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Thermodynamics of Single-Stranded RNA and DNA Interactions with Oligolysines Containing Tryptophan. Effects of Base Composition^{†,‡}

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ABSTRACT: We have examined the thermodynamics of binding of a series of oligolysines (net charge $z = +2$ to $+10$) containing one, two, or three tryptophans to several single-stranded (ss) homo-polynucleotides [poly(A), poly(C), poly(I), poly(dU), poly(dT)] and duplex (ds) DNA in order to investigate the effects of peptide charge, tryptophan content, and polynucleotide base and sugar type. Equilibrium association constants, K_{obs} , were measured as a function of monovalent salt concentration (KCH_3CO_2) and temperature by monitoring the quenching of the peptide tryptophan fluorescence upon interaction with the polynucleotides, from which the dependence of $\Delta G^\circ_{\text{obs}}$, $\Delta H^\circ_{\text{obs}}$, and $\Delta S^\circ_{\text{obs}}$ on $[\text{KCH}_3\text{CO}_2]$ was obtained. As observed previously with poly(U) [Mascotti, D. P., & Lohman, T. M. (1992) *Biochemistry* 31, 8932], the dependence of $\Delta G^\circ_{\text{obs}}$ on $[\text{K}^+]$ for peptide binding to each polynucleotide is entirely entropic in origin (i.e., $\Delta H^\circ_{\text{obs}}$ is independent of $[\text{K}^+]$), consistent with the conclusion that K_{obs} increases with decreasing salt concentration due to the favorable increase in entropy resulting from the displacement of bound cations (K^+) from the nucleic acid upon formation of the complex. For each ss polynucleotide, we find that significantly less than one potassium ion is released thermodynamically per net positive peptide charge, as determined from the value of $\partial \log K_{\text{obs}} / \partial \log [\text{K}^+]$. Interestingly, $(-\partial \log K_{\text{obs}} / \partial \log [\text{K}^+]) / z$ decreases with increasing peptide charge for poly(A), poly(C), and poly(dT), contrary to the behavior observed with poly(U) and ds-DNA, which may reflect a significant release of bound water upon formation of peptide complexes with these ss homo-polynucleotides or an increased binding of K^+ to the ss polynucleotide with increasing $[\text{K}^+]$. Alternatively, there may be conformational differences between the bound states of oligolysines of low charge, relative to oligolysines of higher charge. However, in all cases, peptides with $z < +4$ display different thermodynamics of binding than peptides with $z > +4$. The presence of tryptophan (Trp) within these peptides does not influence the salt dependence of K_{obs} for binding to poly(A), poly(C), or poly(dT). However, the Trp content of the peptide does contribute significantly to the thermodynamics of these interactions: Trp interactions result in a favorable contribution to $\Delta H^\circ_{\text{obs}}$, but an unfavorable contribution to $\Delta S^\circ_{\text{obs}}$, with little effect on $\Delta G^\circ_{\text{obs}}$ due to entropy–enthalpy compensations. Oligolysines containing Trp also display a small, but significant, dependence of K_{obs} on base composition, with K_{obs} decreasing in the order poly(I) \gg poly(dT) \sim poly(U) \sim poly(A) \gg poly(C).

The interaction of proteins with nucleic acids is central to a variety of cellular processes, including gene regulation, replication, recombination, and repair. Many DNA binding proteins display sequence specificity in their binding to duplex DNA. However, there are also a large number of proteins involved in DNA and RNA metabolism that bind preferentially to single-stranded (ss) nucleic acids; hence their binding specificity depends at least partially on nucleic acid conformation. One class of such proteins are the helix destabilizing, or single-stranded binding (SSB), proteins, which are essential for DNA replication, recombination, and repair [for reviews, see Chase and Williams (1986), Lohman *et al.* (1988), Lohman and Bujalowski (1990), and Karpel (1990)]. Although these proteins bind to ss-DNA with high affinities and with no known nucleotide sequence dependence, and hence are referred to as nonspecific DNA binding proteins, they generally display a

wide range of equilibrium binding affinities dependent on ss nucleic acid base and sugar composition.

Equilibrium binding to ss homopolynucleotides has been examined systematically for the *Escherichia coli* SSB protein (Overman *et al.*, 1988; Overman, 1988), the phage fd gene V protein (Sang & Gray, 1989; Porschke & Rauh, 1983; Alma *et al.*, 1983; Bullsink *et al.*, 1985), and the phage T4 gene 32 protein (Kowalczykowski *et al.*, 1981; Newport *et al.*, 1981). For each of these proteins, the equilibrium association binding constant, K_{obs} , shows a large dependence on polynucleotide type (Newport *et al.*, 1981; Overman *et al.*, 1988; Alma *et al.*, 1983; Bullsink *et al.*, 1985; Sang & Gray, 1989). However, the molecular bases for the stability of SSB protein–ss-DNA binding and in particular the origins of the base dependence of K_{obs} have not yet been determined. For these proteins, the intrinsic protein fluorescence due to tryptophan and/or tyrosine is quenched upon binding to ss nucleic acids, and the extent of quenching varies with polynucleotide type (Overman *et al.*, 1988; Newport *et al.*, 1981). In fact, it appears that at least some of the tryptophan residues of the *E. coli* SSB protein interact directly with the polynucleotide (Khamis *et al.*, 1987a,b). Therefore, it is possible that interactions of these aromatic amino acids with the nucleic acid contribute to the stability of these complexes.

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An understanding of the stability and specificity of protein–nucleic acid interactions requires systematic thermodynamic studies. However, the thermodynamics of such systems are complex (Overman *et al.*, 1988; Overman, 1988; Overman, L. B., and Lohman, T. M., manuscript in preparation), and interpretations of such studies can often be facilitated by comparative studies of simpler model systems. The study of small oligopeptides and their interactions with ss and duplex nucleic acids has proven useful for such purposes ((Latt & Sober, 1967a,b; Helene & Dimicoli, 1972; Brun *et al.*, 1975; Lohman *et al.*, 1980; Helene & Maurizot, 1981; Helene & Lancelot, 1982; Rix-Montel *et al.*, 1976; Montenay-Garestier *et al.*, 1982). Although there have been systematic thermodynamic studies of the nonspecific binding of positively charged peptides to duplex RNA and DNA (Latt & Sober, 1967a,b; Lohman *et al.*, 1980), such studies with ss nucleic acids have been lacking. Recently, we have examined the thermodynamics of binding to poly(U) of a series of oligolysines containing one or more tryptophans (Mascotti & Lohman, 1990, 1992). In this paper, we examine the thermodynamics of the interactions of oligolysines (each containing one or more tryptophans) with poly(A), poly(C), poly(I), poly(dT), and double-stranded (ds) plasmid DNA. These results should facilitate the interpretation of thermodynamic studies of more complex interactions between proteins and ss nucleic acids.

MATERIALS AND METHODS

Buffers and Reagents. All solutions were made as described previously (Mascotti & Lohman, 1992). The two low-salt buffers are CB6 + 1.0 mM KCH₃CO₂ (pH 6.0) and CB7 + 1.0 mM KCH₃CO₂ (pH 7.0). The high-salt buffers are identical to the low-salt buffers, except they also contain 2.0 M KCH₃CO₂ and were titrated to pH 6.4 and 7.2, respectively, to maintain constant pH throughout the “salt-back” titrations (Lohman & Mascotti, 1992b; Mascotti & Lohman, 1992).

Peptides. Oligopeptides containing L-lysine (K) and L-tryptophan (W) of the general forms KWK_p-NH₂, KWK_p-CO₂, KWK₃WK-NH₂, and (KW)₃K₂-NH₂ were synthesized by the TAES support laboratory (Texas A&M University, College Station, TX), and KWK-CO₂ was purchased from Serva Fine Chemicals (Westbury, NY). All peptides were purified and verified as described (Mascotti & Lohman, 1990). Stock peptide concentrations were determined spectrophotometrically, as described (Mascotti & Lohman, 1990, 1992; Lohman & Mascotti, 1992b).

Polynucleotides. Poly(dT) (*s*_{20,w} = 10.1 S; ~1000 (±200) nucleotides) and poly(dU) (*s*_{20,w} = ~10 S; ~1000 (±200) nucleotides) were from Midland Certified Reagent Company (Midland, TX; lots 100886 and 021789, respectively); 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; lot 514110) and poly(C) (*s*_{20,w} = 7.8 S; ~430 (±100) nucleotides; lot 0001422001) were from Pharmacia; and poly(I) (*s*_{20,w} = 7.2 S; ~520 (±100) nucleotides) was from P-L Biochemicals (Milwaukee, WI; lot 741-80). pUC8 plasmid DNA was prepared from *E. coli* JM83/pUC8 grown in L broth + 50 mg/mL ampicillin by alkaline lysis, followed by CsCl gradient banding and phenol/chloroform extraction (Maniatis *et al.*, 1982). We discovered that even trace contamination by RNA (below that detectable on an ethidium bromide stained gel) altered the maximal quenching of the tryptophan fluorescence, *K*_{obs}, and the dependence of *K*_{obs} on [KCH₃CO₂] (Mascotti, 1992). The DNA was precipitated with 95% ethanol,

resuspended, and dialyzed extensively against buffer CB + 1.0 mM KCH₃CO₂. Purity was verified by agarose gel electrophoresis and UV absorbance. All nucleic acids were dialyzed extensively against the desired buffer before use, and concentrations were determined spectrophotometrically using the following extinction coefficients on a per nucleotide basis (in 10 mM Tris-HCl, pH 8.1, + 0.1 mM Na₃EDTA + 0.1 M NaCl): poly(A), $\epsilon_{260} = 1.03 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$; poly(C), $\epsilon_{267} = 6.5 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$; poly(I), $\epsilon_{260} = 9.4 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$; poly(dT), $\epsilon_{260} = 8.1 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$; ds-DNA, $\epsilon_{260} = 6.5 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

Fluorescence Titrations and Equilibrium Binding Isotherms. Peptide–polynucleotide binding was monitored by the quenching of the peptide tryptophan fluorescence as described (Mascotti & Lohman, 1990, 1992). The binding density function method of analysis (Bujalowski & Lohman, 1987) was used to determine that the fraction of peptide bound (*L_B/L_T*) is directly proportional to the extent of fluorescence quenching, *Q*_{obs}, for all of the peptide–polynucleotide complexes examined under all conditions reported here (Mascotti & Lohman, 1990, 1992; Lohman & Mascotti, 1992b). Therefore, values of free ligand concentration (*L_F*) and binding density ($\nu = L_B/D_T$, where *D_T* is the polynucleotide concentration in nucleotides) can be calculated for each titration as

$$Q_{\text{obs}}/Q_{\text{max}} = L_B/L_T \quad (1)$$

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

$$\nu = (Q_{\text{obs}}/Q_{\text{max}})(L_T/D_T) \quad (3)$$

Titrations of peptides with polynucleotides (“reverse” titrations) were performed as described (Mascotti & Lohman, 1990; Lohman & Mascotti, 1992b). Fluorescence intensity measurements were made with an SLM Aminco 8000C spectrofluorometer, using excitation wavelengths of 292 nm for poly(U) and poly(A), 296 nm for poly(dT), and 300 nm for poly(C). The isosbestic wavelength of Lys-Trp-Lys-CO₂ binding to poly(A) is 292 nm (Brun *et al.*, 1975); however, different excitation wavelengths for poly(C), poly(dT), and ds-DNA were used in order to reduce absorbance by the polynucleotides and minimize inner filter corrections (Lohman & Mascotti, 1992b). The wavelengths and corresponding extinction coefficients, per nucleotide, used (for inner filter correction purposes) for each polynucleotide were as follows: poly(U) and poly(dU), $\epsilon_{292} = (1.6 \pm 0.5) \times 10^2 \text{ M}^{-1} \text{ cm}^{-1}$; poly(dT), $\epsilon_{296} = (4.0 \pm 0.5) \times 10^2 \text{ M}^{-1} \text{ cm}^{-1}$; poly(A), $\epsilon_{292} = (2.6 \pm 0.5) \times 10^2 \text{ M}^{-1} \text{ cm}^{-1}$; poly(I), $\epsilon_{292} = (2.9 \pm 0.5) \times 10^2 \text{ M}^{-1} \text{ cm}^{-1}$; poly(C), $\epsilon_{300} = (2.6 \pm 0.5) \times 10^2 \text{ M}^{-1} \text{ cm}^{-1}$; ds-pUC8 plasmid DNA, $\epsilon_{296} = (6.0 \pm 0.5) \times 10^2 \text{ M}^{-1} \text{ cm}^{-1}$. The peptide extinction coefficients per tryptophan are: $\epsilon_{292} = (3.3 \pm 0.5) \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$; $\epsilon_{296} = (1.6 \pm 0.5) \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$; $\epsilon_{300} = 8.0(\pm 0.5) \times 10^2 \text{ M}^{-1} \text{ cm}^{-1}$. An emission wavelength of 350 nm was used for titrations with all nucleic acids (2-nm excitation and 8-nm emission bandpasses).

