KINETICS OF PROTEIN-NUCLEIC ACID INTERACTIONS: USE OF SALT EFFECTS TO PROBE MECHANISMS OF INTERACTION

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I. INTRODUCTION

Investigations of protein-nucleic acid interactions are necessary to fully understand the molecular details of many cellular processes. Both equilibrium and kinetic considerations are important, since the strength of a binding interaction as well as its dynamics contribute to the control of gene expression, although one of these features may dominate depending on the particular system and circumstances. Clearly the orchestration in both time and space of the dissociation, association, and lateral movement of proteins along DNA is of central importance to transcription, replication, recombination, repair, and translational events. In fact, the switching of molecular events may often depend on the rates at which one or a set of specific binding sites can be occupied by one repressor protein vs. another rather than their relative binding strengths. The enzymatic functions of many other DNA binding proteins, such as DNA and RNA polymerases and helicases,[1] are intimately linked with their ability to translocate along DNA, the latter at rates of 500 to 1000 base pairs sec^{-1}. Movement of even nonenzymatic DNA binding proteins along nucleic acids may also play an important role in facilitating the location of specific sites on the DNA.[2-6] In vitro kinetic studies of these purified protein-nucleic acid interactions not only enable us to discover how fast these noncovalent binding and rearrangment reactions occur, but, in the best cases, what pathways and translocation abilities are available to these proteins. This information guides our thoughts concerning the types of motions and kinetic processes that are possible in vivo.

The equilibrium, thermodynamic, and structural aspects of protein-nucleic acid interactions have been reviewed recently.[7-11] Here the kinetics of protein-nucleic acid interactions are reviewed with particular emphasis on the dramatic effects of low molecular weight ions (i.e., salt concentration) and how these effects can be used to probe the mechanism of association and dissociation. This review also discusses various features that are of special and unique concern in the kinetics of protein-nucleic acid association (mainly due to the physical nature of the nucleic acid); these include

1. The coiling nature of high molecular weight nucleic acids. (Theoretical estimates of diffusion-controlled rate constants are given for protein association to high molecular weight nucleic acids which are coiled in "domains" as well as the dependence of rate constants on nucleic acid length.)
2. Due to the linear nature of nucleic acids and the fact that each base pair or nucleotide is the start of a potential protein binding site, all of the nonspecific binding sites on the nucleic acid are connected. This allows unique pathways to

be used for the rapid sampling of nucleic acid binding sites by proteins or other ligands.

3. The polyelectrolyte nature of the nucleic acid from which the dramatic salt-dependent equilibrium and kinetic properties arise.
4. The effects of the ratio of nonspecific to specific DNA binding sites on the association of a sequence specific binding protein to its specific site. (The competitive role of nonspecific DNA as well as its role in facilitating the location of the specific site are discussed.)

The review is organized into the following sections.

Section II. Theoretical aspects of nonspecific protein-nucleic acid kinetics — Diffusion-controlled rate constants are considered as well as the effect of nucleic acid length on the nonspecific association of protein to the nucleic acid chain.

Section III. The kinetics of site specific protein-nucleic acid interactions — Emphasis is given to the role of nonspecific DNA in these associations. Mechanisms are described which use nonspecific DNA to facilitate association or dissociation.

Section IV. The polyelectrolyte nature of the nucleic acid — The dramatic salt-dependent equilibrium and kinetic properties of protein-nucleic acid interactions are predominantly due to the high localization of counterions in the vicinity of the nucleic acid and the subsequent release of those ions upon formation of the complex. The effect of salt concentration on the equilibrium binding properties of protein-nucleic acid interactions is reviewed briefly, since it provides the necessary underpinnings for any discussion of salt effects on the kinetics.

Section V. The general features of the effects of salt on the kinetics of protein-nucleic acid binding — Both the qualitative and quantitative effects of salt are discussed (based on polyelectrolyte theory) for one-step vs. multistep reaction pathways.

Section VI. A discussion of the available experimental data on small ligand and protein-nucleic acid kinetics — The focus of this section is on studies in which the salt dependence of the kinetics was examined. This does exclude some interesting protein-nucleic acid systems. However, it should become clear that one should be cautious in drawing very many conclusions about mechanisms without a thorough investigation of the effects of salt concentration on the rate constants.

This review concerns mainly the effects of ion concentration and type on the rate constants and mechanism of protein-nucleic acid kinetics. It is assumed that the rate constants can be measured accurately and over a range of solution conditions and temperatures. It is at this point (with data in hand or at least able to be gathered) that this work describes the interpretation of such data to draw conclusions concerning mechanism. In addition to the review aspects of this work, the fundamental considerations in any study of protein-nucleic acid kinetics are also outlined. What are the important variables which can be used to systematically probe the mechanism of these interactions and at what level can these be interpreted molecularly?

II. RATE CONSTANTS FOR NONSPECIFIC PROTEIN-NUCLEIC ACID INTERACTIONS: THEORETICAL ASPECTS

There are two limiting cases for the rate of association of proteins to nucleic acids:[12] (1)diffusion-controlled associations and (2) reaction-controlled associations. Both of these are familiar concepts. However, they are briefly discussed here since the details of each individual's view sometimes differ and a common ground needs to be established. The term "diffusion-controlled" in its most strict sense refers to the situation in which a reaction (association in this case) occurs between two species every time a

collision occurs. That is, there are no orientation constraints; hence the only controlling element is how fast the two species are able to diffuse to one another.[13] Therefore, by this definition, very few associations between two macromolecules can be truly diffusion-controlled since orientation and steric constraints may prevent the majority of collisions from resulting in effective associations. In discussing protein-nucleic acid associations, I relax the definition to include any association which is *limited* by diffusional processes, although other constraints may also be important. From this point of view, the term "diffusion-limited" is more appropriate.[12] Diffusion-limited protein-nucleic acid associations depend on the spatial correlation of the nucleic acid chain and therefore the rate constants are dependent on the length of the nucleic acid. A more precise mathematical definition of this diffusion-limit has been given by Berg and Blomberg[12] and is discussed below.

The other extreme, a "reaction-controlled" protein-nucleic acid association, refers to one which is independent of the spatial correlation of the nucleic acid chain, i.e., there is no dependence of the association event on the chain segment distribution and hence no effect of diffusion. This latter case might be represented by a process in which the microscopic association rate is small due to the requirement of some slow chemical process or some slow conformational change that must occur in the macromolecule before an association can take place.

Berg and Blomberg[12] have given a precise mathematical description of these two limits. The important term is

$$bk_i \ln (V/v)$$

where b = radius of the nucleic acid chain; k_i = intrinsic association rate constant; V = hydrodynamic volume of the nucleic acid chain ($4/3\ \Pi r_g^3$, where r_g = radius of gyration); v = actual volume of cylindrical chain = $2\Pi Lb^2$ (where 2L = chain contour length).

The diffusion-limit occurs when[12] $bk_i \ln(V/v) \gg 1$ and the reaction-controlled limit occurs when $bk_i \ln (V/v) \ll 1$. For a given nucleic acid, the features which constrain the association are k_i, which is small when some chemistry or conformational rearrangements must occur, and V/v), which is a function of nucleic acid chain length. For a particular protein-nucleic acid complex, in which k_i is constant and small, the length of the nucleic acid can be the feature which determines which limit is approached. For high molecular weight nucleic acids ($V/v \gg 1$ and the nucleic acid forms coiled "domains" (see Section II.A below), so association should approach the diffusion-limited case, whereas for oligonucleotides, $V \simeq v$ and the "domain" effect is negligible. Therefore, in the limit of low molecular weight nucleic acids, the difference between the two limits is due to the intrinsic association rate constant, k_i, since "reaction-controlled" rate constants are independent of nucleic acid length. If k_i is large, then the association will be diffusion-limited, independent of the length of the nucleic acid. Most protein-nucleic acid associations are diffusion-limited, and the association rate constants for these processes are dependent on the molecular weight (length) of the nucleic acid. In the next section a discussion is given of the possible ambiguities that can arise due to a seemingly simple problem of the units used to describe bimolecular rate constants for protein-nucleic acid associations.

A. Bimolecular Rate Constants for Nonspecific Protein-Nucleic Acid Associations

1. The Units Problem

Due to the linear polymeric nature of a polynucleotide, there are two sets of units that can be used to describe the bimolecular association of a protein to the nucleic acid. This results from the two ways to denote the concentration of the nucleic acid: (1) moles of polynucleotide per liter or (2) moles of mononucleotide (or base pairs) per

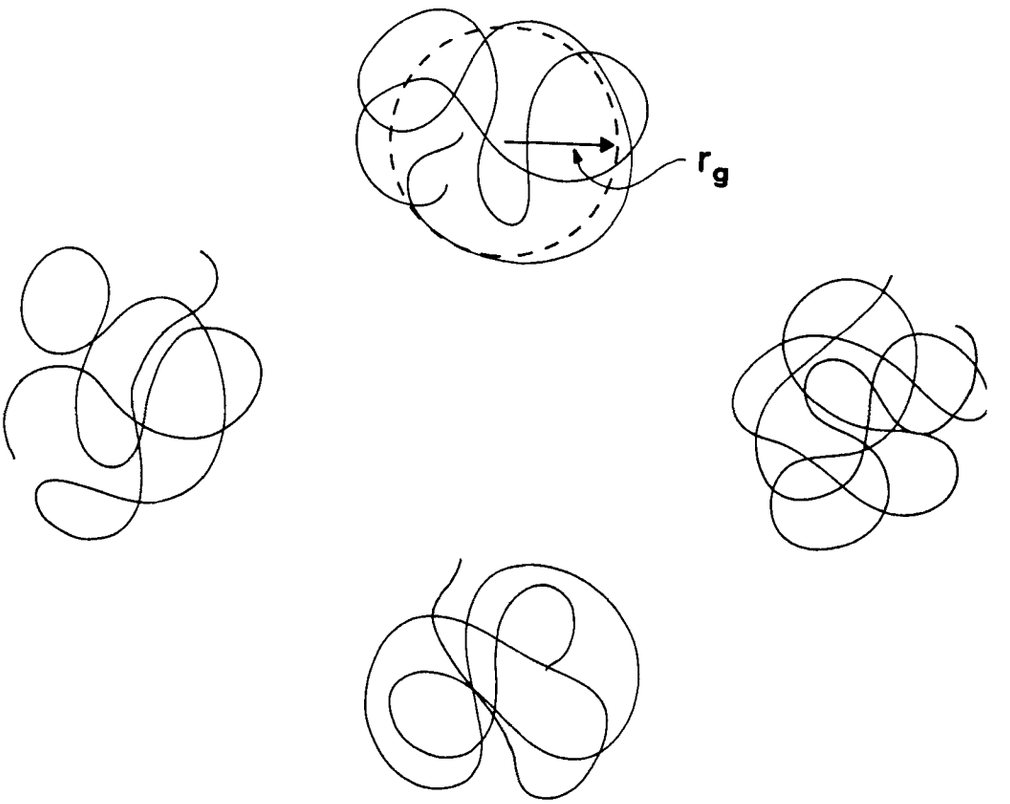

FIGURE 1. A schematic of the "domain" structure of high molecular weight nucleic acids in dilute solution. The radius of gyration, r_g, which is taken to approximate the interaction radius of the polynucleotide is indicated.

liter. The two are simply related by M, the number of nucleotides (or base pairs) per polynucleotide (i.e,, the length of the polynucleotide in base pairs for duplex DNA or nucleotides for single stranded nucleic acids). Since it is not common practice to denote the concentration scale used, but simply to use units of M^{-1} sec^{-1}, an ambiguity often results. The problem is a real one, since many nucleic acid samples that are used in kinetic studies are polydisperse, hence the simplest way to denote concentration is on a nucleotide (or base pair) basis; one needs to know the nucleic acid molecular weight distribution in order to do otherwise. From a physical point of view, however, the nucleotide is not the appropriate concentration unit to use in discussing these bimolecular processes, since the nucleotides are not independently diffusing species, but rather are part of a chain (the polynucleotide). For sufficiently long chains, in dilute solution, each polynucleotide is coiled into its own separate "domain". The bimolecular collision occurs between the protein and the polynucleotide "domain"[12,14,15-17] as shown schematically in Figure 1. If viewed on the nucleotide level, the bimolecular association between protein and a nucleotide (contained within a polynucleotide) must be described as a spatially nonhomogeneous system.[16-18] Of course, the equilibrium constant must be independent of this "domain" effect, so that similar considerations must apply to the dissociation reaction. As the molecular weight of the polynucleotide decreases, the "domain" effect also decreases.[14-17]

Aside from the problem of comparing an experimentally measured association rate constant to that predicted for a diffusion-limited reaction (which is discussed below),

it is important for experimentalists to specify the units which are used when reporting bimolecular association rate constants, since it is not always clear. This is especially true for nonspecific binding events. The average nucleic acid molecular weight and its distribution should also be reported. In this article, the units of the association rate constants will be explicitly specified as M^{-1} (nucleotide) sec^{-1} or M^{-1} (polynucleotide) sec^{-1}, with rate constants k_1 and k_a, respectively, in order to eliminate the potential ambiguity. An observed association rate constant (i.e., experimentally measured, but not yet interpreted) which may be a collection of molecular rate constants will be specified as k_a(app) in this review.

An excellent treatment of the nucleic acid length dependence of protein-nucleic acid associations has been developed by Mazur and Record[16,17] for random coiling polynucleotides. Using a diffuse-sphere model for the nucleic acid and a modification of the von Smoluchowski treatment, they have obtained expressions for nonspecific protein-nucleic acid bimolecular association rate constants as a function of molecular weight using both a site-based [M^{-1}(nucleotide)sec^{-1}] and a molecule-based scale [M^{-1}(polynucleotide)sec^{-1}]. The effects of localizing the nucleic acid sites within a "domain" are explicitly included in the treatment and their expression[16,17] for the rate constant on a polynucleotide scale (molecule-based) is (in our notation):

$$k_a = \frac{Mk}{1 + 0.4\beta^2} \qquad (1)$$

where M is the length of the nucleic acid in nucleotides (or base pairs for duplex DNA); k is the site-based bimolecular rate constant assuming the nucleic acid sites are uniformly distributed in solution; and β^z is a parameter which accounts for the "domain" effect and is proportional to the radius of gyration of the chain. Equation 1 indicates that k_a increases with nucleic acid chain length, but not linearly; for a diffusion-limited reaction, $k_a \propto M^{1/2}$. Mazur and Record[16,17] also show that a measurement of the molecular weight dependence of the association rate constant is necessary to unambiguously determine if a reaction approaches the diffusion-limit or if it is reaction-controlled.

2. The Dependence of Nonspecific Association Rate Constants on Binding Density — the Problem of Overlap of Potential Protein Binding Sites

The discussions above have only dealt with the association of protein to the nucleic acid in the low binding density limit, in which the entire nucleic acid chain is available for binding. As the extent of protein association increases, however, the number of binding sites decreases, but in a nonlinear fashion, since each bound protein occludes many (n) nucleotides or base pairs. The statistical problem of relating association rate constants measured at different binding densities is nontrivial. The overlap problem has been treated for the equilibrium case,[19-21] but has not been generally solved for the association kinetics, except in limiting cases.[15,22-24] An analytic solution is obtainable in the limit of very rapid translocation of the protein (ligand) along the nucleic acid.[22,24] The assumption of a rapid redistribution of the protein on the DNA during the time course of the reaction requires that the instantaneous distribution of protein along the nucleic acid at time t, binding density ν(t), be the same as when the system is at equilibrium at that same binding density. Hence the number of available potential protein binding sites is readily calculated from the equilibrium statistics.[19-21] This limit is referred to as the "IELE" limit for instantaneous establishment of lattice equilibrium and treatments for both noncooperative[22,24] and cooperative[24] binding ligands have been given for reversible binding.

For the noncooperative case, the relaxation equation is[22]

$$\tau^{-1} = k_1[S - f'(\nu)L] + k_{-1} \tag{2}$$

where S is the instantaneous concentration of free potential binding sites on the nucleic acid lattice, L is the free concentration of protein, k_1 is the association rate constant (on a per nucleotide basis), and k_{-1} is the dissociation rate constant,

$$f'(\nu) = \left[\frac{(n\nu(n-1) - 2n + 1)}{(1 - (n-1)\nu)^n}\right](1 - n\nu)^{(n-1)} \tag{3}$$

where ν is the protein binding density (moles of protein bound per mole of base pair or nucleotide) at time t and n is the occluded site size.[19,21] For $n = 1$, $f'(\nu) = -1$ and the standard result for nonoverlapping binding sites is recovered from Equation 2. The above treatment should be most applicable to relaxation experiments in the limit of small perturbations at reasonably low binding densities. The limiting case of irreversible association of large multisite ligands has been solved exactly by Epstein.[23] Approximate methods using matrix-iteration techniques have also been developed by Dateo and Epstein.[25]

In the theoretical treatment of the association kinetics of large ligands (proteins) which have considered the effects of overlap of potential protein binding sites on the DNA[15,22-24] only one treatment has also attempted to incorporate the "domain" effect.[15] That is, having recognized that the bimolecular collision is between the protein and the polynucleotide chain, of radius r_g, how is this "domain" affected upon binding more protein (with overlapping binding sites) thereby removing potential binding sites? Porschke[15] assumes rapid ligand redistribution along the nucleic acid lattice ("IELE" limit) and uses the fraction of free nucleotides, $(1 - \Theta)$, as the weighting function which reduces the effective concentration of polynucleotide chains (domains, D^∞ in Equation 4) as a function of bound ligand, although there is no theoretical basis for this choice. The rate equation for the net formation of complexes is then written as

$$\frac{d[PD]}{dt} = k_A[L][D^x](1 - \Theta) - k_D^\Theta[PD] \tag{4}$$

where k_A is the association rate constant on a polynucleotide basis, Θ is the fraction of occupied nucleotides, and the dissociation rate constant k_D^Θ, is also a function of Θ. This treatment is a useful approximation, but more work is clearly needed in this area. Currently, the two effects of the polynucleotide domain and the overlap of potential binding sites makes it impossible to extrapolate association rate constants obtained at finite binding densities to the zero binding density limit.

B. Theoretical Estimates of Association Rate Constants for Protein-Nucleic Acid Interactions

In order to interpret bimolecular rate constants for protein-nucleic acid associations, it is useful to know the predicted upper limit for these rate constants so that comparisons can be made and conclusions drawn concerning the molecular bases for any deviations from the predicted upper limit. At this point the association process must be specifically defined since most ambiguities and differences in the predicted values are due to mixing comparisons of nonspecific association to the polynucleotide chain with processes which consider the association to a specific site on the chain. The problem of the units for k_a(app) which is discussed above must also be considered here. The process of the nonspecific association of protein to the polynucleotide chain is consid-

ered first, since this is the first association that occurs in any protein-nucleic acid interaction, including those that eventually form sequence specific complexes.

1. Estimates of the Diffusion-Limited Nonspecific Rate Constant (k_a) for Association of Protein to the Polynucleotide Chain

The fastest association reaction that can occur for a particular protein-nucleic acid complex is the nonspecific association of the protein to the polynucleotide chain. As discussed above, the rate constant for this association, k_a has units M^{-1} (polynucleotide) sec^{-1} and usually is diffusion-limited. To exactly calculate k_a for a particular protein to a particular nucleic acid is a difficult task, since one needs to know the orientational constraints of the protein binding site, as well as the long-range electrostatic effects between a polyampholyte and a coiling polyanion. However, one can approximate a value of k_a to compare with experimental values. Furthermore one can predict how k_a should be affected by the nucleic acid chain length, viscosity, temperature, and salt concentration, which should be used as more critical tests of whether an experimental association rate constant is truly a measure of the diffusion-limited rate constant, k_1 (or k_a), for bimolecular association to the chain. None of these variables (chain length, viscosity, temperature, salt), as well as the absolute value of k_a(app) is sufficient to prove that k_a(app) is diffusion-limited, however, they all provide necessary criteria for this to be the case*. Before discussing the case of protein-nucleic acid interactions, we will first examine the simple case of two interacting spheres which yield two criteria for determining if $k_a(app) = k_a$.

The classical von Smoluchowski[26] equation for the steady state diffusion-controlled rate constant, k_a^s for a reaction between two spherical particles A and B is

$$k_a^s = \frac{4\pi N_A}{10^3} (D_A + D_B)(R_A + R_B) \tag{5}$$

where N_A is Avogadro's number and D_A,D_B and R_A,R_B are the diffusion coefficients and radii of each particle, respectively. Equation 5 is valid for uncharged spheres and assumes no orientational constraints. Through substitution of the Stokes-Einstein relation for D_A and D_B, one immediately obtains $k_a^s \alpha T/\eta_o$, where η_o is the solvent viscosity and T is the absolute temperature. If conditions are restricted to dilute aqueous solutions, the temperature dependence of k_a^s is easily calculated from the temperature dependence of the viscosity of water.[27] An Arrhenius plot of this temperature dependence predicts a very small, but positive activation energy of $\approx$ 4 kcal/mol for a diffusion-controlled process.

