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Salt Dependence of the Kinetics of the *lac* Repressor–Operator Interaction: Role of Nonoperator Deoxyribonucleic Acid in the Association Reaction†

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ABSTRACT: The kinetics of binding of *lac* repressor protein and operator deoxyribonucleic acid (DNA) have been studied as a function of monovalent and divalent cation concentration. The salt dependence of the association and dissociation rate constants has been interpreted in light of recent theoretical analyses based on Manning's counterion condensation model. The bell-shaped dependence of the association rate constant on salt concentration evidences a role for nonoperator DNA binding in the repressor's search for the operator site on a large DNA molecule. At intermediate mono- or divalent cation concentrations, the association rate goes through a maximum. At lower cation concentrations, it decreases and becomes dependent on DNA concentration; the high affinity of repressor for nonoperator DNA confines the protein to the DNA. At higher cation concentrations, the association rate decreases and becomes dependent on the weak affinity of repressor for nonoperator DNA. The kinetic data are fit to the theory of Berg and Blomberg [Berg, O. G., & Blomberg, C. (1978) *Biophys. Chem. 8*, 271] for the salt dependence of association kinetics with coupled diffusion, using published values of the affinity for nonoperator DNA. From this fit, one-dimensional diffusion of repressor along the DNA chain is estimated to be about 4 times faster on MgDNA than on NaDNA. At higher cation concentrations, the salt dependence of the association and dissociation rate constants is consistent with a preequilibrium mechanism for the association reaction [Lohman, T. M., deHaseth, P. L., & Record, M. T., Jr. (1978) *Biophys. Chem. 8*, 281]. Agreement between literature values (corrected for the presence of Mg^{2+}) and experimental values of the rate constants in the presence of monovalent salt is quite good.

The *lac* repressor protein and its operator site on deoxyribonucleic acid (DNA), plus inducing sugars, comprise the negative control system of the *lac* operon in *Escherichia coli* [for a comprehensive review, see Miller & Reznikoff (1978)]. The interaction of repressor protein and operator DNA has been extensively studied in vitro by the membrane filter technique. The results are consistent with a simple bimolecular reaction of repressor (R) and operator (O)

$$R + O \underset{k_d}{\overset{k_a}{\rightleftharpoons}} RO \qquad (1)$$

where k_a is the association rate constant and k_d is the dissociation rate constant, and where the observed equilibrium association constant, K_{obsd}, is

$$K_{obsd} = \frac{[RO]}{[R][O]} = \frac{k_a}{k_d} \qquad (2)$$

The repressor binds tightly to the operator. In standard buffer, K_{obsd} is about 1×10^{13} M^{-1}, k_a is about 7×10^9 M^{-1} s^{-1}, and k_d is about 6×10^{-4} s^{-1} (Riggs et al., 1970a,b). The bimolecular association of repressor and operator is very rapid, the value of the rate constant k_a being about 2 orders of magnitude greater than expected for a diffusion-limited reaction. The binding parameters are quite sensitive to ionic conditions. For example, a 10-fold increase in ionic strength (from 0.02 to 0.20 M) results in about a 60-fold decrease in the association rate

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constant k_a and about a 5-fold increase in the dissociation rate constant k_d, corresponding to about a 300-fold reduction in the association constant K_{obsd}.

The *lac* repressor–operator interaction is highly specific: the protein binds operator with roughly 10^7-fold greater affinity than it binds nonoperator DNA sequences (Lin & Riggs, 1972, 1975). The repressor, and probably other regulatory proteins and enzymes which recognize specific sequences, accomplishes the rather formidable task of quickly searching a large DNA molecule for a particular sequence and then binding to it with high affinity. Two models for facilitated diffusion have been put forward to explain the very fast rate of association of repressor and operator. Both invoke a role for nonoperator DNA binding in rate enhancement; the protein first diffuses in solution to the DNA molecule and then travels on DNA to the operator site. In the one-dimensional diffusion or "sliding" model, the repressor diffuses along the DNA chain to the operator. This model has received detailed theoretical analysis (Richter & Eigen, 1974; Berg & Blomberg, 1976, 1977, 1978; Schranner & Richter, 1978). In the direct transfer model, the repressor is transferred between DNA binding sites via transitional binding at two sites (Bresloff & Crothers, 1975; von Hippel et al., 1975).

Dependence of the observed equilibrium constant on salt concentration is a common feature of protein–nucleic acid interactions. Recently, electrostatic effects on the binding of charged ligands to polynucleotides have been treated by application of Manning's polyelectrolyte theory (Record et al., 1976, 1978; Manning, 1978). In the counterion condensation model (Manning, 1969), cations are condensed on the negatively charged polynucleotide; the number of condensed monovalent counterions per phosphate is about 0.75 (Anderson et al., 1978). The remaining unneutralized charges are screened by an ion atmosphere. Binding of ligand, such as a protein, is entropically driven by the counterion diffusion potential, resulting in a large decrease in affinity with increasing salt concentration. The effects of various ions on the binding of *lac* repressor protein to nonoperator DNA were investigated, and the dependence of the observed equilibrium constant K_{obsd}^{RD} on ion concentration was analyzed according to the binding theory (deHaseth et al., 1977a; Revzin & von Hippel, 1977).

In this and the accompanying paper (Barkley et al., 1981), we present a complementary study of the effects of ions on the *lac* repressor–operator interaction.[1] Our approach is to examine a specific protein–nucleic acid interaction in relation to the theoretical and experimental results outlined above. Previous studies have utilized buffers containing both monovalent (K^+) and divalent (Mg^{2+}) cations, which complicates comparison with theory (Riggs et al., 1970a,b; Barkley et al., 1975; Lin & Riggs, 1975). However, these binding data have been interpreted after correction for the presence of Mg^{2+} in the buffers (Record et al., 1977; Lohman et al., 1978). To circumvent this problem, we use buffers containing a single added salt. This paper reports the results for the kinetics of repressor–operator binding. The dependence of the rate constants on salt concentration is analyzed quantitatively by comparison with published theoretical equations, which are summarized below. The following paper (Barkley et al., 1981) deals with the effects of ion concentration and type on the observed equilibrium constant. The findings provide insight into the recognition mechanism and the role of ions in gene regulation.

Berg & Blomberg (1976, 1977, 1978) and Schranner & Richter (1978) have considered association kinetics with coupled three- and one-dimensional diffusion. Though they differ in mathematical formulation and emphasis, the two renditions of the sliding model reach similar conclusions. The rate of association of repressor and operator depends upon the total length of the DNA molecule. It also depends upon the diffusion rate along the chain and the dissociation rate from DNA. Ionic conditions influence the association rate via the affinity of repressor for nonoperator DNA. Berg & Blomberg (1978) derived the ionic strength dependence of the association rate constant k_a for long coiled chains. In the spirit of the counterion condensation model, they visualize association of the protein to DNA as a two-step process: screened electrostatic attraction followed by a discrete binding reaction, which involves displacement of condensed counterions, conformational changes, formation of specific interactions, etc. Transcription of their results into molar concentration units gives

$$k_a = \frac{\Lambda\{L(\Lambda/D_1)^{1/2} \coth [L(\Lambda/D_1)^{1/2}] - 1\}^{-1}}{O(1 + 1/K_{obsd}^{RD}D)} \text{ M}^{-1}\text{ s}^{-1} \quad (3)$$

where $2L$ is the length of a large DNA molecule containing the operator site, D_1 is the one-dimensional diffusion constant of repressor along DNA, O is the molar concentration of operator DNA molecules, D is the molar concentration of DNA nucleotides, K_{obsd}^{RD} is the affinity of repressor for nonoperator DNA expressed in molar concentrations of repressor and nucleotides, and Λ is the global dissociation rate from DNA. Λ is given approximately by eq 4 [cf. eq 2.10, 4.3, and 4.4 of Berg & Blomberg (1978)]

$$\Lambda = [(1.5 \times 10^{-14})K_{obsd}^{RD} \ln (R_c/b)/D_3 + 1/\lambda]^{-1}\text{ s}^{-1} \quad (4)$$

where D_3 is the three-dimensional diffusion constant of the repressor in solution, $\ln (R_c/b)$ is an approximate expression for the screening effect, and λ is the intrinsic dissociation rate from DNA. As discussed by Berg and Blomberg, D_1 and λ are presumably independent of ionic strength, and the screening effect varies only slowly with ionic strength. Thus, the dependence of k_a on ion concentration derives primarily from K_{obsd}^{RD}, which measures the competitive equilibrium between repressor and bound counterions. At low salt concentrations, when K_{obsd}^{RD} is very large, k_a becomes independent of ion concentration:

$$k_a \rightarrow 3D_1/OL^2 \text{ M}^{-1}\text{ s}^{-1} \quad (5)$$

At higher salt concentrations, when K_{obsd}^{RD} is small, k_a is proportional either to $(K_{obsd}^{RD})^{1/2}$ or to K_{obsd}^{RD}, depending on whether association of the protein to DNA is diffusion controlled ($\Lambda \propto 1/K_{obsd}^{RD}$) or reaction controlled ($\Lambda \rightarrow \lambda$).