Equilibrium constants, *K*_{obs}, for peptide–polynucleotide binding were determined by analysis of the equilibrium isotherms using the McGhee and von Hippel (1974) model for non-cooperative large ligand binding to an “infinite”, homogeneous, linear lattice. Since we have established that *Q*_{obs}/*Q*_{max} = *L_B/L_T* and *Q*_{max} (the maximal extent of peptide fluorescence quenching upon saturation with nucleic acid) and *n* (the number of nucleotides occluded by a bound peptide) are independent of salt concentration (Mascotti & Lohman, 1990; 1992), we also obtained *K*_{obs} and the dependence of *K*_{obs}

on salt concentration from analysis of salt-back titrations as described (Overman *et al.*, 1988; Lohman & Mascotti, 1992b).

Analysis of the Dependence of K_{obs} on Monovalent Salt Concentration and pH. The experiments discussed here were performed in the presence of excess monovalent salt, MX. For the binding of short positively charged oligopeptides to linear nucleic acids, K_{obs} is dependent only on the cation concentration, $[M^+]$, due to the release of cations from the nucleic acid upon formation of the complex, and is independent of anion concentration and type (Lohman *et al.*, 1980; Lohman & Mascotti, 1992a; Mascotti & Lohman, 1990; 1992). In general, if binding results in release or uptake of water as well as cations, the dependence of K_{obs} on monovalent salt concentration is given by eq 4 (Tanford, 1969; Record *et al.*, 1978),

$$\partial \log K_{\text{obs}} / \partial \log [M^+] = \Delta c - 2m \Delta w / 55.6 \quad (4)$$

where Δc represents the moles of cations released ($\Delta c < 0$) from the nucleic acid in a thermodynamic sense (Record *et al.*, 1976, 1978), Δw is the moles of waters taken up ($\Delta w > 0$) or released ($\Delta w < 0$) upon peptide binding, m is the salt molality, and $[H_2O]$ is the molality of water (55.6). Upon replacing salt molality by molarity, integration of eq 4 yields eq 5 (Ha *et al.*, 1992).

$$\log K_{\text{obs}} = \log K(1M) + \Delta c \log [M^+] - 0.0156 \Delta w [M^+] \quad (5)$$

The linked effects of monovalent salt concentration and pH on K_{obs} and $\Delta H^{\circ}_{\text{obs}}$ for the binding of charged oligopeptides to nucleic acids were modeled using a titration curve model (Mascotti & Lohman, 1992).

RESULTS

Dependence of K_{obs} on KCH_3CO_2 Concentration for Oligolysines Containing a Single Tryptophan

Poly(A). Figure 1A shows the dependence of K_{obs} on $[KCH_3CO_2]$ for poly(A) binding to the series of oligolysines KWK-CO₂ and KWK_{*p*}-NH₂ (*p* = 1, 2, 4, or 6) which possess net charges ranging from *z* = +2 to +8. These experiments were performed at pH 7.0 (25.0 °C) to avoid protonation of adenine, which facilitates formation of a poly(A) duplex (Holcomb & Tinoco, 1965). KCH_3CO_2 was used to vary the monovalent cation concentration because preferential anion interactions with oligolysines are reduced in acetate, relative to chloride (Mascotti & Lohman, 1990, 1992; Mascotti, 1992). For each peptide, a plot of $\log K_{\text{obs}}$ vs $\log [K^+]$ is linear over the range of salt concentrations investigated and the value of $|\partial \log K_{\text{obs}} / \partial \log [K^+]|$ increases with increasing peptide charge, *z* (see Table I).

The values of $-\partial \log K_{\text{obs}} / \partial \log [K^+]$ from Figure 1A are plotted as a function of *z* in Figure 1B. Since the oligopeptides are not fully protonated at pH 7, the peptide charges were calculated using eq 7 of Mascotti and Lohman (1992) and the average pK values for the α - and ϵ -amino groups of each oligolysine as estimated by Mascotti and Lohman (1992). Each value of *z* is within 7% of the maximum net charge of the peptide that exists at pH 6 (Table I). The values of $|\partial \log K_{\text{obs}} / \partial \log [K^+]|$ increase with increasing peptide charge, although nonlinearly. The dependence of $-\partial \log K_{\text{obs}} / \partial \log [K^+]$ on *z* for peptides with *z* ≤ +4 can be described by a straight line constrained to intersect the origin, with a linear least-squares slope of 0.90 ± 0.08 . Note that $|(-\partial \log K_{\text{obs}} / \partial$

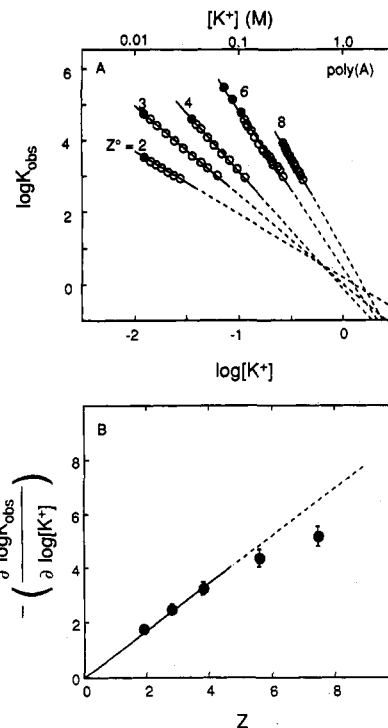


FIGURE 1: (A) Dependence of $\log K_{\text{obs}}$ on $\log [K^+]$ (in terms of total $[K^+]$) for a series of oligolysines containing a single tryptophan binding to poly(A) at 25.0 °C, pH 7.0 (buffer CB). KCH_3CO_2 was used to vary the K^+ concentration. The peptides were KWK_{*p*}-NH₂, with *p* = 1, 2, 4, or 6 ($z^{\circ} = +3, +4, +6, \text{ or } +8$), and KWK-CO₂ ($z^{\circ} = +2$). The maximal net positive charge, z° , is indicated for each line. Data were obtained from titrations at constant salt concentration (●) and from "salt-back" titrations (○). The solid lines are linear least-squares best fits for each peptide, and the parameters are listed in Table I. The dashed lines indicate extrapolations to high salt concentration. (B) Thermodynamic extent of ion release ($-\partial \log K_{\text{obs}} / \partial \log [K^+]$) is plotted as a function of the net positive charge, *z*, on each oligolysine binding to poly(A). The value of *z* was calculated according to eq 7 of Mascotti and Lohman (1992) using the pK values reported in that paper. The solid line drawn is a linear least-squares best-fit line to only the data for *z* ≤ +4, constrained to intersect the origin, and is given by the equation $-\partial \log K_{\text{obs}} / \partial \log [K^+] = (0.90 \pm 0.07)z$. The dashed line is an extrapolation to higher *z*.

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

peptide	<i>z</i> ^b	z^b	$\frac{\partial \log K_{\text{obs}}}{\partial \log [K^+]}$	$\log K_{\text{obs}}(1M)^c$	<i>Q</i> _{max} (%)	$\Delta H^{\circ}_{\text{obs}}^d$
KWK-CO ₂	2	1.9	-1.78 ± 0.20	0.16 ± 0.24	69 ± 2	-5.2 ± 1.5
KWK-NH ₂	3	2.8	-2.50 ± 0.18	-0.03 ± 0.16	76 ± 2	-4.4 ± 1.5
KWK ₂ -NH ₂	4	3.8	-3.26 ± 0.21	-0.13 ± 0.22	81 ± 2	-3.8 ± 1.5
KWK ₄ -NH ₂	6	5.6	-4.38 ± 0.22	0.45 ± 0.22	81 ± 2	-0.2 ± 1.5
KWK ₆ -NH ₂	8	7.5	-5.18 ± 0.25	0.89 ± 0.30	78 ± 2	-0.3 ± 1.5

^a Buffer CB + KCH_3CO_2 , pH 7.0, 25.0 °C. ^b Calculated from eq 7 of Mascotti and Lohman (1992) using the pK values reported in that paper. ^c Obtained from a linear extrapolation of a plot of $\log K_{\text{obs}}$ vs $\log [K^+]$. ^d The average $\Delta H^{\circ}_{\text{obs}}$ within the range of $[K^+]$ examined in units of kcal/mol.

$\log [K^+]) / z$ is lower for the more highly charged peptides; hence 0.90 may be taken as the upper limit of the fraction of monovalent cations displaced thermodynamically per peptide charge upon binding to poly(A).

The data in Figure 1A appear to fall into two groups: the data for peptides with *z* ≤ +4 show a common point of intersection at ~0.6 M K^+ , for which $\log K_{\text{obs}} \sim 0.5$, whereas the data for peptides with *z* > +4 intersect at ~3 M K^+ , where $\log K_{\text{obs}} \sim -2$. This grouping is further supported by Figure 1B, which indicates that the salt dependences for the *z* = +6

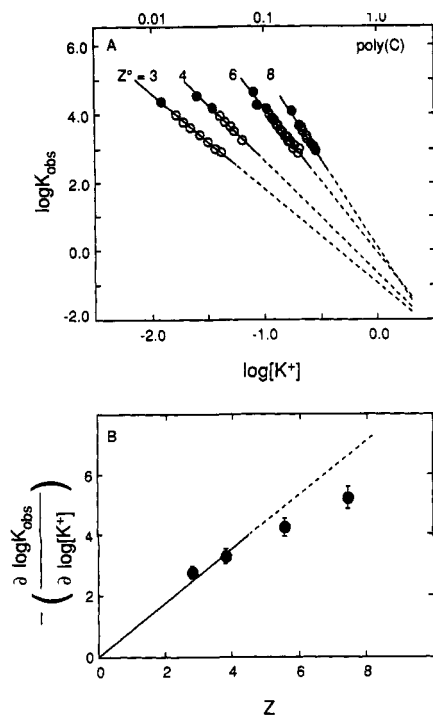


FIGURE 2: (A) Dependence of $\log K_{\text{obs}}$ on $\log [K^+]$ (in terms of total $[K^+]$) for a series of oligolysines containing a single tryptophan binding to poly(C) at 25.0 °C, pH 7.0 (buffer CB). KCH₃CO₂ was used to vary the K^+ concentration. The peptides were KWK_p-NH₂, with $p = 1, 2, 4$, or 6 ($z^0 = +3, +4, +6$, or $+8$). The maximal net positive charge, z^0 , is indicated for each line. Data were obtained from titrations at constant salt concentration (●) and from "salt-back" titrations (○). Smooth lines are linear least-squares best fits for each peptide, and the parameters are listed in Table II. Dashed lines indicate extrapolations to high salt concentration. (B) Thermodynamic extent of ion release ($-\partial \log K_{\text{obs}} / \partial \log [K^+]$) is plotted as a function of the net positive charge, z , on each oligolysine binding to poly(C). The value of z was calculated according to eq 4 using the pK values reported (Mascotti & Lohman, 1992). The solid line drawn is a linear least-squares best-fit line to the data of $z \leq +4$, constrained to intersect the origin, and has a slope of 0.93 ± 0.07 .

and +8 peptides deviate from the straight line describing the salt dependences for the peptides with $z \leq +4$. Similar behavior is also observed for these peptides binding to poly-(C) and poly(dT) (see below) and to poly(U) at 10 °C, although it was not apparent for the binding of the same peptides to poly(U) at 20 and 25 °C (Mascotti & Lohman, 1992). We note, however, that the salt dependence for binding of each peptide could not be determined over the same range of $[KCH_3CO_2]$; more highly charged peptides were examined at higher $[KCH_3CO_2]$. This gradual change in the $[KCH_3CO_2]$ range over which the peptides were studied may contribute to the curvature in Figure 1B. For example, if preferential water release occurs upon formation of the peptide–polynucleotide complex in addition to the release of cations from the polynucleotide, then the value of $[(\partial \log K_{\text{obs}} / \partial \log [M^+])]$ will be lower than if preferential hydration is absent (Tanford, 1969; Record *et al.*, 1978). Furthermore, the relative magnitude of such a preferential hydration effect will increase with increasing $[M^+]$ (see eq 5 and Discussion).