Extensions of Equation 5 have been made to include the influence of electrostatic charge[28,29] and nonuniform reactivity on the surface of the spherical particles[30-33] which are of particular concern in any treatment of macromolecular systems. Since these modifications require a detailed knowledge of the macromolecules and even then cannot be rigorously treated, we simply include them as parameters in a modified von Smoluchowski equation:

$$k_a^s = \left[\frac{4\pi N_A \kappa f_{el}}{10^3}\right] (D_A + D_B) R_{AB} \tag{6}$$

where κ is a unitless steric factor which reflects the orientational constraints for binding, f_{el} represents the electrostatic interactions, and we have written the interaction distance as R_{AB} (= $R_A + R_B$). The electrostatic effects on protein-nucleic acid kinetics

* After this article had been completed, I received a review by Berg and von Hippel[166] which gives an excellent discussion of the varied effects of diffusion on macromolecular interactions.

are most readily studied, experimentally, through observing the effects of salt concentration on the observed association rate constant. Again, it is difficult to calculate f_{el} exactly, even for a macromolecule whose structure is known, however, all calculations indicate that f_{el} for a diffusion-limited association varies only slightly with salt concentration[3,34-36] (see Sections III, IV, and V). It should be emphasized that the majority of observed association rate constants that are measured for protein-nucleic acid binding reflect rate limiting steps that occur after the initial bimolecular association; these rate constants are most usually very salt dependent, but this does not arise from the f_{el} term in Equation 5, but rather from direct release of cations from the nucleic acid[34,35] (and possibly cations or anions from the protein). (These effects are discussed in detail in Sections III, IV, and V.)

The orientation factor, $\varkappa$, is of course dependent on the particular protein and its nucleic acid binding site. For example, if a protein binds with polarity with respect to the 3′ to 5′ polarity of the phosphodiester backbone, $\varkappa$ is at least 1/2. Estimates of $\varkappa$ based on assumed orientational constraints for spheres and cylinders range from $(0.1 - 10^{-4})$[32] to 1/20[9]. Clearly it is difficult to draw general conclusions which are valid for all systems except that orientational constraints will reduce the diffusion-limited rate constant from its value predicted in the absence of these constraints.

In the absence of orientation constraints ($\varkappa = 1$) and assuming $f_{el} = 1$, $D_A = D_B = 5 \times 10^{-7}$ cm²/sec and $R_{AB} = 30$A, one obtains an upper limit of $k_a^* = 2 \times 10^9$ $M^{-1}sec^{-1}$ for two equal-sized spherical proteins (Equation 6). This is the estimate for the maximum diffusion-limited rate constant for two proteins. For a protein associating with a high molecular weight nucleic acid chain, the diffusion coefficient of the nucleic acid is negligible compared to that of the protein; however, the reaction radius is essentially the hydrodynamic radius of the polynucleotide chain. Berg and co-workers[12,36] have developed an expression for the diffusion-limited association rate constant of a protein to any nonspecific binding site within the coiled polynucleotide "domain" which also accounts for the fact that the coiled polynucleotide is not a solid "sphere", rather there is some probability that a protein will move through the coiled "sphere" without colliding with the chain. This expression on a polynucleotide basis is[12,36]

$$k_a = \frac{4\pi D_p r_g N_A}{10^3}\left[1 - \frac{\tanh(\eta r_g)}{\eta r_g}\right] \tag{7}$$

where r_g, the radius of gyration of the polynucleotide, is substituted for the interaction distance, D_p is the three-dimensional diffusion coefficient of the protein. The term (ηr_g) is defined by[12,36]

$$(\eta r_g)^2 = \frac{(3L/r_g)}{(\ln(R_c/b) + 2\pi D_p 1/k_t)} \tag{8}$$

where 2L is the polynucleotide contour length, 1 is the distance between nonspecific binding sites (usually assumed to be either one base pair or one nucleotide, R_c is the average distance between nucleic acid segments within the polynucleotide domain, and the other symbols have been discussed previously. In general, R_c is defined by

$$2\pi L R_c^2 = (4/3)\pi r_g^3 \tag{9}$$

or for a high molecular weight nucleic acid, with persistence length[3], a

$$R_c \simeq 0.60 (La^3)^{1/4} \tag{10}$$

For a diffusion-limited reaction, $k_i \gg 2\pi D_p l$ and the second term in the denominator of Equation 8 is negligible.

In Equation 7, the factor $4\pi D_p r_g$ represents the rate of encounter between the protein and the polynucleotide domain, whereas the term in brackets is an "absorbancy" factor representing the probability that the protein will also bind to a site on the polynucleotide chain, rather than pass through the domain. The "absorbancy" factor varies from 0.77 to 0.87 over the molecular weight range of 300 to 10^4 nucleotides for single-stranded homopolynucleotides, using a value of ≈20 Å for the persistence length.[37] For duplex DNA the calculated transparency factor varies from 0.44 to 0.77 for lengths of 2,000 to 50,000 base pairs at 0.20 *M* NaCl using $a = 500$ Å).[38] Therefore, for flexible coils, there is a finite probability that once the protein reaches the domain of the polynucleotide, it will pass directly through without binding. The probability of the protein passing through the domain without binding is higher for duplex DNA than for single-stranded nucleic acids since the single-stranded polynucleotide coil is more flexible and more compact.

Electrostatic effects will modify both terms in Equation 7, since these will play some role (a minor one) in locating the domain and also in the actual binding event (absorbancy term). Orientation effects should only modify the absorbancy factor since location of the domain is independent of the protein's orientation. Therefore, Equation 7 would be multiplied directly by an electrostatic term (f_{el}) and also Equation 8 would by multiplied by both an electrostatic (f'_{el}) and an orientation ($\varkappa$) term. The electrostatic effects on locating the domain (f_{el}) will depend on the net charge of the protein, whereas the electrostatic effects on binding will depend on both the net charge as well as the positive charge of the DNA binding site. These will all contribute to only small screening effects on k_a, which are observable upon changing the salt concentration (see Section V).

We can now estimate the maximum values of k_a from Equation 7 by neglecting both the orientational and electrostatic effects ($\varkappa = 1$, $f_{el} = 1$), and using $D_p = 5 \times 10^{-7}$ cm²/sec for a typical protein, and $r_g^2 = 2La/3$ for high molecular weight DNA which can be modeled as a flexible coil.[36] These estimates are given in Figure 2 for both single-stranded and duplex polynucleotides as a function of nucleic acid molecular weight. The curves in Figure 2 are applicable to duplex DNA in [NaCl] ≥ 0.10 *M* where $a =$ 500 Å and b = 15 Å. For single-stranded polynucleotides, values of $a = 20$Å and b = 10Å were used. The values of k_a for duplex DNA, in the range 5 to 50×10^3 base pairs (the size of bacteriophage λ DNA), are $\approx 9 \times 10^{10}$ to $\approx 3 \times 10^{11}$ M^{-1} (polynucleotide) sec^{-1}, whereas for single-stranded polynucleotides, k_a has values of $\approx 2 \times 10^9$ to $\approx 6 \times 10^{10}$ M^{-1} (polynucleotide)sec^{-1} over the range 3×10^2 to 5×10^3 nucleotides. The differences in k_a for double- vs. single-stranded nucleic acids are due mainly to differences in the radii of gyration of the coils. Clearly, if these bimolecular diffusion-limited rate constants are normalized on a base pair or nucleotide scale then, k_1 [M^{-1}(nucleotide) sec^{-1}] will decrease as the length of the polynucleotide increases, indicating that those units are not physically meaningful for diffusion-limited rate constants. However, as long as it is clear which unit system is being used (site based vs. molecule based), then conversion between the two is simple.

2. Length Dependence of k_a for Low Molecular Weight, Short Rod-Like Chains

This limit has been treated by Schranner and Richter,[39] Berg et al.[36] and Berg and Ehrenberg,[40] who in the course of treating the length dependence of association to a specific site on the nucleic acid also developed expressions for the diffusion-limited association to the chain. The expression obtained by Berg et al.[36] in the diffusion-limited case is

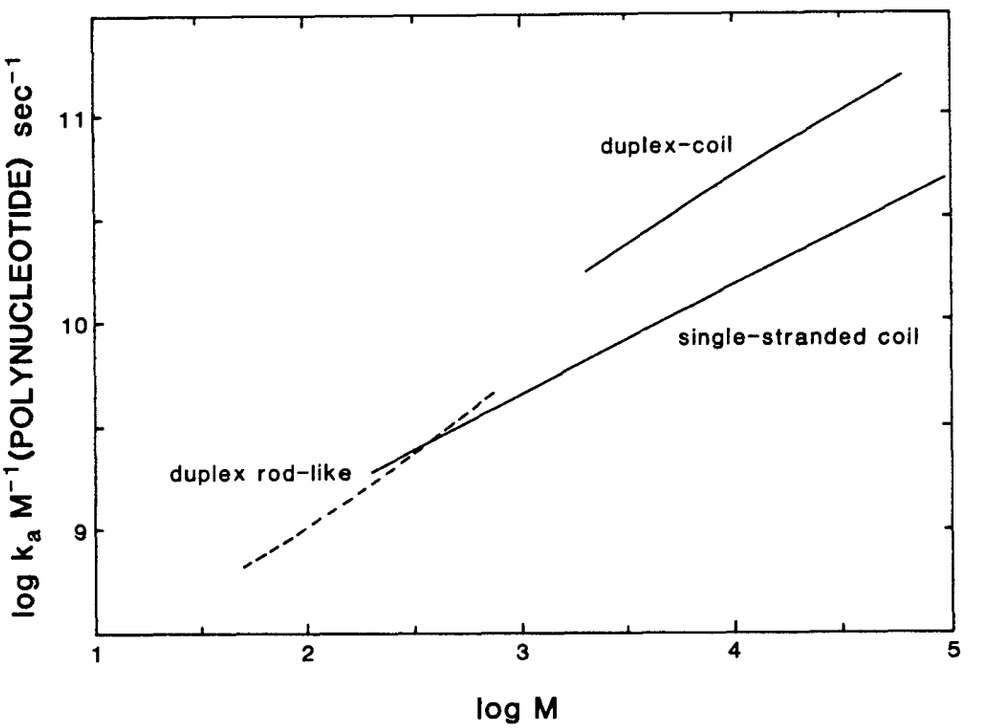

FIGURE 2. The nucleic acid molecular weight dependence of the diffusion-limited bimolecular rate constant, k_a, for protein-polynucleotide association. Duplex and single-stranded DNA are shown in the high molecular weight regime and the short rod-like limit is also shown for duplex DNA. These curves were calculated using Equation 7 from Berg and co-workers[6,28] with $\kappa = 1$ and $f_{el} = 1$, $D_p = 5 \times 10^{-7}$ cm²/sec, and $r_a^2 = 2La/3$ for the high molecular weight region. Values of $a = 500$ Å, $b = 15$ Å were used for duplex DNA and $a = 20$Å, $b = 10$ Å for single-stranded nucleic acids. Equation 11 from Berg et al.[28] was used to calculate the curve for the duplex rod-like association rate constants. M represents the nucleic acid length in base pairs for duplex DNA or in nucleotides for single-stranded nucleic acids.

$$k_a = \frac{2\pi D_p L N_A 10^{-3}}{\ln (2L/b)} \qquad (11)$$

Equation 11 should be valid for duplex DNA up to chain lengths of 700 to 800 base pairs, which is where deviations from the rod-like limit begin to be observed, as judged hydrodynamically.[41] This region has a steeper length dependence than the coil region as shown in Figure 2, and the values of k_a are 10^9 to 10^{10} M^{-1}(polynucleotide) sec^{-1} in the range from 50 to 750 base pairs for duplex DNA.

Figure 2 shows that the bimolecular diffusion-limited association rate constant, k_a can vary by at least as much as a factor of 10^3 depending on the length of the nucleic acid (which determines the interaction radius). Therefore, conclusions about whether a measured nonspecific association rate constant represents a diffusion-limited reaction are difficult to make based only on k_a(app), in the absence of information on the average molecular weight of the nucleic acid.

C. Kinetics of Cooperative Nonspecific Protein-Nucleic Acid Interactions

The effects of cooperative multisite binding on the kinetics of protein-nucleic acid associations have been considered by Epstein.[23,24] In the limit of irreversible binding[23] and for the instantaneous establishment of lattice equilibrium ("IELE") limit,[24] exact

solutions have been obtained (neglecting domain effects). An exact Monte Carlo approach has also been developed[24] so that numerical solutions can be obtained, but this is conveniently done only for short chains due to the need for large amounts of computer time.

The dissociation of cooperatively bound protein-nucleic acid complexes has been treated for the case of irreversible binding. In the limit of high cooperativity (high cooperativity parameter, ω), Lohman[42] has obtained exact solutions to the rate equations for irreversible dissociation. Two cases are considered: (1) fully saturated nucleic acid and (2) fractional saturations (f_{sat}) of the nucleic acid <1. For $f_{sat} = 1$ ($\omega > \approx 500$),

$$\frac{-d[PD]}{dt} = 2k_e [M_N]_o \tag{12}$$

where k_e is the rate constant for dissociation of singly contiguously bound protein from the ends of protein clusters[42] and $[M_N]_o$ is the concentration of nucleic acid molecules (hence $2[M_N]_o$ is the concentration of cluster ends before dissociation). Equation 12 indicates that the dissociation rate is constant (zero order kinetics) when the nucleic acid lattice is initially fully saturated (and ω is large). This behavior results from the fact that when cooperativity is high, only singly contiguous protein molecules dissociate (no proteins from the interior of clusters dissociate). Therefore there is no net change in the fraction of cluster ends throughout the majority of the time course, resulting in a constant rate of dissociation.[42] This behavior has been experimentally observed for the T4 gene 32 protein[43,44] (see Section VI.D.1).

For nucleic acid lattices where $f_{sat} < 1$, only a single exponential decay is observed for irreversible dissociation at intermediate binding densities ($f_{sat} > 0.05$) if ω is large.[42] At sufficiently low binding densities, the population of isolated protein molecules becomes significant and biphasic kinetics are observed, although a stopped-flow experiment will generally not detect the rapid dissociation of isolated molecules.[42-44] Under conditions where $f_{sat} < 1$ and ω is large, the dissociation rate constant for irreversible dissociation of cooperatively bound protein is given by [42]

$$k_d(app) = 2k_e(1 - p_o) \tag{13}$$

where $(1 - p_o)$ represents the fraction of cluster ends before dissociation begins and can easily be calculated using the expressions developed by McGhee and von Hippel.[20] Balazs and Epstein[45] have generalized the above treatment to consider systems with only moderate cooperativities. In this case, analytic solutions are no longer possible, but solutions are obtained which can be evaluated numerically.

III. BIMOLECULAR RATE CONSTANTS FOR SITE SPECIFIC ASSOCIATIONS AND THE ROLE OF NONSPECIFIC DNA

A. General Considerations

The kinetics of association of sequence specific DNA binding proteins to the specific DNA sequence contained within a DNA restriction fragment or within a bacteriophage genome have been studied both experimentally and theoretically. The experimental aspects of sequence specific protein-DNA kinetic studies are discussed in Section VI.C. The theoretical aspects of the association kinetics of a protein to a specific DNA site are discussed first. The theoretical expression for the association rate constant for a protein binding to a specific DNA sequence (length L') which is contained within a DNA molecule of length L depends on

1. The ratio of specific to nonspecific DNA
2. Whether the protein has significant affinity for the nonspecific DNA sites under the conditions of the experiment
3. Whether the protein is capable of undergoing some transfer process, facilitated by the nonspecific DNA, such as one-dimensional translocation (commonly termed "sliding")[2-4] or "direct transfer"[14,46]

If the DNA binding protein can bind to nonspecific sites on the DNA, then these nonspecific sites can either act as traps which impede the binding to the specific site or facilitate binding to the specific site if a mechanism is available for efficient transfer of the protein from the nonspecific sites to the specific site. Generally, one of these two situations will pertain for a particular system (or both depending on the salt conditions) since the only other possibility is that the protein diffuses directly to the specific DNA binding site without interacting with the other DNA sites, which is highly unlikely (except for oligonucleotides). If direct diffusion of the protein to a specific site within high molecular weight DNA did occur, then the maximum bimolecular rate constant (on a specific site basis) for this single step association is $k_a \approx 10^7 - 10^8\ M^{-1}\ sec^{-1}$, assuming a modest orientational constraint of $\kappa = 0.1$ (estimated using Equation 6). If the nonspecific DNA acts solely as a nonfacilitating trap, then k_a(app) will be *lowered* by the factor $(1 + D_T K_{PD})^{-1}$, where K_{PD} is the nonspecific binding constant and D_T is the total concentration of nonspecific binding sites (base pairs). This results because the average number of nonspecific association-dissociation events which must occur before the specific site is located in a random search is $(M - 1)$ where M is the total number of nonspecific sites (base pairs) per DNA chain.[36] This competition by nonspecific DNA is a strong function of the salt concentration (see Section IV). At low salt concentrations where K_{PD} is large, k_a(app) can be reduced considerably if the nonspecific DNA acts solely as a trap.

At moderate salt concentrations, the association rate constant of any protein to a specific site is larger than predicted from Equation 5, even in the absence of any facilitating transfer mechanisms due to an effect that has been termed "hopping". As pointed out by Berg and co-workers,[36,40] "hopping" is solely a consequence of the linear nature of the DNA chain. Once a protein binds nonspecifically, it will undergo a large number of microscopic dissociation-reassociation events before dissociating macroscopically (i.e., to an uncorrelated position away from the chain). For each microscopic dissociation, the protein will reassociate to the same site with high probability. However, due to the linearity of the DNA lattice, there is a finite probability of reassociation to a site one base pair to either side of the original site and a lower probability of reassociating two base pairs away, etc. Therefore, for each macroscopic association, more than one binding "site" can be sampled by the protein due to the large number of intervening microscopic dissociation events.[36,40] It needs to be emphasized that this "hopping" phenomenon occurs for all proteins, since it is a consequence of the linearity of the DNA and therefore is *not* a facilitating mechanism. The expression for the association rate constant to a specific site on a DNA, accounting for this microscopic "hopping", is[36,40]

$$k_a(\text{app}) = \frac{1.5\ \pi\ b\ D_p}{(1 + D_T\ K_{PD})} \tag{14}$$

where the term $(1 + D_T K_{PD})^{-1}$ represents the reduction in k_a due to the fraction of protein that is tied up in nonspecific binding events, as discussed above. In the absence of facilitating mechanisms (e.g., one-dimensional sliding or direct transfer), the failure to consider these "hopping" events will lower the predicted estimate of k_a by approx-

imately a factor of ten.[36] (Remember, however, that "hopping" is a physical reality for all protein-DNA interactions; we are only considering the consequences on the theoretical predictions due to a failure to recognize it.) When facilitating mechanisms such as "sliding" do occur, these effects usually dominate the kinetics so that "hopping" can be neglected.[36]

B. Sliding Mechanisms

The most extensive theoretical work on the role of nonspecific DNA in facilitating the location of a specific DNA sequence by a protein has been done on the "sliding" mechanism.[2-4,12,35,36,40,47-49] The original proposal of a "sliding" mechanism by Riggs et al.[2] was made to explain the extraordinarily large association rate constants observed for *lac* repressor binding to its operator site, contained in phage λ DNA (λplac5). This mechanism was first put in a mathematical framework by Richter and Eigen[3] and subsequently extended to include the coupling of three-dimensional and one-dimensional diffusion of the protein by Berg and Blomberg.[4,12,35,40,48] An excellent summary and extension of this theoretical work has recently been given by Berg et al.,[36] hence only a few of those results need to be reproduced here. The basic idea is that if a protein is able to translocate (slide) while "bound" nonspecifically, it is able to sample the DNA sequences within some average distance on either side of the original site where binding occurred, without having to dissociate and reassociate. This is a direct consequence of the linear nature of the nucleic acid. The sliding process is a one-dimensional random walk which is driven by thermal motion. Therefore, the specific site is "extended" due to the possibility of protein sliding onto the specific site from the flanking nonspecific DNA. The general features and relevance of this type of target extension to biological systems was first discussed by Adam and Delbruck.[50] The extended region of the target (i.e., the minimum flanking distance within which the protein must come in order to be certain of binding specifically) depends on the sliding rate constant, k_s (or equivalently, the one-dimensional diffusion coefficient D_1), as well as the dissociation rate constant for the nonspecific complex, k_{-1}. If able to slide, the average distance (in base pairs) that a protein can cover within the lifetime of the nonspecifically bound complex (the effective target extension) is given by[3,4] $(D_1/k_{-1}l^2)^{1/2}$ where l is the length of the nonspecific binding site (taken as one base pair). The sliding rate constant, k_s, is defined as D_1/l^2 with units of sec^{-1}. The rate constant k_{-1} increases dramatically with increasing salt (see Section V) so that the limits of the extended target decrease with increasing salt.