Lohman et al. (1978) have also derived expressions for the ion concentration dependence of the kinetics of protein–DNA interactions. They formulate the bimolecular association of repressor and operator as a chemical reaction and deal with two possible mechanisms in a steady-state treatment based on the binding theory: (1) a screening-controlled mechanism and (2) a preequilibrium mechanism. Both mechanisms predict a linear log–log relationship between the association rate constant k_a and cation concentration, though the magnitude and significance of the slopes differ in the two cases. In principle, analysis of the effects of cation concentration on the kinetics and equilibrium will distinguish between these limiting mechanisms for the association reaction. The preequilibrium model postulates formation of an intermediate with n ionic

[1] A preliminary account of this work was presented at the Second Biophysical Discussion on Proteins and Nucleoproteins: Structure, Dynamics and Assembly, Airlie House, VA, 1980.

interactions; the rate-limiting step is conversion to the final complex with Z ionic interactions. In this model, the entire salt dependence of k_a is that of the equilibrium constant for formation of the intermediate, which involves both screening and counterion release. Assuming no anion release from the protein, in the presence of only monovalent salt (MX)

$$\frac{\partial \ln k_a}{\partial \ln [M^+]} = -n\psi \quad (6)$$

where ψ is the number of counterions thermodynamically bound per phosphate. For double-helical DNA, $\psi = 0.88$. Combining eq 6 with the salt dependence of K_{obsd} (cf. eq 1 in the following paper) gives

$$\frac{\partial \ln k_d}{\partial \ln [M^+]} = (Z - n)\psi \quad (7)$$

In the presence of only divalent salt (MX_2), similar equations obtain with $\psi = 0.47$.

Materials and Methods

Buffers. To facilitate comparison with the results for non-operator DNA binding, membrane filter experiments were performed under similar conditions. The binding buffers are essentially those described by deHaseth et al. (1977b), except that bovine serum albumin (BSA)[2] [heat treated to inactivate nuclease according to Wang et al. (1974)] was added to stabilize highly dilute repressor protein, and dithioerythritol (DTE) and dimethyl sulfoxide (Me_2SO) were used instead of β-mercaptoethanol and glycerol. At 5% (v/v) dimethyl sulfoxide and glycerol exert the same small effect on the repressor–operator interaction (Pfahl, 1978) and apparently no effect on the repressor–DNA interaction (deHaseth et al., 1977b). The pH was adjusted with the conjugate acid of the added salt or with HCl to within ±0.03 pH unit after addition of all ingredients except dimethyl sulfoxide. (No change in pH was noted upon adding dimethyl sulfoxide.) All chemicals were reagent grade.

Standard binding buffer (BB) is 0.01 M Tris-HCl (pH 7.4 at 20 °C), 0.01 M $Mg(CH_3CO_2)_2$, 0.01 M KCl, 10^{-4} M EDTA, 10^{-4} M DTE, 5% (v/v) Me_2SO, and 25 μg/mL BSA. Standard filtering buffer is BB without DTE, Me_2SO, and BSA.

Phosphate binding buffer (P-BB) is 0.01 M Na_2HPO_4 (pH 7.4 at 20 °C) plus NaCl to give the indicated $[Na^+]$, 10^{-4} M DTE, 5% (v/v) Me_2SO, and 25 μg/mL BSA. Phosphate filtering buffer is P-BB, 0.05 M Na^+, without DTE, Me_2SO, and BSA.

Tris binding buffer (T-BB) is 0.01 M Tris (pH 8.0 at 20 °C) plus salt at the indicated $[M^+]$, or 0.001 M Tris (pH 8.0 at 20 °C) plus $MgCl_2$ at the indicated concentration, 10^{-4} M EDTA (10^{-5} M EDTA for $[MgCl_2] < 0.01$ M), 10^{-4} M DTE, 5% (v/v) Me_2SO, and 25 μg/mL BSA. Tris filtering buffer is T-BB, 0.05 M M^+ or 0.01 M $MgCl_2$, without DTE, Me_2SO, and BSA.

Repressor Protein. Two highly purified preparations of *E. coli lac* repressor were used for membrane filter experiments: one isolated from the i^q strain BMH461 (Müller-Hill et al., 1968) and one isolated from the i^{q1} strain BMH74-12-6 (Müller-Hill et al., 1975). Repressor protein was purified according to Platt et al. (1973) with the following modifications. In all buffers, dithioerythritol concentration was increased to 10^{-3} M, sodium azide was added at 10^{-3} M, and glycerol was omitted. Cell debris and ribosomes were removed by centrifugation at 6×10^4 rpm (70Ti rotor), 4 °C, for 1.5 h. Passage through DEAE-cellulose in 0.2 M potassium phosphate buffer and chromatography on DNA-cellulose [6 mg of salmon DNA/g of cellulose prepared as described by Alberts & Herrick (1971) and UV irradiated as described by Litman (1968)] preceded phosphocellulose chromatography. The pooled peak from the phosphocellulose column was dialyzed against 0.1 M potassium phosphate buffer (pH 7.5 at 4 °C). The protein was >98% pure as judged by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Repressor solutions (0.1–0.2 mg/mL) were stored frozen at −70 °C in 0.1-mL aliquots.

Operator-binding properties of the two repressor preparations were determined by the membrane filter assay under standard conditions (BB, 20 °C) as outlined by Riggs et al. (1970a,b) and described below. The i^q repressor had about 50% of its theoretical operator-binding activity: $k_a = (8.8 \pm 0.8) \times 10^9$ M^{-1} s^{-1}, $k_d = (3.0 \pm 0.4) \times 10^{-4}$ s^{-1} ($\tau_{1/2} = 39 \pm 5$ min), and $K_{obsd} = (2.9 \pm 0.5) \times 10^{13}$ M^{-1}. The i^{q1} repressor had about 80% activity: $k_a = (8.9 \pm 1.8) \times 10^9$ M^{-1} s^{-1}, $k_d = (2.9 \pm 0.3) \times 10^{-4}$ s^{-1} ($\tau_{1/2} = 40 \pm 4$ min), and $K_{obsd} = (3.1 \pm 0.7) \times 10^{13}$ M^{-1}. Thus, the active repressor in the two preparations is identical within experimental error. Prior to use in kinetic experiments, repressor solutions were routinely titrated against operator DNA in BB to determine the active repressor concentration. The rate constants under standard conditions were also checked at periodic intervals. No changes in operator-binding properties were noted in samples stored frozen at −70 °C for 2 years or in thawed samples stored at 4 °C for 5 days.

Operator DNA. Bacteriophage λp*lac*5 DNA (Ippen et al., 1971) was isolated from the temperature-inducible, lysis-defective lysogen BMH782 (obtained from M. Pfahl). The phage DNA was purified according to Miller (1972) with the following modifications. Cells were grown in 2YT medium until $A_{600} = 1.5$–1.8. Phage multiplication was induced by vigorous shaking at 42 °C for 10 min, at 32 °C for 10 min, and again at 42 °C for 10 min, or by addition of an equal volume of hot medium (65 °C) and shaking at 42 °C for 10 min. The CsCl block gradient (6 mL $\rho = 1.7$ CsCl solution; 10 mL $\rho = 1.4$ CsCl solution) was centrifuged at 2.9×10^4 rpm (70Ti rotor), 10 °C, for 1.5 h, and the CsCl equilibrium gradient was centrifuged at 4.5×10^4 rpm (50Ti rotor), 10 °C, for 5 h. The phage suspension was dialyzed against 0.01 M Tris-HCl (pH 7.5), 0.01 M NaCl, 10^{-4} M EDTA and extracted 3 times with 2 volumes of phenol. Residual phenol was removed by ether extraction, and ether was removed by a stream of N_2. The DNA solution was dialyzed against the same buffer and stored at 4 °C over a drop of CCl_4. ^{32}P-Labeled λp*lac*5 DNA was prepared by the same procedure, except that cells were grown in low phosphate medium (Barkley et al., 1975), 10–50 μCi/mL carrier-free [^{32}P]-phosphate was added after 10 min of heat induction, and 1% (v/v) ethanol was included in the final dialysis buffer.

