleotides. Poly(U) [$s_{20,W}$ = 9.5 S, ∼950 (±200) nucleotides] was from Boehringer Mannheim Biochemicals (lot 11088121-42); poly(A) [$s_{20,W}$ = 7.8 S, ∼430 (±100) nucleotides] and poly(C) [$s_{20,W}$ = 7.8 S, ∼430 (±100) nucleotides] were from Pharmacia (lots 514110 and 0001422001, respectively), and poly(I) [$s_{20,W}$ = 7.2 S, ∼520 (±100) nucleotides] was from P-L Biochemicals (Milwaukee, WI; lot 741−80). Average polynucleotide lengths were estimated from sedimentation coefficients using calibrations based on poly(A) and poly(C) (Eisenberg & Felsenfeld, 1967; Inners & Felsenfeld, 1970). Plasmid pUC8 DNA was purified by large scale alkaline lysis and its purity verified as described (Mascotti & Lohman, 1993). All polynucleotides were dialyzed extensively against the desired buffer before use. Polynucleotide concentrations were determined spectrophotometrically as described (Mascotti & Lohman, 1990, 1992, 1993).

Fluorescence Titrations and Determination of Binding Isotherms. Fluorescence titrations were performed by addition of poly(U) to peptide ("reverse" titration) under constant solution conditions using an SLM-Aminco 8000C spectrofluorometer (Spectronic Instruments, Inc., Rochester, NY) as described (Overman et al., 1988; Mascotti & Lohman, 1990; Lohman & Mascotti, 1992b). The intrinsic fluorescence of oligopeptides containing tryptophan is quenched substantially upon binding ss nucleic acids (Dimicoli & Helene, 1972; Helene & Maurizot, 1981; Helene & Lancelot, 1982; Mascotti & Lohman, 1990, 1992, 1993). The tryptophan fluorescence intensity was measured using excitation wavelengths of 292 nm with poly(U) and poly(A), 296 nm with double-stranded (ds) DNA, and 300 nm with poly(C). The isosbestic wavelength for Lys-Trp-Lys-CO_2 binding to poly(A) is 292 nm (Brun et al., 1975); however, different excitation wavelengths for poly(C) and pUC8 DNA were used in order to reduce absorbance by the polynucleotides and minimize inner filter corrections (Lohman & Mascotti, 1992b). The fluorescence intensity at each point in the titration was corrected for dilution and inner filter effects as described (Mascotti & Lohman, 1990, 1992, 1993). The extinction coefficients for the oligoarginines are as follows: $\epsilon_{292} = (3.3 \pm 0.5) \times 10^3$ M^{-1} cm^{-1}, $\epsilon_{296} = (1.6 \pm 0.5) \times 10^3$ M^{-1} cm^{-1}, and $\epsilon_{300} = (8.0 \pm 0.5) \times 10^2$ M^{-1} cm^{-1}. For all experiments, an emission wavelength of 350 nm was used, with excitation and emission band-passes of 2 and 8 nm, respectively. The fluorescence emission spectra and intensity of each peptide containing a single tryptophan were identical (data not shown), indicating a lack of sequence effects. Under all conditions, the fluorescence change resulting from addition of aliquots of polynucleotide to a peptide solution occurred well within the first minute; hence, measurements were taken 2 min after addition of polynucleotide.

The extent of tryptophan fluorescence quenching, Q_{obs}, was calculated from eq 1

$$Q_{obs} = |(F_{init} - F_{obs})|/F_{init} \quad (1)$$

where F_{obs} is the fluorescence intensity measured at total peptide concentration, L_T, and total poly(U) nucleotide concentration, D_T, and F_{init} is the initial fluorescence before addition of polynucleotide (both F_{obs} and F_{init} were corrected for background fluorescence).

The binding density function method (Bujalowski & Lohman, 1987; Lohman & Bujalowski, 1991; Lohman & Mascotti, 1992b), in which multiple titrations of peptide with nucleic acid at several peptide concentrations are analyzed simultaneously, was used to obtain model-independent estimates of the oligopeptide binding density, ν (oligopeptides bound per nucleotide, $\nu = L_B/D_T$), and the free peptide concentration, L_F. Using this approach, we determined that, at the low binding densities used in our studies (<15% saturation of the polynucleotides), Q_{obs} is directly proportional to the fraction of peptide bound to each polynucleotide, L_B/L_T, for each peptide examined in this report (Mascotti & Lohman, 1990; Lohman & Mascotti, 1992b). Therefore, the relationships in eqs 2 can be used rigorously to calculate L_F and the average binding density ν, where Q_{max} is the maximal fluorescence quenching observed at full saturation of peptide

$$Q_{obs}/Q_{max} = L_B/L_T \quad (2a)$$

$$\nu = (Q_{obs}/Q_{max})(L_T/D_T) \quad (2b)$$

$$L_F = (1 - Q_{obs}/Q_{max})L_T \quad (2c)$$

with the nucleic acid.