In general, the association to a specific site which is facilitated by nonspecific DNA can be written as a two-step process[16,34,36] involving the initial association to form a nonspecific complex, PD, followed by transfer of the protein (by some undefined mechanism with rate constant k_2) to form the specific complex, PS:

$$P + D \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PD \tag{15a}$$

$$PD \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} PS \tag{15b}$$

Berg and co-workers[4,12,35,36] have developed an expression for k_2 if the transfer mechanism involves one-dimensional sliding coupled with dissociation and three-dimensional diffusion of the protein between sliding events:

$$k_2 O_T = \frac{k_{-1}}{(k_{-1} L^2/D_1)^{1/2} \coth \left(\frac{k_{-1} L^2}{D_1}\right)^{1/2} - 1} \quad (16)$$

where O_T is the specific site concentration, 2L is the contour length of the DNA, and the other terms have been defined earlier. The overall association rate constant to the specific site is[36]

$$k_a(app) = \frac{k_1}{1 + D_T K_{PD}} \left[\frac{M}{(k_{-1} L^2/D_1)^{1/2} \coth \left(\frac{k_{-1} L^2}{D_1}\right)^{1/2} - 1} \right] \quad (17)$$

where M is the total number of basepairs in the chain and L = (M1/2), l being the length of a basepair. Equation 17 can be simplified in various limits depending on the salt concentration. In the high salt limit, where $D_T K_{PD} \ll 1$ (weak nonspecific binding), Equation 17 reduces to[3,36]

$$k_a(app) = 2k_1[D_1/(k_{-1}l^2)]^{1/2} = 2k_1[k_s/k_{-1}]^{1/2} \quad (18)$$

where k_1 is the nonspecific association rate constant on a per site (base pair) basis. The salt concentration determines the average sliding distance per nonspecific binding event, through the term

$$\left(\frac{D_1}{k_{-1}l^2}\right)^{1/2} = \left(\frac{k_s}{k_{-1}}\right)^{1/2}$$

A priori, one does not know the salt dependence of the one-dimensional diffusion coefficient, D_1, however, the *lac* repressor association kinetics data can be fit with a salt-independent value of $D_1 \sim 10^{-9}$ cm²/sec over a range of [NaCl]. However, a larger value of D_1 is required to fit the data in $MgCl_2$ buffers,[65] although it is still independent of [$MgCl_2$]. Therefore, for a given protein, the effect of salt on the sliding distance arises from the effect on the nonspecific dissociation rate constant, k_{-1}. At very low salt concentrations, Equation 17 overestimates the association rate constant for *lac* repressor-operator (in high molecular weight DNA) since it assumes that the location of the specific site by sliding is rate limiting, but neglects the time for the nonspecific association event. Mazur and Record[16,17] have accounted for this and find better agreement between theory and experiment at these low salt conditions. In the lower molecular weight region, where duplex DNA resembles a flexible rod, Equation 17 reduces to[36,39]

$$k_a(app) = 4\pi D_p \left[\frac{D_1 K_{PD}}{2\pi D_1 l \ln (2L/b)}\right]^{1/2} \tanh \left[\frac{2\pi D_p l L^2}{D_1 K_{PD} \ln(2L/b)}\right] \quad (19)$$

in the diffusion-limited regime.

If dissociation of a protein from a specific site occurs via a sliding pathway, the rate constant can be expressed as[36]

$$k_d(app) = \frac{k_1}{K_{PS} [k_{-1} l^2/(4D_1)]^{1/2} \coth \left(\frac{k_{-1} L^2}{D_1}\right)^{1/2} + K_{PD}} \quad (20)$$

where K_{PS} is the specific binding constant and K_{PD} is the nonspecific binding constant. This can be rewritten as

$$k_d(app) = \frac{k_{-1}}{\gamma\left(\frac{k_{-1}}{4k_s}\right)^{1/2} \coth\left[\frac{M}{2}\left(\frac{k_{-1}}{k_s}\right)^{1/2}\right] + 1} \quad (21)$$

where $\gamma = K_{PS}/K_{PD}$, $k_s = (D_1/l^2)$ and M is the length of the DNA fragment (in base pairs) which contains the specific site. In the limit of weak nonspecific binding ($D_T K_{PD} \ll 1$),

$$k_d(app) = \frac{2\,k_{-1}}{\gamma\left[\frac{k_{-1}}{k_s}\right]^{1/2}} = \frac{2}{\gamma}[k_{-1}k_s]^{1/2} \quad (22)$$

This is also applicable to the dissociation of singly contiguous cooperatively bound proteins from protein cluster ends, if they dissociate by a sliding intermediate. However, in that case $\gamma = \omega$, the cooperativity parameter,[44] and the factor of 2 in Equation 22 should be dropped if dissociation from only one end of the protein cluster is being considered.

IV. SALT EFFECTS ON THE EQUILIBRIUM BINDING OF PROTEINS TO NUCLEIC ACIDS

A. Counterion Condensation Hypothesis

Quantitative treatments of the general effects of changes in salt concentration on protein-nucleic acid equilibria have recently been given.[8,51-55] A brief discussion of these aspects is presented, since it forms the basis for a discussion of the effects of salt on protein-nucleic acid kinetics. Although the interactions of ions with proteins are difficult to model in a general manner since the three-dimensional arrangement of the charged groups on these polyampholytes varies from one structure to another, this is possible for the regularly repeating linear nucleic acid structures. The discussions of salt effects on both the equilibria and kinetics of protein (or any positively charged ligand)-nucleic acid interactions that are presented here are based on the counterion condensation treatments of linear polyelectrolytes developed by Manning[52,56] and Oosawa.[57] The theoretical framework used by Manning[56] in his original treatment of the problem is used rather than the more recent molecular thermodynamic models.[52,58-60] The counterion condensation theory of linear polyelectrolytes, although certainly an approximation to the real situation in solution has been shown to provide an excellent thermodynamic description for the interaction of low molecular weight ions with linear nucleic acids.[8,52,61-64] However, in the limit of low salt, the counterion condensation and Poisson-Boltzmann treatments of linear polyelectrolyte solutions agree very well in their predictions of thermodynamic properties.[65] The linear polyelectrolyte (DNA) is modeled as a linear array of univalent negative point charges separated by an average distance *b* along the contour length of the nucleic acid.[56] The critical parameter of the condensation theory is ξ, which is unitless and proportional to the structural charge density:

$$\xi = \frac{e^2}{\epsilon kTb} \quad (23)$$

where e is the charge on an electron, ε is the bulk dielectric constant, k is Boltzmann's constant, and T is the absolute temperature. Therefore, ξ can be calculated from the known structural charge density of the polyelectrolyte. First let us consider nucleic acid solutions containing only one type of monovalent salt as the supporting electrolyte (e.g., NaCl). If $\xi > 1$ (as it is for both duplex and single-stranded nucleic acids), then low molecular weight monovalent counterions interact with the linear polyelectrolyte in two ways: (1) there is a direct "condensation" of monovalent counterions (e.g., Na^+ or K^+) onto the DNA in order to reduce the net value of ξ to 1 (this includes condensed counterions and structural charges on the DNA).[49] A value of $\xi = 1$ corresponds to one net charge for every 7.14 Å along the contour length. These "condensed" counterions are not site bound, but are mobile along the DNA.[66] In solutions of only monovalent salt, the fraction of a monovalent ion condensed per DNA phosphate, ψ_c, is given by:[8,51,56]

$$\psi_c = 1 - \xi^{-1} \tag{24}$$

(2)After condensation, the remaining unneutralized negative charges on the DNA (1 net negative charge per 7.14 Å) interact with the remaining ions (both counterions and coions) in the bulk solution so that the phosphates are "screened" from one another in the sense of a Debye-Huckel atmosphere. This "screening" effect contributes to the activity coefficient of the DNA,[8,56,63] such that

$$\ln \gamma_D = - N\xi^{-1}\ln\kappa b \tag{25}$$

where γ_D is the activity coefficient of the DNA molecule and κ is the Debye-Huckel screening parameter which is proportional to the square root of the ionic strength. This screening effect is thermodynamically equivalent to the binding of an additional fraction of a counterion, $\psi_s = (2\xi)^{-1}$ per DNA phosphate.[8,51]

Therefore there are two extents of binding of monovalent counterions to linear polyelectrolytes (DNA in this case). These are condensation with $\psi_c = 1 - \xi^{-1}$ and the thermodynamic binding parameter ψ,

$$\psi \equiv \psi_c + \psi_s = 1 - (2\xi)^{-1} \tag{26}$$

which differs from the extent of condensation since the contribution due to screening effects is included.[8,51]

For double helical B form DNA, $\xi = 4.2$, and this value is fairly independent of temperature since for water the product εT has only a slight temperature dependence. Therefore $\psi_c = 0.76$ and the thermodynamic extent of binding, $\psi = 0.88$. For single-stranded nucleic acids, the average internucleotide distance is not an easily measured property, however, estimates of these quantities have been made from the analysis of data on the salt dependence of the helix → coil transition. These estimates of ψ have been tabulated.[8,51,64] The bases of single-stranded nucleic acids are subject to titration over the entire range of pH (as opposed to duplex DNA which only is titrated near pH > 11 or < 5), therefore, ψ_c and ψ vary with pH for single-stranded nucleic acids.[64] In the *absence* of divalent or polyvalent cations (e.g., Mg^{2+}, Ca^{2+}, spermidine), ψ_c and ψ are *constant and independent of the bulk salt concentration.* The results of these treatments[8,51] indicate that even though linear DNA (or RNA) has a very high structural charge density along its contour length, it behaves thermodynamically as if 88% of its structural charge is neutralized by monovalent counterions; this effect is relatively insensitive to the bulk [NaCl].

B. Effects of Monovalent Salt on Protein-Nucleic Acid Equilibria

The effect of changes in the monovalent salt concentration on the equilibrium binding constant, K_{obs}, for a ligand with Z positive charges (or a protein with Z positive charges in its DNA binding site) interacting with a linear polynucleotide has been analyzed by Record et al.[8,51] When a protein binds to a nucleic acid, some number of phosphates (Z) are effectively neutralized. As a result, the condensed counterions which were associated with the Z phosphates are released into solution, as well as the ions which were involved in the long-range screening interactions. This displacement of counterions is the reason for the large dependence of protein-nucleic acid binding constants on salt concentration and in many cases the increase in entropy due to this displacement of counterions provides the driving force for the association.[8] This is the case for oligolysines,[51,55] *lac* repressor-nonspecific DNA[54], and RNA polymerase-nonspecific DNA[67] binding.

The quantitative analysis of these effects is summarized here. For the equilibrium process,

$$L + D \overset{K_{obs}}{\rightleftharpoons} LD \tag{27}$$

we define

$$K_{obs} \equiv \frac{[LD]}{[L][D]} \tag{28}$$

where L is the ligand (protein), D is the nucleic acid, and LD represents the complex. The variation of K_{obs} with monovalent salt concentration, [MX], can be written in its most general form as[8]

$$\frac{d \log K_{obs}}{d \log [MX]} = -\left(\Delta n_M + \Delta n_X - \frac{pm}{55.6}\Delta n_w\right) + \frac{d \log(\gamma_L \gamma_D/\gamma_{LD})}{d \log [MX]} \tag{29}$$

where Δn_M, Δn_X, Δn_W represent the differential binding of cations, anions and water to the free L and D vs. the complex (LD) (e.g., $\Delta n_M = n_{M,L} + n_{M,D} - n_{M,LD}$); m is the molal concentration of MX and p = 2 for a 1:1 salt. Equation 29 includes all of the potential contributions to salt effects on K_{obs} due to differential cation, anion, or water interaction as well as nonideality effects which contribute to salt effects on the activity coefficients. Neglecting differential anion binding and preferential hydration, approximating ($d \log (\gamma_L \gamma_D/\gamma_{LD})/d \log [MX] \sim -Z\psi_c$) and assuming that the only cation release upon forming the complex is from the condensed counterions on the nucleic acid, i.e., $\Delta n_M = Z\psi_c$, then[8,51]

$$\log K_{obs} = \log K_T - Z\psi \log[MX] \tag{30a}$$

$$\left(\frac{d \log K_{obs}}{d \log [MX]}\right)_{T,pH} = -Z\psi \tag{30b}$$

where K_T is the thermodynamic equilibrium constant. (Equation 30 also neglects the salt dependence of the activity coefficients of the small ions M^+, X^-, which is usually quite small.) Therefore the dominant effect of changes in [MX] results from the increase in entropy due to the release of $Z\psi$ thermodynamically bound counterions from the nucleic acid upon forming LD. This is substantial since average values of Z range from five to ten. When the binding reaction occurs in the absence of multivalent cat-

ions, ψ is a constant (0.88 for duplex B form DNA), and log K_{obs} is a linear function of log[MX].

In order to avoid the continual writing of the log-log derivative in Equation 30b, I have defined $S(K_{obs}) \equiv d \log K_{obs}/d \log [MX]$ and this notation is used throughout the review. Similarly, in discussions of salt effects on the kinetic rate constants, $S(k_a) \equiv (d \log k_a/d \log [MX])$ and $S(k_d) \equiv (d \log k_d/d \log [MX])$, where k_a and k_d are the association and dissociation rate constants, respectively (see Section V).

C. Effects of Divalent (or Polyvalent) Cations

If the binding reaction occurs in a buffer containing a mixture of monovalent and multivalent cation salts (e.g., NaCl and $MgCl_2$), then ψ is no longer constant, but is a function of [NaCl] at a constant [$MgCl_2$]. In the absence of any effect of Mg^{2+} on the ligand this results from the fact that Mg^{2+} itself will bind, with a [NaCl] dependent binding constant, to the nucleic acid displacing $2\psi_c$ Na^+ for each bound Mg^{2+}. Therefore, in a mixed Na^+/Mg^{2+} buffer, the nucleic acid has a lower fraction of a Na^+ counterion condensed per phosphate and this fraction varies with both [Na^+] and [Mg^{2+}]. This competitive effect of Mg^{2+} can be quantitatively described and the result is[8,55,68]

$$\log K_{obs} = \log K_T - Z\psi \log[MX] - Z \log [1/2(1 + 4 K_{obs}^{Mg}[Mg^{2+}])^{1/2})] \quad (31)$$

where K_{obs}^{Mg} is the equilibrium constant for Mg^{2+} binding to the nucleic acid, so that

$$\left(\frac{d \log K_{obs}}{d \log [MX]}\right)_{T,pH,Mg} = -Z\psi(1 - 2\Theta_{Mg}) \quad (32)$$

where

$$\Theta_{Mg} \equiv \frac{d \log [1/2(1 + (1 + 4 K_{obs}^{Mg}[Mg^{2+}])^{1/2})]}{d \log [Mg^{2+}]} \quad (33)$$

The value of K_{obs}^{Mg} is dependent on the [NaCl] (just as for any charged ligand) and is described by

$$\log K_{obs}^{Mg} = -1.76 \log [M^+] + \log K_T^{Mg} \quad (34)$$

Hence, when the ligand L binds to the nucleic acid, it displaces both Na^+ and Mg^{2+} from the nucleic acid. At a constant [Mg^{2+}], less Na^+ is condensed on the nucleic acid at lower [Na^+], hence fewer Na ions are displaced when the ligand binds (ψ is reduced by the factor $(1 - 2\theta Mg)$). Therefore, $(d \log K_{obs}/d\log[NaCl])$ which measures Na^+ release varies with the [NaCl] when Mg^{2+} (or other multivalent cations) is present in solution. Hence a plot of log K_{obs} vs. log[MX] will be nonlinear under these conditions.

Values of K_{obs}^{Mg} have been directly measured as a function of monovalent salt in only a few cases;[69,70] however, values for binding to duplex DNA have been estimated from competition studies with lac repressor[68] and pentalysine.[55] The estimates of the slope and intercept of Equation 34 ($S(K_{obs}^{Mg})$ and log K_1^{Mg}) from these two studies are in good agreement with $S(K_{obs}^{Mg}) = -1.66$ to -1.75 and log $K_T^{Mg} = +0.6$ to $+0.35$ (depending of the buffer) from the lac repressor studies[68] and $S(K_{obs}^{Mg}) = -1.7 \pm 0.1$, log $K_T^{Mg} = 0.3 \pm 0.2$ from the pentalysine studies.[55]

It should be emphasized at this point that any process (e.g., conformational change, ligand binding) which changes the extent of counterion condensation on the DNA will be affected by the bulk counterion concentration. However, these effects are *not* determined by the ionic strength of the solution, but rather by the concentrations of

counterions of different type and valence as well as their relative binding affinities to the nucleic acid (e.g., the effect of $MgCl_2$ on protein binding to a nucleic acid is much greater than would be expected on the basis of ionic strength alone). This is a result of the direct binding of cations to the DNA. Most views of processes which are affected by the bulk salt concentration are that they are determined by the ionic strength of the solution. In some instances this is the case, but this is not true for processes which involve nucleic acids (and probably many proteins which directly bind ions).

To illustrate this point, let us examine the binding to DNA of a simple positively charged oligopeptide, pentalysine. The equilibrium binding of pentalysine to duplex T7 DNA has been quantitatively measured in buffers containing only NaCl as the added salt as well as buffers containing various mixtures of NaCl and $MgCl_2$.[55] In the presence of only the 1:1 monovalent salt, NaCl (in excess over the buffer components), the ionic strength (I) of the solution is equal to the [NaCl]. In this case the binding constant for the pentalysine-DNA interaction (K_{obs}^{LD}) is a continuous function of [NaCl] as predicted from condensation theory,[8,51,55] but since [NaCl] = I, K_{obs}^{LD} is also a continuous function of the ionic strength. In a mixed NaCl/$MgCl_2$ solution, the situation is very different as discussed above (Equations 31 to 34). If, as is often incorrectly assumed, the binding constant were simply determined by the ionic strength of the solution, then K_{obs}^{LD} should be the same at an equivalent ionic strength, regardless of the NaCl/$MgCl_2$ composition of the buffer. This is not the case, as shown in Figure 3, where log K_{obs}^{LD} (for pentalysine-T7 DNA) is plotted as a function of ionic strength for three sets of data determined by changing the [NaCl] at three different [$MgCl_2$] (0, 3, and 10 m*M*). Clearly the ionic strength of the solution does not determine the value of K_{obs}^{LD} since it has different values at the same ionic strength depending on the NaCl/$MgCl_2$ composition. As stated above, this is due to the fact that both Na^+ and Mg^{2+} directly bind to the nucleic acid, hence the effect of these ions is more profound than if they only participated in screening. These pentalysine data have been analyzed, correctly accounting for the competitive effects of Mg^{2+}.[55] This is an important point to keep in mind and experiments in mixed monovalent/divalent cation salts can be used to test whether a particular reaction involving macromolecules is solely dependent on ionic strength effects or whether direct binding of ions is important.

Recently an algorithm for calculating protein charge interactions[71] which is simply a summation of screened coulomb potentials has been extended to treat linear polyelectrolytes.[72] This approach has been used to treat the salt effects on protein-nucleic acid equilibria (which can be attempted only if the crystal structure of the protein is known). In their treatment, Matthew and co-workers[73-75] do not account for the entropic contribution due to the release of counterions from the DNA (and possibly other ions from the protein). They also conclude that the value of the binding constant (log K_{obs}) for a protein-DNA complex at a given salt is determined by the square root of the ionic strength, just as predicted for the interactions of low molecular weight ions (in which only Debye-Huckel screening effects are considered). This conclusion results from a neglect of the release of condensed counterions and is clearly incorrect as we show in Figure 3 in the case of a mixed Na^+/Mg^{2+} buffer. Therefore plots of log K_{obs} vs. $I^{1/2}$, although continuous for a given buffer composition, do not yield the same value of K_{obs} when the ratio of Na^+/Mg^{2+} changes, at a constant ionic strength.[68] As a result, log K_{obs} vs. $I^{1/2}$ plots do not have a theoretical interpretation for protein-nucleic acid equilibria, whereas log K_{obs}-log [NaCl] plots do have a theoretical basis and can be interpreted, even in the presence of mixed valence salts.[8,54,68]

The graphical, multicolor depictions which have been made of the electrostatic fields surrounding DNA binding proteins in the vicinity of DNA, using this algorithm,[73-75] are of course dependent upon knowledge of the crystal structures of the macromolecules. The application of the simple summation of screened coulomb potentials[73-75] to

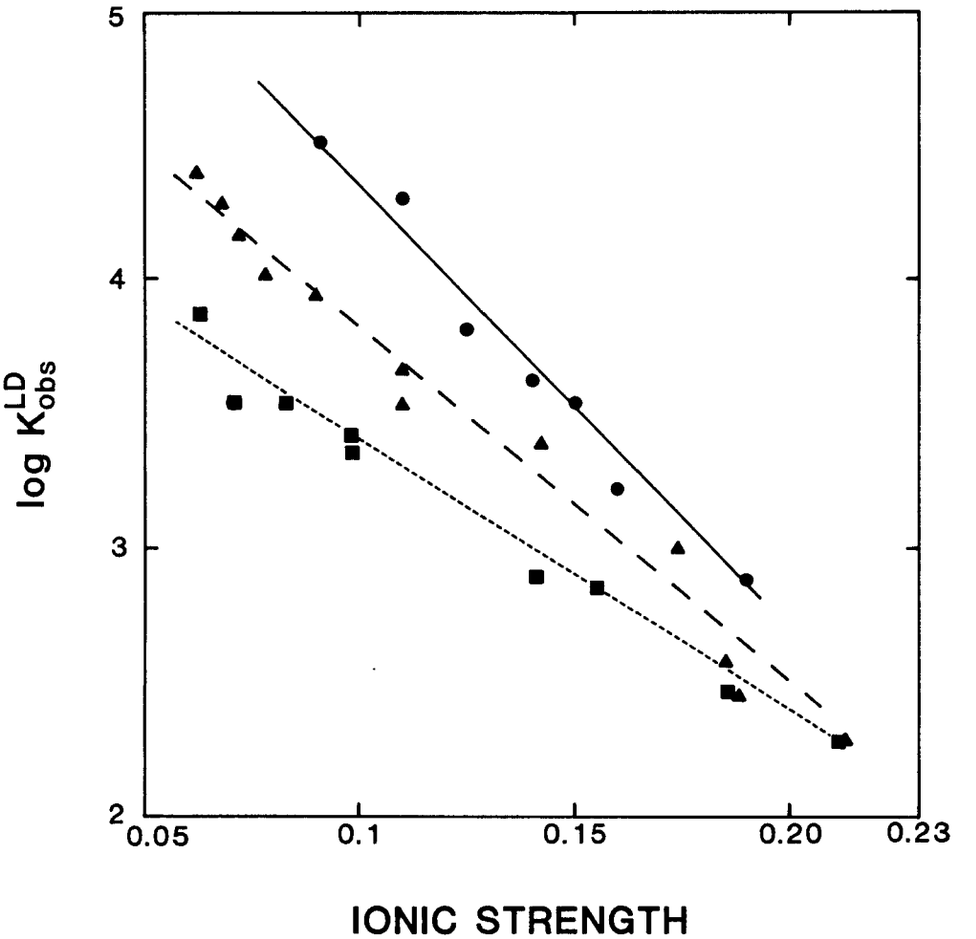

FIGURE 3. The logarithm of the observed binding constant for pentalysine-T7 DNA binding[48] (K_{obs}^{LD}) is plotted as a function of ionic strength for three different sets of buffers which differ in their Na^+/Mg^{2+} content, indicating that K_{obs}^{LD} is *not* determined by the ionic strength for charged ligand-nucleic acid interactions: • (—), varying NaCl only; ▲ (---), 3 m*M* $MgCl_2$, varying NaCl; ■ (...) 10 mm $MgCl_2$, varying NaCl.

show the patches of positive and negative charges on the protein achieves very little more than the representation, obtained from the crystal structure, by simply placing "blue" dots at the positions of the positively charged residues and "red" dots at the positions of the negatively charged residues. This approach[75] cannot be used to quantitatively predict the dependence of protein-nucleic acid binding constants on salt concentration, since it neglects ion release and specific ion binding effects. Thermodynamic analyses of macromolecular interactions must account for all changes which occur in the system.

