Interpretation of Monovalent and Divalent Cation Effects on the *lac* Repressor–Operator Interaction†

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ABSTRACT: We have investigated the effects of mixed Na^+:Mg^{2+} ionic solutions on the stability of the nonspecific *lac* repressor–DNA complex. The effects of Mg^{2+} are simply interpreted in terms of its role as a competitor (with repressor) for DNA sites. From these studies, the binding constant of the Mg–DNA complex can be determined as a function of the concentration of Na^+. We have used this information to interpret the data of Riggs and collaborators (Riggs, A. D., et al. (1970), *J. Mol. Biol. 48,* 67–83; *53,* 401–417) on the ion dependence of the repressor–operator interaction. We find that there are approximately 70% as many ionic interactions in the repressor–operator complex as in the nonspecific complex. Our best estimate is that 8 ± 1 ion pairs are formed. We calculate that the release of counterions in the formation of the specific complex contributes approximately 40% of the favorable free energy change in the association reaction under in vivo ionic conditions. Implications of these findings for the control of the *lac* operon and for the molecular relationship between the specific and nonspecific complexes are considered.

Riggs and co-workers (1970a,b) have investigated the equilibrium and kinetics of the interaction of *lac* repressor with *lac* operator (carried on λφ80dlac DNA) using the filter binding method. Their experiments were carried out in a mixed monovalent–divalent cation buffer, containing K^+, Mg^{2+}, and the Tris cation. In this physiologically relevant system, they found a large effect of monovalent ion concentration on the observed association constant of the repressor–operator (RO) complex (K^{RO}_{obsd}). Smaller effects of temperature and pH on K^{RO}_{obsd} were seen.

Analysis of the dependence of the binding constant of protein–DNA interactions on the concentration of monovalent ions yields useful information on the thermodynamics of the interaction (Record et al., 1976a; deHaseth et al., 1977a). However, the presence of Mg^{2+} in the binding buffer complicates the analysis considerably, because Mg^{2+} itself binds to DNA (competing with the protein for binding sites) with a salt-dependent binding constant (Clement et al., 1973; Krakauer, 1971; Record, 1975; Record et al., 1976a). Here we extend the theory presented in the preceding paper (deHaseth et al., 1977a) to incorporate the effects of monovalent (Na^+, K^+) and divalent (Mg^{2+}) ions when they are simultaneously present in the binding buffers, and present experimental data on the repressor–DNA (RD) nonspecific interaction under these conditions. Combining our theoretical and experimental results, we are then able to quantitatively interpret the repressor–operator binding data of Riggs et al. (1970a,b).[1]

In a subsequent paper, dealing with the effects of ions on the kinetics of charged ligand–nucleic acid interactions, we interpret the kinetic data of Riggs et al. (1970b) in terms of possible mechanisms for R–O association (Lohman, deHaseth, and Record, in preparation.)

Theoretical Section

Addition of Mg^{2+} at a fixed monovalent ion (M^+, X^-) concentration and pH reduces the observed binding constant K^{RD}_{obsd} for the nonspecific interaction between *lac* repressor and DNA. Mg^{2+} binds to DNA in preference to M^+ (M^+ being either Na^+ or K^+), and therefore competes with repressor for sites on the DNA. (A specific interaction between Mg^{2+} and repressor is also possible in principle, though not likely, as discussed below.)

We write

$$R + D \overset{K^{RD}_{obsd}}{\rightleftharpoons} RD \qquad (1)$$

Reaction 1 is the experimentally observed reaction. The thermodynamically relevant reaction, however, is:

$$R_{r,0} + D_0 \overset{K^{RD}_{T}}{\rightleftharpoons} RD + m'_{RD}\psi M^+ \qquad (2)$$

The symbols are as used in the preceding paper (deHaseth et al., 1977a). Briefly: $R_{r,0}$ is repressor with r protons, but no anions bound to the DNA binding region; it is assumed that this is the only repressor species participating in the binding reaction. D_0 is DNA with ψ M^+ ions associated in the thermodynamic sense with each phosphate (Record et al., 1976a), but with no other bound ligands. Finally, m'_{RD} is the number of electrostatic interactions between positive groups on the repressor and negatively charged DNA phosphates, per RD complex. We emphasize that eq 2 is a thermodynamic description of the interaction, which is not dependent on any reaction mechanism (compare deHaseth et al., 1977a). Equations 1 and 2 are readily applied to the repressor–operator reaction by a change of symbols; we keep the notation in terms of the nonspecific (RD) interaction for convenience.

