Nonspecific Interactions of *Escherichia coli* RNA Polymerase with Native and Denatured DNA: Differences in the Binding Behavior of Core and Holoenzyme†

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ABSTRACT: We have investigated the nonspecific interactions of *Escherichia coli* RNA polymerase core and holoenzyme with double-stranded (ds) and single-stranded (ss) DNA. Binding constants for these interactions as functions of such solution variables as monovalent and/or divalent cation concentration, temperature, or pH were determined by the method of deHaseth et al. [deHaseth, P. L., Gross, C. A., Burgess, R. R. and Record, M. T. (1977), *Biochemistry 16,* 4777-4783] from analysis of the elution of the proteins from small columns containing immobilized DNA. This technique, although as yet empirical, has been demonstrated to yield accurate binding constants for the nonspecific interaction of *lac* repressor with ds DNA. We find that observed binding constants (K_{obsd}) are extraordinarily sensitive functions of the monovalent cation concentration for the interactions of both core and holoenzyme with ds DNA. In the absence of divalent cations, the derivatives $-(d \log K_{obsd}/d \log [Na^+])$ are 11 ± 2 for the holo-ds DNA interaction and 21 ± 3 for the core-ds DNA interaction. Consequently, approximately 11 and 21 low-molecular-weight ions are released, in the thermodynamic sense, in the formation of the holo-ds and core-ds complexes, respectively (Record, M. T., Jr., Lohman, T. M., and deHaseth, P. L. (1976), *J. Mol. Biol. 107,* 145-158; Record, M. T., Jr., Anderson, C. F., and Lohman, T. M. (1978), *Q. Rev. Biophys.,* in press). Ion release is a thermodynamic driving force for these nonspecific interactions and causes the stability of the complexes to increase very substantially with a reduction in monovalent ion concentration. Possible molecular models which account for the different salt sensitivities of the holo-ds and core-ds complexes are discussed. Effects of the competitive ligand Mg^{2+} on these interactions are also examined. Substantial ion release (~18 monovalent ions) also accompanies the interaction of either holo or core polymerase with ss DNA. Over the range of ion concentrations investigated, the holo-ss interaction is substantially stronger than the core-ss interaction; furthermore, we conclude that the interactions of polymerase with ss DNA are, in general, stronger than the nonspecific interactions of the enzyme with ds DNA. It is likely that the nonspecific interactions of RNA polymerase with DNA have physiological relevance. Not only is it plausible to assume that the same regions of the protein are involved in both specific and nonspecific interactions, but in addition nonspecific interactions of RNA polymerase and DNA may play a role in determining the availability of this protein, in both the thermodynamic and the kinetic sense, for promoter binding and RNA chain initiation [von Hippel, P. H., Revzin, A., Gross, C. A., and Wang, A. C. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 4808-4812]. Consequently, the strong dependences of the nonspecific interactions of RNA polymerase on ionic conditions suggest the possibility of a modulating role of ion concentrations in the control of transcription.

The nonspecific or general binding of *E. coli* RNA polymerase core and holoenzyme to nonpromoter DNA sequences is thought to be of physiological importance in both the kinetics and thermodynamics of formation of holoenzyme-promoter complexes (Hinkle and Chamberlin, 1972a,b; Chamberlin, 1976; von Hippel et al., 1974). The specific interaction of holo[1] with promoters has been studied in some detail (Hinkle and Chamberlin, 1972a,b; Chamberlin, 1976; Giacomoni, 1976; Giacomoni et al., 1977a,b; Seeburg et al., 1977) but only order of magnitude estimates have been reported for the nonspecific binding constants of holo and core with DNA under any conditions. Therefore, we have investigated the nonspecific interactions of RNA polymerase with DNA under a variety of conditions. Similar studies on the nonspecific interaction of *lac* repressor with DNA have recently been reported by our group (deHaseth et al., 1977a,b; Record et al., 1977) and by von Hippel and co-workers (Butler et al., 1977; Revzin and von Hippel, 1977).

The observed binding constants (K_{obsd}) of many protein-nucleic acid interactions are sensitive functions of such environmental variables as salt concentration, pH, and temperature. By explicitly introducing the consequences of the polyelectrolyte character of the nucleic acid (Manning, 1969, 1972) into the formalism of binding theory (Wyman, 1964; Schellman, 1975), we have interpreted the effects of ions and other ligands on K_{obsd} (Record et al., 1976, 1977, 1978; deHaseth et al., 1977b). Electrostatic interactions between positively charged groups on a protein and negatively charged phosphates on DNA are likely to be a component of most protein-DNA interactions. Formation of these electrostatic interactions results in the release of low-molecular-weight ions previously associated with the binding sites on the polyelectrolytes. The

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[1] Abbreviations used: holo, RNA polymerase holoenzyme; core, core polymerase; ss, single stranded; ds, double stranded; HP, holo-promoter complex; HD, holo-ds DNA; HS, holo-ss DNA; CD, core-ds DNA; CS, core-ss DNA; Bicine, *N,N*-bis(2-hydroxyethyl)glycine; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Pipes, piperazine-*N,N'*-bis(2-ethanesulfonic acid); Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; EDTA, (ethylenedinitrilo)tetraacetic acid.

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entropic effect of counterion release into a dilute salt solution provides the major contribution to the observed favorable free-energy change driving such nonspecific association reactions as the binding of Mg^{2+}, oligolysines, ribonuclease and *lac* repressor to DNA (Record et al., 1976, 1977; deHaseth et al., 1977b).

The amount of counterion release in a protein–nucleic acid interaction, and (in favorable cases) the number of ionic interactions involved, may be determined by measurement of the derivative $d \log K_{obsd}/d \log [M^+]$. In a sufficiently dilute solution of the salt MX, where differential hydration effects can be neglected (cf. Record et al., 1976, 1978):

$$-\frac{d \log K_{obsd}}{d \log [M^+]} = m'\psi + k \qquad (1)$$

where m' is the number of ionic interactions or the number of positively charged groups on the protein that interact with the nucleic acid, ψ is the fraction of an M^+ ion released (in the thermodynamic sense) from the nucleic acid per ionic interaction, and k is the number of ions released from the protein in the formation of the complex. Record et al. (1976, 1978) have derived an analytical expression for ψ from the polyelectrolyte theory of Manning (1969, 1972); ψ is composed of additive contributions from condensed and screening ions and is to a good approximation a function of the axial charge density on the nucleic acid only. For ds DNA, $\psi = 0.88$ monovalent (M^+) ion per phosphate; for ss DNA, $\psi \simeq 0.71$ monovalent ion per phosphate. In particular, ψ is predicted to be independent of the concentration of electrolyte MX in dilute solution. The recent ^{23}Na NMR studies of Anderson et al. (1977) on double-stranded DNA have confirmed that the contribution to ψ from counterion condensation is indeed independent of counterion concentration in the range investigated (0.005–0.5 M).

In those situations so far investigated, it has been assumed, in the absence of evidence to the contrary, that $k \ll m'\psi$ and that the dominant effect of ion concentration on K_{obsd} results from the release of M^+ ions from the nucleic acid. The number of ions released and the maximum number of ionic interactions between protein and nucleic acid are therefore obtainable from the experimental value of the derivative $d \log K_{obsd}/d \log [M^+]$. Since ψ is independent of $[M^+]$, eq 1 can be integrated to yield

$$\log K_{obsd} = \log K_{obsd}\,(1\text{ M}) - m'\psi \log [M^+] \qquad (2)$$

if $k \ll m'\psi$. The quantity K_{obsd} (1 M) is the extrapolated value of K_{obsd} at [MX] = 1 M and is closely related to the thermodynamic, salt-independent equilibrium constant for the protein–nucleic acid interaction in dilute solution (Record et al., 1978). Comparison of the thermodynamic equilibrium constant of a protein–nucleic acid interaction with that determined for a model oligopeptide with the same value of m' permits an estimation of the contribution of nonelectrostatic interactions to the free energy of formation of the protein–nucleic acid complex (Record et al., 1976, 1978).