Poly(C). Figure 2A shows the dependence of K_{obs} on $[KCH_3CO_2]$ for poly(C) binding to the series of oligolysines KWK_p-NH₂ ($p = 1, 2, 4$, or 6) which possess net charges ranging from $z^0 = +3$ to $+8$ (25.0 °C, pH 7.0). We note that poly-(rC) remains single stranded under these solution conditions at pH 7.0 (Fasman *et al.*, 1964; Klump, 1975), as opposed to poly(dC), which undergoes a transition to a duplex form with a pK of ~ 7.5 (Inman, 1964). For each peptide, a plot of $\log K_{\text{obs}}$

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

peptide	z^0	z^b	$\frac{\partial \log K_{\text{obs}}}{\partial \log [K^+]}$	$\log K_{\text{obs}}(1M)^c$	$Q_{\text{max}} (\%)$	$\Delta H^{\circ}_{\text{obs}}^d$
poly(C)						
KWK-NH ₂	3	2.8	-2.78 ± 0.24	-0.96 ± 0.40	62 ± 2	-4.3 ± 1.5
KWK ₂ -NH ₂	4	3.8	-3.30 ± 0.21	-0.66 ± 0.22	61 ± 2	-3.9 ± 1.5
KWK ₄ -NH ₂	6	5.6	-4.25 ± 0.22	-0.07 ± 0.22	62 ± 2	1.1 ± 1.5
KWK ₆ -NH ₂	8	7.5	-5.21 ± 0.25	0.10 ± 0.24	61 ± 2	0.5 ± 1.5
poly(I)						
KWK-CO ₂	2	1.9	-1.43 ± 0.18	1.31 ± 0.22	77 ± 2	-8.3 ± 1.5

^a Buffer CB + KCH₃CO₂, pH 7.0, 25.0 °C. ^b Calculated from eq 7 of Mascotti and Lohman (1992) using the pK values reported in that paper. ^c Obtained from a linear extrapolation of a plot of $\log K_{\text{obs}}$ vs $\log [K^+]$. ^d The average $\Delta H^{\circ}_{\text{obs}}$ within the range of $[K^+]$ examined in units of kcal/mol.

K_{obs} vs $\log [K^+]$ is linear over the range of salt concentrations investigated and the value of $|\partial \log K_{\text{obs}} / \partial \log [K^+]|$ increases with increasing peptide charge (see Table II). As noted for poly(A), the behavior of the peptides appears to fall into two classes ($z = +3, +4$ vs $z = +5, +6$) as defined by the different intersections of the linearly extrapolated values of $\log K_{\text{obs}}$ to high $[K^+]$. These data also indicate that the value of K_{obs} obtained upon linear extrapolation of K_{obs} to 1 M K^+ is highly unfavorable for these peptides binding to poly(C) (Table II).

The slopes of the lines in Figure 2A are plotted in Figure 2B as $-\partial \log K_{\text{obs}} / \partial \log [K^+]$ vs z , where z is the net peptide charge at pH 7.0. The four data points in Figure 2B appear to lie on a straight line with a slope of 0.52 ± 0.07 ; however, the linear least-squares line does not intersect the origin. On the other hand, a straight line defined by the data for the two least charged peptides and constrained to intersect the origin has a slope of 0.93 ± 0.07 (represented by the line drawn in Figure 2B). Clearly, the value of $[(\partial \log K_{\text{obs}} / \partial \log [M^+])]/z$, the salt dependence normalized by the net peptide charge, decreases as the net peptide charge increases in the same manner as for the poly(A) data in Figure 1B.

Poly(dT). Figure 3A shows the dependence of K_{obs} on $[K^+]$ for poly(dT) binding to the series of oligolysines KWK-CO₂ and KWK_p-NH₂ ($p = 1, 2, 4, 6$, or 8) which possess net charges ranging from $z = +2$ to $+10$ (25.0 °C, pH 6.0). Poly(dT) does not titrate at pH 6.0; hence this pH was chosen to maximize the net positive charge on the peptide. For each peptide, a plot of $\log K_{\text{obs}}$ vs $\log [K^+]$ is linear over the range of salt concentrations investigated and the value of $|\partial \log K_{\text{obs}} / \partial \log [K^+]|$ increases with increasing peptide charge (see Figure 3B and Table III). As observed with both poly(A) and poly-(C), the behavior of the peptides appears to fall into two distinct groups based on the basis of the values of $\log K_{\text{obs}}$ extrapolated to 1 M K^+ : those with $z \leq +4$ and those with $z > +4$. In Figure 3B we have plotted the absolute value of the slopes of the lines in Figure 3A as a function of net charge of the free peptides. The solid line in Figure 3B, which has been constrained to intersect the origin, describes the data for peptides of $z \leq +4$ and has a slope of 0.76 ± 0.07 . This slope is significantly lower than the slopes observed for both poly-(A) and poly(C), but it is similar to the slope observed for these peptides binding to poly(U) (Mascotti & Lohman, 1990, 1992). However, for the peptides with $z > +4$, $[(\partial \log K_{\text{obs}} / \partial \log [K^+])]/z$ decreases with increasing z as observed with poly-(A) and poly(C).

Poly(I). We have also examined the dependence of K_{obs} on $[K^+]$ for the binding of KWK-CO₂ to poly(I) (25.0 °C, pH

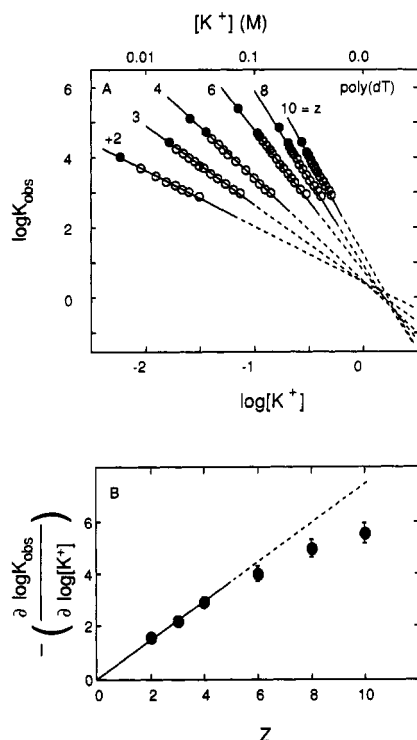


FIGURE 3: (A) Dependence of $\log K_{\text{obs}}$ on $\log [K^+]$ (in terms of total $[K^+]$) for a series of oligolysines containing a single tryptophan binding to poly(dT) at 25.0 °C, pH 6.0 (buffer CB). KCH_3CO_2 was used to vary the K^+ concentration. The peptides were $\text{KWK}_p\text{-NH}_2$, with $p = 1, 2, 4, 6$, or 8 ($z^\circ = +3, +4, +6, +8$, or $+10$), and KWK-CO_2 ($z^\circ = +2$). Data were obtained from titrations at constant salt concentration (●) and from "salt-back" titrations (○). Smooth lines are linear least-squares best fits for each peptide, and the parameters are listed in Table III. Dashed lines indicate extrapolations to high salt concentration. (B) Thermodynamic extent of ion release ($-\partial \log K_{\text{obs}} / \partial \log [K^+]$) is plotted as a function of the net positive charge, z , on each oligolysine binding to poly(dT). The solid line drawn is a linear least-squares best-fit line to only the data for $z \leq +4$, constrained to intersect the origin, and is given by the equation $-\partial \log K_{\text{obs}} / \partial \log [K^+] = (0.76 \pm 0.07)z$. The dashed line is an extrapolation to higher z .

Table III: Dependence of K_{obs} on $[\text{KCH}_3\text{CO}_2]$ and the Thermodynamics of Oligolysines Containing a Single Tryptophan Binding to Poly(dT)^a

peptide	z	$\frac{\partial \log K_{\text{obs}}}{\partial \log [K^+]}$	$\log K_{\text{obs}}(1\text{M})^b$	$Q_{\text{max}} (\%)$	$\Delta H^\circ_{\text{obs}}^c$
KWK-CO_2	2	-1.59 ± 0.20	0.45 ± 0.24	84 ± 2	-2.0 ± 1.5
KWK-NH_2	3	-2.23 ± 0.19	0.42 ± 0.24	91 ± 2	-2.7 ± 1.5
$\text{KWK}_2\text{-NH}_2$	4	-2.94 ± 0.21	0.46 ± 0.22	92 ± 2	-4.7 ± 1.5
$\text{KWK}_4\text{-NH}_2$	6	-4.01 ± 0.22	0.76 ± 0.22	95 ± 2	-3.3 ± 1.5
$\text{KWK}_6\text{-NH}_2$	8	-4.95 ± 0.25	0.93 ± 0.24	94 ± 2	-3.2 ± 1.5
$\text{KWK}_8\text{-NH}_2$	10	-5.54 ± 0.34	1.25 ± 0.27	93 ± 2	-3.0 ± 1.5

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

7.0) and find $\partial \log K_{\text{obs}} / \partial \log [K^+] = -1.43 \pm 0.18$ (Table II). These studies were performed at pH 7.0 since poly(I) remains single stranded and soluble at these concentrations and solution conditions (Thiele & Guschlbauer, 1973). This value of $-\partial \log K_{\text{obs}} / \partial \log [K^+]$ is lower than for any of the other ss polynucleotides (Mascotti & Lohman, 1990; Tables I, II, and III). The limited solubility of poly(I) and its propensity to form triple-stranded structures in solutions of higher salt concentration precluded study of the more highly charged peptides.