The effect of Mg^{2+} on the thermodynamics of the RD interaction is introduced as a competitive equilibrium which reduces the concentration of D_0 sites in eq 2. Mg^{2+} addition therefore causes reaction 2 to shift to the left; this reduces K^{RD}_{obsd}. In the absence of Mg^{2+}, $[D] = [D_0]$ (deHaseth et al., 1977a). In the presence of Mg^{2+}, the ratio $[D]/[D_0]$ can be evaluated by the sequence generating function method of binding theory (Lifson, 1964; Schellman, 1974). The Mg^{2+}

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[1] Preliminary accounts of this work were presented to the Biophysical Society (Seattle, 1976; New Orleans, 1977) and at the Cold Spring Harbor meeting on Molecular Aspects of *lac* Operon Control (1976). Our early calculations on the repressor–operator interaction used an incorrect estimate of the binding constant for the Mg^{2+}–DNA interaction and are superceded by the present analysis.

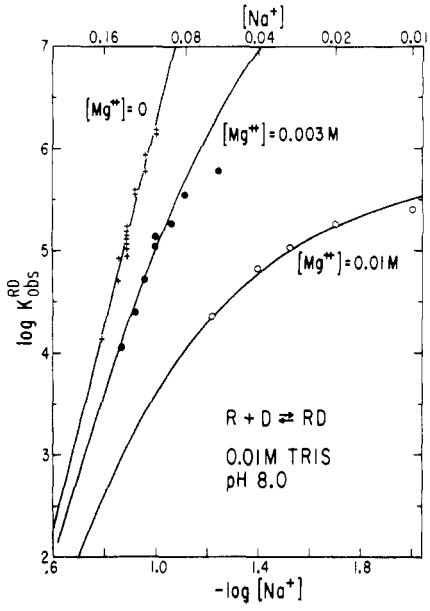

FIGURE 1: The dependence of the observed binding constant of the *lac* repressor–DNA interaction on the concentration of added NaCl, at different concentrations of $MgCl_2$; log-log plots. Elution at 4 °C with buffer T (pH 8.0). (+) $[MgCl_2] = 0$; data from deHaseth et al. (1977a); line drawn is least-squares fit through the data points. (●) $[MgCl_2] = 0.003$ M. (○) $[MgCl_2] = 0.01$ M. The data in $MgCl_2$ were obtained with the Harvard preparation of repressor; 50 μg loaded per run. The curves were calculated using the binding theory developed in this paper and the preceding one (deHaseth et al., 1977a). The buffer and the repressor preparations were described by deHaseth et al. (1977b).

is assumed to bind noncooperatively, and the m'_{RD} phosphates on DNA to which repressor binds in the RD complex are all binding sites for Mg^{2+}. However, each Mg^{2+} covers approximately two sites on binding; i.e., the binding sites overlap (McGhee and von Hippel, 1974; Record et al., 1976a). If $K^{Mg}{}_{obsd}$ is the observed association constant for the binding of Mg^{2+} to a DNA site ($K^{Mg}{}_{obsd}$ being a function of $[M^+]$; see below), we can write:[2]

$$\frac{[D]}{[D_0]} = \{\tfrac{1}{2}[1 + \sqrt{1 + 4K^{Mg}{}_{obsd}[Mg^{2+}]}]\}^{m'_{RD}} \quad (3)$$

Then, by analogy with eq 6 and 10 of deHaseth et al. (1977a), we obtain

$$K^{RD,M}{}_T = K^{RD}{}_{obsd} \frac{[R]}{[R_{r,0}]} \frac{[D]}{[D_0]} [M^+]^{0.88m'_{RD}} \quad (4)$$

and

$$\log K^{RD}{}_{obsd} = \log K^{RD,M}{}_T - r \log \left[\frac{1 + K_H[H^+]}{K_H[H^+]}\right] - 0.88m'_{RD} \log [M^+] - m'_{RD} \log \tfrac{1}{2}[1 + \sqrt{1 + 4K^{Mg}{}_{obsd}[Mg^{2+}]}] \quad (5)$$

where we have introduced $\psi = 0.88$ for double helical DNA, and where K_H is the intrinsic association constant for each of the r protonation reactions. Equation 5 neglects the possible contribution of anion binding and anion release to $K^{RD}{}_{obsd}$, on the basis of the arguments presented in the previous paper (deHaseth et al., 1977a). Introduction of anion effects does not degrade the fit of eq 5 to the experimental data, but introduces two additional parameters (the anion binding constant and the number of sites for anions). Since these parameters are not necessary to obtain a consistent fit to the data, they have not been introduced. We cannot categorically rule out any role of anions in the RD or RO interaction, however.

To use eq 5 in the interpretation of RD and RO binding data in mixed M^+–Mg^{2+} systems, we must evaluate the second (titration effect) and fourth (Mg^{2+} effect) terms of eq 5. We will consider these in this order.

