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Pentalysine–Deoxyribonucleic Acid Interactions: A Model for the General Effects of Ion Concentrations on the Interactions of Proteins with Nucleic Acids†

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ABSTRACT: The interaction of the oligopeptide pentalysine [$(Lys)_5$] with T7 DNA has been investigated by difference boundary sedimentation velocity. The system is of interest as a model for the electrostatic component of the interactions of proteins with nucleic acids. Binding constants for $(Lys)_5$–T7 DNA (K_{obsd}^{LD}) have been determined as a function of [NaCl], [$MgCl_2$], pH, and temperature, under solution conditions such that K_{obsd}^{LD} is in the range 10^2–10^5 M^{-1}. We find that K_{obsd}^{LD} is a sensitive function of the ionic environment but is virtually independent of temperature. In particular, K_{obsd}^{LD} decreases dramatically with increasing [NaCl]. Plots of log K_{obsd}^{LD} vs. log [NaCl] are linear, with slopes that decrease with increasing pH. At constant [NaCl], K_{obsd}^{LD} decreases with an increase in pH or in [$MgCl_2$]. The data, analyzed by binding theory [Record, M. T., Jr., Lohman, T. M., & deHaseth, P. L. (1976) *J. Mol. Biol. 107*, 145–158; Record, M. T., Jr., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103–178] are consistent with an electrostatic interaction between $(Lys)_5$ and T7 DNA, driven by the entropic contribution of counterion release to the free energy of binding. The effects of pH are explained quantitatively by using a simple titration model; as the pH is increased, the net positive charge on the $(Lys)_5$ is reduced, and consequently both K_{obsd}^{LD} and $|(\partial \log K_{obsd}/\partial \log [NaCl])_{pH,T}|$ are reduced. The effects of $MgCl_2$ are explained quantitatively as a competition between Mg^{2+} and $(Lys)_5$ for DNA sites. The lack of a temperature dependence of K_{obsd}^{LD} is consistent with the proposed entropic origin of the binding free energy. The effects of small ions on the $(Lys)_5$–T7 DNA binding equilibrium are similar to those observed in various specific and nonspecific protein–DNA interactions and thereby support the electrostatic interpretation of those binding data given previously. Using the $(Lys)_5$ and other oligopeptide binding data as points of reference, one can decompose observed binding free energies for protein–DNA interactions into electrostatic and nonelectrostatic contributions, distinguish between specific and nonspecific effects of ions on complex formation, and estimate the number of positive charges and the number and nature of titratable groups on the DNA binding site of the protein.

At the molecular level, control of the expression of genetic information resides in the interactions of regulatory proteins and of RNA polymerase with specific and nonspecific sites on DNA [cf. von Hippel & McGhee (1972), Chamberlin (1976), and von Hippel (1979)]. It is reasonable to assume that the same region on the protein is involved in forming both specific and nonspecific complexes with DNA. [Competition experiments suggest that this is the case for *lac* repressor (Lin & Riggs, 1972, 1975) and RNA polymerase (Strauss et al., 1980a,b).] Consequently, the thermodynamic analysis of nonspecific binding is useful in understanding the molecular basis of specificity and the contributions of various noncovalent

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interactions to the stability of the specific complex.

The specific and nonspecific interactions of *Escherichia coli lac* repressor and *E. coli* RNA polymerase with double-helical DNA have been investigated as a function of such variables as temperature, pH, and ion concentrations (Riggs et al., 1970a,b; Lin & Riggs, 1972; Hinkle & Chamberlin, 1972a,b; Revzin & von Hippel, 1977; deHaseth et al., 1977a,b, 1978; Record et al., 1977, 1978; Lohman et al., 1980; Strauss et al., 1980a,b). Both proteins exhibit dramatic dependences of their specific and nonspecific binding constants (K_{obsd}) on the monovalent salt concentration. Analysis of these extremely steep salt dependences, which potentially are of biological significance, provides information about the number of cationic groups on the protein that interact with DNA phosphates in each complex and the ionic and nonionic contributions to the observed binding free energy (Record et al., 1976, 1978). The entropic contribution to the free energy of interaction from the release of low molecular weight ions accounts for the increase in the stability of these complexes with decreasing salt concentration. Determination of the amount of ion release from the dependence of K_{obsd} on the concentration of monovalent salt allows one to estimate the number of cationic residues and phosphates that interact in the complex, since the neutralization of these charges on the reactants by complex formation releases the electrolyte ions previously associated with them [cf. Record et al. (1976, 1978) and Manning (1978)]. Comparisons between the specific and nonspecific complexes of *lac* repressor and RNA polymerase with DNA have been made by Record et al. (1977) and Strauss et al. (1980b), respectively.

The experiments presented here probe the equilibrium properties of pentalysine [$(Lys)_5$]–DNA complexes. Observed binding constants (K_{obsd}^{LD}) for the interaction have been obtained by a difference boundary sedimentation velocity technique (Lohman et al., 1980). We have studied the effects of monovalent and divalent cations, temperature, and pH on the $(Lys)_5$–DNA interaction. We believe that the $(Lys)_5$–DNA interaction provides a model for the electrostatic component of protein–DNA interactions. Complications arising from the details of the geometry of the binding site on the protein, the interactions of the protein with low molecular weight ions, and the difference between the net charge on the protein and the charge on its DNA binding site are less severe for $(Lys)_5$. Effects of environmental variables (T, pH, and electrolyte concentration) on K_{obsd} for the $(Lys)_5$–DNA interaction should provide a standard for comparison of binding data obtained with various proteins and DNA.

The only extensive data previously available on K_{obsd} as a function of monovalent salt concentration, $[M^+]$, for a simple charged oligopeptide system are those of Latt & Sober, (1967a,b), who measured K_{obsd} for a series of oligolysines $(Lys)_N$-ϵ-DNP-Lys ($3 \leq N \leq 8$) by equilibrium dialysis. These data provide a calibration of the difference sedimentation technique for studying the interaction of $(Lys)_5$ with helical nucleic acids. The experiments reported here extend the range of solution conditions examined by Latt & Sober (1967a). From them, a clear picture emerges of the thermodynamics of a nonspecific (electrostatic) ligand–DNA interaction. However, the molecular details of this interaction are still not clearly understood.

Theory

A detailed discussion of the origins and interpretations of ion effects on the interactions of proteins and nucleic acids is given in a recent review (Record et al., 1978). Here we outline the important results of this analysis.

(1) The binding constant K_{obsd}^{LD} for the nonspecific interaction of a ligand (L) with any nucleotide site on the DNA (D) in the limit of zero binding density is equal to the binding constant for the interaction of a single ligand with a DNA molecule to form a 1:1 complex (LD). Consequently, the observed reaction may be written as

$$L + D \overset{K_{obsd}^{LD}}{\rightleftharpoons} LD \tag{1}$$

where $K_{obsd}^{LD} \equiv [LD]/([L][D])$ (brackets representing concentrations).

