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Thermodynamics of Single-Stranded RNA Binding to Oligolysines Containing Tryptophan†

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ABSTRACT: The equilibrium binding to the synthetic RNA poly(U) of a series of oligolysines containing one, two, or three tryptophans has been examined as a function of pH, monovalent salt concentration (MX), temperature, and Mg^{2+}. Oligopeptides containing lysine (K) and tryptophan (W) of the type KWK_p-NH_2 and KWK_p-CO_2 (p = 1–8), as well as peptides containing additional tryptophans or glycines, were studied by monitoring the quenching of the peptide tryptophan fluorescence upon binding poly(U). Equilibrium association constants, K_{obs}, and the thermodynamic quantities $\Delta G°_{obs}$, $\Delta H°_{obs}$, and $\Delta S°_{obs}$ describing peptide–poly(U) binding were measured as well as their dependences on monovalent salt concentration, temperature, and pH. In all cases, K_{obs} decreases significantly with increasing monovalent salt concentration, with $(\partial \log K_{obs}/\partial \log [K^+]) = -0.74\ (\pm 0.04)z$, independent of temperature and salt concentration, where z is the net positive charge on the peptide. The origin of these salt effects is entropic, consistent with the release of counterions from the poly(U) upon formation of the complex. Upon extrapolation to 1 M K^+, the value of $\Delta G°_{obs}$ is observed to be near zero for all oligolysines binding to poly(U), supporting the conclusion that these complexes are stabilized at lower salt concentrations due to the increase in entropy accompanying the release of monovalent counterions from the poly(U). Only the net peptide charge appears to influence the thermodynamics of these interactions, since no effects of peptide charge distribution were observed. The binding of poly(U) to the monotryptophan peptides displays interesting behavior as a function of the peptide charge. The extent of tryptophan fluorescence quenching, Q_{max}, is dependent upon the peptide charge for $z \leq +4$, and the value of Q_{max} correlates with z-dependent changes in $\Delta H°_{obs}$ and $\Delta S°_{obs}$(1 M K^+), whereas for $z \geq +4$, Q_{max}, $\Delta H°_{obs}$, and $\Delta S°_{obs}$(1 M K^+) are constant. The correlation between Q_{max} and $\Delta H°_{obs}$ and $\Delta S°_{obs}$(1 M K^+) suggests a context (peptide charge)-dependence of the interaction of the peptide tryptophan with poly(U). However the interaction of the peptide tryptophan does not contribute substantially to $\Delta G°_{obs}$ for any of the peptides, independent of z, due to enthalpy–entropy compensations. Each of the tryptophans in multiple Trp-containing peptides appear to bind to poly(U) independently, with $\Delta H°_{Trp} = -2.9 \pm 0.7$, although $\Delta G°_{Trp}$ is near zero due to enthalpy–entropy compensations. These studies provide important comparative information for the interpretation of the thermodynamics of protein–ss nucleic acid interactions.

Noncovalent interactions between proteins and nucleic acids are central to the control of cellular processes such as gene expression, replication, recombination, and repair. In order to understand how these processes function in detail, it is necessary to probe quantitatively the equilibria and kinetics of the protein–protein and protein–nucleic acid interactions. Such studies have been initiated for a number of protein–nucleic acid systems [for reviews, see Record et al. (1978, 1990, 1991), Lohman (1986), Lohman and Mascotti (1992a), Lesser et al. (1990), and Lohman and Bujalowski (1990)]. Thermodynamic studies are required to understand the basis for stability and specificity of these complexes; however, these properties are usually sensitive to solution variables such as temperature, pH, salt concentration, and type (both cations and anions) (Record et al., 1991; Lohman & Mascotti, 1992a). In general, this results from the fact that small molecules, such as salt ions, protons, and solvent (H_2O), usually interact preferentially with either the free protein, free nucleic acid, or the complex and thus changes in salt concentration, pH, and temperature can dramatically affect stability and specificity of these complexes. As a result, it is necessary to investigate such linkages by probing these interactions over a wide range of solution variables. However, it is often the case that protein–nucleic acid interactions exhibit complex thermodynamic behavior that is difficult to understand in the absence of comparative studies of simpler model systems. For this reason it is desirable to examine the binding to nucleic acids of relatively simple ligands in order to place in perspective our understanding of the more complex protein–nucleic acid interactions.

The binding of many proteins to nucleic acids is dominated by electrostatic interactions due to the linear polyanionic nature of nucleic acids, which results in the preferential binding of cations to the nucleic acid (Manning, 1969; Record et al., 1976, 1978, 1991; Lohman, 1986; Lohman & Mascotti, 1992a). As a result, there have been a number of studies of the equilibrium binding of small positively charged ligands to linear nucleic acids, with most of these focusing on duplex RNA and DNA (Latt & Sober, 1967a,b; Record et al., 1976, 1978; Lohman et al., 1980; Braunlin et al., 1982; Plum & Bloomfield, 1988). However, there are a large number of proteins involved in DNA and RNA metabolism (replication, recombination, repair, RNA splicing) that interact primarily with single-stranded (ss) forms of nucleic acids and yet there have been relatively few thermodynamic studies of the

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interactions of simple ligands with ss polynucleotides (Brun et al., 1975; Rajeswari et al., 1987; Mascotti & Lohman, 1990). This makes it more difficult to understand some recent examples of stability and specificity that can apparently arise with very short peptides and even amino acids (Weeks et al., 1990; Calnan et al., 1991; Tao & Frankel, 1992).

In order to provide a basis for understanding the more complex protein–ss nucleic acid interactions, we have undertaken a systematic thermodynamic study of the interaction of a series of synthetic oligopeptides with ss polynucleotides. Most previous studies of oligopeptide–ss polynucleotide binding have focused on oligopeptides with charges in the low range from only +1 to +3 (Brun et al., 1975; Helene & Maurizot, 1981; Helene & Lancelot, 1982; Rajeswari et al., 1987). Our previous efforts have focused primarily on experimental determinations of the thermodynamic extent of counterion release which accompanies the binding of the single-stranded (ss) homopolyribonucleotide poly(U) to a series of oligolysines with net positive charges, +2 to +10, and containing a single tryptophan residue (Mascotti & Lohman, 1990). These studies indicated that the binding of these peptides to poly(U) is stabilized by the release of cations from the poly(U) and that the number of cations released is $\sim 0.71 \pm 0.03$ per peptide charge (Mascotti & Lohman, 1990). In this report, we present a systematic thermodynamic characterization of these interactions as a function of pH, temperature, monovalent and divalent cation, oligolysine charge and charge distribution, and tryptophan content. These results should facilitate the interpretation of thermodynamic studies of other charged ligands as well as protein–nucleic acid interactions and provide for more detailed tests of current and future polyelectrolyte theories.

MATERIALS AND METHODS

Buffers and Reagents. Solutions were made with reagent grade salts using distilled H_2O that was further purified by passage through a Milli-Q water purification system (Millipore, Bedford, MA) and Nalgene filters (0.22 μm). The following buffers were used to span the range from pH 6 to 9: 10 mM cacodylic acid, titrated to pH 6.0 (CB6) or pH 7.0 (CB7) with 5 M KOH; 10 mM HEPES [*N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid], titrated to pH 7.5 with 5 M KOH; 10 mM Tris-HCl [tris(hydroxymethyl)aminomethane], titrated to pH 8.0 with 10 M acetic acid, and 10 mM sodium tetraborate ($Na_2B_4O_7$), titrated to pH 8.3 or pH 9.0 with glacial acetic acid. All buffers were titrated to the desired pH at the temperatures used in the titration. Our standard low salt and high salt buffers are CB6 or CB7 + 0.2 mM Na_3EDTA with the addition of 1.0 mM KCH_3CO_2 or 2.0 M KCH_3CO_2, respectively.

Peptides and Polynucleotides. The oligopeptides, KWK_p-NH_2 (p = 1–8), KWK_p-CO_2 (p = 1 and 4), KWK_3WK-NH_2, $(KW)_3K_2$-NH_2, KWG_2K_2-NH_2, containing L-lysine (K), L-glycine (G), and L-tryptophan (W), were synthesized by the TAES support laboratory (Texas A&M University). The peptide KWK-CO_2 was purchased from Serva Fine Chemicals (Westbury, NY). All peptides were purified in our laboratory using heptafluorobutyrate ion-paired reversed-phase HPLC chromatography as described (Mascotti & Lohman, 1990). Purity and composition were verified by fast atom bombardment (FAB) mass spectrometry (University of Texas Health Science Center, Houston, TX). Peptide concentrations were determined by measuring tryptophan absorbance in buffer CB6 + 1.0 mM KCH_3CO_2 at 25 °C using $\epsilon_{280} = 5.69 \times 10^3$ M^{-1} (per Trp) cm^{-1} (Edelhoch, 1967; Mascotti & Lohman, 1990) after verification that the extinction coefficient of tryptophan in all of the peptides studied here is unchanged in the presence of 6 M guanidine hydrochloride/20 mM Tris, pH 6.8 (25 °C).

Poly(U) ($s_{20,w}$ = 9.5 S; $\sim$950 ($\pm$200) nucleotides) was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN), and dialyzed extensively against the desired buffer before use. Poly(U) concentrations were determined spectrophotometrically in 10 mM Tris-HCl, pH 8.1 + 0.1 mM Na_3EDTA + 0.1 M NaCl using $\epsilon_{260} = 9.2 \times 10^3$ M^{-1} (nucleotide) cm^{-1} (Simpkins & Richards, 1967).

Fluorescence Titrations and Determination of Equilibrium Binding Isotherms. Fluorescence titrations were performed using an SLM-Aminco 8000C spectrofluorometer (Urbana, IL) as described (Overman et al., 1988; Mascotti & Lohman, 1990; Lohman & Mascotti, 1992b) by addition of poly(U) to a constant concentration of peptide ("reverse" titration) under constant solution conditions. An excitation wavelength of 292 nm (2-nm bandpass), and an emission wavelength of 350 nm (8-nm bandpass) were used. The fluorescence intensity at each point in a titration was corrected for dilution and inner filter effects as described (Lohman & Mascotti, 1992b) using $\epsilon_{292} = 1.6 \times 10^2$ M^{-1} (nucleotide) cm^{-1} for poly(U) and $\epsilon_{292} = 3.3 \times 10^3$ M^{-1} (tryptophan) cm^{-1} for all tryptophanyl oligolysines. The fluorescence emission spectra and intensities of each peptide containing a single tryptophan were identical (data not shown), indicating a lack of sequence effects. Under all conditions reported here, the fluorescence change resulting from addition of poly(U) to a peptide solution occurred within the first minute, hence measurements were taken 2 min after addition of poly(U) to ensure that equilibrium had been reached.

The intrinsic fluorescence of oligopeptides containing tryptophan is partially quenched upon binding ss nucleic acids (Dimicoli & Helene, 1974; Helene & Maurizot, 1981; Helene & Lancelot, 1982; Mascotti & Lohman, 1990). The extent of tryptophan fluorescence quenching, Q_{obs}, is defined as

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

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 fluorescence intensity before addition of polynucleotide, with both F_{obs} and F_{init} having been corrected for contributions from background fluorescence (Lohman & Mascotti, 1992b).

The binding density function method (Bujalowski & Lohman, 1987; Lohman & Bujalowski, 1991), in which multiple titrations of peptide with nucleic acid at several peptide concentrations are analyzed simultaneously, was used to obtain model-independent determinations of the oligopeptide binding density, ν (oligopeptides bound per nucleotide, $\nu = L_B/D_T$), and the free peptide concentration, L_F, at each value of L_T and D_T. Using this approach, we have determined that at the low binding densities used in our studies (<30% saturation of the poly(U)), Q_{obs} is directly proportional to the fraction of peptide bound to poly(U), L_B/L_T, for all of the peptides studied (Mascotti & Lohman, 1990; Lohman & Mascotti, 1992b). Therefore, on the basis of the fact that we have shown that eq 2a applies, the values of L_F and ν can be calculated at each point in a titration using eqs 2b and 2c where Q_{max} is the maximum fluorescence quenching observed upon saturation of the peptide with the nucleic acid.

Analysis of Equilibrium Binding Isotherms to Obtain K_{obs}. Equilibrium constants, K_{obs}, for peptide–poly(U) binding were obtained by two methods as previously described

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

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

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

(Mascotti & Lohman, 1990). In the first method, a full binding isotherm obtained from a titration of peptide with poly(U) at constant solution conditions was analyzed. In the second method, "salt-back" titrations was analyzed to determine K_{obs} at a series of salt concentrations (Overman et al., 1988; Mascotti & Lohman, 1990; Lohman & Mascotti, 1992b). In both cases, we used a noncooperative model for large ligands binding to a linear homogeneous lattice with overlapping binding sites (McGhee & von Hippel, 1974; Bujalowski et al., 1989). Therefore, the binding constants reported here were obtained in the limit of zero peptide binding density (McGhee & von Hippel, 1974). The value of K_{obs} was obtained from a single-parameter fit using a constant value of the peptide occluded site size, n, equal to the number of residues in the peptide. However, since our binding isotherms were obtained at low binding densities, the estimated value of K_{obs} is not very sensitive to the value of n used (if n is changed by 20%, the K_{obs} needed to fit is only 4% different).