Analysis of Equilibrium Binding Isotherms To Obtain K_{obs}. The intrinsic equilibrium constant, K_{obs}, for the binding of a peptide, L, to a nucleic acid site, D, to form a complex, LD, is defined as K_{obs} = [LD]/[L][D], where [L] = free peptide concentration, [D] = free nucleic acid binding sites concentration, and [LD] = bound peptide concentration. Values of K_{obs} were obtained from analysis of binding isotherms constructed from titrations of peptide with poly(U) at constant

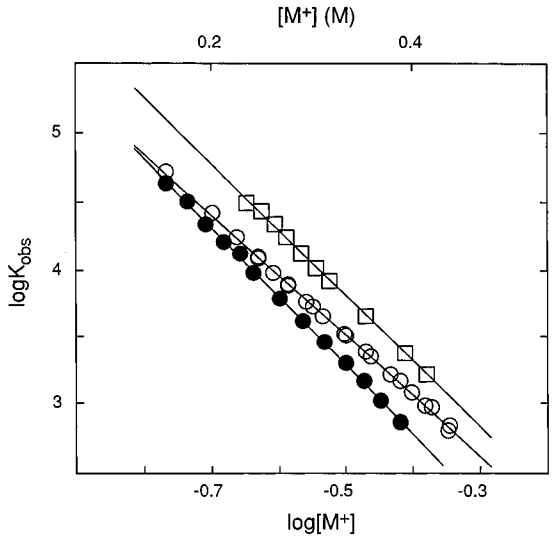

FIGURE 1: Dependence of log K_{obs} on log[M^+] for RWR_4-NH_2 binding to poly(U) in NaCl (●), KCH_3CO_2 (○), and NaF (□) [pH 6.0 (buffer CB) and 25.0 °C]. The data are plotted as a function of total monovalent cation concentration (M^+), and the solid lines are the best fits to the data based on the linear least-squares parameters listed in Table 1.

solution conditions, using a noncooperative overlapping site binding model for large ligands binding to a linear homogeneous lattice (McGhee & von Hippel, 1974; Bujalowski et al., 1989) as described (Overman et al., 1988; Mascotti & Lohman, 1990). Analyzed in this manner, the binding constants reported here have been obtained in the limit of zero-binding density (McGhee & von Hippel, 1974).

Values of K_{obs} as a function of salt concentration were also obtained via analysis of "salt-back" titrations (Overman et al., 1988; Lohman & Mascotti, 1992b), which was possible since eqs 2a−c are valid and the fluorescence intensities of bound and free peptide are independent of salt concentration. The value of Q_{obs} measured at each [KCH_3CO_2] during the salt-back titration was then used to determine ν and L_F using eqs 2b and 2c, and K_{obs} was determined at that [KCH_3CO_2], using eq 3 (McGhee & von Hippel, 1974).

$$K_{obs} = [(\nu/L_F)/(1 - n\nu)]\{(1 - n\nu)/[1 - (n - 1)\nu]\}^{(1-n)} \quad (3)$$

Estimates of ΔH_{obs} and its dependence on [K^+] were obtained by performing a series of salt-back titrations at different temperatures and calculating ΔH_{obs} at each salt concentration from a van't Hoff analysis (Lohman & Mascotti, 1992b).

RESULTS

Dependence of K_{obs} on Monovalent Salt Concentration for Oligoarginines Containing a Single Tryptophan

Poly(U). The dependence of K_{obs} on monovalent salt (MX) concentration was determined for a series of oligoarginines, containing a single tryptophan, (RWR_p-NH_2) binding to poly(U) (pH 6.0 and 25 °C). Figure 1 shows the dependence of log K_{obs} on log[M^+] for the binding of RWR_4-NH_2 to poly(U) in sodium and potassium salts containing different anions. In previous studies with oligolysines, neither K_{obs} nor (∂ log K_{obs}/∂ log[M^+]) show changes upon replacement of Na^+ with K^+ for a constant anion type

Table 1: Dependence of K_{obs} on Monovalent Salt (MX) Concentration for RWR_4-NH_2 Binding to Poly(U) in Different Salt Types[a]

salt	∂ log K_{obs}/∂ log[M^+]	log K_{obs} (1 M)[b]	Q_{max} (%)
KCH_3CO_2	−4.46 (±0.22)	+1.28 (±0.22)	93 (±2)
NaCl	−5.10 (±0.22)	+0.74 (±0.22)	91 (±2)
NaF	−4.89 (±0.24)	+1.36 (±0.25)	92 (±2)

[a] Buffer CB + different salt types as listed at pH 6.0 and 25.0 °C. [b] Obtained from a linear extrapolation of a plot of log K_{obs} *vs* log[M^+].

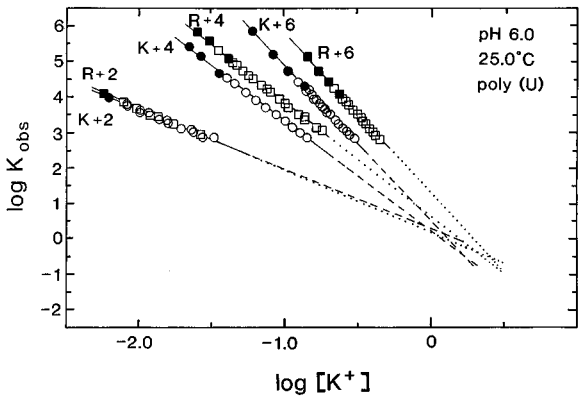

FIGURE 2: Dependence of log K_{obs} on log[K^+] for a series of oligolysines (○ and ●) and oligoarginines (□ and ■) binding to poly(U) [pH 6.0 (buffer CB) and 25.0 °C in KCH_3CO_2]. The data are plotted as a function of the logarithm of the total potassium concentration. The peptides are KWK-CO_2, KWK_2-NH_2, KWK_4-NH_2, RWR-CO_2, RWR_2-NH_2, and RWR_4-NH_2. The net charge of each of the peptides is listed on the figure. Closed symbols represent data from reverse titrations at a constant salt concentration; open symbols represent data from salt-back titrations. Linear least-square lines are shown (see Table 2 for the equations). Dashed and dotted lines are linear extrapolations of the least-square lines for the oligolysines and oligoarginines, respectively.

