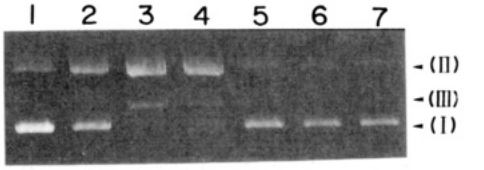

FIGURE 3: Strand scission of ϕX174 RF DNA by trace amounts of iron in the presence of BLM and DTT (lanes 3 and 4). Individual reaction mixtures contained 0.5 μg of DNA, 50 mM sodium cacodylate buffer (pH 7.0), and the following additions: lane 1, none; lane 2, 0.3 μM $FeSO_4$ and 30 μM BLM; lane 3, 0.3 μM $FeSO_4$, 30 μM BLM, and 50 μM DTT; lane 4, 0.06 μM $FeSO_4$, 30 μM BLM, and 50 μM DTT; lane 5, 0.06 μM $FeSO_4$, 30 μM BLM, 50 μM DTT, and 50 μM deferoxamine; lane 6, 0.06 μM $FeSO_4$ and 30 μM BLM; lane 7, 0.3 μM $FeSO_4$ and 50 μM DTT. The reaction samples were incubated at 25 °C for 30 min under aerobic conditions and then analyzed by 1% agarose gel electrophoresis.

of bleomycin is iron not copper.

References

Antholine, W. E., Solariam, D., Sayan, L. A., & Peterring, D. H. (1982) *J. Inorg. Biochem. 17*, 75–94.

Burger, R. M., Freedman, J. H., Horwitz, S. B., & Peisach, J. (1984) *Inorg. Chem. 23*, 2217–2219.

Ehrenfeld, G. M., Rodriguez, L. O., Hecht, S. M., Chang, C., Basus, V. J., & Oppenheimer, N. J. (1985) *Biochemistry 24*, 81–92.

Freedman, J. H., Horwitz, S. B., & Peisach, J. (1982) *Biochemistry 21*, 2203–2210.

Ishizu, K., Murata, S., Miyoshi, K., Sugiura, Y., Takita, T., & Umezawa, H. (1981) *J. Antibiot. 34*, 994–1000.

Kanao, M., Tomita, S., Ishihara, S., Murakami, A., & Okada, H. (1973) *Chemotherapy (Tokyo) 21*, 1305–1310.

Kilkuskie, R. E., Macdonald, T. L., & Hecht, S. M. (1984) *Biochemistry 23*, 6165–6171.

Kuwahara, J., Suzuki, T., & Sugiura, Y. (1985) *Biochem. Biophys. Res. Commun.* (in press).

Melnyk, D. L., Horwitz, S. B., & Peisach, J. (1981) *Biochemistry 20*, 5327–5331.

Oppenheimer, N. J., Chang, C., Rodriguez, L. O., & Hecht, S. M. (1981) *J. Biol. Chem. 256*, 1514–1517.

Sausville, E. A., Peisach, J., & Horwitz, S. B. (1978a) *Biochemistry 17*, 2740–2746.

Sausville, E. A., Stein, R. W., Peisach, J., & Horwitz, S. B. (1978b) *Biochemistry 17*, 2746–2754.

Scheulen, M. E., Kappus, H., Thyssen, D., & Schmidt, C. G. (1981) *Biochem. Pharmacol. 30*, 3385–3388.

Sugiura, Y. (1979) *Biochem. Biophys. Res. Commun. 90*, 375–383.

Sugiura, Y., Ishizu, K., & Miyoshi, K. (1979) *J. Antibiot. 32*, 453–461.

Sugiura, Y., Suzuki, T., Kuwahara, J., & Tanaka, H. (1982) *Biochem. Biophys. Res. Commun. 105*, 1511–1518.

Takahashi, K., Yoshida, O., Matsuda, A., & Umezawa, H. (1977) *J. Antibiot. 30*, 861–869.

Wong, A., Huang, C.-H., & Crooke, S. T. (1984) *Biochemistry 23*, 2946–2952.

Regulation of the Kinetics of the Interaction of *Escherichia coli* RNA Polymerase with the λP_R Promoter by Salt Concentration†

Jung-Hye Roe and M. Thomas Record, Jr.*

Departments of Chemistry and Biochemistry, University of Wisconsin, Madison, Wisconsin 53706

Received May 13, 1985

ABSTRACT: The rate of formation of transcriptionally competent open complexes between *Escherichia coli* RNA polymerase (RNAP) and the λP_R promoter is extraordinarily sensitive to the nature and concentration of the electrolyte ions in the solution. The pseudo-first-order time constant of open complex formation τ_{obsd}, determined in excess RNAP at 25 °C as a function of NaCl concentration, is proportional to the concentration product $[Na^+]^{12}[RNAP]^{-1}$. Consequently, τ_{obsd} is *far* more sensitive to changes in the salt concentration than to changes in the concentration of RNAP. The origin of this effect is the release of the thermodynamic equivalent of 12 monovalent ions in the process of closed complex formation at the λP_R promoter. In more complex ionic mixtures, ion-specific stoichiometric effects on τ_{obsd} are observed. These are *not* ionic strength effects but are instead both valence and species specific. Both the association and dissociation rate constants of RNAP at the λP_R promoter are strongly salt dependent, varying (in NaCl) as $[Na^+]^{-12}$ and $[Na^+]^8$, respectively. Consequently, the equilibrium constant characterizing open complex formation at this promoter varies with $[Na^+]^{-20}$. Electrostatic interactions and counterion release are the major contributors to the binding free energy driving open complex formation in a dilute salt solution. Since the in vivo ionic environment of *E. coli* (and other cells) is highly variable, these large salt effects are almost certainly of physiological significance. Variations in the intracellular concentrations of inorganic and organic ions, including polyamines, must exert both global and also promoter-specific regulatory effects on the initiation of transcription, as well as on numerous other protein–nucleic acid interactions.

