Linkage of pH, Anion and Cation Effects in Protein-Nucleic Acid Equilibria

Escherichia coli SSB Protein-Single Stranded Nucleic Acid Interactions

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We have examined the linkage between pH and monovalent salt concentration (NaCl and NaF) on the equilibrium binding of the Escherichia coli SSB protein to single stranded poly(U) in its (SSB)₄ₛₜ binding mode. In this mode, single-stranded nucleic acid interacts with all four subunits of the SSB tetramer covering ~65 nucleotides and nearest-neighbor cooperative interactions can form between DNA bound SSB tetramers, although protein clusters are limited to dimers of tetramers (octamers). The intrinsic association equilibrium constant, Kₐbs, and the "limited" cooperativity parameter, ω₄ₛₜ, have been determined from titrations that monitor the quenching of the SSB tryptophan fluorescence upon binding poly(U). The cooperativity parameter, ω₄ₛₜ, is independent of salt concentration and type and increases only slightly with increasing pH. However, Kₐbs decreases with increasing salt concentration due to a net release of ions accompanying complex formation. This net ion release has contributions from cation release from the nucleic acid as well as differential binding of both cations and anions to the protein. The dependence of Kₐbs on [NaF] is independent of pH with dlogKₐbs/dlog[NaF] = −4.5(±0.5). However, there is a strong linkage between the effects of [NaCl] and pH, such that (dlogKₐbs/dlog[NaCl]) ranges from −12.0(±0.8) at pH 5.5, to −6.0(±0.5) at pH 9.0 (at 25°C). Thus Cl⁻ release increases with decreasing pH due to a linkage between chloride binding and protonation of the protein, whereas there is essentially no release of F⁻. The linkages of ion concentration and pH on Kₐbs can be described in terms of: (1) cation release from the polynucleotide; (2) release of Cl⁻ from sites on the SSB tetramer that require protonation to bind Cl⁻; (3) binding of cations to sites on the SSB tetramer which require deprotonation for cation binding, and (4) required binding of two-to-three protons by the SSB tetramer in order to form the SSB-poly(U) complex. Thus, the influence of salt concentration on protein-nucleic acid equilibria can be quite complex with contributions from differential ion binding to both the protein and the nucleic acid; however, these can be resolved by examining the linked effects of pH and salt concentration on these interactions.

Keywords: SSB; salt effects; protein-DNA equilibria

1. Introduction

The Escherichia coli single strand binding (SSB§) protein (Sigal et al., 1972; Weiner et al., 1975) is a helix destabilizing protein that is essential for chromosomal replication and repair (Chase & Williams, 1986; Meyer & Laine, 1990; Iahue et al., 1989) and facilitates homologous recombination (Cox & Lehman, 1987). The SSB protein is a stable tetramer in the absence of single-stranded (ss) DNA and the tetramer appears to be the functional form (Chase et al., 1983; Williams et al., 1984; Bujałowski & Lohman, 1991a,b). SSB tetramers bind to ss-DNA and RNA in a number of distinct binding modes that differ in the number of nucleotides occluded per
SSB tetramer (site size, n) as well as the type and magnitude of nearest-neighbor cooperativity (for reviews, see Lohman et al., 1988; Lohman & Bujalowski, 1990).