The λp*lac*5 DNA is full-length λ-DNA as judged by electrophoresis on neutral 0.3% agarose gels (McDonell et al., 1977). The DNA concentration was determined by the absorbance at 260 nm (extinction coefficient 0.02 $cm^2/\mu g$), and the operator concentration was computed by assuming one operator site per λp*lac*5 molecule (molecular weight 3×10^7).

Rate Constants. Association and dissociation kinetics were monitored by the membrane filter assay for the repressor–

[2] Abbreviations used: BSA, bovine serum albumin; DTE, dithioerythritol; Me_2SO, dimethyl sulfoxide; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; UV, ultraviolet; IPTG, isopropyl β-D-thiogalactoside.

operator complex, essentially as described by Riggs et al. (1970a). All experiments were done at 20 °C. Filtering and washing volumes were 0.1 mL for 10-mm filters and 0.5 mL for 25-mm filters. Filtration took about 5–7 s. To conserve filters and counting time, only one aliquot was filtered for each time point. In control experiments in BB, we found that the precision and accuracy of the rate constants were comparable when either one or three aliquots of timed samples were filtered. However, the accuracy of the rate constants is quite sensitive to errors in the background and the conversion factor, so three aliquots of equilibrated samples were filtered and the triplicate values averaged. Rate constants are reported as mean values ± standard deviations of 3–12 experiments. Propagation of errors is computed from standard statistical formulas [see Bevington (1969)].

(*a*) *Association Kinetics.* A variation of a procedure described by Lin & Riggs (1972) was used. Control experiments in BB showed that neither precision nor accuracy was improved by more elaborate procedures, in which the association reaction is stopped prior to filtration by addition of a large excess of unlabeled DNA. Just before the reaction was started, 13 HA Millipore filters were arranged on the filtration disk. A 1- or 2-fold molar excess of repressor was added to ^{32}P-labeled λp*lac*5 DNA [final operator concentration (0.5–100) × 10^{-12} M] in the desired binding buffer and mixed on a magnetic stirrer for about 5 s. Aliquots were filtered at appropriate time intervals (5–20 s). The filters were washed with the corresponding filtering buffer, placed in minivials with scintillation fluid (6.5 g of terphenyl, 1 L of toluene, 50 mL of Beckman Bio-Solv), and counted. To estimate the conversion factor relating the amount of radioactivity retained on the filters to the concentration of repressor–operator complex in solution (Riggs et al., 1970a), three aliquots of a sample containing a 10- or 20-fold excess of repressor were filtered after 15–30 min (1–4 h in the case of low-salt binding buffers). To obtain the background levels of radioactivity retained on the filters in the presence of IPTG, three aliquots of two samples containing appropriate amounts (1- or 2-fold excess and 10- or 20-fold excess) of repressor plus 5 × 10^{-4} M IPTG were also filtered after 15–30 min. The background was subtracted from each point, and the data were analyzed according to the integrated rate equation for a bimolecular reaction [cf. eq 7 of Riggs et al. (1970a)] by linear regression. For reactions in low-salt binding buffers, the kinetic data were also analyzed with the first-order rate equation

$$\frac{d[RO]}{dt} = k_{app}[R] = k_{app}(R - [RO]) \qquad (8)$$

where k_{app} is the apparent first-order rate constant and R is the molar concentration of repressor. The data were analyzed according to the integrated form of eq 8

$$\ln (1 - [RO]/R) = -k_{app}t \qquad (9)$$

by linear regression, and the bimolecular rate constant k_a was computed from $k_a = k_{app}/O$.

(*b*) *Dissociation Kinetics.* A 2–4-fold molar excess of repressor was incubated with ^{32}P-labeled λp*lac*5 DNA [final operator concentration (0.5–100) × 10^{-12} M] in the desired binding buffer for at least 30 min. The dissociation reaction was initiated by addition of a 100-fold excess of unlabeled λp*lac*5 DNA (sonicated for 30 s at 50 W, 10 °C) and mixed on a magnetic stirrer for 15 s. Aliquots were filtered at appropriate time intervals, and the filters were washed and counted as described above. To obtain the background level, three aliquots of a sample containing 5 × 10^{-4} M IPTG were filtered at the end of the reaction. The background was subtracted from each point, and the data were analyzed according to the integrated rate equation for a unimolecular reaction [cf. eq 4 of Riggs et al. (1970a)] by linear regression.

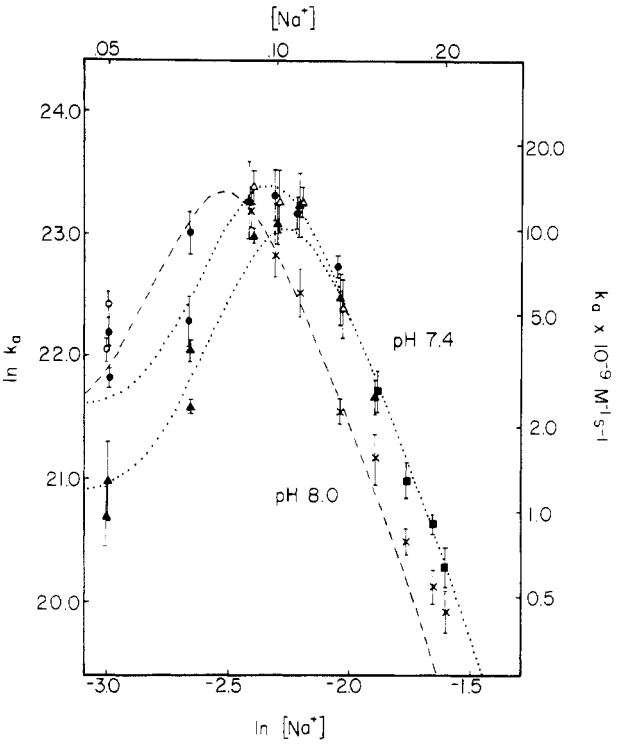

FIGURE 1: Dependence of association rate constant of *lac* repressor protein and λp*lac* DNA on cation concentration for NaCl, pH 7.4 and 8.0. Symbols are experimental values ± SD indicated by error bars; some points are displaced ±0.01 ln [Na^+] unit to avoid overlap of the error bars. Curves are theoretical values calculated according to eq 3 and 4 as described in the text. NaCl, P-BB, pH 7.4: open symbols, $R = O$; solid symbols, $R = 2O$; (○, ●) $O = 5 \times 10^{-13}$ M; (△, ▲) $O = 1 \times 10^{-12}$ M; (■) $O = 2 \times 10^{-12}$ M. The two sets of symbols at 0.05 and 0.07 M Na^+ represent the rate constants obtained by assuming different rate equations: upper symbol, bimolecular reaction; lower symbol, apparent first-order reaction. The data were fit with $K_{obsd}^{RD} = 10^{-3.1}[Na^+]^{-10.3}$ (Revzin & von Hippel, 1977; corrected from pH 7.5 to pH 7.4 by the factor $10^{0.26}$ and converted from base-pair units to nucleotide units by the factor $10^{-0.3}$) and $D_1 = 2.5 \times 10^{-10}$ cm^2 s^{-1}. These data could not be fit by using $K_{obsd}^{RD} = 10^{-5.6}[Na^+]^{-12.0}$ (deHaseth et al., 1977b) for any value of D_1 over a reasonable range of ln (R_c/b) and λ. The upper dotted line was calculated with $O = 5 \times 10^{-13}$ M, and the lower dotted line with $O = 1 \times 10^{-12}$ M. NaCl, T-BB, pH 8.0: (×) mean value for different concentrations of repressor and operator in the range $O = 1 \times 10^{-12}$ M at 0.09 M NaCl to $O = 1 \times 10^{-11}$ M at 0.20 M NaCl. The dashed line was calculated with $O = 1 \times 10^{-12}$ M, $K_{obsd}^{RD} = 10^{-3.9}[Na^+]^{-10.0}$ (deHaseth et al., 1977a; corrected from 4 to 20 °C by the factor $10^{-0.2}$), and $D_1 = 4.5 \times 10^{-10}$ cm^2 s^{-1}.

