Use of Difference Boundary Sedimentation Velocity to Investigate Nonspecific Protein–Nucleic Acid Interactions†

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ABSTRACT: The difference boundary sedimentation velocity technique of Schachman and co-workers is demonstrated to be applicable to the measurement of binding constants (K_{obsd}) in the range 10^2–10^5 M^{-1} for the nonspecific interactions of proteins with DNA. The difference technique can reproducibly detect a 2% change in the sedimentation coefficient of the DNA upon binding ligands, corresponding to average extents of association as low as 10 molecules of protein (in the cases of *Escherichia coli lac* repressor and *E. coli* RNA polymerase) per molecule of bacteriophage T7 DNA. At these low binding densities, it is plausible to assume that the primary effect of ligand binding is on the buoyant mass of the complex and not on the frictional coefficient of the flexible DNA coil. Binding constants calculated by using this assumption agree well with literature values for the nonspecific interactions of RNase and *lac* repressor proteins with double-stranded DNA. Advantages of the method are that it is relatively rapid, requires the optical detection of the DNA only, and can be performed on small amounts of sample. The method appears useful for surveying (to an accuracy of ±50% in K_{obsd} or ±10% in log K_{obsd}) the effects of solution variables on K_{obsd} of protein–DNA interactions. Applications of the method to the nonspecific interactions of RNA polymerase core and holoenzymes with T7 DNA are discussed.

Measurement of binding constants (K_{obsd}) as a function of temperature and solvent conditions permits one to obtain a thermodynamic description of a protein–nucleic acid interaction. Experimental and theoretical investigations of the effects of ion concentrations, pH, and temperature on these interactions have been recently reviewed (Record et al., 1978). The concentration of low molecular weight electrolyte is of major importance in determining the stability of these complexes; generally K_{obsd} decreases dramatically with increasing salt concentration. We have shown that this salt effect results from the release of counterions originally associated with the nucleic acid upon formation of the complex (Record et al., 1976, 1978). Estimates of the magnitude of this entropic contribution to the observed binding free energy and the number of ionic interactions formed between positively charged groups on the ligand and the negatively charged phosphates on the nucleic acid can be obtained by measuring K_{obsd} as a function of salt concentration. However, only a limited number of methods are available to determine K_{obsd} for protein–nucleic acid interactions. Specific interactions with sufficiently small dissociation rate constants have been investigated by the nitrocellulose filter assay (Riggs et al., 1970a,b; Hinkle & Chamberlin, 1972a,b). Nonspecific interactions in these systems can be studied by competition experiments using the filter assay (Lin & Riggs, 1972, 1975). Small DNA columns (deHaseth et al., 1977a,b, 1978) and sucrose gradient band sedimentation (Yamamoto & Alberts, 1974; Draper & von Hippel, 1979) have been used to determine nonspecific binding constants for protein–nucleic acid interactions by analysis of the equilibrium dissociation profile of the complex upon dilution. Both these methods require the determination of free protein concentrations, which limits their range of applicability to small binding constants unless radioactively labeled protein is available. Another sedimentation method to obtain K_{obsd} for nonspecific protein–nucleic acid interactions has been developed by von Hippel & co-workers (Jensen & von Hippel, 1976, 1977; Revzin & von Hippel, 1977). This thermodynamically rigorous technique utilizes sedimentation in the preparative or analytical ultracentrifuge to separate bound and free protein, allowing the determination of the binding density as a function of the free protein concentration. Relatively high binding densities are required, and the method relies on the detection of protein concentration as a function of radial distance in the sedimentation experiment.

Here we investigate a complementary sedimentation technique for studying the nonspecific interactions of proteins with double-helical DNA, in which the sedimentation behavior of the DNA is observed and which is suitable for use at very low binding densities. This method, difference boundary sedimentation velocity, was originally developed by Schachman and co-workers to investigate the binding of small ligands to globular proteins (Schachman, 1959; Richards & Schachman, 1959; Steinberg & Schachman, 1966). Skerrett (1975) and Rees et al. (1977) report further developments in the theory and practice of this technique. Analysis of the difference in sedimentation rates between liganded and free DNA (which can be directly measured with a scanner-equipped ultracentrifuge) yields the binding constant K_{obsd}, subject to assumptions regarding the constancy of the frictional coefficient of the DNA and the additivity of partial specific volumes. Calibration of the method is therefore required. The results of calibration experiments using ribonuclease and *lac* repressor as ligands validate the assumptions and indicate the usefulness of the method. Applications of the technique to the nonspecific interactions of RNA polymerase and pentalysine with T7 DNA are discussed in this and the following paper (Lohman et al., 1980).

Materials and Methods

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

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(*b*) *Buffers.* The experiments with *lac* repressor were done in buffer P (10^{-3} M Na_2HPO_4; 10^{-4} M Na_2EDTA) at pH 7.5. The RNase and RNA polymerase holoenzyme studies were carried out in buffer P, pH 7.7. The core RNA polymerase studies were done in buffer T, which is 0.01 M Tris [2-amino-2-(hydroxymethyl)-1,3-propanediol] and 10^{-4} M Na_2EDTA, pH 7.8. NaCl was added to these buffers in order to vary the monovalent cation concentration. BPES buffer is 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, and 1 mM EDTA.