Upon binding poly(dT) at 25°C, pH 8.1, at least three distinct binding modes can be detected with site sizes of 35(±3), 50(±3), and 65(±3) nucleotides per tetramer (referred to as (SSB)_{3.5}, (SSB)_{5.0}, and (SSB)_{6.5} modes, respectively: Lohman & Overman, 1985; Bujalowski & Lohman, 1986; Bujalowski et al., 1988; Kuil et al., 1990; Wei et al., 1992). In the (SSB)_{3.5} mode, the ss nucleic acid interacts with only two subunits of the SSB tetramer, whereas in both the (SSB)_{5.0} and (SSB)_{6.5} modes, the ss nucleic acid interacts with all four SSB subunits (Lohman & Bujalowski, 1988, Bujalowski & Lohman, 1989a,b). These different binding modes can be selectively populated in vitro by varying the salt concentration and type as well as the extent of DNA saturation with SSB. On poly(dT), the (SSB)_{3.5} mode dominates at [NaCl] ≤ 10 mM and high SSB binding densities, whereas the (SSB)_{6.5} mode dominates at [NaCl] ≥ 200 mM or low SSB binding densities (Lohman & Overman, 1985; Bujalowski & Lohman, 1986). The relative stability of these SSB binding modes are also influenced by pH, temperature (Bujalowski et al., 1988) and polyamines (Wei et al., 1992). Due to the very different DNA binding properties of these SSB binding modes and the fact that the (SSB)_{5.0}/(SSB)_{6.5} modes appear to stimulate RecA activity (Griffith et al., 1984; Muniyappa et al., 1990; Morrical & Cox, 1990), it has been suggested that the (SSB)_{3.5} mode may be used selectively in replication, whereas the (SSB)_{5.0}/(SSB)_{6.5} modes may be used in recombination (Lohman et al., 1988; Lohman & Bujalowski, 1990).

Cooperative binding to ss-DNA in the (SSB)_{6.5} mode differs considerably from binding in the (SSB)_{3.5} mode. Under conditions that favor the (SSB)_{3.5} mode, SSB tetramers bind with high "unitary" cooperativity (Lohman et al., 1986a,b). Similar to that observed for the phage T4 gene 32 protein (Alberts & Frey, 1970; Kowalczykowski et al., 1981; Newport et al., 1981; Lohman, 1984a,b), such that SSB tetramers can form continuous clusters and ultimately saturate the ss-DNA (Rayechal & Wetmur, 1976; Griffith et al., 1984). Recent measurements have indicated that the nearest neighbor cooperativity between adjacent SSB tetramers bound in the (SSB)_{3.5} mode, ω_{3.5}, is at least ~10^3 (Ferrari et al., 1994). However, in the (SSB)_{6.5} mode, only moderate cooperativity is observed (Lohman et al., 1986a,b), in which clustering is limited to the formation of dimers of tetramers (Chrysogoles & Griffith, 1982; Bujalowski & Lohman, 1987a). Therefore, in the (SSB)_{6.5} mode, saturation of the ss-DNA is more difficult due to the inability to form long clusters of tetramers (Bujalowski & Lohman, 1987a). The "limited" cooperativity parameter, ω_{6.5}, which describes the equilibrium between the polynucleotide-bound tetramers and octamers has a value of 420(±80) for the SSB protein-poly(U) interaction at pH 8.1, 25°C in 0.2M NaCl (Bujalowski & Lohman, 1987b; Overman et al., 1988).

In an effort to characterize the properties of these different SSB binding modes, Overman et al. (1988) examined the equilibrium binding of SSB tetramers to form the (SSB)_{6.5} binding mode with poly(A), poly(U), poly(dA) and poly(dT) as a function of monovalent salt concentration and type. At 25°C, pH 8.1, K_{obs} = 410(±120), independent of salt concentration and type for poly(dA), poly(U) and poly(A); however, the equilibrium binding constant, K_{obs}, decreases steeply with increasing salt concentration. A dramatic effect of anion type on both the salt dependence and magnitude of K_{obs} was observed (Overman et al., 1988). These results indicate that electrostatic interactions play a significant role in determining the stability of (SSB)_{6.5} complexes and that a net release of both cations and anions accompanies complex formation, although there are also contributions due to both cation and anion uptake.

To further probe the basis for these anion effects on the stability of the (SSB)_{6.5} complex, we have examined the thermodynamic linkage between effects of pH and anion concentration and type on SSB-poly(U) equilibrium binding. Our results reveal a linkage between pH and the dependence of K_{obs} on [NaCl], but not [NaF]. This is consistent with a model in which a class of anion binding sites on the SSB tetramer requires protonation in order to bind Cl^-, but that F^- binds only weakly to these sites. These studies demonstrate the complex linkages that can exist between pH and salt effects on protein-nucleic acid equilibria, and which can contribute to and modulate protein-nucleic acid stability and specificity.