We have attempted to use the models described by Manning (1978) and Friedman and Manning (1984) to interpret these equilibrium binding isotherms; however, neither model yields an adequate description of the experimental isotherm (see Discussion and Figure 13). Both models (Manning, 1978; Friedman & Manning, 1984) greatly overestimate the apparent negative cooperativity in the binding isotherms for all peptides.

The analysis of a single "salt-back" titration to obtain K_{obs} as a function of salt concentration (Overman et al., 1988; Lohman & Mascotti, 1992b) is valid for the interactions studied here since we have shown that eqs 2a–c apply and the fluorescence intensities of bound and free peptide are independent of salt concentration. In these experiments, a complex of peptide and poly(U), preformed at low KCH_3CO_2 concentration (e.g., CB6 + 1.0 mM KCH_3CO_2), is titrated with a concentrated salt solution (e.g., CB6 + 2.0 M KCH_3CO_2) while the increase in fluorescence accompanying dissociation of the complex is monitored. The value of Q_{obs} measured at each [KCH_3CO_2] during the salt-back titration can be used to determine ν and L_F using eqs 2b and 2c, and K_{obs} can be calculated at each [KCH_3CO_2], using the equation (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 $\Delta H°_{obs}$ and its dependence on salt concentration were obtained by performing a series of salt-back titrations at different temperatures and calculating $\Delta H°_{obs}$ at each salt concentration from a van't Hoff analysis (Lohman & Mascotti, 1992b). Such experiments were performed with identical stock solutions within a single day in order to improve the precision of the data.

An alternative model that can be used to describe our experimental isotherms is the "free-sliding" model (Woodbury, 1981) which differs from the model of McGhee and von Hippel (1974) in that it does not assume "site binding" of the ligands but rather allows translational mobility along the polynucleotide. As was found by Woodbury (1981), estimates of K_{obs} from use of the "free-sliding" model agree with those from use of the model of McGhee and von Hippel (1974) at low binding densities if the ligand site size is slightly smaller (by about 0.5 phosphate) in the "free-sliding" model. Our data, which are obtained at low binding densities, cannot differentiate between these two models.

THEORETICAL BACKGROUND

The intrinsic equilibrium binding constant, for the binding of a peptide, L, to a nucleic acid site, D, to form a complex, LD, is defined as $K_{obs} = [LD]/[L][D]$. The dependence of K_{obs} on monovalent salt concentration, MX, for the interaction of a charged ligand, L, with a polynucleotide, D, to from a complex, LD is given by

$$\frac{\partial \log K_{obs}}{\partial \log [MX]} = \Delta c + \Delta a - \frac{2m\Delta w}{[H_2O]} \quad (4)$$

where m is the molality of monovalent salt and Δc, Δa, and Δw represent the *net* preferential interactions of cations (M^+), anions (X^-), and water with the macromolecular reactants (L and D) or product (LD) (e.g., $\Delta c = (c_{LD} - c_L - c_D)$, where the c_i represent the moles of cations bound thermodynamically per mole of species i) (Record et al., 1978, 1991; Lohman & Mascotti, 1992a). As defined, the preferential interaction parameters represent the net difference in the thermodynamic extent of binding, including contributions from both direct binding and nonideality (activity coefficient) effects. We note that in general, the individual terms Δc, Δa, and Δw can be either positive or negative, indicating a net uptake or release, respectively; however, for positively charged ligands, cation release from the nucleic acid will usually be a major component of Δc (i.e., $\Delta c \ll 0$). We have shown previously that preferential anion interactions with small positively charged oligolysines, such as the ones examined in this study, are negligible (Mascotti & Lohman, 1990). Furthermore, for the binding of charged ligands to polynucleotides the term representing preferential hydration (Δw) is expected to be negligible when compared to the large cation release terms, except at salt concentrations $\geq$ 0.5 M. Therefore, the dependence of K_{obs} on monovalent salt concentration at constant temperature, pressure, and pH for the binding of positively charged peptides to polynucleotides can be simplified to the form in eq 5 (Record et al., 1976, 1978; Lohman et al., 1980; Mascotti & Lohman, 1990; Lohman & Mascotti, 1992a):

$$\log K_{obs} = \log K(1\ M\ M^+) + \Delta c \log [M^+] \quad (5a)$$

$$\left(\frac{\partial \log K_{obs}}{\partial \log [M^+]}\right) = \Delta c \quad (5b)$$

Linked Effects of pH and Monovalent Salt Concentration on K_{obs} and $\Delta H°_{obs}$ for Oligolysine–Poly(U) Interactions. The effects of pH on K_{obs} for oligolysine–poly(U) interactions are well-described by a so-called "titration curve" model, which assumes that the value of K_{obs} is determined by the net charge, z, of the *free* peptide (Lohman et al., 1980) (i.e., the pK's of the peptide amino acids are not altered upon binding nucleic acid). This is based on the observation that the extent of cation release from poly(U) is directly proportional to the net charge on the free oligolysine. Therefore, the model assumes that the peptide, in any protonation state, can bind to poly(U); however, K_{obs} decreases with increasing pH due to the lower net peptide charge, which upon binding to poly(U) results in a lower extent of cation release from the poly(U). The model described by Lohman et al. (1980) is applicable for oligolysine binding to duplex B-form DNA, since duplex DNA is not

titrated within the pH range from 6 to 9. However, this model must be modified slightly as described below to treat the binding to poly(U), since the N-3 of uracil undergoes partial deprotonation at high pH ($pK_U = 9.6$; Simpkins & Richards, 1967; Aylward, 1967).

For the interaction of oligopeptides with ds polynucleotides and ss poly(U), it has been shown that $\Delta c = -z\psi$ in eqs 5a,b to a very good approximation (Record et al., 1976, 1978; Lohman et al., 1980; Braunlin et al., 1982; Plum & Bloomfield, 1988; Mascotti & Lohman, 1990), where z is the net positive charge on the peptide and ψ is the thermodynamic extent of cation binding per phosphate (Record et al., 1976, 1978, 1991; Lohman & Mascotti, 1992a). Therefore, the dependence of K_{obs} on monovalent cation concentration $[M^+]$ for the interaction of a positively charged oligopeptide with a polynucleotide can be described by

$$\log K_{obs} = \log K(1\ M) - z\psi \log [M^+] \quad (6)$$

In the limit of low monovalent salt concentration, the cylindrical Poisson-Boltzmann theory (Anderson & Record, 1980, 1983) and the counterion condensation theory (Manning, 1969; Record et al., 1976) both predict the value of ψ to be $(1 - (2\xi)^{-1})$, where $\xi = e^2/\epsilon kTb$ (e is electronic charge, ϵ is the bulk dielectric constant of the solvent, T is absolute temperature, and b is the axial charge spacing projected onto the polynucleotide contour). In water, $\xi = 7.14/b$, where b is in angstroms and values of ψ are predicted to range from 0.88 for ds B-form DNA to ~0.7 for ss polynucleotides.

There are two potential effects of pH on K_{obs} for an oligopeptide–poly(U) interaction. The major effect is due to protonation of the oligopeptide, which affects the net peptide charge, z; however, since poly(U) is also titratable within the range of our experimental studies, the axial charge spacing and hence ψ is also dependent on pH. The dependence of z on pH is given by

$$z = z^\circ - \Sigma(1 + k_{i,L}a_H)^{-1} \quad (7)$$

where z° is the maximum positive charge on the peptide, observed at low pH, the $k_{i,L}$ are protonation constants for the titratable groups on the peptide, and a_H is the proton activity (Record et al., 1978; Lohman et al., 1980). We assume that the second effect, the pH dependence of ψ, results from the pH-dependent change in the polynucleotide axial charge spacing, b, as given in

$$1/b = [1 + (1 + k_U a_H)^{-1}]/b^\circ \quad (8)$$

where k_U is the protonation constant of uridylate and b° is the average charge spacing of poly(U) at neutral pH ($pH \ll pK_U$). With these effects included, the dependence of K_{obs} on both pH and $[M^+]$ is described by

$$\log K_{obs} = \log K(1\ M) - [z^\circ - \Sigma(1 + k_{i,L}a_H)^{-1}] \times [1 - [14.28\ (1 + (1 + k_U a_H)^{-1})/b^\circ]^{-1}] \log [M^+] \quad (9)$$

Equation 9 predicts that at sufficiently low pH, where the peptide will be fully protonated ($pH \ll pK_{i,L}$), K_{obs} will reach its maximum value and become independent of pH. However, at high pH, near the pK of the base, pK_U, the average charge density of the polynucleotide will increase and thus K_{obs} will also increase. Therefore, at some intermediate pH, a local minimum in K_{obs} should be observed.

This model can also be used to predict the dependence of ΔH°_{obs} on pH. By taking the derivative of eq 9 with respect to $1/T$ (where T = absolute temperature), the following equation is obtained

$$\Delta H^\circ_{obs} = (\Delta H^\circ(1\ M) + z^\circ \log [M^+][(k_U a_H)/(A'(1 + (1 + k_U a_H)^{-1})(1 + k_U a_H)^2)]\Delta h_U - \log [M^+][(\Sigma(k_{i,L}a_H))/(\Sigma(1 + k_{i,L}a_H)^2)]\Delta h_{i,L} - \log [M^+][(\Sigma(1 + k_{i,L}a_H)^{-1})(k_U a_H)/A'(1 + (1 + k_U a_H)^{-1})^2(1 + k_U a_H)^2)]\Delta h_U - \log [M^+][[A'(1 + (1 + k_U a_H)^{-1})]^{-1}(\Sigma(k_{i,L}a_H)/\Sigma(1 + k_{i,L}a_H)^2)]\Delta h_{i,L} \quad (10)$$

where $A' = 14.28/b^\circ$, Δh_U is the enthalpy of uracil protonation, $\Delta h_{i,L}$ is the enthalpy of protonation of the titratable groups of the oligopeptide, and $\Delta H^\circ(1\ M)$ is the value of ΔH°_{obs} at 1 M M^+. Equation 10 predicts that ΔH°_{obs} is independent of pH at both high and low pH and is only slightly pH-dependent at intermediate pH.

Effects of Divalent Cation Concentration on K_{obs}. The effects of Mg^{2+} on K_{obs} and the competitive effects of Mg^{2+} and monovalent cations on the binding of a third ligand (peptide) were analyzed as described (Record et al., 1977; deHaseth et al., 1977; Lohman et al., 1980). In the presence of only Mg^{2+}, the thermodynamic binding equilibria can be written

$$L + D \leftrightarrow LD + z\phi Mg^{2+} \quad (11)$$

where $z\phi$ represents the net thermodynamic release of Mg^{2+} from the poly(U) upon formation of LD and ϕ is the fraction of Mg^{2+} bound per nucleic acid phosphate ($\phi = {}^1/_2(1 - (2\xi)^{-1})$) (de Haseth et al., 1977). The dependence of K_{obs} on $[Mg^{2+}]$ can then be described by

$$\log K_{obs} = \log K(1\ M\ Mg) - (z\phi) \log [Mg^{2+}] \quad (12)$$

where $K(1\ M\ Mg)$ is the true thermodynamic equilibrium constant, which is independent of $[Mg^{2+}]$. On the basis of an estimated value of $\xi = 1.83 \pm 0.3$ for poly(U) (Mascotti & Lohman, 1990), we estimate a value of $\phi = 0.43 \pm 0.01$.

The competitive effects of Mg^{2+} and Na^+ on K_{obs} for oligolysine binding to poly(U) have been analyzed using eq 13. In the absence of preferential anion interactions, the dependence on $[Na^+]$ and $[Mg^{2+}]$ of K_{obs} is given by (Lohman et al., 1980)

$$\log K_{obs} = \log K(1\ M\ Na^+) - (z\psi) \log [Na^+] - \log \{(1 + 4K^{Mg}[Mg^{2+}])^{1/2}[(1/2)[1 + (1 + 4K^{Mg}[Mg^{2+}])^{1/2}]]^{z-1}\} \quad (13)$$

where K^{Mg} is the $[Na^+]$-dependent equilibrium constant for Mg^{2+} binding to poly(U) and z is the net charge on the free peptide.