(*c*) *Ligands.* (*1*) *lac Repressor.* The preparation of the *Escherichia coli lac* repressor used in this study has been described previously (deHaseth et al., 1977a). The protein was purified according to the method of Platt et al. (1973). A partial specific volume of $\bar{v}$ = 0.741 cm^3/g (Butler et al., 1977), a tetramer molecular weight of 148 640 (Beyreuther et al., 1975), and a binding site size of 26 nucleotides (Revzin & von Hippel, 1977) were used in the calculations of K_{obsd}. Concentrations of *lac* repressor were determined by using a molar extinction coefficient for the tetramer of 9.0×10^4 M^{-1} cm^{-1} at 280 nm (Butler et al., 1977).

(*2*) *Ribonuclease.* Crystalline pancreatic ribonuclease A (phosphate and salt free) was purchased from Worthington Biochemical Corp. A partial specific volume of $\bar{v}$ = 0.70 cm^3/g (Ulrich et al., 1964) and a binding site size of eight nucleotides (Jensen & von Hippel, 1976) were used in the calculations of K_{obsd}. Concentrations of RNase were determined by using a molar extinction coefficient of 9.56×10^3 M^{-1} cm^{-1} at 277.5 nm (von Hippel & Wong, 1965).

(*3*) *E. coli RNA Polymerase.* RNA polymerase (core and holoenzyme) from the K-12 strain of *E. coli* was prepared by the method of Burgess & Jendrisak (1975). For the core protein, we used $\bar{v}$ = 0.738 cm^3/g and a M_r of 3.9×10^5 in the calculations. Values of $\bar{v}$ = 0.742 cm^3/g and M_r of 4.8×10^5 were used for holoenzyme (Burgess, 1969). Binding site sizes of $\sim 10^2$ nucleotides were used for both core and holoenzyme [cf. deHaseth et al. (1978)]. The value of K_{obsd} is essentially independent of the choice of site size at the low binding densities used in these experiments (see below). Concentrations were determined by using molar extinction coefficients of 2.1×10^5 M^{-1} cm^{-1} (core) and 3.0×10^5 M^{-1} cm^{-1} (holoenzyme) at 280 nm (Burgess, 1976).

(*d*) *DNA.* Intact T7 bacteriophage DNA was prepared from purified T7 bacteriophage by using the hot phenol method to minimize shear degradation of the DNA (Massie & Zimm, 1965). The intact T7 DNA was stored in BPES buffer and used directly by diluting into the appropriate binding buffer or dialyzed into the binding buffer before use, depending on the concentration of the T7 DNA stock. An apparent partial specific volume of $\bar{v}$ = 0.55 cm^3/g (Cohen & Eisenberg, 1968; Hearst, 1972) and an average nucleotide molecular weight of 331.5 for Na–DNA were used in the calculations. Concentrations of DNA were determined by using an extinction coefficient for DNA at 260 nm of 6.65×10^3 M^{-1} cm^{-1} (per mol of nucleotides).

(*e*) *Difference Boundary Sedimentation Velocity.* Detailed descriptions of the preparation of cells and performance of the centrifuge experiment have been provided elsewhere (Lohman, 1977; Wensley, 1977). Experiments were performed by using a Model E analytical ultracentrifuge equipped with photoelectric scanner and multiplexer. Generally three or five determinations were carried out simultaneously using a four- or six-cell rotor, but the basic experiment involves comparisons of two cells. We have used 12-mm cells with charcoal-filled Epon double-sector centerpieces. Cell 1 contains intact T7 DNA in one sector and buffer in the other sector and is used to follow the sedimentation of the DNA in the binding buffer of interest. Cell 2 contains intact T7 DNA in one sector and T7 DNA plus the protein under investigation in the other sector. DNA concentrations in both cells are identical (10–20 μg/mL); all buffer components are identical in both cells, and volumes of DNA and DNA–protein solutions in cell 2 must be identical. Since this last requirement is difficult to achieve, a double-sector capillary-type synthetic boundary centerpiece is used in cell 2. (Alternatively, two parallel grooves can be scratched in the wall separating the sectors of an ordinary double-sector centerpiece.) The grooves permit transfer of a negligibly small volume of solution from one sector to the other, to equalize the volumes.

The technique is restricted in application to proteins that sediment substantially slower than the DNA to which they bind and to the measurement of binding constants in the range 10^2 M^{-1} < K_{obsd} < 10^5 M^{-1}. The first requirement ensures that no redistribution of free protein occurs during the experiment. The range of binding constants is restricted by the desirability of working at a low binding density and because the free protein concentration at equilibrium is not determined directly but rather as the difference between the total protein concentration and the concentration of bound protein (see below). For most nonspecific DNA binding proteins yet investigated, the binding constant is such a sensitive function of ionic conditions that it is generally possible to obtain binding constants in this range by proper choice of salt concentration (Record et al., 1976, 1978).