(2) Typically, K_{obsd}^{LD} is a strong function of electrolyte concentration (M^+; X^-). In dilute electrolyte solution, this dependence results primarily from the direct stoichiometric participation of electrolyte ions (principally M^+) in the reaction and secondarily from the dependence of the various activity coefficients on the ionic strength. To a good approximation, the molecular reaction corresponding to eq 1 for the localized binding of a Z-valent ligand L which interacts with Z phosphates on D, releasing (in the thermodynamic sense described below) $Z\psi$ cations, is

$$L + D \overset{K^0}{\rightleftharpoons} LD + Z\psi M^+ \tag{2}$$

where K^0 is the equilibrium constant written in terms of concentrations of all species in eq 2. Equation 2 neglects differential titration effects and the possible release of anions from L. The quantity ψ is defined as the fraction of a counterion (M^+) thermodynamically associated with the DNA per phosphate; ψ contains contributions from condensed (Oosawa, 1971; Manning, 1969, 1978) and screening ions. To a good approximation, ψ is a function only of the axial charge density of the nucleic acid and is independent of the added salt concentration. For double-stranded DNA, $\psi = 0.88$ (Record et al., 1976, 1978).

(3) From the above definitions of K_{obsd}^{LD} and K^0, one obtains

$$\log K_{obsd}^{LD} = \log K^0 - Z\psi \log [M^+] \tag{3a}$$

and

$$-\frac{d \log K_{obsd}^{LD}}{d \log [M^+]} = Z\psi \tag{4a}$$

The derivative $-d \log K_{obsd}^{LD}/d \log [M^+]$ is a measure of the stoichiometric coefficient of M^+ in the approximate reaction 2; that is, it is a measure of the amount of ion release in the reaction (Record et al., 1976, 1978). If k anions are released from L in the formation of the LD complex, then (for the case where the only electrolyte ions present are M^+ and X^-) eq 3a and 4a are replaced by

$$\log K_{obsd}^{LD} = \log K^0 - (Z\psi + k) \log [M^+] \tag{3b}$$

and

$$-\frac{d \log K_{obsd}^{LD}}{d \log [M^+]} = Z\psi + k \tag{4b}$$

In eq 3b, K^0 is defined to include the anion participant. For oligoelectrolyte ligands like Lys_5, studied at relatively low electrolyte concentrations, anion effects are not expected to be very important (see below). In situations where anion effects are negligible (or where k is constant), the fact that ψ is independent of electrolyte concentration indicates that log K_{obsd}^{LD} (cf. eq 3a) will be a linear function of log $[M^+]$. Furthermore, since ψ is known from the axial charge density of the nucleic acid (and from comparison of eq 3a with binding data on ligands of known Z), Z for the binding site of an unknown ligand can be estimated from the slope of a plot of

log K_{obsd}^{LD} vs. log [M^+] (Record et al., 1976, 1978).

An alternative, delocalized model for the binding of a Z-valent cationic ligand has been discussed by Manning (1978). Here the ligand is assumed to replace Z M^+ ions in the condensation layer about the polyanion. Consequently, Manning's model yields a stoichiometric coefficient of Z instead of $Z\psi$ for the M^+ term in eq 2 and 3a. Although the molecular difference between the two models is significant, the difference at the thermodynamic level is as yet too small to detect (12% in the coefficient of the log [M^+] term in eq 3a for helical DNA).

(4) Analysis of the data of Latt & Sober (1967a) for the interactions of oligolysines (chain length between three and eight residues) with polyribonucleotides indicated that K^0 is of order unity (log $K^0 \sim 0$) for a purely electrostatic (nonspecific) interaction (Record et al., 1976). [Manning (1978) has demonstrated that this result is expected for the delocalized binding of a cationic ligand to a polyanion.] Consequently, K_{obsd}^{LD} for a purely electrostatic interaction is expected to be of order unity at salt concentrations approaching 1 M; the binding reaction occurs only at lower salt concentration, as a consequence of the entropic contribution to the binding free energy (a free energy of dilution $\Delta G_{dil} = Z\psi RT \ln [M^+]$) of counterions released into the solution at the lower salt concentration.

(5) There is competition between Mg^{2+} and $(Lys)_5$ for sites on the DNA. Experiments on the $(Lys)_5$–T7 DNA interaction were also carried out in buffers containing mixtures of NaCl and $MgCl_2$ in order to investigate the effects on K_{obsd}^{LD} of the competition between Mg^{2+} and $(Lys)_5$ for sites on the DNA. Record et al. (1977, 1978) used binding theory (Schellman, 1974, 1975) to analyze the competitive effects of Mg^{2+} on the nonspecific *E. coli lac* repressor–DNA interaction in mixed NaCl–$MgCl_2$ buffers. They provide an expression which quantitatively accounts for the effect of Mg^{2+} on that interaction (neglecting anion effects):

$$\log K_{obsd} = \log K^0 - Z\psi \log [M^+] - Z \log \frac{[D]}{[D_0]} \quad (5)$$

where

$$\frac{[D]}{[D_0]} = \frac{1}{2}[1 + (1 + 4K_{obsd}^{Mg}[Mg^{2+}])^{1/2}] \quad (6)$$

and K_{obsd}^{Mg} is the observed binding constant for the Mg^{2+}–DNA interaction. $[D_0]/[D]$ represents the probability of finding a nucleotide which has only its complement of M^+ associated with it (and no Mg^{2+}); [D] is the total nucleotide concentration, and $[D_0]$ is the concentration of those nucleotides which are in the same state as they would be in the presence of only M^+ and X^-. In the presence of only counterions of the type M^+, $[D] = [D_0]$ and eq 5 reduces to eq 3a. In the presence of Mg^{2+}, $[D]/[D_0] > 1$ and increases with increasing [Mg^{2+}] and/or decreasing [M^+]. Qualitatively, the addition of Mg^{2+} shifts reaction 2 to the left and decreases K_{obsd}^{LD} by reducing the concentration of DNA sites which have only M^+ associated with them (Record et al., 1977, 1978).

Equation 5 (with the expression for $[D]/[D_0]$ given in eq 6) does not specify the distribution of the Z phosphates on the DNA with which the ligand, L, interacts. Lys_5, for example, may neutralize Z contiguous phosphates since it is effectively a string of contiguous positive charges. An alternate expression to eq 5 in a mixed M^+–Mg^{2+} buffer can be obtained for a site of Z contiguous phosphates having only M^+ associated with them through application of the procedure of McGhee & von Hippel (1974). One calculates the probability that given one nucleotide free of Mg^{2+}, the next $(Z - 1)$ consecutive nucleotides will also be free of Mg^{2+}. The result is (C. F. Anderson, personal communication)

$$\log K_{obsd} = \log K^0 - Z\psi \log [M^+] - \log \{(1 + 4K_{obsd}^{Mg^{2+}}[Mg^{2+}])^{1/2}[(1/2)[1 + (1 + 4K_{obsd}^{Mg^{2+}}[Mg^{2+}])^{1/2}]]^{Z-1}\} \quad (7)$$

For any of the protein– or ligand–DNA interactions which we have investigated [including $(Lys)_5$], eq 5 and 7 (although formally different) yield numerical results which are essentially identical.