Since the ionic environment in vivo and in many in vitro binding assays contains divalent cations, we have examined the effects of Mg^{2+} on the nonspecific interactions of RNA polymerase with ds DNA. If the only effect of Mg^{2+} is as a competitive ligand, $[Mg^{2+}] < [M^+]$, and $k \ll m'\psi$, then eq 2 is replaced by

$$\log K_{obsd} = \log K_{obsd}\,(1\text{ M}) - m'\psi \log [M^+] - m' \log \{\tfrac{1}{2}(1 + \sqrt{1 + 4K^{Mg}{}_{obsd}[Mg^{2+}]})\} \qquad (3)$$

where K_{obsd} (1 M) is as defined in eq 2, and $K^{Mg}{}_{obsd}$ is the observed binding constant for the Mg^{2+}–DNA interaction at the indicated M^+ concentration (Record et al., 1977, 1978). Note that $K^{Mg}{}_{obsd}$ is itself a function of the concentration of M^+; the dependence of $K^{Mg}{}_{obsd}$ on $[M^+]$ has been found to follow eq 2 with $m' \simeq 2$ (Record et al., 1976, 1977, 1978).

We have used the elution of RNA polymerase from columns containing matrix-immobilized DNA to measure the effects of environmental variables ($[M^+]$, $[Mg^{2+}]$, pH, temperature, anion type) on the nonspecific interactions of holoenzyme and core protein with ds and ss DNA. In an investigation of the nonspecific repressor–ds DNA interaction, this column method (which at present lacks a rigorous theoretical basis) was found by deHaseth et al. (1977) to yield binding constants which were in close agreement with those obtained by Revzin and von Hippel (1977) using an entirely different method. To the extent that the interactions of polymerase with nonpromoter DNA are nonspecific, the results should be independent of the choice of DNA, and we have used ds calf thymus DNA and ss wheat germ DNA in these studies. The major conclusions of this investigation have been independently verified using a differential analytical sedimentation velocity method and T7 bacteriophage DNA (Wensley, Burgess, and Record, in preparation).

Materials and Methods

Reagents. Tris base was from Sigma, calf thymus DNA from Worthington, and agarose from Bio-Rad. The cellulose was Whatman CF 11. Other chemicals were reagent grade.

Buffers. Storage buffer for RNA polymerase contained 0.01 M Tris (pH 7.9 at 20 °C), 0.1 M NaCl, 10^{-4} M EDTA, 10^{-4} M DTT, and 50% (v/v) glycerol. The other buffers employed in this study differed from those used by deHaseth et al. (1977b) and Record et al. (1977) to study the interaction between *lac* repressor and DNA only in their pH. Thus, buffer P is 0.01 M Na_2HPO_4 (pH 7.8 at 4 °C), 7×10^{-3} M β-mercaptoethanol, 10^{-4} M EDTA, 5% (v/v) glycerol. Buffer T is similar to buffer P but contains 0.01 M Tris-HCl (pH 8.3 at 4 °C) instead of Na_2HPO_4. When these buffers were used with added NaCl or Na_2SO_4, the total final $[Na^+]$ is indicated as a subscript; e.g., $P_{0.20}$ is buffer P with a total $[Na^+]$ of 0.20 M.

To investigate the pH dependence of the binding reaction, buffers based on Bicine, Hepes, or Pipes (Good et al., 1966) were used, depending on the choice of pH. Investigations of overlapping pH ranges showed that these buffers had no specific effects on the binding reaction (data not shown).

RNA Polymerase. The purification of RNA polymerase holoenzyme was as described previously (Burgess and Jendrisak, 1975). Core protein and σ factor were prepared from purified holoenzyme by passage over Bio-Rex 70 columns (Burgess and Jendrisak, 1975; Burgess, 1976). The σ factor was further purified by gel filtration chromatography on AcA 44 (LKB) (Burgess, 1976). The σ content of the holoenzyme preparations was estimated to be 80 ± 20% from densitometric tracings of Coomassie brilliant blue stained gels (Burgess, 1976). Polymerase was stored at −15 °C in storage buffer until use. Although in general the activity of holoenzyme preparations was not assayed immediately before use, one sample that was assayed by the procedure of Burgess (1969) gave a specific activity of 860 units/mg. The nonspecific binding behavior of RNA polymerase was independent of the preparation used (see Results). Our column-elution profiles (see below) are consistent with the presence of 25 ± 10% core (or other conformation of higher binding constant; cf. Williams and Chamberlin, 1977) in the holo preparations. Similar elution profiles were obtained from experiments where purified σ was added to the

holo preparation to give a σ content of 110 ± 20% (see below).

Protein concentrations were determined spectrophotometrically, assuming the following absorbances at 280 nm (corrected for light scattering) in a 1-cm cell path for a 1 mg/mL solution: holo, 0.62; core, 0.55; σ, 0.88 (Burgess, 1976).

Measurement of Binding Constants for the Interactions of RNA Polymerase with Double-Stranded DNA. In previous papers (deHaseth et al., 1977a,b; Record et al., 1977), we described in detail the use of small DNA cellulose columns containing ds calf thymus DNA (Alberts and Herrick, 1971) to measure nonspecific binding constants for the interaction of *lac* repressor with ds DNA. In brief, the procedure is the following. Protein, bound initially at a low density to DNA sites on the column, is eluted from the column with buffer of constant composition. The elution profile is monitored by protein fluorescence. With *lac* repressor, we observed an exponential decay of the amount of protein retained on the column. Calibration of the technique indicated that the characteristic decay constant was proportional to the binding constant of the repressor–DNA interaction and that indeed the proportionality constant could be calculated by considering elution of the column as a discrete extraction process (deHaseth et al., 1977a), if the amount of accessible DNA on the column was known. This latter quantity was obtained from a saturation experiment using excess repressor under conditions where the binding constant is large ($\sim 10^9$ M^{-1}). For our standard DNA–cellulose columns, containing 150 ± 10 μg of DNA/mL of packed column volume, 46% of the DNA sites was accessible to repressor. For a 1-mL column, this corresponds to 2.1×10^{-7} mol of accessible DNA nucleotides.

We used the same preparation of ds calf thymus DNA–cellulose to investigate polymerase–DNA interactions as that used in studying repressor–DNA interactions (deHaseth et al., 1977a). Therefore, we assume that the same amount of DNA (2.1×10^{-7} mol of nucleotides/mL of column volume) is accessible to RNA polymerase. This assumption is necessary because physical site sizes are not known either for core or for holo in its nonspecific binding mode. As a check, we ran saturation experiments on both forms of the enzyme. Under ionic conditions where the polymerase–ds DNA association constant was $\sim 10^9$ M^{-1} (see below), a 0.5-mL column containing approximately 75 μg of ds DNA bound 360 μg of core (out of 1200 μg loaded) and 520 μg of holo (out of 1550 μg loaded). No detectable amount of polymerase was retained by a column consisting of cellulose only, over the salt range of interest here (data not shown). Using a simple iterative calculation to account for the effects of site overlap (McGhee and von Hippel, 1974), we estimate from these data that the physical site sizes of core and holo are in the range 45–55 base pairs. The difference observed between core and holo (53 vs. 45 base pairs, respectively) is probably within the uncertainty limits of the method. We note that site sizes of this magnitude have been obtained for the specific binding of holo to various promoters in nuclease-protection experiments: 41–44 base pairs (reviewed by Gilbert, 1976), 65 base pairs (B. Meyer, personal communication, 1977). Direct observation of polymerase–promoter complexes in the electron microscope yields a physical site size of approximately 45–55 base pairs [calculated from the results of Hirsh and Schleif (1976)]. Finally, early investigations of the saturation level of nonspecific binding yielded values of 55–70 base pairs per bound polymerase, though the overlap correction would reduce these values (Richardson, 1966; Pettijohn and Kamiya, 1967).

In elution experiments to measure binding constants for nonspecific polymerase–ds DNA interactions, typically a 10–20 μL aliquot of enzyme in storage buffer (140 μg of core or 180 μg of holo) was diluted with 1 mL of elution buffer at 4 °C, gently mixed, and applied to a 1-mL DNA–cellulose column. The column was rinsed with 1 mL of elution buffer and then attached to the buffer reservoir and eluted at a constant rate (deHaseth et al., 1977). The initial binding density was such that no more than 15% of available DNA sites was covered. Approximately 25 fractions (volume 2.0 mL) were collected at an elution rate of 11 mL/h. Protein concentrations were determined by fluorescence; the detection limit was approximately 0.2 μg/mL.