(1) *Titration Effects.* While r is known for the nonspecific interaction, we have no information on K_H. This problem can be circumvented by performing all experiments at the same pH (8.0) as that used in the monovalent cation work (deHaseth et al., 1977a). Then the titration term is constant. We define

$$\log K^{RD,M}{}_T(pH) \equiv \log K^{RD,M}{}_T - r \log \left[\frac{1 + K_H[H^+]}{K_H[H^+]}\right] \quad (6)$$

where $\log K^{RD,M}{}_T(pH)$ is the intercept of a plot of $\log K^{RD}{}_{obsd}$ vs. $\log [M^+]$ extrapolated to $[M^+] = 1.0$ M. $K^{RD,M}{}_T(pH)$ is an apparent thermodynamic constant (at constant pH) but is of course a function of pH.

(2) *The Mg^{2+} Effect.* We now need to know how $K^{Mg}{}_{obsd}$ varies with $[M^+]$, i.e., how the binding of Mg^{2+} to DNA in the absence of *lac* repressor is affected by the salt concentration. The binding of Mg^{2+} to DNA can be represented by

$$Mg^{2+} + D \overset{K^{Mg}{}_{obsd}}{\rightleftharpoons} MgD \quad (7)$$

and

$$Mg^{2+} + D_0 \overset{K^{Mg}{}_T}{\rightleftharpoons} MgD + 2\psi M^+ \quad (8)$$

Equations 7 and 8 are the analogues of eq 1 and 2 with $m' = 2$, the expected number of ionic interactions (ion pairs) for a doubly positively charged Mg^{2+} cation with negatively charged phosphates on the DNA (Record et al., 1976a). From eq 7 and 8 (see also Record et al., 1976a) we expect that

$$\log K^{Mg}{}_{obsd} = \log K^{Mg}{}_T - 2\psi \log [M^+] \quad (9)$$

Thus the slope of a plot of $\log K^{Mg}{}_{obsd}$ vs. $-\log [M^+]$ is expected to be 2ψ, or 1.76 (with $\psi = 0.88$ for double helical DNA). This agrees well with the value of 1.7 obtained from the data of Krakauer (1971) for the binding of Mg^{2+} to poly[r(A)]·poly[r(U)], for which $\psi = 0.89$ (Record et al., 1976a). However, there is no extensive data on $K^{Mg}{}_{obsd}$ for DNA. Estimates of $\log K^{Mg}{}_T$ obtained by use of the theoretical slope to extrapolate literature values of $\log K^{Mg}{}_{obsd}$ (Clement et al., 1973; Sander and T'so, 1971; Willemsen and van Os, 1971) range from 0 to 1. As a result the value of $K^{Mg}{}_T$ suitable to use in eq 9 is not known to within a factor of 10.

To proceed, we have let $K^{Mg}{}_{obsd}$ be a parameter, and obtained its value as a function of $[M^+]$ using eq 5 and experimental values of $\log K^{RD}{}_{obsd}$ at given concentrations of Mg^{2+} and M^+. The quantities $K^{RD,M}{}_T(pH)$ and m'_{RD} are known from the studies of the RD interaction in the absence of Mg^{2+} (deHaseth et al., 1977a). Once values of $\log K^{Mg}{}_{obsd}$ were obtained by the above procedure for various $[Mg^{2+}]$ and $[M^+]$, $\log K^{Mg}{}_{obsd}$ was plotted as a function of $\log [M^+]$. A least-squares line was fit through the data and used to obtain values of $\log K^{Mg}{}_{obsd}$ appropriate to the ionic conditions used by Riggs et al. (1970a,b) to investigate the RO interaction. The slope and intercept of the least-squares line of $\log K^{Mg}{}_{obsd}$ vs. $-\log [M^+]$ can of course be compared with the expected slope (from eq 9) and range of extrapolated intercepts to determine if this

[2] The expression for $[D]/[D_0]$, in the presence of Mg^{2+}, which is given in eq 3 is numerically valid in the limit of low binding density of the protein, so that only the Mg^{2+} binding perturbs the distribution of M^+ between that which is free in solution and the M^+ which is condensed on the DNA.

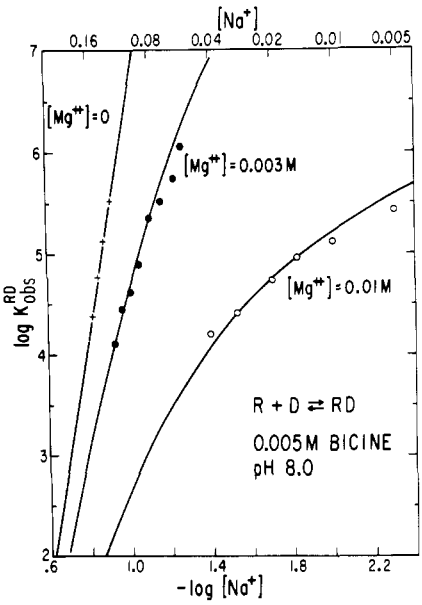

FIGURE 2: See legend to Figure 1. The elution was here with 0.005 M Bicine buffer (pH 8.0), 4 °C. All runs with the Wisconsin prep; 150 μg loaded per run.