(Mascotti & Lohman, 1992). However, as was observed with oligolysines, the value of (∂ log K_{obs}/∂ log[M^+]) is lowest in the presence of $CH_3CO_2^-$, relative to that with Cl^- or F^- (Figure 1 and Table 1). Also, the value of (∂ log K_{obs}/∂ log[K^+]) for the binding of RWR_4-NH_2 to poly(U) in KCH_3CO_2 is −4.47 (±0.22) which is identical to that observed for KWK_4-NH_2 binding to poly(U) in KCH_3CO_2. Hence, the dependence of log K_{obs} on log[K^+] in the presence of KCH_3CO_2 appears to primarily reflect only cation release from poly(U). However, we note that the effect of the different anions shown in Figure 1 is slightly more pronounced for oligoarginines than previously observed for oligolysines (Mascotti & Lohman, 1990). This may reflect slightly higher preferential interactions of anions with the oligoarginine peptides.

Figure 2 shows the dependence of log K_{obs} on log[K^+] for poly(U) binding to oligoarginines with net charges of +2, +4, and +6 in the presence of KCH_3CO_2 and compares these with binding to oligolysines. Table 2 lists Q_{max}, (−∂ log K_{obs}/∂ log[K^+]), and the value of log K (1 M and 25 °C) (linear extrapolation to 1 M K^+) for each oligoarginine studied. The dependence of (−∂ log K_{obs}/∂ log[K^+]) on z for the oligoarginines is described by eq 4 (pH 6.0 and 25.0 °C in KCH_3CO_2):

$$-\partial \log K_{obs}/\partial \log[K^+] = 0.68\ (\pm 0.05)z + 0.42\ (\pm 0.42) \quad (4)$$

These results demonstrate that the charge of the oligopeptide

Table 2: Monovalent Salt (KCH_3CO_2) Dependence and Thermodynamic Parameters for Oligoarginine Binding to Poly(U)[a]

peptide	z	$\partial \log K_{obs}/\partial \log[K^+]$	$\log K_{obs}$ (1 M)[b]	Q_{max} (%)	ΔH_{obs}[c]	$\Delta\Delta G°_{R-K}$[d]	$\Delta\Delta H_{R-K}$[d]
RWR-CO_2	2	−1.75 (±0.20)	+0.16 (±0.24)	82 (±2)	−4.2 (±1.5)	+0.14 (±0.24)	−1.00 (±0.75)
KWK-CO_2	2	−1.68 (±0.20)	+0.26 (±0.24)	80 (±2)	−2.2 (±1.5)	−	−
RWR_2-NH_2	4	−3.21 (±0.21)	+0.61 (±0.22)	91 (±2)	−7.4 (±1.5)	−0.19 (±0.09)	−1.13 (±0.50)
KWK_2-NH_2	4	−3.10 (±0.21)	+0.20 (±0.22)	89 (±2)	−4.0 (±1.5)	−	−
RWR_4-NH_2	6	−4.47 (±0.22)	+1.28 (±0.24)	93 (±2)	−10.4 (±1.5)	−0.22 (±0.05)	−1.10 (±0.30)
KWK_4-NH_2	6	−4.36 (±0.22)	+0.49 (±0.22)	92 (±2)	−4.9 (±1.5)	−	−

[a] Buffer CB + KCH_3CO_2 at pH 6.0 and 25.0 °C. [b] Obtained from a linear extrapolation of a plot of log K_{obs} *vs* log[K^+] to 1 M KCH_3CO_2. [c] The average ΔH_{obs} within the range of K^+ concentrations examined in units of kilocalories per mole. [d] $\Delta\Delta G°_{R-K}$ and $\Delta\Delta H_{R-K}$ are defined in the text (eqs 5 and 6) and have units of kilocalories per mole.

is the primary determinant of the salt concentration dependence of K_{obs}. The values of Q_{max} and $(-\partial \log K_{obs}/\partial \log[K^+])$ are comparable for both the oligoarginines and oligolysines; however, except when $z = +2$, the absolute value of K_{obs} is generally larger for the oligoarginines, with the difference between oligoarginines and oligolysines becoming more apparent with increasing peptide charge. Table 2 lists the relative differences in $\Delta G°$ (1 M and 25 °C) for oligoarginines and oligolysines binding to poly(U), where $\Delta\Delta G°_{R-K}$ is defined in eq 5, and p is the number of lysines and/or arginines (*e.g.*, $p = 2$ for both RWR-CO_2 and RWR-NH_2).

$$\Delta G°_{R-K} = [\Delta G°_{Arg}\,(1\ M\ K^+ \text{ and } 25\ °C) - \Delta G°_{Lys}\,(1\ M\ K^+ \text{ and } 25\ °C))/p \quad (5)$$

Within the range of KCH_3CO_2 concentrations examined, the values of K_{obs} are identical for RWR-CO_2 and KWK-CO_2. However, for the longer peptides, K_{obs} is larger for the arginine peptides than for the lysine peptides of the same net charge. Comparison of the oligoarginine-oligolysine peptides when $p = 3$ and 5, where the difference in K_{obs} is significant, yields $\Delta\Delta G°_{R-K} = -0.2$ (±0.1) kcal/mol (Table 2). Therefore, within the uncertainty of the data, the free energy change upon replacing a lysine residue with an arginine residue is independent of peptide length (i.e., $\Delta\Delta G°_{R-K}$ is additive). This value of $\Delta\Delta G°_{R-K}$ is consistent with the apparent lack of a difference between K_{obs} for KWK-CO_2 and RWR-CO_2, where it would be predicted that log K_{obs} should be only about 0.15 higher for RWR-CO_2, which is well within the uncertainty of the two measurements.