With T7 DNA, rotor speeds were in the range 20 000–28 000 rpm. Rotor temperatures were controlled at or near 20 °C by using the RTIC unit. Cells were scanned at 265 nm at 8-min intervals. From cell 1, the boundary position r_0 of T7 DNA is obtained as a function of time (cf. Figure 1a). From cell 2, one obtains a series of difference peaks, which increase in area with time (cf. Figure 1b). The difference peaks originate from the more rapid sedimentation of the DNA–ligand complex than of the free DNA (schematically illustrated in Figure 1c). Boundary positions of the complex (r_B) at any time are obtained from the areas of the difference peaks (A) and the appropriate values of r_0 from cell 1 according to

$$r_B = r_0 + A/h \tag{1}$$

where h is the height of the T7 DNA plateau region as shown in Figure 1c. In eq 1, the difference peak is approximated by a triangle, which is justified for the sharp boundaries and small boundary separations used here (cf. Figure 1b). Areas of difference peaks were routinely determined by cutting them out and weighing them on an analytical balance.

(*f*) *Calculation of Binding Constants from Difference Sedimentation Data.* To obtain K_{obsd} from the difference sedimentation technique, it is necessary to evaluate the sedimentation coefficient ratio S_B/S_0 of the complex to the free DNA. This is most accurately accomplished as follows. At a constant time in the run

$$\frac{1}{S_B} \ln \frac{r_B}{r_B^0} = \frac{1}{S_0} \ln \frac{r_0}{r_0^0} \tag{2}$$

where r_B^0 and r_0^0 are the appropriate radial distances at t = 0. Substitution of eq 1 into eq 2 yields

$$\ln\left(1 + \frac{A}{r_0 h}\right) = \left(\frac{S_B}{S_0} - 1\right) \ln r_0 - \frac{S_B}{S_0} \ln r_0^0 + \ln r_B^0 \tag{3}$$