(6) There are effects of titration of $(Lys)_5$ on K_{obsd}^{LD}. Although a dependence of K_{obsd}^{LD} on pH can arise, in principle, from titration of either the ligand or the nucleic acid, we consider only the ligand since helical DNA should not undergo titration in the pH range of interest (pH 6–9). There are a number of possible ways in which titration of a ligand, L, can affect the binding interaction between L and the nucleic acid (Record et al., 1978). One possibility is that protonation of the ligand may be required for it to bind to the nucleic acid. This model has been applied by deHaseth et al. (1977b) to interpret pH effects on the nonspecific interactions of *E. coli lac* repressor with helical DNA. In that system, it appears that protonation of two amino acid residues is required for binding.

The pH dependence of the $(Lys)_5$–DNA interaction does not fit the above model which requires protonation. It can, however, be described by a model (the titration curve model) in which protonation facilitates binding of $(Lys)_5$ to DNA by increasing the net positive charge on the pentalysine. This increases its binding affinity by increasing the number of electrostatic interactions and the number of counterions released from the DNA upon complex formation.

The effect of titration of the ligand is introduced by letting Z be a function of pH. In the simplest case, we let

$$Z = Z_0 - \sum_{i=1}^{r}(1 - \bar{\theta}_{i,L}) \quad (8)$$

where Z_0 is the maximum net number of positive charges on the binding site of the ligand at the low pH end point of the titration, r is the number of titratable groups in the binding site of the ligand, and $1 - \bar{\theta}_{i,L}$ is the average fractional extent of deprotonation of the ith group on the ligand. For i independent titratable groups (characterized by equilibrium constants for protonation $k_{i,L}$)

$$1 - \bar{\theta}_{i,L} = (1 + k_{i,L}a_{H^+})^{-1} \quad (9)$$

where a_{H^+} is the proton activity. Therefore, from eq 3a, 8 and 9, neglecting anion effects and the small (experimentally negligible) dependence of K^0 on Z

$$\log K_{obsd}^{LD} = \log K^0 - [Z_0 - \sum_{i=1}^{r}(1 + k_{i,L}a_{H^+})^{-1}]\psi \log [M^+] \quad (10)$$

The dependences of log K_{obsd}^{LD} on log [M^+], pH, and T are readily obtained from eq 10 and are given in eq 11, 12, and 13:

$$-\left(\frac{\partial \log K_{obsd}^{LD}}{\partial \log [M^+]}\right)_{pH,T} = [Z_0 - \sum_{i=1}^{r}(1 + k_{i,L}a_{H^+})^{-1}]\psi \quad (11)$$

$$\left(\frac{\partial \log K_{obsd}^{LD}}{\partial \text{ pH}}\right)_{T,[M^+]} = \sum_{i=1}^{r}\frac{k_{i,L}a_{H^+}}{(1 + k_{i,L}a_{H^+})^2}\psi \log [M^+] \quad (12)$$

$$\left(\frac{\partial \log K_{obsd}^{LD}}{\partial\, 1/T}\right)_{pH,[M^+]} = \left(\frac{\partial \log K^0}{\partial\, 1/T}\right)_{pH,[M^+]} - \psi \log [M^+] \sum_{i=1}^{r} \frac{k_{i,L} a_{H^+}}{(1 + k_{i,L} a_{H^+})^2} \frac{\partial \log k_{i,L}}{\partial\, 1/T} \quad (13)$$

From eq 13, one obtains the observed enthalpy change ΔH°_{obsd} for the association of L with D as

$$\Delta H^\circ_{obsd} = \Delta H^\circ - \psi \log [M^+] \sum_{i=1}^{r} \frac{k_{i,L} a_{H^+}}{(1 + k_{i,L} a_{H^+})^2} \Delta h_{i,L} \quad (14)$$

where $\Delta h_{i,L}$ is the enthalpy of protonation of the ith group on L and ΔH° is the intrinsic enthalpy change upon interaction of L with D (at low pH and/or high salt).

Equations 10–14 predict the general behavior expected for binding processes following the titration curve model.

(a) Log K_{obsd}^{LD} will be a linear function of log $[M^+]$ at constant pH, with a slope which decreases in magnitude with increasing pH and an intercept which is independent of pH. Therefore, plots of log K_{obsd}^{LD} vs. log $[M^+]$ should converge at high salt. [For the model in which titration is a prerequisite for binding, log K_{obsd}^{LD} is also predicted to be a linear function of log $[M^+]$ at constant pH, but in this case the intercept decreases with increasing pH and the slope is independent of pH [cf. deHaseth et al. (1977b) and Record et al. (1978).]

(b) K_{obsd}^{LD}, although in general a function of pH, will become invariant to pH at low pH (where all r groups are protonated), at high pH (where all r groups are deprotonated), and at high salt (since the dependences of K_{obsd}^{LD} on pH and salt in the titration curve model are coupled). (By contrast, in the model in which titration is required, the binding constant will not become independent of pH at high pH or high salt.)

(c) ΔH°_{obsd} will be relatively insensitive to pH, except at very low salt, and generally will be dominated by the intrinsic binding enthalpy ΔH°. (In the model in which titration is required, ΔH°_{obsd} is predicted to be a sensitive function of pH and can be dominated by the enthalpy of titration of groups on the ligand.)

The general difference between the titration curve model described above and the model in which protonation is necessary for the association of L with D is that the magnitude of proton titration effects is a function of salt concentration in the titration curve model. Consequently, one can determine which model describes the system better by examining the effects of salt, pH, and temperature on K_{obsd}^{LD}. The results of these studies on the interaction of $(Lys)_5$ with DNA are described below.

Materials and Methods

(*a*) *Reagents.* All chemicals were reagent grade. All solutions were prepared with doubly distilled deionized water.

(*b*) *Buffers.* The following buffers were used in this work. Buffer C (pH 6.05) is 5 mM sodium cacodylate and 5 mM cacodylic acid. Buffer P (pH 6.8) is 5 mM $Na_2HPO_4 \cdot 7H_2O$ and 5 mM $NaH_2PO_4 \cdot H_2O$. Buffer P (pH 7.6) is 10 mM $Na_2HPO_4 \cdot 7H_2O$ titrated with concentrated H_3PO_4. Buffer B is 10 mM $Na_2B_4O_7 \cdot 10H_2O$ and 0.1 mM Na_3EDTA, titrated to pH 8.4 or 8.8 with concentrated HCl. Buffer T (pH 7.5) is 10 mM Tris [tris(hydroxymethyl)aminomethane] titrated with concentrated HCl.

(*c*) *DNA and Pentalysine.* The preparation of intact T7 DNA for these experiments is described in the preceding paper (Lohman et al., 1980). Crystalline pentalysyl pentaacetate was purchased from Bachem, Inc. (Marina del Rey, CA). According to the manufacturer, the $(Lys)_5$ was judged to be homogeneous by electrophoresis and thin-layer chromatography. A molecular weight of 658 and a partial specific volume of 0.82 cm^3/g were used in the calculations for the $(Lys)_5$ cation.