To obtain binding constants from the elution data, the logarithm of the percentage of the initial load of protein remaining on the column after elution of fraction i was plotted vs. i, the fraction index (deHaseth et al., 1977a). For *lac* repressor, linear plots were usually obtained, and the slope λ was related to the binding constant, K_{obsd}, using the relationship

$$\lambda = -\log\left(1 + \frac{V_f}{D_T K_{obsd}}\right) \tag{4}$$

where D_T is the total accessible DNA content and V_f is the fraction volume (deHaseth et al., 1977a). For our standard 1-mL ds DNA–cellulose columns, $D_T = 2.1 \times 10^{-7}$ mol of nucleotide and $V_f = 2 \times 10^{-3}$ L. In some cases, curved plots were obtained with repressor, and K_{obsd} was evaluated from the tangent line to the curve at the point where 60% of the initial load of protein remained on the column. Although the *magnitude* of the estimated binding constants varied somewhat with the choice of point on the elution profile through which the tangent lines were drawn, the *dependence* of the binding constants on solution variables (i.e., the relative binding constants) did not (deHaseth et al., 1977a,b). In the present study, curvature was observed in the semilog plots of the data for the elution of both core and holo. For reasons we do not understand, the extent of curvature increases somewhat with increasing flow rate in the range 4.5 to 57 mL/h. At the standard flow rate of 11 mL/h, curvature was more pronounced when more than 30% of the accessible DNA was occupied; the extent of curvature was constant for binding densities below this value (data not shown). Consequently, we worked at low binding densities (≤15% coverage of the DNA), and the lowest flow rate (11 mL/h) deemed practical.

Binding constants for polymerase–DNA interactions were calculated using eq 4 and the slopes (λ) of tangent lines to elution curves at the point where 60% of the initial protein load (held constant within a series of experiments) remained on the column. As a result of the curvature in semilogarithmic plots of elution data (cf. the above discussion and Figures 1 and 5 below), there is an apparent variation in the calculated binding constant of about a factor of 4 between the 90% point and the 30% point on the elution profile. At this stage in the development of the column technique, we are unable to say whether this reflects a real variation in K_{obsd} or not. Our choice of the 60% point was motivated in part by experimental convenience and in part because binding constants calculated at this point for *lac* repressor and RNA polymerase are in agreement with those determined by independent methods (cf. deHaseth et al., 1977a,b; Wensley, Burgess, and Record, in preparation). Values of log K_{obsd} at the 60% point are reproducible to ±0.15 log unit. Values of log K_{obsd} for *lac* repressor calculated by eq 2 from elution profiles agree with the thermodynamically rigorous results of Revzin and von Hippel (1977) to within ±0.15 log unit (deHaseth et al., 1977a). The curvature of the elution profiles for RNA polymerase introduces an additional absolute uncertainty of ±0.3 log unit; consequently, we estimate that absolute binding constants calculated for RNA

polymerase from the 60% point of elution profiles have an absolute accuracy of ±0.45 log unit. Our results should therefore be considered as no more than order of magnitude estimates of the actual nonspecific binding constants. However, in view of the *extreme* sensitivity of these binding constants to monovalent and divalent ion concentrations (see below), this absolute uncertainty is not as serious as it would otherwise appear. Moreover, the major conclusions of this work are obtained from the analysis of relative binding constants, which, if consistently determined, have an accuracy of ±0.15 log unit. As was the case with *lac* repressor, the choice of the standard point on the elution profile at which all binding constants are calculated does not affect the dependence of the binding constants on solution variables, within experimental error.

Measurement of Binding Constants for the Interactions of RNA Polymerase with ss DNA. The interactions of RNA polymerase with single-stranded DNA were investigated using small 2% (w/v) agarose columns containing 1.5 mg of immobilized denatured wheat germ DNA per mL of column volume. The ss DNA-agarose was prepared by the method of Schaller et al. (1972) and was a gift of Dr. J. Jendrisak. Since the method of preparation involves gelation of the agarose under denaturing conditions, little DNA renaturation should occur. ss DNA-agarose was selected in preference to ss DNA-cellulose for this reason and because the agarose matrix is superior to cellulose in the retention of ss DNA (Jendrisak and Burgess, unpublished). However, in trial experiments, ss calf thymus DNA-cellulose and ss calf thymus DNA-agarose gave similar elution profiles to those obtained on the ss wheat germ DNA-agarose columns (data not shown).

Direct determination of the capacity of the ss DNA-agarose column for RNA polymerase was not practical because of the quantities of material required. Richardson (1966) found that fd DNA in solution bound approximately 11 μg of RNA polymerase per μg of DNA. Schaller et al. (1972) found that fd DNA in an agarose matrix bound 7.4 μg of holoenzyme per μg of DNA. Therefore, we assume that two thirds of the column-immobilized DNA (1.0 mg/mL of column volume) is accessible to polymerase. Thus, in the application of eq 4 to the calculation of binding constants from elution data on 1-mL ss DNA-agarose columns, a value of $D_T = 3 \times 10^{-6}$ mol of nucleotides was used.

In the use of ss DNA-agarose columns to determine binding constants, 140 μg of either core or holo was typically applied to a 1.0-mL column. This quantity of protein should cover only ~2% of the accessible ss DNA nucleotides. A flow rate of 17 mL/h was used to elute the columns. Little effect of flow rate on the elution profile was observed over the range 11-37 mL/h.

Comparable or slightly greater curvature was observed in semilogarithmic plots of elution data from ss DNA-agarose columns as compared to ds DNA-cellulose columns (cf. Figure 5 below). As before, binding constants were calculated from slopes of tangent lines to the elution profiles at the point where 60% of the applied protein remained on the column. In view of the curvature of these plots, and the uncertainty in the amount of ss DNA on the column available for polymerase binding (see above), we estimate the uncertainty in absolute values of log K_{obsd} to be ±0.6 log unit. The reproducibility and relative accuracy are again about ±0.15 log unit, however.

Results

Binding Constants for the Nonspecific Interactions of RNA Polymerase with Double-Stranded DNA Are Very Sensitive Functions of the NaCl Concentration. A series of profiles describing the elution of holo and core from ds DNA-cellulose columns as a function of NaCl concentration is given in Figure 1. In both cases, the elution behavior is qualitatively similar to that reported by deHaseth et al. (1977a,b) for the nonspecific interaction of *lac* repressor protein with ds DNA, except that (as discussed above) a somewhat greater curvature is apparent in the polymerase elution data. Binding constants for the nonspecific holo-ds DNA (HD) interaction (K^{HD}_{obsd}) and the core-ds DNA (CD) interaction (K^{CD}_{obsd}) were calculated from tangent lines to the elution profiles at the point where 60% of the applied protein remained on the column, as described under Materials and Methods. For a small number of elution curves not reaching the 60% point (e.g., core at 0.2 M Na^+) a linear extrapolation of the data was used. This provides a minimum estimate of the binding constant at this NaCl concentration. Logarithms of these binding constants are plotted in Figure 2 as a function of the logarithm of the Na^+ concentration. Also included in Figure 2 are values from earlier series of experiments. In the case of holo, the earlier experiments were performed on a sample which was not σ saturated; the molecular ratio of σ factor to core protein was 0.80 ± 0.2 (see above). The two sets of data are virtually coincident, probably due to the fact that core is somewhat more strongly bound in the salt range studied (cf. Figure 1) and consequently does not interfere with the determination of K^{HD}_{obsd}. Equations of the least-squares lines plotted on the figure are

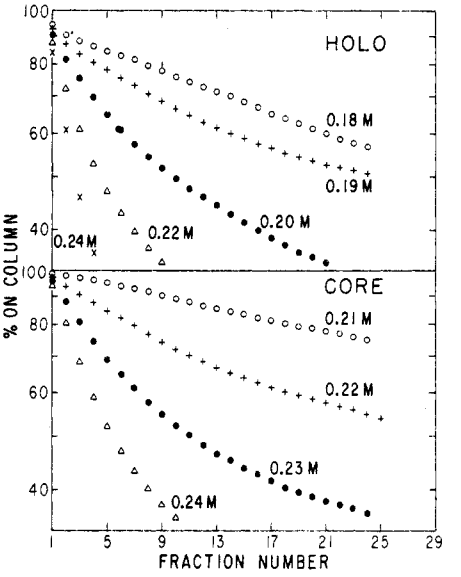

FIGURE 1: The elution of RNA polymerase from ds DNA-cellulose columns; semilog plots. Columns were loaded with polymerase in buffer P containing NaCl to the indicated concentrations and eluted with the same buffer at 4 °C. Top: 115 μg of σ saturated holo (σ content 110 ± 20%) applied. Bottom: 100 μg of core applied.

$$\log K^{HD}_{obsd} = -10.8 \log [Na^+] - 2.5 \quad (5)$$

$$\log K^{CD}_{obsd} = -21.2 \log [Na^+] - 8.5 \quad (6)$$

In the least-squares calculation for holo, the two points at the extremes of the salt range (0.16 and 0.26 M) were omitted; likewise, the data point at 0.18 M for core was omitted from the analysis.