Effects of pH on K_{obs} for Oligoarginines Binding to Poly(U). Between pH 6 and 7, where the pK of the α-amino group of the peptides is approached, changes in pH exert an influence on the salt dependence of K_{obs} (Lohman et al., 1980; Mascotti & Lohman, 1992). We examined the binding of oligoarginines to poly(U) as a function of [KCH_3CO_2] and temperature at both pH 6.0 and 7.0 (buffer CB6 and CB7). Previously, by analyzing the dependence of ΔH_{obs} on pH [using eq 10 of Mascotti and Lohman, (1992)], we have estimated the values of pK_α for several oligolysines binding to poly(U). These estimates of the pK_α can be used to calculate z (at a given pH) for each peptide using eq 7 of Mascotti and Lohman (1992). Using the same approach, we have estimated the pK_α for the oligoarginines and find them to be identical, within experimental error, to those determined for the equivalently charged oligolysines (Mascotti, 1992). The pK values of the η-amino groups of the oligoarginines could not be determined but are likely to be above 9, since the pK of the guanidinium group of arginine is 12.48 (Dawson et al., 1986).

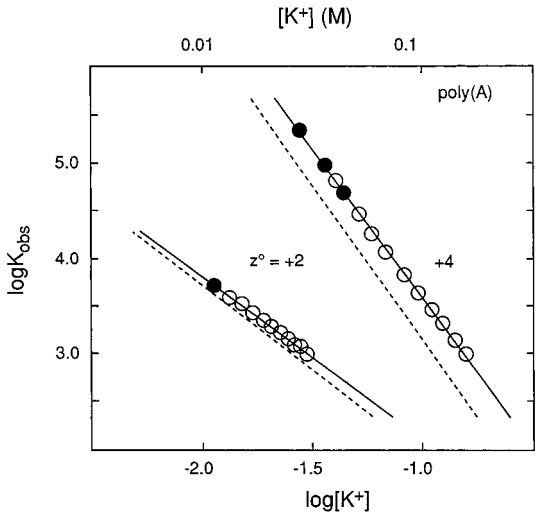

FIGURE 3: Dependence of log K_{obs} on log[K^+] for a series of oligoarginines binding to poly(A) [pH 7.0 (buffer CB) and 25.0 °C in KCH_3CO_2). The data are plotted as a function of the logarithm of the total potassium concentration. The peptides were RWR-CO_2 and RWR_2-NH_2. The net maximal charge of each peptide is listed on the figure. Closed symbols represent data from reverse titrations at a constant salt concentration; open symbols represent data from salt-back titrations. The solid lines are linear least-squares fits to the data (see Table 3 for the parameters which describe these lines). For comparison, the dashed lines show the linear least-squares best fits for oligolysines of the same charge binding to poly(A) under identical conditions [data from Mascotti and Lohman (1993)].

Poly(A). The dependence of K_{obs} on [K^+] was also determined for poly(A) binding to the oligoarginines (containing a single tryptophan) with net charges of +2 and +4 (pH 7.0 and 25 °C in KCH_3CO_2). Figure 3 compares these with our previous studies of the same peptide, but containing lysine (see also Table 3). As with binding to poly(U), the values of $(\partial \log K_{obs}/\partial \log[K^+])$ are identical for the peptides of the same net charge, but the absolute value of $(\partial \log K_{obs}/\partial \log[K^+])$ increases with net peptide charge. However, the affinities (K_{obs}) of the oligoarginines are larger than those for the oligolysines of equivalent net charge, and this difference increases with increasing peptide charge. Table 3 shows that $\Delta\Delta G°_{R-K} \sim -0.2$ (±0.1) kcal/mol (defined in eq 5) for oligoarginines where $z° = +2$ and +4, indicating that each arginine−phosphate interaction is independent and identical. Also, the increase in K_{obs} for the oligoarginines binding to poly(A) over that observed for the oligolysines is similar to that observed for these peptides binding to poly(U) (Table 3).

Poly(C). The dependence of K_{obs} on [K^+] was also determined for RWR_2-NH_2 binding to poly(C) (pH 7.0 and 25 °C in KCH_3CO_2). Only this oligoarginine, with a formal charge of +4, was examined because the more highly charged oligoarginines caused precipitation of poly(C) at the

Table 3: Monovalent Salt (KCH_3CO_2) Dependencies and Thermodynamic Parameters for Oligoarginines Binding to Poly(A), Poly(C), and Poly(I)[a]

peptide	z^b	$\partial \log K_{obs}/\partial \log[K^+]$	$\log K$ (1 M)	Q_{max} (%)	$\Delta H_{obs}{}^c$	$\Delta\Delta G°_{R-K}{}^d$	$\Delta\Delta H_{R-K}{}^d$
poly(A)							
RWR-CO_2	1.9	−1.72 (±0.24)	+0.39 (±0.37)	71 (±2)	−6.5 (±1.5)	−0.16 (±0.15)	−0.65 (±0.75)
KWK-CO_2	1.9	−1.78 (±0.20)	+0.16 (±0.24)	69 (±2)	−5.2 (±1.5)	−	−
RWR_2-NH_2	3.8	−3.17 (±0.21)	+0.34 (±0.22)	78 (±2)	−5.2 (±1.5)	−0.18 (±0.08)	−0.67 (±0.38)
KWK_2-NH_2	3.8	−3.26 (±0.21)	−0.13 (±0.22)	81 (±2)	−3.8 (±1.5)	−	−
poly(C)							
RWR_2-NH_2	3.8	−3.41 (±0.21)	−0.40 (±0.22)	60 (±2)	−5.4 (±1.5)	−0.13 (±0.08)	−0.50 (±0.38)
KWK_2-NH_2	3.8	−3.30 (±0.21)	−0.66 (±0.22)	61 (±2)	−3.9 (±1.5)	−	−
poly(I)							
RWR-CO_2	1.9	−1.50 (±0.16)	+1.44 (±0.22)	82 (±2)	−9.6 (±1.5)	−0.10 (±0.15)	−0.65 (±0.75)
KWK-CO_2	1.9	−1.43 (±0.18)	+1.31 (±0.22)	77 (±2)	−8.0 (±1.5)	−	−