Numerous physical and chemical variables act to regulate the rate of open complex formation and transcription initiation at a promoter. For the P_R promoter of bacteriophage λ, these include the concentration of free RNA polymerase (RNAP) holoenzyme (Hawley & McClure, 1980; Roe et al., 1984), the extent of repression by the cI gene product (λ repressor) or by cro protein (Johnson et al., 1981; Ptashne et al., 1980; Shea & Ackers, 1985), the temperature (Roe et al., 1984, 1985),

† This work was supported by NIH Grant GM23467.

* Address correspondence to this author at the Department of Chemistry.

0006-2960/85/0424-4721$01.50/0

and the salt concentration (Roe et al., 1984). Relatively small effects of DNA superhelicity (Botchan, 1976) and of pH (Roe, 1984) have also been observed. Recently, a physical–chemical model for the behavior of the λP_R promoter and the entire λO_R control system as a function of the concentrations of λ and cro repressors and of RNA polymerase has been proposed (Shea & Ackers, 1985). One purpose of this paper is to demonstrate that the concentrations of ionic solutes have the potential to play an equally important role in regulation of the kinetics of formation of transcriptionally competent complexes at promoters.

At a thermodynamic level, protein–nucleic acid interactions may be modeled as ion-exchange reactions, in which the neutralization of polyelectrolyte charge that occurs upon forming the noncovalent complex is accompanied by the release of large numbers of electrolyte ions to the bulk solution (Record et al., 1976, 1978, 1985):

$$\text{protein} + \text{nucleic acid} \rightleftarrows \text{complex} + n \text{ ions} \quad (1a)$$

where typically $n \gg 1$. Consequently, the equilibrium extent of interaction is a sensitive function of the bulk salt concentration. The partitioning of this effect between the apparent rate constants for the association and dissociation reactions depends on the details of the mechanism and has been used by us as the basis for the proposal of a minimal three-step mechanism for formation of an open complex (RP_o) between RNA polymerase (R) and the promoter (P) (Roe et al., 1984, 1985):

$$R + P \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} I_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} I_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} RP_o \quad (2)$$

The intermediate I_1 is a "closed" complex. Formation of RP_o involves sequential conformational changes in the protein (to form the intermediate I_2 and nucleate DNA opening) and then in the DNA.

In this paper we quantify the effects of monovalent salt on the kinetic parameters for formation and dissociation of open complexes at the λP_R promoter. By comparison with previous results obtained under other ionic conditions (Roe et al., 1984), we demonstrate that the large salt effects on these parameters are not general ionic strength effects but rather ion-specific effects that can be modeled by multiple-equilibrium binding theory and a stoichiometric formulation (cf. eq 1a) of the protein–DNA ion-exchange reaction. We cite evidence to indicate the variability of the in vivo ionic environment of *Escherichia coli* and of cells of other organisms and argue that the extraordinary sensitivity of protein–DNA interactions to the ionic environment provides both a global and also a promoter-specific control mechanism in the regulation of transcription, at the same time that the ionic interactions which give rise to this effect provide a major source of binding free energy under the usual range of physiological ionic conditions.

Materials and Methods

E. coli RNA Polymerase (RNAP). RNA polymerase holoenzyme (80 ± 10% σ saturated) was the gift of Dr. R. R. Burgess. The enzyme was 40 ± 10% active, as judged by transcription (Chamberlin et al., 1979) or equilibrium binding (Roe et al., 1984) assays performed at the time of the experiments reported here. All polymerase concentrations are expressed in terms of the concentration of active enzyme.

λP_R Promoter. The isolation of the *Hae*III 890 base pair (bp) fragment containing the λP_R promoter has been previously described (Roe et al., 1984). Purified fragments were labeled in vitro with [^{3}H]dCTP by the method of O'Farrell et al. (1980). Specific activities of the labeled DNA fragment were $\sim 2 \times 10^6$ cpm/μg.

Association and Dissociation Kinetic Experiments. All experiments were performed at 25 °C in a Mg^{2+}-free buffer containing 0.01 M sodium 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (NaHepes) (pH adjusted to 7.5 at 25 °C with HCl), 0.18–0.26 M NaCl, 10^{-4} M dithiothreitol (DTT), and 100 μg/mL bovine serum albumin (BSA).