V. SALT EFFECTS ON THE KINETICS OF PROTEIN-NUCLEIC ACID INTERACTIONS

As a result of the polyelectrolyte nature of nucleic acids, the kinetics of the interactions of proteins (or any positively charged ligand) with nucleic acids are also very sensitive to the bulk salt concentration.[34] This results from the same considerations that are discussed above for the equilibrium binding properties. As in the case of the

equilibrium constant, the rate constants describing protein-nucleic acid kinetics are defined in terms of the macromolecular species only. The low molecular weight salt ions which also participate in the reaction are not explicitly considered in the reaction scheme. As a result, the observed rate constants will in general depend on the salt concentration,[34] whenever ions are released or bound before the rate limiting step. From a study of the salt dependences of the observed association rate constant, k_a, dissociation rate constant, k_d, and the equilibrium constant, K_{obs}, it is possible to obtain fundamental information about the basic mechanism of the interaction.[34,35] As is always the case, a study of the salt dependence of the kinetics (or K_{obs}) should not substitute for other means of investigating the mechanism, but it does provide an extremely powerful supplement to more standard variables which are used to probe a mechanism (e.g., temperature, viscosity, etc.). A systematic study of the effects of changes in salt concentration and type on the kinetics of protein-nucleic acid interactions is particularly useful due to the extreme salt sensitivity of these interactions and because we can interpret such effects due to Manning's polyelectrolyte theory[56] as well as Poisson-Boltzmann theory of charged cylinders[65] and their successful application to the interpretation of salt effects on protein-nucleic acid equilibria.[8,51] In most cases, it is necessary to have detailed information on the salt dependence of the equilibrium binding constant, K_{obs}, in order to correctly interpret the salt effects on the rate constants.[34] Although we use the quantitative predictions of Manning's polyelectrolyte theory to interpret the effects of salt on protein-nucleic acid kinetics, the qualitative conclusions that are reached are independent of the details of Manning's model.

In the discussion of the interaction of ions with nucleic acids (Section IV), it was stated that the interactions can be viewed as originating from two effects. In the discussion of salt effects on protein-nucleic acid kinetics, it is useful to maintain this separation of ion interactions into the categories of (1) screening effects, which are determined by the ionic strength, I, of the solution, and (2) effects due to the direct binding (condensation) of cations to the nucleic acid (and possibly anions and/or cations to the protein) which are *not* determined by the ionic strength as discussed above. The reason for maintaining this separation of the different effects (at least from an interpretive viewpoint) is that protein-nucleic acid rate constants which are solely affected by Debye-Huckel screening are not very salt sensitive when compared to rate constants for pathways involving the displacement or reacquisition of condensed counterions on the nucleic acid. Due to these effects, the salt sensitivity of the rate constants (relative to the salt dependence of K_{obs}) can be used to differentiate between single-step reactions and those reactions which involve intermediates.

This is very different than for interactions between low molecular weight charged species,[76] where direct binding of ions to the reactants or products does not usually occur. In that case, all salt effects are determined by the ionic strength; and the rate constants although sensitive to the salt concentration, do not vary as dramatically, when compared to the salt effects that are observed for protein-nucleic acid kinetics and equilibria.[8,34,51]

Lohman et al.[34] have divided protein-nucleic acid kinetics into two categories based on the quantitative effects of salt on the kinetic rate constants: (1)single-step associations (no observable intermediates) and (2) multiple-step associations (distinct intermediates).

The relative salt dependences, $S(k_a)$ and $S(K_{obs})$, can be used diagnostically to determine the complexity of a particular protein-nucleic acid association reaction as categorized above.[34] The effects of salt on k_a and k_d for single-step, diffusion-limited reactions are relatively simple to describe quantitatively.[34] The dependence of k_a and k_d on [NaCl] for a multistep pathway will depend on the particular protein and the pathway. However, the magnitude of the salt effects, particularly on k_a (compared to K_{obs}),

can be predicted and in this way, one can determine if the pathway is likely to involve distinct intermediates.[34] Furthermore, if an expression for k_a(app) is available for a proposed mechanism, one can readily write down the expected [NaCl] dependence based on the salt dependence of K_{ob} and the predicted salt dependences of the *single-step* association and dissociation rate constants. Since the rate constants for multistep mechanisms are generally very sensitive to the [NaCl], a comparison of the predicted and observed salt dependences provides a good test of a proposed mechanism involving proteins and nucleic acids.

A. Single-Step Reactions

Consider the association of a protein (ligand), L, to a nucleic acid, D, which occurs in a single step:

$$L + D \underset{k_d}{\overset{k_a}{\rightleftharpoons}} LD \tag{35}$$

Lohman et al.[34] have obtained expressions for the dependence of the single step rate constants on [NaCl] by analogy with the Bronsted-Bjerrum treatment of the kinetics of interaction of low molecular weight ions[76] and application of Manning's polyelectrolyte theory.[56] In this treatment, the effects of salt on k_a derive from the dependence of the activity coefficient of the reaction site on the DNA, consisting of Z phosphates. The result is[34]

$$\log k_a = - Z\psi_s \log I + \log k_o \tag{36}$$

where $\log k_o$ combines all of the salt independent terms, and I is the ionic strength of the solution. Equation 36 describes the salt effects due to the "release" of ions participating in the screening of the Z phosphates which make up the DNA site, hence association reactions which can be described by Equation 36 have been called "screening-controlled" associations.[34] From Equation 36, k_a is seen to have only a weak dependence on ionic strength (k_a decreases slightly as ionic strength increases) since ψ_s is small (0.12 for B form DNA). Even for a protein which forms ten ionic interactions with the nucleic acid (Z = 10), a log-log plot of k_a vs. I has a slope of only −1.2 for a one-step association. Equation 36 does not consider effects on the rate of association due to the protein's overall net charge; however, this effect should also yield only a weak salt dependence. If the protein has a net negative charge, the screening effects due to this may offset or even dominate the dependence given in Equation 36 yielding a slightly positive value of (d log k_a/d log I). The major points, however, are that (d log k_a/d log [NaCl]) is predicted to be small and k_a is determined by the ionic strength of the solution (rather than direct ion binding effects). As we see below, this is only the case for the *association* rate constant of a single-step reaction.

The salt effects on the dissociation rate constant for a single-step reaction are more complex than for the association reaction since the major effect results from the need to recondense cations on the nucleic acid upon dissociation of the protein. As a result, k_d is much more sensitive to the monovalent salt concentration and is also *not* determined by the ionic strength of the solution, when buffers with multivalent cations are used, due to the competition between monovalent and multivalent cations for the DNA, as discussed for the equilibrium case. Through a comparison of Equations 30a and 36, Lohman et al.[34] find *in the absence of multivalent cations*):

$$\log k_d = Z \psi_c \log [M^+] + \log k_o' \tag{37}$$

for a single-step dissociation of a ligand, breaking Z ionic interactions with the nucleic acid, where k_o' represents all salt independent terms. Therefore, k_d increases dramatically with increasing $[M^+]$, since $\psi_c = 0.76$ (for duplex B DNA) and a plot of log k_d vs. log$[M^+]$ is predicted to be linear with a slope equal to $Z\psi_c$. In the case of a protein with $Z = 10$, (d log k_d/d log $[M^+]$) = 7.6 (as opposed to −1.2 for k_a), indicating that k_d increases by a factor of $10^{7.6}$ for each tenfold increase in $[M^+]$. This results solely from the requirement to recondense 7.6 monovalent counterions per dissociated ligand.

The effects of a mixed monovalent/divalent cation buffer (e.g., Na^+/Mg^{2+}) indicate that ionic strength is *not* the appropriate variable to describe salt effects on the dissociation rate constant, for the same reasons that apply to K_{obs} (see Section IV). Using the same procedure as was used to quantitatively describe the effects of $[Mg^{2+}]$ on K_{obs}[8,55,68] (Equation 31), Lohman et al.[34] have shown:

$$\log k_d = Z\psi_c \log [M^+] + Z\log [1/2(1 + (1 + 4 K_{obs}^{Mg} [Mg^{2+}])^{1/2})] \quad (38)$$

where the symbols are the same as in Equation 31. As a result, in a mixed Na^+/Mg^{2+} buffer, log k_d is no longer a linear function of log$[M^+]$ due to the dependence of K_{obs}^{Mg} on $[M^+]$ as described by

$$\log K_{obs}^{Mg} = -1.7(\pm 0.1) \log[M^+] + 0.3(\pm 0.2) \quad (39)$$

(Equation 39 is based on competition studies between Mg^{2+} and pentalysine.)[55] Therefore, log k_d will vary less dramatically with $[M^+]$ since Mg^{2+} competes with the monovalent counterion, M^+, for rebinding to the nucleic acid. (Binding constants for Mg^{2+} to various single-stranded nucleic acids have not been extensively measured, which limits the quantitive usefulness of mixed Na^+/Mg^{2+} comparisons for these nucleic acids.)

B. Multistep Reactions

The most useful way to differentiate between a pathway involving intermediates and one which occurs in a single step is to investigate the salt effects on the association rate constant. Let us consider the simplest case where a pathway involves a single intermediate I_1:

$$L + D \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} I_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} LD \quad (40)$$

where I_1 represents an intermediate protein-nucleic acid complex which is different from the product, LD. If the rate limiting step occurs after I_1 (e.g., the step with rate constant k_2 in Equation 40, then the observed association rate constant, k_a(app), will be a function of k_1, k_{-1}, and k_2 (at least). Even if we neglect any salt dependence associated with k_2, it is clear that k_a(app) will have a large salt dependence due to its dependence on k_{-1}.[34] Recall that k_{-1} is the dissociation rate constant for the protein-nucleic acid intermediate, I_1, and it is quite salt dependent due to the minimum requirement to recondense counterions onto the nucleic acid (Equation 37). From Equations 36 and 30a it follows that if $|S(k_a)| \gg 0.15|S(K_{obs})|$ (for B form DNA, since $\psi_\bullet = 0.12$ and $\psi = 0.88$), then the association pathway involves an intermediate and cannot be described by a single-step reaction.[34] Of course whether the reaction involves multiple intermediates is a more difficult question which cannot be answered simply from this qualitative argument, although quantitative analysis of $S(k_a)$, $S(k_d)$, and $S(K_{obs})$, along with other information can yield these answers in some cases. In making comparisons of the salt dependencies of k_a, k_d, and K_{obs}, it is always important to check whether K_{obs}

$= k_a/k_d$. This is not necessarily the case for a multistep reaction where the rate limiting step in the association pathway may be different from the rate limiting step in the pathway for dissociation. This will also depend on the conditions (temperature, salt, pH) of the experiment, since the rate limiting step will generally not be the same under all experimental conditions.

C. Qualitative Aspects

The major interpretive uses of studies of the [NaCl] dependence (or any other monovalent salt) of protein-nucleic acid rate constants are (1) from a comparison of $S(k_a)$ and $S(K_{obs})$ one can determine whether the association involves a single step or is more complex, and (2) given a proposed mechanism for a protein-nucleic acid association (or dissociation) one of the best ways to test the mechanism is by comparing the observed [NaCl] dependence of the rate constants vs. that predicted on the basis of the experimental $S(K_{obs})$ and the predicted [NaCl] dependences of the single-step association and dissociation rate constants, k_1 and k_{-1}, respectively (Equations 36, 37, and 38 above).[34]

1. Salt Dependences for Single-Step Reactions

The following features should apply to this case, in the absence of multivalent cations and in the absence of ion release from the protein:

1. $|S(k_a)| \simeq 0.1|S(K_{obs})|$
2. $S(K_{obs}) \simeq -S(k_d)$

In the presence of multivalent cations:

3. (d log k_a/d log I) should remain small and k_a will be determined by the ionic strength. (N.B.-This is *only* true for a single-step association reaction.)
4. $S(k_d)$ will be substantially smaller in magnitude than in the absence of multivalent cations. Larger Mg^{2+} concentrations will decrease $S(k_d)$ (and $|S(K_{obs})|$) more significantly (compared to $S(k_d)$, in the absence of Mg^{2+}). This will also hold for multistep reactions.

If these features are not observed, then it is likely that the reaction is not a simple single step reaction.

With respect to item 1 above, for a single-step association the characteristics of the particular protein will determine whether $S(k_a)$ is slightly positive or negative. If diffusion of the protein to the nucleic acid is rate limiting, then the net charge on the protein will influence the sign of $S(k_a)$, $S(k_a) > 0$ if net charge < 0; $S(k_a) < 0$ if net charge >0. However, if the process of orienting the positively charged nucleic acid binding site with respect to the nucleic acid is rate limiting, then $S(k_a) < 0$. Many DNA binding proteins are acidic (pI < 7), although the DNA binding site is of course positively charged which probably aids in the proper orientation of the DNA binding site toward the DNA. Therefore these two effects, with opposing salt dependences, may generally cancel, leaving $S(k_a) \approx 0$. The point is, however, that $|S(k_a)|$ is small in comparison to $|S(K_{obs})|$ (on the order of 10% or less) for a single-step association.[34] On the other hand, the [NaCl] dependence of k_d is generally large, comparable in magnitude to $S(K_{obs})$, and positive since in order for the protein to dissociate, counterions must reassociate (recondense) with the DNA site. Therefore, as the salt concentration increases, the dissociation rate will increase. Since $S(k_a) \simeq 0$ for a single step association, then $S(k_d) \simeq -S(K_{obs})$. These salt dependences are characteristic of single step associations, with diffusion-limited association rate constants, k_a.[34] This also points out the

need to have accurate values of $S(K_{obs})$, in order to draw conclusions concerning the salt dependences of k_a and k_d.

2. Salt Dependences For Multistep Reactions

In order to predict the salt dependence for the observed rate constant for a multistep reaction, one needs an expression for the observed rate constant in terms of the elementary, single-step rate constants and any equilibrium constants. Once this expression has been obtained, i.e., we have $k_a(app) = f(k_1, k_{-1}, K_{obs})$, then a prediction of the salt dependences can be made, if $S(K_{obs})$ is known. In the simple cases where preferential hydration and preferential ion binding to the protein do not contribute to the salt dependences (see Record et al.[8,51] for a discussion), then this is done in the following manner (in the absence of multivalent cations): (1) obtain Z from $S(K_{obs})$ and Equation 30b, and (2) use Equations 36 and 37 (or their derivatives with respect to log [NaCl]) to obtain the predicted monovalent salt dependences of the single step rate constants, k_1 and k_{-1}.

Multistep association reactions involving protein-nucleic acid intermediates most often occur in sequence specific binding reactions, although this is not always the case. Examples include binding reactions which require the melting out of a stretch of duplex DNA as in the case of RNA polymerase-promoter associations.[77-80] Any association of a protein to a specific DNA sequence which occurs via nonspecific binding as in sliding[2,5,6,34,81-83] or direct transfer[14,84-87] mechanisms also involves detectable intermediates. Even the association of nonspecific binding proteins, particularly those that bind cooperatively, can involve distinct intermediates along the pathway.[43,44,88,89] Aside from the comparisons of $S(k_a)$, and $S(K_{obs})$, which should both be determined in buffers containing only one type of monovalent salt, one can also use mixed Na^+/Mg^{2+} buffers to evaluate whether an observed association rate constant is diffusion-limited (screening-controlled).[34] For a diffusion-limited association, the value of k_a should be independent of the cation composition of the buffer at a given ionic strength (neglecting ion effects on the protein).[34] However, as stated above, for an association involving protein-nucleic acid intermediates along the pathway, $k_a(app)$ is a function of k_1 and k_{-1} (at least). The effects of Mg^{2+} on k_{-1} are the same as described above for K_{obs}, and are quite dramatic due to the direct competition between Mg^{2+} and Na^+ for binding to the nucleic acid. As a result any association involving intermediates (Equation 36) will have a dramatically different value of $k_a(app)$ in solutions which have the same ionic strength, but which differ in NaCl and $MgCl_2$ concentrations[34] (examples are given below). Therefore, measuring $k_a(app)$ at constant ionic strength, but varying NaCl/$MgCl_2$ ratios, can be used to determine whether the observed rate constant is for a single-step, diffusion-limited association.

The analysis and predictions of the relative salt dependences of k_a, k_d, and K_{obs} that are discussed above assume that the protein binds to the nucleic acid in the same mode over the entire salt concentration range. If this is not the case, then the analysis is not applicable over the entire salt range. In fact, deviations from these predictions can result from a number of possibilities, e.g., a change in rate limiting step or a change in the binding mode or a structural change in the protein. The latter two effects will generally be reflected by a change in the behavior of the equilibrium binding constant, whereas the former will not. For this reason, it is important to measure K_{obs} over as much of the salt range as possible since this will indicate whether the mode of binding is affected by the ionic environment.

D. Salt Effects on Kinetic Processes Involving Sliding

The salt dependence of the rate constants can be used to determine if a one-dimensional translocational ("sliding") mechanism is likely. For a single-step association, k_a

is essentially independent of salt, as discussed above. If an association occurs via a nonspecifically bound intermediate which is able to slide, then the term $(D_1/k_{-1}l^2)^{1/2}$ is always a part of the expression for the apparent association rate constant.[3,4,35,36] For example, in the moderate to high salt limit (weak nonspecific binding $D_T K_D \ll 1$), the expression for k_a(app) is given by Equation 18. In this limit, assuming that the sliding rate constant, k_s, is independent of salt, the [NaCl] dependence is

$$\frac{d \log k_a(app)}{d \log [NaCl]} \approx \frac{1}{2}\left(\frac{d \log k_1}{d \log [NaCl]}\right) + \frac{1}{2}\left(\frac{d \log K_{PD}}{d \log [NaCl]}\right) \quad (41)$$

with $K_{PD} = k_1/k_{-1}$. Since k_1 is the nonspecific association rate constant (single step reaction), it is essentially independent of [NaCl] and Equation 41 reduces to

$$\frac{d \log k_a(app)}{d \log [NaCl]} \approx \frac{1}{2}\left(\frac{d \log K_{PD}}{d \log [NaCl]}\right) \quad (42)$$

i.e., the salt dependence (log-log) of an association rate constant which involves sliding is approximately one half the salt dependence of the nonspecific equilibrium constant, K_{PD}, in the limit of weak nonspecific binding.[28,35] Therefore a comparison of $S(k_a)$ and $S(K_{PD})$ will indicate whether a sliding mechanism is likely.

The salt dependence of the dissociation rate constant can also be used to identify a protein which dissociates from a specific site via sliding. From the expression for k_d(app) in the limit of weak nonspecific binding[36] (Equations 21 and 22, Section III.B), one finds:

$$\frac{d \log k_d(app)}{d \log [NaCl]} \approx -\left(\frac{d \log K_{PS}}{d \log [NaCl]}\right) + \frac{1}{2}\left(\frac{d \log K_{PD}}{d \log [NaCl]}\right) \quad (43)$$

where the nonspecific binding constant $K_{PD} = k_1/k_{-1}$ and K_{PS} is the specific binding constant. Equation 43 must be used if $S(K_{PS})$ is different than $S(K_{PD})$ as in the case of *lac* repressor.[68] However, if $S(K_{PS}) = S(K_{PD})$, then Equation 43 reduces to [36]

$$\frac{d \log k_d(app)}{d \log [NaCl]} \approx -\frac{1}{2}\left(\frac{d \log K_{PD}}{d \log [NaCl]}\right) \quad (44)$$

which is the characteristic salt dependence for sliding mechanisms in the high salt limit. In light of Equation 43, one must be careful if information about the relative salt dependences of K_{PS} and K_{PD} is not known. Of course, one must carefully examine as wide a range of [NaCl] as possible to be sure that the weak binding limit is applicable. If not, the full expression for k_a or k_d must be used[36] to analyze the experiments. A final warning is that the discussion above has assumed that all salt dependences have been determined in solutions containing only monovalent salt. In mixed mono- and divalent salt solutions, the analysis can become complicated and caution should be used, particularly since K_{PD} decreases significantly in the presence of Mg^{2+} (Section IV.C), so that the lifetime of the nonspecific complex is lowered (k_{-1} increases) and sliding effects are diminished.