(*d*) *Sedimentation Technique.* The $(Lys)_5$–T7 DNA equilibrium was investigated by using a difference boundary sedimentation velocity technique which has been described in the preceding paper (Lohman et al., 1980). It is an application of the difference sedimentation methods developed by Schachman and associates (Schachman, 1959; Richards & Schachman, 1959; Steinberg & Schachman, 1966).

One obtains the ratio of the sedimentation coefficients of the $(Lys)_5$–T7 DNA complex, S_B, to that of the T7 DNA, S_0, which can be related to the binding density, ν (moles of bound Lys_5 per mole of nucleotide) (Jensen & von Hippel, 1976; Lohman et al., 1980):

$$\frac{S_B}{S_0} - 1 = \nu \frac{M_L(1 - \bar{v}_L \rho)}{M_D(1 - \bar{v}_D \rho)} \quad (15)$$

where M is molecular weight, $\bar{v}$ is partial specific volume, ρ is the solution density, and the subscripts L and D refer to $(Lys)_5$ and NaDNA nucleotides, respectively. Equation 15 assumes additivity of partial specific volumes upon formation of the equilibrium complex and that the frictional coefficient of the T7 DNA does not change when $(Lys)_5$ binds. The applicability of the latter assumption to the $(Lys)_5$–DNA binding equilibrium is discussed below. Moreover, eq 15 neglects various thermodynamic complexities of the actual multicomponent system, which may become important when the molecular weight of the electrolyte is not negligible in comparison with that of the ligand (Eisenberg, 1976). In view of these assumptions, the method was calibrated as described below.

From the value of ν, the observed binding constant for association is calculated as

$$K_{obsd}^{LD} = \frac{1}{[L]}(1/\nu - n)^{-1} \quad (16)$$

where [L] is the free Lys_5 concentration and n is the number of nucleotides occluded by the $(Lys)_5$ when it is bound to the DNA. We have used $n = 6$ for Lys_5 based on the analysis, by McGhee & von Hippel (1974), of the equilibrium dialysis experiments of Latt & Sober (1967a) on the binding of $(Lys)_N$-ε-DNP-Lys to poly(rA)·poly(rU), where N varied from 3 to 8.

Results and Discussion

In our application of the difference boundary sedimentation velocity technique to measure binding constants of nonspecific protein–nucleic acid interactions, binding densities such that less than 1% of the DNA was covered by protein were routinely used (Lohman et al., 1980). Comparison of binding constants measured in this way for the nonspecific interactions of RNase and *lac* repressor with double-stranded DNA with those of Jensen & von Hippel (1976) and Revzin & von Hippel (1977) indicated that the only detectable effect of ligand binding at low binding density was on the buoyant mass of the complex and not on the frictional properties of the flexible DNA coil (Lohman et al., 1980).

The binding of a $(Lys)_5$ molecule, however, has a much smaller effect on the mass of the complex than does the binding of a protein. Consequently, binding densities of $(Lys)_5$ such that ~30% of the DNA nucleotides were covered had to be used in this work in order to detect the effect of the ligand on the sedimentation coefficient of the DNA. This made it necessary to examine the effects of binding density on the

Table I: $(Lys)_5$-T7 DNA Binding Parameters[a]

[Na^+] (M)	$S_B/S_0 - 1$	$\nu \times 10^2$	[DNA] $\times 10^5$ (M) in nucleotides	$(Lys)_5 \times 10^6$ (M) bound	$(Lys)_5 \times 10^6$ (M) free	log K_{obsd}
0.091	0.061	7.6	4.80	3.65	4.34	4.51
0.110	0.047	5.9	2.44	1.44	4.66	4.30
0.125	0.036	4.5	4.67	2.10	9.6	3.81
0.140	0.051	6.4	4.48	2.87	25.1	3.62
0.150	0.030	3.8	2.37	0.90	14.0	3.54
0.160	0.024	3.0	4.47	1.34	22.0	3.22
0.190	0.020	2.5	4.24	1.06	38.6	2.88

[a] Conditions: buffer C; pH 6.0; 20 °C.

calculation of binding constants and to calibrate the technique by comparison with other studies.

Binding constants K_{obsd}^{LD} were measured as a function of the apparent binding density ν (calculated from the sedimentation data) under two sets of solution conditions (buffer T, 0.1 M Na^+; buffer C, 0.12 M Na^+). In each case, no significant dependence of the calculated value of K_{obsd}^{LD} on ν was obtained over the range studied ($0.016 \leq \nu \leq 0.11$, corresponding to saturation of from 10 to 66% of the DNA phosphates). Within experimental error (±50% in K_{obsd}^{LD}; ±10% in log K_{obsd}^{LD}), K_{obsd}^{LD} was found to be independent of ν. Since the site exclusion effect (McGhee & von Hippel, 1974) should reduce K_{obsd}^{LD} by approximately a factor of 4 over this range of binding densities, it appears likely that a compensation is occurring between site exclusion and a decrease in the frictional coefficient of the complex with increasing ν. In the experiments reported below, the range of binding densities $0.02 \leq \nu \leq 0.08$ was used, and no corrections to zero binding density were attempted. In view of the additional assumptions required to apply the difference sedimentation method to the binding of $(Lys)_5$ to DNA, it was necessary to calibrate the technique. This could be done by comparison with the data of Latt & Sober (1967a) for the binding of $(Lys)_5$-ε-DNP-Lys to synthetic helical polyribonucleotides, obtained at pH 7.0, 4 °C, by equilibrium dialysis. We find that K_{obsd}^{LD} is essentially independent of temperature but dependent upon pH (see below). At pH 7 and 20 °C, were estimate by interpolation that log $K_{obsd}^{LD} = 3.8 \pm 0.2$ and $-(\partial \log K_{obsd}^{LD}/\partial \log [Na^+])_{pH,T} = 5.0 \pm 0.5$. For comparison, after correcting the data of Latt & Sober (1967a) for the interaction of the ε-DNP group (Record et al., 1976), we obtain log $K_{obsd} = 3.6$ [for $(Lys)_5$-poly(A)·poly(U)] and log $K_{obsd} = 3.9$ [for $(Lys)_5$-poly(I)·poly(C)]. In both cases, $-(\partial \log K_{obsd}/\partial \log [Na^+])_{pH,T} = 4.4 \pm 0.4$. The agreement in both log K_{obsd} and its derivative is within experimental uncertainty, and we conclude that the difference sedimentation method, although in part empirical as applied to this system, yields good estimates of K_{obsd}^{LD}.