[a] Buffer CB + KCH_3CO_2 at pH 7.0 and 25.0 °C. [b] Calculated at pH 7.0 using the pK_α values from Mascotti and Lohman (1992). [c] The units on ΔH_{obs} are kilocalories per mole. [d] $\Delta\Delta G°_{R-K}$ and $\Delta\Delta H_{R-K}$ are defined in the text (eqs 5 and 6) and have units of kilocalories per mole.

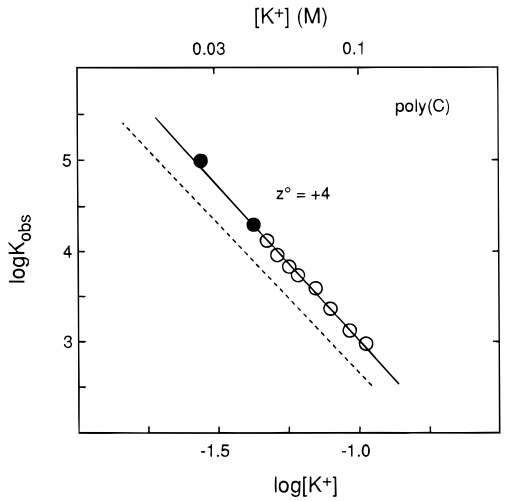

FIGURE 4: Dependence of log K_{obs} on log[K^+] for RWR_2-NH_2 binding to poly(C) [pH 7.0 (buffer CB) and 25.0 °C in KCH_3CO_2]. The data are plotted as a function of the logarithm of the total potassium concentration. The net maximal charge of the peptide is listed on the figure. Closed symbols represent data from reverse titrations at a constant salt concentration; open symbols represent data from salt-back titrations. The solid line describes the linear least-squares fit to the data (see Table 3 for the parameters which describe this line). For comparison, the dashed line is the linear least-squares best fit for KWK_2-NH_2 binding to poly(C) under identical conditions [data from Mascotti and Lohman (1992)].

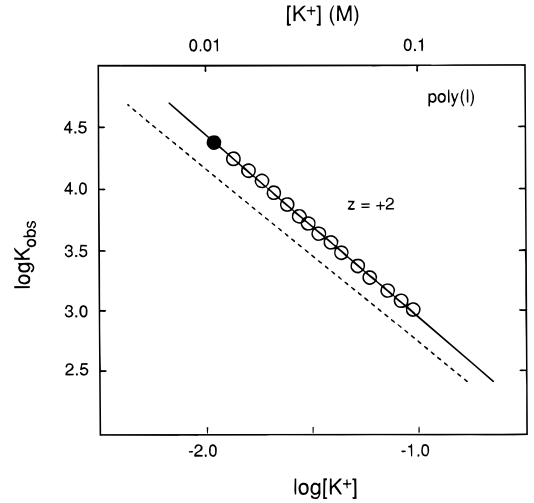

FIGURE 5: Dependence of log K_{obs} on log[K^+] for RWR-CO_2 binding to poly(I) [pH 7.0 (buffer CB) and 25.0 °C in KCH_3CO_2]. The data are plotted as a function of the logarithm of the total potassium concentration. The net maximal charge of the peptide is shown in the figure. The closed symbol represents data from reverse titrations at a constant salt concentration; open symbols represent data from salt-back titrations. The solid line shows the linear least-squares fit to the data (see Table 3 for the parameters which describe this line). For comparison, the dashed line is the linear least-squares best fit for KWK-CO_2 binding to poly(I) in the same conditions [data Mascotti and Lohman (1992)].

binding densities required for accurate determination of K_{obs}. On the other hand, the affinities of the less charged oligoarginines for poly(C) were too low to measure by fluorescence techniques at the salt concentrations used. Figure 4 shows the dependence of log K_{obs} on log[K^+] for RWR_2-NH_2 binding to poly(C) and compares it to that observed for KWK_2-NH_2 (dashed line). Table 3 lists Q_{max}, ($\partial \log K_{obs}/\partial \log[K^+]$), and log K (1 M and 25 °C) for both peptides. The values of z were calculated using eq 7 of Mascotti and Lohman (1992). Again, the values of ($\partial \log K_{obs}/\partial \log[K^+]$) for both RWR_2-NH_2 and KWK_2-NH_2 are identical, although the arginine peptide shows a slightly higher affinity for poly(C), with a $\Delta\Delta G°_{R-K}$ of −0.13 (±0.08) kcal/mol (Table 3).