2. Materials and Methods

(a) Reagents and buffers

All chemicals were reagent grade and all solutions were made with distilled and deionized (Milli-Q) water. Buffer T is 10 mM tris(hydroxymethyl)aminomethane (Tris) (pH 8.1), 0.1 M trisodium ethylenediaminetetraacetate (Na₃EDTA); Buffer H (pH 7.0, pH 7.5, or pH 8.1) is 10 mM Heps, 0.1 M Na₃EDTA, Buffer M (pH 5.5 or pH 6.5) is 10 mM Mes, 0.1 M Na₃EDTA. Buffer C (pH 8.6 or pH 9.0) is 10 mM Ches, 0.1 M Na₃EDTA. The pH of the buffers did not vary by more than ±0.1 over the range of salt concentrations examined.

(b) E. coli SSB protein and nucleic acids

SSB protein was purified as described (Lohman et al., 1986a) and prepared for use in fluorescence titrations as described (Overman et al., 1988). SSB concentration was determined spectrophotometrically in buffer T + 0.2 M NaCl (ε₂₅₀ = 1.33 x 10⁴ M⁻¹ cm⁻¹ (tetramer) cm⁻¹) (Lohman & Overman, 1985). Sedimentation coefficients (s₂₀,w) were determined as described (Overman et al., 1988). Stocks of poly(U) had s₂₀,w = 81 S and 9.5 S, corresponding to
average lengths of 900±100) and 1100±200) nucleotides, respectively. A fractionated sample of poly(U) having $\lambda_{260} = 14.8$ gave identical results in binding assays (Lohman et al., 1986b). The poly(U) was extensively dialyzed versus Buffer T+0.1 M NaCl and concentrations were determined spectrophotometrically using $\varepsilon_{260} = 9.2 \times 10^4$ M$^{-1}$ (nucleotide) cm$^{-1}$.

(c) Equilibrium binding isotherms

Equilibrium titrations ("reverse" titrations) of SSB protein with poly(U), monitoring the quenching of the SSB tryptophan fluorescence ($\lambda_{ex} = 282$ or 290 nm; $\lambda_{em} = 347$ nm) were performed in an SLM 8000 spectrofluorometer as described (Overman et al., 1988) with temperature controlled at 25.0(±0.1)°C. All measurements were corrected for dilution, photobleaching and inner filter effects (Lohman & Overman, 1985; Bujalski & Lohman, 1987a).

Equilibrium binding isotherms were constructed as previously described (Overman et al., 1988) using a model-independent binding density function analysis (Bujalski & Lohman, 1987a; Lohman & Bujalski, 1991; Lohman & Mascotti, 1992). This method makes no assumptions concerning the relationship between the extent of fluorescence quenching, $Q_{obs}$ and the fraction of bound ligand, but allows one to rigorously determine this relationship. Using this method, we have shown that binding to ss nucleic acids in the (SSB)$_{65}$ binding mode, $Q_{obs}$ is directly proportional to the fraction of bound SSB tetramers (i.e. $L_{\infty}/L_T = Q_{obs}/Q_{max}$) in buffers containing NaCl, NaCH$_3$CO$_2$ and NaF salts (25°C, pH 8.1), but not in NaBr or KCl salts (Overman et al., 1988). Under conditions such that $L_{\infty}/L_T$ is directly proportional to $Q_{obs}$, one can determine $Q_{max}$, the maximum extent of fluorescence quenching when SSB is fully saturated with nucleic acid, from a linear extrapolation of a plot of $Q_{obs}$ versus $L_{\infty}/L_T$ (Bujalski & Lohman, 1987a; Bujalski & Lohman, 1989). Under such conditions, one can simply calculate the concentration of bound and free SSB, $L_B$ and $L_F$, respectively, and the binding density, $v$, using eqns (1) to (3):

$$L_B = L_T(Q_{obs}/Q_{max}).$$

$$v = (Q_{obs}/Q_{max})L_T/D_T.$$

In NaBr and KCl salts, where $L_{\infty}/L_T \neq Q_{obs}/Q_{max}$ (Overman et al., 1988), one can still rigorously determine the average degree of binding and hence obtain the binding isotherm using the binding density function method (Bujalski & Lohman, 1987a; Lohman & Bujalski, 1991; Lohman & Mascotti, 1992).