RESULTS

Equilibrium Binding to Poly(U) of Oligolysines Containing a Single Tryptophan

Effects of Monovalent Salt Concentration. Figure 1 shows the results of titrations with poly(U) of three concentrations of KWK_2-NH_2 (37 mM NaCl, pH 6.0, 25.0 °C), plotted as the observed tryptophan fluorescence quenching, Q_{obs}, vs log [poly(U)]. The smooth curves are simulated isotherms based on eq 3 (McGhee & von Hippel, 1974). Each titration is well-described by the same interaction parameters for this model ($K_{obs} = 5.4 \times 10^4\ M^{-1}$, $n = 4$ nucleotides, $Q_{max} = 0.885$),

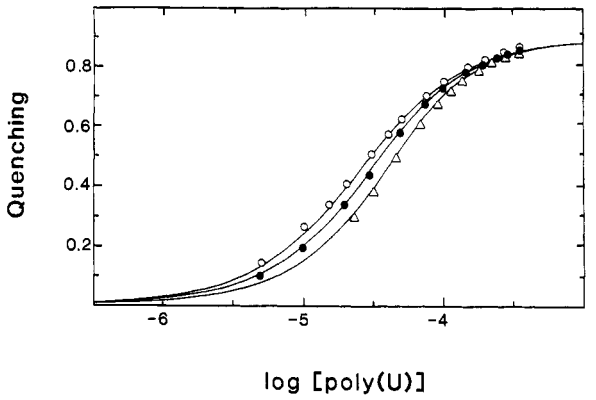

FIGURE 1: Titrations of the oligopeptide, KWK_2-NH_2, with poly(U). Titrations at three peptide concentrations (1.8 μM (○), 3.2 μM (●), and 6.0 μM (△)) were monitored by the quenching of the peptide tryptophan fluorescence in 37 mM NaCl, pH 6.0 (buffer CB), 25.0 °C. The [poly(U)] is given as moles of nucleotides per liter (M). Solid lines are simulated isotherms generated using eqs 2 and 3, with Q_{max} = 0.885, n = 4 nucleotides, and K_{obs} = 5.4 × 10^4 M^{-1}.

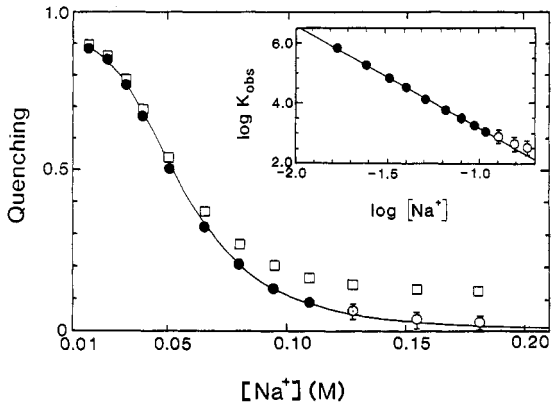

FIGURE 2: Salt-back titration performed by titrating a preformed poly(U)–KWK_2-NH_2 complex with NaCl (25.0 °C, pH 6.0) and monitoring the resulting increase in tryptophan fluorescence (decrease in Q_{obs}) that accompanies dissociation of the peptide–poly(U) complex. Initial peptide and poly(U) concentrations were 1.48 and 100.5 μM. The squares (□) represent raw data based on observed fluorescence quenching. Circles (○ and ●) are the same data after correction for dilution and inner filter effects as described in the text. The inset shows a plot of of log K_{obs} vs log [Na^+], where the values of K_{obs} were determined from the salt-back titration using eqs 2 and 3. The linear least-squares line, determined only using the data represented by the closed symbols (●), is log K_{obs} = −3.42 log [Na^+] − 0.26. The smooth curve representing the raw data was generated on the basis of eqs 2 and 3.

indicating that aggregation of the peptide and/or peptide–nucleic acid complexes does not occur under these conditions at these peptide concentrations (1.8–6 μM). This is consistent with previous studies showing that fluorescence polarization of the free peptides is independent of peptide concentration in this concentration range (Mascotti & Lohman, 1990). We note that we have determined independently for each peptide that the extent of tryptophan fluorescence quenching is directly proportional to the fraction of bound peptide, hence eqs 2 are valid.

The dependence of K_{obs} on monovalent salt concentration (MX) was obtained by performing a series of titrations at different [MX] as well as by analyzing salt-back titrations (see Materials and Methods) in order to determine more precisely the dependence of K_{obs} on [MX]. Figure 2 shows an example of a salt-back titration using NaCl to dissociate a preformed KWK_2-NH_2–poly(U) complex (pH 6.0, 25 °C). The resulting values of K_{obs} at each [NaCl], obtained using eqs 2a–c and 3 are shown in the inset to Figure 2. The values

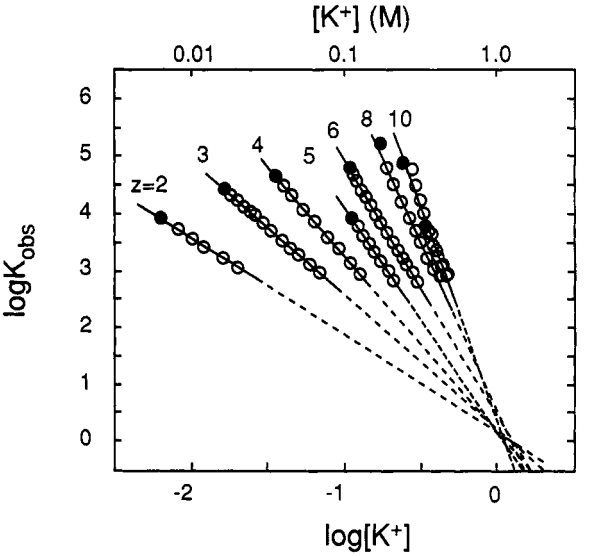

FIGURE 3: Plot of log K_{obs} vs log [K^+] for a series of oligolysines containing a single tryptophan binding to poly(U) (20.0 °C, buffer CB, pH 6.0). KCH_3CO_2 was used to vary the [K^+] concentration, and the data are plotted as a function of the total potassium concentration (M). The peptides with z = +3, 4, 6, 8, and 10 were KWK_p-NH_2 (p = 1, 2, 4, 6, and 8), and the peptides with z = +2 and 5 were KWK_p-CO_2 (p = 1 and 4), where the net positive charge of the peptide is indicated for each data set. Symbols: (●) K_{obs} determined from analysis of a full isotherm performed at a constant KCH_3CO_2 concentration; (○) K_{obs} determined from salt-back titrations. Linear least-squares lines are shown. Dashed lines are extrapolations of the least-squares (solid) lines.

of K_{obs} determined by both procedures are in excellent agreement at each [NaCl]; however, the salt-back titration method provides a more accurate estimate of the dependence of K_{obs} on salt concentration (Lohman & Mascotti, 1992b). Figure 2 indicates that K_{obs} decreases with increasing [NaCl], consistent with previous studies (Mascotti & Lohman, 1990). The dependence of log K_{obs} on log [NaCl] for this +4 peptide is linear over this range of [NaCl], with $\partial \log K_{obs}/\partial \log$ [NaCl] = −3.42 (±0.25).

We have shown previously for the interactions of KWK_4-NH_2 with poly(U), that the salt dependence of K_{obs} ($\partial \log K_{obs}/\partial \log [M^+]$), is independent of monovalent anion type (F^-, Cl^-, $CH_3CO_2^-$, Br^-) (Mascotti & Lohman, 1990). However, we find a slight decrease in K_{obs} upon replacing acetate with chloride or bromide (K_{obs} is ~40% lower at 0.15 M), which is more apparent with peptides of higher charge (e.g., KWK_8-NH_2) (Mascotti, 1992), whereas the values of K_{obs} and $\partial \log K_{obs}/\partial \log [M^+]$ are identical in fluoride and acetate salts for all peptides studied here, suggesting that preferential anion binding to the peptides is minimized in the presence of acetate and fluoride salts. Therefore, the experiments reported here have been performed in the presence of KCH_3CO_2.

Figure 3 shows the dependence of K_{obs} (20.0 °C, pH 6.0) on [KCH_3CO_2] for poly(U) binding to the series of oligolysines KWK_p-CO_2 (p = 1 and 4) and KWK_p-NH_2 (p = 1, 2, 4, 6, and 8) which possess net charges ranging from z = +2 to +10. For each peptide, a plot of log K_{obs} vs log [KCH_3CO_2] is linear over the range of salt concentrations investigated and the value of $|\partial \log K_{obs}/\partial \log [K^+]|$ increases with increasing peptide charge (see Table I). These results are similar to previous experiments performed at 25 °C (Mascotti & Lohman, 1990).

The dependence of K_{obs} on [K^+] was also determined at five temperatures between 10 °C and 40 °C and the values of ∂

Table I: Dependence of K_{obs} on $[KCH_3CO_2]$ and the Thermodynamics of Oligolysines Containing a Single Tryptophan Binding to Poly(U)[a]

peptide	z	$\partial \log K_{obs}/\partial \log [K^+]$	$\log K_{obs}$ (1 M)[b]	Q_{max} (%)	$\Delta H°_{obs}$[c]
KWK-CO_2	2	−1.68 (±0.20)	+0.26 (±0.24)	80 (±2)	−2.2 (±1.5)
KWK-NH_2	3	−2.30 (±0.19)	+0.36 (±0.24)	85 (±2)	−3.2 (±1.5)
KWK_2-NH_2	4	−3.10 (±0.21)	+0.20 (±0.22)	89 (±2)	−4.0 (±1.5)
KWG_2K_2-NH_2	4	−3.48 (±0.21)	−0.24 (±0.22)	85 (±2)	−4.0 (±1.5)
KWK_4-CO_2	5	−3.76 (±0.22)	+0.37 (±0.22)	90 (±2)	−4.9 (±1.5)
KWK_4-NH_2	6	−4.36 (±0.22)	+0.49 (±0.22)	92 (±2)	−4.9 (±1.5)
K_4WK-NH_2	6	−4.71 (±0.22)	+0.14 (±0.20)	89 (±2)	−4.9 (±1.5)
KWK_6-NH_2	8	−5.95 (±0.25)	+0.46 (±0.24)	92 (±2)	−5.7 (±1.5)
KWK_8-NH_2	10	−7.02 (±0.34)	+0.77 (±0.27)	91 (±2)	−5.4 (±1.5)

[a] Buffer CB, pH 6.0, 25.0 °C in KCH_3CO_2. [b] Obtained from a linear extrapolation of a plot of log K_{obs} vs log $[K^+]$. [c] The average $\Delta H°_{obs}$ within the range of $[M^+]$ examined in units of kilocalories per mole.

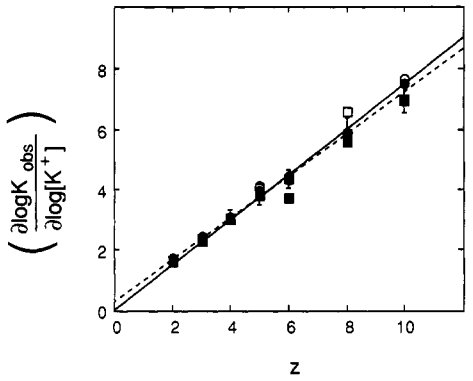

FIGURE 4: The experimental value of $-(\partial \log K_{obs}/\partial \log [K^+])$, representing thermodynamic extent of cation release from poly(U) upon binding the oligopeptide, is directly proportional to the net positive charge on the oligopeptide, z, and independent of temperature over the range from 10 °C to 40 °C. The temperatures for each determination are (○) 10.0 °C, (●) 20.0 °C, (▲) 25.0 °C, (□) 30.0 °C, and (■) 40.0 °C. The solid straight line representing the data was constrained to intersect the origin (slope = 0.75 ± 0.04), whereas the slope and intercept of the linear least-squares line (dashed) equal 0.72 ± 0.04 and 0.23 ± 0.25, respectively.

$\log K_{obs}/\partial \log [K^+]$ for each peptide are plotted as a function of the net peptide charge, z, in Figure 4. These data indicate that $|\partial \log K_{obs}/\partial \log [K^+]|$ for each peptide is independent of temperature and directly proportional to z under these conditions (pH 6.0). The average value of $(-\partial \log K_{obs}/\partial \log [K^+])/z$ for the data shown in Figure 4 is 0.76 ± 0.04; however, the slope of a linear least-squares line through the data is 0.72 ± 0.04, with an intercept of 0.23 ± 0.25 at $z = 0$. Therefore, we conclude that $|\partial \log K_{obs}/\partial \log [K^+]|$ is directly proportional to the peptide charge, z, and that the thermodynamic extent of cation release from poly(U) per oligolysine charge is 0.74 ± 0.04, independent of temperature. The values of $\partial \log K_{obs}/\partial \log [K^+]$ and K_{obs} extrapolated to 1 M KCH_3CO_2, for binding to poly(U) of the oligolysines containing a single tryptophan are listed in Table I (in KCH_3CO_2 at 25.0 °C).

Figure 3 also indicates that upon linear extrapolation of log K_{obs} to high $[KCH_3CO_2]$ the data for each peptide intersect, within experimental error, at a salt concentration close to 1 M K^+ with a value of $\log K_{obs} = 0$, independent of z (see also Table I). These values of $\log K$(1 M K^+) increase by less than 0.5 from 40 °C to 10 °C and hence appear to be independent of temperature, within our experimental error. Thus, the $\Delta G°_{obs}$ for binding of these oligolysines to poly(U) is approximately zero at 1 M K^+.