Poly(I). Figure 5 and Table 3 compare the dependence of log K_{obs} on log[K^+] for KWK-CO_2 and RWR-CO_2 binding to poly(I) (25.0 °C and pH 7.0). These studies were performed at pH 7.0 in order to maximize the solubility of poly(I) and avoid the formation of multistranded forms of poly(I) (Thiele & Guschlbauer, 1973). The values of z were calculated on the basis of the pK_α for RWR-CO_2 and KWK-CO_2 using eq 7 of Mascotti and Lohman (1992). Interestingly, the dependence of log K_{obs} on log[K^+] for both peptides is slightly smaller (by ∼15%) than that for any other polynucleotide examined [Tables 2 and 3; see also Mascotti and Lohman (1993)]. Furthermore, at 30 mM KCH_3CO_2, K_{obs} for the binding of either KWK-CO_2 or RWR-CO_2 to poly(I) is approximately 4−5-fold higher than that for binding of the same peptides to poly(A). The molecular basis for this difference is not known, although it may reflect a larger inter-phosphate spacing within poly(I), relative to other ss polynucleotides. However, as observed with binding to other polynucleotides, the affinity of poly(I) for RWR-CO_2 is greater than that for KWK-CO_2. Upon saturation of KWK-CO_2 and RWR-CO_2 with poly(I), we observe maximal tryptophan quenchings of 77 ± 2% and 82 ± 2%, respectively. Unfortunately, the limited solubility of poly(I) in solutions of higher salt concentration precluded studies of the more highly charged peptides.

Table 4: Monovalent Salt (KCH_3CO_2) Dependence and Thermodynamic Parameters for Oligolysines and Oligoarginines Containing a Single Tryptophan Binding to Supercoiled pUC8 DNA[a]

peptide	z	$\partial \log K_{obs}/\partial \log[K^+]$	$\log K$ (1 M)	Q_{max}	ΔH_{obs}[b]	$\Delta\Delta G°_{R-K}$[c]	$\Delta\Delta H_{R-K}$[c]
RWR_2-NH_2	4	−3.29 (±0.30)	+0.29 (±0.32)	33 (±2)	−4.1 (±1.5)	−0.50 (±0.08)	−0.93 (±0.38)
KWK_2-NH_2	4	−3.94 (±0.30)	−0.68 (±0.32)	47 (±2)	−1.3 (±1.5)	−	−

[a] Buffer CB + KCH_3CO_2 at pH 6.0 and 25.0 °C. [b] The units are kilocalories per mole for ΔH_{obs}. [c] $\Delta\Delta G°_{R-K}$ and $\Delta\Delta H_{R-K}$ are defined in the text (eqs 5 and 6) and have units of kilocalories per mole.

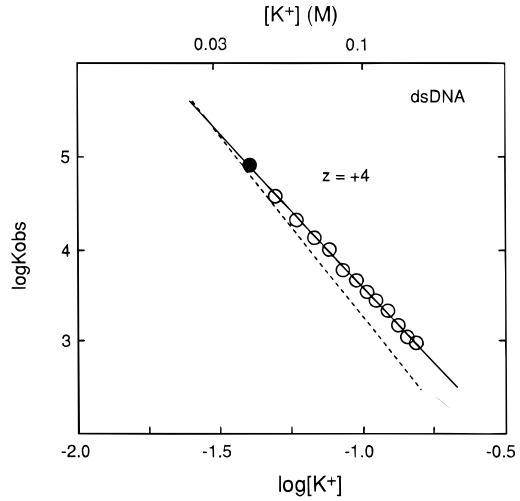

FIGURE 6: Dependence of log K_{obs} on log[K^+] for RWR_2-NH_2 binding to supercoiled double-stranded (ds) DNA [pH 6.0 (buffer CB) and 25.0 °C in KCH_3CO_2]. The data are plotted as a function of the logarithm of the total potassium concentration. The net maximal charge of the peptide is shown in the figure. The closed symbol represents data from reverse titrations at a constant salt concentration; open symbols represent data from salt-back titrations. The solid line shows the linear least-squares fit to the data (see Table 4 for the parameters which describe this line). For comparison, the dashed line is the linear least-squares best fit for KWK_2-NH_2 binding to supercoiled dsDNA under identical conditions. The parameters describing the best-fit line for the KWK_2-NH_2 data are also listed in Table 4.

Duplex B-Form DNA. We have also examined the effect of [K^+] on K_{obs} for the binding of the oligoarginine, RWR_2-NH_2, to a natural dsDNA, supercoiled ds pUC8 plasmid DNA (with ∼10% nicked circular). The results are shown in Figure 6 (and Table 4) and compared with the results for KWK_2-NH_2 binding to the same dsDNA. These experiments were performed at pH 6.0 in order to maximize the peptide charge, whereas duplex DNA is not titrated at pH 6 (Record, 1967). The values of ($\partial \log K_{obs}/\partial \log[K^+]$) are −3.29 ± 0.3 and −3.94 ± 0.30 for KWK_2-NH_2 and RWR_2-NH_2, respectively, both of which have a net charge of +4 at pH 6.0. The binding of the arginine peptide, RWR_2-NH_2, to dsDNA is slightly more favorable than that for the lysine peptide, and we calculate a $\Delta\Delta G°_{R-K}$ of −0.50 (±0.08) kcal/mol from extrapolation to 1 M salt (see Table 4).