Results

Association Kinetics. The rate of association of *lac* repressor protein and operator DNA was measured as a function of cation concentration for various salts and buffers: NaCl in phosphate buffer, pH 7.4; NaCl in Tris buffer, pH 8.0; $NaCH_3CO_2$ in Tris buffer, pH 8.0; and $MgCl_2$ in Tris buffer, pH 8.0. As depicted in Figures 1–3, the qualitative dependence of the association rate constant k_a on cation concentration is the same in all cases. The rate constant *increases* with increasing cation concentration at lower salt concentrations, reaches a maximum at intermediate salt concentrations ([Na^+] ≃ 0.1 M or [$MgCl_2$] ≃ 0.006 M), and then *decreases* with increasing cation concentration at higher salt concentrations. This unusual salt dependence is consistent with reaction mechanisms in which the repressor binds to nonoperator regions of the DNA prior to binding at the operator site. Two

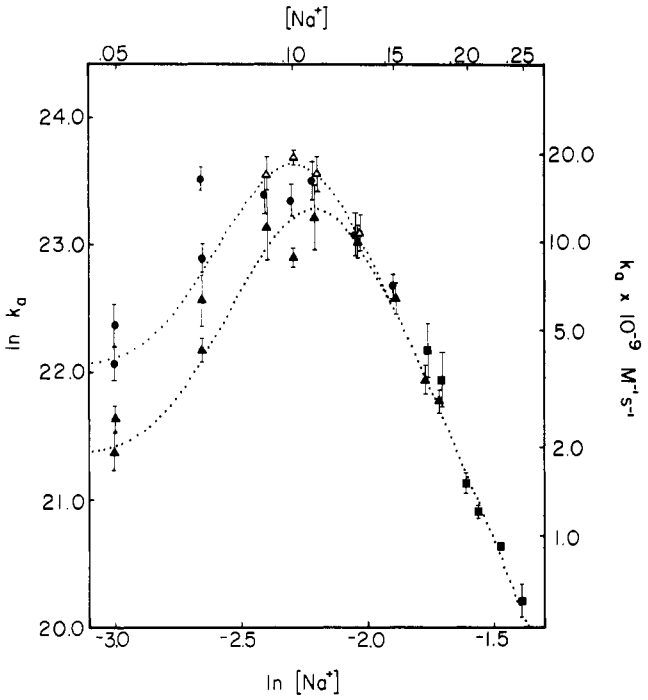

FIGURE 2: Dependence of association rate constant of *lac* repressor protein and λp*lac* DNA on cation concentration for $NaCH_3CO_2$, T-BB, pH 8.0. Symbols are experimental values ± SD indicated by error bars; some points are displaced ±0.01 ln [Na^+] unit to avoid overlap of the error bars. Open symbols, $R = O$; solid symbols, $R = 2O$; (●) $O = 5 \times 10^{-13}$ M; (△, ▲) $O = 1 \times 10^{-12}$ M; (■) $O = 2 \times 10^{-12}$ M. The two sets of symbols at 0.05 and 0.07 M $NaCH_3CO_2$ represent the rate constants obtained by assuming different rate equations: upper symbol, bimolecular reaction; lower symbol, apparent first-order reaction. Curves are theoretical values calculated according to eq 3 and 4 as described in the text. The data were fit with $K_{obsd}^{RD} = 10^{-1.7}[Na^+]^{-9.1}$ (deHaseth et al., 1977a; corrected from 4 to 20 °C by the factor $10^{-0.2}$) and $D_1 = 4.0 \times 10^{-10}$ cm^2 s^{-1}. The upper dotted line was calculated with $O = 5 \times 10^{-13}$ M, and the lower dotted line with $O = 1 \times 10^{-12}$ M.

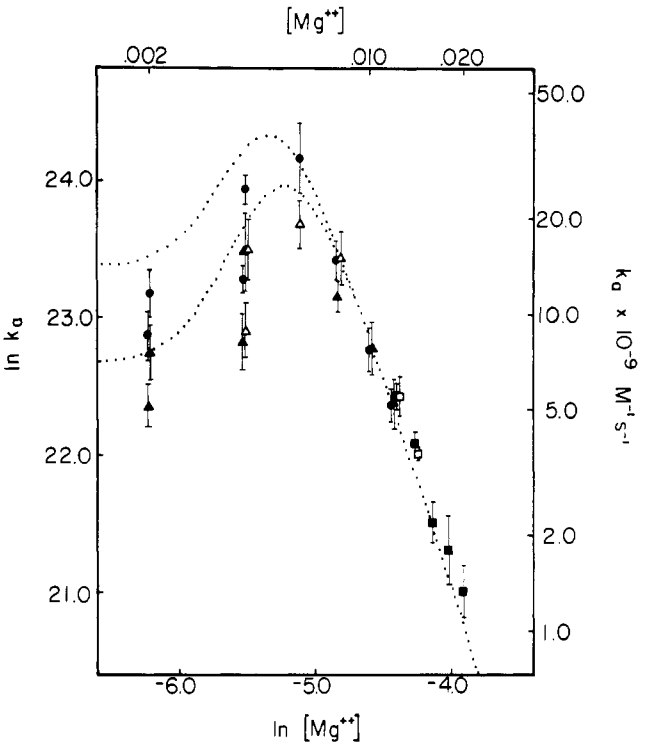

FIGURE 3: Dependence of association rate constant of *lac* repressor protein and λp*lac* DNA on cation concentration for $MgCl_2$, T-BB, pH 8.0. Symbols are experimental values ± SD indicated by error bars; some points are displaced by ±0.02 ln [Mg^{2+}] unit to avoid overlap of the error bars. Open symbols, $R = O$; solid symbols, $R = 2O$; (●) $O = 5 \times 10^{-13}$ M; (△, ▲) $O = 1 \times 10^{-12}$ M; (□, ■) $O = 2 \times 10^{-12}$ M. The two sets of symbols at 0.002 and 0.004 M $MgCl_2$ represent the rate constants obtained by assuming different rate equations: upper symbol, bimolecular reaction; lower symbol, apparent first-order reaction. Curves are theoretical values calculated according to eq 3 and 4 as described in the text. The data were fit with $K_{obsd}^{RD} = 10^{-6.1}[Mg^{2+}]^{-5.8}$ (deHaseth et al., 1977a; corrected from 4 to 20 °C by the factor $10^{-0.2}$) and $D_1 = 1.5 \times 10^{-9}$ cm^2 s^{-1}. The upper dotted line was calculated with $O = 5 \times 10^{-13}$ M, and the lower dotted line with $O = 1 \times 10^{-12}$ M.

kinetic consequences of a transitional binding to DNA are manifest. At higher salt concentrations, the interaction with DNA effectively concentrates the protein in the vicinity of the DNA molecule, enhancing the probability of its reaching the operator. Increasing the relatively low affinity of repressor for DNA by decreasing the cation concentration accelerates the association rate. At lower salt concentrations, however, the much stronger interaction with DNA traps the protein on nonoperator regions and retards the association rate.