Poly(dU). We have also measured K_{obs} as a function of $[K^+]$ for the binding of $\text{KWK}_4\text{-NH}_2$ to poly(dU) (25.0 °C,

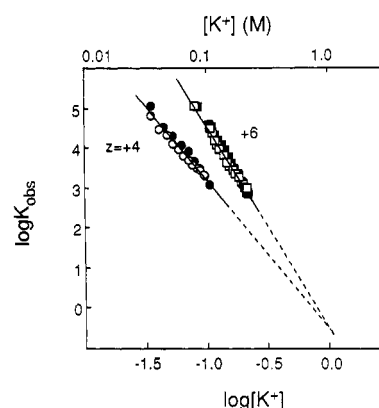


FIGURE 4: Dependence of $\log K_{\text{obs}}$ on $\log [K^+]$ (in terms of total $[K^+]$) for a series of oligolysines containing a single tryptophan binding to ds-DNA [supercoiled (●, ■) and linear (○, □)] at 25.0 °C, pH 6.0 (buffer CB). KCH_3CO_2 was used to vary the K^+ concentration. The peptides were $\text{KWK}_p\text{-NH}_2$, with $p = 2$ or 4 ($z^\circ = +4$ or $+6$). Solid lines are linear least-squares best fits for each peptide–ds-DNA interaction (the parameters are listed in Table IV as the average of supercoiled and linear DNA), and the dashed lines indicate extrapolations to high salt concentration.

Table IV: Dependence of K_{obs} on $[\text{KCH}_3\text{CO}_2]$ and the Thermodynamics of Oligolysines Containing a Single Tryptophan Binding to Supercoiled and Linear pUC8 DNA^a

peptide	z	$\frac{\partial \log K_{\text{obs}}}{\partial \log [K^+]}$	$\log K(1\text{M})^b$	$\Delta H^\circ_{\text{obs}}$ (kcal/mol)	$Q_{\text{max}} (\%)$
supercoiled					
$\text{KWK}_2\text{-NH}_2$	4	-3.94 ± 0.30	-0.68 ± 0.32	-2.0 ± 1.5	47 ± 2
$\text{KWK}_4\text{-NH}_2$	6	-5.41 ± 0.33	-0.68 ± 0.30	0.7 ± 1.5	50 ± 2
linear					
$\text{KWK}_2\text{-NH}_2$	4	-3.54 ± 0.30	-0.34 ± 0.32	ND ^c	47 ± 2
$\text{KWK}_4\text{-NH}_2$	6	-5.16 ± 0.33	-0.52 ± 0.30	ND ^c	50 ± 2

^a Buffer CB + KCH_3CO_2 , pH 6.0, 25.0 °C. ^b Obtained from a linear extrapolation of a plot of $\log K_{\text{obs}}$ vs $\log [K^+]$. ^c Not determined.

pH 6.0). Over the $[\text{KCH}_3\text{CO}_2]$ range examined (0.07–0.26 M), $\log K_{\text{obs}}$ shows a linear dependence on $\log [K^+]$, described by eq 6.

$$\log K_{\text{obs}} = (-4.29 \pm 0.22) \log [K^+] + (0.43 \pm 0.22) \quad (6)$$

The values of K_{obs} for binding of $\text{KWK}_4\text{-NH}_2$ to poly(U), poly(dU), and poly(dT) appear to be the same within experimental error, although the salt dependence for binding to poly(dU) is closer to that of poly(U) (-4.36 ± 0.22) than to poly(dT) (-4.01 ± 0.22) (Mascotti & Lohman, 1990). The values of the maximal extent of quenching, Q_{max} , are nearly identical ($93 \pm 2\%$) for the interaction of $\text{KWK}_4\text{-NH}_2$ with poly(U), poly(dU), and poly(dT), suggesting that the tryptophan interacts similarly with each of these polynucleotides.

Duplex B-Form DNA. A linear dependence of $\log K_{\text{obs}}$ on $\log [K^+]$ was observed for the binding of $\text{KWK}_2\text{-NH}_2$ and $\text{KWK}_4\text{-NH}_2$ to the duplex plasmid DNA, pUC8 (pH 6.0, 25 °C), as shown in Figure 4 (see Table IV). Identical binding behavior was observed for linear and negatively supercoiled pUC8. The values of K_{obs} obtained upon linear extrapolation to 1 M K^+ indicate that the intrinsic interaction of oligolysines with ds-DNA, excluding contributions from counterion release from the DNA, is small and slightly unfavorable, in agreement with previous reports (Lohman *et al.*, 1980). If the salt dependence of K_{obs} ($\partial \log K_{\text{obs}} / \partial \log [K^+]$) is plotted vs z (from the data in Table IV) and the data are fit to a linear least-squares line which is constrained to intersect the origin, the line has a slope of 0.91 ± 0.07 . This value is larger than the values obtained for binding to ss polynucleotides and is

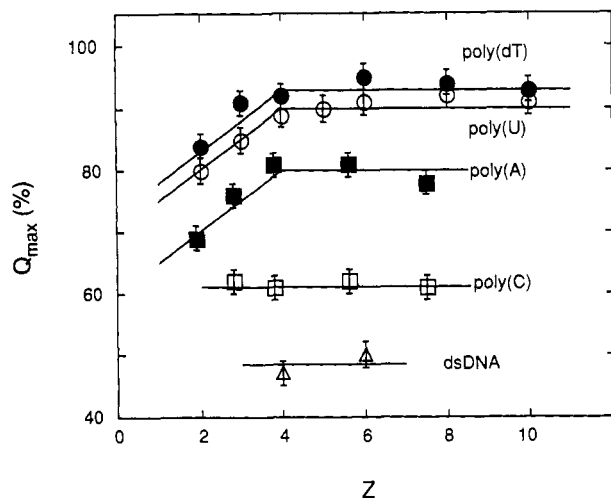


FIGURE 5: Dependence of maximal quenching of the tryptophan fluorescence, Q_{\max} , on the peptide charge, z , for the interactions of oligolysines which contain one tryptophan with poly(dT) (●), poly(U) (○), poly(A) (■), poly(C) (□), and ds-DNA (△). Q_{\max} is independent of KCH_3CO_2 .

consistent with the predicted value of 0.88 monovalent counterions associated thermodynamically per phosphate in ds-DNA (Record *et al.*, 1976).

Correlation between Net Peptide Charge and Tryptophan Fluorescence Quenching. The maximal extents of quenching, Q_{\max} , for saturation of oligolysines containing a single tryptophan with nucleic acid are dependent upon the net charge of the peptide as well as the polynucleotide type, as shown in Figure 5. For binding to poly(A), Q_{\max} increases from $69 \pm 2\%$ for $z = +2$ to $81 \pm 2\%$ for $z = +4$, where it remains constant for $z \geq +4$. This dependence is qualitatively similar to the dependence observed for the binding of these same peptides to poly(U) and poly(dT), as shown in Figure 5, although the values of Q_{\max} are lower for the poly(A) interaction than for poly(U) or poly(dT). The behavior of these peptides appears to fall into two classes, with Q_{\max} dependent on z for $z \leq +4$, whereas Q_{\max} is independent of z for $z > +4$. This change in Q_{\max} at $z = +4$ seems to correlate with the charge-dependent differences in the salt dependence of K_{obs} (Figures 1–3) and $\Delta H^\circ_{\text{obs}}$ (see below).

For the binding of monotryptophanyl oligolysines to poly(C), Q_{\max} is $\sim 61\%$, independent of z (Figure 5). However, the value of Q_{\max} for the KWK-CO_2 -poly(C) interaction was not determined due to low affinity of the peptide at the salt concentrations used for the binding studies, although a value of 51% has been reported (Brun *et al.*, 1975) in ~ 3 mM NaCl (pH 7.0, 26 °C). If this data point were included in Figure 5, it would suggest that the interaction of poly(C) with oligolysines containing a single Trp also shows the same trend in Q_{\max} as observed for poly(A), poly(U), and poly(dT). However, we note that the absolute magnitude of Q_{\max} for binding to poly(C) is lower than for the other ss homopolynucleotides and that this correlates with the lower affinity of poly(C) for these peptides.

For KWK-CO_2 binding to poly(I), we observe $Q_{\max} \sim 77\%$ (Table II), which is approximately equal to Q_{\max} for the interaction of KWK-CO_2 with poly(U) under similar conditions (Figure 5). However, the affinity of this peptide for poly(I) is significantly higher than for poly(U); hence Q_{\max} shows no correlation with affinity in this case. The value of Q_{\max} for oligolysines with $z = +4$ and $+6$ binding to duplex DNA is lower ($\sim 50\%$) than for binding to any of the ss polynucleotides.

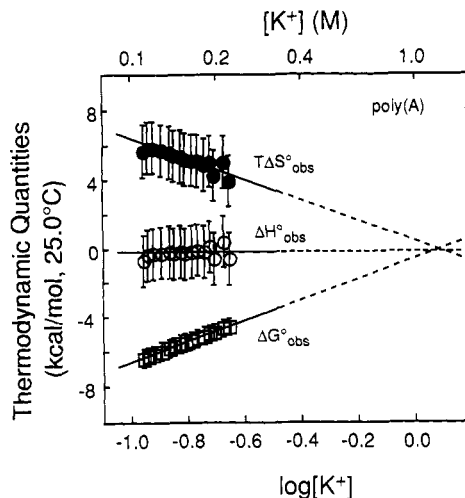


FIGURE 6: Dependence of $\Delta G^\circ_{\text{obs}}$ (□), $\Delta H^\circ_{\text{obs}}$ (○) and $T\Delta S^\circ_{\text{obs}}$ (●) on $\log[K^+]$ for the interaction of $\text{KWK}_4\text{-NH}_2$ with poly(A), pH 7.0 (buffer CB), 25.0 °C. KCH_3CO_2 was used to vary $[K^+]$. $\Delta H^\circ_{\text{obs}}$ is independent of $[K^+]$ ($\Delta H^\circ_{\text{obs}} = -0.2$ kcal/mol). The solid line through the $\Delta G^\circ_{\text{obs}}$ data is a linear least-squares best fit with the equation $\Delta G^\circ_{\text{obs}}$ (kcal/mol) = $-0.61 + 2.60 \log[K^+]$. $T\Delta S^\circ_{\text{obs}}$ was calculated from $(\Delta H^\circ_{\text{obs,ave}} - \Delta G^\circ_{\text{obs}})$. The solid line drawn through the $T\Delta S^\circ_{\text{obs}}$ data is a linear least-squares best-fit with the equation $T\Delta S^\circ_{\text{obs}}$ (kcal/mol) = $0.51 - 2.60 \log[K^+]$. The dashed lines are extrapolations to high salt concentration.

