Biophysical Chemistry 8 (1978) 281–294


# ANALYSIS OF ION CONCENTRATION EFFECTS ON THE KINETICS OF PROTEIN-NUCLEIC ACID INTERACTIONS. APPLICATION TO *LAC* REPRESSOR-OPERATOR INTERACTIONS

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Received 4 May 1978

The effects of monovalent and divalent cations on the bimolecular rate constant of the reaction of a positively charged ligand with a nucleic acid polyanion are analyzed for two possible reaction mechanisms. One mechanism postulates that the association reaction occurs without intermediates, and that ion effects on the rate constant result entirely from the screening of the charged reactants by ionic atmospheres of low molecular weight ions (a screening-controlled mechanism). This mechanism is analyzed by analogy with the Bronsted-Bjerrum theory for the kinetics of interaction of low molecular weight ions. The second mechanism to be considered here postulates the existence of a ligand-DNA intermediate which is in rapid equilibrium with the reactants (pre-equilibrium mechanism). Ion concentration effects on the association rate constants for the pre-equilibrium mechanism result mainly from the release of counterions from the DNA upon formation of the intermediate. Both of the above mechanisms predict that the logarithm of the association rate constant, $k_a$, will be a linear function of the logarithm of the monovalent cation concentration, $[M^+]$ (in the absence of competition by divalent cations or anions). Knowledge of the salt dependences of $k_a$ and of the observed equilibrium constant $K_{obs}$ of the ligand-nucleic acid interaction should usually be sufficient to determine whether a screening-controlled mechanism or a pre-equilibrium mechanism is suitable to describe the process. If the association reaction can be described by a pre-equilibrium mechanism, the number of ionic interactions involved in the ligand-nucleic acid intermediate can be estimated. This analysis, extended to include the effects of divalent cations on screening or on the pre-equilibrium step, is applied to literature data on the salt dependence of the kinetics of the interaction of *lac* repressor with *lac* operator DNA. When the operator is present on bacteriophage λ DNA, the observed reaction kinetics are consistent with the formation of an intermediate repressor-DNA complex in a pre-equilibrium step. On the other hand, the kinetics of association of *lac* repressor with synthetic *lac* operator fragments may be an example of a screening-controlled reaction.

## 1. Introduction

The interactions of proteins with nucleic acids are being studied intensely, with the goal of understanding the molecular basis of specific sequence recognition in gene expression. In the past few years, it has been recognized that the interactions of proteins with non-specific DNA sequences may play an important *in vivo* role in controlling the free concentration of regulatory proteins such as repressors and RNA polymerase [1]. The study of the equilibrium properties of these non-specific complexes is being pursued in order to understand their sources of stability and to compare them with the specific interactions.

Also of interest are kinetic questions, such as the mechanisms by which a protein, initially distributed non-specifically along the DNA or free in solution, locates its specific DNA site. How is the protein able to sample DNA sequences in order to locate this site? There have been various suggestions including one dimensional diffusion of the protein along the DNA lattice [2,3] and a direct transfer mechanism in which a protein having two DNA binding sites may be transferred from one region of the DNA to another, without having to dissociate [4].

In general, equilibrium binding constants for the interactions of proteins and other cationic ligands with

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nucleic acids show a large dependence on salt concentration. This results from the release of salt ions in the formation of the ligand-nucleic acid complex; a quantitative interpretation of the salt dependence in terms of the number of ionic interactions in the equilibrium complex has been developed [5,6]. The purpose of the present paper is to predict the effects of salt on the association rate constant for two possible mechanisms for the interaction of charged ligands with nucleic acids, and to demonstrate that the experimental determination of the salt dependences of association (or dissociation) rate constants can be helpful in elucidating the mechanism of the reaction.

The two reaction mechanisms considered are:

1) a single-step association mechanism, in which the salt dependence of the bimolecular rate constant results entirely from the screening effect of the ionic atmospheres about the reactants (a screening-controlled mechanism); and

2) an association mechanism involving a ligand-nucleic acid intermediate in rapid equilibrium with the reactants (a pre-equilibrium mechanism).

The salt effect on the association rate constant for the second mechanism results from screening (small effect) and the release of counterions previously bound to the DNA upon formation of the intermediate (large effect). These two mechanisms can be considered to be the limiting cases of a general steady-state treatment of the association reaction. The rate limiting step in the screening-controlled mechanism is the initial encounter of the reacting species. This screening-controlled situation is analogous to the Bronsted-Bjerrum treatment of the salt dependence of the kinetics of interaction of low molecular weight ions [7]. However, the polyelectrolyte nature of the nucleic acid introduces a functional dependence of the association rate constant $k_a$ (and also the dissociation rate constant $k_d$) on the ionic conditions which differs from that predicted and observed for low molecular weight ions. For the polyelectrolyte case, in the absence of competing ligands (e.g., divalent cations, interacting anions), $\log k_a$ and $\log k_d$ are predicted to be linear functions of the logarithm of the monovalent cation concentration, $[M^+]$ (or, equivalently, of the logarithm of the ionic strength, $I$). In contrast, for the interactions of low molecular weight ions, $\log k_a$ is a linear function of $I^{1/2}$.

The pre-equilibrium mechanism postulates the formation of an intermediate in rapid equilibrium with the reactants. The rate limiting step is the formation of the stable product from this intermediate. This mechanism serves as a mathematically tractable alternative to the screening-controlled mechanism. Analysis of the data of Riggs et al. [8] and Barkley et al. [9] on the ion concentration dependences of the kinetics of interaction of *lac* repressor with the *lac* operator region carried on phage λ DNA, which we present here, indicates that this interaction can be described by the pre-equilibrium model. We show that, in general, the salt dependences of $k_a$ and $k_d$ can be used to distinguish between the two mechanisms for a ligand-nucleic acid interaction. The pre-equilibrium treatment presented here is similar to the pre-equilibrium analysis of the effects of cations on DNA renaturation developed by Manning [10].

## 2. Review of ion effects on ligand–nucleic acid equilibria

The polyelectrolyte theory of Manning [11,12] predicts that if the mean axial charge density of a linear polyelectrolyte, such as DNA, in the presence of excess uni-univalent salt only, is greater than a critical value, then counterions (e.g. $Na^+$) will condense along the DNA backbone until the net charge density is lowered to this critical value (1 charge/7.1 Å in water at 25°C). Under these conditions the extent of counterion condensation is theoretically determined solely by the ratio of the structural charge density to the critical charge density. The results of a recent $^{23}Na$ nmr study by Anderson et al. [13] indicate that the extent of sodium association with DNA is constant over a wide range of NaCl concentrations (0.005 M–0.5 M) at the value predicted by the condensation model. After condensation the DNA is still highly charged. Interactions between the remaining unneutralized charges are screened by ion atmosphere formation by mobile ions.