Comparison of eq 5 and eq 1 and 2 indicates that 11 ± 2 monovalent ions (Na^+ and possibly Cl^-) are released in the nonspecific interaction of holo with ds DNA (Record et al., 1976, 1978; deHaseth et al., 1977b). From the numbers of ions released upon complex formation, the number of ionic interactions (m') can be estimated if an assumption is made about the anion release term in eq 1. (At constant pH and $[Mg^{2+}]$

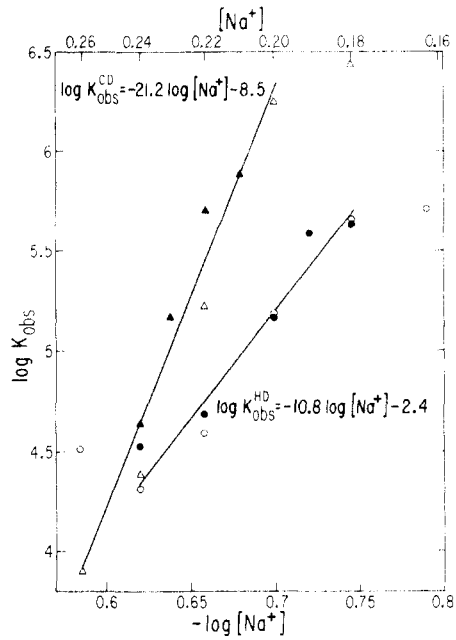

FIGURE 2: The dependences of the observed binding constants for the HD and CD interactions on the concentration of added NaCl; log–log plots. Elution at 4 °C in buffer P: (○) Holo (σ content 80 ± 20%), 180 μg loaded; (●) holo (σ content 110 ± 20%), 115 μg loaded; (△) core, 140 μg loaded; (▲) core, 100 μg loaded.

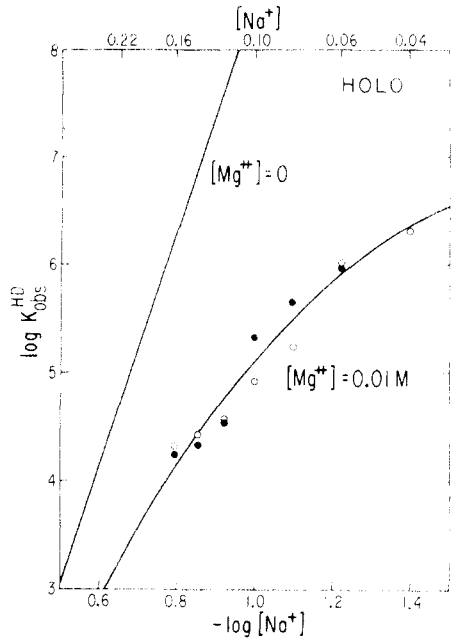

FIGURE 3: The dependence of the observed binding constant of the HD interaction on the concentration of added NaCl in buffer T containing 0.01 M $MgCl_2$; log–log plots. Elution at 4 °C: (○) 180 μg of holo (σ content 80 ± 20%) loaded; (●) 60 μg of holo (σ content 110 ± 20%) loaded. The curve drawn through the data points was calculated as described in the text. The least-squares line (eq 3) describing the binding of holo to DNA in the absence of $MgCl_2$ is shown for comparison.

= 0 this is the only other term that can contribute to the slope of a plot of log K_{obsd} vs. log [Na^+].) As a first approximation, we assume that the anion-release term is zero, i.e., that no anions were bound to the protein in the region of the DNA binding site. Then it can be estimated (by dividing the number of ions released by ψ = 0.88, the number of Na^+ ions thermodynamically bound per phosphate in ds DNA) that a maximum of 12 ± 2 phosphate groups on the DNA are involved in ionic interactions with groups on the protein. The effect of Cl^- release (if any occurs) would be to reduce this estimate.

From eq 6, we observe that approximately 21 ± 3 monovalent ions are released in the interaction of core with ds DNA. This corresponds to a maximum of 24 ± 4 ionic interactions between positively charged groups on the protein and negatively charged phosphates on the DNA. The number of ions released in the core–ds DNA interaction is approximately *twice* as large as that observed in the nonspecific holo–ds DNA interaction. However, the core–ds DNA binding constants are not as large relative to the holo–ds DNA binding constants at fixed NaCl concentrations as one might expect on the basis of this result. If, for example, all the interactions in both cases were ionic and all the ions released were Na^+ ions from the nucleic acid, then one would predict $K^{CD}_{obsd} = (K^{HD}_{obsd})^2$ from a comparison of the numbers of ions released. Instead, K^{CD}_{obsd} is of comparable magnitude to K^{HD}_{obsd} in the salt range investigated, and slight extrapolation of the data in Figure 2 reveals that, at ~0.26 M NaCl, $K^{CD}_{obsd} = K^{HD}_{obsd}$. Above 0.26 M NaCl, $K^{HD}_{obsd} > K^{CD}_{obsd}$. Binding studies by Wensley, Burgess, and Record (in preparation) using a differential analytical sedimentation velocity method confirm these results. Possible physical explanations for these effects include (a) a larger DNA binding site on the core protomer than on holoenzyme, coupled with a conformational change in core or other thermodynamically unfavorable factors (e.g., anion binding) and/or (b) the binding of core dimers to DNA. These possibilities will be considered under Discussion.

An additional set of binding data on the core–DNA interaction was obtained in 0.01 M Tris buffer, pH 8.3, with KCl as the variable monovalent salt (data not shown). Binding constants under these conditions agreed with those in Figure 2 and eq 6 within experimental error. We conclude that the effects of the K^+ and Na^+ ions are similar and that the Tris cation has minimal effect on the interaction at the high K^+ concentrations used in the experiments.

The Effects of pH and Temperature Are Small. In the range 4 to 37 °C, pH 7.8, no significant effect of temperature is seen on either K^{HD}_{obsd} (in $P_{0.20}$) or K^{CD}_{obsd} (in $P_{0.22}$) (data not shown). Since we expect the temperature and pH effects on nonspecific protein–DNA interactions to result primarily from the same molecular events (deHaseth et al., 1977b)—protonation of groups on the protein—it is not surprising that only weak pH dependences are seen as well.

For holo, log K^{HD}_{obsd} decreases by less than 0.5 log unit over the pH range 7.1–8.3 (data not shown), with the slope of a linear least-squares fit to the data, $\partial \log K^{HD}_{obsd}/\partial$ pH = −0.4. For core, a consistent picture is observed, with a slope of −0.3 through data in the pH range of 6.4–9.0 (data not shown). Since the weak pH dependences (with fractional slopes) extend over a wide pH region, they cannot be interpreted in terms of a direct requirement for protonation of groups on the polymerase that participate in DNA binding. In contrast to this, a rather large effect of both pH and temperature was found on the *lac* repressor–ds DNA interaction (deHaseth et al., 1977b), and the binding data were fit by assuming a requirement for protonation of two groups on repressor with p*K*s near 7. Instead, it appears that titration of polymerase changes the free energy of binding merely by permitting the formation of additional ionic interactions, as is the case with pentalysine (cf. Record et al., 1978; Lohman and Record, unpublished).

The Interactions of Holo and Core with Double-Stranded DNA Are Affected Differently by Mg^{2+} ions. Figure 3 summarizes the effect of 0.01 M $MgCl_2$ on the binding constant K^{HD}_{obsd} of the holo–ds DNA interaction. The presence of the

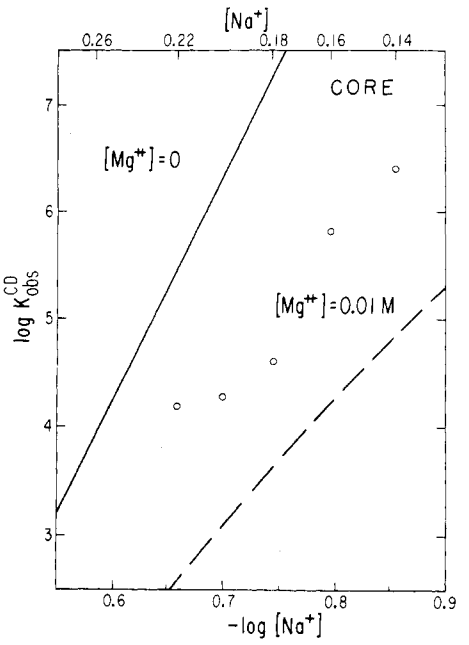

FIGURE 4: The dependence of the observed binding constant of the CD DNA interaction on the concentration of added NaCl in buffer T containing 0.01 M $MgCl_2$; log-log plot. Elution at 4 °C: 140 μg of core loaded. The curve below the data points represents the predicted behavior of the binding constant for the CD interaction in the presence of 0.01 M $MgCl_2$; see text. The least-squares line (eq 4) describing the binding of core to ds DNA in the absence of $MgCl_2$ is shown for comparison.