Therefore, the quantity S_B/S_0 is obtained from the slope of

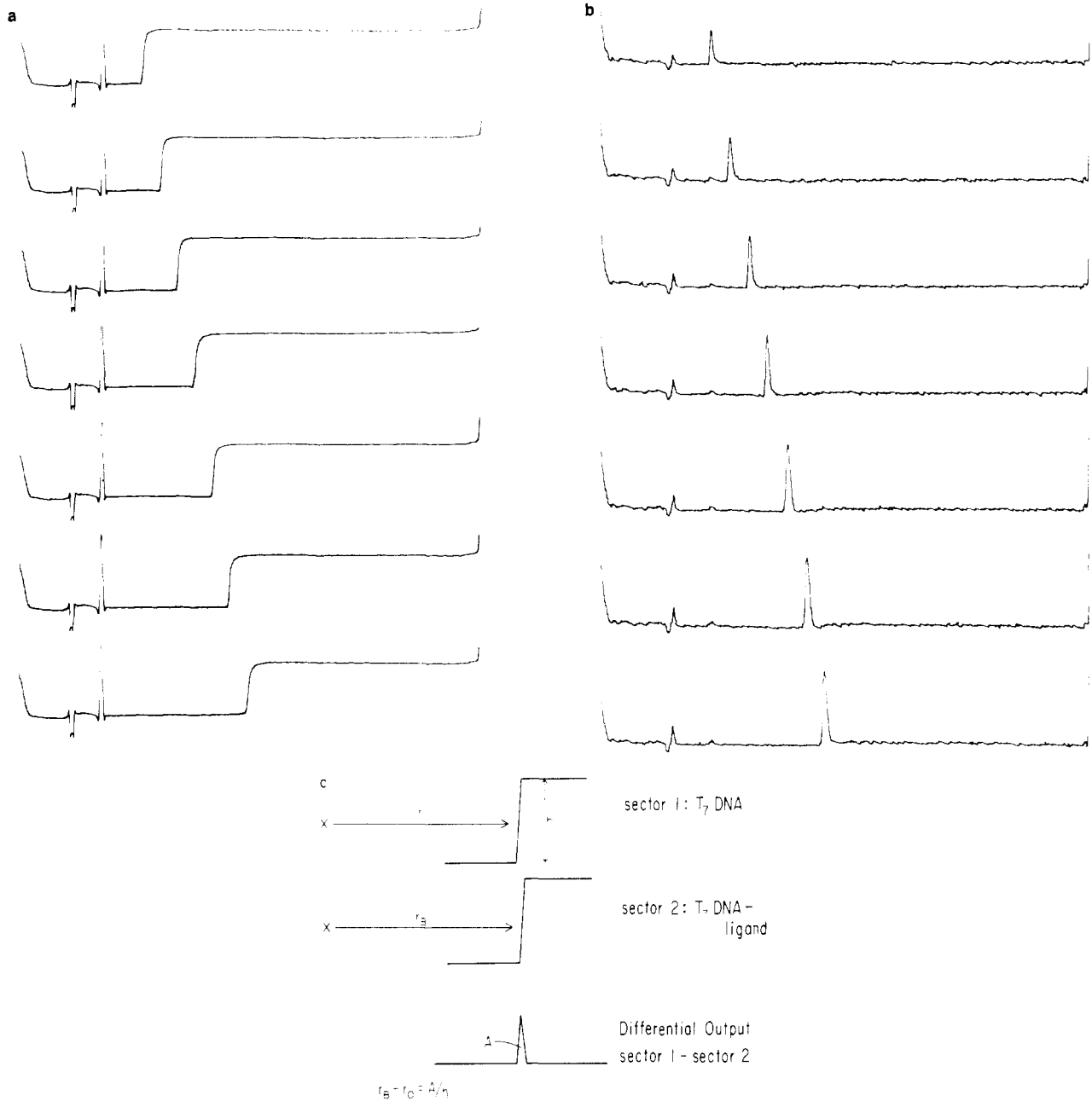

FIGURE 1: (a) A typical time series of scans of cell 1 (T7 DNA only) at 8-min intervals. (b) A typical time series of scans of cell 2 (difference cell) at 8-minute intervals for the interaction of T7 DNA with the ligand pentalysine [cf. Lohman et al. (1980)]. The scans shown here are from the same experiment as the scans shown in (a). (c) The origin of the difference peaks shown in Figure 1b; r_0 and r_B are the radial distances from the center of the rotor to the midpoint in the DNA boundary for the DNA only and the ligand–DNA complex, respectively; h is the height of the plateau region and A is the peak area.

a plot of $\ln (1 + A/(r_0 h))$ vs. $\ln r_0$ for the data of a given run.

The sedimentation coefficient ratio S_B/S_0 is related to molecular parameters by

$$\frac{S_B}{S_0} = \frac{M_B(1 - \bar{v}_B\rho)f_0}{M_0'(1 - \bar{v}_0\rho)f_B} \tag{4}$$

In eq 4, M_B, $\bar{v}_B$, and f_B are the molecular weight, apparent partial specific volume, and frictional coefficient of the complex, M_0', $\bar{v}_0$, and f_0 are the corresponding values for the unliganded Na–DNA, and ρ is the solvent density. The difference between M_B and M_0' is a measure of the equilibrium amount of bound ligand, from which the binding density and binding constant can be evaluated. Assumptions must be made regarding $\bar{v}_B$ and f_B to obtain this information from the measured sedimentation coefficient ratio.

(1) We evaluate $\bar{v}_B$ by assuming additivity of partial specific volumes (Jensen & von Hippel, 1976a), i.e.

$$\bar{v}_B = \frac{\nu' M_L \bar{v}_L + M_0' \bar{v}_0}{\nu' M_L + M_0'} \tag{5}$$

where M_L is the molecular weight of the ligand and ν' is the binding density expressed in ligands bound per DNA molecule. This assumption is reasonable unless there is a substantial volume change upon formation of the complex. Revzin & von Hippel (1977) found that K_{obsd} for the nonspecific interaction of *lac* repressor with DNA was independent of pressure, indicating that there is no detectable volume change in this interaction.

(2) We assume that the frictional coefficient of the DNA coil is unperturbed by binding the compact ligand, so that

$$f_B = f_0 \quad (6)$$

The primary justification for this assumption is the agreement with experiment which its use provides (see below). It seems plausible that, at low binding densities of a nonspecifically bound globular ligand whose interaction with the DNA is primarily electrostatic, there should be little effect on either the persistence length of the DNA or its effective hydrodynamic volume (which is much larger than the volume of the ligand).

Equations 4–6 may be combined to yield a relationship between the binding density ν' and the sedimentation coefficient ratio S_B/S_0:

$$\nu' = \left(\frac{S_B}{S_0} - 1\right)\left(\frac{M_0'}{M_L}\right)\left(\frac{1 - \bar{v}_0\rho}{1 - \bar{v}_L\rho}\right) \quad (7a)$$

An equivalent form of eq 7a is

$$\nu = \left(\frac{S_B}{S_0} - 1\right)\left(\frac{M_0}{M_L}\right)\left(\frac{1 - \bar{v}_0\rho}{1 - \bar{v}_L\rho}\right) \quad (7b)$$

where ν is the reduced binding density in moles of protein bound per mole of DNA nucleotides and M_0 is the molecular weight of the average DNA nucleotide (331.5 for the sodium salt). From ν, one can obtain K_{obsd} from the equation of McGhee & von Hippel (1974):

$$K_{obsd} = \frac{\nu}{[L]} \frac{[1 - (n-1)\nu]^{n-1}}{(1 - n\nu)^n} \quad (8a)$$

where n is the site size (in nucleotides covered by a bound ligand) and [L] is the concentration of free ligand. Experiments reported here were carried out at sufficiently low binding density that the binding constant can in practice be estimated (to within 25% in K_{obsd} and 2% in log K_{obsd}) by

$$K_{obsd} \simeq \nu/[L] \quad (8b)$$

in which a knowledge of the binding site size is not required. In the sedimentation experiments, [L] is evaluated as the difference between the total ligand concentration $[L_T]$ and the concentration of bound ligand $\nu[D_T]$, where $[D_T]$ is the total concentration of DNA nucleotides:

$$[L] = [L_T] - \nu[D_T] \quad (9)$$

Estimation of the Accuracy of the Difference Sedimentation Velocity Technique. Although the absolute values of either S_0 or S_B are accurate to only ~4%, as determined by comparison among a number of experiments, the ratio S_B/S_0 has an accuracy of ~1%, as estimated from the standard deviation of the linear least-squares line used to determine $S_B/S_0 - 1$ (see eq 3). The higher accuracy of the ratio results from the direct determination of S_B/S_0 in one experiment, thereby eliminating the error associated with reproducing the rotor speed and temperature in two independent sedimentation experiments.