Association kinetic experiments were performed by adding excess RNAP (final active concentration 0.7–50 nM) to a solution of the ^{3}H-labeled 890-bp λP_R fragment (final concentration 0.1–0.4 nM). The extent of open complex formation was monitored as a function of time by heparin-competition or nucleotide-stabilization filter binding assays (Roe et al., 1984). In nucleotide-stabilization assays, samples (10–20 μL) were added to nucleotide triphosphate (NTP) buffer [140–180 μL, containing 10^{-4} M CpA, UTP, and GTP, 0.04 M tris(hydroxymethyl)aminomethane (Tris) (pH 8), 0.01 M $MgCl_2$, 0.12 M KCl, 10^{-4} M DTT, and 100 μg/mL BSA] and incubated for 10 s at 37 °C, followed by filtration and a subsequent high-salt wash (10 mM Tris and 0.8 M NaCl) (Roe et al., 1984). Maximum extents of filter retention were 85 ± 5% (heparin assay) and 70 ± 8% (nucleotide-stabilization assay). Background retentions in the two assays were 3 ± 2% and 10 ± 3% of the total radioactivity, respectively.

For dissociation kinetic experiments, open complexes were formed at the λP_R promoter by incubating the ^{3}H-labeled 890-bp DNA fragment (0.2 nM) and RNAP (3 nM) at 25 °C for 10 min in 0.19 M Na^+ buffer. An equal volume of heparin solution (final concentration 5 μg/mL), containing NaCl to achieve the desired final salt concentration, was added. Duplicate samples were taken as a function of time after heparin addition and assayed by heparin-competition filter binding or by nucleotide stabilization, followed by filtration and a high-salt wash. The dissociation reaction followed irreversible first-order kinetics over at least 80% (as judged by the heparin assay) to 95% (as judged by nucleotide stabilization) of the reaction. The small percentage of more slowly dissociating complexes detected by heparin competition but not by nucleotide stabilization probably does not represent functional open complexes at λP_R.

Results

Kinetics of Association of RNAP with the λP_R Promoter Are Extraordinarily Salt Dependent. Association kinetic experiments were performed at 25 °C in at least a 7-fold excess of active RNAP, so that the reaction was pseudo first order (Hawley & McClure, 1980; Roe et al., 1984, 1985). A τ plot (McClure, 1980) of the reciprocal of the pseudo-first-order rate constant ($k_{obsd}^{-1} \equiv \tau_{obsd}$, in seconds) as a function of the reciprocal of the total concentration of active RNAP (in nM^{-1}) is shown in Figure 1 for association kinetic studies at 0.21 and 0.23 M Na^+. There are two principal features of the τ plot:

(1) Within experimental error (±10–20 s), the intercept of the τ plot at 25 °C is zero at both salt concentrations. At the other salt concentrations investigated (0.19–0.27 M), the τ intercepts were also scattered around zero without any apparent systematic change with [Na^+] (data not shown). [In previous studies with a Mg^{2+}-containing buffer, we found that the τ intercept for λP_R was distinguishably different from zero only at or below 20 °C (Roe et al., 1985).] Consequently, over the range of salt concentrations and RNAP concentrations investigated, values of τ_{obsd} are inversely proportional to the product of the second-order association rate constant k_a and the active RNAP concentration R_T:

$$\tau_{obsd} = (k_a R_T)^{-1} \qquad (\tau_{obsd} \gtrsim 20 \text{ s}) \quad (3)$$

(In other words, the isomerization steps occurring subsequent

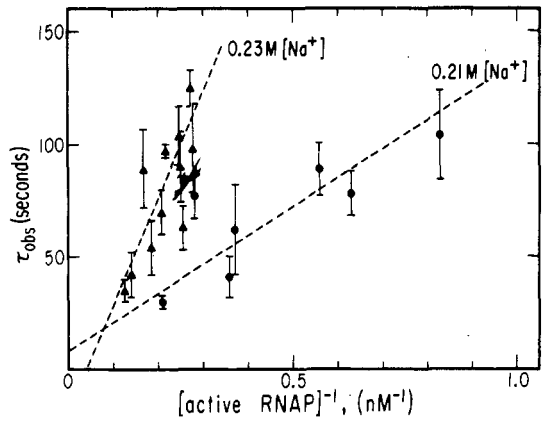

FIGURE 1: τ plots for formation of open complexes at the λP_R promoter at 0.21 M Na^+ and 0.23 M Na^+. The pseudo-first-order time constant τ_{obsd} is plotted as a function of the reciprocal of the active RNA polymerase concentration. Conditions: 25 °C, 0.01 M NaHepes (adjusted with HCl to pH 7.5), 10^{-4} M DTT, 0.1 mg/mL BSA, and either 0.20 (●) or 0.22 M (▲) NaCl. The dotted lines are weighted linear least-squares fit to the data: at 0.23 M Na^+, $\tau_{obsd} = -(20 \pm 20) + [(2.1 \pm 0.5) \times 10^6]^{-1}[RNAP]^{-1}$ s, and at 0.21 M Na^+, $\tau_{obsd} = (8 \pm 12) + [(7.8 \pm 1.6) \times 10^6]^{-1}[RNAP]^{-1}$ s, where, according to the three-step mechanism (Roe et al., 1984, 1985), $\tau_{obsd} = k_2^{-1} + k_3^{-1} + k_a^{-1}[RNAP]^{-1}$.

to the initial binding step at λP_R are relatively fast at 25 °C and do not contribute to the observed association kinetics.)