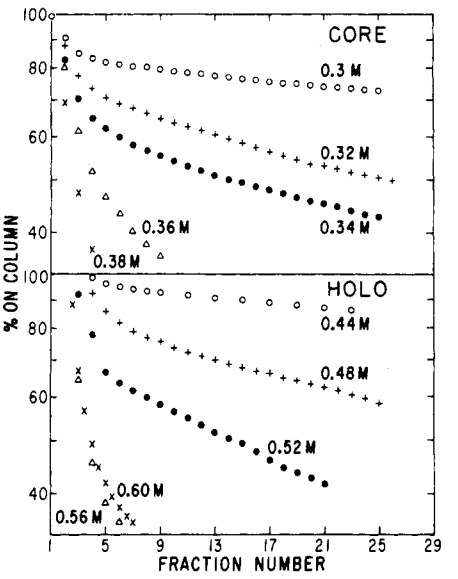

FIGURE 5: The elution of RNA polymerase from ss DNA-agarose columns; semilog plots. Columns were loaded with polymerase in buffer P containing added NaCl to the concentrations indicated and eluted with the same buffer at 4 °C. Top: 140 μg of core loaded. Bottom: 140 μg of holo (σ content 80 ± 20%) loaded. The elution curves for holo have been normalized to reflect the behavior of the 55% of the protein which binds more strongly than the rest; see text.

competitive DNA-binding ligand, Mg^{2+}, reduces K^{HD}_{obsd} at any fixed NaCl concentration and introduces curvature into the log K_{obsd} − log $[Na^+]$ plot. Qualitatively, these effects are the same as those reported by Record et al. (1977) for the nonspecific interaction of *lac* repressor with DNA, and a similar analysis can be made of the binding data. We have used values of $m'\psi = 10.8$ and log K^{HD}_{obsd} (1 M) $\simeq -2.5$ obtained in the absence of Mg^{2+} (eq 5), and calculated from eq 3 the Mg^{2+}–ds DNA binding constants K^{Mg}_{obsd} as a function of NaCl concentration that would be necessary to yield the observed constants K^{HD}_{obsd} in the presence of Mg^{2+}. (This calculation neglects possible anion release by the protein.) The resulting values of K^{Mg}_{obsd} give rise to the least-squares line:

$$\log K^{Mg}_{obsd} = 0.50 - 1.71 \log [Na^+] \qquad (7)$$

Equation 7 is in good agreement with the equations needed to fit the data on the interactions of *lac* repressor (Record et al., 1977) and pentalysine (Lohman and Record, unpublished) with DNA in the presence of 0.003 or 0.01 M $MgCl_2$. The solid curve in Figure 3 is calculated from the binding theory of Record et al. (1977) using eq 7 for K^{Mg}_{obsd}. This curve is in good agreement with the experimental data and permits us to conclude that the only effect of Mg^{2+} on the holo–ds DNA interaction is as a competitor for DNA sites.

Figure 4 compares values of K^{CD}_{obsd} in the presence and absence of 0.01 M $MgCl_2$ as a function of NaCl concentration. It is noteworthy that the presence of Mg^{2+} in this system does not have a large effect on either the magnitude or linearity of log K^{CD}_{obsd} as a function of log $[Na^+]$. The dashed curve in Figure 4 shows the predicted effect of 0.01 M $MgCl_2$ on the core protein–DNA interaction. This curve was calculated from eq 3 using eq 7 to obtain K^{Mg}_{obsd} and eq 6 for the binding behavior of core protein in the absence of Mg^{2+}. The theory in its present form neglects anion binding by the DNA site on the polymerase and specific effects of Mg^{2+} in complex formation.

The measured binding constants K^{CD}_{obsd} are between one and two orders of magnitude larger than those predicted by the theory. A discrepancy of this magnitude suggests that (for core) one or both of our assumptions is incorrect and that either anion effects or a specific role of Mg^{2+} must be included to treat the interaction. These possibilities are considered more fully in the discussion.

Both Core and Holoenzyme Interact More Strongly with Single-Stranded DNA Than with Double-Stranded DNA under Fixed Ionic Conditions. Semilogarithmic plots of data from elutions of core and holoenzyme from ss wheat germ DNA-agarose columns as a function of NaCl concentration are shown in Figure 5. From a comparison of Figures 5 and 1, one sees that the elution of both forms of RNA polymerase from ss DNA columns is shifted to substantially higher NaCl concentrations than those required to elute the enzyme from columns containing ds DNA. In part, this results simply from the fact that there is 14-fold more accessible DNA per milliliter on the ss DNA-agarose columns. Upon calculating binding constants from the elution profiles using eq 4, however, it is clear that the interactions of core and holo with ss DNA are much stronger than the interactions with ds DNA would be at the same NaCl concentrations (see below). Moreover, inspection of Figure 5 shows that the holo–ss DNA interaction is stronger than the core–ss DNA interaction at fixed NaCl concentration.

Binding constants calculated from slopes of tangent lines at the 60% point from the data of Figure 5 and other series of experiments for core enzyme are shown in Figure 6. In a few cases where the elution was not carried out to the 60% point, a linear extrapolation of the semilogarithmic plots was again used to obtain a minimum estimate of the binding constant under these conditions.

The least-squares line through the core data in Figure 6 has the equation

$$\log K^{CS}_{obsd} = -17.6 \log [Na^+] - 4.4 \qquad (8)$$

The holo data can be fit by a line of the same slope, displaced

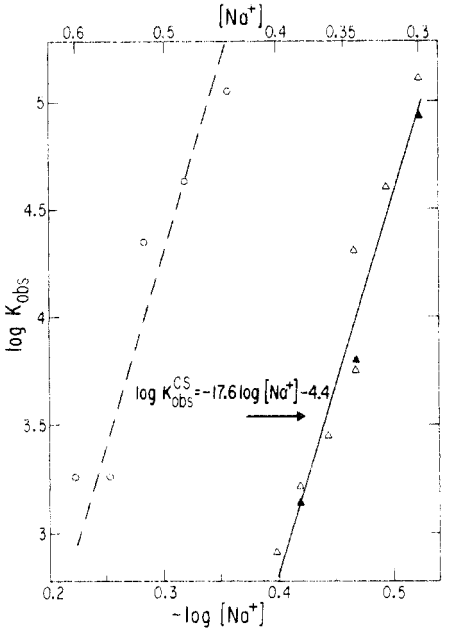

FIGURE 6: The dependences of the observed binding constants for the interactions of holo and core with ss DNA on the concentration of added NaCl; log-log plots. Elution at 4 °C in buffer P. (△) (▲) 140 μg of core loaded. Different symbols apply to different series of experiments: (○) 140 μg of holo (σ content 80 ± 20%) loaded; binding constants calculated for the strong-binding fraction only; see text.

from the core data by +3.4 log units. This is shown as a dashed line in Figure 6 (not a least-squares line).

An anomaly associated with the interaction of holo with ss wheat germ or calf thymus DNA appeared in these studies. After application of holo to the ss DNA–agarose column at an initial low density (coverage of approximately 2% of the available DNA sites) in the salt range 0.44–0.6 M NaCl, approximately 40–50% of the protein eluted immediately (in the first three fractions). The remaining material eluted as expected; elution profiles for holo in Figure 6 reflect the (normalized) elution of the remaining material. This behavior was observed either for ordinary holo preparations (containing approximately 80% of the stoichiometric amount of σ factor) or for a σ-saturated sample and was not changed by a 1-h equilibration period prior to elution of the column. Similar behavior of the enzyme was also observed when the columns were initially loaded at low salt (0.25 M) and then eluted with buffer containing higher salt concentrations. Gel electrophoresis of aliquots from selected fractions showed that the early eluting material had a σ content similar to that eluted later. This raises the possibility that the early eluting enzyme contains partially inactive σ factor. The behavior of holo on ss DNA columns is being further investigated.