What are the necessary molecular features that enable a nucleic acid binding protein to "slide" while "bound" to the nucleic acid? At this point so few proteins have been unambiguously shown to be involved in sliding processes that generalizations are impossible. As discussed by Hill and Tsuchiya,[164] the important requirement for proteins to slide is not the strength of binding (or weakness), but rather that the activation energy for lateral movement be low. As long as this constraint is met, even proteins

which have a high affinity for the nucleic acid should be capable of sliding. Both a high affinity for the nucleic acid and likely a low activation energy for lateral motion along the nucleic acid can be maintained if the binding free energy is exclusively electrostatic[6,44] although this has not been proven for the latter. As Winter et al.[6] suggest, the protein would view the nucleic acid as an isopotential surface with very little barrier to lateral movement. The *lac* repressor-nonspecific DNA binding[54] as well as RNA polymerase holoenzyme nonspecific DNA binding[67] seem to be exclusively electrostatic, based on quantitative analyses of the [NaCl] dependence of their binding constants.[54,67] From this point of view, these proteins are good candidates for sliding abilities and the evidence supporting this possibility is discussed in Section VI.C.

VI. EXPERIMENTAL KINETIC DATA

For the purposes of this review, the experimental aspects of protein and ligand-nucleic acid kinetics have been divided into the following categories:

1. Small molecule — single-stranded nucleic acids
2. Small molecule — duplex DNA
3. Protein — duplex DNA
4. Protein — single-stranded nucleic acids

Although there have been a number of experimental investigations of ligand and protein-nucleic acid kinetics, not all of them are discussed in this review. The emphasis is on the effects of salt, which immediately limits the number of systems since most experimental studies of protein (or ligand) nucleic acid kinetics have either neglected salt as a variable or have not varied it in a sufficiently systematic manner to draw meaningful conclusions. Even some kinetic studies which have systematically varied the monovalent salt concentration are difficult to interpret since accurate data on the salt dependence of the equilibrium constant are not available. The small molecule experiments (ions, intercalative dyes, oligopeptides) are discussed from the point of view of model systems and also to indicate the upper limit of the diffusion-limited rate constant for a ligand-nucleic acid association. The available data on the salt dependences of protein-nucleic acid kinetics are then discussed.

A. Small Ligand-Single-Stranded Nucleic Acid Kinetics

Porschke has studied the binding kinetics of ions and oligopeptides to synthetic single-stranded oligo- and polynucleotides (mainly RNA). The equilibria and kinetics of Mg^{2+} and Ca^{2+} binding to the single-stranded polynucleotides,[90] poly(A) and poly(C), as well as a series of single-stranded oligonucleotides[91] [$A(pA)_n$, (n = 4–17), $C(pC)_5$ $U(pU)_5$, $I(pI)_5$, and $d\{A(pA)_5\}$] have been investigated by Porschke using field jump relaxation techniques. The Mg^{2+}-oligonucleotide experiments were carried out in 0.5 m*M* Tris, pH 8.0 (no added salt), and the Ca^{2+}-oligonucleotide experiments in 1 m*M* Na Cacodylate, pH 5.9. No salt dependent data were collected since the field jump technique requires an extremely low salt concentration. The association kinetics for both Mg^{2+} and Ca^{2+} binding to the oligonucleotides are best described by a two-step process:

$$M^{2+} + D \underset{k_{10}}{\overset{k_{01}}{\rightleftharpoons}} (MD)_0 \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} MD \qquad (45)$$

Table 1
RATE CONSTANTS FOR SMALL LIGAND INTERACTIONS WITH SINGLE-STRANDED OLIGONUCLEOTIDES (EQUATION 45)

Ligand	Oligo-nucleotide	Salt	k_{01} (M^{-1}(oligo) sec^{-1})	k_{10} (sec^{-1})	k_{12} (sec^{-1})	k_{21} (sec^{-1})
Mg^{2+}	$A(pA)_{17}$	0.5 m*M* Tris	3.5×10^{10}	1.9×10^{5}	10^{3}	2.7×10^{4}
Ca^{2+}	$A(pA)_4$	1 m*M* Na cacodylate, pH 5.9	4.6×10^{10}	1.0×10^{7}	2.9×10^{6}	1.8×10^{6}
$(Arg)_3$	$A(pA)_6$	1 m*M* Na cacodylate, pH 6.55	2.0×10^{10}	4.0×10^{5}	5.8×10^{6}	8.9×10^{5}
	$I(pI)_6$		2.5×10^{10}	5.4×10^{5}	—	—
	$A(pA)_4$		1.5×10^{10}	1.2×10^{6}	—	—
$(Lys)_3$	$A(pA)_6$		1.4×10^{10}	1.4×10^{6}	1.7×10^{6}	2.4×10^{6}
	$I(pI)^5$		1.5×10^{10}	1.4×10^{6}	—	—
	$A(pA)_4$		9.4×10^{9}	1.2×10^{6}	—	—
Lys-Tyr-Lys	$A(pA)_6$	1 m*M* Na cacodylate, pH 5.9	5.6×10^{9}	1.9×10^{6}	—	—
Lys-Phe-Lys	$A(pA)_6$		7.0×10^{9}	1.9×10^{6}	—	—
Lys-Gly-Lys	$A(pA)_6$		4.6×10^{9}	2.3×10^{6}	—	—

Table 2
RATE CONSTANTS FOR SMALL LIGAND - SINGLE STRANDED POLYNUCLEOTIDE BINDING

Ligand	Polynucleotide	k_1 (M^{-1}(nucleotide)sec^{-1})	k_{-1} (sec^{-1})	Salt
Mg^{2+}	Poly(C)	1.3×10^{10}	8.5×10^{4}	0.5 m*M* Tris, pH 8.0
Mg^{2+}	Poly(A)	2.0×10^{10}	4.0×10^{4}	0.5 m*M* Tris, pH 8.0
Ca^{2+}	Poly(A)	1.0×10^{10}	2.0×10^{4}	0.5 m*M* Tris, pH 8.0

where k_{01} represents the bimolecular rate constant for formation of an outer sphere complex (i.e., none of the six H_2O molecules hydrating the divalent cation are affected by the interaction). These rate constants are diffusion-limited with values of $k_{01} = 3$ to 5×10^{10} M^{-1} (oligo) sec^{-1} (Table 1). Mg^{2+}, but not Ca^{2+}, seems to subsequently form an inner sphere complex (loss of some waters of hydration) in the second step, with rate constant k_{12}, in its interaction with oligo A(pA)n,[90] but not polynucleotides.[91] The extent of inner sphere complexation also decreases as the length of the A(pA)n increases.[90] The inner sphere complexes seem to be only formed with Mg^{2+} and oligo *ribo*adenylates, since these were not observed with $C(pC)_5$, $U(pU)_5$, $I(pI)_5$, or the deoxy form $d\{A(pA)_5\}$.

The field jump relaxation kinetics of Mg^{2+} and Ca^{2+}-polynucleotide complexes are simpler than the oligomer case in that only a single relaxation process is observed,[91] and binding seems to occur via a single-step process. In 0.5 m*M* Tris, pH 8.0(20°C), Porschke has found $k_1 = 1$ to 2×10^{10} M^{-1}(nucleotide) sec^{-1} for Mg^{2+} and Ca^{2+} association to the synthetic polynucleotides poly(A) and poly(C) (Table 2). The average length of these polynucleotides is not stated by Porschke,[91] so the association rate constant cannot be converted to a polynucleotide scale. However, assuming a minimum length of 400 nucleotides yields an estimate of $k_a \approx 4$ to $8 \times 10^{12} M^{-1}$(polynucleotide) sec^{-1}. For Mg^{2+} and Ca^{2+} binding to single-stranded polynucleotides, there is no indication of

Table 3
ASSOCIATION RATE CONSTANTS FOR OLIGOPEPTIDE-POLYNUCLEOTIDE BINDING (IN 1 m*M* TRIS, pH 8.0).

Oligopeptide	Polynucleotide	k_a (M^{-1}(polynucleotide)sec^{-1}) "sphere" model[15]	k_1 M^{-1}(nucleotide)sec^{-1}
$(Arg)_3$	Poly(A)	4.4×10^{11}	4.1×10^{9}
	Poly(C)	5.1×10^{11}	4.5×10^{9}
	Poly(I)	4.1×10^{11}	9.1×10^{8}
	Poly(U)	2.8×10^{11}	1.5×10^{9}
$(Lys)_3$	Poly(A)	2.6×10^{11}	2.5×10^{9}
	Poly(C)	4.5×10^{11}	3.7×10^{9}
	Poly(I)	3.4×10^{11}	9.4×10^{8}
	Poly(U)	2.6×10^{11}	9.7×10^{8}

inner sphere complex formation.[91] Since binding is exclusively electrostatic and does not involve localization or site binding, there should be few steric constraints. Thus, a value of $k_a \approx 10^{12}$ to $10^{13} M^{-1}$(polynucleotide) sec^{-1} should represent an upper limit for the diffusion-limited association rate constant of a small ligand to the domain of a polynucleotide chain of moderate size.

Porschke has also investigated the binding kinetics of small positively charged oligopeptides to single-stranded oligonucleotides[92] by electric field jump relaxation techniques. The association and dissociation rate constants for some of the oligopeptides are listed in Table 1. The bimolecular association rate constants for $(Arg)_3$ and $(Lys)_3$ binding to oligonucleotides are in the range 1 to 2×10^{10} M^{-1}(oligo) sec^{-1}. The $(Arg)_3$-$A(pA)_5$ and $(Lys)_3$-$A(pA)_5$ relaxations can be described by a two-step mechanism, consisting of binding followed by some fast unstacking of the A residues.[92] The association to all other oligonucleotides is consistent with a single-step reaction pathway. The association rate constants for Lys-X-Lys (where X = Tyr, Phe, or Gly) binding to oligonucleotides are smaller than for $(Lys)_3$ by factors of 2 to 3 in the range 4 to 7×10^{9} M^{-1}(oligo) sec^{-1}. In general, the rate constants for the positively charged oligopeptide-oligonucleotide associations are diffusion-limited and any differences in the equilibrium binding constant for the different peptides originates in the dissociation rate constant.[92,93]

The kinetics of charged oligopeptide single-stranded polynucleotides has also been investigated.[15,94] Porschke used a "sphere" model[15] (Section II.A, Equation 4) to account for the domain effect of the polynucleotide in the analysis of the bimolecular diffusion-limited association of a series of oligoarginines and oligolysines with poly(A), poly(C), poly(U), and poly(I). The data are consistent with a single-step association and the results are summarized in Table 3. Porschke also analyzed his data in the standard fashion using a M^{-1}(nucleotide) sec^{-1} scale (accounting for large ligand site exclusion effects) and these values are listed in Table 3 for comparison. In general, the association rate constants obtained from the "sphere" model analysis are factors of ≈200 larger than given by the analysis assuming binding on a nucleotide site basis.[15] One problem with studies using synthetic homopolynucleotides is the extreme polydispersity of the sample, unless a size fractionation is performed. The average lengths of the homopolynucleotides used in Porschke's study ranged from 750 nucleotides for poly(C) to 1700 for poly(I). The association rate constants, for the oligopeptides are approximately a factor of ten lower than those measured for Mg^{2+} and Ca^{2+} (Table 2),[91] although this comparison can only be done on a nucleotide site basis. The rate constants for association to the polynucleotide domain range from 2.6 to 5.1×10^{11} M^{-1}(polynucleotide) sec^{-1} for $(Arg)_3$ and $(Lys)_3$[15] in 1 m*M* Tris pH 8.0 (no temperature

specified). Unfortunately, salt dependent data are not available since the field jump technique is limited to solutions of low conductivity. As discussed above, k_a is expected to be fairly insensitive to salt, although the dissociation rate constant should increase with increasing [NaCl] with $S(k_d) \approx 1.7$ ($= Z\psi_c$, where $\psi_c = 0.56$ for polyA).[8,64]

The relaxation kinetics of polynucleotide-oligopeptide complexes can not be fit to a single-step pathway when the oligopeptides contain aromatic residues and a two-step mechanism has been used to interpret the data.[93,94] This two-step mechanism is necessary to describe the binding of Lys-Trp-Lys to single-stranded poly(A)[94] (in 1 m*M* Na Cacodylate, 1 m*M* NaCl, 0.2 m*M* EDTA, pH 7.0) where the first step is the diffusion-limited association of the oligopeptide to the polynucleotide chain. The second step has been interpreted as an insertion of the aromatic residue of the peptide between adjacent bases with rate constant $k_{12} = 1.5 \times 10^5$ sec^{-1}.

B. Small Ligand-Duplex DNA Kinetics

As with single-stranded nucleic acids, very few studies have been undertaken on the salt dependence of the kinetics of small ligand-duplex DNA interactions. This is an area which needs more work since salt-dependent data on model systems would facilitate the interpretation of the generally more complex protein-nucleic acid kinetics.

1. Oligopeptides

The relaxation kinetics of Lys-Tyr-Lys and Lys-Trp-Lys-calf thymus DNA complexes have been investigated by Porschke and Ronnenberg[90] using electric field jump techniques (in 1 m*M* Na Cacodylate, 1 m*M* NaCl, pH 7.0). Both high molecular weight (3×10^4 base pairs) and sonicated ($\approx$ 500 base pairs) DNA were used. In all cases, the data were best described by a two-step association reaction. A determination of the bimolecular association rate constant could only be made in the case of Lys-Trp-Lys - high molecular weight DNA (3×10^4 base pairs), where $k_{01} = 3.2 \times 10^8 M^{-1}$(base pair) sec^{-1} (Equation 45) with the dissociation rate constant $k_{10} = 4 \times 10^3$ sec^{-1}. This value of k_{01} corresponds to a rate constant of $k_a = 9.6 \times 10^{12} M^{-1}$(polynucleotide) sec^{-1} for association to the DNA domain.

The second step, presumably involving insertion of the aromatic group between the base pairs in an intercalative fashion is characterized by $k_{12} = 6.4 \times 10^3$ sec^{-1} and $k_{21} = 4.4 \times 10^2$ sec^{-1} (Equation 45). This scheme is identical to the one used to interpret the interaction of Lys-Trp-Lys to single-stranded poly(A) (see above), however, the second step (k_{12}) is 20 times slower for intercalation of the tryptophan ring into duplex DNA. No salt dependence data is available.

2. Intercalative Dyes

An enormous amount of work has been given to the investigation of the equilibrium and kinetic binding properties of dyes and drugs to duplex DNA, particularly those that intercalate. In only a few cases, however, have salt dependent effects been studied. The kinetics of binding of ethidium to duplex DNA have been studied by a number of groups, yielding different results depending mainly on the technique or signal used to monitor binding.[22,46,84,85,95,96] Intercalative dyes, which are positively charged, are not simply analyzed since they bind to DNA in a number of different modes. In the case of ethidium, three binding modes have been described, when absorbance changes in the ethidium are used to monitor binding:[46] a nonintercalative binding to the outside of the duplex, which is mainly electrostatic, as well as two distinct intercalative binding modes. The electrostatic, outside binding is considerably weakened at high salt (Section IV), therefore most investigations of the intercalative mode have been done in 1 *M* Na^+ salts,[46] where the intercalative mode still possesses a high affinity for duplex DNA. The experiments of Bresloff and Crothers[46] have recently been reanalyzed[87] to account for

excluded site binding and to test additional models for direct transfer mechanisms. In these studies, three distinct relaxation times are resolved.[46] The association rate constant for outside binding of ethidium is $k_o = 1.36 \times 10^7 M^{-1}$ (base pair) s^{-1}, and for the two intercalative species, $k_{int1} = 1.4 \times 10^6 M^{-1}$ (base pair) sec^{-1} and $k_{int2} = 2.6 \times 10^5 M^{-1}$ (base pair) sec^{-1} (all determined in 1 *M* $NaNO_3$, pH 6.5, 23°C).[46,87] When the enhancement of ethidium fluorescence is used to monitor binding to duplex DNA, only a single relaxation time is observed corresponding to a single-step reaction.[22] Data from fluorescence correlation spectroscopy[96] have also been analyzed by a single-step reaction pathway. The association rate constants obtained from these fluorescence studies are $k_1 = 1.7 \times 10^7 M^{-1}$ (base pair) s^{-1} (0.1 *M* KCl, pH 7.5, 25.3°C)[46] to poly (dA-T) and $k_1 = 1.8 \times 10^7 M^{-1}$ (base pair) sec^{-1} (0.10 *M* NaCl, pH 8.0 22°C)[96] to duplex calf thymus DNA. These values from the fluorescence studies compare well to the outside binding rate constant obtained from absorption studies and may be measuring the same bimolecular step. Mandal et al.[95] have measured $k_1 = 1.6 \times 10^6 M^{-1}$ (base pair) sec^{-1} for ethidium-calf thymus DNA (1 *M* NaCl, pH 6.5, 20°C) by hydrogen-deuterium exchange, which compares well to the association rate constant for intercalative binding measured by Bresloff and Crothers[46] but is lower than the outside binding association rate constant. Other investigations of singly charged dyes, some of which do not intercalate, have determined the following association rate constants to duplex DNA; proflavine, $k_1 = 1.7 \times 10^7 M^{-1}$ (base pair) sec^{-1} (0.10 *M* Na^+;[97] di-*t*-butylproflavine (nonintercalative), $k_1 = 1.5 \times 10^8 M^{-1}$ (base pair) s^{-1} (0.2 *M* Na^+, 25°C).[98]

The only salt dependent kinetic data for dye-duplex DNA binding is for the bis-intercalator, A_cD_i1, – poly(dA-T) interaction.[99] A_cD_i1 is a dimer of acridine connected by a spermine analogue linker, having a charge of Z = +4. The association reaction, under conditions of excess poly(dA-T), is described by a single exponential with $k_1 = 3 \times 10^7 M^{-1}$ (base pair) sec^{-1}, which is independent of [NaCl] from 0.1 to 0.30 *M* (pH 5.0, 20°C).[99] The lack of a [NaCl] dependence for k_1 (0.1 $M \leqslant$ [NaCl] $\leqslant$ 0.3 *M*) as well as the absolute magnitude of k_1 are consistent with the conclusion that k_1 reflects a diffusion-limited, screening-controlled association in this [NaCl] range.[99] In the range 0.30 to 1 *M* NaCl, the association rate constant decreases with increasing [NaCl] with $S(k_a) = -1.4$. This is slightly larger than expected for a screening controlled reaction with Z = 4, although recall that the predictions of Equation 36 do not consider salt dependent effects on the ligand which may occur with A_cD_i1 (such as self-stacking). The change in salt dependence may also reflect contributions from a monointercalated intermediate. Shafer and Waring[100] have shown that monointercalation of echinomycin, another bis-intercalator, is favored at high [NaCl].

C. Protein-Duplex DNA Kinetics

The majority of the avaiable data concerning the effects of salt concentration on protein-duplex DNA kinetics has been obtained for the interaction of three *E. coli* encoded proteins with specific sequences on the DNA: *lac* repressor-operator,[2,5,6,101] RNA polymerase-promoter, [77,102,103] and the restriction endonuclease, *Eco* RI, with its six base pair recognition sequence.[82,83] The only salt dependent kinetic studies of nonspecific binding of proteins to duplex DNA are those for protamine-calf thymus DNA (see Section VI.C.4 below), although the salt dependence of the kinetics of the nonspecific, cooperative binding of the bacteriophage T4 gene 32 protein and *E. coli* SSB protein to single-stranded homopolynucleotides[88,104,105] have been examined and they are discussed in Section VI.D.

1. Lac Repressor-Operator Kinetics

The *Lac* repressor-operator interaction is the most studied protein-nucleic acid system from most points of view, including kinetics. Interestingly, the first investigation

of the kinetics of this interaction by Riggs et al.[2] included a study of the dependence of the rate constants on [KCl], at constant [Mg^{2+}], as well as pH and temperature. Additional dissociation rate studies were performed by Barkley et al.[106] as a function of [KCl] (at 3 m*M* Mg^{2+}) for repressor-operator and repressor-inducer-operator complexes. As discussed by Lohman et al.[34] (Section V), the presence of a mixture of mono- and divalent cations in the buffer complicates the analysis and interpretation of the dependence of the rate constants on [KCl]. The studies of Riggs et al.[2] have been repeated and expanded by Barkley[5] and Winter et al.[6] to include experiments as a function of monovalent salt in the absence of divalent cations, which simplifies the interpretation. In addition, Barkley[5] has investigated the effects of pH, anions, and $MgCl_2$ (in the absence of monovalent salts). Winter et al.[6] have also examined the association of *lac* repressor to operator contained in DNA fragments of variable length, 203 base pairs, 6700 base pairs, and full length λ plac5 (5×10^4 base pairs). A complementary study of the salt dependence of the kinetics of wild-type (and a tight binding mutant) *lac* repressor binding to two synthetic operator fragments, 21 and 26 base pairs, has been done by Goeddel et al.[101] although the interpretation of these is also complicated since they were performed in a mixed Na^+/Mg^{2+} buffer. Both the association and dissociation kinetics were examined in all four studies[2,5,6,101] using standard nitrocellulose filter binding techniques.