We have also examined the effect of different monovalent cations (K^+, Na^+, and (n-butyl)-NH_3^+) on K_{obs} of the interaction of KWK_4-NH_2 with poly(U) (pH 6.0, 25.0 °C), and these data are shown in Figure 5. The (n-butyl)-NH_3^+ is fully protonated at pH 6.0 (Robinson & Stokes, 1959). The values of K_{obs} and its dependence on salt concentration are

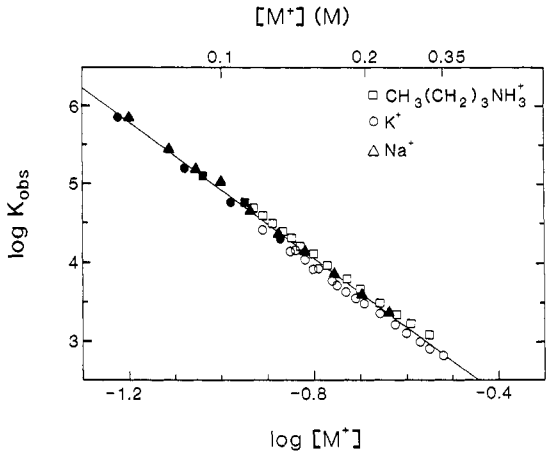

FIGURE 5: The monovalent salt dependence of K_{obs} is independent of the cation type for the binding of KWK_4-NH_2 to poly(U). $\log K_{obs}$ is plotted vs log $[M^+]$ where the monovalent salts used are (△, ▲) $NaCH_3CO_2$, (○, ●) KCH_3CO_2, and (□, ■) n-butyl-NH_4-CH_3CO_2 (plotted in terms of total $[M^+]$), pH 6.0 (buffer CB), 25.0 °C. Closed symbols represent K_{obs} obtained from reverse titrations, and the open symbols represent data obtained from salt-back titrations. The linear least-squares through all three data sets is $\log K_{obs} = -4.36 \log [M^+] + 0.56$. The parameters for each individual salt type are listed in Table II.

Table II: Effects of Cation Type on the Salt Dependence of Binding of KWK_4-NH_2 to Poly(U)[a]

salt	$\partial \log K_{obs}/\partial \log [M^+]$	$\log K_{obs}$ (1 M)[b]	Q_{max} (%)
KCH_3CO_2	−4.36 (±0.22)	+0.49 (±0.22)	92 (±2)
$NaCH_3CO_2$	−4.45 (±0.22)	+0.50 (±0.22)	91 (±2)
(n-butyl)-$NH_3CH_3CO_2$	−4.28 (±0.22)	+0.68 (±0.22)	94 (±2)

[a] Buffer CB + different acetate salt types, pH 6.0, 25.0 °C. [b] Obtained from a linear extrapolation of a plot of log K_{obs} vs log $[M^+]$.

independent of cation type, even for the bulky (n-butyl)-NH_3^+ (see also Table II). This is somewhat surprising, since it is expected that these cations would bind with different affinities to poly(U). Certainly, when a divalent cation, such as Mg^{2+}, is present, the value of K_{obs} is affected dramatically (see below).

K_{obs} and Its Dependence on Cation Concentration Are Independent of Peptide Charge Spacing. The free energy change ($\Delta G°_{obs}$) for the binding of the monotryptophan oligolysines to poly(U) appears to be dependent primarily on the net positive charge of the peptide, based on experiments with amidated vs carboxylated (zwitterionic) oligolysines of the same net charge (Mascotti & Lohman, 1990). We have investigated this further by examining the effect on K_{obs} and $\partial \log K_{obs}/\partial \log [K^+]$ of varying the oligolysine charge spacing and distribution. For this purpose, we compared two amidated oligopeptides, KWG_2K_2-NH_2 and KWK_2-NH_2, both of which possess $z = +4$ at pH 6 but differ in their charge spacing. We

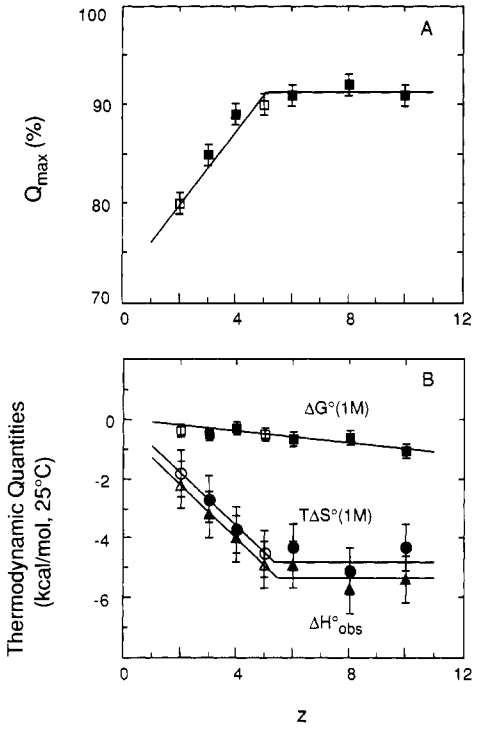

FIGURE 6: (A) The dependence of tryptophan fluorescence quenching, Q_{max}, on oligopeptide net charge, z, for binding to poly(U) in KCH_3CO_2 (pH 6.0 (buffer CB), 25.0 °C). All values of Q_{max} are independent of $[KCH_3CO_2]$. (B) The thermodynamic quantities, $\Delta G°_{obs}$, $\Delta H°_{obs}$, $T\Delta S°_{obs}$, for oligolysines binding to poly(U) in KCH_3CO_2 (pH 6.0 (buffer CB), 25 °C) were extrapolated to 1 M KCH_3CO_2 and plotted as a function of oligopeptide net charge, z.

find that K_{obs}, $(\partial \log K_{obs}/\partial \log [K^+])$, and the thermodynamic parameters, $\Delta H°_{obs}$ and $\Delta S°_{obs}$, derived from an analysis of the temperature dependence of K_{obs}, are identical for each peptide (see Table I). The only possible difference between the poly(U) binding properties of these two peptides is a slightly lower value of Q_{max} for KWG_2K_2-NH_2 (85 ± 2%) than for KWK_2-NH_2 (89 ± 2%), although the free fluorescences of each peptide are identical at 1.5 μM.

Tryptophan Fluorescence Quenching by Poly(U) Is Dependent upon Peptide Charge. We observe that the extent of tryptophan fluorescence quenching, Q_{max}, upon binding to poly(U) of a series of oligolysines containing a single tryptophan is dependent on the net peptide charge as shown in Figure 6A. Q_{max} is only 80 ± 2% for a peptide with z = +2, but it increases as z increases to +4, reaching a constant value of Q_{max} = 91 ± 2% for $z \geq +4$. This indicates that the tryptophan interaction with poly(U) is context-dependent, at least when contained within an oligolysine. A value of Q_{max} = 78% has previously been reported for the interaction of KWK-CO_2 with poly(U) (26 °C) (Brun et al., 1975), which is consistent with our results for the same peptide under similar conditions (80 ± 2%) (Table I and Figure 6A).

Effects of Temperature on K_{obs}. The temperature dependence of K_{obs} and the effect of salt concentration on $\Delta H°_{obs}$ were examined by performing a series of salt-back titrations at different temperatures (10 °C to 40 °C, pH 6.0) (Lohman & Mascotti, 1992b). As discussed above, we find that for KWK_4-NH_2, $\partial \log K_{obs}/\partial \log [K^+]$ = −4.39 ± 0.22, independent of temperature. van't Hoff analyses of these data were performed to obtain $\Delta H°_{obs}$ and $\Delta S°_{obs}$ as a function of $[KCH_3CO_2]$. Each van't Hoff plot is linear over the temperature range from 10 °C to 40 °C, suggesting that $\Delta C_p°$ = 0 over this temperature range (Lohman & Mascotti, 1992b).

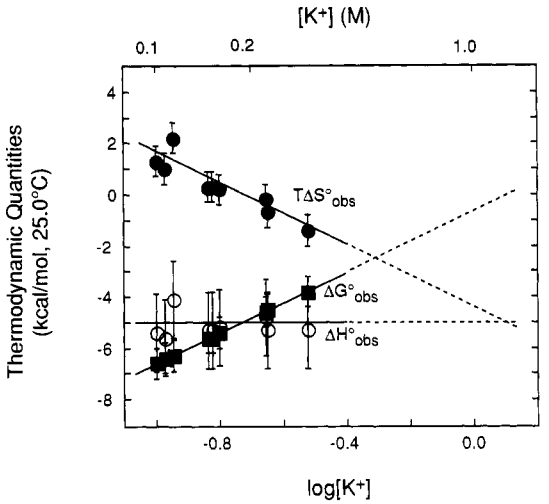

FIGURE 7: Dependence of $\Delta G°_{obs}$ (■), $\Delta H°_{obs}$ (○), and $T\Delta S°_{obs}$ (●), on log $[K^+]$ for the equilibrium binding of KWK_4-NH_2 with poly(U) (25.0 °C, pH 6.0 (buffer CB), where KCH_3CO_2 was used to vary the $[K^+]$). $T\Delta S°_{obs}$ was calculated from $(\Delta H°_{obs,av} - \Delta G°_{obs})$. Solid lines are the linear least-squares lines, and the dashed lines are extrapolations to high salt concentrations.

The values of $\Delta G°_{obs}$, $\Delta H°_{obs}$, and $T\Delta S°_{obs}$ are plotted in Figure 7 as a function of log $[KCH_3CO_2]$ for the KWK_4-NH_2–poly(U) interaction (pH 6.0, 25.0 °C). For this peptide, $\Delta H°_{obs}$ is independent of $[KCH_3CO_2]$ over the range from ~0.1 to ~0.3M, with an average value of $\Delta H°_{obs}$ = −5.0 ± 1.5 kcal/mol. However, $\Delta G°_{obs}$ and $\Delta S°_{obs}$ are strongly dependent on $[KCH_3CO_2]$. $\Delta G°_{obs}$ increases from −6.7 ± 1.5 kcal/mol at ~0.1 M KCH_3CO_2 to −3.0 ± 1.5 kcal/mol at ~0.3 M KCH_3CO_2, whereas $T\Delta S°_{obs}$ decreases from +1.5 ± 1.5 kcal/mol at ~0.1 M KCH_3CO_2 to −1.4 ± 1.5 kcal/mol at ~0.3 M KCH_3CO_2.

These results indicate that $\Delta G°_{obs}$ for KWK_4-NH_2–poly(U) binding is enthalpically-driven under all of the experimental conditions tested; however, the dependence of $\Delta G°_{obs}$ on $[KCH_3CO_2]$ has its origins in the dependence of $\Delta S°_{obs}$ on $[KCH_3CO_2]$. The same trends in $\Delta G°_{obs}$, $\Delta H°_{obs}$, and $\Delta S°_{obs}$ as functions of $[KCH_3CO_2]$ were observed for poly(U) binding to all of the tryptophan-containing oligolysines examined. As we discuss below, the favorable $\Delta H°_{obs}$ is due primarily to the interaction of tryptophan with poly(U), whereas we interpret the salt dependence of $\Delta G°_{obs}$ and $\Delta S°_{obs}$ as originating from the polyelectrolyte effect, $(\Delta G°_{PE} = RT \ln [K^+])$, i.e., the release of counterions (K^+) from the poly(U) upon formation of the complex. This polyelectrolyte effect diminishes as the $[K^+]$ increases since the entropy change diminishes for the release of K^+ from the poly(U) into a solution containing a higher $[K^+]$ (Record et al., 1976; Record, 1988).

The values of $\Delta S°_{obs}$ and $\Delta G°_{obs}$ for the various peptide–poly(U) interactions are dependent on the salt concentration as well as the oligolysine net charge. This makes direct comparisons of $\Delta S°_{obs}$ and $\Delta H°_{obs}$ among the different peptides difficult since the data for the oligolysines possessing different net charges have been necessarily obtained over different ranges of $[K^+]$. However, the polyelectrolyte effect that is responsible for these salt dependences is eliminated at 1 M KCH_3CO_2 (Record et al., 1976). Therefore, we have extrapolated the values of $\Delta S°_{obs}$ and $\Delta G°_{obs}$ to 1 M KCH_3CO_2 in order to compare them for the different oligopeptides. Values of $\Delta G°_{obs}$, and $T\Delta S°_{obs}$ at 1 M K^+ were determined by linear extrapolation to log $[K^+]$ = 0 as shown in Figure 7.

Figure 6B shows the dependence of $\Delta G°_{obs}$(1 M), $\Delta H°_{obs}$, and $T\Delta S°_{obs}$(1 M) (25.0 °C, pH 6.0) on oligopeptide net

charge, z, for the interaction of poly(U) with the series of monotryptophan oligolysines, KWK_p-NH_2 and KWK_p-CO_2 (see also Table I). At 25 °C, $\Delta G°_{obs}$(1 M) shows only a slight dependence on z, whereas both $\Delta H°_{obs}$ and $T\Delta S°_{obs}$(1 M) show a complex dependence on z. For $z \geq +4$, both $\Delta H°_{obs}$ and $T\Delta S°_{obs}$(1 M) are independent of z, with values of −5.3 ± 1.5 and −4.9 ± 1.5 kcal/mol, respectively, whereas for $z < +4$, both $\Delta H°_{obs}$ and $T\Delta S°$(1 M) become more negative with increasing z. We also note that $\Delta H°_{obs}$ and $T\Delta S°_{obs}$(1 M, 25 °C) nearly compensate at each value of z, resulting in the slight dependence of $\Delta G°_{obs}$(1 M, 25 °C) on z. This enthalpy–entropy compensation phenomenon is observed at all temperatures from 10 °C to 40 °C (data not shown).