From Figure 6 we infer that 18 ± 3 monovalent ions (Na^+, Cl^-) are released in the interaction of core with ss DNA. Using $\psi = 0.71$ for ss DNA (Record et al., 1976), one can estimate a *maximum* number of 25 ± 4 ionic interactions between core and phosphate groups on ss DNA. This number is similar to that estimated above for the interaction of core with ds DNA. In both cases, if anions are released from the DNA site on polymerase, this estimate must be reduced. To attempt to assess the importance, if any, of anion binding, elution experiments were performed in Na_2SO_4 using core and ss DNA–agarose columns. We anticipated that the use of sulfate would alter the contribution (if any) of an anion effect to the slope of eq 8. Elution data were analyzed as above; the least-squares relationship between log K^{CS}_{obsd} and log $[Na^+]$ is

$$\log K^{CS}_{obsd} = -15.8 \log [Na^+] - 2.8 \quad (9)$$

Within experimental error, the slope appears unaffected. If anion binding is a factor, the number of SO_4^{2-} ions released must be comparable, within experimental error, to the number of Cl^- ions released. Comparison of eq 8 and 9 indicates the core–DNA interaction is somewhat stronger at a given Na^+ concentration in Na_2SO_4 than in NaCl. However, this apparent difference can be completely accounted for by correcting for incomplete dissociation of $NaSO_4^-$ (Marcus and Kertes, 1969; Robinson and Stokes, 1955) which has an association constant of 5.0 M^{-1} in the limit of zero ionic strength.

Similar estimates of the numbers of ions released (~18) and maximum ionic interactions (~25) appear reasonable (on the basis of our more limited data) for the holo–ss DNA interaction. The complex of holo with ss DNA is substantially more stable than the complex of core with ss DNA at any salt concentration: $K^{HS}_{obsd}/K^{CS}_{obsd} \simeq 2500$ at 0.48 M NaCl.

The complexes of both core and holo with ss DNA are substantially more stable than their complexes with ds DNA. For core, extrapolation of eq 6 and 8 to 0.28 M NaCl, which represents the smallest extrapolation of the two sets of data possible, gives $K^{CS}_{obsd}/K^{CD}_{obsd} \simeq 100$. (This ratio may decrease with decreasing NaCl concentration.) A much longer extrapolation of one or both sets of holo binding data is required to estimate $K^{HS}_{obsd}/K^{HD}_{obsd}$ at constant NaCl concentration. Moreover, comparison of Figures 2 and 6 indicates that this ratio changes substantially with changing NaCl concentration. At 0.48 M NaCl, we measure log $K^{HS}_{obsd} = 4.6$. By extrapolation we estimate log $K^{HD}_{obsd} = 1.0$ at 0.48 M NaCl. Therefore, $K^{HS}_{obsd}/K^{HD}_{obsd} \simeq 4 \times 10^3$. At 0.28 M NaCl, this ratio appears to approach 10^5. It is clear that both holo and core are, in principle, melting proteins (von Hippel and McGhee, 1972; McGhee, 1976).

Discussion

The Nonspecific Interaction of Holo with Double-Stranded DNA Appears to be Purely Electrostatic. Of the various association reactions between forms of RNA polymerase and DNA, the interaction between holo and ds DNA appears to be the most straightforward thermodynamically. The dependences of K^{HD}_{obsd} on the Na^+ concentration in the presence and absence of Mg^{2+} are consistent with a situation in which the association reaction is driven by the release of ions from the DNA and in which a maximum of 12 ± 2 ionic interactions between protein and ds DNA are formed in the complex (m'_{HD} = 12 ± 2). Krakow et al. (1976) report studies by Krakow, and Jovin and Scheit, which show that DNA binding protects a substantial number of lysine groups on polymerase from chemical modification. This suggests that at least some of the charge interactions might be between lysine groups on the protein and phosphate groups on the DNA. The logarithm of the extrapolated association constant obtained from eq 5, log K^{HD}_{obsd} (1 M) = −2.5, is within experimental error of the value estimated by Record et al. (1976, 1978) from model oligopeptide data for the formation of 12 ionic interactions involving lysine groups [log K_{obsd} (1 M) = −1.6]. The binding data in Mg^{2+} are fit by assuming that log K^{HD}_{obsd} (1 M) = −2.5 and $m'_{HD} = 12$. There is very little effect of pH or temperature on K^{HD}_{obsd}. In a sense the situation is comparable to, but less complex than, the nonspecific interaction of *lac* repressor with ds DNA, which is driven by ion release from the DNA and results in the formation of 12 ± 2 ionic interactions but in which there is a requirement for protonation of two groups on repressor. This affects the apparent value of the

thermodynamic binding constant obtained at a given pH (deHaseth et al., 1977b; Record et al., 1977). In both cases, the data are consistent with a picture of the interaction in which anion and Mg^{2+} effects on the protein are unimportant and the only role of Mg^{2+} is as a competitive ligand (with the proteins) for DNA sites.

The Interaction of Core with Double-Stranded DNA Is Thermodynamically Complex. The experiments of this paper suggest that the core polymerase–ds DNA interaction is both qualitatively different and somewhat more complex thermodynamically than the holoenzyme–DNA interaction discussed above. In NaCl, approximately twice as many ions are released in the CD interaction as in the HD interaction. The thermodynamic complexity of the CD interaction is shown by the unfavorable intercept of eq 6 in the 1 M NaCl standard state and by our inability to fit the CD binding data in 0.01 M MgCl with the same model and approximations used successfully in the HD interaction (above) and in the nonspecific interaction of *lac* repressor with ds DNA (deHaseth et al., 1977b; Record et al., 1977), e.g., no anion effects and no specific effects of Mg^{2+} on the protein–DNA complex.

Two possible explanations for the CD thermodynamic binding data are the following.

(a) Although holo may bind to DNA as a monomer under all salt conditions investigated (Krakow and von der Helm, 1970; Zarudnaya et al., 1976), it is possible that core binds to ds DNA as a dimer, using holo-like binding sites on both protomers to interact with the DNA. In this model, the two-fold increase in number of low-molecular-weight ions released in the CD as compared to the HD interaction is readily explained. Furthermore, the unfavorable intercept is interpreted in terms of the free energy expended to convert protomer to dimer, since protomer is the predominant form at the NaCl and core concentrations used in this work (Berg and Chamberlin, 1970; Wensley, Burgess, and Record, in preparation). The specific effect of Mg^{2+} could be explained as a stabilization of the aggregated form; polymerase aggregation is indeed favored in the presence of Mg^{2+} (Wensley, Burgess, and Record, in preparation). However, the dimer model is made less tenable by the observation of Wensley, Burgess, and Record (in preparation) that $K^{CD}{}_{obsd} < K^{HD}{}_{obsd}$ above 0.27 M NaCl in the absence of Mg^{2+}. If core were binding as a dimer of two holo-like sites at lower salt, one would expect a change in core binding behavior at this point. The simple dimer model should not permit $K^{CD}{}_{obsd}$ to become less than $K^{HD}{}_{obsd}$.

(b) The DNA binding site on the core protomer may be approximately twice as large (i.e., contain twice as many positively charged groups) as the site on holo. This would imply that the σ subunit either occupies or otherwise excludes approximately half of the DNA binding site when it binds to core. The question then arises as to the origin of the unfavorable nonelectrostatic term in the CD binding free energy. This could be accounted for if a conformational change in either protein or DNA were required for the interaction to occur. Although this possibility is rendered less likely by the lack of a temperature dependence of $K^{CD}{}_{obsd}$ and our failure to detect any fluorescence change in the CD interaction (data not shown), it has a partial analogy in the hemoglobin field. The effector ligand 2,3-diphosphoglycerate interacts electrostatically with positively charged groups on hemoglobin; only in the deoxy conformation is the relative orientation of these groups proper for strong binding of the ligand (Benesch et al., 1969). It is possible that core undergoes an analogous conformational change in order to bind to DNA. The anomalous effect of Mg^{2+} on the CD interaction could be accounted for if Mg^{2+} shifted the putative core conformational equilibrium to favor the DNA binding form. One can further speculate that the σ subunit locks the molecular conformation of RNA polymerase in the form that binds to DNA but at the same time excludes part of the binding site accessible on core. Indeed, Wu et al. (1976), using a fluorescent probe on σ, observed biphasic kinetics in the σ–core reaction and attributed the slower phase to a unimolecular conformational change following the bimolecular interaction of core and σ.