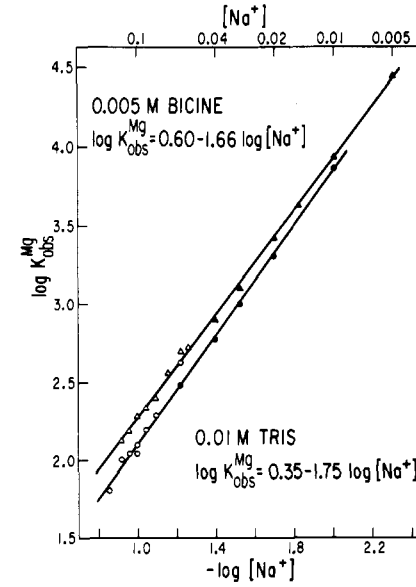

FIGURE 3: The dependence of the binding constants for the interaction between Mg^{2+} and DNA, as calculated from the data in Figures 1 and 2 (see text), on the concentration of added NaCl; log-log plots. (Circles) Data in 0.01 M Tris; (○) $[MgCl_2]$ = 0.003 M; (●) $[MgCl_2]$ = 0.01 M. (Triangles) data in 0.005 M Bicine; (△) $[MgCl_2]$ = 0.003 M; (▲) $[MgCl_2]$ = 0.01 M.

approach is reasonable. The results demonstrate that the approach is valid.

Materials and Methods

The experimental procedures have been described in the previous two papers of this series (deHaseth et al., 1977a,b).

Results

(1) *Mg^{2+} Effects on the Nonspecific RD Interaction.* Figures 1 and 2 illustrate the effects of Mg^{2+} on the association constant K^{RD}_{obsd} in two different buffers at pH 8. In Figure 1, 0.01 M Tris[3] was used; Tris was the buffer used by Riggs et al. (1970a,b) in investigating the repressor-operator interaction. Since an interaction between the Tris cation and DNA is possible, a second set of experiments (Figure 2) was carried out in 0.005 M Bicine, a dipolar ion buffer, at the same pH. The results in the two buffer systems were very similar. At a constant monovalent ion concentration (here Na^+), addition of Mg^{2+} reduces K^{RD}_{obsd}, as is expected from eq 5 and the role of Mg^{2+} as a competitive ligand. The reduction in K^{RD}_{obsd} obtained by adding Mg^{2+} is greatest at low $[Na^+]$.

In Figure 1, the data in the absence of Mg^{2+} is taken from deHaseth at al. (1977a). The least-squares equations of the lines through these data were used to obtain values of log K^{RD,M_T}(pH), defined in eq 6, and of m'_{RD}. In 0.01 M Tris, pH 8, log K^{RD,M_T}(pH 8) = −3.72 and m'_{RD} = 11.4. In 0.005 M Bicine, pH 8, log K^{RD,M_T}(pH 8) = −5.46 and m_{RD}' = 13.8. We attribute the apparent differences in these quantities primarily to experimental uncertainty, although an interaction of the Tris cation with the DNA would reduce the estimate of m'_{RD} in that system. (For the purposes of calculations using eq 5 it made no significant difference whether the above parameters were used as calculated or whether log K^{RD,M_T}(pH) was evaluated forcing m' to be an integer (12 ± 2) and fitting the mid-range of the binding data accordingly.)

Proceeding as outlined in the theoretical section, we obtained values of log K^{Mg}_{obsd} at 0.003 and 0.01 M Mg^{2+} in each buffer, which are plotted as a function of −log $[Na^+]$ in Figure 3. The data from the two buffers are in quite close agreement, although there is a systematic difference (less than 0.2 log unit) between the two sets of data. We have no explanation for this effect. The data at 0.003 M Mg^{2+} and 0.01 M Mg^{2+} in each buffer fall on a common line, indicating that the effects of Mg^{2+} are adequately treated by eq 5. The slopes of the least-squares lines through the data in the two buffers are 1.75 and 1.66, in good agreement with the expected value of 1.76 (eq 9). This also agrees well with the Mg^{2+}-DNA binding constants obtained from measurements of the effect of Mg^{2+} on the pentalysine-T_7 DNA interactions (Lohman and Record, unpublished data) by an analysis similar to the one presented here. This too indicates to us that our theoretical description of the RD interaction in mixed M^+-Mg^{2+} systems is adequate, and thus that specific interactions between Mg^{2+} and the repressor do not play a significant role.

Using the least-squares equations for log K^{Mg}_{obsd} as a function of log $[Na^+]$ from Figure 3, theoretical curves were drawn through the data of Figures 1 and 2. These fits to the data are quite good, as would be expected from Figure 3. The fit is, however, very sensitive to the parameters in the equation for log K^{Mg}_{obsd}, and is significantly degraded if an average least-squares line for log K^{Mg}_{obsd} is constructed from the data of Figure 3.