Effects of Temperature, $[\text{KCH}_3\text{CO}_2]$, and Peptide Charge

Poly(A). The thermodynamic quantities $\Delta H^\circ_{\text{obs}}$ and $\Delta S^\circ_{\text{obs}}$ were determined as a function of $[\text{KCH}_3\text{CO}_2]$ from van't Hoff analysis of K_{obs} . Figure 6 shows the dependence of $\Delta G^\circ_{\text{obs}}$, $\Delta H^\circ_{\text{obs}}$ and $T\Delta S^\circ_{\text{obs}}$ on $[\text{KCH}_3\text{CO}_2]$ for $\text{KWK}_4\text{-NH}_2$ binding to poly(A) (pH 7.0, 25.0 °C). Over the range of temperatures examined (5–45 °C), the van't Hoff plots were linear, indicating that $\Delta C_p^\circ \sim 0$ under these conditions. Furthermore, $\Delta H^\circ_{\text{obs}} = 0$ (-0.2 ± 1.5) kcal/mol, independent of $[K^+]$, whereas both $\Delta G^\circ_{\text{obs}}$ and $T\Delta S^\circ_{\text{obs}}$ are highly dependent on $[K^+]$, becoming less favorable as $[K^+]$ increases. Therefore, the dependence of $\Delta G^\circ_{\text{obs}}$ on $[K^+]$ is entirely entropic in origin as was also observed for binding to poly(U) (Mascotti & Lohman, 1992). The $[K^+]$ dependence of the thermodynamic quantities shown in Figure 6 are well-described by the linear least-squares lines given in eqs 7a–c.

$$\Delta G^\circ_{\text{obs}} = (-0.6 \pm 0.4) + (6.0 \pm 0.4) \log[K^+] \quad (7a)$$

$$\Delta H^\circ_{\text{obs}} = -0.2 \pm 1.5 \quad (7b)$$

$$T\Delta S^\circ_{\text{obs}} = (0.4 \pm 1.5) - (6.0 \pm 1.5) \log[K^+] \quad (7c)$$

The thermodynamic quantities for oligolysine–polynucleotide binding are dependent upon the bulk salt concentration due to the entropic contribution from counterion (K^+) release from the nucleic acid upon peptide binding (Record *et al.*, 1976; Lohman, 1980; Mascotti & Lohman, 1990, 1992). The entropic contribution to ΔS° and ΔG° due to counterion release (polyelectrolyte effect; Record, 1988) is predicted to be negligible at 1 M K^+ , since the free energy of dilution due to counterion release is 0 at the 1 M K^+ standard state (Record *et al.*, 1976, 1978). Therefore, we estimate $\Delta G^\circ_{\text{obs}}(1\text{M})$ by extrapolation of $\log K_{\text{obs}}$ ($\Delta G^\circ_{\text{obs}}$) to 1M K^+ . $T\Delta S^\circ(1\text{M})$ is then obtained by subtraction of $\Delta H^\circ_{\text{obs}}$, which is independent of $[K^+]$, from $\Delta G^\circ(1\text{M})$. The values of $\Delta G^\circ_{\text{obs}}(1\text{M})$, $\Delta H^\circ_{\text{obs}}$, and $T\Delta S^\circ_{\text{obs}}(1\text{M})$ for oligolysines binding to poly(A) are shown as a function of z in Figure 7B and Table I.

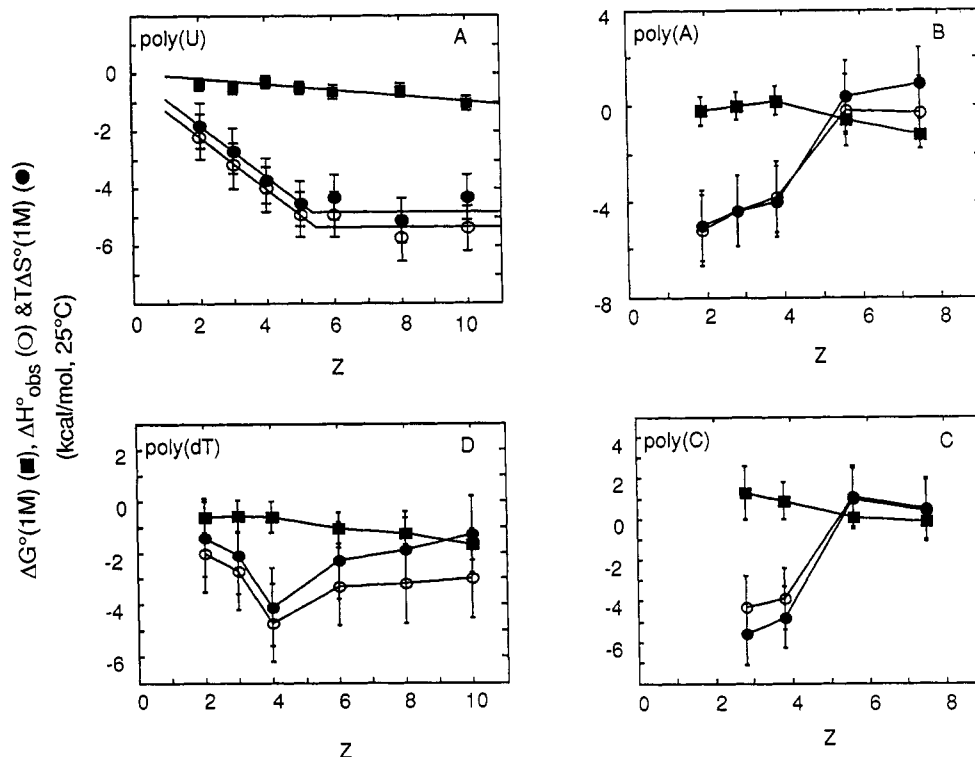


FIGURE 7: (A) Thermodynamic quantities $\Delta G^\circ(1M)$, $\Delta H^\circ_{\text{obs}}$, and $T\Delta S^\circ(1M)$ for oligolysines binding to poly(U) in KCH_3CO_2 , (pH 6.0 (buffer CB), 25.0 °C, extrapolated to 1 M KCH_3CO_2 and plotted as a function of oligopeptide net charge, z (data from Mascotti & Lohman, 1992). The observed thermodynamic parameters have been extrapolated to 1 M $[K^+]$. (B) Dependence of $\Delta G^\circ(1M)$, $\Delta H^\circ_{\text{obs}}$, and $T\Delta S^\circ(1M)$ on z for oligolysines binding to poly(A) at pH 7.0 (buffer CB), 25.0 °C. The observed thermodynamic parameters have been extrapolated to 1 M $[K^+]$. The value of z was calculated according to eq 7 of Mascotti and Lohman (1992) using the pK values reported in that paper. (C) Dependence of $\Delta G^\circ(1M)$, $\Delta H^\circ_{\text{obs}}$, and $T\Delta S^\circ(1M)$ on z for oligolysines binding to poly(C) at pH 7.0 (buffer CB), 25.0 °C. The observed thermodynamic parameters have been extrapolated to 1 M $[K^+]$. The value of z was calculated according to eq 7 of Mascotti and Lohman (1992) using the pK values reported in that paper. (D) Dependence of $\Delta G^\circ(1M)$, $\Delta H^\circ_{\text{obs}}$, and $T\Delta S^\circ(1M)$ on z for oligolysines binding to poly(dT) at pH 6.0 (buffer CB), 25.0 °C. The observed thermodynamic parameters have been extrapolated to 1 M $[K^+]$.

Figure 7B indicates that at 25 °C both $\Delta H^\circ_{\text{obs}}$ and $T\Delta S^\circ_{\text{obs}}(1M)$ become less favorable with increasing z , from ~ -5 kcal/mol at $z^\circ = +2$ to $\sim +1$ kcal/mol for $z^\circ = +8$. However, neither $\Delta H^\circ_{\text{obs}}$ nor $T\Delta S^\circ_{\text{obs}}(1M, 25^\circ C)$ varies uniformly with z , and in fact they appear to fall into two groups, relative to the value for $z = +4$. This is consistent with the dependence of Q_{max} and $(-\partial \log K_{\text{obs}}/\partial \log [K^+])/z$ on z (Figures 1B and 5). Furthermore, $\Delta G^\circ_{\text{obs}}(1M, 25^\circ C)$ is nearly independent of peptide charge due to enthalpy–entropy compensations.

Poly(C). As with poly(A) and poly(U), $\Delta H^\circ_{\text{obs}}$ is independent of $[K^+]$ and the dependence of $\Delta G^\circ_{\text{obs}}$ on $[K^+]$ is entropic in origin for the mono-Trp-oligolysines binding to poly(C) (data not shown). The dependences of $\Delta G^\circ(1M, 25^\circ C)$, $\Delta H^\circ_{\text{obs}}$ and $T\Delta S^\circ(1M, 25^\circ C)$ on z are shown in Figure 7C and Table II. $\Delta H^\circ_{\text{obs}}$ becomes less favorable with increasing z , but this is compensated by the $T\Delta S^\circ(1M, 25^\circ C)$ term, which becomes more favorable with increasing z , such that $\Delta G^\circ_{\text{obs}}(1M, 25^\circ C)$ varies only slightly, becoming more favorable with increasing z . Again, as with poly(U) and poly(A), there appears to be a difference in thermodynamic behavior between peptides with $z^\circ \leq +4$ and peptides with $z^\circ > +4$. Both $\Delta H^\circ_{\text{obs}}$ and $T\Delta S^\circ(1M, 25^\circ C)$ are significantly more positive for the more highly charged peptides ($z^\circ = +6$ and $+8$).

Poly(I). Values of $\Delta H^\circ_{\text{obs}}$ and $T\Delta S^\circ(1M, 25^\circ C)$ for KWK- CO_2 binding to poly(I) are shown in Table II. As noted with poly(A) and poly(C), is independent of $[K^+]$ and the salt-dependence of $\Delta G^\circ_{\text{obs}}$ is entropic in origin. Note that $\Delta H^\circ_{\text{obs}}$ for the poly(I) interaction is much more favorable (-8.3 ± 1.5 kcal/mol) than for the other ss polynucleotides.

Poly(dT). The values of $\Delta G^\circ(1M, 25^\circ C)$, $\Delta H^\circ_{\text{obs}}$ and $T\Delta S^\circ(1M, 25^\circ C)$ are shown in Figure 7D and Table III. As observed with poly(A), poly(C), and poly(I), $\Delta H^\circ_{\text{obs}}$ is independent of $[K^+]$, and the dependence of $\Delta G^\circ_{\text{obs}}$ on $[K^+]$ is entropic in origin, for poly(dT) (data not shown). As with the other polynucleotides, $\Delta G^\circ(1M, 25^\circ C)$ shows only a slight dependence on z due to enthalpy–entropy compensations; however, $\Delta H^\circ_{\text{obs}}$ and $T\Delta S^\circ(1M, 25^\circ C)$ show a distinct minimum at $z = +4$. Q_{max} increases with increasing z for $z \leq +4$ and becomes independent of z for $z > +4$, which correlates with the thermodynamics (Figure 5). Although the variation of Q_{max} with z for binding to poly(dT) resembles the behavior observed with poly(U), the dependence of the thermodynamic quantities for the KWK peptides binding to these two ss polynucleotides differ in the region $z > +4$.

Duplex DNA. Plasmid pUC8 DNA was used to examine the interaction of two oligolysines with duplex as a function of $[KCH_3CO_2]$ (see Table IV). Within experimental error, $\Delta H^\circ_{\text{obs}}$ is 0 for both KWK₂-NH₂ and KWK₄-NH₂ binding to ds-DNA, independent of $[K^+]$, whereas $\Delta G^\circ_{\text{obs}}$ (K_{obs}) is strongly dependent on $[K^+]$ due to a large dependence of $\Delta S^\circ_{\text{obs}}$ on $[K^+]$ (data not shown). This behavior is similar to that observed for pentalysine binding to ds-DNA (Lohman *et al.*, 1980).

Oligolysines Containing Multiple Tryptophans

Poly(A). The dependence of $\log K_{\text{obs}}$ on $\log [KCH_3CO_2]$ (pH 7.0, 25.0 °C) for the binding to poly(A) of a series of oligolysines of constant charge ($z = +6$) containing one, two, or three tryptophans (KWK₄-NH₂, KWK₃WK-NH₂, (KW)₃K₂-NH₂) is shown in Figure 8 (see also Table V). For

Table V: Comparison of the Dependence of K_{obs} on $[\text{KCH}_3\text{CO}_2]$ and the Thermodynamics of Multi-Tryptophan Oligolysines Binding to Poly(A)^a

peptide	$\partial \log K_{\text{obs}} / \partial \log [\text{K}^+]$	$\log K_{\text{obs}}(1\text{M})^b$	Q_{max} (%)	$\Delta H^\circ_{\text{obs}}^c$	$T\Delta S^\circ(1\text{M})^d$
KWK ₄ -NH ₂	-4.38 ± 0.22	0.45 ± 0.22	81 ± 2	-0.2 ± 1.5	0.4 ± 1.5
KWK ₃ WK-NH ₂	-4.56 ± 0.27	0.51 ± 0.27	72 ± 2	-3.4 ± 1.5	-2.7 ± 1.5
(KW) ₃ K ₂ -NH ₂	-4.85 ± 0.33	0.47 ± 0.33	72 ± 2	-5.7 ± 1.5	-5.1 ± 1.5

^a Buffer CB + KCH_3CO_2 , pH 7.0, 25.0 °C. ^b Obtained from a linear extrapolation of a plot of $\log K_{\text{obs}}$ vs $\log [\text{K}^+]$. ^c The average of $\Delta H^\circ_{\text{obs}}$ within the range of $[\text{K}^+]$ examined in units of kcal/mol. ^d The value of $T\Delta S^\circ_{\text{obs}}$ extrapolated to 1 M K^+ , in units of kcal/mol.