(d) Determination of equilibrium binding parameters

A "limited" cooperativity (tetramer/octamer) model (Bujalski & Lohman, 1987a) was used to analyze the equilibrium binding of SSB tetramers to poly(U). This model accounts for the observation that SSB protein, when bound in its (SSB)$_{65}$ mode, forms protein clusters that are limited in length to dimers of tetramers (octamers). The tetramer/octamer model assumes: (1) that SSB tetramers bind to the ss nucleic acid lattice with intrinsic equilibrium constant $K_{obs}$; (2) nucleic acid bound SSB tetramers interact with other tetramers through nearest-neighbor interactions to form octamers (dimers of tetramers), but higher order clusters are not formed, i.e. bound SSB tetramers do not interact with both nearest neighbor tetramers simultaneously. The model accounts for the overlap of potential protein binding sites on the nucleic acid and is defined by 3 parameters: the number of nucleotides occluded by the bound tetramer (site size, $n$), the cooperativity parameter, $\omega_{tet}$, and the equilibrium constant for isolated binding of SSB tetramers, $K_{obs}$. The cooperativity parameter, $\omega_{tet}$, represents the equilibrium constant for formation of a nucleic acid bound SSB octamer from 2 isolated, nucleic acid bound SSB tetramers. This model provides a better description of SSB tetramer-as polynucleotide binding in the (SSB)$_{65}$ binding mode over the entire range of binding densities (Bujalski & Lohman, 1987b) than a model which assumes the formation of SSB clusters of unlimited length (e.g. see McGhee & von Hippel, 1974, 1976; Schellman, 1974). The closed-form expression for the limited cooperativity "tetramer-octamer" binding isotherm is given in eqn (4) in Scatchard form, where $q = [1-(n-1)v] + [(1-(n-1)v)^2 - v(1-\omega)(2-2(n-1)v)]^{1/2}$.

$$v/L_T = (K_{obs}q^2 - 2\eta q + (1-\omega)q^2)^{1/2}/(q^{2-1}).$$

(4)

Non-linear least-squares analysis of the equilibrium isotherms according to eqn (4), with the site size constrained to $n = 65$ nucleotides per SSB tetramer, was used to determine the best-fit values of $K_{obs}$ and $\omega_{tet}$ (Overman et al., 1988).

For experiments performed in NaCl, KCl, NaF or NaCH$_3$CO$_2$ salts, where $Q_{max}$, $n$ and $\omega_{tet}$ and the free SSB protein fluorescence are known and are independent of salt concentration, and $L_{\infty}/L_T = Q_{obs}/Q_{max}$, the dependence of $K_{obs}$ on salt concentration was obtained with high precision from analysis of a "salt-back" titration as described (Overman et al., 1988). We emphasize that this procedure can not be used with confidence unless $Q_{max}$, $n$ and $\omega_{tet}$ are independent of salt concentration and $L_{\infty}/L_T = Q_{obs}/Q_{max}$. For example, this procedure can not be used in NaBr and KCl salts since the fluorescence of free SSB protein changes with the concentration of these salts (Overman et al., 1988).

(e) Anion competition experiments

We have performed a number of experiments in buffers containing a mixture of different anion types at constant concentration and concentration to characterize the binding of anions to SSB. The observed equilibrium constant, $K_{obs}$, for the binding of a protein, P, to a nucleic acid site, D, to form a complex, PD, can be written as in eqn (5):

$$K_{obs} = K^*\Sigma_{\text{rep}}/\Sigma_{\text{dep}},$$

where $K^*$ is the apparent protein-nucleic acid equilibrium constant in the limit of low salt concentration (i.e. no anion binding), and the $\Sigma_i$ are the binding polynomials for the 3 macromolecule species, P, D and PD (Wyman, 1964; Schellman, 1975). Even though it is likely that anions also bind to the complex, PD, we are only interested in describing the anions that are released upon formation of PD, since it is only these anions that will affect $K_{obs}$ upon changing the salt concentration. Hence, we specify $\Sigma_{\text{rep}} = \Sigma_{\text{dep}} = 1$, which assumes anions bind only to the free protein, P.