An essential component for the analysis of salt effects on the kinetics is a thorough study of the salt dependences of both the specific and nonspecific binding constants as discussed above. Each of the kinetic studies on the *lac* repressor-operator interaction was accompanied by a thorough salt dependent study of the observed binding constant for the specific repressor-operator interaction (K_{obs}^{RO}).[107,108] Although the determinations differ slightly, within experimental error, $((d \log K_{obs}^{RO}/d \log [M^+]) = -7 \pm 1$, in the absence of divalent cations,[107,108] which is identical to the prediction made by Record et al.[68] based on the mixed Na^+/Mg^{2+} data of Riggs et al.[2] From this and application of Equation 30b (with $\psi = 0.88$ for duplex B form DNA), an estimate of $Z = 8 \pm 1$ is obtained for repressor-operator, which is a maximum value, assuming no contribution to the salt dependence due to ion binding or release from repressor.[8,51] The [NaCl] dependence of the nonspecific *lac* repressor-DNA binding constant (K_{obs}^{RD}) has also been thoroughly investigated, in the absence of divalent cations[54,109,110] and also as a function of [$MgCl_2$] (in the absence of monovalent cations)[54] and in mixed Na^+/Mg^{2+} buffers.[68] These studies indicate that $S(K_{obs}^{RD}) = -10 \pm 2$ corresponding to $Z_{max} = 11 \pm 2$ for the nonspecific interaction. Note that the nonspecific repressor-DNA complex forms ≈3 additional ionic interactions than the specific complex.[68]

a. Association Kinetics

One approach to the analysis of the salt dependence of the association rate constant of a protein-nucleic acid interaction is to (1) compare $S(k_a)$ with $S(K_{obs})$, both obtained in the absence of multivalent cations, and (2) compare ($d \log k_a/d \log I$) and k_a, in different salt conditions, where the contributions to the ionic strength, I, are from only one type of monovalent salt vs. a mixture of monovalent and divalent or a single type of divalent salt. These comparisons are sensitive indicators of whether associations occur via intermediates. Averaging the data of Barkley[5] and Winter et al.[6] one finds $S(k_a) = -4.8 \pm 0.5$ for the association of *lac* repressor to operator in λ plac5 DNA in the range $0.125\ M \leq [M^+] \leq 0.20M$. The predicted value of $S(k_a)$ if the association were a single-step, diffusion-limited reaction is ≈ -1.0 ($= -Z\psi_a$). On the basis of the observed salt dependence of k_a, Lohman et al.[34] suggested that the reaction pathway is more complex than a single step association. The proposed association pathway, which fits the majority of the data involves a two-step mechanism. Repressor first binds nonspecifically to the DNA domain, followed by a one-dimensional translocation (sliding)

along the DNA, coupled with intermittent dissociations, until contact with the operator is made.[3,5,6,35,36] The predicted salt dependence of k_a for such a mechanism in the high salt limit is given by Equation 42[3,4,35,36] which equals −5 for *lac* repressor-nonspecific DNA.[54] At lower salts, the salt dependence becomes more complex[5,6] as discussed by Berg et al.;[36] however, the behavior in the high salt limit can be used diagnostically. A log-log salt dependence for k_a which is one half the log-log salt dependence of the nonspecific binding constant provides good evidence in support of a sliding mechanism (Section V.D).

Additional salt dependent data indicating the involvement of an intermediate in the *lac* repressor association comes from a comparison of association rate constants in NaCl or KCl only,[5,6] $MgCl_2$ only,[5] and in a mixed KCl/$MgCl_2$ buffer.[2] When rate constants for the *lac* repressor-λ plac5 DNA association are compared at a constant ionic strength, I = 0.15, it is clear that the rate constants in the three different buffer systems differ dramatically, e.g., $k_a = 6 \times 10^8$ M^{-1} s^{-1} (in NaCl)[5]; 3×10^9 M^{-1} s^{-1} (in KCl/mg acetate);[2] $\approx 7 \times 10^7$ M^{-1} s^{-1} (in $MgCl_2$, extrapolated to I = 0.15)[5]. If this were a single-step association, these rate constants would be the same, regardless of the salts used to obtain an ionic strength of 0.15.

At low monovalent salt concentrations, the agreement between the observed [NaCl] dependence[6] of k_a and that predicted by Berg et al.[36] on the basis of a "sliding" model is not as good as in the high [NaCl] regions for both λ plac 5 DNA and the 6700 base pair operator containing fragment. Mazur and Record[16,17] have shown that inclusion of the time for the nonspecific association event, which is significant at low [NaCl], rather than the use of a steady state treatment,[36] yields much better agreement between the "sliding" theory and the experimental results. In addition, Mazur and Record[16,17] suggest that the quantitative differences between the experimental values[6] of k_a and those predicted for the mechanism which includes only sliding[36] may be due to the contribution of a "direct transfer" pathway in addition to sliding. Winter et al.[6] argue convincingly that a mechanism which only incorporates a "direct transfer" mechanism is not supported by the experiments. However, it is entirely possible that at low salt, where sliding becomes less effective as a facilitating mechanism, the direct transfer mechanism (if it exists) would begin to contribute.[16,17]This possibility was also discussed qualitatively by Winter et al.[6] If both mechanisms are available to *lac* repressor, the sliding mechanism will dominate at high salt.[16,17,36]

Studies of the salt dependence of *lac* repressor association to 21 and 26 base pair synthetic operator fragments[101] indicate that this behaves as a diffusion-limited, or screening-controlled association, as suggested by Lohman et al.[34] The association rate constant decreases only slightly with increasing salt with $(d \log k_a/d \log I) = -0.5$. The predicted slope from Equation 36 is −1.0, which agrees well with the experiments. This salt dependence is considerably smaller than observed for the association to λ plac5 DNA, in which a nonspecifically bound intermediate has been proposed. The oligo-operator experiments[101] were performed in buffers containing 10 m*M* Mg^{2+} (using KCl to vary the ionic strength); no data was obtained in buffers containing only monovalent salt. The largest value of $k_a = 2 \times 10^9$ M^{-1} sec at I = 0.05 is a reasonable value for a diffusion-limited association of a protein to an oligonucleotide, since it is only a factor of 3 below the k_a for the Lys-Tyr-Lys association to $A(pA)_5$ (Table 1). No comparable data are available for oligopeptide-duplex oligonucleotide model systems.

From their analysis of the *lac* repressor-operator data, Winter et al.[6] also determined the nonspecific association rate constant for *lac* repressor binding to any site on the polynucleotide. Using Equation 7, Winter et al.[6] calculate $k_1 = 3.1 \times 10^6$ M^{-1} (base pair) s^{-1} for λ plac DNA (5×10^4 base pairs) and $k_1 = 6.3 \times 10^6$ M^{-1} (base pair) s^{-1} for a 6700-base pair fragment. On a domain basis, these rate constants have values of $k_a = 1.6 \times 10^{11}$ and 4.2×10^{10} M^{-1} (polynucleotide) sec^{-1}, respectively, assuming $k_a = Mk_1$.[36] Al-

though this rate constant was not directly measured over the [KCl] range of their experiments, Winter et al.[6] found that a constant, salt independent value of k_1 fit the data very well. The small salt dependence of the nonspecific association rate constant, k_1, is the expected behavior for a simple single step reaction (Section V).[35,36]

The recent *lac* repressor-operator kinetics data has been fully analyzed by Barkley,[5] Berg et al.[36] Winter et al.[6] and Mazur and Record[16,17] in terms of the sliding mechanism for operator location, including the effects of DNA length on the association reaction. The reader is referred to the original papers for a full discussion of this mechanism, since only the salt effects have been discussed here.

b. Dissociation Kinetics

A number of salt dependence studies of the dissociation of *lac* repressor-operator complexes have been done, both in mixed K^+/Mg^{2+} buffers[2,101,106] and in buffers containing only monovalent salt.[5,6] The analysis of this data has not been as extensive as the analysis of the corresponding association rate constants. Lohman et al.[34] analyzed the [KCl] dependence of k_d(app) from experiments performed in mixed K^+/Mg^{2+} buffers for both λ plac5 DNA[2,106] and synthetic operator fragments (21 and 26 base pairs)[101] before data in the absence of Mg^{2+} were available. In all cases, k_d(app) increases with increasing [KCl], although a log-log plot of k_d(app) vs. [KCl] is not linear in the presence of Mg^{2+}.[2,34,106] This occurs since the Mg^{2+}-DNA interaction itself is salt dependent so that the effect of Mg^{2+} is greater at low [KCl] (Equation 23).[34] From data[2,106] obtained in buffers containing 3 m*M* Mg^{2+} and 10 m*M* Mg^{2+}, Lohman et al.[34] conclude that Mg^{2+} acts only as a competitive ligand and does not exert a specific effect on the repressor itself. After accounting for the competitive effect of Mg^{2+}, using Equation 38, Lohman et al.[34] predicted the monovalent dependence of k_d(app) for the *lac* repressor-λ plac5 DNA dissociation, in the absence of Mg^{2+} as

$$\log k_d = 1.76 \log [K^+] - 1.57 \quad (46)$$

Barkley[5] and Winter et al.[6] have since measured this dependence and find slightly different results in each case. In the high monovalent salt region (0.1 to 0.2 *M* KCl or NaCl), Barkley[5] finds a higher salt dependence such that $S(k_d) = 3.1$ at pH 8.0 and 3.8 at pH 7.4 for repressor dissociation from λ plac5. Winter et al.[6] have measured $S(k_d) = 2.5 \pm 0.5$ for λ plac5 (pH 7.5), 1.7 ± 0.3 for the 6700-base pair operator containing fragment, and 2.2 ± 0.4 for an 80-base pair operator containing fragment. From application of Equation 43, which is valid in the weak nonspecific binding limit, the sliding mechanism predicts $S(k_d) \approx 2$. In all cases, however, $|S(k_a)| > |S(k_d)|$ and $|S(k_d)| \ll |S(K_{obs})|$, indicating that dissociation does not occur in a single step. The results are consistent with the interpretation that repressor slides off the operator and subsequently dissociates from a nonspecific site.[6] The DNA length dependence of k_d is not completely consistent with this mechanism, however.[6]

The association of *lac* repressor to the 21 and 26 base pair *lac* operator fragments[101] which is likely to be diffusion-controlled also yields $|S(k_a)| > |S(k_d)|$, however, as Lohman et al.[34] discuss, this can be explained due to the presence of Mg^{2+} in the buffer. However, one must be cautious in any analysis of effects of salt on protein-oligonucleotide interactions since fewer ions interact with oligonucleotides due to the reduced electrostatic potential (compared to polynucleotides).[111] In the case of the 21 and 26 base pair operator, the salt dependence is consistent with a single-step dissociation of repressor.[34]

The log-log plots of k_d(app) vs. [NaCl] (or [KCl]) still show curvature at lower salt concentrations, even in the absence of divalent cations.[5,6] In the absence of preferential ion effects on the repressor itself, this deviation from a linear log-log plot must reflect

multiple dissociation processes, or a change in rate limiting step as the monovalent salt concentration is lowered. Winter et al.[6] suggest that as the salt is lowered ([KCl] in their case), the rate limiting step in the two-step dissociation process becomes the nonspecific dissociation step rather than the first step of repressor sliding off the operator to form the nonspecific complex. An additional possibility, however, is a competition between the sliding mechanism and direct dissociation of repressor from the operator (by-passing the nonspecifically bound intermediate). Each of these processes has a different [NaCl] dependence, so that as the [NaCl] is changed the fraction of molecules which dissociate by each of the two mechanisms changes (sliding is favored at low salt), thereby yielding a nonlinear log k_d - log [NaCl] plot.

2. Kinetics of Eco RI Restriction Endonuclease Binding to its Specific Cleavage Site

The interaction of the type II restriction endonuclease *Eco* RI with its specific recognition sequence has been studied under conditions which eliminate the cleavage reaction. In the absence of divalent cations, the endonucleolytic cleavage reaction is eliminated, but not the ability of *Eco* RI to bind specifically to its recognition site, 5′-GAATTC.[112] Just as with other sequence specific binding proteins, *Eco* RI endonuclease also has a substantial affinity for nonspecific DNA sites.[112,113]

The equilibrium constant for the specific binding of *Eco* RI to the recognition site, contained in the plasmid pBR322, has been measured as a function of [NaCl], in the absence of divalent cations, over the range 0.088 to 0.17 *M*. A constant value of (d log K(specific)/d log [NaCl]) = -7.1 ± 0.6 was measured[114] from which $Z_{max} = 8.1$, assuming no preferential ion release from the protein. A quite similar result was obtained for the specific interaction of *Eco* RI with the self-complementary dodecadeoxynucleotide d(CGCGAATTCGCG),[115] i.e., (d log K (specific)/d log[KCl]) = -8.2 ± 0.3. This estimate of 8 to 9 ionic interactions corresponds very well to the finding that 8 phosphates are protected from ethylation in the specific *Eco* RI-DNA complex.[116] Unfortunately, studies of the salt dependence of the nonspecific binding constant have not been performed.

There is evidence which suggests that the *Eco* RI endonuclease makes use of nonspecific DNA sites in its pathway for locating and leaving its specific binding site. Jack et al.[82] have measured the dissociation rate constants for *Eco* RI endonuclease from a series of duplex DNA fragments ranging in size from 34 to 6200 base pairs, each containing a single *Eco* RI recognition sequence. The apparent dissociation rate constant increases from 5×10^{-3} min^{-1} ($t_{1/2}$ = 140 min) for the 34-base pair fragment to 4.6×10^{-2} min^{-1} ($t_{1/2}$ = 15 min) for full-length pBR322 (4362 base pairs) in 0.073 *M* NaCl, pH 7.6 (no divalent cations). Although the association rate constants were not measured directly, relative rates of association to the various fragments were approximately the same as in the dissociation, whereas the equilibrium constants for specific complex formation were found to be independent of fragment length.[82] Jack et al.[82] interpret this in terms of a "sliding" mechanism, suggesting that *Eco* RI dissociates from its specific site by first "sliding" to form an intermediate nonspecific complex followed by dissociation from the nonspecific site. The length dependent dissociation data have been fit[82] to the expression for k_d(app) from Berg et al.[36] (Equations 20 and 21, Section III.B).

The value of $(D_1/k_{-1}l^2)^{1/2} = (k_s/k_{-1})_{1/2} = 1.3 \times 10^3$ base pairs provides the best fit to the data at 0.073 *M* NaCl.[82] Recall that this term represents the effective sliding distance (in base pairs) during the macroscopic lifetime of the nonspecific *Eco* RI complex and that this is [NaCl] dependent due to the [NaCl] dependence of k_{-1}. The fit of Equation 20 to the data is good; however, a value of $k_1 = 3.1 \times 10^4$ M^{-1} (base pair) sec^{-1} for the nonspecific association rate constant is necessary to attain the fit. This is nearly a factor of 100 lower than expected for a diffusion-limited rate constant (according to

Equation 7 and when compared to the value of 3 to 6×10^6 M^{-1} (base pair) sec^{-1} found for *lac* repressor.[6]

Jack et al.[82] have also measured the [NaCl] dependence of the dissociation rate constant of *Eco* RI from the 34 base pair fragment and full length pBR322 (which contains only one recognition site). Above 0.15 *M* NaCl, the k_d for both DNA molecules have the same [NaCl] dependence, within experimental error. However, below 0.15 *M* NaCl, the dependences differ significantly. For dissociation from the 34-base pair fragment, $S(k_d) = 8.5$, whereas for the 4362-base pair intact pBR322, $S(k_d) = 4.1$.[82] Since the 34-base pair fragment is not a polymer, it may not have its full complement of condensed counterions,[111] which means that 8.5 may be a slight underestimate. Upon comparing these values of $S(k_d)$ to the salt dependence of the specific binding constant, $S(K_{obs}) = -8.2 \pm 0.2$, we see that $|S(k_d)\ (34b.p)| = |S(K_{obs})|$, but $|S(k_d)\ (4362)| = 1/2\ |(SK_{obs})|$. These salt dependences are consistent with the following picture. Dissociation from the 34-base pair fragment behaves as a single-step dissociation, since k_d has the same absolute value for its [NaCl] dependence as K_{obs} (opposite sign). The salt dependence of dissociation from the *Eco* RI site contained in pBR322 is only $1/2|S(K_{obs})|$ which is expected if a sliding step is involved (Equations 22 and 44), assuming salt independent values for D_1 (k_s) and γ. There is not enough data above 0.15 *M* NaCl to confirm this, but the prediction is that as the [NaCl] is raised above 0.15 *M* NaCl dissociation from both DNA molecules would follow the single-step direct dissociation since this is faster at higher salts. This type of switchover from dissociation via a sliding pathway to a single-step dissociation seems to occur for the dissociation of singly contiguously bound bacteriophage T4 gene 32 protein from the ends of cooperatively bound protein clusters[43,44] (Section VI.D). Therefore, above 0.15 *M* NaCl, the log-log salt dependence for *Eco* RI dissociation from the site on pBR322 is expected to have a slope of ≈8 rather than 4, if it switches to a direct dissociation pathway at high salts.

The experiments of Jack et al.[82] were done in the absence of divalent cations. Langowski et al.[83] have performed kinetics experiments designed to investigate the processivity of the *Eco* RI endonuclease in order to directly test its ability to translocate or slide along duplex DNA. These latter experiments used an enzymatic cleavage assay which requires Mg^{2+}, hence they were performed in buffer containing 80 m*M* NaCl and 10 m*M* $MgCl_2$ pH 7.2. Variations in the salt composition of the buffers were not investigated.[83] Under these conditions, Langowski et al.[83] conclude that *Eco* RI cutting is totally distributive (i.e., only one restriction site is cut per binding event); their data indicate that no linear diffusion, or sliding, of *Eco* RI occurs in their experiments.[83] Although at face value this result is inconsistent with the conclusions of Jack et al.,[82] the major difference between the two sets of experiments is the salt composition of the buffers. As stated above, the effective sliding distance for a protein is given by the expression[3,4] $(D_1/k_{-1}l^2)^{1/2}$ or $(k_s/k_{-1})^{1/2}$. If D_1 is assumed to be independent of [NaCl] (although it may be a function of the cation, e.g., Na^+ vs. Mg^{2+}),[5] then the sliding distance is only affected by changes in salt through k_{-1}. As the salt concentration (either Na^+ or Mg^{2+}) is increased, so does k_{-1} ($k_{-1} \propto [NaCl]^8$ for *Eco* RI), hence the sliding distance decreases dramatically with increasing salt. The ratio of k_{-1} for nonspecific dissociation of *Eco* RI under the conditions used by Langowski et al.[83] (80 m*M* NaCl, 10 m*M* $MgCl_2$) vs. those of Jack et al.[82] (73 m*M* NaCl) can be calculated using Equation 38 which assumes that Mg^{2+} acts only as a competitor for *Eco* RI binding and neglects preferential ion binding to the protein. Since the [NaCl] dependence of the nonspecific binding constant has not been measured, it is assumed to have the same [NaCl] dependence as the specific complex ($S(K_{obs}) = -7.13$).[114] With these assumptions the dissociation rate constant under the conditions of Jack et al.[82] is a factor of ≈200 smaller than k_{-1}, under the conditions of Langowski et al.[83] As a result, the sliding distance under the Langowski et al.[83] conditions is predicted to be only 90 base pairs

(reduced by a factor of 10). (The value of k_{-1} calculated for the 80 m*M* NaCl, 10 m*M* $MgCl_2$ conditions is equivalent to the value predicted in 152 m*M* NaCl alone). It is not clear whether reduction of the sliding distance by this amount would be sufficient for the experiments of Langowski et al.[83] to appear nonprocessive, however, it is clear that the addition of 10 m*M* $MgCl_2$ will reduce the sliding distance considerably simply due to its effect on k_{-1}. Recent experiments by Maas and co-workers[169] at lower $MgCl_2$ concentrations do detect processive action by *Eco* RI. This suggests that both sets of experiments[82,83] are correct and the different results are due to the nonspecific competitive effect of Mg^{2+} which reduces the lifetime of the nonspecific *Eco* RI-DNA complex and hence its average sliding distance. Of course, increases in [NaCl] would achieve the same effect.

3. RNA Polymerase-Promoter Kinetics

Both the equilibrium and kinetic properties of the sequence specific RNA polymerare holoenzyme-promoter binding have been extensively studied (for a recent review, see von Hippel et al.)[117] As in the case of *lac* repressor, the observed association rate constant for promoter binding is unusually high in some cases. For the bacteriophage T5 early promoters, k_a exceeds 10^{10} M^{-1} (promoter) s^{-1} in 10 m*M* Mg^{2+}, 0.12 *M* KCl, pH 8.0.[118] Mainly on the basis of these large association rate constants and by analogy with the well-studied *lac* repressor-operator kinetics, it has been proposed that location of the promoter by RNA-polymerase involves a pathway which is facilitated by nonspecific DNA.[80] However, there is very little experimental evidence that the facilitation of the association rate is due to a sliding mechanism. The interaction of RNA polymerase holoenzyme with a promoter to form an "open" promoter complex (i.e., RNA polymerase melting a region of the DNA sequence at the promoter after which mRNA initiation occurs) is a complicated process involving several steps: association to nonspecific DNA, promoter location, and recognition ("closed" promoter complex), melting of the DNA to form the "open" promoter complex. Hinkle and Chamberlin[102,119,120] examined the effects of [NaCl] on the dissociation of RNA polymerase holoenzyme from T7 DNA, however, the effects of salt have only recently been used to analyze the details of the kinetic pathways of RNA polymerase-promoter interactions.[77,103] The quantitative NaCl concentration dependences of the equilibrium binding properties of nonspecific RNA polymerase-DNA complexes[67,110,121] (including so-called "tight binding" complexes[122-124] and end binding[125]) as well as specific promoter binding[126,127] have been studied. The nonspecific holoenzyme-duplex DNA interaction is extremely salt dependent. In the absence of divalent cations, the [NaCl] dependence of the nonspecific association equilibrium constant is[67,110,121] $S(K_{obs}^{RD}) = -10 \pm 2$. This can be compared to the salt dependence of the specific binding constant, K_{obs}^{RP} measured for binding to the T7 A1 promoter, which is also temperature dependent.[127] At 0°C, $S(K_{obs}^{RP}) = -10.5 \pm 1.5$, whereas at 37°C, $S(K_{obs}^{RP}) = -13.7 \pm 1.0$. These different [NaCl] dependences (at 0 and 37°C) presumably reflect the formation of "closed" and "open" promoter complexes, respectively.[127] As proposed by Strauss et al.,[127] the increased [NaCl] sensitivity of the binding at 37°C may be due to the melting of the duplex DNA with its added contribution to counterion release.[8] The full molecular interpretations of these salt dependences have been given in the original papers.