The accuracy of the calculated binding constants, K_{obsd}, depends on the binding density ν and the free ligand concentration, [L], which in turn depends on the total ligand concentration in solution (cf. eq 9). In the limit of detectability (namely, a 2% change in S), a 1% error in S_B/S_0 yields, in this "worst case" situation, a ±50% uncertainty in the determination of ν (eq 7) and in relative values of K_{obsd}. Absolute values of K_{obsd} are subject to somewhat greater uncertainty as a result of errors in the estimation of the active protein concentration. This may be less than the total protein concentration due to protein adsorption to surfaces during the preparation of the sample (we used plastic test tubes and pipets to minimize this) as well as the possible presence of inactive protein in the sample. If we assume that the total concentration of active protein in the centrifuge cell is known to within ±50% and combine this with the uncertainty in ν (±50% in the worst case), the absolute uncertainty in K_{obsd} should be no more than a factor of 2. (In our thermodynamic analysis, we are interested in log K_{obsd} and a factor of 2 in K_{obsd} is only a 10% error in log K_{obsd}.) We find that the relative error in a series of binding constants determined as a function of salt concentration is ~20% as judged by the reproducibility in the slopes of the log K_{obsd} vs. log $[Na^+]$ plots (see Results).

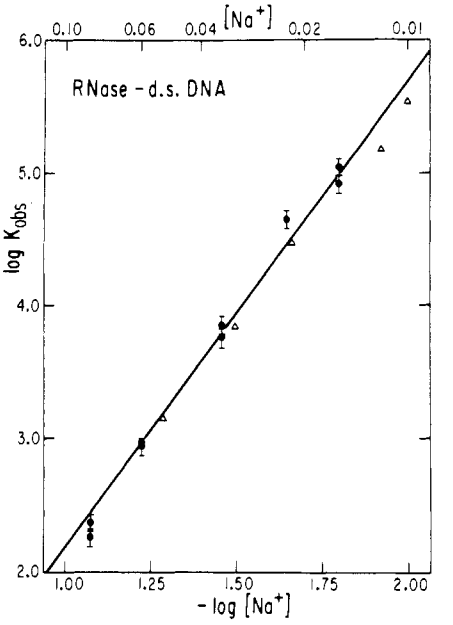

FIGURE 2: Dependence of the observed RNase–double-stranded DNA binding constant, K_{obsd}, on [NaCl]; log–log plot. (●) Difference measurements with T7 DNA (20 °C; pH 7.7); (△) data of Jensen & von Hippel (1976) on RNase–calf thymus DNA (24 °C; pH 7.7).

Table I: RNase–T7 DNA Binding Parameters[a]

$[Na^+]$ (M)	$S_B/S_0 - 1$	$\nu \times 10^3$	[RNase] × 10^7 (M) bound	[RNase] × 10^7 (M) free	log K_{obsd}
0.016	0.128	4.6	2.2	0.64	4.91
	0.261	9.5	4.5	1.1	5.04
0.023	0.208	7.5	3.5	2.1	4.65
0.035	0.083	3.0	1.4	5.6	3.76
	0.178	6.5	3.1	10.9	3.85
0.060	0.034	1.2	0.6	13.4	2.97
	0.061	2.2	1.0	27.0	2.94
0.085	0.018	0.65	0.3	27.7	2.37
	0.029	1.1	0.5	55.5	2.26

[a] [DNA] = 4.75×10^{-5} M in nucleotides. Conditions: buffer P; pH 7.7; 20 °C.

Results

RNase. In Figure 2 association binding constants for the RNase–T7 DNA interaction obtained by the difference boundary sedimentation velocity technique are compared with those obtained by Jensen & von Hippel (1976, 1977). At each [NaCl], experiments were done at two different RNase concentrations and identical results were obtained, within experimental error. Sedimentation coefficient ratios (S_B/S_0) for the RNase–T7 DNA experiments are listed in Table I, along with the calculated values of ν and K_{obsd}. The sedimentation coefficient of T7 DNA at 20 °C, pH 7.7, and 0.15 M NaCl

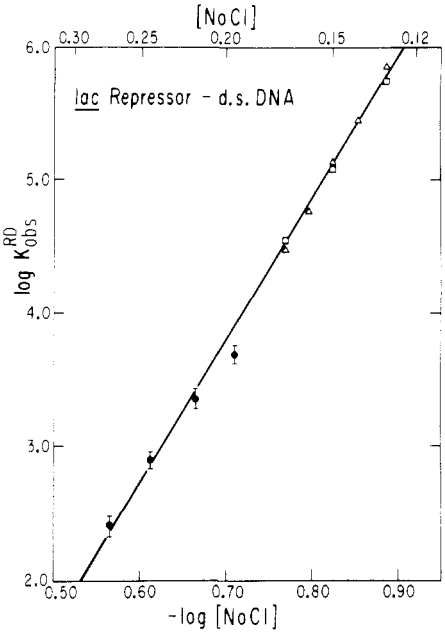

FIGURE 3: Dependence of the observed *lac* repressor–double-stranded DNA binding constant, K_{obsd}^{RD}, on [NaCl]; log–log plot (20 °C; pH 7.5). (●) Difference measurements with T7 DNA; (△) data from deHaseth et al. (1977a); (□) Data from Revzin & von Hippel (1977).