Record et al. [5] have defined $\psi$ as the fraction of a counterion thermodynamically bound per phosphate, where $\psi$ is composed of both condensation, $\psi_c$, and screening, $\psi_s$, terms. Manning has shown [11] that the fraction of a monovalent counterion condensed per phosphate, which we call $\psi_c$ [5], is given by

$$\psi_c = 1 - \xi^{-1}, \qquad (1)$$

where $\xi = e^2/\epsilon kTb$; $e$ is the charge on an electron, $\epsilon$ is the dielectric constant of the bulk solvent, $k$ is Boltzmann's constant, $T$ is the absolute temperature and $b$ is the mean axial phosphate spacing projected along the DNA backbone. The screening effect of mobile ions on the unneutralized DNA charges is thermodynamically equivalent to the association of the additional fraction $\psi_s$ of a counterion with the DNA, per phosphate. The quantity $\psi_s$ is obtained from the salt dependence of the polyelectrolyte activity coefficient, $\gamma_D$, which is given by [5,6,12]

$$\log \gamma_D = -N\xi^{-1} \log \kappa b, \quad (2)$$

where $N$ is the number of phosphate groups on the DNA molecule, $b$ is defined as above, and $\kappa$ is the Debye–Hückel screening parameter, which is proportional to the square root of the ionic strength. In the presence of excess monovalent salt [5,6]

$$\psi_s = -(1/N)(\partial \log \gamma_D/\partial \log [M^+])_{T,pH} = (2\xi)^{-1}. \quad (3)$$

Therefore $\psi$, the fraction of a monovalent counterion thermodynamically associated per phosphate, is given by

$$\psi = \psi_c + \psi_s = 1 - (2\xi)^{-1}. \quad (4)$$

The effect of monovalent ion concentration on the equilibrium between a charged ligand and a nucleic acid has been analyzed by Record et al. [5,6]. When a charged ligand (protein), L, binds to a DNA molecule, D, and neutralizes $m'$ phosphates, $m'\psi$ monovalent counterions are released from the DNA. The observed equilibrium constant, $K_{obs}$, for the reaction

$$L + D \overset{K_{obs}}{\rightleftharpoons} LD$$

defined as

$$K_{obs} \equiv [LD]/[L][D],$$

varies with counterion concentration as

$$(\partial \log K_{obs}/\partial \log [M^+])_{T,pH} = -m'\psi. \quad (5)$$

When the reaction occurs in the absence of competitive anions or polyvalent cations, $\psi$ is a constant (0.88 for double stranded DNA [5,14]) and $\log K_{obs}$ is a linear function of $\log [M^+]$ [5]. Eq. (5) pertains if the interaction does not result in a net release of ions from L. Ion release from L is not considered in this communication since our only application is to the *lac* repressor-operator interaction which (on the basis of our prior studies [15,16]) can be treated adequately without including such effects.

## 3. Theoretical

### *3.1. Ion concentration effects on screening-controlled reactions*

Consider the possibility that the interaction of a positively charged ligand (L) with $m'$ phosphates on a nucleic acid (D), in the presence of excess monovalent salt, occurs in a single step (without intermediates). This mechanism has not been demonstrated to exist for any ligand-polyelectrolyte interaction, but is presented by analogy with the classical mechanism used to interpret ionic strength effects on the interaction of low molecular weight ions [7]. In both cases, the only effect of salt on the bimolecular rate constant is assumed to result from the screening effect of the atmospheres of mobile ions which surround the reactants and reduce the coulombic forces between them; we call these screening-controlled reactions. The diffusion controlled reaction between two charged species is an example of a screening-controlled reaction. We expect the observed *association* rate constant for a screening-controlled reaction to exhibit a relatively weak dependence on counterion concentration, resulting from screening effects alone. Conversely, we expect the observed dissociation rate constant for this mechanism to exhibit a strong dependence on monovalent counterion concentration, the consequence of the fact that counterions condense on the polyion as the ligand dissociates (see section 2).

To show this, we use a conventional collision or activated complex formulation, similar to that assumed in the Bronsted-Bjerrum treatment of the kinetics of reactions between low molecular weight ions [7]. The elementary reaction between a single ligand L and a polynucleotide D is assumed to be:

$$L + D \overset{K_T^{\ddagger}}{\rightleftharpoons} C^{\ddagger} \overset{k_f}{\rightleftharpoons} \text{Complex}, \quad (6)$$

where $K_T^{\ddagger}$ is the thermodynamic equilibrium constant for formation of the collision complex $C^{\ddagger}$, and $k_f$ is the rate constant for formation of the stable product complex. We define $C^{\ddagger}$ to be a transient species in which L has penetrated the ionic atmosphere surrounding D, but in which the $m'$ ionic interactions which

help to stabilize the complex have not yet formed. The step with rate constant $k_f$ involves formation of the $m'$ ionic interactions. $K_T^{\pm}$ is written in terms of activities, $a$, as

$$K_T^{\pm} = a_C^{\pm}/a_L a_D. \quad (7)$$

From eq. (6), the rate of this screening-controlled association can be written:

$$d(\text{Complex})/dt = -\,d[C^{\pm}]/dt = k_f[C^{\pm}]. \quad (8)$$

From eq. (7) and the definition of activity as a product of an activity coefficient, $\gamma_i$ and a concentration, we can obtain the concentration of $C^{\pm}$ and substitute into eq. (8) to obtain:

$$d(\text{Complex})/dt = k_f K_T^{\pm}\,[L]\,[D]\;\gamma_L\gamma_D/\gamma_{C^{\pm}}. \quad (9)$$

The observed rate equation is written in terms of concentrations of reactive species and an observed rate constant, $k_a$:

$$d(\text{Complex})/dt = k_a[L]\,[D]. \quad (10)$$

By comparison of eqs. (9) and (10), the observed rate constant is seen to be

$$k_a = k_f K_T^{\pm}\,\gamma_L\gamma_D/\gamma_{C^{\pm}}. \quad (11)$$

We are interested in the dependence of $k_a$ on ion concentration. We assume that $k_f$ is independent of counterion concentration, $[M^+]$, since otherwise by definition the association reaction would not be screening-controlled. Since $K_T^{\pm}$ is only a function of temperature and pressure, the ion dependence of $k_a$ is confined to the activity coefficient term. As we have already discussed [6], all factors in the ratio $\gamma_L\gamma_D/\gamma_{C^{\pm}}$ should cancel except for the contributions from the reaction site of the ligand, $\gamma_{L,site}$ and the $m'$ phosphates which comprise the reaction site on the DNA. Incorporating this into eq. (11) yields:

$$\log k_a = \log k_0 - m'\psi_s \log I + \log \gamma_{L,site}, \quad (12a)$$

where $\psi_s$ is the component of $\psi$ attributable to the Debye–Hückel screening effect (see eq. (3)). We have combined all salt independent terms in eq. (12a) in $k_0$. In the presence of only monovalent cations, $M^+$, the ionic strength $I$ is equal to $[M^+]$.

In general, there is no available theoretical expression to evaluate $\gamma_{L,site}$ (which should depend on the particular ligand under consideration). At this stage we are forced to neglect its contribution to the salt dependence of $k_a$ and write

$$(\partial \log k_a/\partial \log I) \approx -\,m'\psi_s. \quad (12b)$$

In neglecting the contribution of $\gamma_{L,site}$, eq. (12b) can be considered only as a qualitatively correct expression. That is to say, $(\partial \log k_a/\partial \log I)$ should be small for a screening-controlled association, since $\psi_s$ is small (0.12 for double stranded DNA). However, it is quite possible that $(\partial \log \gamma_{L,site}/\partial \log I)$ may be of comparable magnitude to $m'\psi_s$ and hence eq. (12b) may not be quantitatively correct. There is no available data on $(\partial \log \gamma_{L,site}/\partial \log I)$ at this time.

The observed rate constant for the dissociation reaction is $k_d = k_a/K_{obs}$, where $K_{obs}$ is the observed equilibrium constant for the association reaction. From eqs. (12) and the salt dependence of $K_{obs}$, in the absence of divalent cations, as given in eq. (5), we conclude that

$$\log k_d = \log k_0' + m'\psi_c \log [M^+], \quad (13a)$$

and

$$(\partial \log k_d/\partial \log [M^+]) = m'\psi_c. \quad (13b)$$

The term $\gamma_{L,site}$ has again been neglected in eqs. (13). As stated above, the $[M^+]$ dependence of $k_d$ is a result of the fact that counterions recondense on the DNA upon dissociation of the ligand.