(1) Dependence of K_{obsd}^{LD} on Monovalent Salt Concentration in the Low pH Limit. K_{obsd}^{LD} was determined by the difference sedimentation technique as a function of NaCl concentration in buffer C at pH 6.05 and 20 °C. Table I summarizes the data obtained. Under the binding conditions employed here, Lys_5 is fully protonated with a net charge of +5. The data of Table I are plotted in Figure 1, which shows that log K_{obsd}^{LD} is a linear function of log [NaCl]. The least-squares fit to the data points is given by

$$-\log K_{obsd}^{LD} = (5.3 \pm 0.5) \log [NaCl] + (0.9 \pm 0.3) \quad (17)$$

Comparison of eq 16 with eq 3a or 3b indicates that 5.3 ± 0.5

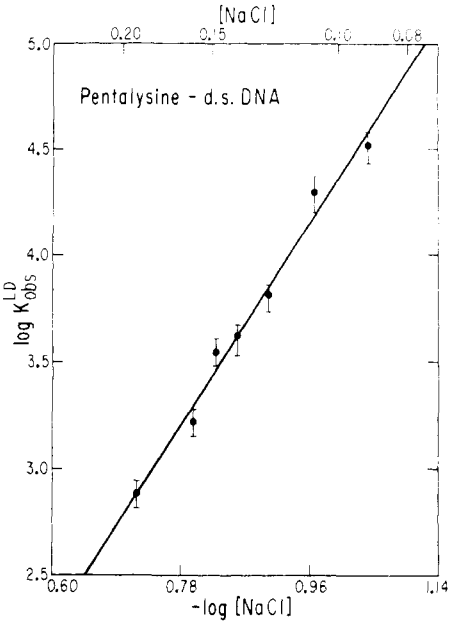

FIGURE 1: Dependence of the observed pentalysine–T7 DNA binding constant on [NaCl] (buffer C; pH 6.05; 20.0 °C).

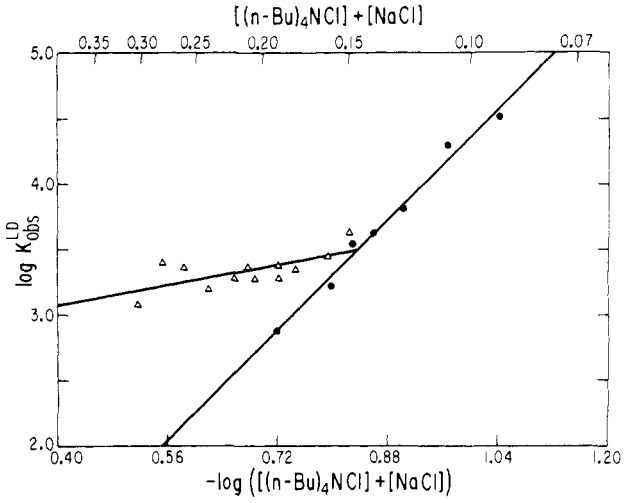

FIGURE 2: Dependence of the observed pentalysine–T7 DNA binding constant on [(n-Bu)$_4$NCl] (buffer C; pH 6.05; 20.0 °C). (△) Variable [(n-Bu)$_4$NCl] in constant (0.15 M) NaCl; (●) variable [NaCl], data from Figure 1.

electrolyte ions are released (in the thermodynamic sense) upon formation of the LD complex. Since the contribution of cation release from the DNA to this total is expected to be the range 4.4–5.0 (see above), it appears that the contribution of anion release from the $(Lys)_5$ is small. Two sets of experiments were carried out to investigate further the role (if any) of anions.

(a) From competition experiments using ^{23}Na NMR, Anderson et al. (1978) estimated that the tetra-n-butylammonium cation (n-Bu)$_4N^+$ has an affinity for double-helical DNA which is ~20-fold less than that of Na^+. We have measured K_{obsd}^{LD} as a function of [(n-Bu)$_4$NCl] at a fixed [NaCl]. The extent of Na^+ condensation should be only slightly reduced by addition of (n-Bu)$_4$NCl. The major effect of (n-Bu)$_4$NCl upon the DNA should be on the screening of the electrostatic interactions between DNA phosphates. Addition of (n-Bu)$_4$NCl therefore provides a way to increase [Cl^-] without substantially affecting the extent of Na^+ condensation along the DNA. If there were appreciable binding of Cl^- to $(Lys)_5$, K_{obsd}^{LD} should be affected by the increase in [Cl^-]. The results

of this experiment are shown in Figure 2. The behavior of K_{obsd}^{LD} as a function of [NaCl] is shown for comparison. The experiments were done at constant [NaCl] (0.15 M) and variable [$(n$-Bu)$_4$NCl], up to 0.155 M. From Figure 2 one observes that K_{obsd}^{LD} changes only slightly as the [$(n$-Bu)$_4$NCl] is increased. The slope of the least-squares straight line in the mixed system is -1.0 ± 0.3. This slope can be explained by the increase in ionic strength and its effect on the screening of the DNA phosphates. If the decrease in log K_{obsd}^{LD} as a function of log ([$(n$-Bu)$_4$NCl] + [NaCl]) is due only to screening interactions, the slope should be approximately $-0.12Z$ or 0.6 (Record et al., 1976, 1978; see below) which is in reasonable agreement with the observed value. Alternatively, the slope can be interpreted to indicate the release of up to one chloride ion (if differential screening effects are negligible) when $(Lys)_5$ binds to DNA at pH 6.05. Consequently, at least 80% of the low molecular weight ions released in the binding reaction originate from the DNA.

The second experiment performed to investigate the anion binding properties of $(Lys)_5$ was the measurement of K_{obsd}^{LD} as a function of [CH_3CO_2Na] to compare with the behavior in [NaCl] (data not shown). Within experimental error, there is no detectable difference in the stability of the $(Lys)_5$–DNA complex when the acetate ion is substituted for the chloride ion. If $(Lys)_5$ did bind anions, one might expect a difference in binding affinity between Cl^- and $CH_3CO_2^-$ which would result in a shift in K_{obsd}^{LD} upon substitution of one anion for the other. For example, deHaseth et al. (1977b) found that the nonspecific binding constant for the interaction of *lac* repressor with DNA was 40 times larger in sodium acetate than in sodium chloride at the same [Na^+]. However, since Cl^- and $CH_3CO_2^-$ occupy neighboring positions in the Hofmeister series [cf. Record et al. (1978)], one cannot rule out the possibility that these anions bind to an equal extent to $(Lys)_5$.

If the data discussed above were of higher accuracy, it would be possible to use them to distinguish between the localized and delocalized models of $(Lys)_5$ binding and to determine the precise roles of anion effects and differential screening effects on the thermodynamics of the reaction. Although these questions cannot be answered quantitatively at present, we can conclude from the data that cation release is the dominant feature in the interaction of $(Lys)_5$ with DNA. The binding reaction is driven at low salt concentrations predominantly by cation release; at high salt concentrations where the entropic contribution of cation release to the observed free energy of binding is less, the reaction does not occur to an appreciable extent. From the amount of cation release, we can obtain a reasonable estimate of the valence of the ligand binding site. Neglecting anion release and assuming the localized binding model upon which eq 4a is based, we obtain $Z = 6.0 \pm 0.6$. The deviation of this value from the expected valence (+5) may be caused by a small amount of anion release, by systematic sources of experimental error, and/or by a deficiency in the model. If we neglect anion release and apply the delocalized binding model of Manning (1978), we obtained a valence of 5.3 ± 0.5, in agreement with the expected result.