Belintsev et al.[103] have measured the rate of *E. coli* RNA polymerase specific complex formation with intact bacteriophage T7 DNA (which contains several promoters) as a function of [$MgCl_2$], at a constant [$MgCl_2$] of 10 m*M* (pH 8.0, 25°C). A nitrocellulose filter binding assay was used and formation of nonfilterable complexes was monitored. The observed association rate constants show a larger [NaCl] dependence than predicted for a single-step association; k_a varies from 10^9 to 3×10^7 M^{-1} (promoter) sec^{-1} over the range 0 to 150 m*M* NaCl (at 10 m*M* $MgCl_2$). Belintsev et al.[103] also

observe an optimum value for k_a, near 100 m*M* NaCl. By analogy with the *lac* repressor-operator association kinetics[5,6,35,36] Belintsev et al.[103] conclude that RNA polymerase uses a sliding mechanism in its pathway for location of the promoter. The experiments of Belinstsev et al.[103] are difficult to interpret for several reasons. First of all there are several inconsistencies in the reported data. The [NaCl] dependence shown in Figure 3 of their paper[103] does not correspond exactly to the data in Figures 1 and 2 (the 50-, 75-, and 100-m*M* points are mislabeled in at least one of their figures and Table 1). As a result, the data in Figure 3 of their paper are incorrect and the quantitative [NaCl] dependence cannot be calculated. Furthermore, the data at the lowest salt concentration were obtained in 10 m*M* $MgCl_2$ (no NaCl), whereas all other measurements were in mixed NaCl/$MgCl_2$ buffers. Therefore the data which are plotted as a function of [NaCl] in order to show an optimum in k_p (minimum in T) are misleading since the point at zero NaCl is meaningless on this scale, due to the competitive effects of Mg^{2+} (Section IV). The value of the nonspecific equilibrium constant used by Belintsev et al.[103] to calculate k_p at 10 m*M* $MgCl_2$ (no NaCl) is also overestimated as listed in their Table 1. This has not been measured under these conditions for RNA polymerase holoenzyme and as shown by de Haseth et al.[54] and Record et al.[68] for *lac* repressor, it is not a simple process to extrapolate data obtained in mixed Na^+/Mg^{2+} buffers to conditions in the absence of Na^+. Since the major evidence used by Belintsev et al.[103] to propose a sliding mechanism is the "bell-shaped" dependence of k_a on NaCl concentration, this conclusion must be considered as very preliminary. This is especially the case since changes in the aggregation state of RNA polymerase at low salt are well documented[128,129] and this effect may contribute to the decrease in k_a at low salt. This point should be emphasized, since changes in salt concentration can affect the solubility and aggregation states of various proteins. Hence conclusions concerning apparent changes in binding constants or rate constants as a function of salt should be approached cautiously until studies of the salt-dependent effects on the protein itself have been performed.

Aside from the large rate constants measured by Bujard et al.[118] the best experimental evidence for the role of nonspecific DNA in facilitating the location of promoters by RNA polymerase is from Park et al.[81] who measured the time-dependent distribution of RNA polymerase along T7 DNA using a rapid mixing photocrosslinking technique.[130] These experiments were performed under constant ionic conditions (10 m*M* $MgCl_2$, 50 m*M* NaCl, 50 m*M* Tris, pH 8.0).[81] Park et al.[81] conclude that the entire T7 DNA molecule serves as the target for RNA polymerase binding and that the nonspecific DNA facilitates the rate of encounter of polymerase with the promoter. Through comparisons of the experimental data with computer simulations, Park et al.[81] conclude that uncorrelated dissociations and reassociations cannot explain the observed rate enhancement, but a sliding mechanism could. Park et al.[81] did not consider correlated association-dissociation events, (e.g., "hopping",[36,88] as a means of explaining their observations. The one-dimensional diffusion coefficient which Park et al.[81] estimate from their data ($D_1 \approx 6.2 \times 10^{-9}$ cm²/sec) is larger than the theoretical estimate for *lac* repressor,[49] a protein which is considerably smaller than RNA polymerase. The analysis by Park et al.[81] can only be considered qualitative at this point since mechanisms other than sliding can explain the transfer of RNA polymerase from nonspecific DNA sites to the promoter. Although sliding may be important for promoter location, the Park et al.[81] data does not prove it.

Roe et al.[77] have investigated the [NaCl] and temperature dependences of the dissociation and association of *E. coli* RNA polymerase holoenzyme with DNA fragments containing the bacteriophage λ P_R promoter. These nitrocellulose filter binding experiments were done in buffers containing 10 m*M* $MgCl_2$, pH 8.0, and variable [KCl] to facilitate comparisons with results obtained by other techniques. As a result, Roe et

al.[77] correct for the competitive effects of Mg^{2+} through use of Equation 29 in order to interpret the effects of [KCl]. The observed association rate constant (10 m*M* $MgCl_2$, 0.12 *M* KCl, pH 8.0) under pseudo first-order conditions of excess RNA polymerase) is $k_a(app) \approx 2.6 \times 10^6\ M^{-1}\ (RNAP)s^{-1}$, much smaller than the diffusion-controlled limit. The [NaCl] dependence of $k_a(app)$ (corrected for the competitive Mg^{2+} effect) was found to be $-S(k_a) \geq 9$, whereas the dissociation rate constant varied as $S(k_d) \geq 6$. Roe et al.[77] conclude from this as well as the temperature dependences of $k_a(app)$ and $k_d(app)$ that multiple intermediates are involved in the association and dissociation pathways. (The observations that $|S(k_a)| > |S(k_d)|$ and $|S(k_a)| \gg 0.1\ |S(K_{obs})|$ are unambiguous indicators of intermediates along the pathway.) Roe et al.[77] propose the following mechanism:

$$R + P \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} I_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} I_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} RP_o \tag{47}$$

where R ≡ RNA polymerase, P ≡ promoter, RP_o ≡ "open" promoter complex, and I_1 and I_2 represent transient intermediates which do not accumulate significantly. The rate limiting step, under the conditions of Roe et al.[77] is the I_1 to I_2 conversion (k_2 is limiting in the association and k_{-2} is limiting in the dissociation). Using a steady state analysis, Roe et al.[77] propose that $k_a(app) = k_2K_1$ and $k_d(app) = k_{-2}/K_3$. As a result, the major contribution to the [NaCl] dependence of $k_a(app)$ is from the equilibrium constant $K_1 = k_1/k_{-1}$, which is large[77,128] ($S(K_1) \approx -8$). The salt dependence of K_3, which presumably involves ion release due to melting approximately 10 to 17 base pairs in forming RP_o, could account for most of the salt dependence of $k_d(app)$.[77] This complex mechanism also seems to accommodate the observations of Hawley and McClure[131,132] if an additional intermediate is included in Equation 47.[77]

Roe et al.[133] have also determined that the forward rate constants for *E. coli* RNA polymerase holoenzyme interactions with different promoters (e.g., λP_R, λP_{RM}, $\lambda P_R'$) have quantitatively different [NaCl] dependences. This has several significant ramifications. First of all, as Roe et al.[133] suggest, transcription initiation could possibly be regulated by changes (fluctuations) in ion concentration which may be associated with different phases of the cell cycle, since the different salt sensitivities possessed by different promoters will affect the relative occupancy of these promoters. Secondly, from the point of view of interpreting salt effects on the kinetics of RNA polymerase-promoter interactions, one must not assume that $S(K_{obs}^{RP})$ is the same for different promoters when comparing $S(k_a)$, $S(k_d)$, and $S(K_{obs})$ values.

4. Protamine Nonspecific DNA Kinetics

The kinetics of a protamine, clupeine Z, binding nonspecifically to duplex calf thymus DNA has been studied as a function of NaCl concentration by Watanabe and Schwarz.[134] This protamine binds cooperatively to duplex DNA, and relaxation kinetics experiments were performed by inducing dissociation through NaCl concentration jumps, monitoring the decrease in fluorescence of a fluorescein label on the protamine.[134] The data were analyzed by measuring initial relaxation times.[134,135] At 0.40 *M* NaCl (pH 7.5, 20°C), $k_1 = 1.7 \times 10^7\ M^{-1}$ (base pair) sec^{-1}, and for dissociation of singly contiguous protein from a cluster end, $k_d(app) \approx 0.29\ sec^{-1}$. Experiments performed between 0.3 and 0.5 *M* NaCl show the expected behavior; i.e., $k_d(app)$ increases with increasing [NaCl] ($S(k_d) = 2.74$) and k_a decreases with increasing NaCl ($S(k_a) = -0.82$). Over this same range of [NaCl], $S(K\omega) = -3.6$; however, the cooperativity parameter, ω, also increases with increasing [NaCl], leveling off above 0.40 *M*. Both the magnitude of k_1 and the [NaCl] dependence suggests that the association rate constant is

diffusion-limited for the protamine-DNA reaction, under these conditions.[134] More extensive data are required to confirm this.

D. Protein-Single Stranded Nucleic Acid Kinetics

A variety of nucleic acid binding proteins interact selectively with single-stranded conformations of DNA or RNA. Most notably are the class of proteins referred to as helix destabilizing proteins (previously called melting proteins and frequently referred to simply as single strand binding proteins). An extensive review of these proteins has recently been given.[136] The most-studied examples of this class of proteins are the bacteriophage T4 encoded gene 32 protein,[137] the filamentous phage gene 5 protein,[138] and the *E. coli* encoded SSB protein.[139] These three proteins bind selectively and cooperatively to single-stranded DNA and RNA (although their affinity for DNA is generally higher than for RNA) and possess only a weak affinity for duplex DNA even at low salt concentrations. From the point of view of detailed structural information, the filamentous gene 5 protein is the best studied since crystal structures of the protein and its complexes with oligonucleotides have been determined (for a review see Brayer and McPherson).[140] Quantitative solution studies of the equilibrium nucleic acid binding properties of the gene 5 protein[89,141,142] and the SSB protein[143-150] have been undertaken to varying degrees, however, the T4 gene 32 protein has been the most extensively studied[151-158] from this point of view. Investigations of the single-stranded nucleic acid binding kinetics of *E. coli* SSB protein,[104,147,159] fd gene 5 protein,[89] and T4 gene 32 protein[43,44,88,160-163] have been carried out, however, the only extensive studies of the salt dependences of the rate constants are those of T4 gene 32 protein.[43,44,88,161,162]

1. T4 Gene 32 Protein-Single Stranded Nucleic Acid Kinetics

From studies of a number of single-stranded homopolynucleotides, von Hippel and colleageus have determined that the nearest neighbor equilibrium cooperativity parameter,[19,20] ω, has a value in the range of 10^3 to 5×10^3, independent of [NaCl] and polynucleotide type[151,155,156] even though the binding constant, K, shows a large polynucleotide specificity. Furthermore, the [NaCl] dependence in the absence of divalent cations is $S(K\omega) = -6.5 \pm 1$, which also is independent of the polynucleotide lattice, within the error limits given.[155,156] Due to the salt independence of ω, both the cooperative binding constant, $K\omega$, and the isolated binding constant for T4 gene 32 protein-single stranded nucleic acid interactions possess the same salt dependence. The salt dependence of gene 32 protein binding has definite contributions from both cation and anion effects, which complicates the molecular interpretation. However, valid conclusions can still be drawn from comparisons among $S(K_{obs})$, $S(k_a)$, and $S(k_d)$ as discussed in Sections IV and V.

a. Association Kinetics of T4 Gene 32 Protein

Lohman and Kowalczykowski[88] studied the association of T4 gene 32 protein to single-stranded homopolynucleotides by fluorescence stopped-flow techniques, monitoring the quenching of the intrinsic tryptophan fluorescence of the protein upon binding to excess nucleic acid (on a site basis). At high nucleic acid concentrations, only a single exponential decay was observed, whereas at lower concentrations, at least two exponentials were apparent. For all polynucleotides studied, an optimum k_a(app) was measured near 0.10 *M* NaCl, and two qualitatively different types of association behavior were observed on the low [NaCl] vs. high [NaCl] sides of the optimum. On the low [NaCl] side, the association behaves as single-step reaction; k_a(app) is independent of polynucleotide type (i.e., no specificity) and inversely proportional to the solution viscosity; the activation energy (E_a) is + 4 kcal/mol and the [NaCl] dependence is given by:

$$\log k_a(\text{app}) = 1.5 \pm (0.3) \log [\text{NaCl}] + 7.95 \quad (48)$$

That is, k_a(app) increases with increasing [NaCl] below 0.10 *M* NaCl. Above the [NaCl] optimum the association appears to be a multistep process (τ^{-1} levels off at high nucleic acid concentrations) and the [NaCl] dependence is

$$\frac{d \log k_a(\text{app})}{d \log [\text{NaCl}]} = -5 \pm 1 \quad (49)$$

At high NaCl concentrations, k_a(app) does display polynucleotide specificity and $E_a = -24 \pm 6$ kcal/mol. Furthermore, for the association reaction with poly(dT), k_a(app) is observed to be essentially independent of [NaCl] in the range 0.10 to 0.30 *M* NaCl. All of these observations can be incorporated into the following three-step mechanism:[88]

$$P + D \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PD_{is} \quad \text{(nucleation)} \quad (50a)$$

$$PD_{is} + P \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} PD \quad \text{(growth)} \quad (50b)$$

$$PD_{\text{contiguous}} \xrightarrow{k_r} PD_{\text{equilibrium cluster distribution}} \quad \text{(redistribution)} \quad (50c)$$

where the first step with rate constant k_1 (on a per nucleotide basis) is the bimolecular, diffusion-limited association of protein to form a noncooperative, isolated T4 gene 32 protein-nucleic acid complex, PD_{is} (nucleation step). The second step with rate constant k_2 represents the formation of the first small cooperative complexes (growth step). The last step is the redistribution of the gene 32 protein clusters to form their equilibrium cluster distribution.

The reason that different association behaviors are observed in the low salt vs. high salt regions is due to the large [NaCl] dependence associated with the single-step dissociation rate constant, k_{-1} (Equation 50a). At low [NaCl], k_{-1} is small and $k_{-1} < 2k_2[PD_{is}]$, so that the bimolecular association in Step 1 (Equation 50a) is rate limiting, therefore k_a(app) = k_1. Hence the properties of a bimolecular, diffusion-limited rate are observed at low salt. (The increase in k_a(app) with increasing [NaCl], below 0.10 *M* NaCl, may reflect a screening contribution due to the fact that gene 32 protein has a net negative charge, or it may be due to a salt-dependent protein conformational change which places some fraction of the protein population in a nonbinding form.[88]) In the range 0.1 to 0.3 *M* NaCl, $k_1 = 3$ to 4×10^6 M^{-1} (nucleotide) sec^{-1}, independent of salt.[88] As the [NaCl] is increased above 0.10 *M* NaCl, k_{-1} increases [$S(k_{-1}) \approx 6 \pm 1$], so that eventually $k_{-1} > 2k_2[PD_{is}]$, at which point a pre-equilibrium exists and k_a(app) = $2k_2K_1P_{tot}$, where P_{tot} is the total T4 gene 32 protein concentration and $K_1 = k_2/k_{-1}$. Therefore k_a(app) reflects the properties of the equilibrium constant, K_1 (e.g., nucleic acid specificity, $\Delta H \ll 0$, and a large negative [NaCl] dependence due to direct ion release.[88]

The bimolecular rate constant for the noncooperative association of T4 gene 32 protein to the polynucleotide chain ($k_a = Mk_1$) is in the range 9×10^8 to 1.5×10^9 M^{-1} (polynucleotide) sec^{-1} which compares well to the estimate based on Equation 7,[36,88] but is a factor of 100 smaller than the domain rate constants for oligopeptides[15] (Table 3).

In the analysis of the second growth step (Equation 50b), Lohman and Kowalcyzkowski[88] conclude that the formation of cooperative, contiguous protein complexes, with rate constant $k_2 \approx 2.5 \times 10^8$ M^{-1}(contiguous site) sec^{-1}, involves some facilitated transfer of noncooperatively bound protein, since $k_2 \gg k_1$. One possibility is that the growth step occurs by a pathway in which free gene 32 protein binds noncooperatively and then "slides" until it encounters a previously bound, isolated protein or a cluster end, to which it binds forming the cooperative interaction. The arguments follow the same lines as those discussed for the *lac* repressor-operator association.[2,3,5,6,36] The expression for k_2 in the high salt limit, if sliding of gene 32 protein monomers occurs, is

$$k_2 = \frac{k_1(k_s/k_{-1})^{1/2}}{(1 + D_T K_1)} \tag{51}$$

where D_T is the nucleotide concentration and the term $(1 + D_T K_1)$ accounts for the competition of noncooperative binding sites for the protein. A sliding rate constant, $k_s = D_1/l^2$) equal to 2.3×10^6 sec^{-1}, is needed to fit the association data with this sliding model.[88] This value is similar to the one obtained for *lac* repressor.[5,6] Although it is clear that the pathway for formation of cooperative complexes must involve a process other than three-dimensional diffusion of T4 gene 32 protein directly to an isolated bound protein or a cluster end, the association data do not prove that sliding is involved. However, combined with the evidence from irreversible dissociation experiments by Lohman[43,44] (see below), sliding of gene 32 protein monomers does seem to be a likely possibility.

In principle, the salt dependence of k_2 can be used to distinguish between a "sliding" mechanism and an unfacilitated transfer such as "hopping", which occurs for all nucleic acid binding proteins as a consequence of microscopic dissociations and the connectivity of the nonspecific binding sites. However, the [NaCl] dependence of k_s is needed for this. If it is assumed that k_s is independent of [NaCl] as it seems to be for *lac* repressor on duplex DNA, then (d log k_1/d log [NaCl]) is predicted to be −3 for a sliding process (i.e., $k_2 \propto K_1^{1/2}$) or zero for a hopping process. The [NaCl] dependence of k_2 has not been determined with great accuracy, but $S(k_2) \simeq -0.9 \pm 0.3$, which does not agree with either case. Other possibilities are discussed by Lohman and Kowalczykowski.[88]

b. Dissociation Kinetics of T4 Gene 32 Protein

The dissociation kinetics of cooperatively bound T4 gene 32 protein-single stranded nucleic acid complexes have been studied by a number of groups, using fluorescence stopped-flow techniques to monitor the dissociation. Irreversible dissociation of the complexes at high salt was induced by NaCl concentration jumps[43,44,160-163] or at lower [NaCl] by trapping the dissociated protein with an excess of a higher affinity nucleic acid (whose complex with gene 32 protein possesses a different fluorescence quantum yield than the original complex).[44] The irreversible dissociation of cooperatively bound T4 gene 32 protein from natural single-stranded DNA[43,160,161] or RNA[163] is complex, yielding a sum of at least two exponential decays when the nucleic acid is less than fully saturated with gene 32 protein. However, only a single exponential decay is observed under these same conditions when synthetic homopolynucleotides are used to form the complex (either DNA or RNA).[43,44,161,162] In addition, the [NaCl] induced irreversible dissociation from high molecular weight synthetic homopolynucleotides which are initially fully saturated with T4 gene 32 protein yields a *constant rate* of dissociation (i.e., zero order kinetics).[43] This observation as well as the results of a quantitative analysis of the increase of the apparent dissociation rate constant, k_d(app), with decreasing

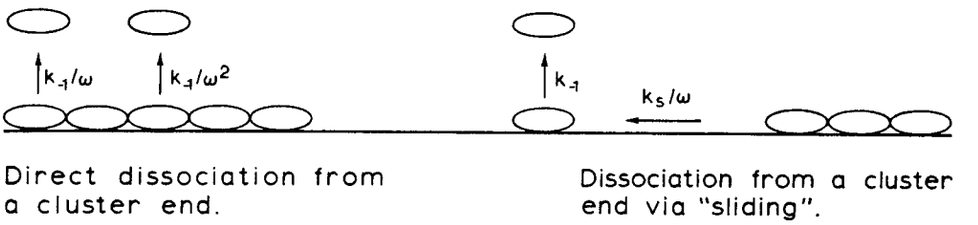

FIGURE 4. Schematic depicting the four possible dissociation kinetic processes for a cooperatively bound protein nucleic acid complex: (1) direct dissociation of doubly contiguous protein (k_{-1}/ω^2); (2) direct dissociation of singly contiguous protein (k_{-1}/ω); (3) direct dissociation of isolated protein (k_{-1}); (4) formation of an isolated protein from a singly contiguous protein by a sliding process (k_s/ω).

initial binding density, indicate that the only species which dissociate under all salt conditions studied are singly contiguously bound gene 32 protein from the ends of protein clusters.[43,44] The dissociation of doubly contiguous molecules from the interior of protein clusters was never observed. Isolated proteins are either a negligible fraction of the bound protein due to the high value of ω or they dissociate within the dead time of the stopped-flow experiments. These data were analyzed using a quantitative model for the irreversible dissociation of highly cooperative nonspecifically bound protein-nucleic acid complexes[42] (Section II.C).