From the above approximate model for a screening-controlled reaction we see that both $\log k_a$ and $\log k_d$ are predicted to be linear functions of $\log [M^+]$ if $(\partial \log \gamma_{L,site}/\partial \log [M^+])$ is negligible. Furthermore, an increase in $[M^+]$ reduces the observed equilibrium constant, $K_{obs}$, by both increasing $k_d$ and decreasing $k_a$ (the major effect is on $k_d$). We note that for double helical DNA, in the presence of only monovalent salt, where $\psi_c = 0.76$ and $\psi_s = 0.12$, eqs. (12) and (13) predict that the magnitude of $(\partial \log k_d/\partial \log [M^+])$ is approximately six times that of $(\partial \log k_a/\partial \log [M^+])$ for the screening-controlled mechanism. Insofar as we are aware, no data for model compounds are available to test eqs. (12) or (13).

The screening-controlled model which has been developed in this section is applicable to ligands which associate with nucleic acids by three dimensional diffusion to the nucleic acid site. The qualitative result for such association reactions is that only a small dependence of $k_a$ on salt concentration should be observed. This result should be valid for any ligand (oligopeptide or large protein)-nucleic acid association. The quantitative treatment presented above should be most

applicable for small ligands such as charged oligopeptides. The analysis may require modification in order to provide a quantitative treatment of possible diffusion-controlled protein-nucleic acid association reactions because of the difference between the net charge on the protein and the charge on its binding site. Indeed, some DNA binding proteins have a net negative charge at neutral pH so that screening at large distances would actually facilitate the approach of ligand and nucleic acid [17,18].

It is important to contrast the predicted linear dependence of log $k_a$ and log $k_d$ on log $[M^+]$ for screening-controlled ligand-nucleic acid interactions (in the absence of competitive effects of anions and divalent cations) with the case of low molecular weight ions, where log $k_a$ and log $k_d$ are predicted to be linear functions of $I^{1/2}$, the square root of the ionic strength [7]. This difference arises simply from the fact that the logarithm of the activity coefficient of a low molecular weight ion, log $\gamma$, is proportional to the Debye–Hückel screening parameter, $\kappa$; however, for linear polyelectrolytes, log $\gamma$ is a linear function of log $\kappa$ [6, 12]. Furthermore in the case of low molecular weight ion association kinetics, the derivative, $(\partial \log k_a / \partial I^{1/2})$ is proportional to the product of the charges on the two associating ions [7]. No corresponding product of charges appears in eq. (12) for the $[M^+]$ dependence of $k_a$ predicted for the screening-controlled association of an oligocation with a macromolecular nucleic acid.

### *3.2. Ion concentration effects on rate constants of reactions involving an intermediate*

In this mechanism, the interaction of a cationic ligand, L, with a DNA molecule D is assumed to form an intermediate complex, $I_n$, with $n$ ionic interactions. The intermediate is subsequently converted to the final complex. We make the pre-equilibrium approximation, i.e. we assume that the equilibrium between $I_n$ and reactants (with equilibrium constant $K_I$) is established rapidly on the time scale of the conversion of $I_n$ to the product complex, LD. Then

$$L + D \overset{K_I}{\rightleftharpoons} I_n, \qquad I_n \xrightarrow{k_f} LD. \tag{14}$$

The molecular reaction for formation of $I_n$ is written:

$$L + D \overset{K_{T,I}}{\rightleftharpoons} I_n + n\psi M^+, \tag{15}$$

where

$$K_{T,I} \approx [I_n]\,[M^+]^{n\psi}/[L]\,[D], \tag{16}$$

$K_{T,I}$ is only a function of temperature and pressure. The $n\psi$ counterions appearing in the pre-equilibrium of eq. (15) include the $n\psi_c$ counterions released by the formation of the intermediate as well as the $n\psi_s$ screening counterions. The latter are introduced, as in section 3.1, through the activity coefficient of the $n$ phosphates in the DNA binding site (recall $\psi = \psi_c + \psi_s$). The activity coefficients of the ligand L and ions $M^+$ are neglected, since these contributions should to an extent cancel one another [6], such that their residual ion concentration dependence should be small in comparison to that of the activity coefficient of the DNA site [5,6].

From eq. (14) the rate of formation of LD from the intermediate can be written:

$$d[LD]/dt = k_f [I_n] \tag{17}$$

or, with the pre-equilibrium approximation for $[I_n]$,

$$d[LD]/dt = k_f K_{T,I}/[M^+]^{n\psi}\ [L]\,[D]. \tag{18}$$

From eq. (18), the experimentally observed rate constant, $k_a$, is:

$$k_a = k_f K_{T,I}/[M^+]^{n\psi}, \tag{19}$$

or

$$\log k_a = \log k_0 - n\psi \log [M^+], \tag{20}$$

where $k_f$ is assumed to be independent of salt concentration and has been included with all other salt independent terms in $k_0$. Therefore, the $[M^+]$ dependence of $k_a$ is given by:

$$(\partial \log k_a / \partial \log [M^+]) = -n\psi. \tag{21}$$

Consequently this pre-equilibrium analysis predicts that, in the absence of competitive effects of anions or divalent cations, a log-log plot of $k_a$ versus $[M^+]$ will again be linear, as predicted for the screening-controlled mechanism discussed in section 3.1. However, the slopes will differ, not only in magnitude but in significance, for the two case (cf. eqs. (12) and (21)).

As in section 3.1, the ion concentration dependence of the observed dissociation rate constant $k_d$ (for the pre-equilibrium model) is readily evaluated from the corresponding expressions for the observed equilibrium constant and the association rate constant. If there are $m'$ ionic interactions formed in the equilibrium com-

plex, and $n$ ionic interactions formed in the intermediate, then from eqs. (5) and (21)

$$(\partial \log k_d/\partial \log [M^+]) = (m' - n)\psi. \quad (22)$$

If $m' > n$, eq. (22) predicts that $k_d$ should increase with increasing $[M^+]$. This results from the recondensation of counterions onto the DNA upon dissociation of the charged ligand.

From eqs. (21) and (22) one observes that if $n > \frac{1}{2}m'$, the ion concentration dependence of the association rate constant will be larger *in magnitude* than that of the dissociation rate constant. [Recall that this situation cannot arise for a screening-controlled reaction as long as the buffer contains no polyvalent cations capable of competing with the ligand.]

### *3.3. Relative magnitudes of ion concentration effects on screening-controlled reactions and reactions involving intermediates*

For double helical DNA, in the absence of divalent cations, $\psi_s = 0.12$ and $\psi = 0.88$ [5]. Consider the association reaction of an oligocationic ligand with double helical DNA. If the association step is screening-controlled, then $(\partial \log k_a/\partial \log [M^+]) \approx -0.12m'$, where $m'$ is the number of ionic interactions in the equilibrium complex (cf. eq. (12)). On the other hand, if the association reaction proceeds by way of an intermediate with $n$ ionic interactions, then $(\partial \log k_a/\partial \log [M^+]) = -0.88n$ (cf. eq. (21)). If $m'$ is known from analysis of the dependence of the equilibrium constant for the interaction on ion concentrations (cf. eq. (5)), then one can estimate the salt dependence of $k_a$ for a screening-controlled mechanism of association (eq. (12)). If the observed salt dependence substantially exceeds that predicted by eq. (12), then it is reasonable to postulate the existence of an intermediate, $I_n$. From eq. (21), the number of ionic interactions in the intermediate can be estimated from the experimental quantity $(\partial \log k_a/\partial \log [M^+])$.