If two different anions, X and Y, bind competitively to a single class of "n" identical and independent anion
Table 1  
Effect of pH on SSB tetramer-poly(U) equilibrium binding in the (SSB)$_{65}$ binding mode in NaCl (25°C)

<table>
<thead>
<tr>
<th>pH</th>
<th>$Q_{max}$</th>
<th>$\log K_{obs}$</th>
<th>$K_{obs}$ (M$^{-1}$)</th>
<th>$\omega_{20}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(100% NaCl)</td>
<td>(0.35 M NaCl)</td>
<td>(1 M NaCl)</td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td>0.64(±0.01)</td>
<td>-12.9(±0.8)</td>
<td>03(±0.3)</td>
<td>27(±0.6) x 10$^3$</td>
</tr>
<tr>
<td>6.5</td>
<td>0.57(±0.01)</td>
<td>-8.4(±0.2)</td>
<td>1(±0.1)</td>
<td>6(±1) x 10$^3$</td>
</tr>
<tr>
<td>7.0</td>
<td>0.62(±0.01)</td>
<td>-7.8(±0.2)</td>
<td>0.1(±0.1)</td>
<td>4.8(±1) x 10$^3$</td>
</tr>
<tr>
<td>7.5</td>
<td>0.61(±0.01)</td>
<td>-8.0(±0.2)</td>
<td>0.2(±0.1)</td>
<td>2.5(±0.1) x 10$^3$</td>
</tr>
<tr>
<td>8.0</td>
<td>0.58(±0.01)</td>
<td>-7.4(±0.8)</td>
<td>0.2(±0.2)</td>
<td>9.3(±0.6) x 10$^2$</td>
</tr>
<tr>
<td>8.5</td>
<td>0.56(±0.01)</td>
<td>-6.7(±0.2)</td>
<td>0.6(±0.1)</td>
<td>2(±0.1) x 10$^3$</td>
</tr>
<tr>
<td>9.0</td>
<td>0.54(±0.01)</td>
<td>-6.0(±0.3)</td>
<td>0.8(±0.2)</td>
<td>8(±0.6) x 10$^2$</td>
</tr>
</tbody>
</table>

† Estimated from a linear extrapolation of a plot of log $K_{obs}$ versus log [NaCl].
‡ $Q_{max}$ is dependent on the NaCl concentration at this pH.

3. Results

(a) Stability of the SSB tetramer

The E. coli SSB protein remains tetrameric over the range of protein concentrations and salt concentrations and types examined in this study (Overman et al., 1988). Sedimentation velocity studies yield a constant $s_{20w} = 4.4(±0.3) S$, reflecting an SSB tetramer, over the pH range used in these studies (pH 5.5 to 9.0) (data not shown). Studies of guanidine hydrochloride-induced denaturation of the SSB tetramer (0.2 M NaCl, pH 8.1, 37°C) (M. Ferrari, unpublished results) also indicate that 98% of the SSB protein remains tetrameric under these conditions at 0.02 M (tetramer). This is tenfold lower than the average SSB concentration used in our studies and fivefold lower than the lowest SSB concentration used. Furthermore, the equilibrium constant for assembly of the mutant SSB-1 tetramer is independent of salt concentration between 50 mM and 1 M NaCl (Bujalowski & Lohman, 1991a,b). Therefore, the effects of pH and salt concentration and type reported here reflect changes in the SSB tetramer-polynucleotide complex stability and are not due to changes in the assembly state of the SSB tetramer.

(b) Conditions favoring formation of the (SSB)$_{65}$ complex with poly(U)

The number of nucleotides occluded by the SSB tetramer (site size, n) when bound to ss nucleic acids is dependent upon ion concentration and type and pH (Lohman & Overman, 1985; Bujalowski & Lohman, 1986; Bujalowski et al., 1988; Wei et al., 1992) as well as polynucleotide type (W. Bujalowski & T.M.L., unpublished results). The apparent site size for the SSB tetramer-poly(U) interaction was studied over the range of pH, salt conditions and types examined here (data not shown) and a salt dependent change in the site size was observed under all conditions, although the relative midpoints for these transitions changed as a function of pH as observed for the SSB-poly(dT) interaction (Bujalowski et al., 1988). However, for the studies reported here, the SSB-poly(U) complexes form the (SSB)$_{65}$ binding mode exclusively, hence the effects of salt concentration and pH discussed here reflect changes in the energetics of formation of the (SSB)$_{65}$ binding mode from free poly(U) and free SSB tetramers and do not reflect changes in the mode of SSB binding to poly(U).