Comparison of panels A and B of Figure 6 reveals a striking correlation among the dependences of Q_{max}, $\Delta H°_{obs}$, and $T\Delta S°_{obs}$(1 M, 25 °C) on net peptide charge, z. All three quantities vary linearly with z in the range $z \leq +4$, whereas they are constant for $z \geq +4$. As Q_{max} increases, both $\Delta H°_{obs}$ and $T\Delta S°$(1 M, 25 °C) become more negative in a nearly compensating manner. Recall that the thermodynamic quantities plotted in Figure 6 have been obtained by extrapolation to 1 M K^+ and hence do not include contributions from the polyelectrolyte effect. Therefore, this correlation suggests that the negative enthalpy and entropy changes result from interactions involving the tryptophan and that these are dependent on the net peptide charge for $z \leq +4$ but are constant for $z \geq +4$. The fact that $T\Delta S°_{obs}$ and $\Delta H°_{obs}$ are nearly compensating also indicates that tryptophan binding to poly(U) contributes very little to the net $\Delta G°_{obs}$(1 M, 25 °C), independent of peptide charge. This correlation among Q_{max}, $\Delta H°_{obs}$, $T\Delta S°$(1 M K^+) is observed at all temperatures ranging from 10 °C to 40 °C.

We have also investigated the thermodynamics of binding KWK-CO_2 to poly(U) at pH 7.0 and estimate $\Delta H°_{obs}$ = −5.2 ± 1.5 kcal/mol at ~6 mM K^+ (KCH_3CO_2), in good agreement with the value of −3.7 ± 2 kcal/mol reported by Brun et al. (1975) at ~3 mM Na^+ (NaCl). Although our experiments and those of Brun et al. (1975) were obtained in different salts, this does not seem to have an effect, consistent with our observation that $\Delta H°_{obs}$ for oligolysines binding to poly(U) is unchanged in KCH_3CO_2 vs NaCl (Mascotti, 1992).

Linkage of pH and Temperature Effects. The dependence of log K_{obs} on pH for the binding of KWK_4-NH_2 to poly(U) at three [KCH_3CO_2] is shown in Figure 8. At each pH, log K_{obs} decreases as the [K^+] increases. At each salt concentration, K_{obs} is constant for pH ≤ 6; however, K_{obs} decreases with increasing pH until it reaches an apparent plateau value above pH 8. The smooth curves in Figure 8 were generated using eq 9 with two peptide protonation constants as adjustable fitting parameters. These were designated K_α and K_ϵ, representing the α-amino and an average value for all the ε-amino groups, respectively. Values of pK_α = 7.2 and pK_ϵ = 8.8 gave the best fits to the experimental data; the use of additional pK's did not improve the fits. The other parameters required in eq 9 were constrained with the following values: log K(1 M) = 0.49, $z°$ = 6, and b = 3.7 Å (see Table I), pK_U = 9.6 (Simpkins & Richards, 1967; Aylward, 1967)).

We also determined the pH dependence of the van't Hoff enthalpy change, $\Delta H°_{obs}$, which is shown in Figure 9 for the interaction of KWK_4-NH_2 with poly(U) (25.0 °C, 0.1 M KCH_3CO_2). These data can be described as being roughly independent of pH, with an average $\Delta H°_{obs}$ = −5.2 ± 2.5 kcal/mol. However, these data are well-described by the solid curve in Figure 9, which was generated using eq 10 with the same parameters as those used to simulate the data in Figure 8, as well as $\Delta h°_\alpha$ = −12.7 kcal/mol, $\Delta h°_\epsilon$ = −11.35 kcal/mol (Greenstein, 1933), and $\Delta h°_U$ = −8.6 kcal/mol (Aylward, 1967). A value of $\Delta H°$(1 M) = −3.0 kcal/mol was used to obtain the curve in Figure 9, which is only slightly lower than the experimental value of $\Delta H°_{obs}$ = −4.9 ± 1.5 kcal/mol (see Table I).

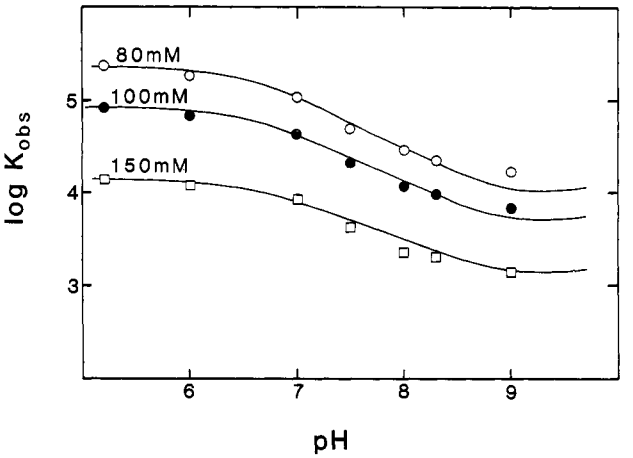

FIGURE 8: The dependence of log K_{obs} on pH for the binding of KWK_4-NH_2 to poly(U) in 80, 100, and 150 mM [KCH_3CO_2] at 25.0 °C. (Cacodylate buffer was used for pH 6.0 and 7.0; HEPES buffer was used for pH 7.5; Tris–acetate buffer was used for pH 8.0; sodium tetraborate buffer was used for pH 8.3 and pH 8.8.) The total K^+ concentrations are indicated. The solid lines are simulations based on eq 9 with the following parameters: log K(1 M) = 0.49, $z°$ = 6, $b°$ = 3.7 Å, pK_α = 7.2, pK_ϵ = 8.8, pK_U = 9.6.

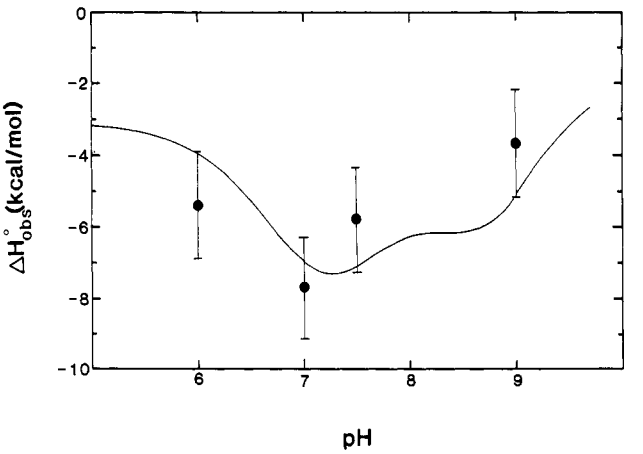

FIGURE 9: The dependence of $\Delta H°_{obs}$ on pH for the binding of KWK_4-NH_2 to poly(U) in KCH_3CO_2 (the total [K^+] is 0.1 M) at 25.0 °C (buffers are described in Materials and Methods). The solid curve is a simulation based on eq 10 using the parameters given in Figure 8 and the following additional parameters: $\Delta H°$(1 M) = −3.0 kcal/mol, $\Delta h°_\alpha$ = −12.7 kcal/mol, $\Delta h°_\epsilon$ = −11.35 kcal/mol, $\Delta h°_U$ = −8.6 kcal/mol.

All of the oligolysine–poly(U) interactions were studied as functions of [KCH_3CO_2] and temperature at both pH 6.0 and pH 7.0 (buffer CB6 and CB7). On the basis of the values of $\Delta H°_{obs}$ measured at pH 6 and pH 7, we have estimated pK_α for binding of each oligolysine to poly(U) using eq 10, since the region between pH 6 and 7 is most sensitive to the value of pK_α. A rough lower estimate of pK_ϵ could also be obtained from fitting the data for $\Delta H°_{obs}$ as a function of pH. For the lower charged oligolysines ($z \leq +8$), a single pK could account for the difference in $\Delta H°_{obs}$ at pH 6 vs pH 7, suggesting that pK_ϵ must be at least one unit higher than pK_α. For the most highly charged oligolysine ($z = +10$), the difference in $\Delta H°_{obs}$ could not be accounted for by only a single protonation

Table III: Apparent Values of pK_α and pK_ϵ for Oligolysines Binding to Poly(U)[a]

peptide	pK_α	pK_ϵ
KWK-CO_2	7.85 (±0.2)	≥9.2
KWK-NH_2	7.57	≥8.6
KWK_2-NH_2	7.62	≥8.6
KWK_4-NH_2	7.19	≥8.8
KWK_6-NH_2	7.50	≥8.8
KWK_8-NH_2	6.97	≥7.6

[a] Measured in buffers containing KCH_3CO_2 at 25.0 °C.

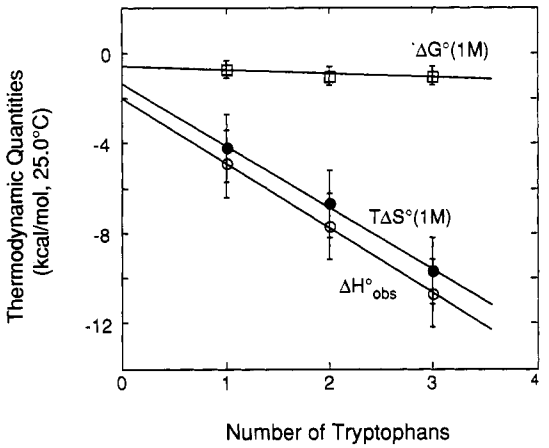

FIGURE 10: The thermodynamic quantities, ΔG°_{obs}(1 M), ΔH°_{obs}, and $T\Delta S^\circ_{obs}$(1 M), for poly(U) binding to oligolysines all with net charge $z = +6$, but containing 1, 2, or 3 tryptophans plotted vs the number of tryptophans (buffer CB, pH 6.0, 25.0 °C). The oligopeptides are (○) KWK_4-NH_2, (●) KWK_3WK-NH_2, and (□) $(KW)_3K_2$-NH_2, and the thermodynamic data are listed in Table IV. The equations of the linear least-squares lines through the data are given in eqs 14a–c.

constant; thus a lower estimate of pK_ϵ was necessary to describe the data for this peptide using eq 10.

The estimates of pK_α and pK_ϵ are listed in Table III and were used in eq 7 to calculate z for the peptides in the pH range from 6 to 7. The apparent values of pK_α decrease with increasing oligolysine charge from ~7.9 to ~7.1, while the apparent values of pK_ϵ decrease with increasing charge from ~9.2 to ~7.6. The values of pK_α agree quite well with previous estimates based on potentiometric studies. However, our estimates of the apparent pK_ϵ are 1.5–2.5 units lower than previous values obtained from direct potentiometric measurements (Ellenbogen, 1952; Yaron et al., 1972), which is not surprising since these estimates represent an average over all the ϵ-amino groups. Previous estimates of pK_α and pK_ϵ for oligolysines of various lengths were not used in our simulations since the oligolysines used in those studies did not contain tryptophan and were determined at only one salt concentration, whereas our data span a range of $[KCH_3CO_2]$ and this range differs for each peptide.

Oligolysines Containing Multiple Tryptophans

We have compared the binding to poly(U) of three oligolysines, KWK_4-NH_2, KWK_3WK-NH_2, $(KW)_3K_2$-NH_2, each of which possess $z = +6$, but contains either one, two, or three tryptophans. The results of these studies are shown in Figure 10 and Table IV. As a control, we also compared K_4WK-NH_2 and KWK_4-NH_2 and found no differences indicating that the position of a single tryptophan does not influence the peptide binding properties under these conditions (pH 6.0, KCH_3CO_2) (see also Table I). We also find that the values of Q_{max} upon poly(U) binding, as well as the

Table IV: Dependence of K_{obs} on $[KCH_3CO_2]$ for Poly(U) Binding to Oligolysines Containing Multiple Tryptophans[a]

peptide	$\partial \log K_{obs}$ / $\partial \log [K^+]$	$\log K_{obs}$ (1 M)[b]	Q_{max} (%)	ΔH°_{obs}[c]
K_5-NH_2[d]	−4.36 (±0.22)	+0.0 (±0.22)		−2.0 (±1.5)
KWK_4-NH_2	−4.36 (±0.22)	+0.49 (±0.22)	92 (±2)	−4.9 (±1.5)
KWK_3WK-NH_2	−4.55 (±0.22)	+0.74 (±0.22)	90 (±2)	−7.7 (±1.5)
$(KW)_3K_2$-NH_2	−4.60 (±0.23)	+0.76 (±0.24)	92 (±2)	−10.7 (±1.5)

[a] Buffer CB + KCH_3CO_2, pH 6.0, 25.0 °C. [b] Obtained from a linear extrapolation of a plot of log K_{obs} vs log $[K^+]$. [c] The average ΔH°_{obs} within the range of $[K^+]$ examined in units of kilocalories per mole. [d] Values predicted from extrapolation to zero tryptophans.

fluorescence of the free peptides, are identical for these three peptides, independent of the number of tryptophans, suggesting that each tryptophan binds to poly(U) in a similar environment and is quenched equivalently even for the multiple tryptophan-containing peptides. Table IV indicates that the values of K_{obs} for KWK_3WK-NH_2 and $(KW)_3K_2$-NH_2 are only slightly greater (less than a factor of ~2) than K_{obs} for the single Trp peptides. Furthermore, within experimental error, the value of $(\partial \log K_{obs}/\partial \log [K^+])$ is independent of the number of tryptophans indicating that the tryptophan interaction with poly(U) does not result in any detectable ion release (Table IV). No differences were observed in the binding properties of KWK_3WK-NH_2 with poly(U) upon replacing KCH_3CO_2 with KCl suggesting that preferential anion interactions were unaffected by the presence of multiple tryptophans.