The intercept of eq 18 (log $K^0 = -0.9 \pm 0.3$) is related to the intrinsic free energy of formation of five lysine–phosphate interactions. From this quantity, we estimate the intrinsic free energy of a lysine–phosphate interaction to be $\sim 0.2 \pm 0.1$ kcal. Record et al. (1976, 1978) obtained a similar estimate from the equilibrium dialysis data of Latt & Sober (1967a). The fact that this intrinsic free energy is small and positive indicates that a lysine–phosphate interaction is not intrinsically favored

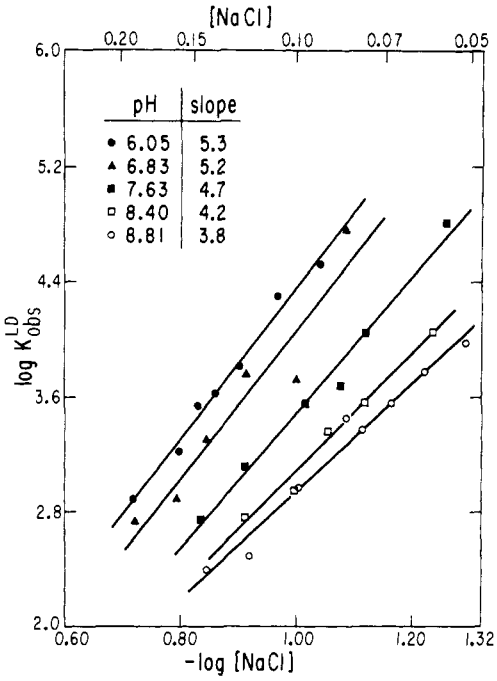

FIGURE 3: Dependence of the observed pentalysine–T7 DNA binding constant on [NaCl] at various values of pH (20.0 °C). (●) Buffer C, pH 6.05; (▲) buffer P, pH 6.83; (■) buffer P, pH 7.63; (□) buffer B, pH 8.40; (○) buffer B, pH 8.81. Lines through data are linear least-squares lines.

Table II: ∂ Log K_{obsd}^{LD}/∂ Log [Na^+] as a Function of pH for the $(Lys)_5$–T7 DNA Interaction

pH	∂ log K_{obsd}^{LD}/∂ log [Na^+]	extrapolated intercept at 1 M Na^+
6.05	-5.3 ± 0.5	-0.92 ± 0.3
6.83	-5.2 ± 0.5	-1.13 ± 0.3
7.63	-4.7 ± 0.5	-1.24 ± 0.3
8.40	-4.2 ± 0.4	-1.09 ± 0.3
8.81	-3.8 ± 0.3	-0.82 ± 0.3

relative to a Na^+–phosphate interaction but that the binding of an oligolysine to DNA is driven at low Na^+ concentrations by the entropic contribution to the binding free energy from the release of Na^+ ions into the dilute salt solution.

(2) Dependence of K_{obsd}^{LD} on [NaCl] as a Function of pH. K_{obsd}^{LD} was determined as a function of [NaCl] over a range of pH (6.05–8.81) at 20 °C by using the difference sedimention method. Values of log K_{obsd}^{LD} as a function of log [NaCl] are plotted in Figure 3 for the the various values of pH investigated. The slopes and intercepts of the linear least-squares analyses of the data at each pH are given in Table II. Log K_{obsd}^{LD} is a linear function of log [NaCl] at each pH; the slope $-(\partial \log K_{obsd}^{LD}/\partial \log [NaCl])_{pH,T}$ decreases with increasing pH, although the intercept (log K^0) is independent of pH within experimental uncertainty. At a constant [NaCl], K_{obsd}^{LD} decreases with increasing pH; this effect is more pronounced at low [NaCl].

These qualitative aspects of the behavior of K_{obsd}^{LD} as a function of pH and [NaCl] are in agreement with the titration curve model discussed above. In this model, various titration states of $(Lys)_5$ can bind to DNA; the effect of pH on K_{obsd}^{LD} results from a decrease in the average valence Z of $(Lys)_5$ with increasing pH. Figure 4 demonstrates the fit of the titration curve model to the $(Lys)_5$ binding data. In the analysis, we have used the experimental values of log K^0 and $(\partial \log K_{obsd}^{LD}/\partial \log [NaCl])_{pH,T}$ determined at pH 6.05 (the low pH

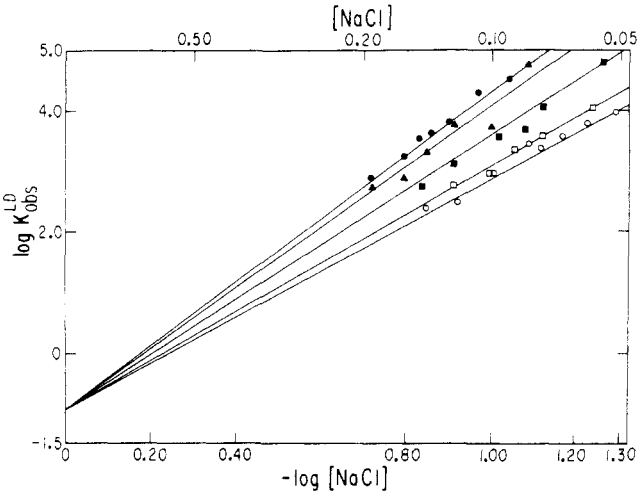

FIGURE 4: Theoretical fit of data in Figure 5 to the titration curve model developed in the theoretical section. Fit was obtained assuming two titratable groups on pentalysine with pK values of 7.2 and 8.4 and log K^0 = −0.92.

limit) as parameters in eq 10. Consequently, we are examining the behavior of K_{obsd}^{LD} and $(\partial \log K_{obsd}^{LD}/\partial \log [NaCl])_{pH,T}$ as functions of pH, relative to their behavior at pH 6.05. Use of the experimental value of $(\partial \log K_{obsd}^{LD}/\partial \log [NaCl])_{pH,T}$ is equivalent to a choice of Z_0 = +6, whereas the theoretical maximum net valence of $(Lys)_5$ is +5. To the extent that this difference reflects a systematic error in our determination of $(\partial \log K_{obsd}^{LD}/\partial \log [NaCl])_{pH,T}$ or a systematic contribution from anion release, use of Z_0 = +6 is an appropriate parameterization.