The results of sections 3.1 and 3.2 may be used to make qualitative predictions concerning mechanisms of protein-nucleic acid interactions. For a particular protein, one should observe only a small dependence of $k_a$ on $[M^+]$ if it associates purely by three dimensional diffusion, whereas a much larger dependence of $k_a$ on $[M^+]$ should be seen if it associates with a specific site via an intermediate. (Other mechanisms in which conformational changes in the ligand or the lattice are rate-limiting are of course also possible.) These qualitative predictions may be useful in the study of the association reactions of a site specific gene regulatory protein. The association of a site specific protein with nonspecific DNA (DNA containing no specific binding sites) should exhibit only a small salt dependence of $k_a$ if the rate limiting step is three dimensional diffusion. A comparison of $(\partial \log k_a/\partial \log [M^+])$ for the association of the same protein to its specific DNA binding site (in a large DNA molecule containing many nonspecific sites) with $(\partial \log k_a/\partial \log [M^+])$ for the nonspecific reaction should determine whether the association to the specific site involves a mechanism more complex than three dimensional diffusion. The above comparisons should be made only if the reactions have been studied in the absence of competing ligands such as $Mg^{++}$, since this will complicate matters significantly (cf. section 3.4).

### *3.4. The presence of $Mg^{++}$ affects $(\partial \log k_a/\partial \log [M^+])$ for a screening-controlled association differently than for a pre-equilibrium mechanism involving an intermediate*

Consider the common experimental situation in which $Mg^{++}$ or some other divalent cation, which does not specifically bind to the bases of the nucleic acid, is present along with $M^+$ in the binding buffer used to investigate the kinetics of interaction of a cationic ligand with a nucleic acid. The effect of $Mg^{++}$ on $(\partial \log k_a/\partial \log [M^+])$, as well as on the magnitude of $k_a$, may indicate whether the association reaction is screening-controlled or an intermediate is involved.

*Screening-controlled reactions.* If the association reaction is screening-controlled, the dependence of $\log k_a$ on $\log I$ will be linear and relatively small (cf. section 3.1). The effect of $Mg^{++}$ on $k_a$ will be only the result of its contribution to the ionic strength; i.e., $\log k_a$ is predicted to remain a linear function of $\log I$. This results from the dependence of the activity coefficient of the DNA on ionic strength. The effect of addition of $Mg^{++}$ on the dissociation reaction of a screening-controlled system is obtained from the relationship $k_d = k_a/K_{obs}$, eq. (12a) and our previous treatment of the dependence of $K_{obs}$ on $[M^+]$ and $[Mg^{++}]$ [6,15]. The result is:

$$\log k_d = \log k'_0 + m'\psi_c \log [M^+] + m' \log S, \quad (23)$$

where

$$S \equiv \tfrac{1}{2}\,(1 + (1 + 4\,K_{obs}^{Mg^{++}}\,[Mg^{++}])^{1/2}), \quad (24)$$

(cf. discussion of eq. (29) below). Qualitatively, upon addition of $Mg^{++}$ at constant $[M^+]$, $k_d$ will increase dramatically. Furthermore, $(\partial \log k_d/\partial \log [M^+])_{Mg^{++}}$ will no longer be constant and will also be reduced in magnitude. The decrease in $(\partial \log k_d/\partial \log [M^+])_{Mg^{++}}$ is a result of the binding of $Mg^{++}$ to DNA in preference to $M^+$ (however, $Mg^{++}$ does not bind infinitely more strongly than $M^+$) so that when the ligand L dissociates, less $M^+$ recondenses onto the DNA resulting in a smaller dependence of $k_d$ on $[M^+]$. From eqs. (23) and (24) one observes that $(\partial \log k_d/\partial \log [M^+])_{Mg^{++}}$ is a function of both $[M^+]$ and $[Mg^{++}]$ (recall that $K_{obs}^{Mg^{++}}$ is also dependent on $[M^+]$). Therefore in a mixed $Na^+$–$Mg^{++}$ buffer, it is quite possible that $|(\partial \log k_a/\partial \log [M^+])_{Mg^{++}}| > |(\partial \log k_d/\partial \log [M^+])_{Mg^{++}}|$ for a screening-controlled reaction, even though this is not possible if $M^+$ is the only counterion in solution.

*Pre-equilibria involving an intermediate.* In contrast to the screening-controlled case, the effect of $Mg^{++}$ on an *association* reaction involving a pre-equilibrium is complex. Qualitatively, this is the consequence of the competitive effect of $Mg^{++}$ on the formation of the pre-equilibrium complex. Here a quantitative treatment of this effect of $Mg^{++}$ is presented. We neglect other possible roles of $Mg^{++}$ in the mechanism of association of the ligand with the nucleic acid. We have previously demonstrated that the effects of $Mg^{++}$ on the equilibria between the protein *lac* repressor and its specific and nonspecific DNA complexes can be interpreted entirely in terms of competition by $Mg^{++}$ for DNA sites [16].

To introduce the effects of $Mg^{++}$ competition into the rate equations for associations involving an intermediate, we note that the experimental rate equation for the formation of LD is written in terms of the total free DNA site concentration, $D_T$, regardless of its state of ligation by $Mg^{++}$.

$$d[LD]/dt = k_a [L]\,[D_T]. \quad (25)$$

In sections 3.1 and 3.2 we treated situations where there was no $Mg^{++}$ or other competitive ligand in the system. Therefore, all of the DNA sites were in the same state of ion binding; i.e. only $M^+$ condensed on the DNA. This state will be represented by the symbol $D_0$. In section 3.1 and 3.2, $D_0 = D_T$. When $Mg^{++}$ is present in the buffer, $D_0 \leqslant D_T$, Because $D_T$ includes those sites with $Mg^{++}$ ions associated with them [16].

Consider a ligand-nucleic acid association involving an intermediate, $I_n$, in the presence of $Mg^{++}$ and excess $M^+$. In the pre-equilibrium model, the observed reaction sequence is

$$L + D_T \overset{K_I}{\rightleftharpoons} I_n, \qquad I_n \xrightarrow{k_f} LD, \quad (26)$$

from which we can derive an expression for the observed rate constant $k_a$ (eq. (25)). Since the intermediate species $I_n$ is in equilibrium with reactants, this first step of the reaction, characterized by the equilibrium constant $K_I$, can be treated using equilibrium thermodynamics. The analysis of this first step, therefore, does not imply any mechanism for the formation of $I_n$, but will enable us to extract the salt dependence of $k_a$ assuming that $k_f$ is independent of $[M^+]$. The thermodynamic reaction for formation of the intermediate, $I_n$ is written as

$$L + D_0 \overset{K_{T,I}}{\rightleftharpoons} I_n + n\,\psi\,M^+, \quad (27)$$

where

$$K_{T,I} = [I_n]\,[M^+]^{n\psi}/[L]\,[D_0]. \quad (28)$$

In eqs. (27) and (28), we have made the same assumptions which led to eqs. (15) and (16) in section 3.2. Again, $\psi$ contains both the condensation effect and the screening effect of $M^+$ ions.