(2) *The Repressor-Operator Interaction.* The preceding analysis provides the basis for interpreting the data of Riggs et al. (1970a,b) on the ion dependences of the repressor-operator equilibrium. However, there are two unknown parameters in the repressor-operator interaction: m'_{RO} and K^{RO}_T. To minimize the possibility of ambiguity in the calculation, we have made use of the observation of Riggs et al. (1970a,b) that log K^{RO}_{obsd} is a linear function of the square root of the ionic strength ($I^{1/2}$). A similar relationship holds in all our series of experiments. This functional dependence appears coincidental because it has no basis in theory except in the case of the interactions of low molecular weight ions, and because a different linear relationship is obtained at each Mg^{2+} concen-

[3] Abbreviations used: Bicine, *N,N*-bis(2-hydroxyethyl)glycine; Tris, tris(hydroxymethyl)aminomethane.

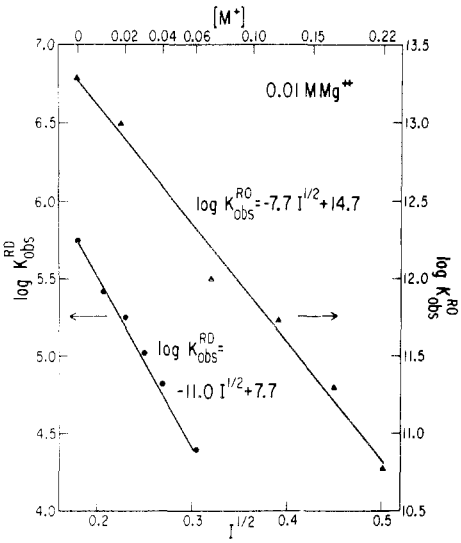

FIGURE 4: Comparison of the dependences of the specific and nonspecific interactions of *lac* repressor with DNA in buffer containing 0.01 M Mg^{2+} on the concentration of added monovalent salt. Plots of the logarithm of the observed binding constants vs. the square root of the ionic strength. (▲) Specific interaction; data of Riggs et al. (1970a); 0.01 M Tris, pH 7.4, 20 °C. (●) Nonspecific interaction; data from Figure 1.

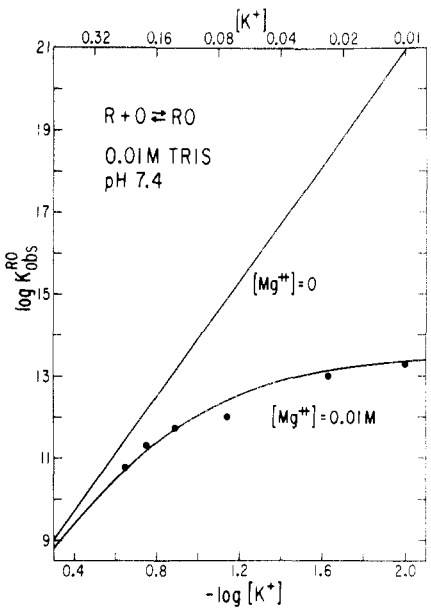

FIGURE 5: The dependence of the observed binding constant for the specific interaction between *lac* repressor and operator DNA on the concentration of added KCl. Data of Riggs et al. (1970a) obtained in buffer containing 0.01 M Mg^{2+}; the curve drawn through the data points is the best fit to these data points. The line labeled $[Mg^{2+}] = 0$ is calculated based on the parameters obtained for the fit; see text.

tration (data not shown). Nevertheless, when the ionic strength is varied by changing the MX concentration only (at constant Mg^{2+}), then it is easily shown that

$$\frac{m'_{RO}}{m'_{RD}} = \frac{(\partial \log K^{RO}{}_{obsd}/\partial I^{1/2})_{Mg^{2+}}}{(\partial \log K^{RD}{}_{obsd}/\partial I^{1/2})_{Mg^{2+}}} \quad (10)$$

where I is the ionic strength. Thus the ratio of the slopes of log K_{obsd} vs. $I^{1/2}$ plots equals the ratio of the numbers of ionic interactions formed in the complexes. Equation 10 only holds either in the absence of anion binding to the DNA-binding site of the repressor, or, if anion binding does occur, if the number of anions released in each binding mode (specific and nonspecific) is proportional to the number of ionic interactions formed in that binding mode.

Figure 4 shows the data of Riggs et al. (1970a) in 0.01 M Mg^{2+}, 0.01 M Tris, pH 7.4, and the data of Figure 1 in the same buffer, pH 8.0, plotted in the above manner. (Experiments performed on the RD interaction as a function of [NaCl] at both pH 8.0 and pH 7.4 gave identical slopes.) From the ratio of slopes in Figure 4, we estimate $m'_{RO} = 0.66\ m'_{RD}$. We have concluded that $m'_{RD} = 12 \pm 2$ (deHaseth et al., 1977b). Therefore $m'_{RO} = 8 \pm 2$. Lin and Riggs (1975) report data on $K^{RO}{}_{obsd}$ and $K^{RD}{}_{obsd}$ in 0.003 M Mg^{2+}. From these data, we calculate $m'_{RO} = 0.74 m'_{RD}$, or $m'_{RO} = 9 \pm 2$. With m'_{RO} narrowed down to this range, an evaluation of $K^{RO}{}_T$ can be obtained by fitting the data of Riggs et al. (1970a).