Since decreases in the association rate constant k_a at lower ionic strength had not been reported previously (Riggs et al., 1970a; Barkley et al., 1975), we were concerned about possible artifacts of the filter assay under these conditions. For example, instability of *lac* repressor in the low-salt binding buffers would reduce the apparent bimolecular rate constant. We performed numerous equilibrium binding measurements as controls. Titration experiments yielded the expected values for the active repressor concentration, even when the protein was kept at high dilution in low-salt binding buffers at 4 °C for 30 min before titration. Moreover, the precision of the association rate constant is essentially independent of cation concentration, which argues against irregularities in the assay at lower salt concentrations. Furthermore, inspection of Figure 3 explains why decreases in k_a at lower ionic strength had been missed in earlier work. Most experiments varying ionic strength were performed in the presence of 0.01 M Mg^{2+} (BB, KCl concentration variable). As evident in Figure 3, further increases in salt concentration, whether by addition of $MgCl_2$ or KCl, decrease the rate constant. The few measurements at lower Mg^{2+} concentration were performed in the presence of 0.002 or 0.003 M Mg^{2+} plus low concentrations of KCl (<0.03 M). Under these conditions, the values of the rate constant would be greater than the values in the presence of 0.01 M Mg^{2+}, though less than the maximum value. Another possible explanation for reductions in the rate constant k_a at lower salt concentrations is a conformational change in the repressor, which affects its interaction with DNA. For example, the affinity of a low-salt conformer could be less than the maximum affinity of a high-salt conformer. Though we think it unlikely, we cannot rule out such an interpretation since neither the salt dependence of repressor conformation nor the affinities of repressor for operator and nonoperator DNA at lower salt concentrations are known.

The association rate was also measured over a limited range of repressor and operator concentrations. Figures 1–3 show the rate constants for different concentrations of operator DNA (circles, $O = 5 \times 10^{-13}$ M; triangles, $O = 1 \times 10^{-12}$ M; squares, $O = 2 \times 10^{-12}$ M) and for different ratios of repressor and operator concentrations (open symbols, $R/O = 1$; solid symbols, $R/O = 2$). At higher salt concentrations, the rate constant is independent of the concentration of repressor and operator, as expected for a simple bimolecular reaction. On the other hand, at lower salt concentrations, the rate constant depends on the concentration of operator DNA: k_a is decreased when the operator concentration is increased. The rate constants k_a were determined by assuming rate equations for

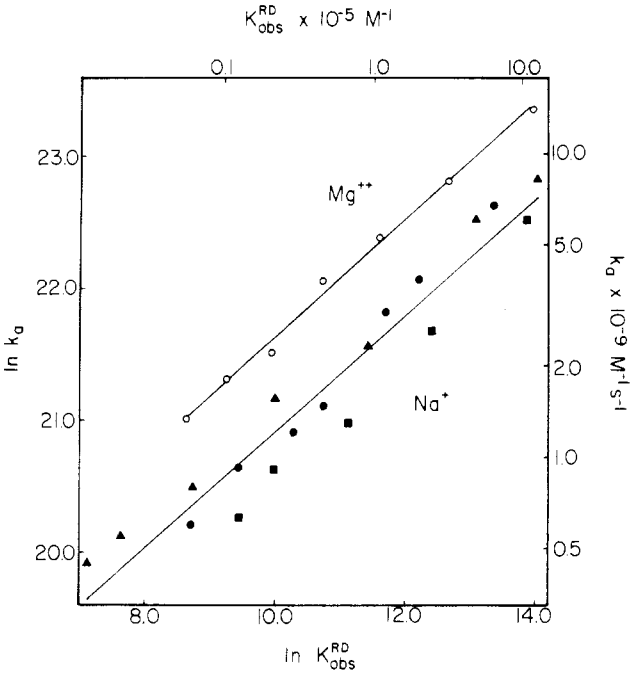

FIGURE 4: Dependence of association rate constant of *lac* repressor protein and λp*lac* DNA on the repressor–DNA equilibrium constant at higher salt concentration. Symbols represent mean values of k_a from Figures 1–3 plotted against literature values of K_{obsd}^{RD} (deHaseth et al., 1977a; Revzin & von Hippel, 1977) at corresponding salt concentrations: (■) NaCl, P-BB, pH 7.4, $[Na^+] \geq 0.13$ M; (▲) $[NaCl] \geq 0.10$ M, T-BB, pH 8.0; (●) $[NaCH_3CO_2] \geq 0.15$ M, T-BB, pH 8.0; (○) $[MgCl_2] \geq 0.008$ M, T-BB, pH 8.0. Lines drawn are least-squares fit through the points: upper line, Mg^{2+} data; lower line, combined Na^+ data.

a bimolecular reaction (upper symbols) and for an apparent first-order reaction (lower symbols). Although the two rate laws give comparable fits to the kinetic data (correlation coefficients ~0.98), the second-order analysis yields somewhat larger values of k_a than the first-order analysis. For intermediate salt concentrations where the association rate is fastest, we do not find a significant dependence of the rate constant on operator concentration. However, these data are probably the least accurate because of the problems associated with measuring such rapid reaction rates. The rate constant also appears to be independent of repressor concentration at lower and intermediate salt concentrations.

(*a*) *One-Dimensional Diffusion Is Faster on MgDNA Than on NaDNA.* Berg & Blomberg's (1978) analysis of the ionic strength dependence of the association reaction predicts the essential features of the experimental findings. The fit to their theory is illustrated in Figures 1–3. The curves drawn in the figures were generated from eq 3 and 4, taking $2L = 1.6 \times 10^{-3}$ cm for λ-DNA, $D_3 = 5 \times 10^{-7}$ cm^2 s^{-1} for the repressor (molecular weight 1.5×10^4), ln $(R_c/b) = 3$, and $\lambda = 10^4$ s^{-1}, and using literature values of K_{obsd}^{RD}. The upper dotted lines were calculated with $O = 5 \times 10^{-13}$ M, the lower dotted lines with $O = 1 \times 10^{-12}$ M. Values of D_1 were adjusted to give a reasonable fit to the data. The agreement between Berg and Blomberg's theory and the experimental results is satisfying. For intermediate and higher salt concentrations, the theoretical curves fit the data within experimental error, with one exception. For NaCl at pH 8.0, the theoretical curve (dashed line) falls off more rapidly at higher salt concentrations than the data (crosses). The fit can be marginally improved by using larger values of λ. (Increasing λ to 10^6–10^7 s^{-1} removes the small amount of downward curvature in the tail of the theoretical curve; further increases have no effect.) Because the predicted rate constant depends primarily on K_{obsd}^{RD} at higher salt concentrations, this discrepancy may reflect uncertainty in the values of K_{obsd}^{RD}. For lower salt concentrations, the theoretical curves are in qualitatve accord with the data. As anticipated from eq 5, both the predicted and observed rate constants exhibit an inverse dependence on the concentration of operator DNA. The nature of the reaction changes from bimolecular to apparent first order at low salt concentrations. For NaCl at pH 7.4 and for $NaCH_3CO_2$, the values of k_a obtained from the first-order analysis give more quantitative fits to the theory than those obtained from the second-order analysis. Moreover, the apparent first-order rate constants k_{app} for 0.05 and 0.07 M Na^+ are independent of operator concentration. For $MgCl_2$, the values of k_a obtained from the first-order analysis lie below the theoretical curves. However, the values of k_{app} for 0.002 and 0.004 M $MgCl_2$ are slightly dependent on operator concentration, suggesting that the reaction is not completely first order at these salt concentrations. Because *lac* repressor protein does not adhere well to membrane filters at very low salt concentrations, we have not been able to verify Berg and Blomberg's prediction that k_a levels off and becomes independent of salt concentration at low salt (see eq 5). Our inability to detect the predicted dependence on operator concentration at intermediate salt concentrations may be due to limited accuracy of the data.

Table I: Parameters for Sliding Model

conditions[a]	one-dimensional diffusion constant,[b] D_1 (cm^2 s^{-1})	slope of plot[c] ln k_a vs. ln K_{obsd}^{RD}
NaCl, P-BB, pH 7.4	2.5×10^{-10}	0.49 ± 0.03
NaCl, T-BB, pH 8.0	4.5×10^{-10}	0.43 ± 0.01
$NaCH_3CO_2$, T-BB, pH 8.0	4.0×10^{-10}	0.53 ± 0.02
$MgCl_2$, T-BB, pH 8.0	1.5×10^{-9}	0.45 ± 0.01

[a] Measurements at 20 °C. [b] Estimated by fitting eq 3 and 4 to the data in Figures 1–3 as described in the text. [c] The least-squares slope ± SD of plots in Figure 4.