A more mundane explanation of the apparent unfavorable nonelectrostatic term in the CD interaction would be anion binding. If approximately four to six anions are released in the formation of the CD complex, then m' is reduced from 24 to 18–20 and the unfavorable term can be accounted for (calculations not shown). However this explanation does not readily account for the Mg^{2+} effect on the CD interaction and seems inconsistent with the remainder of the polymerase–DNA binding data, in which anion effects do not appear to be of importance.

We conclude that the CD interaction involves substantially more ionic interactions than does the HD interaction. The probable explanation of this effect is that the core protomer has more ionic groups accessible to bind to DNA; these groups may be covered or otherwise made inaccessible by the presence of σ subunit in holo. Since the physical site size for the CD interaction appears to be approximately 50 base pairs or 100 nucleotides (cf. Materials and Methods), this large number of ionic interactions (18–24) is certainly not unreasonable.

Comparison of the Nonspecific Interactions of RNA Polymerase with Single-Stranded and Double-Stranded DNA. Application of the column method (deHaseth et al., 1977a) to measure binding constants for RNA polymerase–DNA interactions required us to select ionic conditions such that $10^4\ M^{-1} \leq K_{obsd} \leq 10^6\ M^{-1}$ (ds DNA–cellulose columns) or $10^3\ M^{-1} \leq K_{obsd} \leq 10^5\ M^{-1}$ (ss DNA–agarose columns); the difference in range is due to the fact that the two matrices contain different amounts of DNA (see above). Extrapolations of the binding data are required to compare binding constants for the various interactions at the same ion concentrations. Since the existence of anion binding would introduce curvature in the log–log plots, linear extrapolation of these plots (Figures 2 and 6) is only justified if anion binding can be ignored. With this reservation, we proceed to a comparison of the nonspecific interactions of holo and core with ds and ss DNA.

(a) Comparison of the HS and HD Interactions. The HD interaction involves the release of 11 ± 2 monovalent ions, the formation of a maximum of 12 ± 2 ionic interactions, and no substantial nonelectrostatic component (see above). On the other hand, the HS interaction involves the release of substantially more monovalent ions ($\sim$18), as many as twice the number of ionic interactions (a maximum of $\sim$24), and a binding constant $K^{HS}{}_{obsd}$ which is sufficiently larger than $K^{HD}{}_{obsd}$ at any NaCl concentration to indicate that there is a favorable nonelectrostatic component to the holoenzyme–ss DNA interaction. We estimate a minimum favorable nonelectrostatic contribution to log $K^{HS}{}_{obsd}$ of approximately 3 log units (or $\sim$4 kcal of free energy) and note that if the observed binding constants include a contribution from the displacement of anions then the actual nonelectrostatic contribution is larger in magnitude. This nonelectrostatic component may involve interactions between holoenzyme and ss DNA which are related to those stabilizing the open complexes between holoenzyme and promoters (Chamberlin, 1976; see below). We attribute the greater number of ionic interactions in the HS complex than in the HD complex to the greater flexibility of ss DNA than ds DNA. It is of course unknown whether the additional interactions are with miscellaneous sites

on the surface of the protein or if the ss DNA binds to several physiologically relevant sites on the protein. Since the ss DNA saturation data of Richardson (1966) suggest a physical site size of ~120 nucleotides for RNA polymerase on ss fd DNA, the existence of up to 25 ionic interactions is not surprising.

(b) Comparison of the CS and CD Interactions. Whereas the CD interaction appears thermodynamically complex (see Results), the CS interaction more nearly fits the simple picture of an electrostatic protein–nucleic acid interaction. Neither anion effects nor protein–protein interactions need to be introduced to explain the magnitudes and [NaCl] dependence of K^{CS}_{obsd}. Replacement of Cl^- by SO_4^{2-} has only a minor effect, if any. Equations 8 and 9 may be interpreted in terms of the formation of approximately 25 ionic interactions, the release of approximately 17 monovalent ions, and no nonelectrostatic component to the CS interaction.

It is therefore difficult at this stage in the investigation of these interactions to propose a consistent physical model for the CS and CD interactions. We note, however, that the results on the CS interaction do not necessarily invalidate either model (dimer binding or protomer binding) suggested to explain the thermodynamic data on the CD interaction. The CS interaction was of necessity investigated at higher NaCl concentrations than those used in the study of the CD interaction. Higher salt concentrations shift the core monomer–dimer equilibrium in the direction of dissociation; therefore, the binding of dimers may not be thermodynamically feasible (Berg and Chamberlin, 1970; Wensley, Burgess, and Record, in preparation). This would imply that the similarity in the calculated number of ionic interactions in the CD and CS complexes would be only coincidental. Alternatively, the greater flexibility of ss DNA may mean that the CS complex can form the same number of ionic interactions as the CD complex without the appearance of a thermodynamically unfavorable factor (as observed in the CD interaction). Or, if an anion effect were the origin of the unfavorable contribution to the CD interaction, that effect might be coincidentally masked by a compensating term in the CS interaction. Since the release of as few as four to six anions could explain the CD binding data, the replacement of Cl^- by SO_4^{2-} might make too small a difference in the number of anions released to detect experimentally.

Comparison of the Specific and Nonspecific Interactions of RNA Polymerase with DNA. Only limited data are available on the interactions of RNA polymerase holoenzyme with specific promoter sites. Hinkle and Chamberlin (1972a) estimated a binding constant for the holo–T7 promoter (HP) interaction of 10^{12}–10^{14} M^{-1} in 0.01 M Mg^{2+}, 0.05 M K^+, 37 °C. For five fd RF promoters, present on restriction fragments in the size range 380–1500 base pairs, Seeburg et al. (1977) calculated equilibrium constants K^{HP}_{obsd} from ratios of rate constants for the holo–promoter interactions, determined using the filter-binding method. At 0.01 M $MgCl_2$, 0.12 M KCl, values of K^{HP}_{obsd} ranged from 2×10^8 M^{-1} to 2×10^{11} M^{-1}. Increasing the KCl concentration to 0.16 and 0.20 M decreased the stabilities of the complexes by 3- to 5-fold and 20- to 30-fold, respectively. For comparison, in 0.01 M $MgCl_2$ we obtain $K^{HD}_{obsd} \simeq 4 \times 10^4$ M^{-1} at 0.12 M NaCl and $K^{HD}_{obsd} \simeq 2 \times 10^4$ M^{-1} at 0.16 M NaCl (cf. Figure 3). Under these ionic conditions, which should be in the physiological region, the specificity ratio $K^{HP}_{obsd}/K^{HD}_{obsd}$ therefore ranges from approximately 10^4 to 10^7 for the various fd promoters. Note that only the strongest binding fd promoter complexes appear to approach the relative specificity of the *lac* repressor–operator interaction, for which the ratio of specific and nonspecific association constants is in the range 10^8–10^9 (von Hippel et al., 1974; Lin and Riggs, 1975; Record et al., 1977).

Comparisons with Previous Work. Estimates of binding constants for the nonspecific interactions of holo and core with ds DNA have been provided by Hinkle and Chamberlin (1972a,b) and Chamberlin (1976). At 0.01 M $MgCl_2$, 0.05 M NaCl, they estimate $K^{HD}_{obsd} \simeq 10^6$–$10^7$ M^{-1} and $K^{CD}_{obsd} \simeq 10^{11}$ M^{-1}. We measure $K^{HD}_{obsd} \simeq 10^6$ M^{-1} under these conditions and estimate by extrapolation that $K^{CD}_{obsd} \simeq 10^{10}$ M^{-1} (cf. Figure 4). Recently, Williams and Chamberlin (1977) have investigated the binding of RNA polymerase holoenzyme to a promoterless fragment of T7 DNA by electron microscopy. Under the above ionic conditions, they report a nonspecific binding constant of approximately 3×10^4 M^{-1} at 37 °C, obtained upon slow dilution of the enzyme from its storage buffer and brief (<5 min) incubation with the DNA fragment. They also report that "if dilution is rapid and occurs in a single step, or if incubation extends beyond 5 min, a substantial portion [~25% in their experiments] of RNA polymerase is converted to a form that binds randomly with much greater affinity (about 10^8 M^{-1})." Although the relationship between these results and ours is unclear, it is conceivable that their tight-binding form of holoenzyme corresponds to the 25 ± 10% of the holoenzyme preparation used in our work which binds more tightly to nonspecific ds DNA than the remainder and which we assumed to be core polymerase. However, there is a significant difference between the nonspecific binding constant for holoenzyme reported by Williams and Chamberlin (1977) and that observed in this investigation under the same ionic conditions.