Figure 5 shows the fit of eq 5 to the repressor–operator data for $m'_{RO} = 8$ and $K^{RO}{}_T = 8 \times 10^6\ M^{-1}$ (log $K^{RO}{}_T = 6.9$). The fit is significantly degraded for $m' = 6$ or 10, no matter what value of $K^{RO}{}_T$ is used. In these calculations, the values of $K^{Mg}{}_{obsd}$ obtained in Tris buffer (Figure 3) were used, but $m'_{RO} = 8$ is the best fit for either set of values of $K^{Mg}{}_{obsd}$ (cf. Figure 3). Therefore we conclude that $m'_{RO} = 8 \pm 1$ and log $K^{RO}{}_T = 6.9 \pm 1.0$, where the error limits on log $K^{RO}{}_T$ encompass the range of values obtained using either expression for $K^{Mg}{}_{obsd}$ and $7 \leq m'_{RO} \leq 9$. Figure 5 also gives the behavior of log $K^{RO}{}_{obsd}$ predicted in the absence of Mg^{2+} (from eq 5). Without Mg^{2+} present as a competitor, the binding of *lac* repressor to operator would be essentially irreversible at monovalent cation concentrations below 0.05–0.10 M.

Discussion

(1) *Effects of Mg^{2+} on Repressor–DNA Interactions in the Presence of Monovalent Cations.* The theoretical analysis given above appears to account for the effects of Mg^{2+} (in the presence of monovalent cations) on the specific and nonspecific interactions of repressor with DNA. Mg^{2+} is treated strictly as a competitor for DNA sites; no specific interaction between repressor and Mg^{2+} is required to interpret the binding data. In the thermodynamic analysis of the system, it proved convenient to analyze a particular path for the repressor–DNA interaction, in which the effects of Mg^{2+} were introduced as a competing reaction for available DNA sites. The conclusions drawn from that analysis regarding Mg^{2+} effects on $K^{RD}{}_{obsd}$ are of course independent of the choice of path, and the actual reaction path, or mechanism, is presumably a displacement of Mg^{2+} and monovalent cations (Na^+ or K^+, as appropriate) by the protein. This mechanistic picture provides a suitable vehicle for explaining the experimentally observed effects of Mg^{2+} on the binding constant $K^{RD}{}_{obsd}$ (see Figures 1 and 2). The following description is of relevance for any ligand–DNA interaction for which ionic forces contribute to the binding free energy.

(a) DNA binds Mg^{2+} in preference to Na^+ or K^+, although the preference is not absolute and varies with the monovalent cation concentration, as expressed by eq 7–9. Consequently the addition of Mg^{2+} to the repressor–DNA binding buffer (at constant $[M^+]$, pH, and temperature) must reduce $K^{RO}{}_{obsd}$ and $K^{RD}{}_{obsd}$, since Mg^{2+} is harder to displace from the DNA than are monovalent cations. This effect of Mg^{2+} as a direct participant in the association reaction is quite different and much more substantial than would be expected from its contribution to the ionic strength of the solution. Therefore empirical representations of binding data such as Figure 4 (cf. Riggs et al., 1970a,b) are only applicable at a fixed Mg^{2+} concentration, and will have different slopes and intercepts at each Mg^{2+} concentration.

(b) The extent of saturation of DNA sites with Mg^{2+} (in place of M^+) varies with both $[Mg^{2+}]$ and $[M^+]$ according to

eq 7-9, since M^+ ions are displaced when Mg^{2+} ions bind to the DNA. Therefore the binding of repressor to DNA will displace numbers of Mg^{2+} and M^+ ions from the DNA which are functions of $[Mg^{2+}]$ and $[M^+]$. Thus the derivative $-(\partial \log K^{RD}_{obsd}/\partial \log [M^+])_{Mg^{2+}}$, which measures the number of M^+ ions released from the DNA (ignoring anion binding), will be itself a function of $[M^+]$. In other words, curvature is seen in a plot of $\log K^{RD}_{obsd}$ (or $\log K^{RO}_{obsd}$) vs. $\log [M^+]$. At fixed $[Mg^{2+}]$, the binding of M^+ to DNA decreases with decreasing $[M^+]$, so the number of M^+ ions released when repressor binds is small and the magnitude of $\partial \log K^{RD}_{obsd}/\partial \log [M^+]$ is small. At higher $[M^+]$, there is less binding of Mg^{2+}, and the binding of repressor releases more M^+ ions than at low $[M^+]$. Consequently the magnitude of $\partial \log K^{RD}_{obsd}/\partial \log [M^+]$ increases with increasing $[M^+]$, approaching the value obtained in the absence of Mg^{2+}. Recall that the reason linear plots of $\log K^{RD}_{obsd}$ vs. $\log [M^+]$ are observed in the absence of Mg^{2+} is that then the binding of M^+ to DNA is independent of $[M^+]$ (Record et al., 1976a).