Although the presence of multiple tryptophans within the oligolysines does not affect $\Delta G^\circ_{obs}(K_{obs})$ for the interaction with poly(U), both ΔH°_{obs} and ΔS°_{obs}(1 M) are influenced as shown in Table IV and Figure 10. The observed value of ΔG°(1 M, 25 °C) is essentially independent of the number of tryptophans (N) and is well-described by

$$\Delta G^\circ(1\ M\ K^+, 25\ ^\circ C)\ (kcal/mol) = -0.6\ (\pm 0.4) - 0.15\ (\pm 0.15)N \quad (14a)$$

The values of both ΔH°_{obs} and $T\Delta S^\circ_{obs}$(1 M, 25 °C) decrease with increasing number of tryptophans as shown in Figure 10 and eqs 14b and 14c.

$$\Delta H^\circ_{obs}\ (kcal/mol) = -2.0\ (\pm 1.5) - 2.85\ (\pm 0.7)N \quad (14b)$$

$$T\Delta S^\circ_{obs}(1\ M\ K^+, 25\ ^\circ C)\ (kcal/mol) = -1.4\ (\pm 1.5) - 2.7\ (\pm 0.7)N \quad (14c)$$

Clearly, the independence of ΔG°(1 M K^+, 25 °C) on N results from dramatic enthalpy–entropy compensations. Extrapolation of ΔG°(1 M K^+, 25 °C) to $N = 0$ yields an estimate of ΔG°(1 M K^+, 25 °C) = −0.6 (±0.4) kcal/mol for a hexavalent oligolysine, containing no tryptophans. This extrapolation appears to be justified since under the conditions of these experiments, each Trp within the three peptides appears to interact with poly(U) equivalently and independently. As in the case of oligolysines containing a single tryptophan, the van't Hoff plots do not exhibit any noticeable curvature indicating that ΔC_p° for these reactions is negligible.

Effects of Mg^{2+} and Temperature on KWK_4-NH_2–Poly(U) Binding

We have measured K_{obs} for KWK_4-NH_2 binding to poly(U) in the presence of only added $MgCl_2$ (in the absence of added monovalent salt, buffer CB, pH 6.0, 25.0 °C) in order to examine the effect of Mg^{2+} binding to poly(U). Recall that

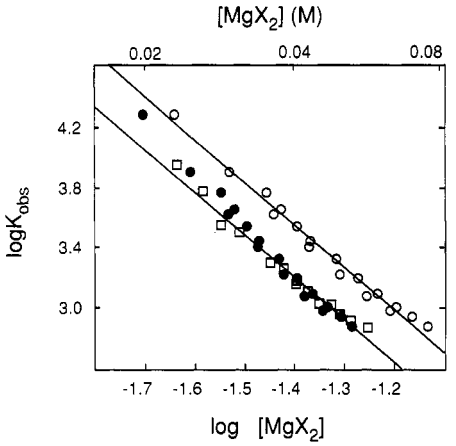

FIGURE 11: The dependence of log K_{obs} on log [Mg^{2+}] (total [M^{2+}]) for the binding of KWK_4-NH_2 to poly(U). Symbols: (□) $MgCl_2$ and (○) $Mg(CH_3CO_2)_2$ (buffer CB, pH 6.0, 25.0 °C). The equations of the linear least-squares lines are listed in Table V. The solid circles (●) represent the $Mg(CH_3CO_2)_2$ data replotted as $[Mg^{2+}]_{free}$ after correction for the presumed formation of ion pairs between Mg^{2+} and CH_3CO_2, based on an ion-pairing constant, K_{IP} = 8 M^{-1}, assuming formation of $Mg(CH_3CO_2)^+$.

Table V: Dependence of K_{obs} on Magnesium Concentration for the Interaction of KWK_4-NH_2 with Poly(U)[a]

salt type	$\partial \log K_{obs}/\partial \log [M^{+2}]$[b]	log K (1 M)[b]	Q_{max} (%)	$\Delta H°_{obs}$[c] (kcal/mol)[c]
$MgCl_2$	−2.82 (±0.25)	−0.74 (±0.40)	91 (±2)	ND[d]
$Mg(CH_3CO_2)_2$	−2.82 (±0.25)	−0.39 (±0.45)	91 (±2)	−10.1 (±1.5)
$Mg(CH_3CO_2)_2$[e]	−3.42 (±0.25)	−1.57 (±0.45)	91 (±2)	−10.1 (±1.5)

[a] Buffer CB + $MgCl_2$ or $Mg(CH_3CO_2)_2$, pH 6.0, 25.0 °C. [b] Obtained from a linear extrapolation of a plot of log K_{obs} vs log [K^+]. [c] Determined as an average of $\Delta H°_{obs}$ within the range of [Mg^{2+}] examined. [d] Not determined. [e] The salt dependence of K_{obs} and log K (1 M), in the presence of $Mg(CH_3CO_2)_2$, are based on free [Mg^{2+}], which was calculated using an ion pairing constant of 8 M^{-1}.

under these conditions chloride does not bind appreciably to KWK_4-NH_2 (Mascotti & Lohman, 1990); therefore, the slope of a plot of log K_{obs} vs log [$MgCl_2$] should reflect only the thermodynamic release of Mg^{2+} from the poly(U) upon binding of the peptide (see eq 12). The dependence of log K_{obs} on log [$MgCl_2$] for the binding of KWK_4-NH_2 to poly(U) is shown in Figure 11; the linear least-squares line describing the data is log K_{obs} = −2.82 (±0.25) log [$MgCl_2$] − 0.74 (±0.40) (see Table V). The relative values of $\partial \log K_{obs}/\partial \log [Mg^{2+}]$ = −2.82 (±0.25) and $\partial \log K_{obs}/\partial \log [K^+]$ = −4.36 (±0.22) for the same peptide, indicate that the number of Mg^{2+} that are released is approximately half the number of K^+. In fact, the ratio of the two slopes is 0.65 ± 0.07, in excellent agreement with the value of 0.60, predicted from simple counterion condensation principles (see Theoretical Background section).

Also shown in Figure 11 is the dependence of log K_{obs} on log [$Mg(CH_3CO_2)_2$] for the binding of the same peptide, KWK_4-NH_2 to poly(U) (see also Table V). At the same total [Mg^{2+}], the values of K_{obs} measured in $Mg(CH_3CO_2)_2$ are larger than those measured in $MgCl_2$. These differences are not due to differences in preferential interactions of the anions with the oligopeptide, based on our studies in KCl vs KCH_3CO_2 (Mascotti & Lohman, 1990). Therefore, the higher K_{obs} in the presence of acetate is likely due to the tendency of Mg^{2+} and $CH_3CO_2^-$ to form ion pairs to yield $Mg(CH_3CO_2)^+$ (Cannon & Kibrick, 1938; Nancollas, 1956; Archer & Monk, 1964). The partial formation of such ion pairs would result in a lowering of the free [Mg^{2+}] in the presence of acetate, thus at the same total [Mg^{2+}], the apparent values of K_{obs} would be higher in the presence of acetate. If we correct the

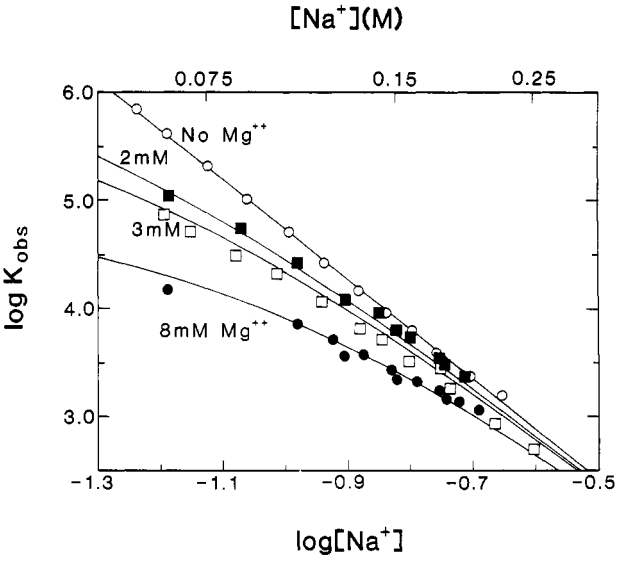

FIGURE 12: The dependence of log K_{obs} on log [Na^+] (total [Na^+]) for the interaction of KWK_4-NH_2 (z = +6) with poly(U) in the presence of 0, 2.0, 3.0, and 8.0 mM $MgCl_2$ (25.0 °C, buffer CB, pH 6.0). The solid lines are simulated curves based on eqs 13 and 15 and log K(1 M) = 0.28, z = 6, ψ = 0.768.

$Mg(CH_3CO_2)_2$ data to account for such ion pairing by using an ion-pairing constant of 8 M^{-1} to calculate the free Mg^{2+} concentration, then the two sets of data (Cl^- vs $CH_3CO_2^-$) overlap as shown in Figure 11. Ion pairing between Mg^{2+} and $CH_3CO_2^-$ has been reported, and an ion-pairing constant of 8 M^{-1} is within the limits of previously published values for this constant (Nancollas, 1956; Cannon & Kibrick, 1938; Emara, 1979; Archer & Monk, 1964).

We have also determined the van't Hoff $\Delta H°_{obs}$ in the presence of [$Mg(CH_3CO_2)_2$] (pH 6.0) for the binding of KWK_4-NH_2 to poly(U) and find $\Delta H°_{obs}$ = −10.1 ± 1.5 kcal/mol, independent of the $Mg(CH_3CO_2)_2$ concentration (data not shown). (The use of free vs total [Mg^{2+}] does not affect this estimation of $\Delta H°_{obs}$.) The value of $\Delta H°_{obs}$ determined in $Mg(CH_3CO_2)_2$ is more negative by 5–6 kcal than in KCH_3CO_2 ($\Delta H°_{obs}$ = −4.9 ± 1.5 kcal/mol) (see Table I). This extra enthalpic contribution to the binding likely reflects the excess enthalpy change accompanying the release of Mg^{2+} (relative to K^+) from the poly(U) upon binding KWK_4-NH_2 (see Discussion).

We have measured K_{obs} for the KWK_4-NH_2–poly(U) interaction as a function of [NaCl] in the presence of varying concentrations of $MgCl_2$, and the results are shown in Figure 12. The results of these experiments are in qualitative agreement with behavior observed previously for the interaction of pentalysine with duplex B-form DNA (Lohman et al., 1980) and nonspecific binding to *Escherichia coli lac* repressor to duplex DNA (Record et al., 1977). Qualitatively, at constant [NaCl], K_{obs} decreases as the [Mg^{2+}] increases and this effect is more pronounced at lower [NaCl]. This results in curvature in the plots of log K_{obs} vs log [Na^+] at constant total [Mg^{2+}], with the curvature increasing as the total [Mg^{2+}] increases (cf. 2 mM vs 8 mM Mg^{2+} in Figure 12). This can be quantitatively explained by the competition between Na^+ and Mg^{2+} for binding to poly(U) and the fact that the equilibrium binding constants of both Mg^{2+} and KWK_4-NH_2 are functions of [Na^+]. The derivative ($\partial \log K_{obs}/\partial \log [Na^+]$) always measures only the release of Na^+ upon formation of the oligopeptide–poly(U) complex, even when both Na^+ and Mg^{2+} are released as is the case in buffers containing a mixture of mixed Na^+ and Mg^{2+}. Therefore,

this derivative is at its maximum (in absolute value) in the absence of Mg^{2+} or at high $[Na^+]$ and decreases under conditions such that Mg^{2+} competes better with Na^+ (low $[Na^+]$ and high $[Mg^{2+}]$).