The decrease in K_{obsd}^{LD} obtained from an increase in pH from 6.05 to 8.81 at constant [NaCl] proved to be too large to be accounted for by the titration of one $-NH_2$ group on $(Lys)_5$. Consequently, the independent titration of two $-NH_2$ groups on $(Lys)_5$ was assumed to occur over this pH range, and pK values for these groups were determined by fitting eq 10 to the experimental data of Figure 3. The best agreement between theory and experiment was obtained by using pK values of 7.2 and 8.4; this fit is shown in Figure 4. We assume that these pK values refer to the α-NH_2 group and a composite of the ϵ-NH_2 groups on $(Lys)_5$, respectively. Ellenborgen (1952) investigated the titration behavior of lysine, dilysine, and trilysine and showed that the pK of the α-amino group decreased with increasing chain length from 9.2 to 7.5 to 7.1 for the three oligolysines, respectively. Yaron et al. (1972) examined the titration behavior of a series of oligolysines from dilysine to decalysine and also found a decrease in the pK of the α-amino group with increasing chain length. Yaron et al. (1972) obtained a pK of 6.9 for the α-amino group of pentalysine in 0.2 M KCl at 25 °C. Our best fit value of 7.2 for the pK of the α-amino group of pentalysine at 20.0 °C compares well with those mentioned above. The pK of 8.4 which is necessary to fit the data at high pH is lower than typical pK values for ϵ-amino groups by ~1 pK unit and probably reflects some composite or average of all the ϵ-amino groups. A more detailed calculation does not seem warranted at this time.

The dependence of K_{obsd}^{LD} on pH at constant [NaCl] is depicted in Figure 5 for three different [NaCl] (0.083, 0.123, and 0.19 M). The smooth curves through the data are theoretical curves calculated from eq 10 by using the same parameters as in Figure 4 (log K^0 = −0.9, $-(\partial \log K_{obsd}^{LD}/\partial \log [NaCl])_{pH,T}$ = 5.3, and pK values of 7.2 and 8.4 for the two titration events). Data points were obtained by interpolation

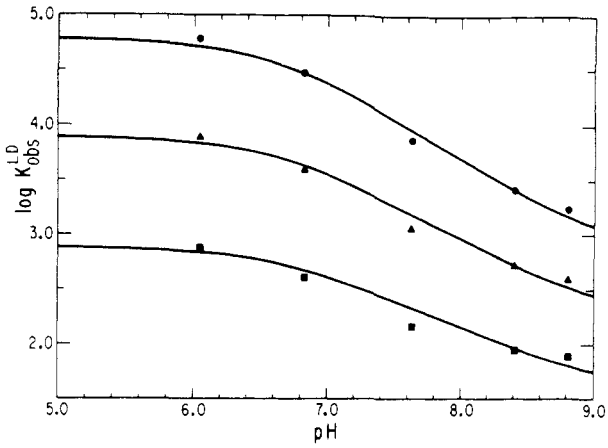

FIGURE 5: Dependence of the observed pentalysine–T7 DNA binding constant on pH at constant [NaCl] (20.0 °C). (●) 0.083 M NaCl; (▲) 0.123 M NaCl; (■) 0.191 M NaCl. Smooth curves are calculated by using the titration curve model developed in the text and assuming two titratable groups on pentalysine with pK values of 7.2 and 8.4 and log K^0 = −0.92. Data points were interpolated from least-squares lines in Figure 4.

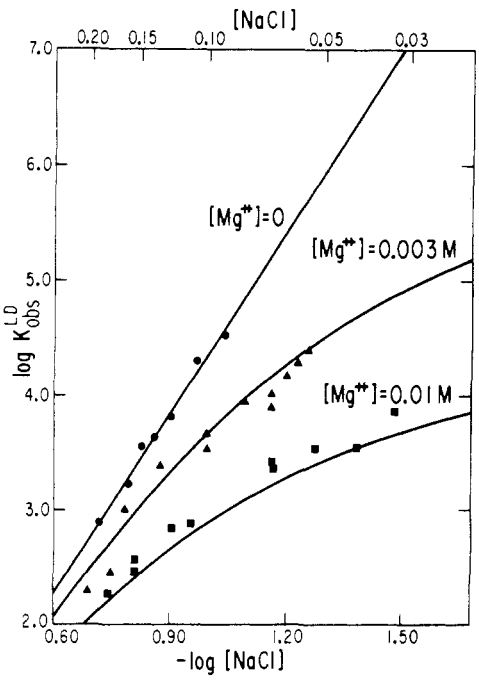

FIGURE 6: Dependence of the observed pentalysine–T7 DNA binding constant on [NaCl], in the presence of Mg^{2+} (buffer C; pH 6.05; 20.0 °C). (●) No Mg^{2+}, variable [NaCl]; (▲) 0.003 M Mg^{2+}, variable [NaCl]; (■) 0.01 M Mg^{2+}, variable [NaCl]. Smooth curves are theoretical curves using eq 5 or 7.

using the equations of the least-squares lines from Figure 3. Agreement between theory and experiment is good. Note that the pH dependence of K_{obsd}^{LD} is more pronounced at low [NaCl], as also illustrated in Figure 4. This behavior is characteristic of the titration curve model for describing pH effects on K_{obsd}^{LD} and distinguishes this model from that in which protonation is required.

(3) Dependence of K_{obsd}^{LD} on [NaCl] in the Presence of the Competitive Ligand Mg^{2+}. K_{obsd}^{LD} was determined at pH 6.05 and 20 °C as a function of [NaCl] at concentrations of $MgCl_2$ of 0.003 and 0.01 M. These results are plotted in Figure 6, in which the dependence of K_{obsd}^{LD} on [NaCl] in the absence of Mg^{2+} is shown for comparison (cf. Figure 1). Addition of $MgCl_2$ reduces K_{obsd}^{LD} and the magnitude of $(\partial \log K_{obsd}^{LD}/\partial$

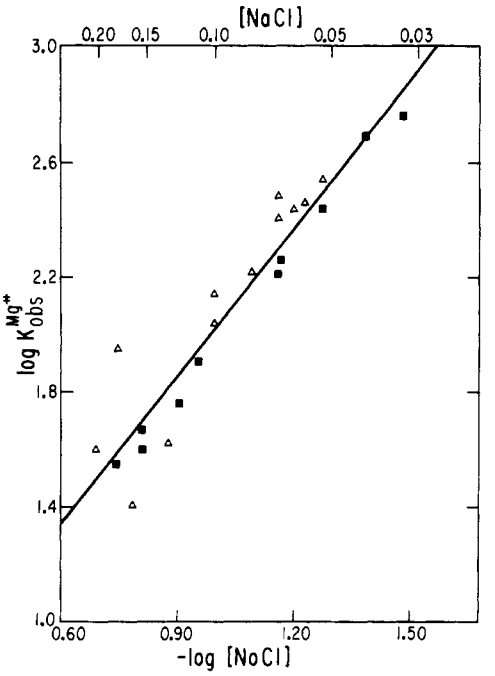

FIGURE 7: Dependence of the Mg^{2+}–T7 DNA binding constant on [NaCl] calculated from the data in Figure 7 by using eq 5 and 7. (▲) Calculated from 0.003 M Mg^{2+} data; (■) calculated from 0.01 M Mg^{2+} data.