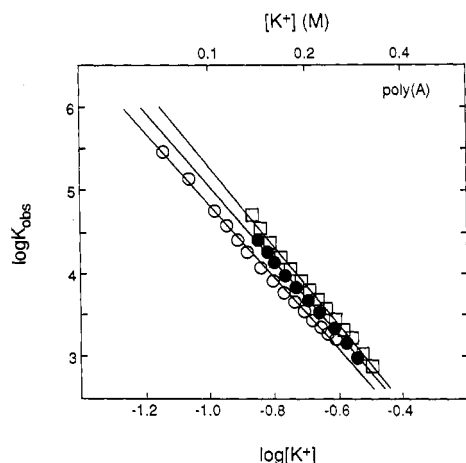


FIGURE 8: Comparison of the dependence of $\log K_{\text{obs}}$ on $\log [\text{K}^+]$ (plotted in terms of total $[\text{K}^+]$) for oligolysines of charge $z = +6$ containing multiple tryptophan residues binding to poly(A) in KCH_3CO_2 , pH 7.0 (buffer CB), 25.0 °C. The peptide sequences and corresponding symbols are KWK₄-NH₂ (○), KWK₃WK-NH₂ (●) and (KW)₃K₂-NH₂ (□). The lines drawn are linear least-squares fits to each peptide–poly(A) interaction, and the parameters for each line are listed in Table V.

all peptides, $\log K_{\text{obs}}$ is linearly dependent on $\log [\text{K}^+]$ over the $[\text{K}^+]$ range studied, with slopes that are identical within experimental error (-4.5 ± 0.3); however, a slight but systematic increase in K_{obs} is observed with increasing number of tryptophans.

On the basis of these data, we can estimate the thermodynamic properties of the interaction of poly(A) with an oligolysine with $z = +6$, which does not possess any tryptophan (e.g., K₅-NH₂). The estimated values are $-\partial \log K_{\text{obs}} / \partial \log [\text{K}^+] = 4.36 \pm 0.22$ and $\log K(1\text{M}) = 0$, which are close to the values estimated for binding of K₅-NH₂ to poly(U) (Mascotti & Lohman, 1992). However, these estimations must be viewed with some caution, since with poly(A) the values of Q_{max} are lower for peptides containing two and three tryptophans ($72 \pm 2\%$), relative to those containing one ($81 \pm 2\%$), indicating that each Trp in KWK₃WK-NH₂ and (KW)₃K₂-NH₂ does not interact in the same way as the tryptophan in KWK₄-NH₂. By contrast, each tryptophan in these same oligolysines appears to interact identically and independently upon binding to poly(U) (Mascotti & Lohman, 1992).

Values of $\Delta H^\circ_{\text{obs}}$ and the dependence of $\Delta H^\circ_{\text{obs}}$ on $[\text{KCH}_3\text{CO}_2]$ were estimated from van't Hoff analysis (pH 7.0, KCH_3CO_2). No detectable curvature was noted in the van't Hoff plots, within the temperature range from 10 to 40 °C, indicating $\Delta C_p^\circ \sim 0$. The thermodynamic quantities determined from this analysis (extrapolated to 1 M K^+) are shown in Figure 9A (see also Table V). Both $\Delta H^\circ_{\text{obs}}$ and $\Delta S^\circ_{\text{obs}}(1\text{M}, 25^\circ\text{C})$ are dependent upon the number of tryptophans, N , but compensate such that $\Delta G^\circ(1\text{M}, 25^\circ\text{C})$ is independent of N . $\Delta H^\circ_{\text{obs}}$ becomes more favorable with increasing N , whereas $T\Delta S^\circ_{\text{obs}}(1\text{M}, 25^\circ\text{C})$ becomes less favorable with increasing N (see eqs 8a,b). This enthalpy–entropy compensation was also observed for the binding of

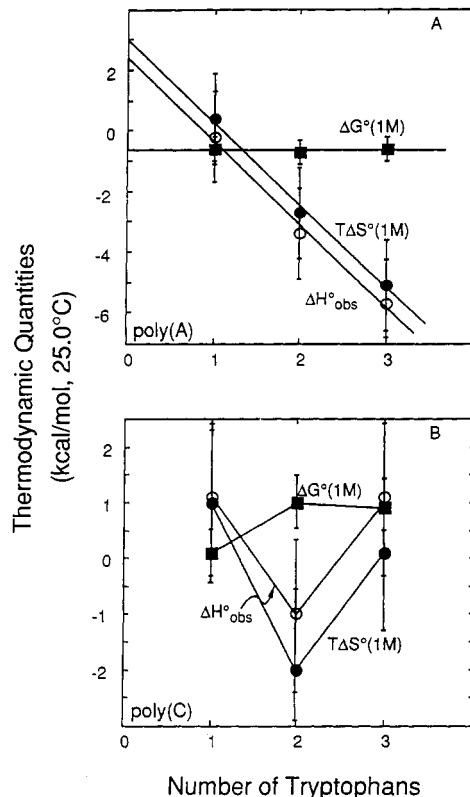


FIGURE 9: (A) Thermodynamic quantities $\Delta G^\circ_{\text{obs}}(1\text{M})$, $\Delta H^\circ_{\text{obs}}$, and $T\Delta S^\circ_{\text{obs}}(1\text{M})$ for poly(A) binding to oligolysines, all with net charge $z = +6$ but containing 1, 2, or 3 tryptophans, are plotted vs the number of tryptophans (buffer CB, pH 7.0, 25.0 °C). The peptides used are given in the caption to Figure 8, and the thermodynamic data are listed in Table V. The equations of the linear least-squares lines through the data are given in eqs (8a,b). (B) Thermodynamic quantities $\Delta G^\circ_{\text{obs}}(1\text{M})$, $\Delta H^\circ_{\text{obs}}$, and $T\Delta S^\circ_{\text{obs}}(1\text{M})$ for poly(C) binding to oligolysines, all with net charge $z = +6$ but containing 1, 2, or 3 tryptophans, are plotted vs the number of tryptophans (buffer CB, pH 7.0, 25.0 °C). The peptides used are given in the caption to Figure 8, and the thermodynamic data are listed in Table VI.

these same oligopeptides to poly(U). However, for the binding of these peptides to poly(U), both Q_{max} and $\Delta H^\circ_{\text{obs}}/N$ are independent of N , whereas this is not the case for poly(A), which suggests that the tryptophans within these peptides do not interact identically and independently with poly(A).

$$\Delta H^\circ_{\text{obs}} = (2.4 \pm 1.5) - (2.75 \pm 0.65)N \quad (8a)$$

$$T\Delta S^\circ(1\text{M}, 25.0^\circ\text{C}) = (2.9 \pm 1.5) - (2.75 \pm 0.65)N \quad (8b)$$

Poly(C). Table VI shows the results of studies of the dependence of $\log K_{\text{obs}}$ on $\log [\text{KCH}_3\text{CO}_2]$ (pH 7.0, 25.0 °C) for the binding to poly(C) of a series of oligolysines of constant charge ($z = +6$) containing one, two or three tryptophans (KWK₄-NH₂, KWK₃WK-NH₂, (KW)₃K₂-NH₂). A very slight difference in $\partial \log K_{\text{obs}} / \partial \log [\text{K}^+]$ is observed for the peptide with one Trp (-4.25 ± 0.22) compared to the peptides with two and three Trp residues (-5.02 ± 0.30).

Table VI: Comparison of the Dependence of K_{obs} on $[\text{KCH}_3\text{CO}_2]$ and the Thermodynamics of Multi-Tryptophan Oligolysines Binding to Poly(C)^a

peptide	$\partial \log K_{\text{obs}} / \partial \log [\text{K}^+]$	$\log K_{\text{obs}}(1\text{M})^b$	$Q_{\text{max}} (\%)$	$\Delta H^\circ_{\text{obs}}^c$	$T\Delta S^\circ(1\text{M})^d$
KWK ₄ -NH ₂	-4.25 ± 0.22	-0.07 ± 0.22	62 ± 2	1.1 ± 1.5	1.0 ± 1.5
KWK ₃ WK-NH ₂	-5.02 ± 0.30	-0.75 ± 0.30	54 ± 2	-1.0 ± 1.5	-2.0 ± 1.5
(KW) ₃ K ₂ -NH ₂	-5.02 ± 0.33	-0.64 ± 0.30	54 ± 2	1.0 ± 1.5	0.1 ± 1.5

^a Buffer CB + KCH₃CO₂, pH 7.0, 25.0 °C. ^b Obtained from a linear extrapolation of a plot of $\log K_{\text{obs}}$ vs $\log [\text{K}^+]$. ^c The average of $\Delta H^\circ_{\text{obs}}$ within the range of $[\text{K}^+]$ examined in units of kcal/mol. ^d The value of $T\Delta S^\circ_{\text{obs}}$ extrapolated to 1 M K⁺, in units of kcal/mol.

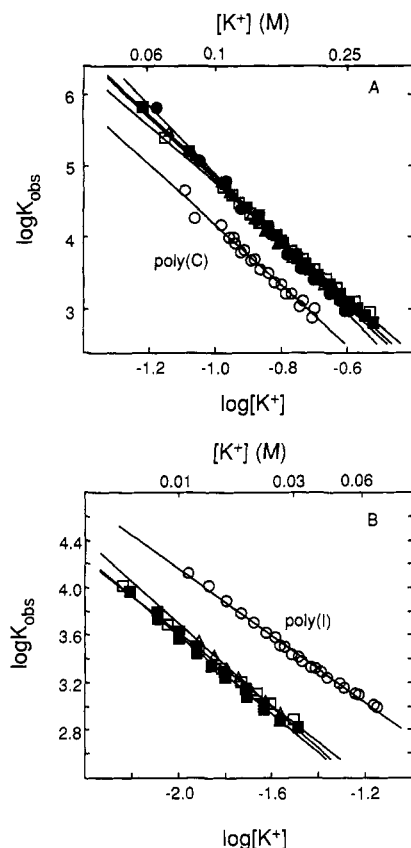


FIGURE 10: (A) Value of $\log K_{\text{obs}}$ as a function of $\log [\text{K}^+]$ for the interaction of KWK₄-NH₂ with poly(A) (Δ), poly(C) (\circ), poly(U) (\blacksquare), poly(dT) (\square), and poly(dU) (\bullet) in KCH₃CO₂, 25.0 °C, at pH 6.0 (buffer CB) for poly(U), poly(dT), and poly(dU) and at pH 7.0 (buffer CB) for poly(C) and poly(A). (B) Value of $\log K_{\text{obs}}$ as a function of $\log [\text{K}^+]$ for the interaction of KWK-CO₂ with poly(A) (Δ), poly(I) (\circ), poly(U) (\blacksquare), and poly(dT) (\square) in KCH₃CO₂, 25.0 °C, at pH 6.0 (buffer CB) for poly(U) and poly(dT) and at pH 7.0 (buffer CB) for poly(I) and poly(A).