The data in Figure 12 can be analyzed to obtain values of K_{obs}^{Mg} for the interaction of Mg^{2+} with poly(U) using the approach described previously (Lohman et al., 1980) using eq 13. In this approach, K_{obs}^{Mg} is obtained as a fitting parameter. The values of K_{obs}^{Mg} that were obtained by this fitting procedure are dependent upon the $[Na^+]$ as expected for the binding of a charged ligand and are described by

$$\log K_{obs}^{Mg} = (-1.56 \pm 0.30) \log [Na^+] + 0.28 \pm 0.30 \quad (15)$$

Equation 15 indicates that $\log K_{obs}^{Mg}$ is a linear function of $\log [Na^+]$ with a slope of -1.56 ± 0.30. This value of $(\partial \log K_{obs}^{Mg}/\partial \log [Na^+])$ indicates that only a fraction of a Na^+ is released from poly(U) upon binding a magnesium ion and is essentially identical to the salt dependence observed for the binding KWK-CO_2 ($z = +2$) to poly(U) (see Table I). The smooth curves in Figure 12 were simulated using eqs 13 and 15 and describe the data very well suggesting that the effects of Mg^{2+} on peptide–poly(U) binding can be explained solely by the competition between Na^+ and Mg^{2+} for the poly(U) and that K_{obs}^{Mg} is dependent upon $[Na^+]$.

As previously discussed (de Haseth et al., 1977), if the effects of Na^+ vs Mg^{2+} on K_{obs} for the binding of KWK_4-NH_2 to poly(U) can be described by the simple competitive binding of Na^+ and Mg^{2+} in the context of a counterion condensation model, then to a first approximation, the relationship in eq 16 is predicted to hold. In fact, we find that this relationship

$$\log K(1\text{ M NaCl}) - \log K(1\text{ M } MgCl_2) = (z\phi) \log K^{Mg}(1\text{ M}) \quad (16)$$

holds within experimental error, since $\log K$(1 M NaCl) – $\log K$(1 M $MgCl_2$) = 0.12 (±0.22) – (–0.74 (±0.40)) = 0.86 (±0.46) (Table V), whereas $(z\phi) \log K^{Mg}(1\text{ M}) = 0.79$ (±0.90). Thus, the effect of Mg^{2+} on the monovalent salt concentration dependence of oligolysine–poly(U) interactions is well-described by a model in which Mg^{2+} and Na^+ bind to poly(U) in primarily delocalized modes (de Haseth et al., 1977).

DISCUSSION

Determinations of K_{obs} in the Limit of Zero Peptide Binding Density. The equilibrium constants, K_{obs}, that we report here for oligopeptide–poly(U) binding were determined by extrapolation to the limit of zero peptide binding density. The experimental equilibrium binding isotherms were determined using a model-independent method (Bujalowski & Lohman, 1987). These isotherms were then analyzed to obtain K_{obs} using a model for noncooperative binding of large ligands to a homogeneous infinite linear lattice (McGhee & von Hippel, 1974). This model provides an accurate quantitative description of the experimental isotherms for these interactions as discussed previously (Mascotti & Lohman, 1990; Lohman & Mascotti, 1992b). Furthermore, the experimental isotherms used for this extrapolation were obtained at low binding density (<30% saturation of the poly(U)) as shown in Figure 13. As a result, only a short extrapolation to the zero binding density limit is required. Therefore, although we have used the model of McGhee and von Hippel (1974) to perform this extrapolation, the zero binding density values of K_{obs} reported here should be largely independent of the model used to perform the extrapolation, since the isotherms were obtained at such low binding densities.

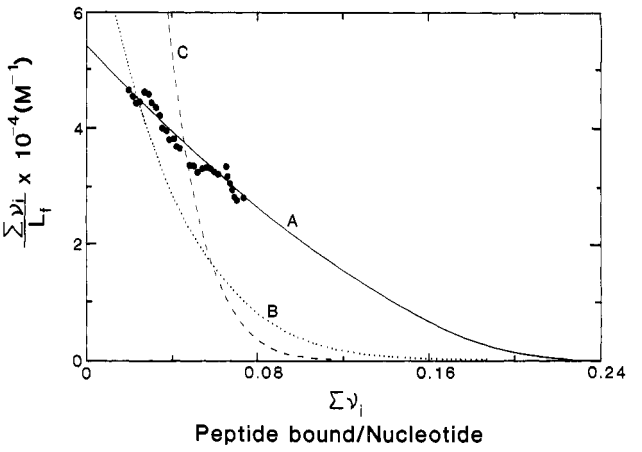

FIGURE 13: The experimental model-independent equilibrium binding isotherm (●) for KWK_2-NH_2 ($z = +4$) binding to poly(U) (25.0 °C, pH 6.0, 37 mM Na^+) plotted according to Scatchard (1949) compared with three theoretical models. The continuous curve (A) represents the best-fit isotherm generated using the noncooperative model of McGhee and von Hippel (1974) (see eq 3 in the text) with $K_{obs} = 5.4 \times 10^4$ M^{-1} and $n = 4$. The dotted line (B) represents the two-variable model of Manning (1978) using the following parameters: $N = 4$, $b = 3.8$ Å, and $[Na^+] = 37$ mM. The dashed line (C) represents the model of Friedman and Manning (1984) with the parameters $K_{obs} = 4.4 \times 10^5$ M^{-1} and $n = N = 4$.

Manning and colleagues (Friedman & Manning, 1984; Ray & Manning, 1992) have argued that use of the McGhee and von Hippel (1974) model to obtain K_{obs} at zero binding density is not valid for highly charged ligands such as the oligolysines studied here, since this model does not incorporate electrostatic effects that are dependent upon ligand binding density. However, our expectations are that such binding density dependent electrostatic effects should be small at the low binding densities that we have used in our studies and that the model of McGhee and von Hippel (1974) provides an accurate description of the experimental binding isotherms at least at the low binding densities used in our experiments. Furthermore, although alternative models have been proposed (Friedman & Manning, 1984; Ray & Manning, 1992), none of these accurately account for such electrostatic effects as we discuss below.

In Figure 13, we compare an experimental binding isotherm for the KWK_2-NH_2–poly(U) interaction with theoretical binding isotherms generated using three different models. As shown previously (Mascotti & Lohman, 1990), the McGhee & von Hippel (1974) model describes the experimental isotherm very well in the low binding density region covered by the experimental isotherm. However, Manning's "two-variable" model (Manning, 1978) as well as a model that incorporates both electrostatic and ligand overlap effects (Friedman & Manning, 1984) yields theoretical isotherms that do not describe the experimental isotherm well at all. In fact, use of either of these models severely overestimates the zero binding density value of K_{obs}, since both models greatly overestimate the apparent negative cooperativity effects of ligand binding due to an inappropriate procedure for calculating the effects of bound ligands on binding of additional ligands.

A more recent attempt to incorporate electrostatic repulsive effects among oligocations bound to a linear lattice (Ray & Manning, 1992) modifies the Manning (1978) two-variable theory by assuming that compaction of the polynucleotide charge spacing occurs upon binding oligocations. This "compaction" model contains two adjustable parameters: $db/d\theta_N$ (the dependence of the average polynucleotide charge

spacing on the binding density of the oligocation) and K' (an empirical constant which adjusts for undefined favorable contributions to the free energy of binding). As a result, this model differs from Manning's previous models (Manning, 1978; Friedman & Manning, 1984) in that it lacks predictive value. Upon binding an oligocation, which neutralizes the charge on z phosphates, the "compaction" model recalculates a new polynucleotide charge density by averaging over the entire polynucleotide (including the bound oligocation). This reaveraging approach appears to be physically unrealistic and results in an overestimation of the binding density dependent electrostatic effects due to oligocation binding, especially at low binding densities. This model can achieve reasonable agreement with experimental isotherms for oligolysine–poly(U) binding only by assuming that binding causes a 50% compaction of the poly(U) along its contour length (Ray & Manning, 1992), which appears unrealistic. Although poly(U) is quite flexible, we have no experimental evidence suggesting compaction of poly(U) by oligolysines at the low binding densities used in our studies [see Mascotti and Lohman (1990), Mascotti (1992), and the Materials and Methods section]. Some compaction of poly(U) may occur at higher peptide binding densities outside of our experimental regime.

As a further test of the compaction model (Ray & Manning, 1992), we have used it to analyze experimental isotherms for oligolysine (D. P. Mascotti, unpublished) and polyamine (Braunlin et al., 1982) binding to duplex DNA. Only marginal agreement can be obtained between the model and experiment, and this requires the assumption that the duplex DNA becomes compacted by as much as 40% along its contour length (i.e., $db/d\theta_N = -0.7$ Å per oligolysine charge) (D. P. Mascotti and T. M. Lohman, unpublished calculations). This degree of compaction is even more unrealistic than the compaction required to fit the poly(U) isotherms since duplex DNA is considerably more rigid than poly(U) and there is no experimental support for such compaction at the low binding densities used in the experiments. It appears that this model (Ray & Manning, 1992) overestimates the electrostatic effects at low peptide binding densities, and compaction of the nucleic acid must be assumed in order to balance this effect. As a result, we conclude that these models do not provide a useful description of our experimental isotherms, whereas the McGhee and von Hippel (1974) model provides an excellent description of these isotherms, at least in the low peptide binding density range used experimentally. However, we reemphasize that the values of K_{obs} reported here and previously (Mascotti & Lohman, 1990) were obtained at such low binding densities that extrapolations to zero binding density should be accurate even in the absence of a model.

Comparison with an Intercalative Model for the Interaction of Tryptophan with Polynucleotides. Helene and colleagues (Brun et al., 1975; Rajeswari et al., 1987) have analyzed the binding of single-stranded and duplex polynucleotides to short oligolysines containing a single tryptophan using a two-step binding model. This model assumes the existence of two types of polynucleotide-bound peptides: Complex A in which only coulombic interactions stabilize the peptide–polynucleotide complex, so that the peptide tryptophan fluorescence is identical to that of the free peptide, and complex B, in which the peptide tryptophan contributes to stability by forming stacking interactions with adjacent bases which results in complete quenching of the tryptophan fluorescence. The main support for this model is the observation that the fluorescence lifetime of nucleic acid-bound KWK-CO_2 is the same as that of the free peptide (Brun et al., 1975; Montenay-Garestier et al., 1982).

In this two-step model (Brun et al., 1975), the coulombic complex (A) forms with equilibrium constant K_1, whereas K_2 represents the isomerization step for forming the stacked complex (B) as shown in eq 17.

$$L + D \overset{K_1}{\leftrightarrow} A \overset{K_2}{\leftrightarrow} B \quad (17)$$

Our equilibrium constant, K_{obs}, which includes all bound peptide species (i.e., both complexes A and B in eq 17 (Bujalowski & Lohman, 1987; Lohman & Bujalowski, 1991), is related to K_1 and K_2 by $K_{obs} = K_1(1 + K_2)$, where $K_2 = Q_{max}/(1 - Q_{max})$. Therefore, our values of K_{obs} and Q_{max} can be used to calculate K_1 and K_2 for the two-step model. Where data have been obtained for the same peptide under similar conditions, our values of K_{obs} and Q_{max} agree with those reported by Brun et al. (1975). However, Brun et al. (1975) have measured the temperature dependences of K_1 and K_2 and report that K_1 has a larger temperature dependence than K_2, which does not seem consistent with the view that K_1 reflects the formation of only electrostatic interactions, since this type of interaction is expected to have a smaller ΔH° than for a stacking or intercalation reaction. As a result, we have not attempted to interpret our values of K_{obs} in terms of this two-step model.

Salt Dependence of K_{obs} for Oligolysine–Poly(U) Binding. As in our previous studies (Mascotti & Lohman, 1990), we observe a linear dependence of log K_{obs} on log [K^+] for the interaction of poly(U) with oligolysines of net charge $z = +2$ to $+10$, over the range from ~6 mM to 0.5 M K^+ or Na^+. We find no evidence for preferential anion binding to the oligolysines studied here, even for oligolysines containing multiple tryptophans, hence the observed salt effects are dominated by preferential cation binding. Furthermore, the effects of salt are entirely entropic in origin and we interpret these as resulting primarily from the release of monovalent cations that were previously bound to the poly(U).

The number of monovalent cations (K^+) released thermodynamically, as estimated from the measured values of $\partial \log K_{obs}/\partial \log [K^+]$, is 0.74 ± 0.04 per net positive charge on each oligolysine, independent of temperature. This value of 0.74 is significantly less than the value of ~0.88, that is obtained upon binding the same oligolysines to duplex DNA (Mascotti & Lohman, 1990; D. P. Mascotti & T. M. Lohman, in preparation). This difference appears to be due to the higher linear charge density of the duplex DNA, which thus binds an increased number of monovalent cations (Manning, 1972), which are subsequently released upon binding the oligolysine (Record et al., 1976). The apparent release of only a fraction of a monovalent cation per charge on the oligolysine is consistent with previous expectations (Record et al., 1976; Mascotti & Lohman, 1990). For these simple peptides, we also find that the magnitude of the monovalent cation displacement is controlled by the net positive charge on the oligopeptide rather than the distribution of charges, since the binding of KWG_2K_2-NH_2 to poly(U) is accompanied by the same cation release as the isovalent, KWK_2-NH_2 (Table I). Furthermore, the series of oligolysines with increasing numbers of tryptophans (1, 2, or 3) display salt dependences that are independent of tryptophan content. However, we emphasize that the overall net charge on more complex ligands such as proteins does not control binding to nucleic acids, since some proteins (e.g., *E. coli lac* repressor) bind strongly to DNA even though they possess an overall net negative charge.