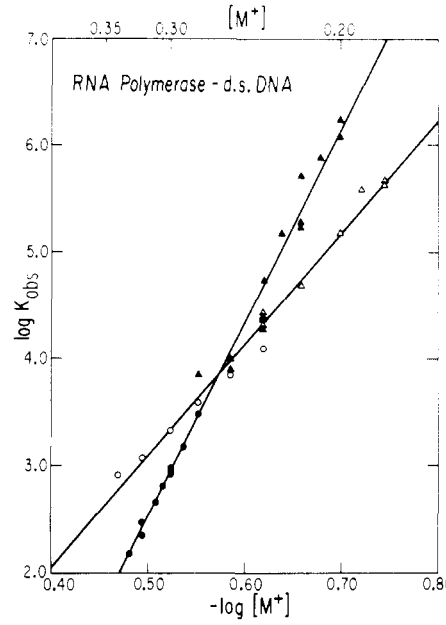

FIGURE 4: Dependence of the observed RNA polymerase holoenzyme and core binding constants, K_{obsd}, on [NaCl]; log–log plot. Filled symbols: core–double-stranded DNA. Open symbols: holoenzyme–double-stranded DNA. (●) Difference measurements on core–T7 DNA; (▲) data from deHaseth et al. (1978) on core–calf thymus DNA; (○) difference measurements on holoenzyme–T7 DNA; (△) data from deHaseth et al. (1978) on holoenzyme–calf thymus DNA.

and ~18 μg/mL DNA was 27.0 ± 0.5 S. (Recall that the accuracy of the experiment lies in the fact that the protein–T7 DNA complex is compared directly with the T7 DNA in the difference cell, so that the ratio of S values (S_B/S_0) is much more accurate than the absolute value of S obtained from cell 1.)

Both the magnitudes of K_{obsd} and the slopes of the log K_{obsd} vs. log [Na^+] plots of the two sets of data for the RNase–double-stranded DNA equilibrium shown in Figure 2 are in good agreement. The linear least-squares line for the difference sedimentation data, obtained at 20 °C in buffer P, pH 7.7, is

$$\log K_{obsd} = -(3.7 \pm 0.3) \log [Na^+] - (1.6 \pm 0.5) \quad (10)$$

The linear least-squares line obtained by Jensen & von Hippel (1976) for the interaction of RNase with double-stranded calf thymus DNA at 24 °C in 10^{-3} M Na_2HPO_4 and 10^{-4} M Na_2EDTA, pH 7.7, is given by

$$\log K_{obsd} = -3.3 \log [Na^+] - 1.0 \quad (11)$$

The least-squares line through both sets of data is the one shown in Figure 2 and is given by

$$\log K_{obsd} = -(3.5 \pm 0.3) \log [Na^+] - (1.3 \pm 0.5) \quad (12)$$

From this comparison, we conclude that the difference sedimentation technique measures binding constants for the RNase–double-stranded DNA interaction which are, within experimental uncertainty, identical with the absolute binding constants measured by Jensen & von Hippel (1976).

E. coli lac Repressor. The data obtained by the difference sedimentation technique for the nonspecific interaction of *E. coli lac* repressor–T7 DNA interaction are given in Figure 3, where they are compared with values of K_{obsd} obtained by deHaseth et al. (1977a,b) and Revzin & von Hippel (1977). The difference sedimentation experiments were done in buffer P at 20 °C, identical with the conditions of Revzin & von Hippel (1977). The data of deHaseth et al. (1977a,b) shown in Figure 3 were corrected for a slight pH difference of 0.13 pH unit by using the experimentally determined dependence of K_{obsd} on pH for the nonspecific *lac* repressor–DNA interaction ($\partial \log K_{obsd}/\partial$ pH = −2.1; deHaseth et al., 1977b). The difference sedimentation binding data do not overlap with those obtained by the other techniques (deHaseth et al., 1977b; Revzin & von Hippel, 1977). It was necessary to perform our experiments in buffers containing a higher [Na^+] to obtain K_{obsd} within the measurable range for this technique (10^2–10^5 M^{-1}). However, on the basis of the experiments of deHaseth et al. (1977b) we believe there is little, if any, anion release when *lac* repressor binds nonspecifically to DNA. Therefore, the log K_{obsd}–log [Na^+] plot should be linear over a wide range of [Na^+] (Record et al., 1976). The difference sedimentation data follow the linear extrapolation of the data of deHaseth et al. (1977a,b) and Revzin & von Hippel (1977). The linear least-squares line which describes the difference sedimentation data is

$$\log K_{obsd} = -(8.7 \pm 1.0) \log [Na^+] - (2.4 \pm 2.0) \quad (13)$$

The linear least-squares line for the three sets of data shown in Figure 3 is given by

$$\log K_{obsd} = -(10.7 \pm 1.0) \log [Na^+] - (3.7 \pm 1.0) \quad (14)$$

Through application of the analysis of the salt dependence of ligand–nucleic acid equilibria (Record et al., 1976), eq 14 is consistent with the formation of 12 ± 2 ionic interactions in the *lac* repressor–nonspecific DNA equilibrium complex. This is in agreement with the extensive analysis of the *lac* repressor–nonspecific DNA interaction given by deHaseth et al. (1977a,b). On the basis of Figure 3, we feel confident that the difference sedimentation technique measures absolute binding constants for the *lac* repressor–nonspecific DNA system.