From a linear extrapolation of these binding parameters to $N = 0$, we predict that an oligolysine with $z = +6$, but containing no tryptophan (e.g., K₅-NH₂), will bind to poly(C) with values of $\partial \log K_{\text{obs}} / \partial \log [\text{K}^+] = -3.99 \pm 0.36$ and $\log K(1\text{M}, 25^\circ\text{C}) = 0$ (0.08 ± 0.45). However, as observed with poly(A), the extent of fluorescence quenching of the individual Trp residues upon binding poly(C) is dependent upon the number of Trp residues in the peptide ($Q_{\text{max}} = 54 \pm 2\%$ for $N = 2$ and 3; $Q_{\text{max}} = 61 \pm 2\%$ for $N = 1$). Therefore, the linear extrapolation to $N = 0$ used to estimate these quantities may not be valid, and therefore these should be viewed only as approximations.

The value of $\Delta H^\circ_{\text{obs}}$ and its dependence on $[\text{KCH}_3\text{CO}_2]$ were estimated from van't Hoff analysis (pH 7.0, KCH₃CO₂). No detectable curvature was noted in the van't Hoff plots, within the temperature range from 10 to 40 °C, indicating $\Delta C_p^\circ \sim 0$. The thermodynamic quantities determined from this analysis (extrapolated to 1 M K⁺) are shown in Figure 9B (see also Table VI). The dependence of

the thermodynamic quantities on the number of Trp residues is clearly different for the peptides binding to poly(C) and those binding to poly(A) and poly(U). For poly(C), $\Delta G^\circ(1\text{M}, 25^\circ\text{C}) \sim 0$ kcal/mol for $N = 1$; however, $\Delta G^\circ(1\text{M}, 25^\circ\text{C})$ becomes positive (+1 kcal/mol) for both $N = 2$ and 3 (Table VI and Figure 9B). Within experimental error, $\Delta H^\circ_{\text{obs}}$ and $T\Delta S^\circ(1\text{M}, 25^\circ\text{C})$ are nearly 0 and are independent of the number of tryptophans contained within each oligolysine (Table VI and Figure 9B). In this case, there is no apparent correlation between Q_{max} and $\Delta H^\circ_{\text{obs}}$ (or $T\Delta S^\circ(1\text{M}, 25^\circ\text{C})$); therefore, we conclude that the tryptophan interactions with poly(C) may not be identical and/or independent.

Comparison of the Binding of Oligolysines to Different Single-Stranded Polynucleotides. We have used two peptides, KWK₄-NH₂ and KWK-CO₂, to assess the dependence of K_{obs} on nucleotide base composition since the K_{obs} for these peptides could be measured with the greatest number of different ss polynucleotides. Figure 10A compares the dependence of $\log K_{\text{obs}}$ on $\log [\text{K}^+]$ for KWK₄-NH₂ binding to poly(U), poly(dU), poly(dT), poly(C), and poly(A). Among the different polynucleotides, binding to poly(C) is weakest at all salt concentrations, whereas K_{obs} and its salt dependence are indistinguishable for binding to poly(U), poly(dU), poly(dT) and poly(A). Therefore, the $\Delta G^\circ_{\text{obs}}$ for binding of KWK₄-NH₂ to poly(C) is unfavorable (by approximately 0.7 kcal/mol) relative to the other ss polynucleotides examined.

Figure 10B compares the salt dependences of K_{obs} for KWK-CO₂ binding to poly(A), poly(U), poly(I), and poly(dT). K_{obs} is the same within error for binding to poly(A), poly(U), and poly(dT) at each salt concentration, whereas K_{obs} for the binding to poly(I) is significantly larger and increases with increasing salt concentration ($\Delta G^\circ_{\text{obs}}$ is approximately 0.6 kcal/mol greater for poly(I) at 0.02 M K⁺). Thus the tryptophan-containing oligolysines bind to ss polynucleotides with the following hierarchy: poly(I) > poly(U) \sim poly(dU) \sim poly(dT) \sim poly(A) > poly(C).

DISCUSSION

The Dependence of K_{obs} on Salt Concentration is Entropic in Origin for Oligolysines Binding to Single-Stranded Polynucleotides. For each oligolysine examined, the equilibrium binding constant, K_{obs} , increases with decreasing $[\text{KCH}_3\text{CO}_2]$, such that $\partial \log K_{\text{obs}} / \partial \log [\text{K}^+]$ remains constant over the salt concentration range examined. Furthermore, the absolute magnitude of $\partial \log K_{\text{obs}} / \partial \log [\text{K}^+]$ increases with increasing peptide positive charge, z , for the binding to all polynucleotides, similar to our previous report with poly(U) (Mascotti & Lohman, 1990). This effect is entirely entropic in origin and is due to changes in cation (K⁺) concentration (Mascotti & Lohman, 1990). Upon linear extrapolation of the plots of $\log K_{\text{obs}}$ ($\Delta G^\circ_{\text{obs}}$) to 1 M K⁺, the resulting values of $\Delta G^\circ_{\text{obs}}$ are approximately 0 for all peptides, consistent with the proposal that the increased binding of the peptides to the nucleic acid at lower $[\text{K}^+]$ is driven primarily by cation release from the nucleic acid into bulk solution, which results in an

increased entropy of dilution (Record *et al.*, 1976, 1978). However, whereas for oligolysines binding to poly(U) $\partial \log K_{\text{obs}}/\partial \log [K^+]$ is nearly directly proportional to z , for oligolysines binding to poly(A), poly(C), and poly(dT), $(-\partial \log K_{\text{obs}}/\partial \log [K^+])/z$ decreases with increasing peptide charge.

Thermodynamics of Peptide Binding to Single- and Double-Stranded Polynucleotides are Dependent upon Peptide Charge for Oligolysines Containing a Single Tryptophan. The value of $\Delta H^\circ_{\text{obs}}$ is dependent upon the tryptophan content of the oligolysine, the peptide charge, and the polynucleotide type, although it is insensitive to the salt concentration. For oligolysines containing a single tryptophan, $\Delta H^\circ_{\text{obs}}$ becomes more positive with increasing z for poly(A) and poly(C), while the opposite is true for poly(U) and poly(dT). The dependence of $\Delta H^\circ_{\text{obs}}$ on peptide charge may reflect differences in the extent of base stacking within the ss polynucleotide–oligopeptide complexes, since it is known from calorimetric and spectroscopic studies that the base stacking within poly(A) and poly(C) is enhanced by the binding of magnesium and oligolysines with $z = +2$ or $+3$ (Willemsen & van Os, 1971; Krakauer, 1972; Durand *et al.*, 1975; Porschke, 1976, 1978).

The tryptophan fluorescence quenching of these oligopeptides upon binding ss polynucleotides is also dependent on nucleotide base composition and peptide charge. Q_{max} increases with increasing z for peptides with $z \leq +4$, but is independent of z for $z > +4$, upon binding poly(A), poly(U), and poly(dT). Similar correlations exist for the interaction of these same peptides with poly(A), poly(C), and poly(dT). In the ranges of z where Q_{max} is dependent on z , $\Delta H^\circ_{\text{obs}}$ is also dependent on z . These observations suggest that the mode of peptide binding to ss polynucleotides changes with net peptide charge up to $z = +4$.

Possible Origins of the Nonlinear Dependence of $|\partial \log K_{\text{obs}}/\partial \log [K^+]|$ on Peptide Charge. For the binding of oligopeptides ($z = +2$ to $+10$) to poly(A), poly(C), and poly(dT), the salt dependence, normalized per peptide charge, $(-\partial \log K_{\text{obs}}/\partial \log [K^+])/z$, decreases with increasing peptide charge, whereas this quantity is essentially constant for the same peptides binding to poly(U) (Mascotti & Lohman, 1992). Furthermore, the dependence of $(-\partial \log K_{\text{obs}}/\partial \log [K^+])/z$ on z differs depending on the particular ss polynucleotide. The difference in the modes of binding of peptides with $z \leq +4$, relative to those with $z > +4$, could contribute to the dependence of $|\partial \log K_{\text{obs}}/\partial \log [K^+]|/z$ on z ; however, the fact that we observe very little dependence of $-\partial \log K_{\text{obs}}/\partial \log [K^+]$ on z with poly(U) suggests that this may not be the explanation. Another possible explanation is that the extent of K^+ binding per phosphate may increase with increasing $[K^+]$ and this may be polynucleotide-specific. A third possibility is that preferential water release occurs upon formation of the peptide–polynucleotide complex and the extent of water release differs depending on the polynucleotide. These latter two possibilities are considered below.

Increase in the Fraction of Monovalent Counterions Bound to the Single-Stranded Polynucleotides with Increasing Salt Concentration. If the fraction of monovalent cations (K^+) bound per nucleic acid phosphate increases with increasing $[K^+]$, a nonlinear dependence of $\log K_{\text{obs}}$ on $\log [K^+]$ would result as indicated by eq 4. Although the extent of counterion binding to B-form ds-DNA appears to be constant over the range from 5 mM to 0.5 M NaCl (Anderson *et al.*, 1978), this has not yet been examined experimentally for ss polynucleotides. Therefore, this remains a possible explanation.

Preferential Hydration. Even if the extent of K^+ released per peptide charge is constant upon formation of a peptide–

polynucleotide complex, a decrease in $|\partial \log K_{\text{obs}}/\partial \log [M^+]|$ with z can still occur if a net release of H_2O accompanies formation of the oligopeptide–polynucleotide complex (*i.e.*, if $\Delta w < 0$ in eqs 4 and 5). Furthermore, the magnitude of this effect will increase with increasing $[M^+]$ (see eq 4 and Ha *et al.*, 1992). This effect is a direct consequence of the Gibbs–Duhem equation, reflecting the fact that the activity of water decreases as the salt concentration (activity) increases (Tanford, 1969; Record *et al.*, 1978). A plot of $\log K_{\text{obs}}$ vs $\log [K^+]$ for a single peptide may not display observable curvature since the range of salt concentration over which K_{obs} can be measured for a single peptide is narrow; however, this effect might be observable upon comparing the salt dependences of a series of peptides differing in z , since these will necessarily be measured over a wider range of salt concentrations. If this is the case, then peptides of higher charge (which are necessarily studied at higher $[M^+]$ in order to lower K_{obs} into a measurable range) would be expected to have a lower value of $|\partial \log K_{\text{obs}}/\partial \log [M^+]|/z$, which is the behavior shown in Figure 11.

In Figure 11, we have plotted $-\partial \log K_{\text{obs}}/\partial \log [K^+]$ as a function of z for the set of mono-Trp oligolysines binding to poly(U), poly(A), poly(dT), and poly(C). The dashed lines through the poly(A), poly(dT), and poly(C) data are linear least-square lines representing the data for the oligolysines with $z \leq +4$, constrained to intersect the origin. The dashed line through the data for poly(U) in Figure 11A represents the linear least-squares best fit for all points ($+2 \leq z \leq +10$), constrained to intersect the origin, since these data are linear within our uncertainty over the entire range of z . The slopes of the dashed lines shown in Figure 11 are listed in Table VII. The continuous lines in Figure 11 were simulated using eq 4 and the values of Δc and Δw listed in Table VII. The midpoint of the molar salt concentration range examined for each peptide was used to calculate the salt molality (m) in eq 4. In each case, use of eq 4 with the assumptions that Δc is constant for each peptide and bound water is released upon complex formation provides a good description of the data for each polynucleotide. However, these fits also require the assumption that Δw is dependent on peptide charge, z (see Table VII). With poly(U), the dependence of $-\partial \log K_{\text{obs}}/\partial \log [K^+]$ on z is nearly linear; therefore only a small value of Δw ($= -2z$) is needed to describe the data. For poly(A) and poly(dT), $\Delta w = -15z$, whereas for poly(C), $\Delta w = -25z$. Therefore, if preferential hydration is the cause of the decrease in $|\partial \log K_{\text{obs}}/\partial \log [K^+]|/z$ with increasing z , then the extent of water release upon binding an oligolysine decreases in the order poly(C) > poly(A) \sim poly(dT) > poly(U).