It is appropriate to discuss at this point the extent to which Na^+ and K^+ can be treated interchangeably as monovalent cations. Latt and Sober (1967) investigated the effects of K^+ and Na^+ on the observed association constants of oligolysine–polyribonucleotide interactions. No differences between K^+ and Na^+ were noted with poly[r(I)]·poly[r(C)]. With poly[r(A)]·poly[r(U)], a difference was noted; the binding of oligolysines with 4–7 residues was 2.5–3 times weaker in NaCl than in KCl. However, this effect may be due to a specific interaction between Na^+ and either the 2′-HO- group or the uracil base and, therefore, not be a factor when DNA is the lattice. Shapiro et al. (1969) found no base compositional preference on the part of either Na^+ or K^+ in competition equilibrium dialysis studies using naturally occurring DNA. Moreover, we see no difference in the effects of Na^+ and K^+ on the observed binding constants for the nonspecific interactions of RNA polymerase with DNA (deHaseth et al., in preparation). The experiments of Riggs et al. (1970a,b) on the RO interaction were performed in KCl; our experiments on the RD interaction were performed in NaCl. We expect that there will be only minor differences in either the relative or absolute association constants in the two salts, and that the two sets of experiments can be compared as we have done in this paper.

(2) *Comparison of the Thermodynamics of RO and RD Complex Formation.* In the previous paper (deHaseth et al., 1977), we estimated that $m'_{RD} = 12 \pm 2$ ionic interactions from the findings that 11 ± 2 monovalent ions are released in formation of the RD complex and that most of these ions appear to be cations previously associated with the DNA. At pH 8, 4 °C, we found that $\log K^{RD}_T$ was in the range -3.7 to -5.5; this value could be accounted for entirely by the thermodynamic constant for formation of 12 ± 2 ionic interactions and the requirement for protonation of two groups on repressor at a pH substantially higher than their normal pK.

On the other hand, in the present analysis we find $m'_{RO} = 8 \pm 1$ ionic interactions, and on this basis obtain $\log K^{RO}_T = 6.9$ at pH 7.4 in 0.01 M Tris, 24 °C (the conditions used by Riggs et al., 1970a). There are fewer ionic interactions in the RO complex and the thermodynamic binding constant contains a very large and favorable contribution (as it should) from factors other than ion release which presumably give rise to the specificity of the R–O interaction. Nevertheless we calculate that, under the free ion concentrations usually assumed for the in vivo situation (~0.2 M monovalent cation, 0.003 M divalent cation), counterion release contributes substantially to the net free energy change on formation of the RO complex. Applying eq 5 to the RO interaction in 0.003 M Mg^{2+} and 0.2 M M^+, we obtain $\log K^{RO}_{obsd} = 11.5$. By comparison with $\log K^{RO}_T = 6.9$, we see that approximately 40% of the free energy change accompanying complex formation under physiological conditions is the result of the entropic contribution of counterion release.[4]

(3) *Implications for Control of the lac Operon.* In view of the hypothesis of von Hippel et al. (1974, 1975) and Lin and Riggs (1975) that nonspecific binding of repressor is an integral part of the control of the *lac* operon, it is important to estimate K^{RO}_{obsd} and K^{RD}_{obsd} under physiological conditions.[5] The comparison of K^{RO}_{obsd} and K^{RD}_{obsd} will be different at each salt concentration, pH, and temperature, since these variables affect the two binding constants differently. (Effects of salt concentration and pH are less on K^{RO}_{obsd} than on K^{RD}_{obsd}; an increase in temperature increases K^{RO}_{obsd} but decreases K^{RD}_{obsd}.) At pH 7.4, use of $\Delta H^{RO}_{obsd} = 8.5$ kcal/mol (Riggs et al., 1970b) gives $\log K^{RO}_{obsd} = 11.75$ at 37 °C, 0.003 M Mg^{2+} and 0.20 M M^+. At pH 8, 4 °C, 0.003 M Mg^{2+}, 0.20 M M^+, $\log K^{RD}_{obsd} = 2.8$, as estimated using eq 5 and the parameters of the 0.01 M Tris buffer system. Correcting to pH 7.4 and 37 °C using the pH and temperature dependences of $\log K^{RD}_{obsd}$ determined by deHaseth et al. (1977a) and Revzin and von Hippel (1977), we estimate $\log K^{RD}_{obsd} = 3.25$. Therefore $K^{RO}_{obsd}/K^{RD}_{obsd} \simeq 3 \times 10^8$. We note that our estimate of this ratio is intermediate between the estimates of Lin and Riggs (1975), who obtained 10^8, and von Hippel et al. (1974, 1975) who used 10^9. The magnitudes calculated for the individual binding constants are about two orders of magnitude less than those used by von Hippel et al. (1974, 1975) and are similar to the values used by Lin and Riggs (1975). Since the ratio of $K^{RO}_{obsd}/K^{RD}_{obsd}$ is the important quantity in the calculations of von Hippel et al. (1974, 1975), our revision of the binding constants does not affect their conclusions. In particular, using their assumptions regarding cell volume (10^{-15} L) and number of repressor molecules per cell (10), we estimate that the ratio of free repressor to total repressor is 0.04, and the ratio of free operator to total operator is only 2.5×10^{-3}. Upon induction, if the binding of inducer to repressor reduces the RO binding constant by 10^3, the fraction of repressor free in solution is essentially unchanged, but the ratio of free operator to total operator increases to 0.7. Therefore the accessibility of this control DNA region to RNA polymerase increases about 300-fold upon induction. Since the ratio of constitutive to basal rates of synthesis of *lac* enzymes is about 10^3, the equilibrium calculation is in order-of-magnitude agreement with the in vivo results.