Determination of the Enthalpic Contributions to Binding

The temperature dependence of K_{obs} was examined for the binding of oligoarginines (containing a single tryptophan) to poly(U), poly(A), poly(C), poly(I), and duplex pUC8 plasmid DNA, from which estimates of the ΔH_{obs} for binding were obtained by van't Hoff analysis. The results are summarized in Tables 2−4, which also give the relative differences in ΔH_{obs} per residue for the oligoarginines *vs* oligolysines of the same type (also containing a single tryptophan) that we have studied previously (Mascotti & Lohman, 1992, 1993). This difference, $\Delta\Delta H_{R-K}$, is defined in eq 6, where p is the number of charged side chains in the oligopeptide.

$$\Delta\Delta H_{R-K} = (\Delta H_{Arg,obs} - \Delta H_{Lys,obs})/p \qquad (6)$$

For the binding to each polynucleotide, ΔH_{obs} is negative and becomes more negative with increasing oligoarginine chain length. Furthermore, the ΔH_{obs} is always more negative for oligoarginine binding than for oligolysine binding, generally by at least 0.5−1.0 kcal/mol (per arginine), indicating that oligoarginine binding has a more favorable enthalpic contribution to binding. For each polynucleotide, we find that ΔH_{obs} per residue and $\Delta\Delta H_{R-K}$ are both independent of peptide length within our experimental uncertainty (i.e., the contributions of each arginine can be considered additive). Since ΔH_{obs} was measured as a function of [K^+], we extrapolated these values to 1 M K^+ as described (Mascotti & Lohman, 1992, 1993) which facilitates comparison of peptide binding thermodynamics measured in various [K^+] ranges. These 1 M K^+ extrapolations reflect the thermodynamic quantities in the absence of cation release (Record et al., 1976; Lohman & Mascotti, 1992).

The poly(U) studies were performed at pH 6.0 and 25.0 °C, and we find $\Delta\Delta H_{R-K}$ = ca. −1.1 kcal/mol per residue (Table 2), independent of peptide length. The poly(A) studies were performed at pH 7.0 and 25.0 °C, and we find $\Delta\Delta H_{R-K}$ = ca. −0.7 kcal/mol (Table 3). The poly(C) studies were performed only with RWR_2-NH_2 and KWK-CO_2 (pH 7.0 and 25 °C), and we find $\Delta\Delta H_{R-K}$ = −0.50 (±0.38) (Table 3). The poly(I) studies were also performed only with RWR_2-NH_2 and KWK-CO_2 (pH 7.0 and 25 °C), and we find $\Delta\Delta H_{R-K}$ = −0.65 (±0.75) (Table 3). The studies with duplex plasmid DNA (pUC8) were also performed only with KWK_2-NH_2 and RWR_2-NH_2. As with all of the polynucleotides, ΔH_{obs} is more favorable for the binding of RWR_2-NH_2 than for that of KWK_2-NH_2, with a $\Delta\Delta H_{R-K}$ of −0.93 (±0.38) kcal/mol (Table 4). Attempts to examine binding of the longer oligoarginines possessing higher net charge failed due to the onset of aggregation. We note that Q_{max} is considerably lower for RWR_2-NH_2 (33 ± 2%) compared to that for KWK_2-NH_2 (47 ± 2%) when binding to dsDNA. The origin of this difference is unknown.

In addition to the points mentioned above, we emphasize that we observed no apparent effect of monovalent salt concentration on ΔH_{obs}, at least over the range of salt concentrations examined (up to ∼0.5 M). This contrasts with the observation that ΔH_{obs} for *Escherichia coli* SSB tetramer binding to ssDNA and RNA is a strong function of salt concentration (with dramatic anion effects) in the range above 0.2 M (Lohman et al., 1996).

DISCUSSION

Proteins that bind to DNA and RNA generally possess some subset of the positively charged amino acids, lysine, arginine, and histidine, within their nucleic acid binding sites. Therefore, an understanding of the thermodynamic basis of protein−nucleic acid binding requires quantitative information on the relative contributions of these different charged amino acids to the thermodynamics of these interactions. Such studies are needed to determine if the different positively charged amino acid side chains have different relative contributions to binding stability and specificity. Many DNA and RNA binding proteins contain all three positively charged residues; however, there are also numerous examples of DNA binding proteins that are rich in one particular positively charged amino acid. For example, some histones (e.g., H1) are very lysine-rich, whereas others are slightly lysine-rich (e.g., H2A and H2B) or slightly arginine-rich (e.g., H3 and H4) (Bradbury, 1977; Johns, 1977). Protamines, which bind to DNA during meiosis, are mostly arginine-rich (Oliva & Dixon, 1991; Porschke, 1991).

Although the contribution of the positive charge from lysine and arginine is likely to be similar, it is clear that binding specificity can result from the different hydrogen bonding capabilities of the side chains. A particularly striking example is a peptide fragment of the HIV-1 Tat protein, which binds with specificity to its mRNA recognition site, TAR. This specificity is dependent upon an arginine at a specific position within the nonomer peptide (Weeks et al., 1990; Calnan et al., 1991; Tao & Frankel, 1992). However, the relative contributions of the other positively charged amino acids to the thermodynamics of binding have not been established. This is partly due to the absence of quantitative studies of well-defined model peptides containing arginine and histidine, since most previous studies have been performed with peptides containing only lysine. The thermodynamic studies of oligoarginines binding to linear ss and duplex nucleic acids reported here were performed to compare with our previous thermodynamic studies of oligolysines (Mascotti & Lohman, 1990, 1992, 1993).