Contributions of Multiple Tryptophans to the Thermodynamics of Charged Oligopeptide–Single-Stranded Polynucleotide Interactions. We have examined the thermodynamics of binding oligolysines containing multiple tryptophans to poly(A) and poly(C) and compare the results with our previous studies of the same peptides interacting with poly(U) (Mascotti & Lohman, 1992). For these studies we used a series of oligolysines with a constant net charge of $z = +6$. In all cases, the salt dependence, $\partial \log K_{\text{obs}}/\partial \log [K^+]$, is independent of the number of tryptophans contained within the oligolysines, indicating that the presence of these tryptophans does not influence net cation or water release. However, the thermodynamics of oligopeptide binding is definitely influenced by the number of tryptophans contained within the oligolysine. For binding to each polynucleotide, we observe significant changes in $\Delta H^\circ_{\text{obs}}$ and $\Delta S^\circ_{\text{obs}}$; however, the enthalpy and entropy changes appear to compensate so

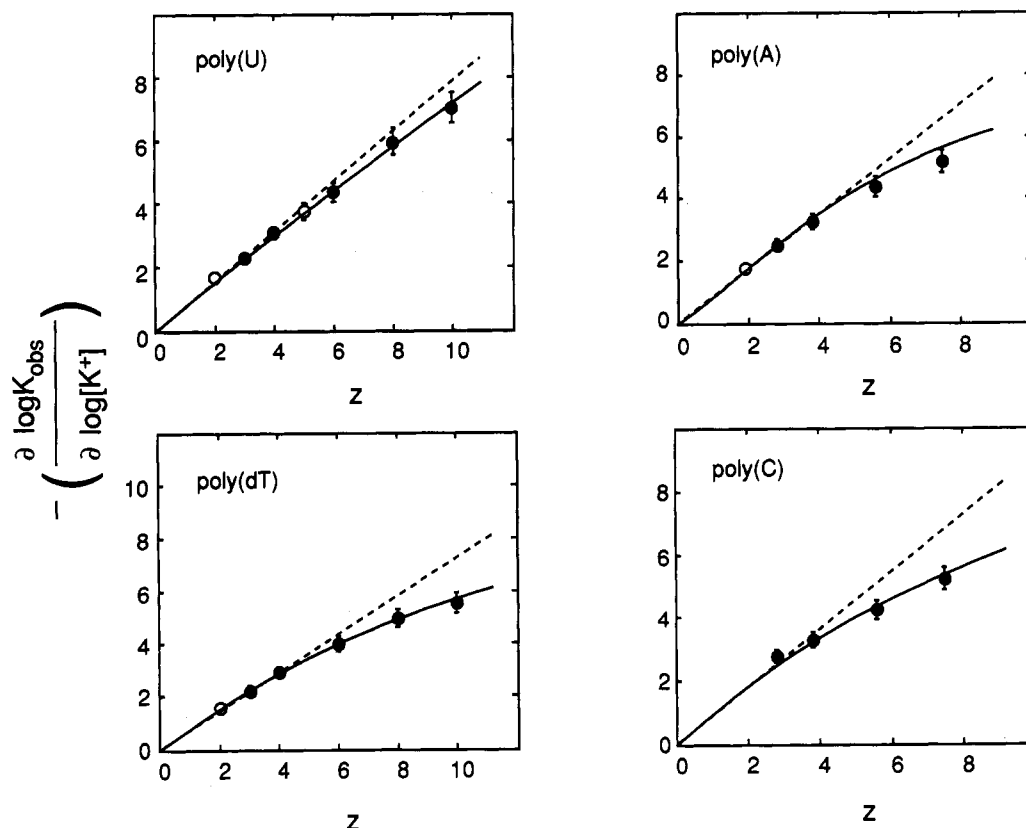


FIGURE 11: (A, top, left) Thermodynamic extent of ion release ($-\partial \log K_{\text{obs}}/\partial \log [K^+]$) plotted as a function of the net positive charge, z , on each oligolysine binding to poly(U) (Mascotti & Lohman, 1990). The dashed line drawn is a linear least-squares best-fit line to all of the data, constrained to intersect the origin, and is given by the equation $-\partial \log K_{\text{obs}}/\partial \log [K^+] = (0.74 \pm 0.04)z$. The solid line is a best fit to eq 4 using $\Delta w = -2z$, $\Delta c = 0.74z$, and the midpoint of the salt concentration range examined for each polynucleotide (Mascotti & Lohman, 1990). (B, top, right) Thermodynamic extent of ion release ($-\partial \log K_{\text{obs}}/\partial \log [K^+]$) plotted as a function of the net positive charge, z , on each oligolysine binding to poly(A). The value of z was calculated according to eq 7 of Mascotti & Lohman (1992) using the pK values reported in that paper. The dashed line drawn is a linear least-squares best-fit line to only the data for $z \leq +4$, constrained to intersect the origin, and is given by the equation $-\partial \log K_{\text{obs}}/\partial \log [K^+] = (0.90 \pm 0.07)z$. The solid line is a best fit to eq 4 using $\Delta w = -15z$, $\Delta c = 0.90z$, and the midpoint of the salt concentration range examined for each polynucleotide (Figure 1A). (C, bottom, left) Thermodynamic extent of ion release ($-\partial \log K_{\text{obs}}/\partial \log [K^+]$) is plotted as a function of the net positive charge, z , on each oligolysine binding to poly(dT). The dashed line drawn is a linear least-squares best-fit line to only the data for $z \leq +4$, constrained to intersect the origin, and is given by the equation $-\partial \log K_{\text{obs}}/\partial \log [K^+] = (0.76 \pm 0.07)z$. The solid line is a best fit to eq 3 using $\Delta w = -15z$, $\Delta c = 0.76z$, and the midpoint of the salt concentration range examined for each polynucleotide (Figure 3A). (D, bottom, right) Thermodynamic extent of ion release ($-\partial \log K_{\text{obs}}/\partial \log [K^+]$) plotted as a function of the net positive charge, z , on each oligolysine binding to poly(C). The value of z was calculated according to eq 7 of Mascotti & Lohman (1992) using the pK values reported in that paper. The dashed line drawn is a linear least-squares best-fit line to the data of $z \leq +4$, constrained to intersect the origin, and has a slope of 0.93 ± 0.07 . The solid line is a best fit to eq 4 using $\Delta w = -25z$, $\Delta c = 0.93z$, and the midpoint of the salt concentration range examined for each polynucleotide (Figure 2A).

Table VII: Calculation of Δw and Δc for Oligolysine–Polynucleotide Interactions^a

polynucleotide	Δw^b	Δc^b
poly(U)	$(-2 \pm 1)z$	$(0.74 \pm 0.04)z^c$
poly(A)	$(-15 \pm 5)z$	$(0.90 \pm 0.08)z$
poly(C)	$(-25 \pm 5)z$	$(0.93 \pm 0.07)z$
poly(dT)	$(-15 \pm 5)z$	$(0.76 \pm 0.07)z$

^a Buffer CB + KCH_3CO_2 , 25.0 °C, pH 6.0, for poly(dT) and poly(U); pH 7.0 for poly(A) and poly(C). ^b Parameters used in eq 4 to fit Figure 11. ^c Taken from Mascotti and Lohman (1992).

that $\Delta G^\circ_{\text{obs}}(K_{\text{obs}})$ changes very little with tryptophan content. The effect of the number of Trp residues is most complex for the interaction of these peptides with poly(C), since we observe a minimum in both ΔH° and ΔS° at $N = 2$ tryptophans, whereas for both poly(U) and poly(A), ΔH° and ΔS° decrease linearly with N .

Q_{max} is independent of the number of tryptophans, N , for the binding of oligolysines to poly(U) (Mascotti & Lohman, 1992), which suggests that each tryptophan interacts identically and independently with poly(U). However, for these same peptides binding to poly(A) and poly(C), Q_{max} is

dependent on N (see Tables V and VI), indicating that multiple Trp residues interact differently with poly(A) and poly(C) than does a single tryptophan, reflecting context-dependent effects.

Base Composition Dependence of Single-Stranded Polynucleotide Binding to Oligolysines Containing Tryptophan. We have compared the interactions of two peptides, $\text{KWK}_4\text{-NH}_2$ and KWK-CO_2 , with several ss homopolynucleotides in order to assess any dependence of binding on nucleotide base composition. These comparisons, shown in Figure 10, indicate the following hierarchy in order of decreasing affinity: poly(I) > poly(U) \sim poly(dU) \sim poly(dT) \sim poly(A) > poly(C). Poly(U), poly(A), poly(dU), and poly(dT) bind with essentially the same K_{obs} and salt dependence; however, a definite higher affinity is observed for poly(I), whereas a definite lower affinity is observed for poly(C).

The base specificity of Trp binding that is reflected in Figure 10 may partially explain the base specificities that have been observed in the equilibrium binding to ss nucleic acids of a number of ss-DNA binding proteins, such as *E. coli* SSB protein (Overman *et al.*, 1988) and the phage T4 gene 32 protein (Newport *et al.*, 1981). The *E. coli* SSB tetramer

contains 16 tryptophans and 16 tyrosines, although only eight tryptophans are believed to participate in stacking interactions with the ss polynucleotide (Khamis *et al.*, 1987a,b). Gene 32 protein contains five tryptophans per monomer, at least one of which interacts with the polynucleotide (Toulme & Helene, 1980). The following hierarchy of K_{obs} is observed for *E. coli* SSB protein binding ss polynucleotides: poly(dT) > poly(dC) > poly(I) > poly(U) > poly(dA) > poly(A) > poly(C) (Overman *et al.*, 1988). For T4 gene 32 protein, the hierarchy is poly(dT) > poly(dI) > poly(I) ~ poly(dU) > poly(dC) > poly(dA) > poly(A) > poly(U) > poly(C) (Newport *et al.*, 1981). The only general correlation among these data is that these proteins as well as the mono-Trp oligolysines bind with higher affinity to poly(I) and with lowest affinity to poly(C). The most notable difference is that the proteins bind with considerably higher affinity to poly(dT) than to poly(I), whereas the opposite is true for the mono-Trp oligolysines. This discrepancy may reflect the fact that the SSB and gene 32 protein binding studies were both conducted at salt concentrations in excess of 0.2 M (Overman *et al.*, 1988; Newport *et al.*, 1981), which are conditions that favor the formation of triple-stranded helices in poly(I) (Thiele & Guschlbauer, 1973). Assuming the triple-stranded structures do not bind to these proteins, this would lower the apparent affinity, possibly inverting the affinity of these proteins for poly(I) vs poly(dT). These few correlations suggest the possibility that some component of this base specificity is due to tryptophan interactions, although it is clear that the specificities are not due entirely to tryptophan interactions. The phage fd gene V protein also displays base composition dependence in its equilibrium binding to ss polynucleotides (Sang & Gray, 1989; Porschke & Rauh, 1983): poly(dT) > poly(dU) > poly(dI) ~ poly(I) > poly(U) > poly(A) ~ poly(dC) ~ poly(dA) > poly(C) (Sang & Gray, 1989; Alma *et al.*, 1983; Porschke & Rauh, 1983; Bullsink *et al.*, 1985); however, the gene V protein does not contain tryptophan, although it does contain tyrosine.

It is clear that even the relatively simple oligopeptides examined here show complex thermodynamics in their interactions with ss polynucleotides. The interactions of these peptides with some ss polynucleotides show clear context dependence, whereas interactions appear to be context-independent for others (e.g., poly(U)). In any case, these systematic thermodynamic studies of model peptide systems provide an important set of reference data against which thermodynamic studies of proteins binding to ss polynucleotides can be compared.

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