In addition, Berg and Blomberg's theory predicts an explicit dependence of the association rate constant k_a on the repressor–DNA equilibrium constant K_{obsd}^{RD}. As mentioned in the introduction, the rate constant should be a linear function of either $(K_{obsd}^{RD})^{1/2}$ or K_{obsd}^{RD} at higher salt concentrations. Thus, we have plotted ln k_a vs. ln K_{obsd}^{RD} for the data at higher salt concentrations in Figure 4. Here, as well as in following sections, the term "higher salt concentration" refers to cation concentrations at which both the predicted and observed association rate constants are independent of the operator DNA concentrations investigated. For the various conditions, this is NaCl at pH 7.4, $[Na^+] \gtrsim 0.13$ M; NaCl at pH 8.0, $[NaCl] \gtrsim 0.10$ M; $[NaCH_3CO_2] \gtrsim 0.15$ M; and $[MgCl_2] \gtrsim 0.008$ M. In Figure 4, each point represents the mean value of k_a at a particular salt concentration taken from Figures 1–3; the corresponding value of K_{obsd}^{RD} is estimated as described in the legends. The solid symbols are data for Na^+ (squares, NaCl at pH 7.4; triangles, NaCl at pH 8.0; circles, $NaCH_3CO_2$), and the open circles are data for $MgCl_2$. In every case, ln k_a is a linear function of ln K_{obsd}^{RD}, with the least-squares slope given in the last column of Table I. The average slope of 0.47 ± 0.04 implies that $k_a \propto (K_{obsd}^{RD})^{1/2}$ at higher salt concentrations, as predicted for diffusion-controlled association of *lac* repressor to the DNA molecule. Furthermore, according to eq 3 and 4, plots of ln k_a vs. ln K_{obsd}^{RD} for various conditions should be superimposable, other factors being equal. Any

Table II: Parameters for Preequilibrium Model

conditions[a]	$n\psi$[b]	ln k_a (1 M)[c]	no. of ionic interactions in intermediate,[d] n	$(Z-n)\psi$[e]	ln k_d (1 M)[f]	$Z-n$[g]
NaCl, P-BB, pH 7.4, $[Na^+] \geq 0.13$ M	5.2 ± 0.3	11.9 ± 0.5	5.9 ± 0.3	4.3 ± 0.3	0.1 ± 0.5	4.9 ± 0.3
$[NaCl] \geq 0.10$ M, T-BB, pH 8.0	4.3 ± 0.1	13.0 ± 0.3	4.8 ± 0.2	3.5 ± 0.2	−1.1 ± 0.4	4.0 ± 0.3
$[NaCH_3CO_2] \geq 0.15$ M, T-BB, pH 8.0	4.8 ± 0.2	13.5 ± 0.4	5.5 ± 0.3	2.7 ± 0.3	−4.5 ± 0.5	3.0 ± 0.3
$[MgCl_2] \geq 0.008$ M, T-BB, pH 8.0	2.49 ± 0.09	11.3 ± 0.4	5.3 ± 0.2	1.5 ± 0.2	−0.8 ± 0.7	3.2 ± 0.4

[a] Measurements at 20 °C. [b] The negative least-squares slope ± SD of plots in Figures 1–3 analyzed according to eq 6. [c] The least-squares intercept ± SD of plots in Figures 1–3. [d] Computed from the values in column 2, with $\psi_{M^+} = 0.88$ and $\psi_{M^{2+}} = 0.47$. [e] The least-squares slope ± SD of plots in Figures 5 and 6 analyzed according to eq 7. [f] The least-squares intercept ± SD of plots in Figures 5 and 6. [g] Computed from the values in column 5, with $\psi_{M^+} = 0.88$ and $\psi_{M^{2+}} = 0.47$.

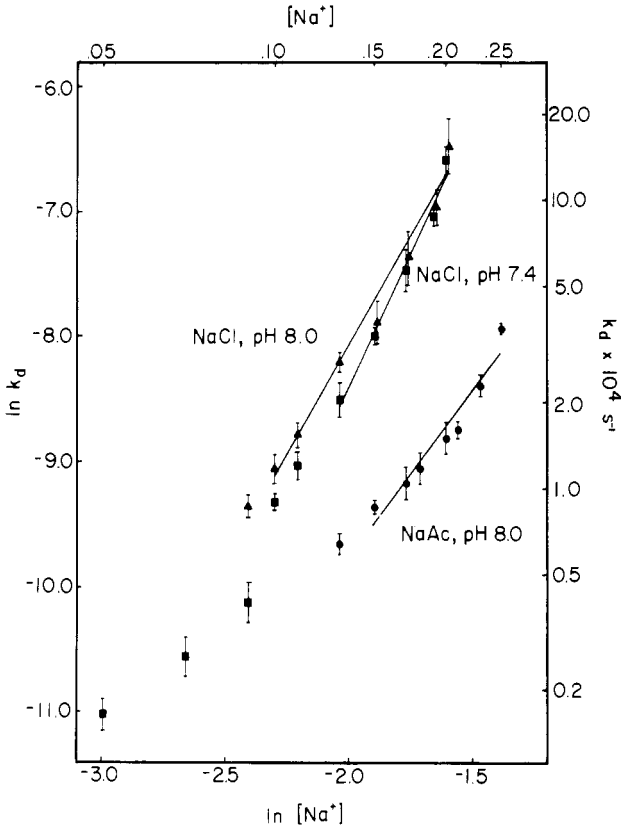

FIGURE 5: Dependence of dissociation rate constant of *lac* repressor protein and λp*lac* DNA on cation concentration for NaCl, pH 7.4; NaCl, pH 8.0; and $NaCH_3CO_2$, pH 8.0. Symbols are experimental values ± SD indicated by error bars; some points are displaced ±0.01 ln $[Na^+]$ unit to avoid overlap of the error bars: (■) NaCl, P-BB, pH 7.4; (▲) NaCl, T-BB, pH 8.0; (●) $NaCH_3CO_2$, T-BB, pH 8.0. Lines drawn are least-squares fit through the points at higher salt concentrations.

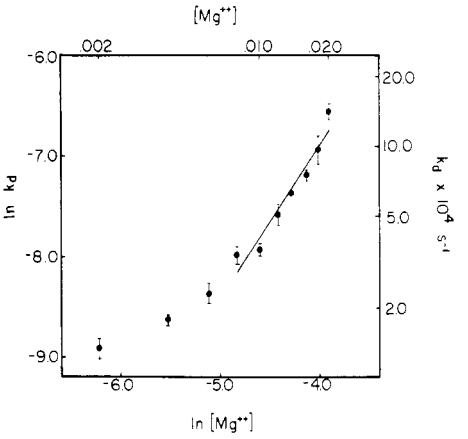

FIGURE 6: Dependence of dissociation rate constant of *lac* repressor protein and λp*lac* DNA on cation concentration for $MgCl_2$, T-BB, pH 8.0. Symbols are experimental values ± SD indicated by error bars. Line drawn is the least-squares fit through the points at higher salt concentrations.

differences may be attributed to differences in the one-dimensional diffusion constant D_1. Figure 4 shows the least-squares lines through all the data for Na^+ and through the data for Mg^{2+}. Admittedly, there is considerable scatter in the combined data for Na^+, encompassing uncertainties in both k_a and K_{obsd}^{RD}. Nevertheless, the difference between the two lines is consistent with more rapid diffusion of *lac* repressor on MgDNA than on NaDNA. The values of D_1 for various conditions are summarized in column 2 of Table I. The one-dimensional diffusion constant of repressor along DNA is about 4×10^{-10} cm^2 s^{-1} for NaDNA and about 1.5×10^{-9} cm^2 s^{-1} for MgDNA.

(*b*) *Association Mechanism Involves a Preequilibrium Step at Higher Salt Concentrations.* As evident in Figures 1–3, the log–log plots of the association rate constant vs. cation concentration appear linear at higher salt concentrations. This behavior is predicted by the treatment of Lohman et al. (1978).