Both ds and ss DNA columns have been used in the purification of holo and core RNA polymerase (cf. Burgess, 1976, for a review). In most cases, these columns have been eluted with a relatively steep salt gradient or with a discontinuous step gradient. Nevertheless, the results of such experiments are in general agreement with ours. On a ds DNA–cellulose column in the absence of Mg^{2+}, holo and core elute together in a broad peak from 0.30 to 0.45 M K^+ or Na^+ (Mukai and Iida, 1973; Burgess and Jendrisak, 1975; Burgess, 1976). We find (cf. Figure 1) that below 0.26–0.27 M Na^+, $K^{HD}_{obsd} < K^{CD}_{obsd}$. However, above 0.27 M Na^+, $K^{HD}_{obsd} > K^{CD}_{obsd}$ (Wensley, Burgess, and Record, in preparation). In view of this crossover at a salt concentration just below the point of elution from the preparative column, the lack of separation of core and holo is reasonable. Using a shallow salt gradient, we have found (data not shown) that core and holo elute together over the range 0.25–0.32 M NaCl, with the peak centered at 0.27 M NaCl, which is very near the crossover salt concentration. Clearly, the salt concentration at which polymerase will elute from a preparative column will depend on the steepness of the gradient relative to the size and DNA content of the column. Since core and holo elute together, however, we may conclude that elution from a typical ds DNA cellulose column occurs at an effective binding constant of approximately 10^4 M^{-1} (cf. Figure 1).

Mukai and Iida (1973) found that in the presence of 0.01 M $MgCl_2$ separation of holo and core was obtained on ds DNA–cellulose, with holo eluting at 0.15 M NaCl and core at 0.25 M NaCl in the gradient. From Figures 3 and 4, we find that these salt concentrations correspond to values of K^{HD}_{obsd} and K^{CD}_{obsd} of approximately 10^4 M^{-1}. Our results agree with the order of elution observed by Mukai and Iida (1973) and suggest again that elution in a salt gradient occurs at a binding constant near 10^4 M^{-1} on a typical ds DNA–cellulose column.

Richardson (1966) found that the ratio of binding constants for the interactions of RNA polymerase with ss fd DNA and ds T7 DNA was approximately 20:1 on a weight or nucleotide basis (37 °C, 0.05 M KCl, 0.005 M $MgCl_2$). Although neither

TABLE I: RNA Polymerase-DNA Interactions.

Complex	No. of monovalent ions released	Max no. of ionic interact.	Binding constant K_{obsd} (M^{-1}) in 0.2 M NaCl or KCl		Nonelectrostatic binding free energy[d]
			No $MgCl_2$	0.01 M $MgCl_2$	
HD	11 ± 2	12 ± 2	1×10^5	(3×10^3)[c]	None
HS	18 ± 5	25 ± 7	(2×10^{11})[b]		Favorable
HP[a]				$\sim 10^7$ to $\sim 10^{10}$	
CD	21 ± 3	24 ± 3	2×10^6	1×10^4	Unfavorable
CS	18 ± 3	25 ± 4	(8×10^7)[b]		None

[a] Data of Seeburg et al. (1977); fd RF promoters, 37 °C, pH 8. [b] Estimated by linear extrapolation of Figure 6. [c] Estimated from Figure 3. [d] Obtained from the sign and magnitude of the nonelectrostatic component of log K_{obsd} after correction for the contribution of the appropriate number of lysine-like ion pairs (see text).

the extent of σ saturation of the polymerase nor the extent of promoter binding to T7 DNA in these experiments is known, this result is at least qualitatively consistent with our findings that, at all reasonable salt conditions, both holo and core interact substantially more strongly with ss DNA than with nonspecific sites on ds DNA.

Nüsslein and Heyden (1972) purified RNA polymerase on ss DNA-agarose columns. In a KCl gradient, core eluted at 0.5 M and holo at 0.7 M. Arndt-Jovin et al. (1975) found that core eluted at 0.3 M NaCl and holo at 0.8 M NaCl from a ss DNA-Sepharose column. These results, too, are in qualitative agreement with our findings (Figure 6), which also suggest that elution occurs at a somewhat lower binding constant from a ss DNA column ($K_{obsd} \sim 10^2$–10^3 M^{-1}). This is not unreasonable, in view of the substantially higher DNA concentrations of the ss DNA column matrices.

Possible Biological Significance of the Observed Steep Salt Dependences. The facts that the DNA binding constants for both core and holo are extremely sensitive to ionic conditions and that the HD and CD interactions are differentially affected by alterations in the ionic environment open up the possibility that transcription may be controlled in part by variations in the cellular ionic environment. Drastic changes in the pattern of gene expression resulting from a large alteration in the intracellular ionic conditions have been postulated to occur as a result of alterations in the cell membrane upon infection of cultured mouse cells by picorna viruses (Carrasco and Smith, 1976).

Conclusions

Many of the principal conclusions of this investigation are summarized in Table I, which serves to emphasize the major role of the release of low-molecular-weight ions in driving these association reactions. It is worthwhile to mention again that the interpretation of these data in terms of numbers of ionic interactions and nonelectrostatic binding components (columns 3 and 6) must remain tentative until the assumptions in the calculations (most notably regarding the absence of anion binding) are tested. However, the general conclusions of this work are independent of these assumptions. (1) At 0.01 M $MgCl_2$, 0.20 M Na^+ or K^+ (this ionic condition is chosen as the best approximation to physiological conditions possible with the available data), the nonspecific HD and CD interactions are of comparable strength, although the dependences of the two interactions upon ion concentrations are very different. (At low salt concentrations, the CD interaction is much stronger; see above.) (2) The CD complex involves approximately twice the number of ionic interactions as the HD complex. (3) Both core and holo bind more strongly to ss DNA than to ds DNA and are, in principle, melting proteins. (4) The HS interaction is sufficiently stronger than the CS interaction at any salt concentration to indicate a favorable nonelectrostatic component of the interaction, which may be physiologically relevant. (5) The specificity ratio $K^{HP}_{obsd}/K^{HD}_{obsd}$ of 10^4–10^7 is less for any fd RF promoter under assumed physiological conditions than the specificity ratio (10^8–10^9) calculated for *lac* repressor protein (von Hippel et al., 1974; Lin and Riggs, 1975; Record et al., 1977).

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Nucleotide Sequence of Phenylalanine Transfer RNA from *Schizosaccharomyces pombe*: Implications for Transfer RNA Recognition by Yeast Phenylalanyl-tRNA Synthetase†

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ABSTRACT: The nucleotide sequence of *Schizosaccharomyces pombe* $tRNA^{Phe}$ was determined to be pG-U-C-G-C-A-A-U-G**-G*-U-G-ψ-A-G-D-D-G-G-G-A-G-C-A-ψ-G*-A-C-A - G - A - Cm-U-Gm-A-A-Y-A-ψ-m^5C-U-G-U-U-G-m^7G-U* - C - A - U-C-G-G-T-ψ-C-G-A-U-C-C-C-G-G-U-U-U-G-U-G-A-C-A-C-C-A_{OH}. This sequence differs from that of *S. cerevisiae* $tRNA^{Phe}$ in 27 nucleotides. *Saccharomyces cerevisiae* phenylalanyl-tRNA synthetase aminoacylates both the homologous $tRNA^{Phe}$ and *S. pombe* $tRNA^{Phe}$; the reactions have similar K_m and V_{max} values. However, the nucleotide sequence in the D stem is different in the two tRNAs. This region was proposed by Roe, B., et al. [(1973) *Biochemistry 12,* 4146-4154] to be the major recognition site for yeast phenylalanyl-tRNA synthetase, but the present results cast doubt on the validity of this hypothesis.

Mischarging, the aminoacylation of tRNA with the wrong amino acid, has often been observed in in vitro reactions, especially when aminoacyl-tRNA synthetases and tRNA from different sources are used (Jacobson, 1971). Dudock et al. (1970) and Taglang et al. (1970) discovered that yeast phenylalanyl-tRNA synthetase will charge *E. coli* $tRNA^{Val}$ with phenylalanine. The ability of this enzyme to charge many cognate and noncognate tRNA species has been used by Dudock in an approach to define the nucleotide sequence responsible for proper recognition by yeast phenylalanyl-tRNA synthetase. Eleven pure tRNA species could be aminoacylated by this enzyme (Roe et al., 1973). A comparison of their nucleotide sequences revealed some common structural features (the same base found in the same position in the different

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