In general, our studies show that the dependence of the equilibrium binding constant, K_{obs}, on monovalent salt concentration for oligoarginines binding to ss as well as duplex polynucleotides is the same as that observed for oligolysines possessing the same net charge (Mascotti & Lohman, 1990, 1992, 1993). For both oligopeptides, the magnitude of the salt dependence of K_{obs} ($\partial \log K_{obs}/\partial \log[MX]$) is dependent upon the axial charge density of the polynucleotide under study as observed previously (Mascotti & Lohman, 1990). This suggests that the contribution to binding free energy due to the formal positive charge on the peptides is the same for both arginine and lysine. However, although we previously observed only a very slight effect of anion type on the magnitude of K_{obs} for oligolysines binding to ss polynucleotides, the effect of anion type is more pronounced with the oligoarginines, although the effect is still small (see Figure 1). The fact that K_{obs} decreases in proceeding from F^- to $CH_3CO_2^-$ to Cl^-, following the Hofmeister series (Hofmeister, 1888; von Hippel & Schleich, 1969; Record et al., 1978; Collins & Washabaugh, 1985), suggests that the effect is due to weak preferential interactions of anions with the oligoarginines. However, in the presence of $CH_3CO_2^-$, the salt dependences ($-\partial \log K_{obs}/\partial \log[K^+]$) for KWK_4-NH_2 and RWR_4-NH_2 binding to poly(U) are identical [compare Table 2 with Mascotti and Lohman (1990)], indicating that these preferential anion interactions are minimized when monovalent salts containing acetate are used. Since our studies do not indicate any charge-independent cation release resulting from the interaction of tryptophan with poly(U) for either oligolysines (Mascotti & Lohman, 1990, 1992) or oligoarginines, we conclude that the large negative value of ($\partial \log K_{obs}/\partial \log[KCH_3CO_2]$) reflects mainly K^+ release from polynucleotides due to phosphate charge neutralization upon oligopeptide binding. The number of cations (K^+) released from poly(U) per net peptide charge is identical within experimental uncertainty for the oligoarginines (0.68 ± 0.03) and the oligolysines (0.71 ± 0.03) (Mascotti & Lohman, 1990, 1992).

Although the salt dependences of K_{obs} are identical for oligoarginines and oligolysines possessing the same net charge, the oligoarginines bind systematically with a higher affinity to each polynucleotide. On average, we measure an additional $\Delta G°$ of -0.2 ± 0.1 kcal/mol per arginine. This higher affinity (lower $\Delta G°$) is enthalpic in origin, with a ΔH_{obs} of -0.83 ± 0.52 kcal/mol per arginine. Since these effects are independent of base composition and polynucleotide type (Tables 2−4), we conclude that it results from arginine interactions with the phosphate backbone and that these interactions are enthalpically more favorable. Furthermore, within our experimental uncertainty, this additional stability of oligoarginine over oligolysine appears to be additive with respect to the number of charged residues substituted. It seems most likely that hydrogen bonding of the guanidinium group of arginine to the nucleic acid phosphates contributes to this increased stability.

Comparison with Previous Studies. There have been a number of previous studies that have attempted to determine the relative binding affinities for DNA and RNA of lysine *vs* arginine contained within both oligo- and polypeptides. Most of the studies used polydisperse polyarginine and polylysine, rather than the well-defined shorter oligopeptides used in this study. The results of the studies with longer polypeptides are difficult to interpret due to the fact that the binding of longer polypeptides to DNA and RNA causes aggregation and precipitation. Therefore, in studies with longer polypeptides, it has generally not been possible to separate effects due to binding from effects on the aggregation phenomenon, which has generally been monitored. For example, Leng and Felsenfeld (1966) showed that polyarginine precipitates duplex DNA more readily than polylysine at all salt concentrations examined and the precipitation processes displayed macroscopic cooperativity. However, this cooperativity for precipitation does not reflect a cooperativity of binding *per se*, since the precipitation step occurs after peptide binding. We find no evidence for cooperative binding of either oligolysines or oligoarginines to ss- or dsDNA or RNA (Mascotti & Lohman, 1990). Therefore, one cannot separate the differential effects of the side chains on binding *vs* the aggregation process. In fact, even with the relatively short peptides used in the present study, we observe the formation of complexes that scatter light upon exceeding a critical binding density of bound peptides [~30% saturation of poly(U) phosphates for oligolysines and ~10−15% for oligoarginines] (D. P. Mascotti, unpublished observations). These observations are qualitatively consistent with earlier studies of charged oligopeptides

binding to ss and ds nucleic acids (Porschke, 1979) and polyamines binding to poly(A) (Yen et al., 1983). To avoid these complications, we have intentionally limited our studies to low peptide binding densities.

In apparent contrast to the above results, Olins et al. (1967, 1968) observed that polylysine preferentially stabilizes the dsDNA against thermal denaturation, relative to polyarginine. However, these results are also difficult to interpret in terms of binding affinity due to the use of polypeptides, whose complexes with DNA formed turbid solutions, suggesting that the bound complexes were in an aggregated form. Furthermore, any effects on the melting temperature of the duplex DNA could potentially result from differential interactions with either ss- or dsDNA, since the polylysine and polyarginine could bind to either.

Our results agree qualitatively with those of Wehling et al. (1975), who examined charged polypeptides binding to dsDNA using polypeptide affinity columns to which dsDNA solutions were bound at low salt concentrations. The resultant complexes were then eluted with NaCl, and the peak salt concentration where the maximal amount of DNA was eluted was taken as a qualitative indication of the affinity. On the basis of these studies, it was concluded that polyarginine binds with higher affinity to dsDNA than does polylysine. Previous studies of charged oligopeptides binding to duplex DNA (Standke & Brunnert, 1975; Porschke, 1978, 1979) also concluded that oligoarginines bind with higher affinity than oligolysines. The studies reported here have expanded these studies to ss polynucleotides and also explored the salt and temperature dependence of K_{obs} for oligoarginine−nucleic acid interactions, which enabled us to determine the relative contributions of ΔH_{obs} and $\Delta S°_{obs}$ to its higher binding affinity, relative to lysine.

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