An expression for $D_T/D_0$ in the presence of $Mg^{++}$ has been obtained [6,16] from the $Mg^{++}$ binding polynomial, $S$, [19] on a per nucleotide basis, evaluated by the sequence generating function method [20,21]. The $n$ phosphates on the DNA which are neutralized by the ligand L in the intermediate, $I_n$, are all potential sites for $Mg^{++}$ binding. Each bound $Mg^{++}$ neutralizes two phosphates so that the sites overlap [22]. The intrinsic binding constant for the $Mg^{++}$–DNA interaction is $K_{obs}^{Mg^{++}}$, which has an $[M^+]$ dependence of its own [16]. From this analysis we obtain [6,16]

$$[D_T]/[D_0] = \tfrac{1}{2}\,[1 + (1 + 4K_{obs}^{Mg^{++}}\,[Mg^{++}])^{1/2}]^n \equiv S^n. \quad (29)$$

Eq. (29) is applicable when the distribution of $M^+$ between the free solution and the DNA lattice is per-

turbed only by the presence of $Mg^{++}$ (i.e. at low binding density of the associating ligand, L). This is the situation which exists for the *lac* repressor-operator studies of Riggs et al. [8]. Substituting eq. (29) into eq. (28) one obtains an expression for $[I_n]$. The remainder of the steps are identical to those outlined in section 3.2 and yield:

$$\log k_a = \log k_0 - n \log S - n\psi \log [M^+]. \quad (30)$$

For a reaction occurring by a pre-equilibrium mechanism, eq. (30) indicates that $\log k_a$ will no longer be a linear function of $\log [M^+]$ when $Mg^{++}$ (or any competitive ligand) is present in the solution. This is a consequence of the complex $[M^+]$ dependence of $S$, and holds whether or not the $[Mg^{++}]$ makes a significant contribution to the ionic strength. Recall from above that this will not be the case for a screening-controlled reaction, where $\log k_a$ is a linear function of the logarithm of the ionic strength, $I$, even in the presence of $Mg^{++}$. In fact, the addition of $Mg^{++}$ may provide a way to test for an association mechanism which involves a DNA-ligand intermediate. If an intermediate is involved, modest increases of $Mg^{++}$ at constant $[M^+]$ should drastically reduce the magnitude of the observed $k_a$, whereas only a slight decrease in $k_a$ due to an increase in total ionic strength should be observed for a screening-controlled association.

An expression for the variation of $k_d$ with $[M^+]$ in the presence of $Mg^{++}$ can be derived for the pre-equilibrium mechanism by combining eq. (30) with the analogous expression [6,16] for the $[M^+]$ dependence of $K_{obs}$ in the presence of $Mg^{++}$. If there are $m'$ ionic interactions in the equilibrium complex, then for the pre-equilibrium model one predicts that:

$$\log k_d = \log k_0' + (m' - n) \log S + (m' - n)\psi \log [M^+], \quad (31)$$

where $k_0'$ includes all salt independent quantities.

## 4. Application to *lac* repressor-operator kinetics

### *4.1. Ion concentration effects on the rate constants of lac repressor–operator interactions*

Kinetic data are available for only a few ligand-nucleic acid systems. To our knowledge, no rate measurements as a function of salt concentration have been made on simple cation-nucleic acid interactions. Measurements on simple systems will be necessary to provide a thorough test of the analysis presented in the preceding sections. Of the systems investigated, rate studies as a function of salt concentration have been carried out only for the interaction of *lac* repressor with operator [8,9,23] and of *E. coli* RNA polymerase with promoters [24,25]. All of these studies were performed in buffers containing $Mg^{++}$, using the filter binding assay.

The *lac* repressor-operator interaction represents the only system in which both $k_a$ and $k_d$ have been determined as a function of ionic conditions [8,9,23]. Riggs et al. measured $k_a$ and $k_d$ for the interaction of wild type *lac* repressor with the *lac* operator region of λ h80dlac DNA as a function of KCl concentration in the presence of 0.01 M $Mg^{++}$. Barkley et al. [9] investigated the dependence of $k_d$ for this system on KCl concentration in the presence of 0.003 M $Mg^{++}$. Recently, Goeddel et al. [23] have determined $k_a$ and $k_d$ for the interactions of wild type and a tight binding (X86) mutant of *lac* repressor with synthetic *lac* operator fragments containing little if any nonspecific DNA (21 and 26 base pair fragments). The Goeddel et al. [23] measurements were made in 0.01 M $Mg^{++}$ at two KCl concentrations in the range investigated by Riggs et al. [8]. The approach outlined in section 3 can be used to examine whether the salt dependences of these rate constants are consistent with either a screening-controlled or pre-equilibrium mechanism for these interactions. In no case are we able to establish a mechanism, although analysis of the data on the interaction of wild type repressor with λh80dlac DNA does support some ideas which have already been proposed [2–4]. Our main purpose in considering these data is to illustrate the effects of monovalent and divalent cations on the rate constants of protein-nucleic acid interactions, and to demonstrate how the analysis of kinetic data as a function of ionic conditions can contribute to an understanding of the mechanism of the interaction.

It is useful to consider first the data of Goeddel et al [23] on the interaction of repressor with synthetic operator fragments. The association rate constants are at or above the theoretical estimate of the diffusion-limited association of uncharged particles the size of repressor and operator, and decrease with increasing KCl concentration. Since the operator fragments are so small and are not linked to any nonspecific DNA,

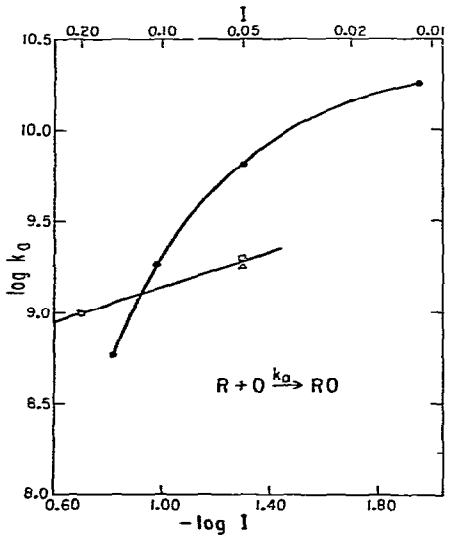


Fig. 1. The dependence of the observed association rate constant for the *lac* repressor-operator interaction on the ionic strength, I, of the solution (log–log plot). (●) – Data of Riggs et al. [8] – operator contained in λh80dlac DNA; (□, △) – Data of Goeddel et al. [23] – operator fragments; (□) – 26 base pair fragment, (△) – 21 base pair fragment. Both the Riggs et al. [8] and Goeddel et al. [23] data were obtained in buffers containing 0.01 M $Mg^{++}$ (except for the Riggs et al. [8] point at $I \approx 0.01$ which contained 0.0033 M $Mg^{++}$); the ionic strength was varied by adding KCl.

the RO (fragment) association [23] may be an example of a screening-controlled association. We have plotted $k_a$ for the wild type *lac* repressor–operator (fragment) association measured by Goeddel et al. [23] as a function of ionic strength (log–log plot) in fig. 1 (also shown in fig. 1 are the $k_a$ values from Riggs et al [8]). Two points suggest that the RO (fragment) association [23] is screening-controlled. First of all, the largest $k_a$ for the wild type repressor associating with either fragment at 0.05 ionic strength (10 mM $Mg^{++}$, 10 mM KCl, pH 7.4) is $\sim 2 \times 10^9\ M^{-1}s^{-1}$ [23]. Under identical conditions, $k_a$ in the RO (λh80dlac) system [8] is $\sim 7 \times 10^9\ M^{-1}s^{-1}$ and increases to $> 10^{10}$ at lower ionic strength [8]. Goeddel et al. [23] point out that the measured $k_a$ for the RO (fragment) association compares well with the $k_a$ estimated by Richter and Eigen [2] for the diffusion-controlled association of repressor with a prolate ellipsoid. The calculation [2] includes electrostatic effects but considers the operator to be stationary and also neglects orientation effects. Secondly, the ionic strength dependence of $k_a$ in the wild type repressor–operator (fragment) system [23] is in agreement with our predictions for a screening-controlled association. For wild type *lac* repressor, which has $m' = 8 \pm 2$ [16], we predict $(\partial \log k_a/\partial \log I) \approx -1.0$ (cf. eq. (12b); recall that this neglects the contribution from $\gamma_{L,site}$ and therefore is probably only correct to within a factor of 2). Unfortunately, $k_a$ was measured at only two ionic strengths [23] so we are unable to determine if there is curvature in the log $k_a$–log $I$ plot. From fig. 1 we calculate $(\partial \log k_a/\partial \log I) \approx -0.5$, which is small (compare it with the Riggs et al. [8] data on RO (λh80dlac) which is also shown in fig. 1) and consistent with our expectations of the salt dependence of a screening-controlled association.