(2) Both τ_{obsd} at a given R_T and also k_a for association of RNAP with the λP_R promoter are *exquisitely* sensitive to salt concentration. For example, at $R_T = 4.8$ nM, τ_{obsd} is approximately 30 s at 0.21 M Na^+ but increases to approximately 70 s at 0.23 M Na^+ (cf. Figure 1). Values of k_a determined from the slopes of the weighted linear least-squares fits are $(7.8 \pm 1.6) \times 10^6$ M^{-1} s^{-1} at 0.21 M Na^+ and $(2.1 \pm 0.5) \times 10^6$ M^{-1} s^{-1} at 0.23 M Na^+. (Values of k_a estimated from eq 3 are not significantly different from these values within experimental uncertainty.)

Values of k_a determined from τ analyses at five Na^+ concentrations (0.19–0.27 M Na^+) are listed in Table I; k_a decreases by almost 2 orders of magnitude over this range of Na^+ concentrations. Since the total range of second-order rate constants reported by McClure and co-workers (Mulligan et al., 1984) for different promoters at a fixed salt concentration is approximately 4 orders of magnitude in k_a (from 10^4 M^{-1} s^{-1} to 10^8 M^{-1} s^{-1}), clearly *small variations in the ionic environment have as large an effect on the association kinetics as is observed for changes in promoter sequence investigated at a constant salt concentration.*

The logarithm of k_a is plotted as a function of the logarithm of the [Na^+] in Figure 2. The plot is linear with a slope of $-(11.9 \pm 1.1)$, indicating that the thermodynamic equivalent of approximately 12 Na^+ ions is released in the initial binding equilibrium (K_1) that forms the closed complex at this promoter. [This interpretation assumes that monovalent ions are not reactants in the conformational change in the second step of mechanism 2 (Roe et al., 1984; Lohman et al., 1978).]

Dependence of the Dissociation Rate Constant k_d and Equilibrium Constant K_p of the RNAP–λP_R Interaction on [Na^+]. Figure 2 and Table I summarize the behavior of the first-order dissociation rate constant k_d as a function of [Na^+]; k_d increases by slightly more than 1 order of magnitude over this range. The slope of the log–log plot of k_d vs. [Na^+] is 7.7 ± 0.2, indicating that the net uptake of the thermodynamic equivalent of approximately eight ions occurs prior to or as part of the rate-limiting step of the dissociation reaction (k_{-2}) (Roe et al., 1984; Lohman et al., 1978). We infer that much

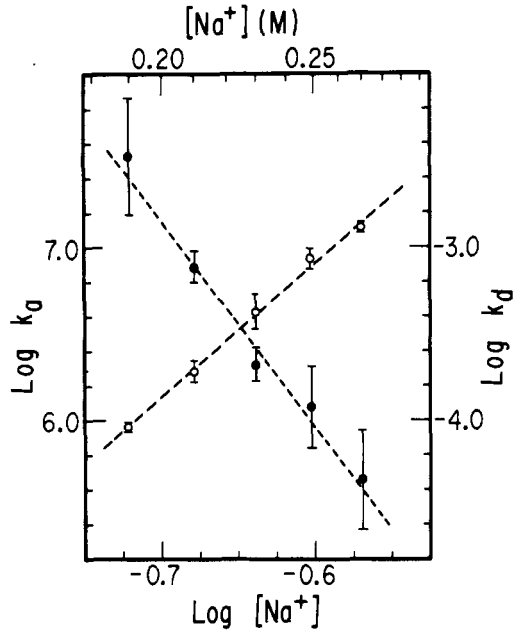

FIGURE 2: Dependence of association and dissociation rate constants k_a (●) and k_d (○) on [Na^+]. Experimental conditions are the same as those of Figure 1. The dotted lines are weighted linear least-squares fits to the data: $\log k_a = -(1.2 \pm 0.7) - (11.9 \pm 1.1) \log [Na^+]$; $\log k_d = (1.5 \pm 0.1) + (7.7 \pm 0.2) \log [Na^+]$.

Table I: Dependence of Rate and Equilibrium Constants of λP_R–RNA Polymerase Interactions on [Na^+][a]

[Na^+] (M)	k_a (M^{-1} s^{-1})	k_d (s^{-1})	$K_p = k_a/k_d$ (M^{-1})
0.19	$(3.4 \pm 2.2) \times 10^7$	$(9.1 \pm 0.5) \times 10^{-5}$	$(3.7 \pm 2.6) \times 10^{11}$
0.21	$(7.8 \pm 1.6) \times 10^6$	$(1.9 \pm 0.3) \times 10^{-4}$	$(4.1 \pm 1.5) \times 10^{10}$
0.23	$(2.1 \pm 0.5) \times 10^6$	$(4.2 \pm 0.9) \times 10^{-4}$	$(5.0 \pm 1.9) \times 10^9$
0.25	$(1.2 \pm 0.6) \times 10^6$	$(8.6 \pm 1.4) \times 10^{-4}$	$(1.4 \pm 1.0) \times 10^9$
0.27	$(4.6 \pm 2.7) \times 10^5$	$(1.3 \pm 0.1) \times 10^{-3}$	$(3.5 \pm 2.4) \times 10^8$

[a] Binding buffer: 0.01 M NaHepes (pH 7.5), 10^{-4} M DTT, 0.1 mg/mL BSA, and 0.18–0.26 M NaCl.

of this ion effect occurs in the rewinding (renaturation) of DNA from the open complex to form the native helical structure in the initial step of the dissociation reaction (Roe et al., 1984).