E. coli RNA Polymerase. The binding constants obtained by the difference sedimentation technique for the nonspecific binding of the core and holoenzyme forms of *E. coli* RNA polymerase to T7 DNA are given in Figure 4. The data for core RNA polymerase are summarized in Table II. Owing

Table II: Core RNA Polymerase–T7 DNA Binding Parameters[a]

$[Na^+]$ (M)	$S_B/S_0 - 1$	$\nu \times 10^5$	[DNA] $\times 10^5$ (M) in nucleotides	[core] $\times 10^9$ (M) bound	[core] $\times 10^9$ (M) free	log K_{obsd}
0.28	0.085	12.4	2.88	3.6	46.4	3.48
0.29	0.080	11.6	2.88	3.3	86.7	3.18
0.29	0.054	7.9	1.44	1.14	58.9	3.18
0.30	0.039	5.7	1.44	0.82	69.2	2.97
0.30	0.047	6.9	2.88	1.99	88.0	2.94
0.30	0.035	5.1	5.76	2.94	67.1	2.93
0.30	0.043	6.3	7.20	4.54	85.5	2.92
0.305	0.034	5.0	5.76	2.88	87.1	2.81
0.31	0.049	7.2	5.76	4.15	176	2.66
0.32	0.024	3.5	5.76	2.02	178	2.35
0.32	0.065	9.5	5.76	5.47	354	2.48
0.33	0.033	4.8	5.76	5.47	354	2.17

[a] Conditions: buffer T; pH 7.8; 20 °C.

to the high molecular weight of RNA polymerase, the experiment could be performed under conditions where an average of 10 or fewer RNA polymerase molecules were bound per T7 DNA molecule. Since wild-type T7 DNA contains as many as eight strong-binding or promoter sites for RNA polymerase holoenzyme (Hinkle & Chamberlin, 1972a,b), the possibility must be considered that interaction with these sites is the dominant binding mechanism rather than the weaker nonspecific interaction of holoenzyme with all regions of the DNA. However, the experiments of Strauss et al. (1980a,b) indicate that binding constants for the interaction of polymerase holoenzyme with promoter sites on T7 DNA are no more than 3 orders of magnitude larger than nonspecific binding constants under the conditions of these studies (0.24–0.36 M NaCl; pH 7.7; 20 °C). Since there are $\sim 10^4$ as many nonspecific sites as promoter sites per T7 DNA molecule, more than 90% of the binding of holoenzyme should occur at nonspecific sites, even at low binding densities. In support of this, we find no significant effect of temperature or pH on the binding of holoenzyme to T7 DNA (data not shown) as expected for nonspecific binding (deHaseth et al., 1978). The binding of holoenzyme to promoters on T7 DNA is both temperature and pH dependent (Strauss et al., 1980a,b).

The dependence on [NaCl] of K_{obsd}, obtained by the difference sedimentation technique, for the binding of core and holoenzyme RNA polymerase to T7 DNA are given by the following linear least-squares lines (20 °C; pH 7.7): for holoenzyme

$$\log K_{obsd} = -(8.1 \pm 1.0) \log [NaCl] - (0.9 \pm 2.0) \quad (15)$$

for core

$$\log K_{obsd} = -(18.2 \pm 2.0) \log [NaCl] - (6.6 \pm 2.0) \quad (16)$$

Analysis of the slopes of these lines (Record et al., 1976) indicates that approximately twice as many ions are released when core interacts nonspecifically with DNA as when holoenzyme interacts nonspecifically with DNA. If anion release from RNA polymerase can be neglected, then the slopes indicate that a maximum of 9 ± 1 and 21 ± 2 ionic interactions are formed for the holoenzyme- and core–nonspecific DNA complexes, respectively. This is in agreement with the corresponding values of 12 ± 2 and 24 ± 4 obtained by deHaseth et al. (1978) using the quantitative DNA–cellulose chromatography technique. Moreover, the magntidues of K_{obsd} obtained from the difference sedimentation technique agree quite well with the magnitudes of K_{obsd} obtained on the same system by deHaseth et al. (1978). These data are compared in Figure 4. The linear least-squares lines through both sets of data are as follows: for holoenzyme

$$\log K_{obsd} = -(10.5 \pm 1.0) \log [NaCl] - (2.1 \pm 2.0) \quad (17)$$

for core

$$\log K_{obsd} = -(18.0 \pm 2.0) \log [NaCl] - (6.5 \pm 2.0) \quad (18)$$

Discussion of the differences in the nonspecific binding properties of holoenzyme and core RNA polymerase inferred from these data is given by deHaseth et al. (1978).