We must also comment on the observed salt dependence of $k_d$ for the RO (fragment) system. Goeddel et al. [23] observed that $k_a$ was more sensitive to ionic strength than $k_d$. (Recall that the KCl concentration was varied at constant $Mg^{++}$ concentration (0.01 M).) This does not rule out a screening-controlled mechanism since, as mentioned in section 3.4, $|(\partial \log k_a/\partial \log [M^+])|$ can be greater than $|(\partial \log k_d/\partial \log [M^+])|$ even for a screening-controlled reaction, when $Mg^{++}$ is also present in the buffer. Assuming that the RO (fragment) data of Goeddel et al. [23] is screening-controlled, we have used eq. (23) which describes the salt dependence of $k_d$ in the presence of $M^+$ and $Mg^{++}$ to predict $(\partial \log k_d/\partial \log [M^+])$ in the absence of $Mg^{++}$. We used $m' = 8$, from our previous analysis of the equilibrium studies of Riggs et al. [26] on the *lac* repressor–operator system [16]. The use of eq. (23) as well as eqs. (29), (30), and (31) requires an expression for $K_{obs}^{Mg^{++}}$ (which is itself a function of $[M^+]$). Since all of the data discussed here were determined in 0.01 M Tris buffer, we have used the equilibrium binding data of Record et al. [16] for the $Mg^{++}$–DNA interaction in the presence of 0.01 M Tris in which values of $K_{obs}^{Mg^{++}}$ were determined from equilibrium binding studies of the nonspecific interaction of *lac* repressor with DNA in the presence of $Mg^{++}$. The association equilibrium constant for the $Mg^{++}$–DNA interaction, $K_{obs}^{Mg^{++}}$, follows the equation

$$\log K_{obs}^{Mg^{++}} = -1.74 \log [NaCl] + 0.37. \qquad (32)$$

In using eq. (32) to interpret the RO kinetic data,

which were obtained in the presence of $K^+$, we assume that $Na^+$ and $K^+$ act equivalently [16]. From this analysis, we predict that in the absence of $Mg^{++}$, $(\partial \log k_d/\partial \log [M^+]) \approx 5.0$ for the RO (fragment) system. In the absence of $Mg^{++}$, therefore, we predict that $|(\partial \log k_a/\partial \log [M^+])| \ll |(\partial \log k_d/\partial \log [M^+])|$ for the RO (fragment) studies, since $(\partial \log k_a/\partial \log [M^+])$ for a screening-controlled association is not affected significantly by the presence of $Mg^{++}$ at the concentrations used in this study.

In studying the salt dependences of reactions involving oligonucleotides, as in the Goeddel et al. [23] experiments, one also has to keep in mind that the ion association properties of oligonucleotides may be different from those of polynucleotides. On the basis of previous work by Record and Lohman [27], the ion association properties of a 21-base pair fragment should not be substantially different from those of polynucleotides.

Goeddel et al. [23] also measured $k_a$ for the X86 *lac* repressor–operator (fragment) association. The magnitudes of $k_a$ for the X86-fragment association are lower than those for the wild type-fragment system at both ionic strengths studied [23]. The differences in magnitude are difficult to explain. The measured value of $(\partial \log k_a/\partial \log I)$ for the X86-operator (fragment) is $\approx -1.0$. Although $(\partial \log k_a/\partial \log I)$ for X86 is approximately twice that for wild type repressor, it is still qualitatively consistent with our predictions for screening-controlled associations.

Having discussed the *lac* RO (fragment) data [23] and presented evidence that its behavior is consistent with a screening-controlled association, we will compare it to the Riggs et al. [8] data on wild type *lac* RO (λh80dlac). In binding buffer containing 0.01 M $Mg^{++}$, Riggs et al. [8] have determined $k_a$ and $k_d$ for the *lac* RO (λh80dlac) system as a function of [KCl]. They observed linear relationships between the logarithms of both $k_a$ and $k_d$ and the square root of the ionic strength, $I^{1/2}$, which we have represented by the empirical equations:

$$\log k_a = -6.2\, I^{1/2} + 11.2, \tag{33a}$$

$$\log k_d = 2.3\, I^{1/2} - 3.7. \tag{33b}$$

Although a plot of $\log k$ versus $I^{1/2}$ does represent the data well, the slope of such a plot apparently has a simple theoretical interpretation only for the case of the interaction between two low molecular weight ions (cf. section 3.3). We have plotted the association rate constants, $k_a$, of Riggs et al. [8] as a function of ionic strength, $I$ (log–log plot) in fig. 1 where they can be directly compared with the *lac* RO (fragment) association data of Goeddel et al. [23]. Three aspect of the Riggs et al. [8] data should be noted: 1) at 0.05 ionic strength, $k_a = 7 \times 10^9\, M^{-1}s^{-1}$ and increases to $\sim 1.5 \times 10^{10} M^{-1}s^{-1}$ at 0.01 ionic strength. These values are much too high to be explained by three dimensional diffusion of *lac* repressor to operator, especially since operator in λh80dlac DNA is not capable of diffusing on the time scale of association; 2) there is substantial curvature in the $\log k_a$–$\log I$ plot of fig. 1 indicating that the *lac* RO (λh80dlac) association is not screening-controlled; 3) the salt dependence of $k_a$ in the Riggs et al. [8] system is much greater than that of the Goeddel et al. [23] system.

On the basis of the above, it seems possible that the *lac* RO (λh80dlac) association involves a DNA-repressor intermediate. We therefore have analyzed the RO kinetics measurements of Riggs et al. [8] using the preequilibrium model developed in sections 3.2 and 3.4. We assume that the *lac* repressor forms an intermediate with the nonspecific DNA sites and is subsequently transferred (mechanism unspecified) to the operator site. Since kinetic data are not available for the RO interaction in the absence of the competitive ligand $Mg^{++}$, we assume that the only effect of $Mg^{++}$ is as a competitor for sites on the DNA and apply the analysis of section 3.4. This assumption regarding $Mg^{++}$ is supported by our studies of the equilibrium binding of *lac* repressor to nonspecific DNA in the presence and absence of $Mg^{++}$ [15,16]. Competitive effects of anions have not been included since it has been shown that these are not necessary to explain the salt dependence of the *lac* repressor–nonspecific DNA equilibrium constants [15].

We have used eqs. (30) and (31) and the Riggs et al. [8] data to predict the monovalent ion concentration dependences of $k_a$ and $k_d$ of the *lac* RO (λh80dlac) interaction, if the competitive ligand $Mg^{++}$ were not present in the binding buffers of Riggs et al. [8]. Eq. (32) for the $[M^+]$ dependence of $K_{obs}^{Mg^{++}}$ was used in these calculations.