The NaCl concentration dependence of the irreversible dissociation kinetics of cooperative T4 gene 32 protein-single stranded homopolynucleotide complexes has been thoroughly studied by Lohman[43,44] over a wide range of [NaCl], from 50 mM to 1M for poly(rA) complexes. (The effects of polynucleotide specificity, anions, temperature, and pH were also examined.[43,44]) For all polynucleotides, it was found that k_d(app) for cooperative gene 32 protein complexes is a sensitive function of the [NaCl]: k_d(app) increases with increasing [NaCl].[43,44] However, the log-log dependence of k_d(app) on [NaCl] is not constant, as expected for a dissociation which occurs by a single-step pathway. In fact, for gene 32 protein complexes, with high molecular weight poly (rA) ($S_{20,w}$ = 9.9 S), $S(k_d) \approx 6.5 \pm 0.5$ in the range 0.5 to 0.6 M NaCl and decreases to $\approx$ 2.5 below 0.1 M NaCl.[44] The limiting value of $S(k_d) \approx 6.5 \pm 0.5$ is identical to the absolute value of $S(K\omega)$, i.e., $S(k_d) = -S(K_{obs})$. This suggests that the dissociation of T4 gene 32 protein from the ends of protein clusters occurs as a single step with rate constant k_{-1}/ω in this [NaCl] region.[44] The low salt limiting value of $S(k_d) = 2.5 \pm 0.3$ ($\approx 1/2\ |S(K_{obs})|$) suggests that the mechanism or rate limiting step in the dissociation of singly contiguous protein changes at low salt. Additional evidence such as an increase in the activation energy from 14 ± 1 kcal/mol at 0.6 M NaCl to 27.0 ± 1 kcal/mol at 0.2 M NaCl, as well as polynucleotide specificity of the break point in the [NaCl] dependence of k_d, also suggests a change in mechanism at low salt. Lohman[44] proposed the following mechanism to explain these observations which is shown schematically in Figure 4. Singly contiguously bound gene 32 protein can dissociate from the end of a protein cluster by two pathways: (1) direct dissociation with rate constant k_{-1}/ω and (2) a two-step process involving the sliding of gene 32 protein off the cluster end to form an isolated bound protein intermediate which subsequently dissociates.[44] The overall rate constant for this second process is given by $(k_s/\omega)(k_{-1}/k_s)^{1/2}$ which is identical to Equation 22, with $\gamma = \omega$. (The factor of 2 is missing because dissociation from only one cluster end is considered here.) Therefore, the net dissociation rate constant from a cluster end, k_e, is the sum of these two pathways:[44]

$$k_e = \frac{1}{\omega}\left[k_{-1} + (k_s k_{-1})^{1/2}\right] \tag{52}$$

Equation 52 yields the experimentally observed [NaCl] dependence if the sliding rate constant, k_s, is independent of [NaCl], since $S(k_{-1}) = 6 \pm 0.5$, where k_{-1} is the rate constant for the single-step dissociation. Therefore the dissociation data are consistent with a mechanism in which gene 32 protein dissociates directly from a cluster end at high [NaCl], but dissociates via a sliding pathway at low [NaCl].[44] The sliding rate constant which best fits the data is $2 \times 10^6 sec^{-1}$ which is surprisingly close to the value of k_s needed to fit the association data for gene 32 protein[88] (see above). These two analyses of data from different experimental approaches both suggest that monomers of T4 gene 32 protein are capable of one-dimensional translocation along single-stranded nucleic acids.[44,88] This also illustrates how small changes in salt concentration can affect the mechanism of a protein-nucleic acid interaction in addition to its rate of binding. One caveat in this analysis is the lack of accurate binding constants for the gene 32 protein-poly(rA) association in the low salt region (<0.10 *M* NaCl), where quantitative comparisons between $S(k_d)$ and $S(K\omega)$ need to be made. However, other data[43,44,88,155,156] indicate that $S(K\omega)$ remains ≈ -6 in this low [NaCl] region and that the change in $S(k_d)$ reflects a real change in the dissociation mechanism. Evidence for an additional dissociation pathway is also based on the fact that the observed dissociation rate constant for singly contiguous protein, k_d, is consistently greater than that predicted from $K\omega$ and the measured association rate constant,[44] i.e., $k_d > k_{-1}/\omega$.

2. fd Gene 5 Protein-Single Stranded Nucleic Acid Kinetics

The association kinetics of gene 5 protein to the synthetic homopolynucleotides poly(dT) and poly(A) have been investigated by Porschke and Rauh[89] using fluorescence stopped-flow techniques. The mixing experiments were done with excess polynucleotide in a manner similar to the T4 gene 32 protein experiments of Lohman and Kowalczykowski,[88] discussed above. Porschke and Rauh[89] performed their experiments at 0.1 *M* NaCl for poly(dT) and 0.15 *M* NaCl for poly(rA) and did not examine the kinetics as a function of salt. However, it is interesting to compare their findings with the T4 gene 32 protein kinetics.[88] The association mechanism of gene 5 protein[89] in its formation of cooperatively bound complexes is similar to that found for T4 gene 32 protein[88] in that it also follows the kinetic scheme outlined in Equation 50. The association to poly(dT) in 0.10 *M* NaCl is described by a single exponential decay, in the presence of excess poly(dT), when the gene 5 protein fluorescence quenching is monitored. At lower poly(dT) concentrations (higher ratios of gene 5 protein/DNA), multiple exponential decays are observed corresponding to excluded binding site effects (overlap) as well as redistribution of cooperatively bound gene 5 protein.[89] Analysis of the poly(dT) concentration dependence of the relaxation times indicates that the fastest relaxation process corresponds to the reaction in Equation 50a, such that $\tau_f^{-1} = k_1[\text{poly(dT)}] + k_{-1}$, just as for the T4 gene 32 protein association under "strong binding" (low salt) conditions.[88] This corresponds to the limit where $2k_2[PD_{ii}] > k_{-1}$, so that k_1 is measured (Equation 50). A value of $k_1 = 2.7 \times 10^7\ M^{-1}$(nucleotide) sec^{-1} was obtained for the formation of isolated (noncooperative) complexes.[89] This corresponds to $k_a = 8.1 \times 10^9\ M^{-1}$ (polynucleotide) sec^{-1}, since Porschke and Rauh[89] used poly(dT) which was ≈ 300 nucleotides long.

The association kinetics of gene 5 protein with poly(A) in 0.15 *M* NaCl shows the "weak binding" pre-equilibrium behavior[89] which Lohman and Kowalczykowski[88] observed for the T4 gene 32 protein association to all homopolynucleotides at sufficiently high salts. Therefore Equations 50a and 50b are needed to describe the association of gene 5 protein to poly(A) in 0.15 *M* NaCl, with $k_{-2} = 0$. This corresponds to the limit $k_{-1} \gg 2k_2[PD]_{ii}$, as discussed above for gene 32 protein.[88] Under these conditions one observes only the growth process (Equation 50b) coupled to the nucleation step (Equation 50a).[88,89] Porschke and Rauh[89] used both Monte Carlo simulations, following the

work of Epstein[24] and also the method described by Lohman and Kowalczykowski[88] to analyze the growth step in order to obtain the rate constant k_2, for the gene 5 protein-poly(A) association (see Equation 50b); this rate constant is termed k_a^c by Porschke and Rauh.[89] The Monte Carlo simulations neglected gene 5 protein dimerization which does occur under the experimental conditions used. Upon comparison of the two methods, Porschke and Rauh[89] conclude that the pre-equilibrium nucleation analysis[88] overestimates the growth rate constant, although the model is a good approximation at short times.[88,89] (For T4 gene 32 protein, $k_2 \gg k_1$, such that this overestimate should not affect the conclusion that gene 32 protein monomers undergo limited translocation.[88]) Although the analysis of the growth step does not indicate that a facilitated mechanism (such as sliding) is used by gene 5 protein, other experiments do suggest that such a mechanism may be operative.[89] This is based on the absence of a very slow phase in the gene 5 protein-poly(dT) association in the presence of excess gene 5 protein (only a single exponential decay is observed.[89] If some fast facilitated mechanism for redistribution of gene 5 protein did not occur, then the redistribution of protein, which is necessary to fully saturate the DNA,[23,24] would be limited by the dissociation of singly contiguous gene 5 protein and a biphasic association reaction would be observed.[23,24,89]

The association rate constant to the polynucleotide chain, poly(dT), is greater for gene 5 protein [8×10^9 M^{-1} (polynucleotide) sec^{-1}] than for T4 gene 32 protein [1.5×10^9 M^{-1} (polynucleotide) sec^{-1}] in 0.10 *M* NaCl.[88,89] Correcting for the difference in protein diffusion coefficients (a factor of 1.8), then $k_a(g5p) = 3\ k_a(g32p)$. This may reflect additional orientational constraints on gene 32 protein binding (e.g., gene 32 protein has been shown to bind with polarity along the sugar phosophate backbone,[152] which introduces a steric factor of at least $x = 1/2$ in Equation 7.[88] If dimers of gene 5 protein (with two DNA binding sites) are involved in the association reaction, then this may also contribute to its larger value of k_a. The association rate constant for *E. coli* SSB to poly (dT)[104,105] is comparable to that of gene 5 protein.[151]

The relaxation kinetics of gene 5 protein-oligo $d(pT)_4$ complexes have also been studied by temperature jump experiments at constant salt conditions (but the exact conditions are not stated).[89] A two-step process, such as Equation 45, has been used to describe the data, with $k_{01} = 5 \times 10^8$ M^{-1} s^{-1}, $k_{10} = 5 \times 10^4$ sec^{-1}, $k_{12} = 5 \times 10^3$ sec^{-1}, and $k_{21} = 8 \times 10^3$ sec^{-1}.

3. E. coli SSB-Single Stranded Nucleic Acid Kinetics

A few studies of the nucleic acid binding kinetics of the *E. coli* single strand binding protein (SSB) have been reported. Krauss et al.[147] have performed temperature jump and stopped-flow experiments to study the kinetics of SSB-single stranded oligonucleotide complex formation. In 0.20 *M* KCl, pH 7.4 (no salt dependence was performed), rate constants for SSB association to $d(pT)_8$, $d(pT)_{16}$, and $d(pT)_{30-40}$ are 7×10^7, 5×10^7, and 3×10^8 M^{-1} (oligo) sec^{-1}, respectively; the dissociation rate constants are 1.7×10^3 s^{-1}, 40 s^{-1}, and <1 s^{-1}, respectively. The association rate constants are considerably smaller than those measured for oligopeptide-oligonucleotide interactions. These kinetics studies and equilibrium binding experiments[147] indicate that an SSB tetramer has four binding sites for $d(pT)_8$, only two binding sites for $d(pT)_{16}$, and only one binding site for $d(pT)_{30-40}$ From these and other experiments, Krauss et al.[147] conclude that single-stranded DNA of sufficient length can wind around the SSB tetramer. A similar model, but with DNA wrapping around an octamer of SSB, has been reached independently by Chrysogelos and Griffith[149] using electron microscopy, nuclease digestion, and density banding studies.

The association rate constants for SSB-polynucleotide binding have been only briefly studied. Romer et al.[104] have measured the noncooperative, nonspecific association of SSB to a poly(dT) domain at two [NaCl]: $k_a = 2.4 \times 10^9$ M^{-1}(polynucleotide) sec^{-1} in

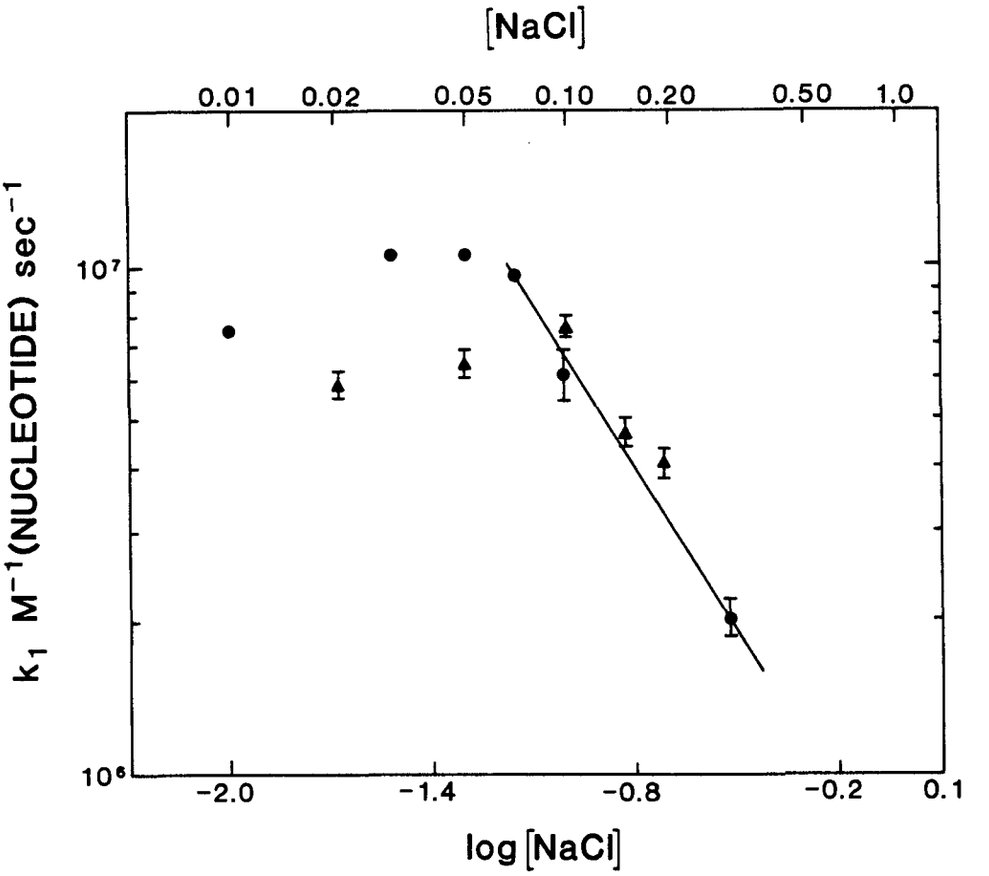

FIGURE 5. The bimolecular association rate constant, k_1[M^{-1}(nucleotide)sec^{-1}] vs. [NaCl] (log-log plot) for the *E. coli* SSB-poly(U) (▲) and poly(rεA) (•) association (Lohman, unpublished). This shows the [NaCl] dependence which is predicted for a diffusion-limited, screening-controlled association.

0.2 *M* NaCl and 1.1×10^9 in 0.95 *M* NaCl. Based on these two points, $S(k_a) = -0.5$. Since $S(K_{obs})$ has been estimated as −4.5 by Krauss et al.[147] for $d(pA)_{40}$ binding and $S(K_{obs}) = -7.9$ for the SSB-poly(U) equilibrium,[170] it is clear that $S(k_a) \ll S(K_{obs})$, suggesting that these are screening-controlled, diffusion-limited associations. The association kinetics of SSB to poly(U) and poly(rεA) have also been measured over a range of [NaCl] (pH 8.0, 25.0°C) using stopped-flow techniques, monitoring the quenching of the SSB intrinsic tryptophan fluorescence.[108] Under conditions of excess polynucleotide, a single exponential decay is observed and the reciprocal relaxation time is directly proportional to the polynucleotide concentration.[105] The association of SSB to both poly(U) and poly(rεA) seems to behave as a screening-controlled reaction. Figure 5 shows the bimolecular association rate constant, k_1 (M^{-1}(nucleotide) sec^{-1}) as a function of [NaCl] (log-log plot). In the range 10 m*M* ≤ [NaCl] ≤ 75 m*M*, for poly(rεA) and 20 m*M* ≤ [NaCl] ≤100 m*M* for poly(U), k_1 is essentially independent of [NaCl] with maximum values of 1.0×10^7 and 7.5×10^6 M^{-1}(nucleotide) sec^{-1}, respectively. Above 0.10 *M* NaCl, both sets of rate constants decrease with increasing [NaCl], but only slightly, with $S(k_a) = -1.1$. This is the expected behavior for a screening-controlled association reaction. On a polynucleotide scale, the maximum rate constants are $k_a = 4.5 \times 10^9$ M^{-1}(polynucleotide) sec^{-1} for poly(rεA) ($S_{20,w} = 6.5S$; ≈450 nucleotides) and 4.7×10^9 for poly(U) ($S_{20,w} = 8.5$; ≈620 nucleotides). These are only slightly larger than

the values obtained by Romer et al.[104] for the SSB-poly(dT) association. After correcting for the difference in protein diffusion coefficients, these values are essentially identical to those found for gene 5 protein,[89] but are larger than those measured for gene 32 protein.[88]

Romer et al.[104] have also investigated the interaction of SSB with oligo- and polynucleotides using 270 MH_z ^{1}H-NMR spectroscopy. SSB binding to $d(pT)_8$, poly(dT), and poly(dA) was monitored by observing the line broadening of the methyl protons of thymine and H2 and H8 on adenine as a function of binding density. Romer et al.[104] find that the methyl protons undergo rapid exchange (>100 s^{-1}) between the bound and free states, even at low binding densities. The macroscopic dissociation rate for SSB-poly(dT) complexes under these conditions is very slow ($\ll 1 s^{-1}$),[138] hence the fast exchange observed in the NMR experiment cannot be due to macroscopic dissociation-reassociation events.[104] To explain these observations, Romer et al.[104] suggest that the fast exchange rate reflects the ability of SSB to move one dimensionally along the poly(dT). They propose a rolling mechanism (rather than sliding), whereby the poly(dT) segments which enter and exit the SSB tetramer at nearly the same point can exchange quickly and compete for the same DNA binding site on one of the four SSB protomers; this can result in rolling of the tetramer along the DNA.

Schnieder and Wetmur[159] have studied the kinetics of transfer of SSB protein from 375 nucleotide single-stranded DNA molecules as a function of the recipient DNA length and NaCl concentration by a nitrocellulose filter binding technique. The transfer kinetics are oberved to be first order and the same rate is observed for the loss of nonfilterable donor DNA-SSB complexes as for the appearance of nonfilterable recipient DNA-SSB complexes. (Heat denatured DNA was used both as donor and recipient.) This has been interpreted as indicating an all or none transfer of SSB.[159] The transfer rate seems to be diffusion-limited due to the inverse dependence of the rate on solution viscosity. The transfer rate is independent of [NaCl] in the range 30 to 150 m*M*, but increases by a factor of 2 from 150 to 300 m*M*. This increase in rate occurs in the same [NaCl] region where a change in SSB binding mode (increase in site size)[148] and decrease in cooperativity[145] are also observed. It is not known whether these observations have a common molecular explanation. Schneider and Wetmur[159] propose that SSB exchange between single-stranded DNA molecules occurs by the direct transfer of clusters of cooperatively bound SSB, with no free SSB intermediate. If this is the case, then SSB may have the ability to participate in a number of different types of facilitated transfer mechanisms[151] (see above).

VII. SUMMARY AND CONCLUSIONS

Although it is largely appreciated that protein-nucleic acid kinetics (and equilibria) are influenced by the salt concentration, as a result changes in salt concentration and type have generally been avoided rather than systematically varied as a means of investigating the mechanism. This point is underscored by the limited number of experimental kinetics studies that are reviewed here in which the salt concentration is used as a probe of the kinetics. In addition to reviewing the available experimental and theoretical studies, I have also attempted to point out the various ways in which salt dependent studies of protein-nucleic acid kinetics can be used to decipher a mechanism. I reemphasize that these types of studies should not replace the standard methods, but do provide a powerful supplement since protein-nucleic acid complexes are so sensitive to the ion concentration and composition of the media.

It is also emphasized that the ionic strength is *not* the controlling variable with respect to salt effects on protein-nucleic acid kinetics (and equilibria) as it is in the case of low molecular weight ionic interactions. The exception to this is the case of diffu-

sion-limited association rate constants, whose salt dependence is determined by the ionic strength. In general, the effects of multivalent cations on the protein-nucleic acid rate constants are much larger than expected on the basis of their contribution to the ionic strength of the solution, due to their direct competitive binding to the nucleic acid. As a result, experiments performed in the presence of multivalent cations must be analyzed carefully, accounting for both the competition between cation and protein for DNA binding, as well as the NaCl concentration dependence of multivalent cation binding to the DNA.

It is clear from this review that there is a considerable need for studies of salt effects on the kinetics of model systems, such as positively charged oligopeptide binding. Our understanding of the effects of salt on protein-nucleic acid kinetics is dependent on our ability to understand these model systems.

Finally, although the dramatic effects of changes in salt concentration on both the kinetic and equilibrium binding properties of protein-nucleic acid complexes have been discussed as a means of probing the interaction, one must also consider the possibility that salt effects are likely to play a regulatory role in gene expression and nucleic acid metabolism. This possibility has most recently been discussed by Record and colleagues[77,133] on the basis of their studies of *E. coli* RNA polymerase holoenzyme binding to a number of different bacteriophage λ promoters. They find that the salt dependence of both the kinetic and equilibrium binding properties to these different promoters varies considerably such that small changes in salt concentration can drastically vary the ratio of specific binding constants and rate constants, affecting their occupancy and rate of occupancy by RNA polymerase. The implications for control are obvious. Fluctuations in ion concentration are known to occur during the cell cycle[165-167] and may be effectively used to control processes involving protein-nucleic acid interactions. On the other hand, if these changes in ion concentration do not affect the protein-nucleic acid interactions in the cell, then there must be an extremely effective mechanism to uncouple these effects, which seems unlikely.

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