$\log [NaCl])_{pH,T}$ at fixed [NaCl]. Both effects are more pronounced at low [NaCl]. Qualitatively similar behavior was observed by Record et al. (1977) for the nonspecific binding of *E. coli lac* repressor protein to DNA and by deHaseth et al. (1978) and Strauss et al. (1980a,b) for the nonspecific and specific interactions of *E. coli* RNA polymerase holoenzyme with DNA. The explanations of these effects of Mg^{2+} on K_{obsd}^{LD} are the following. (a) Mg^{2+} binds to DNA to an extent which is dependent upon the concentrations of both Mg^{2+} and Na^+, as expected from the formalism of eq 1–4a above. (b) Mg^{2+} competes with Lys_5 for DNA phosphates, and so reduces K_{obsd}^{LD}. For a given [$MgCl_2$], the competition is more effective at low [NaCl] where the Mg^{2+} binding density is greater. (c) The derivative $-(\partial \log K_{obsd}^{LD}/\partial \log [NaCl])_{pH,T}$ is predominantly a measure of the release of Na^+ ions from the DNA when $(Lys)_5$ binds. In the presence of Mg^{2+}, less Na^+ is bound to the DNA and therefore less is released when $(Lys)_5$ binds.

The curves drawn through the $(Lys)_5$ binding data in the presence of Mg^{2+} in Figure 6 are theoretical curves utilizing either eq 5 or 7 to describe the competitive effect of Mg^{2+}. Once again, as in the fitting procedure used to describe the interdependence of pH and [NaCl] effects on K_{obsd}^{LD}, the slope $(\partial \log K_{obsd}^{LD}/\partial \log [NaCl])_{pH,T}$ and intercept ($\log K^0$) of the plot of $\log K_{obsd}^{LD}$ vs. log [NaCl] in the absence of Mg^{2+} are used in the analysis of Mg^{2+} effects on K_{obsd}^{LD}. In addition, knowledge of the Mg^{2+}–DNA binding constant as a function of [NaCl] is required. Since the fit is quite sensitive to this quantity, we have let K_{obsd}^{Mg} be a parameter, and determined it as a function of [NaCl] from the measured K_{obsd}^{LD} using eq 5 or 7. Within experimental uncertainty, values of K_{obsd}^{Mg} determined by this procedure from data at Mg^{2+} concentrations of 0.003 and 0.01 M fall on a common line; K_{obsd}^{Mg} is a function of [NaCl] but not of [$MgCl_2$]. This is illustrated in Figure 7. Log K_{obsd}^{Mg} is a linear function of log [NaCl], as might be expected from eq 3a. The least-squares line through the data of Figure 7 is

$$\log K_{obsd}^{Mg} = -(1.7 \pm 0.1) \log [NaCl] + (0.3 \pm 0.2) \quad (18)$$

Table III: Temperature Dependence of the $(Lys)_5$–T7 DNA Interaction

pH	[NaCl] (M)	t (°C)	K_{obsd} (M^{-1})[a]	ΔH°_{obsd} [kcal/mol of $(Lys)_5$]
6.05	0.138	10.0	4.7×10^3*	−0.6 ± 0.8
		20.0	4.7×10^3	
		25.0	4.5×10^3*	
		30.0	4.5×10^3*	
7.63	0.080	9.3	1.2×10^4	−1.9 ± 0.8
		10.0	1.2×10^4	
		20.0	1.4×10^4	
		25.0	9.2×10^3	
8.81	0.082	10.3	2.6×10^3	−1.5 ± 0.8
		20.0	2.3×10^3	
		25.0	2.3×10^3	

[a] All values are the average of two measurements *except* those marked with an asterisk.

Equation 18 is in close agreement with expressions for the NaCl dependence of K_{obsd}^{Mg} determined by competition binding experiments with other ligands under other solution conditions (cf. Record et al., 1978). We therefore conclude that the effects of Mg^{2+} on the $(Lys)_5$–DNA binding reaction are successfully described by the simple competitive binding models developed above and by Record et al. (1977, 1978).

(4) Temperature Dependence of the Pentalysine–DNA Interaction. The dependence of K_{obsd}^{LD} on temperature at constant [NaCl] and pH was determined at pH 6.05, 7.63, and 8.81. The results are shown in Table III. At each pH, the binding constant is essentially independent of temperature. The values of ΔH°_{obsd} obtained from van't Hoff plots and listed in Table III are all the same within the accuracy of the experiments, and all are close to zero. We interpret this result to mean that both the intrinsic enthalpy change (ΔH°) and the contribution to ΔH°_{obsd} from the protonation reactions are small (cf. eq 14), since the latter term is expected to be small for the titration curve model (except at very low [NaCl]). The small values of ΔH°_{obsd} are consistent with our model for the Lys_5–DNA binding reaction as an entropy-driven process, which occurs at low electrolyte concentration as a result of the entropic contribution to the binding free energy from counterion release. The small ΔH°_{obsd} is also consistent with the titration curve model and indicates that protonation of the ϵ-amino groups of the lysine side chains is not directly coupled to ΔH°_{obsd} [so that full protonation of $(Lys)_5$ is not necessary for binding to DNA]. Hence, the Δh for protonation of the ϵ-NH_2 groups of $(Lys)_5$ does not contribute strongly to ΔH°_{obsd} (see eq 14). If full protonation of $(Lys)_5$ were required for binding, a large ΔH°_{obsd} due to direct coupling of protonation with binding would be seen [see deHaseth et al. (1977b)].

Conclusion

The interaction of $(Lys)_5$ with DNA serves as a model system for the electrostatic component of protein–nucleic acid interactions. We have shown that K_{obsd}^{LD} is a sensitive function of ion concentrations and pH but is insensitive to temperature. The binding reaction is entropically driven by the release of electrolyte ions (predominantly cations released from the DNA) into a dilute salt solution when $(Lys)_5$ binds. With increasing pH, the net positive charge on $(Lys)_5$ decreases as a result of deprotonation of the α- and ϵ-NH_2 groups. This reduces the amount of ion release that accompanies complex formation, and consequently both K_{obsd}^{LD} and $-(\partial \log K_{obsd}^{LD}/\partial \log [NaCl])_{pH,T}$ decrease with increasing pH. We have obtained a quantitative description of the coupled effects of pH

and [NaCl] using binding theory and a model for the interaction in which the protonation events are not required for binding but merely increase the binding affinity of the ligand. Effects of Mg^{2+} are quantitatively treated by using a competition binding model in which Mg^{2+} and $(Lys)_5$ compete for DNA phosphate sites.

For ligands like $(Lys)_5$ where it can be inferred that anion effects on the binding reaction are small or absent, we have shown that the derivative $-(\partial \log K_{obsd}^{LD}/\partial \log [NaCl])_{pH,T}$ is a measure of the number of Na^+ released in the reaction and consequently of the number of positive charges in the binding site on the ligand. In addition, the value of the intrinsic binding constant ($\log K^0 = -0.9 \pm 0.3$) provides an estimate of the contribution of all other factors besides Na^+ release to the free energy of the $(Lys)_5$–DNA interaction. This intrinsic constant, which is similar to those obtained by Record et al. (1976, 1978) from the oligolysine binding data of Latt & Sober (1967a), can be used to estimate the contribution of a known number of cationic groups in the binding site of a protein to the binding free energy of a protein–nucleic acid interaction under any ionic condition [cf. Record et al. (1976, 1978), deHaseth et al. (1977b, 1978), and Strauss et al. (1980a,b)].

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