We have also analyzed the kinetic data according to their theory of ion concentration effects on the association kinetics, assuming no anion release. The negative slope and intercept of the least-squares lines through the data at higher salt concentrations (not shown in the figures) are given in columns 2 and 3 of Table II. Comparison of the slopes in columns 2 and 5 shows that the salt dependence of k_a is larger in magnitude than that of k_d. As pointed out by Lohman et al., in the presence of only mono- or divalent cations, this situation is inconsistent with a screening-controlled mechanism and points to a preequilibrium mechanism for the association reaction. The number of ionic interactions, n, in the intermediate repressor–DNA complex is computed according to eq 6 from the slopes $\partial \ln k_a/(\partial \ln [Na^+])$ with $\psi = 0.88$ and $\partial \ln k_a/(\partial \ln [Mg^{2+}])$ with $\psi = 0.47$. The values of n for various conditions are given in column 4 of Table II. There are about six ionic interactions in the intermediate complex at pH 7.4, and closer to five at pH 8.0.

Dissociation Kinetics. The rate of dissociation of the *lac* repressor–operator complex was measured as a function of cation concentration for the same salts and buffers. Figures 5 and 6 illustrate the dependence of the dissociation rate constant k_d on cation concentration. In all cases, the log–log plots exhibit continuous upward curvature. Dissociation of repressor–operator complex appears to be a simple unimolecular reaction at all salt concentrations investigated. For NaCl and $MgCl_2$, most of the rate constants plotted in the figures are mean values for different initial concentrations of repressor and operator. The dissociation rate constant is independent of the concentration of repressor and labeled operator DNA in the incubation mixture, as well as of the relative excess of unlabeled operator DNA added to start the reaction, over the limited range of concentrations investigated. However,

for $[Na^+] = 0.05$ M, the dissociation kinetics were slightly biphasic at higher operator concentration ($O = 2 \times 10^{-12}$ M). The small fast component, which could come from dissociation of the repressor–DNA complex, was not observed at lower operator concentration ($O = 5 \times 10^{-13}$ M). The rate constant estimated for the slow component agreed with the rate constant obtained at lower operator concentration. On the basis of our results for the association rate constants, microscopic reversibility would require that the dissociation kinetics depend on operator concentration at lower salt concentrations. However, the values of k_d reported here were not measured under reversible conditions. The dissociation rate constants represent rate constants for transfer of repressor from labeled to unlabeled operator DNA.

In order to estimate the magnitude of the salt dependence of k_d at higher salt concentrations, we have analyzed the kinetic data according to the theory of Lohman et al., assuming no anion release. The least-squares lines through the data at higher salt concentrations are shown in Figures 5 and 6: the slope and intercept of the lines are given in columns 5 and 6 of Table II. As noted before, the salt dependence of k_a and k_d is consistent with a preequilibrium mechanism. The values of $Z - n$ for various conditions, estimated according to eq 7 from the slopes $\partial \ln k_d/(\partial \ln [Na^+])$ and $\partial \ln k_d/(\partial \ln [Mg^{2+}])$ with the appropriate value of ψ, are given in the last column of Table II.

Discussion

This study of the dependence of the association rate constant k_a on cation concentration clearly demonstrates a role for nonoperator DNA binding in the repressor's quest for its operator site. The unusual salt dependence is qualitatively consistent with both proposed mechanisms for facilitated diffusion: the sliding model and the transfer model. However, the good quantitative fit of the data provided by Berg & Blomberg's (1978) theory lends strong support to the sliding model.[3] The data confirm two predictions of their theory: an inverse dependence of k_a on operator DNA concentration at lower salt concentrations, and a linear dependence of k_a on $(K_{obsd}^{RD})^{1/2}$ at higher salt concentrations. The minor deviations from the theory noted at lower salt concentrations might be due to systematic errors, such as errors in the extrapolated values of K_{obsd}^{RD} used to fit the data or buffer effects. Although we saw no evidence of buffer effects at higher salt concentrations ($[Na^+] = 0.13$ M; Barkley et al., 1981), they could become significant at lower salt concentrations. The λp*lac*5 DNA used in the experiments contains two pseudooperator sites proximate to the *lac* operator site: a "second" operator with 10–30-fold lower affinity, which lies 375 base pairs into the *Z* gene (Reznikoff et al., 1974; Gilbert et al., 1975), and a "third" operator with 100-fold lower affinity, which lies at the end of the *I* gene (Gilbert et al., 1976). Pfahl et al. (1979) have constructed λd*lac* phages deleted for each of the pseudooperators and have shown that the presence of these sites has only small effects on the rate of association of repressor and operator. The published version of the sliding model does not take these sites into account. Unpublished results (O. Berg, unpublished calculations) indicate that inclusion of the "second" operator does not affect the predicted values of k_a at lower salt concentrations, but could increase the predicted apparent k_a as much as 2-fold at higher salt concentrations. For example, if the "second" operator had only 10-fold lower affinity than the operator, it could contribute as much as 50% extra to the predicted value of k_a at ~0.13 M M^+. The absence of a conspicuous bulge in the data of Figures 1 and 2 suggests that the "second" operator has less of an effect, and thus probably somewhat lower affinity.

Using the sliding model to interpret our data, we deduce that the repressor diffuses about 4 times faster on MgDNA than on NaDNA. Berg and Blomberg obtained a similar result. They estimated the one-dimensional diffusion constant $D_1 = 1.7 \times 10^{-9}$ cm^2 s^{-1} in the presence of Mg^{2+} (BB, variable KCl concentration) from the data of Riggs et al. (1970a), which is very close to the value $D_1 = 1.5 \times 10^{-9}$ cm^2 s^{-1} we obtain for $MgCl_2$. In fitting their theory, they took values of K_{obsd}^{RD} for a buffer with lower Mg^{2+} concentration [BB, 0.003 M in place of 0.01 M $Mg(CH_3CO_2)_2$, variable KCl concentration; Lin & Riggs, 1975]. Analysis of the data of Riggs et al. with values of K_{obsd}^{RD} for a buffer with the same Mg^{2+} concentration (~T-BB, 0.01 M $MgCl_2$, variable NaCl concentration) does not change their conclusion. The kinetic data were fit as described under Results with $K_{obsd}^{RD} = 10^{7.5} \times 10^{-11}$ $(I - 0.01)^{1/2}$ (corrected from 4 to 20 °C by the factor $10^{-0.2}$, Record et al., 1977; *I* refers to ionic strength counting the Tris, Riggs et al., 1970a) and $D_1 = 1.5 \times 10^{-9}$ cm^2 s^{-1}. We note, however, that the values of D_1 that best fit the data are quite sensitive to the function K_{obsd}^{RD}. For example, if the values of K_{obsd}^{RD} from deHaseth et al. (1977a) and Record et al. (1977) were not corrected for the difference in temperature, the values of D_1 would be $D_1 = 3.5 \times 10^{-9}$ cm^2 s^{-1} for NaCl at pH 8.0 and also for $NaCH_3CO_2$ and $D_1 = 1.0 \times 10^{-9}$ cm^2 s^{-1} for $MgCl_2$, instead of the values given in Table I, and $D_1 = 1.0 \times 10^{-9}$ cm^2 s^{-1} for the data of Riggs et al. Thus, it is possible that errors in the affinities of repressor for nonoperator DNA could account for apparent differences in one-dimensional diffusion on MgDNA and on NaDNA. Independent of any analysis, though, the experimental data for the association rate constants in Figures 1–3 attest to a difference between Mg^{2+} and Na^+. The maximal rate constants measured are $k_a \sim 3 \times 10^{10}$ M^{-1} s^{-1} for $MgCl_2$, compared to $k_a \sim (1.2–1.4) \times 10^{10}$ M^{-1} s^{-1} for Na^+. The intriguing possibility that the *lac* repressor, and perhaps other proteins, has greater mobility on MgDNA may have physiological significance. Since the cellular environment contains about 0.002–0.003 M Mg^{2+}, it is likely that the repressor encounters MgDNA in vivo. The results presented in this paper provide no basis for speculation about the physical reason for differential mobility of a protein on DNA in the presence of mono- or divalent cations. Because there are well-established theoretical and experimental differences in DNA with various counterions, we have tacitly attributed the differential mobility to the properties of the DNA. However, we cannot exclude the possibility of a cation effect on the protein.