The kinetics calculations were performed by picking an integral value of $n$, the number of ionic interactions formed in the intermediate, and calculating the

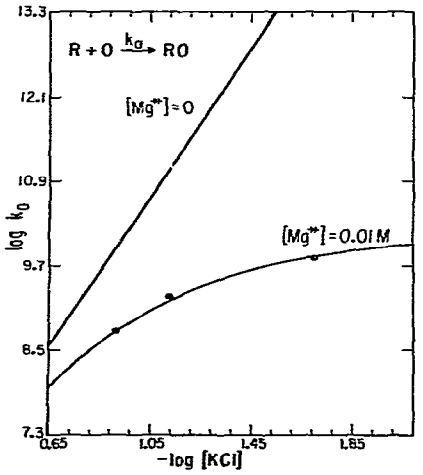


Fig. 2. The dependence of the observed *association* rate constant for the *lac* repressor–operator interaction on the concentration of added KCl. Data of Riggs et al. [8] obtained in the presence of 0.01 M $Mg^{++}$. The smooth curves show the predicted behavior in the absence of $Mg^{++}$ and in the presence of 0.01 M $Mg^{++}$, as explained in the text.

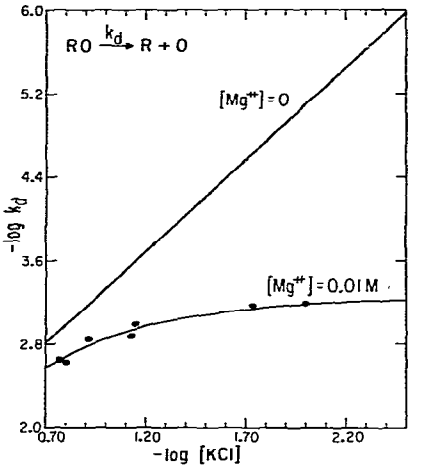


Fig. 3. The dependence of the observed *dissociation* rate constant for the *lac* repressor–operator interaction on the concentration of added KCl. Data of Riggs et al. [8] obtained in the presence of 0.01 M $Mg^{++}$. The smooth curves show the predicted behavior in the absence of $Mg^{++}$ and in the presence of 0.01 M $Mg^{++}$, as explained in the text.

expected behavior for each point from Riggs et al. [8], in the absence of $Mg^{++}$. This was done until a value for $n$ was found which yielded the least deviation from linearity in a log $k$ versus log $[K^+]$ plot. A value of $\psi = 0.88$ for double stranded DNA was used in the calculations. The best fit values obtained were $n = 6$ for the association data and $(m' - n) = 2$ for the dissociation data. These two values are consistent, as they must be, with the finding that $m'$, the number of ionic interactions formed in the equilibrium repressor–operator complex has the value $8 \pm 2$ [16]. The predicted linear plots from this calculation are shown in figs. 2 and 3, along with the actual rate constants measured by Riggs et al. [8] in the presence of 0.01 M $Mg^{++}$. The smooth curves through the Riggs et al. [8] data points were then recalculated using eqs. (30) and (31) and the predicted linear behavior in the absence of $Mg^{++}$. The *predicted* linear equations for the behavior of the repressor–operator rate constants as a function of $[K^+]$, in the absence of $Mg^{++}$ are given in eqs. (34).*

$$\log k_a = -5.28 \log [K^+] + 5.13 \qquad (34a)$$

$$\log k_d = 1.76 \log [K^+] - 1.57. \qquad (34b)$$

* Footnote, see next column.

Some qualitative features of the $[M^+]$ dependences of $k_a$ and $k_d$ for the RO (λh80dlac) interaction [8] in the presence of $Mg^{++}$ may be noted. In figs. 2 and 3, one observes the large decrease in $k_a$ and increase in $k_d$ at constant $[K^+]$ upon addition of $Mg^{++}$ into the system. This is due to the competition between $Mg^{++}$ and repressor for DNA sites. The curvature in the log–log plots in the presence of $Mg^{++}$ is due to the fact that the $Mg^{++}$–DNA interaction is itself salt dependent (eq. (32)). As a result, there is more effective competition between repressor and $Mg^{++}$ at low salt than at high salt, thereby depressing $k_a$ more at low

* In the absence of $Mg^{++}$, $k_a$ will not increase indefinitely as eq. (34a) would imply. An upper limit to $k_a$ can be estimated from the expected diffusion-controlled association of repressor to nonspecific DNA sites [35], using the relationship $k_a \approx 4\pi D r_g N \times 10^{-3}$, where $D$, the diffusion coefficient of repressor, is approximately $5 \times 10^{-7}$ $cm^2$ $s^{-1}$, $r_g$, the radius of gyration of λ DNA, is approximately $1.2 \times 10^{-5}$ cm, and $N$ is Avogadros number. This estimate yields an upper limit for $k_a$ of $4 \times 10^{10}$ $M^{-1}$ $s^{-1}$, which from eq. (34a) would be the $k_a$ expected in 0.09 M KCl with no $Mg^{++}$. Hence if one were to do experiments in the absence of $Mg^{++}$, one would only expect to see the behavior described in eq. (34a) above ~ 0.1 M $K^+$.

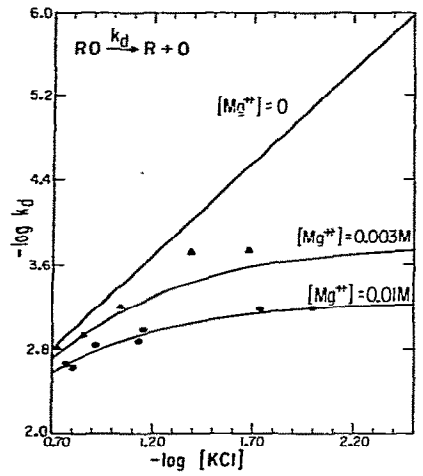


Fig. 4. The dependence of the observed dissociation rate constant for the *lac* repressor–operator interaction on the concentration of added KCl, at different $Mg^{++}$ concentrations. (•) – Data of Riggs et al. [8] in 0.01 M $Mg^{++}$; (▲) – Data of Barkley et al. [9] in 0.003 M $Mg^{++}$. The smooth curves show the predicted behavior in the various [$Mg^{++}$], as a function of added KCl, as calculated in the text.

[$K^+$], and increasing $k_d$ more at low [$K^+$], with respect to the situation in the absence of $Mg^{++}$.

In more recent kinetic experiments in buffer containing 0.003 M $Mg^{++}$, Barkley et al. [9] have examined the effect of [$K^+$] on the dissociation rate constants, $k_d$, of the repressor–operator (λdlac) (RO) and repressor–inducer–operator (RIO) complexes. These data provide a test of the analysis, since they were obtained in buffer containing 0.003 M $Mg^{++}$, rather than the 0.01 M $Mg^{++}$ RO data obtained by Riggs et al. [8]. The RO dissociation data are plotted in fig. 4 along with the 0.01 M $Mg^{++}$ data of Riggs et al. [8]. The smooth curves are the predicted dependences of log $k_d$ on log [$K^+$] in the presence of 0.003 M $Mg^{++}$ and 0.01 M $Mg^{++}$, based on the dependence in the absence of $Mg^{++}$ (eq. (34b)), eq. (31) and a value of $(m' - n) = 2$. The fit predicts with reasonable accuracy the decrease in $k_d$ when [$Mg^{++}$] is lowered from 0.01 M $Mg^{++}$ to 0.003 M $Mg^{++}$. The presence of $Mg^{++}$ therefore has a large effect on the magnitudes of the dissociation rate constants for RO complexes; the difference in the values of $k_d$ obtained by Riggs et al. [8] in 0.01 M $Mg^{++}$ and Barkley et al. [9] in 0.003 M $Mg^{++}$ can be accounted for solely by the increased $Mg^{++}$ binding to DNA in 0.01 M $Mg^{++}$, at a constant [$K^+$]. The data of Barkley et al. [9] on the dissociation of RIO also yield $(m' - n) = 2 \pm 1$, which is consistent with the conclusion that inducer binds to repressor at some position other than the DNA binding site and exerts an allosteric effect on the repressor to cause induction [28].