Although neither the dissociation rate constant nor the overall equilibrium constant $K_p = k_a/k_d$ (Roe et al., 1984, 1985) characterizing the RNAP–promoter interaction is likely to be of direct physiological relevance in the regulation of transcription initiation, determination of the behavior of K_p as a function of solution variables is necessary in order to understand the contributions of ionic and other noncovalent interactions to the overall binding free energy under specified conditions. Table I summarizes the dependence of K_p on [Na^+]; K_p decreases by 3 orders of magnitude from 0.19 M Na^+ to 0.27 M Na^+. Clearly, almost any desired value of K_p can be achieved by appropriate choice of ionic conditions. From a plot of $\log K_p$ vs. $\log [Na^+]$ (cf. Figure 3), it is apparent that the ion-exchange stoichiometry for the overall process of open complex formation at λP_R in NaCl is approximately 20 (cf. eq 1a):

$$\text{RNAP} + \lambda P_R \rightleftarrows \text{complex} + 20 \text{ monovalent ions} \qquad (1b)$$

This dependence of the equilibrium constant on the 20th power of the salt concentration is one of the largest salt dependences yet observed for a site-specific protein–DNA interaction. [Comparably large salt effects have been reported for the nonspecific interactions of core RNAP with native and denatured DNA and for the nonspecific interactions of RNAP

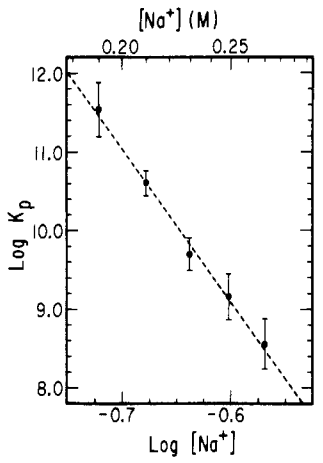

FIGURE 3: Dependence of the equilibrium constant $K_p = k_a/k_d$ on $[Na^+]$. Experimental conditions are the same as those of Figure 1. The dotted line is the weighted linear least-squares fit to the data: $\log K_p = -(2.7 \pm 0.8) - (19.6 \pm 1.3) \log [Na^+]$.

holoenzyme with denatured DNA (deHaseth et al., 1978; Lohman et al., 1980a).] From the steep slope and small intercept of the log–log plot of Figure 3, one may conclude that electrostatic interactions and counterion release make the dominant contribution to the binding free energy of the RNAP–λP_R open promoter complex under the solution conditions investigated. Strauss et al. (1980a,b) and Shaner et al. (1983) reached a similar conclusion in the case of the open complex between RNAP and the T7A1 promoter, where the stoichiometry of ion release is approximately 12–14. For λP_R, Figure 2 indicates that approximately 60% of the salt dependence of K_p originates in k_a and 40% is contributed by k_d, in agreement with the results of Roe et al. (1984) in a $Mg^{2+}/K^+/Tris^+$ buffer.

Discussion

Comparison of Effects of Ion Concentrations and RNA Polymerase Concentration on τ_{obsd}. Gene expression is controlled at the level of the noncovalent interactions of proteins and nucleic acids (Record et al., 1985; von Hippel, 1979). Regulation may occur at the thermodynamic level, as in the control of expression of the *E. coli* lac operon by the equilibrium binding of lac repressor protein (von Hippel, 1979; von Hippel et al., 1974), or at the kinetic level. The control of initiation of transcription in *E. coli* occurs at the level of the kinetics of formation of transcriptionally competent open complexes between RNA polymerase and promoter sequences (Hawley et al., 1982; McClure, 1983; Roe et al., 1984, 1985). McClure (1983) has argued that the concentration of *free* RNAP holoenzyme under standard growth conditions in vivo is approximately 30 nM, which is sufficiently in excess of the concentration of any single promoter site so that the pseudo-first-order time constant τ_{obsd} at this RNAP concentration is likely to be the relevant association kinetic variable to predict the relative utilization of different promoters. At temperatures above 20 °C, for the range of monovalent salt concentrations ($0.19 \text{ M} \leq [Na^+] \leq 0.27$ M) and RNAP concentrations (1–100 nM) investigated (such that $\tau_{obsd} > 20$ s), we find for the λP_R promoter that (cf. eq 3)

$$\tau_{obsd} \propto (k_a R_T)^{-1} \propto [Na^+]^{12} R_T^{-1} \quad (4)$$

Consequently, τ_{obsd} for this promoter is *far* more sensitive to changes in the salt concentration than to changes in the RNAP concentration; a 6% decrease in $[Na^+]$ has the same effect on τ_{obsd} (i.e., decreases τ by a factor of 2) as would be obtained by doubling the concentration of RNAP. In addition, the mathematical model for regulation of the λP_R promoter by cI protein proposed by Shea & Ackers (1985) may be interpreted to indicate that τ_{obsd} for this promoter varies as the first or second power of the total cI repressor concentration, in the region of control by cI. While it is premature to attempt to incorporate ion effects into these relatively qualitative models for regulation at the macromolecular level, it is worthwhile to note that τ_{obsd} for λP_R is also intrinsically more sensitive to changes in salt concentration than to changes in cI concentration. Moreover, it is clearly necessary that such modeling exercises be based on internally consistent sets of interaction parameters obtained under the same physiologically relevant ionic conditions.