We should note here that a simple calculation shows that, in the absence of nonspecific binding, all the operator would be complexed with repressor both before and after induction. Hence the interaction of repressor with nonspecific DNA is necessary for induction to occur.

(4) *Molecular Differences in the RO and RD Interactions.* Whereas it appears likely that the same site on *lac* repressor

[4] W. Gilbert (personal communication, 1977) has determined the number of repressor–phosphate contacts in the repressor–operator complex using chemical modification of phosphates with ethylnitrosourea. A total of 7 phosphates are involved. The agreement between this number and our calculated value of 8 ± 1 phosphates obtained from the data of Riggs et al. (1970a) suggests that our assumptions regarding anion effects, etc., are reasonable.

[5] Through recent measurements of the distribution of repressor molecules bound to operator and non-operator DNA using minicells, Huang, Revzin, Butler, O'Connor, Noble, and von Hippel have estimated the effective cationic activity of the interior of the *E. coli* cell to be between 0.17 and 0.24 M Na^+ equivalents (personal communication).

is involved in the nonspecific and operator binding processes (Bourgeois and Pfahl, 1976), the interaction with operator is not simply the superposition of specific interactions between nucleic acid bases and repressor and the nonspecific interactions of the RD complex. Instead a comparison of the RO and RD results indicates that possibly one less protonation event is required in the RO interaction (Riggs et al., 1970b), and the RO complex contains only ~70% of the ionic interactions of the RD complex.

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Composition and Template Activity of Chromatin Fractionated by Isoelectric Focusing†

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ABSTRACT: HeLa cell interphase chromatin has been sheared and fractionated by isoelectric focusing. Chromatin fractions are obtained with a wide range of isoelectric points. No free DNA is observed. While protein/DNA ratios are similar in the various fractions, they appear to contain different nonhistone chromosomal proteins. A minor chromatin fraction with isoelectric point ≃7.0 does not contain histone H1. This fraction is considerably more active as template with different RNA polymerases than the other fractions. Kinetic studies, in which RNA polymerase activity is assayed at various concentrations of chromatin, indicate that the greater activity of *Escherichia coli* RNA polymerase is due to an increased rate of transcription at saturating concentrations of template (V_{max}) and is not due to a lower concentration required for half-maximal rate of transcription (K_m). In contrast, the increased rate of transcription by calf-thymus RNA polymerases II and III is due to a decrease in chromatin concentration required for half-maximal rate of transcription rather than an increased rate of transcription at saturating concentrations of template. These results suggest that chromatin with isoelectric point ≃7 offers a greater frequency of binding sites for mammalian RNA polymerases, as would be expected for a "transcriptionally active" fraction.

Recent observations on the fine structure of chromatin (Axel et al., 1974; Kornberg and Thomas, 1974; Olins and Olins, 1974) and the reconstitution of active transcribing chromatin (Barrett et al., 1974; Chan et al., 1973; Gilmour and Paul, 1969) have made it increasingly desirable to separate transcriptionally active chromatin from total chromatin. Fractionation has been attempted by a variety of techniques including mechanical breakage (Arnold and Young, 1974; Chesterton et al., 1974; Clark and Felsenfeld, 1971) or nuclease digestion (Gottesfeld et al., 1974) followed by fractionation techniques based on differences in size (Janowski et al., 1972), density (Frenster et al., 1963; McCarthy et al., 1974; Henner et al., 1975), and charge (Simpson, 1974).

Isoelectric focusing (IEF)[1] has been used extensively in the

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[1] Abbreviations used are: IEF, isoelectric focusing; p*I*, isoelectric point; $PhCH_2SO_2F$, phenylmethanesulfonyl fluoride; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.