Pentalysine. The difference sedimentation technique appears most suitable for studies involving large protein–DNA interactions since one is able to work at very low binding densities and still obtain measurable differences in sedimentation coefficients between DNA and the protein–DNA complex. However, we show in the following paper (Lohman et al., 1980) that the method is also applicable to the investigation of the binding of a small cationic ligand (pentalysine) to T7 DNA.

Conclusion

We have shown that in the cases where data are available for comparison (*lac* repressor, RNA polymerase, and RNase) (Jensen & von Hippel, 1976; Revzin & von Hippel, 1977; deHaseth et al., 1977a,b, 1978) the binding constants obtained by the difference boundary sedimentation velocity technique are in good agreement with those obtained by other methods. For protein–nucleic acid interactions, the binding constants are determined at very low binding density, since the method can detect a small number of protein molecules bound to an intact T7 DNA molecule. Moreover, the method enables one to measure binding affinities for ligand–nucleic acid interactions by monitoring only the presence of the nucleic acid. Therefore, the interactions of nucleic acids with ligands which cannot be detected by absorbance or fluorescence can be studied by this technique.

References

Beyreuther, K., Adler, K., Fanning, E., Murray, C., Klemm, A., & Geisler, N. (1975) *Eur. J. Biochem. 59*, 491–509.

Burgess, R. R. (1969) *J. Biol. Chem. 244*, 6168–6176.

Burgess, R. R. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M. J., Eds.) pp 69–100, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Burgess, R. R., & Jendrisak, J. J. (1975) *Biochemistry 14*, 4634–4638.

Butler, A. P., Revzin, A., & von Hippel, P. H. (1977) *Biochemistry 16*, 4757–4768.

Cohen, G., & Eisenberg, J. (1968) *Biopolymers 6*, 1077–1100.

deHaseth, P. L., Gross, C. A., Burgess, R. R., & Record, M. T., Jr. (1977a) *Biochemistry 16*, 4777–4783.

deHaseth, P. L., Lohman, T. M., & Record, M. T., Jr. (1977b) *Biochemistry 16*, 4783–4790.

deHaseth, P. L., Lohman, T. M., Burgess, R. R., & Record, M. T., Jr. (1978) *Biochemistry 17*, 1612–1622.

Draper, D. E., & von Hippel, P. H. (1979) *Biochemistry 18*, 753–760.

Hearst, J. E. (1972) *J. Mol. Biol. 4*, 415–417.

Hinkle, D., & Chamberlin, M. J. (1972a) *J. Mol. Biol. 70*, 157–185.

Hinkle, D., & Chamberlin, M. J. (1972b) *J. Mol. Biol. 70*, 187–195.

Jensen, D. E., & von Hippel, P. H. (1976) *J. Biol. Chem. 251*, 7198–7214.

Jensen, D. E., & von Hippel, P. H. (1977) *Anal. Biochem. 80*, 267–281.

Lin, S., & Riggs, A. D. (1972) *J. Mol. Biol. 72*, 671–690.

Lin, S., & Riggs, A. D. (1975) *Cell 4*, 107-111.
Lohman, T. M. (1977) Doctoral Dissertation, University of Wisconsin, Madison.
Lohman, T. M., deHaseth, P. L., & Record, M. T., Jr. (1980) *Biochemistry* (fourth paper of four in this issue).
Massie, H. R., & Zimm, B. H. (1965) *Proc. Natl. Acad. Sci. U.S.A. 54*, 1636-1641.
McGhee, J. D., & von Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469-489.
Platt, T., Files, J. G., & Weber, K. (1973) *J. Biol. Chem. 248*, 110-121.
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.
Rees, A. W., DeBuysere, M. S., & Lewis, E. A. (1977) *Arch. Biochem. Biophys. 182*, 478-487.
Revzin, A., & von Hippel, P. H. (1977) *Biochemistry 16*, 4769-4776.
Richards, E. G., & Schachman, H. K. (1959) *J. Phys. Chem. 63*, 1578-1591.
Riggs, A. D., Bourgeois, S., & Cohn, M. (1970a) *J. Mol. Biol. 53*, 401-417.
Riggs, A. D., Suzuki, H., & Bourgeois, S. (1970b) *J. Mol. Biol. 58*, 67-83.
Schachman, H. K. (1959) *Ultracentrifugation in Biochemistry*, pp 170-174, Academic Press, New York.
Skerret, R. J. (1975) *Anal. Biochem. 66*, 1-11.
Steinberg, I. Z., & Schachman, H. K. (1966) *Biochemistry 5*, 3728-3747.
Strauss, H. S., Burgess, R. R., & Record, M. T., Jr. (1980a) *Biochemistry* (first paper of four in this issue).
Strauss, H. S., Burgess, R. R., & Record, M. T., Jr. (1980b) *Biochemistry* (second paper of four in this issue).
Ulrich, D. V., Kupke, D. W., & Beams, J. W. (1964) *Proc. Natl. Acad. Sci. U.S.A. 52*, 349-356.
von Hippel, P. H., & Wong, K. Y. (1965) *J. Biol. Chem. 240*, 3909-3923.
Wensley, C. G. (1977) Doctoral Dissertation, University of Wisconsin, Madison.
Yamamoto, K. R., & Alberts, B. (1974) *J. Biol. Chem. 249*, 7076-7086.

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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