Ion Effects on the RNAP–λP_R Interaction Are Not Ionic Strength Effects. The ionic environment of *E. coli* is of course more complex than that used here to investigate the RNAP–λP_R interaction. A common mixed ionic buffer used to approximate more closely the in vivo cationic environment is that of Hawley & McClure (1980): 0.12 M KCl, 0.01 M $MgCl_2$, and 0.04 M Tris (pH 8). (At pH 8 the concentration of Tris cation in this buffer is approximately 0.02 M.) Roe et al. (1984) examined the kinetics of association of RNAP with the λP_R promoter as a function of [KCl] in this buffer. At 25 and 37 °C, over the range of concentrations investigated, τ_{obsd} was found to be inversely proportional to the product $k_a R_T$. Partial dissection of the ion effect on k_a and τ_{obsd} for this association reaction indicated that, for the choice of ionic conditions investigated

$$\tau_{obsd} \propto (k_a R_T)^{-1} \propto [K^+]^x [Mg^{2+}]^y [Tris^+]^z R_T^{-1} \quad (5)$$

where $x \simeq 5 \pm 1$ and $y \simeq 2 \pm 1$ and where (cf. eq 4) we infer that $x + 2y + z \simeq 12$. (Equation 5 assumes that anion exchange does not contribute to the salt dependence of τ_{obsd}, a point that is currently being investigated.) Competitive interactions between K^+, Mg^{2+}, and $Tris^+$ cations for the vicinity of the DNA (which may be modeled as competitive ion-exchange equilibria) act to reduce the extent of K^+ release that accompanies formation of the closed RNAP–λP_R complex. At the same time, however, release of Mg^{2+} and $Tris^+$ cations becomes a factor, so that τ_{obsd} and k_a become functions of these concentrations as well. Consequently, the presence of a complex mixture of ions reduces the dependence of τ_{obsd} on the concentration of any single type of ion but increases the number of ways in which τ_{obsd} can be regulated by ionic variables in vitro or in vivo.

There is a fundamental distinction between salt effects on the kinetics of reactions of small ionic solutes and the salt effects we describe above. In the classical Debye–Hückel–Brønsted–Bjerrum (DHBB) description of salt effects on the kinetics of association of small ions, the effect of added salt is to screen the long-range electrostatic interactions of the reactants [cf. Moelwyn-Hughes (1971)]. This screening effect is expressed in terms of the so-called Debye length, which is inversely proportional to the square root of the ionic strength (I) of the salt solution. The DHBB model predicts a linear relationship between the log of the association rate constant and $I^{1/2}$:

$$\log k_a = \log k_a^* + 1.02 z_1 z_2 I^{1/2}$$

where z_1 and z_2 are the valences of the reactants and k_a^* is the rate constant at $I = 0$. According to the DHBB theory, divalent ions are distinguished from monovalent ions only on the basis of their contribution to I, in which ion concentrations are weighted by the square of their valence. All ions of the same valence are predicted to contribute identically to the screening effect on a reaction rate constant. Screening effects

of salt ions are typically relatively small, involving changes in k_a of less than an order of magnitude over a wide range of ionic strengths.

By contrast, salt effects on the kinetics (and equilibria) of reactions of proteins with DNA are very large ion-specific effects, which are introduced by the polyelectrolyte character of the reactants, are therefore fundamentally different from the DHBB screening effect in solutions of small ionic solutes, and consequently are not describable in terms of an ionic strength effect (Lohman et al., 1978; Lohman, 1985; Record et al., 1985). In the case of the RNAP–λP_R interaction, the net overall charge on both macromolecules is negative at the pH of the experiments reported here. Hence the global screening effect of salt should cause k_a to increase with increasing salt concentration. Instead, a precipitous decrease of k_a with increasing salt concentration is observed (cf. Figure 2). As discussed above, these salt effects on k_a and τ_{obsd} of the RNAP–λP_R interaction are stoichiometric effects that result from the competitive ion-exchange equilibria that characterize the interaction of both small ions and large proteins with the DNA polyanion. A direct comparison of the kinetic data of Roe et al. (1984), obtained in the KCl–$MgCl_2$–Tris buffer, with those of this paper, obtained in NaCl, indicates that values of k_a are between 1 and 2 orders of magnitude greater in NaCl than in the KCl–$MgCl_2$–Tris mixture at a specified ionic strength. (Relatively small and to an extent compensating effects of differences in the temperature and pH of these measurements were taken into account in making this comparison.) Clearly, ionic strength and the classical DHBB analysis of salt effects are completely inappropriate for analysis of these ion-exchange reactions.