The sliding model and the preequilibrium model of Lohman et al. (1978) represent alternative treatments of transitional binding to DNA in the association reaction. We have analyzed our kinetic data in terms of the preequilibrium model and computed the number of ionic interactions *n* in the repressor–DNA intermediate from the slope of the log–log plots at higher salt concentrations. Although the preequilibrium model makes no assumptions about the mechanism of conversion of the intermediate to repressor–operator complex, Lohman et al. assume that the conversion rate is independent of salt concentration. However, reformulation of the sliding model as a two-state reaction yields a conversion rate that is highly salt dependent (O. Berg, unpublished calculations). Also, it is possible that the rate of conversion of the repressor–DNA complex to the repressor–operator complex may depend on

[3] Unpublished theoretical work of O. Berg shows that the transfer model cannot explain the data.

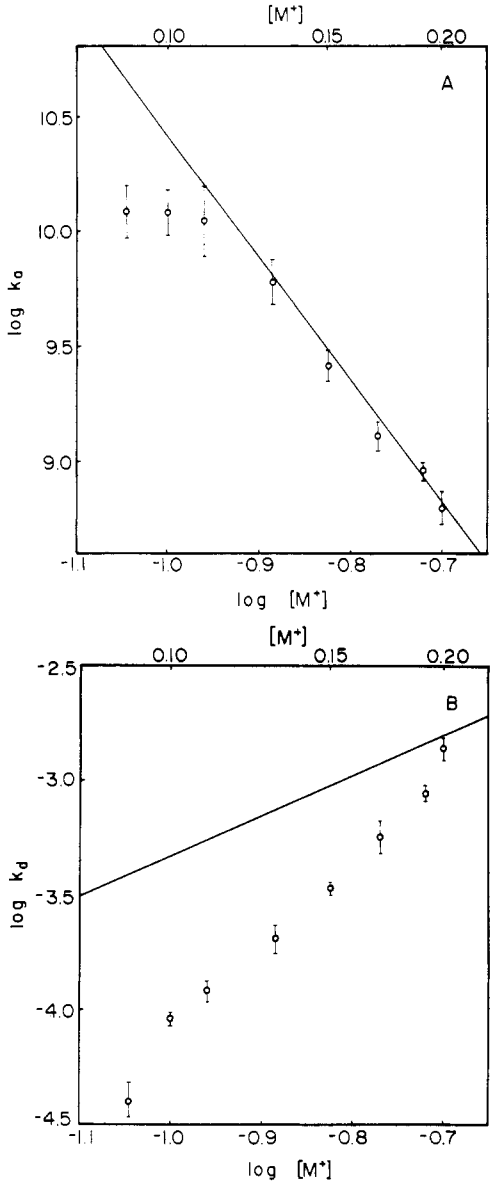

FIGURE 7: Comparison of predicted and experimental rate constants for *lac* repressor–operator binding in the presence of monovalent cations. Symbols represent experimental values for NaCl, T-BB, pH 7.4; curves represent predicted values for KCl, BB without Mg^{2+}. (A) Dependence of association rate constant on monovalent cation concentration. Points represent mean values of k_a from Figure 1. The line is calculated according to log k_a = 5.28 log [K^+] + 5.13 (Lohman et al., 1978). (B) Dependence of dissociation rate constant on monovalent cation concentration. Points represent mean values of k_d from Figure 5. The line is calculated according to log k_d = 1.7 log [K^+] − 1.57 (Lohman et al., 1978).

salt concentration for any mechanism. Thus, no physical meaning may be associated with the parameter n. Nevertheless, we can compare our kinetic data with the rate constants in the presence of monovalent cation predicted by Lohman et al. Assuming that Mg^{2+} competes with repressor for binding to DNA, they have corrected the data of Riggs et al. (1970a) for the presence of Mg^{2+} in the buffers. As pointed out by the authors, the Mg^{2+} correction is a parameterization and should be valid independently of the interpretation of the parameter n. Figure 7 shows the predictions of Lohman et al. and the experimental data. The solid lines in panels A and B denote the corrected association and dissociation rate constants for KCl in Tris buffer at pH 7.4; the points represent the mean values of the rate constants for NaCl in phosphate buffer at pH 7.4 from Figures 1 and 5. At higher salt concentrations, the agreement between the corrected and experimental values of k_a is excellent. The slopes are identical, giving $n = 6 \pm 2$ for the corrected data and $n = 5.9 \pm 0.3$ for the experimental data at higher salt concentrations. As noted by Lohman et al., their analysis is only expected to apply above ~0.1 M M^+. On the other hand, the agreement between the corrected and experimental values of k_d is less satisfactory. The slopes are quite different, giving $Z - n = 2$ for the corrected data and $Z - n = 4.9 \pm 0.3$ for the experimental data at higher salt concentrations. The excellent fit of the association rate constants as well as the poor fit of the dissociation rate constants may be partly fortuitous. For example, the concentrations of KCl used in the parameterizations are slightly higher than the concentrations in the experiments of Riggs et al., who included a contribution of 0.01 M from the Tris in computing the ionic strength. Inspection of the fit between the experimental and calculated values in the presence of Mg^{2+} shown by Lohman et al., however, suggests that this would make only minor differences in the predictions. Effects of Na^+ or K^+ and possible differences in the repressor preparations would also cause systematic discrepancies. Adjusting for the small K^+ ion effect [cf. Table II of Barkley et al. (1981)] would raise the experimental k_a and k_d values by 0.25 and 0.12 log unit, respectively; adjusting for a difference in the repressor [Riggs et al. reported $k_a = (7 \pm 0.9) \times 10^9$ M^{-1} s^{-1} and $k_d = (6.2 \pm 1.3) \times 10^{-4}$ s^{-1} under standard conditions] would lower the experimental k_a values by −0.10 log unit and raise the experimental k_d values by 0.32 log unit. Compensating for both K^+ and the repressor would raise the experimental k_a and k_d values by about 0.15 and 0.44 log unit, respectively. These changes slightly degrade the fit for the association rate constants. However, they substantially improve the agreement between the values of the dissociation rate constants, though not the slopes. This comparison, plus the one described in the following paper (Barkley et al., 1981), indicates that the primary effect of Mg^{2+} on the repressor–operator interaction is a competitive effect.

Acknowledgments

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Ion Effects on the *lac* Repressor–Operator Equilibrium†

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ABSTRACT: The effects of ions on the interaction of *lac* repressor protein and operator DNA have been studied by the membrane filter technique. The equilibrium association constant was determined as a function of monovalent and divalent cation concentrations, anions, and pH. The binding of repressor and operator is extremely sensitive to the ionic environment. The dependence of the observed equilibrium constant on salt concentration is analyzed according to the binding theory of Record et al. [Record, M. T., Jr., Lohman, T. M., & deHaseth, P. L. (1976) *J. Mol. Biol. 107*, 145]. The number of ionic interactions in repressor–operator complex is deduced from the slopes of the linear log–log plots. About 11 ionic interactions are formed between repressor and DNA phosphates at pH 7.4 and about 9 ionic interactions at pH 8.0, in reasonable agreement with previous estimates. A favorable nonelectrostatic binding free energy of about 9–12 kcal/mol is estimated from the extrapolated equilibrium constants at the 1 M standard state. The values are in good accord with recent results for the salt-independent binding of repressor core and operator DNA. The effects of pH on the repressor–operator interaction are small, and probably result from titration of functional groups in the DNA-binding site of the protein. For monovalent salts, the equilibrium constant is slightly dependent on cation type and highly dependent on anion type. At constant salt concentration, the equilibrium constant decreases about 10 000-fold in the order $CH_3CO_2^- \geq F^- > Cl^- > Br^- > NO_3^- > SCN^- > I^-$. The wide range of accessible equilibrium constants provides a useful tool for in vitro studies of the repressor–operator interaction.

The interaction of *lac* repressor protein with DNA is influenced by ionic conditions. In the accompanying paper (Barkley, 1981), we examined the salt dependence of the kinetics of binding of the repressor and its operator site on a large DNA molecule. The unusual dependence of the association rate on cation concentration is consistent with mechanisms involving transitional binding of the protein to nonoperator DNA. Using Berg & Blomberg's (1978) theory for the sliding model, we deduced that diffusion of repressor along the DNA chain is about 4 times faster for MgDNA than for NaDNA. This paper reports an investigation of the effects of ions on the *lac* repressor–operator equilibrium. The affinity of repressor for operator is determined as a function of the concentration and type of salt and of pH, and the ion effects are interpreted according to the binding theory outlined below. The findings for the repressor–operator interaction are compared to published results for the repressor–DNA interaction.

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