We have also analyzed the salt dependence of K_{obs} for oligolysine binding to poly(U) by plotting K_{obs} vs mean ion activity ($a_{\pm}$) rather than salt concentration. We find that $(-\partial \log K_{obs}/\partial \log a_{\pm})$ is well-described as a linear function of the oligolysine charge, z, with a linear least-squares slope = 0.67 ± 0.05 and an ordinate intercept of 0.60 ± 0.60 (25.0 °C). These values are essentially the same, within experimental error, as those obtained when the salt dependence is analyzed using salt concentration (see Figure 4). Therefore, our conclusion that the number of monovalent cations displaced thermodynamically upon formation of an oligolysine–poly(U) complex is significantly less than one per oligolysine net charge (~0.7) is independent of the use of salt concentration or mean ion activity in this analysis. This result is consistent with recent Monte-Carlo calculations (Olmsted et al., 1992).

Extent of Tryptophan Fluorescence Quenching Correlates with ΔH°_{obs} for Peptide–Poly(U) Binding. Figure 6 indicates that the extent of tryptophan fluorescence quenching (Q_{max}) upon binding the mono-Trp oligolysines to poly(U) is correlated with the values for both ΔH°_{obs} and $T\Delta S^{\circ}_{obs}(1\ M\ K^+)$. [Recall that the 1 M K^+ designation for $\Delta S^{\circ}_{obs}(1\ M\ K^+)$ indicates that this represents the value of ΔS°_{obs} obtained by extrapolation to 1 M K^+, hence this quantity does not include the favorable contributions from cation release from the poly(U) (polyelectrolyte effect); however, ΔH°_{obs} is independent of $[K^+]$ (at pH 6).] All three quantities, Q_{max}, ΔH°_{obs}, and $T\Delta S^{\circ}_{obs}(1\ M\ K^+)$, show a linear dependence on z for $z \leq +4$, whereas they are constant for $z \geq +4$. ΔH°_{obs} becomes more favorable with increasing z, until $z = \sim+4$, while $T\Delta S^{\circ}(1\ M\ K^+)$ becomes less favorable with increasing z. The transition at $z = +4$ clearly indicates that the interaction of the tryptophan with poly(U) is influenced by the net charge on the oligolysine; however, the molecular basis for these context-dependent effects is not known. The enthalpy–entropy compensation results in a $\Delta G^{\circ}_{obs}(1\ M\ K^+)$ that is nearly independent of z.

Even though ΔG°_{obs} is also nearly independent of the number of Trp residues in the oligolysines studied here, the interaction of these Trp residues with the poly(U) is clearly indicated by the dependence of both ΔH°_{obs} and ΔS°_{obs} on the number of Trp residues, as well as the fact that the Trp fluorescence is quenched upon binding poly(U). For the three isovalent ($z = +6$) peptides, KWK_4-NH_2, KWK_3WK-NH_2, and $(KW)_3K_2$-NH_2, Q_{max} is observed to be independent of the number of tryptophan residues. However, ΔH°_{obs} shows a linear dependence on the number of Trp residues, N, becoming more negative with increasing N (see Figure 10 and eq 14b). Each Trp interaction contributes ~ −2.9 ± 0.7 kcal/mol to ΔH°_{obs}. The constant value of Q_{max} and the fact that each Trp residue contributes equivalently to ΔH°_{obs} suggests that each Trp residue in each of these oligolysines binds to poly(U) in a nearly independent and identical manner. However, Figure 10 also indicates that each Trp contributes very little to ΔG°_{obs} ($\Delta G^{\circ}_{Trp}(1\ M, 25\ °C) < -1$ kcal/mol) due to significant enthalpy–entropy compensation (see also Figure 6B).

Coulombic Contributions to ΔG°_{obs} for Oligolysine–Poly(U) Binding and Estimation of $\Delta G^{\circ}_{Lys\text{-}phos}$. The ΔG°_{obs} for oligolysine–poly(U) binding can be separated into contributions from counterion release (the polyelectrolyte effect (ΔG°_{PE}) (Record, 1988)), lysine–phosphate interactions ($\Delta G^{\circ}_{Lys\text{-}phos}$), and tryptophan–poly(U) interactions (ΔG°_{Trp}). Following eq 5a, ΔG°_{obs} can be written as in eq 18 (Record et al., 1976, 1978; Lohman et al., 1980)

$$\Delta G^{\circ}_{obs} = \Delta G^{\circ}_{obs}(1\ M\ M^+) - \Delta cRT \ln [M^+] \quad (18)$$

where $\Delta G^{\circ}_{PE} = -\Delta cRT \ln[M^+]$ (Record, 1988) and $\Delta G^{\circ}_{obs}(1\ M\ M^+)$ is the observed free energy change in the absence of cation release from the polynucleotide. As discussed previously (Record et al., 1976; Lohman et al., 1980; Lohman & Mascotti, 1992a), $\Delta G^{\circ}_{obs}(1\ M\ M^+)$ generally contains contributions due to coulombic (ΔG°_{Coul}) and noncoulombic interactions (e.g., ΔG°_{Trp}). If we assume these contributions to be independent, then we can write $\Delta G^{\circ}_{obs}(1\ M\ M^+)$ as in

$$\Delta G^{\circ}_{obs}(1\ M\ M^+) = \Delta G^{\circ}_{Trp} + \Delta G^{\circ}_{Coul} \quad (19)$$

For the oligolysine–poly(U) interactions studied here, we find that $\Delta G^{\circ}_{Trp} \sim 0$, due to enthalpy–entropy compensation, hence ΔG°_{Coul} is simply equal to $\Delta G^{\circ}_{obs}(1\ M\ M^+)$. If the lysine–phosphate interactions are equivalent, then we can substitute $\Delta G^{\circ}_{Coul} = z\Delta G^{\circ}_{Lys\text{-}phos}$. This assumption can be tested by calculating $\Delta G^{\circ}_{Lys\text{-}phos}$ for each oligolysine (i.e., $\Delta G^{\circ}_{Coul}/z$). If the value of $\Delta G^{\circ}_{Lys\text{-}phos}$ is constant for the series of oligolysines differing in charge, it is likely that the $\Delta G^{\circ}_{Lys\text{-}phos}$ is independent of peptide charge.

For each oligolysine–poly(U) interaction studied here, we calculate $\Delta G^{\circ}_{Lys\text{-}phos} = -0.1 \pm 0.2$ kcal/mol, independent of peptide charge, z. This result suggests that $\Delta G^{\circ}_{Coul} = z\Delta G^{\circ}_{Lys\text{-}phos}$ is a good approximation; however, due to the small values of $\Delta G^{\circ}_{Lys\text{-}phos}$, it would be difficult to state this unequivocally. The fact that $\Delta G^{\circ}_{Lys\text{-}phos}$ is zero within experimental error is likely due to the fact that the free energy gain resulting from the formation of lysine–phosphate interactions upon peptide binding is offset by the fact that these interactions replaced potassium–phosphate interactions. For the case of oligolysines binding to duplex RNA or DNA, it has been estimated that $\Delta G^{\circ}_{Lys\text{-}phos}$ is slightly unfavorable, although possibly zero within experimental error as well (Record et al., 1976; Lohman et al., 1980).

Thermodynamic Behavior of Oligolysine–Poly(U) Interactions in the Absence of Tryptophan. In order to examine the binding of an oligolysine that does not contain tryptophan, we attempted to perform competition studies using K_5-NH_2 or KGK_4-NH_2 and the tryptophan-containing peptides. Unfortunately, this was not possible for technical reasons, since high KGK_4-NH_2 binding densities were required to displace the oligolysines containing tryptophan from the poly(U) and this induced precipitation of the poly(U). However, on the basis of our studies of poly(U) binding to oligolysines containing differing numbers of Trp, we can make a reasonable prediction for the thermodynamic behavior of an oligolysine containing no tryptophans.

For a peptide with net charge $z = +6$ (e.g., K_5-NH_2 or KGK_4-NH_2) binding to poly(U), we predict that $\partial \log K_{obs}/\partial \log [K^+] = -4.36 \pm 0.22$, since there is no contribution of Trp to cation release. Furthermore, on the basis of our results using oligolysines of varying charge, as well as oligolysines containing multiple tryptophan residues, we find that $\Delta G^{\circ}_{Lys\text{-}phos}$ is approximately zero. Thus, we predict that log $K(1\ M\ K^+)$ should be approximately zero for K_5-NH_2 binding to poly(U) in accord with previous estimations (Record et al., 1976, 1978; Lohman et al., 1980). This predicted dependence of K_{obs} on $[K^+]$ for K_5-NH_2 is given by

$$\log K_{obs} = -4.36 \log [K^+] + 0 \quad (20)$$

Upon extrapolation of the values of ΔH°_{obs} and $T\Delta S^{\circ}$ (1 M K^+, 25 °C) for KWK_4-NH_2, KWK_3WK-NH_2, and

$(KW)_3K_2$-NH_2 to $N = 0$ (no Trp) (see Figure 10 and eqs 14) we predict $\Delta H°_{obs} = -2.0 \pm 1.5$ and $T\Delta S° = -1.4 \pm 1.5$ kcal/mol (1 M K^+, 25 °C) for K_5-NH_2 binding to poly(U). The near zero estimation for $\Delta H°_{obs}$ is in general agreement with the van't Hoff measurement of $\Delta H°_{obs} = -1.0 \pm 0.5$ kcal/mol for K_5-CO_2 ($z = +5$) binding to duplex DNA (Lohman et al., 1980) and is expected for a ligand that binds solely electrostatically to a polynucleotide (Record et al., 1978).

Effect of Magnesium on Ligand–Single-Stranded Nucleic Acid Interactions. The effects of Mg^{2+}, either alone or in competition with monovalent cations such as K^+ or Na^+, on the interaction of oligolysines with poly(U) are well described by a counterion condensation model as seems to be true for Mg^{2+} binding to duplex B-form DNA (de Haseth et al., 1977; Record et al., 1977; Lohman et al., 1980). On the basis of these previous models, we have analyzed the Na^+–Mg^{2+} competition studies to obtain quantitative estimates of the intrinsic binding constant for Mg^{2+}–poly(U) binding, K_{obs}^{Mg} as a function of $[Na^+]$ (see eq 17). These values of K_{obs}^{Mg} will be useful for interpreting the effects of Mg^{2+} on protein–poly(U) interactions.

We have also noted that the $\Delta H°_{obs}$ for KWK_4-NH_2 binding to poly(U) differs depending on whether it is measured in the presence of $Mg(CH_3CO_2)_2$ (−10.1 ± 1.5 kcal/mol) or K^+ (−4.9 ± 1.5 kcal/mol) (Tables I and V). The extra exothermic contribution to the binding enthalpy in the presence of Mg^{2+} likely reflects the excess enthalpy change accompany the release of Mg^{2+} (relative to K^+) from the poly(U) upon binding KWK_4-NH_2. Since ~3 Mg^{2+} are released upon binding KWK_4-NH_2, this difference could be explained if the binding of Mg^{2+} to poly(U) is more endothermic by ~2 kcal/mol relative to K^+, thereby contributing an additional ~ −6 kcal/mol to $\Delta H°_{obs}$ for the binding of KWK_4-NH_2 to poly(U) above that observed in the presence of K^+. In fact, Krakauer (1972) measured a value of $\Delta H° \sim +2$ kcal/mol, calorimetrically, for Mg^{2+} binding to poly(U) in the limit of zero binding density at ~30 mM NaCl (near the midpoint of our data, Figure 11), which supports this explanation. Such a large enthalpic change would only seem possible if the Mg^{2+} undergoes partial dehydration upon binding poly(U). Partial dehydration of Mg^{2+} has been observed upon binding to short oligoadenylates, although it diminishes as the oligonucleotide length increases (Proschke, 1979). Such effects have not been observed for Mg^{2+} binding to other RNA oligonucleotides, including $U(pU)_5$, or for poly(A) and poly(C); however, poly(U) has not been examined (Porschke, 1979, 1976).

Finally, we have noted differences in K_{obs} for oligopeptide–poly(U) binding determined at the same total $[Mg^{2+}]$, depending on whether the anion is Cl^- or $CH_3CO_2^-$. These differences seem to be due to the tendency of Mg^{2+} and $CH_3CO_2^-$ to form ion pairs. The interaction of KWK_4-NH_2 with poly(U) provides an ideal test of the relative effects of $MgCl_2$ and $Mg(CH_3CO_2)_2$ on the interaction of a charged ligand with a polynucleotide, since we know that there is no preferential interaction of either chloride or acetate with KWK_4-NH_2. We have estimated empirically an ion-pairing constant of ~8 M^{-1} for $Mg(CH_3CO_2)^+$ (25 °C, pH 6). This value is in good agreement with published estimates for this ion-pairing constant (Nancollas, 1956; Cannon & Kibrick, 1938; Emara, 1979; Archer & Monk, 1964). This suggests that studies of protein–nucleic acid interactions performed in the presence of magnesium acetate should also be analyzed and interpreted carefully by taking into account the ion-pairing propensity of magnesium and acetate (Zou & Richardson, 1991).

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