Regulatory Role of the Ionic Environment in Controlling Protein–Nucleic Acid Interactions. The thermodynamic stability and the kinetics of conformational transitions and binding interactions of nucleic acids are such strong functions of the in vitro ionic environment that, within reasonable limits, virtually any extent of reaction or rate of reaction can be achieved by appropriate choice of ion concentrations (Record et al., 1976, 1978, 1981, 1985). Consequently, ions play a critical role in the control or regulation of these processes in vitro, serving both as perturbants of the system and as probes of the thermodynamic basis of stability (Record et al., 1976, 1977, 1978, 1981; deHaseth et al., 1977; Lohman et al., 1980b; Shaner et al., 1983) and of the mechanism of the interaction (Lohman et al., 1978; Lohman, 1985; Roe et al., 1984, 1985).

In particular, electrostatic interactions and counterion release provide much of the net favorable binding free energy of formation of both closed and open RNAP–promoter complexes (Roe et al., 1984, 1985; Shaner et al., 1983; Strauss et al., 1980b) and give rise to the extraordinary dependences of equilibrium constants and rate constants on ion concentrations discussed above. Since the association rate constant k_a for open complex formation at the λP_R promoter is an important determinant of the rate of transcription initiation from this promoter under physiological conditions [since $\tau_{obsd} \propto (k_a R_T)^{-1}$ for this case], then the nature of the in vivo ionic environment must play a major role in determining the rate of initiation from this promoter. For the intracellular ionic environment also to play a regulatory role in varying the rate of initiation of transcription at λP_R or other salt-dependent promoters, it is only required that the in vivo ionic environment change in response to external stimuli. Substantial evidence presently exists to demonstrate that large changes in the intracellular concentrations of both inorganic and organic ions occur both in *E. coli* (see below) and more generally in prokaryotic and eukaryotic cells, either in response to changes in the external environment or to accompany changes in the growth rate or stage of development of the cell [see, for example, Boynton et al. (1982)].

Ion-Specific Variations in the Ionic Environment of E. coli. The intracellular ionic environment of *E. coli* undergoes substantial variation as a function of growth conditions (Epstein & Schultz, 1965; Measures, 1975; Munro et al., 1972; Record et al., 1985). The inorganic cations K^+, Na^+, and Mg^{2+} and the polyamine putrescine (2+) are all present at nonnegligible (free) concentrations; lower levels of free Ca^{2+} and spermidine (3+) are also present. Both inorganic (e.g., Cl^-, phosphate) and organic (e.g., glutamate, acetate, organic phosphate) anions are present. It appears that the total concentration of organic anions is typically higher than that of Cl^-, the dominant inorganic anion. In *E. coli*, the intracellular concentrations of K^+, glutamate, putrescine, and in some cases Na^+ and other ions are regulated to respond to general and/or solute-specific changes in the external osmotic environment. In particular, the internal free K^+ concentration varies over a wide range (from ~0.1 to ~0.6 M) in response to variations in the external osmolarity (Epstein & Schultz, 1965). At high external osmolarity, the dominant organic anion in *E. coli* is glutamate (Measures, 1975; Munro et al., 1972). Under these conditions it appears that the glutamate concentration substantially exceeds the Cl^- concentration in *E. coli*. [The Cl^- concentration is always relatively low, though it increases with increases in the external Cl^- concentration (Schultz et al., 1962). It is not clear to us whether Cl^- or glutamate is the major monovalent anion in *E. coli* at very low external osmolarity.] Na^+ and Cl^- concentrations in *E. coli* can be varied specifically by varying the external NaCl concentration (Schultz et al., 1962). Under all external osmotic conditions and NaCl concentration, the major intracellular monovalent cation is K^+ (Epstein & Schultz, 1965). The intracellular putrescine concentration decreases as the intracellular K^+ concentration increases (Munro et al., 1972). Presumably this reflects an attempt by the cell to adjust its osmolarity without totally upsetting its ionic balance, although no extent of putrescine excretion would appear sufficient to compensate for the effects of high levels of K^+ on protein–nucleic acid interactions.

Conclusion

The large effects of ion concentrations that we observe on the rate of open complex formation between RNA polymerase and the λP_R promoter are not an isolated phenomenon but instead are a dominant characteristic of the kinetics and equilibria of protein–nucleic acid interactions and nucleic acid conformational equilibria. Given the magnitude of these ion effects and the variability of the intracellular ionic environment, it is clear that these changes in in vivo ion concentrations *must* play a global regulatory role in the initiation of transcription and on numerous other steps in gene expression. In addition, specific regulatory roles of ion concentrations on transcription initiation at specific promoters are readily envisioned, as a result of the different magnitudes and different salt dependences of the association kinetic parameters. [Indeed, significant effects of salt concentration on relative promoter strengths (Kajitani & Ishihama, 1983a,b) and on the efficiency of promoter utilization (Dausse et al., 1976; Miller & Burgess, 1978) in in vitro mixed transcription assays have been reported.] Promoter-specific effects of ion concentrations at the level of the defined kinetics of open complex formation have been observed by us and will be described later (Roe and Record, unpublished results).

ACKNOWLEDGMENTS

We thank Dick Burgess for providing the RNA polymerase used in these experiments and for his long-standing interest in this work. Mike Mossing and Kelli Kellbach made key contributions to the development of the ideas discussed in this paper. We also thank Sheila Aiello for her assistance in preparing the manuscript.