It should be noted here that the correction for the presence of $Mg^{++}$ in the RO system is a parameterization; the best value for $n$ being that which predicts the best straight line in the absence of $Mg^{++}$. Our interpretation of $n$ has been that it is the number of ionic interactions formed in a DNA-repressor intermediate before formation of the equilibrium complex. As will be discussed in the next section, even if this molecular interpretation is incorrect, the mathematical correction for the effect of the competing ligand, $Mg^{++}$, should still be valid. The predicted slope in the absence of $Mg^{++}$ will remain the same, but its molecular interpretation will depend on the mechanism used to analyze the data. The agreement between our calculations and the data of Riggs et al. [8] in 0.01 M $Mg^{++}$ (and variable KCl) and Barkley et al. [9] in 0.003 M $Mg^{++}$ (and variable KCl) gives us confidence in our ability to account for the effects of competing ligands, such as $Mg^{++}$, on the kinetics of protein-DNA interactions.

We have applied this same analysis to the RNA polymerase-promoter dissociation kinetic studies of Hinkle and Chamberlin [24], as recently revised by Giacomoni [29]. After correction for the competitive effects of the 0.01 M $Mg^{++}$ in the binding buffer [24], a value of $(m' - n) \approx 6 \pm 2$ is obtained from these data, if a pre-equilibrium mechanism is assumed. The interpretation of this result must await the determination of the salt dependences of the association rate constant and/or equilibrium constant for RNA polymerase-promoter binding.

### *4.2. Possible models for lac repressor–operator association*

There have been various proposals to explain the observations that the *lac* RO (λh80dlac) association rate constants measured by Riggs et al. [8] are larger than the apparent diffusion limit for association by three-dimensional diffusion of two species the size of *lac* repressor and operator. The original calculation

[8], however, did not account for the large amount of nonspecific DNA on λh80dlac DNA molecules containing the operator region. Two recent calculations [2,3] have shown that inclusion of this nonspecific DNA as a means of trapping repressor with subsequent one-dimensional diffusion of the protein to the operator can qualitatively account for the large observed $k_a$. One complication is that the experimental values of $k_a$ were measured in the presence of 0.01 M $Mg^{++}$, which, as shown previously, will reduce $k_a$ from its value in only monovalent salt, if a DNA-repressor intermediate is involved in the association mechanism. If the association mechanism is the same in the presence and absence of $Mg^{++}$, the values of $k_a$ may be even larger at a given salt concentration when only KCl is present in the buffer (cf. fig. 2 and the footnote to eq. (34a)).

Another model which has been proposed by von Hippel et al. [4] also uses nonspecific DNA as a means of trapping the repressor. It has been proposed that *lac* repressor may contain two DNA binding sites [30–32]. If this is true, the repressor may sample various sites on the DNA by direct transfer when a part of the DNA chain contacts a second DNA binding site on repressor and dislodges the protein from that part of the DNA to which it was previously bound [4]. Bresloff and Crothers [33] have invoked a similar transfer model to explain their association kinetic data for the ethidium cation–DNA interaction. This type of mechanism would also enhance the observed rate constant for RO association, although no quantitative calculations have been made.

Our analysis cannot differentiate between these two models, although we do provide supporting evidence for the participation of a repressor–DNA intermediate in the reaction. The interpretation of the value of $n = 6 \pm 2$ ionic interactions formed in the association intermediate is not clear to us. The most plausible intermediate would seem to be a nonspecific DNA–repressor complex. However, the nonspecific DNA–repressor binding mode has been shown to have $12 \pm 2$ ionic interactions in its equilibrium complex [15,34]. A possible explanation is that this value of 6 represents binding to only one of the two putative DNA binding sites of repressor and that the association mechanism may be similar to the direct transfer model proposed by von Hippel et al. [4], although there are insufficient data to allow further speculation on this point.

As mentioned in the previous section, the interpretation of the slope of a $\log k_a$–$\log [M^+]$ plot in terms of the number of ionic interactions involved in an association intermediate is strictly limited to a reaction which involves a pre-equilibrium mechanism and in which the entire salt dependence is that of the equilibrium constant for formation of the intermediate. The *lac* repressor–operator association reaction may be more complicated than this. Although the method of analysis presented here predicts the effects of the competitive ligand $Mg^{++}$ on the RO interaction, the behavior of $\log k_a$ as a function of $\log [M^+]$ (in the absence of $Mg^{++}$) predicted by this analysis may not have an unambiguous molecular interpretation. For example, Berg and Blomberg [3] have proposed a mechanism for the RO interaction involving initial nonspecific binding followed by one-dimensional diffusion, coupled with dissociation and reassociation of the repressor until the operator is located. The Berg and Blomberg analysis [3] predicts that the salt dependence of $k_a$ is of the form

$$\frac{\partial \log k_a}{\partial \log [M^+]} = \frac{\partial \log \sqrt{K_{obs}^{RD}}}{\partial \log [M^+]}, \tag{35}$$

where $K_{obs}^{RD}$ is the nonspecific association equilibrium constant. It has been shown that $K_{obs}^{RD}$ has the following dependence on monovalent salt concentration in the absence of $Mg^{++}$ [15]:

$$(\partial \log K_{obs}^{RD} / \partial \log [M^+]) = -11 \pm 2.$$

This result, along with eq. (35) predicts that

$$(\partial \log k_a / \partial \log [M^+]) = -5.5 \pm 1, \tag{36}$$

for the RO association reaction if it follows the one-dimensional diffusion model. Eq. (36) agrees quite well with our estimation of $(\partial \log k_a / \partial \log [M^+])$ in the absence of $Mg^{++}$ (eq. (34)). Therefore, we cannot make a choice between these two possible models, but can only say that they both seem consistent with the available data.

The calculations presented in this section on the *lac* repressor–operator kinetics are intended primarily to illustrate the use of salt dependences of rate constants to analyze mechanisms. To understand the mechanism of the repressor–operator interaction, further experiments must be performed. One important series of experiments will be the measurement of $(\partial \log k_a / \partial \log [M^+])$ in the absence of any competitive ligands

such as $Mg^{++}$. Also, as suggested by Richter and Eigen [2], $k_a$ should be determined as a function of DNA chain length with operator-containing fragments. The results of such studies might differentiate between the direct transfer model, the one-dimensional diffusion model, and three dimensional diffusion, if only one mechanism is operative over the entire range of ionic conditions and DNA molecular weights.

## Acknowledgement

We thank Dr. Charles Anderson for a critical reading of the manuscript and useful suggestions. We also thank Drs. G.S. Manning, P.H. von Hippel and R.L. Baldwin for their comments on an earlier version of this manuscript. One of us (T.M.L.) acknowledges on Amoco Foundation fellowship (1976–1977), issued through the University of Wisconsin, Department of Chemistry. Support from the NSF (PCM76-11016) is also gratefully acknowledged.

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