REFERENCES

Botchan, P. (1976) *J. Mol. Biol. 105*, 161–176.

Boynton, A. L., McKeehan, W. L., & Whitfield, J. R., Eds. (1982) *Ions, Cell Proliferation and Cancer*, Academic Press, New York.

Chamberlin, M. J., Nierman, W. C., Wiggs, J., & Neff, N. (1979) *J. Biol. Chem. 254*, 10061–10069.

Dausse, J.-P., Sentenac, A., & Fromageot, P. (1976) *Eur. J. Biochem. 65*, 387–393.

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

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

Epstein, W., & Schultz, S. G. (1965) *J. Gen. Physiol. 49*, 221–239.

Hawley, D. K., & McClure, W. R. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 6381–6385.

Hawley, D. K., Malan, T. P., Mulligan, M. E., & McClure, W. R. (1982) in *Promoters: Structure and Function* (Rodriguez, R., & Chamberlin, M. J., Eds.) pp 54–68, Praeger, New York.

Johnson, A. D., Poteete, A. R., Lauer, G., Sauer, R. T., Ackers, G. K., & Ptashne, M. (1981) *Nature (London) 294*, 217–223.

Kajitani, M., & Ishihama, A. (1983a) *Nucleic Acids Res. 11*, 671–686.

Kajitani, M., & Ishihama, A. (1983b) *Nucleic Acids Res. 11*, 3873–3888.

Lohman, T. M. (1985) *CRC Crit. Rev. Biochem.* (in press).

Lohman, T. M., deHaseth, P. L., & Record, M. T., Jr. (1978) *Biophys. Chem. 8*, 281–294.

Lohman, T. M., Wensley, C. G., Cina, J., Burgess, R. R., & Record, M. T., Jr. (1980a) *Biochemistry 19*, 3516–3522.

Lohman, T. M., deHaseth, P. L., & Record, M. T., Jr. (1980b) *Biochemistry 19*, 3522–3530.

McClure, W. R. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5634–5638.

McClure, W. R. (1983) in *Biochemistry of Metabolic Processes* (Lennon, D. L. G., Stratman, F. W., & Zahlten, R. N., Eds.) pp 207–217, Elsevier, New York.

Measures, J. C. (1975) *Nature (London) 257*, 398–400.

Miller, J. S., & Burgess, R. R. (1978) *Biochemistry 17*, 2064–2069.

Moelwyn-Hughes, E. A. (1971) *Chemical Statics and Kinetics of Solutions*, Academic Press, New York.

Mulligan, M. E., Hawley, D. K., Entriken, R., & McClure, W. R. (1984) *Nucleic Acids Res. 12*, 789–800.

Munro, G. F., Hercules, K., Morgan, J., & Sauerbier, W. (1972) *J. Biol. Chem. 247*, 1272–1280.

O'Farrell, P. H., Kutter, E., & Nakanish, M. (1980) *Mol. Gen. Genet. 179*, 421–435.

Ptashne, M., Jeffrey, A., Johnson, A. D., Maurer, R., Meyer, B. J., Pabo, C. O., Roberts, T. M., & Sauer, R. T. (1980) *Cell (Cambridge, Mass.) 19*, 1–11.

Record, M. T., Jr., deHaseth, P. L., & Lohman, T. M. (1976) *J. Mol. Biol. 107*, 145–158.

Record, M. T., Jr., deHaseth, P. L., & Lohman, T. M. (1977) *Biochemistry 16*, 4791–4796.

Record, M. T., Jr., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103–178.

Record, M. T., Jr., Mazur, S. J., Melancon, P., Roe, J.-H., Shaner, S. L., & Unger, L. (1981) *Annu. Rev. Biochem. 50*, 997–1024.

Record, M. T., Jr., Anderson, C. F., Mills, P., Mossing, M., & Roe, J.-H. (1985) *Adv. Biophys.* (in press).

Roe, J.-H. (1984) Doctoral Dissertation, University of Wisconsin, Madison.

Roe, J.-H., Burgess, R. R., & Record, M. T., Jr. (1984) *J. Mol. Biol. 176*, 495–521.

Roe, J.-H., Burgess, R. R., & Record, M. T., Jr. (1985) *J. Mol. Biol.* (in press).

Schultz, S., Wilson, N., & Epstein, W. (1962) *J. Gen. Physiol. 46*, 159–166.

Shaner, S. L., Melancon, P., Lee, K. S., Burgess, R. R., & Record, M. T., Jr. (1983) *Cold Spring Harbor Symp. Quant. Biol. 47*, 463–472.

Shea, M. A., & Ackers, G. K. (1985) *J. Mol. Biol. 181*, 211–230.

Strauss, H. S., Burgess, R. R., & Record, M. T., Jr. (1980a) *Biochemistry 19*, 3496–3504.

Strauss, H. S. Burgess, R. R., & Record, M. T., Jr. (1980b) *Biochemistry 19*, 3504–3515.

von Hippel, P. H. (1979) in *Biological Regulation and Development* (Goldberger, R. F., Ed.) pp 279–347, Plenum Press, New York.

von Hippel, P. H., Revzin, A., Gross, C., & Wang, A. C. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4808–4812.