We have used a model-independent method (Bujalowski & Lohman, 1987a) to analyze SSB-poly(U) titrations in which the SSB tryptophan fluorescence quenching, $Q_{obs}$, is monitored upon complex formation and have determined that $L_{eq}/L_T = Q_{obs}/Q_{max}$ in buffers containing NaCl, KCl, NaH$_2$CO$_3$, and NaF at all pH values (5.5 to 9.0), but not in NaBr or KCl (pH 8.1, 25°C) (Overman et al., 1988). $Q_{max}$ is independent of salt concentration above 0.2 M (except for NaBr and KCl); however, $Q_{max}$ is dependent upon ion type (Overman et al., 1988) and is also dependent upon pH, increasing from 50% at pH 9.0 to 72%, at pH 5.5 (see Table 1).

(c) Anion competition studies

Formation of the (SSB)$_{65}$ complex with poly(U) is accompanied by a net release of both cations and anions (Overman et al., 1988). To further examine the characteristics of these anion effects, we
measured $K_{obs}$ in Buffer T (pH 8.1, 25°C) containing a mixture of NaCl and a second Na$^+$ salt of a different anion (acetate, thioric, bromide or glutamate) such that the total Na$^+$ concentration remained constant while the mole fraction of chloride ($x_{Cl}$) varied from 0 to 1. The dependence of $K_{obs}$ on the mole fraction of a particular anion is sensitive to the competitive effects of chloride versus the second anion on the SSB-poly(U) equilibrium. To analyze the isotherms in the presence of a mixture of anions we used the weighted average of the $Q_{max}$ values determined in buffers containing only a single anion (see Table 2). Non-linear least-squares analyses of these isotherms yielded $\omega_{T=0} = 413 (\pm 120)$, independent of anion type (pH 8.1, 25°C). Therefore, we constrained $\omega_{T=0} = 413$ and $n = 65$ and obtained $K_{obs}$ from non-linear least-squares analysis of the isotherms.

Figure 1 shows plots of log $K_{obs}$ versus $x_{Cl}$ for four sets of experiments performed in mixtures of

<table>
<thead>
<tr>
<th>pH</th>
<th>$Q_{max}$</th>
<th>$\left( \frac{\Delta \log K_{obs}}{\Delta \log [NaF]} \right)$</th>
<th>log $K_{obs}$</th>
<th>$K_{obs}(M^{-1})$</th>
<th>$\omega_{T=0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>0.63 (±0.01)</td>
<td>-4.0 (±1.6)</td>
<td>3.9 (±0.1)</td>
<td>N.D.</td>
<td>160</td>
</tr>
<tr>
<td>8.1</td>
<td>0.66 (±0.01)</td>
<td>-4.3 (±2.4)</td>
<td>3.9 (±0.3)</td>
<td>1.8 (±0.6) x 10^5</td>
<td>410</td>
</tr>
<tr>
<td>9.0</td>
<td>0.54 (±0.01)</td>
<td>-4.4 (±0.7)</td>
<td>3.2 (±0.7) x 10^5</td>
<td>560</td>
<td></td>
</tr>
</tbody>
</table>

† Extrapolated.
‡ The observed binding constant at 0.35 M NaF.
§ Constrained to the value determined in NaCl at pH 5.5.
|| $Q_{max}$ is dependent on the NaF concentration under these conditions.
N.D., not determined.

Figure 1. Competitive effects of anions at constant Na$^+$ concentration on the SSB-poly(U) equilibrium binding in the (SSB)$_{65}$ mode in buffer T, 25°C. (a) Chloride versus chloride competition (0.35 M NaX). $K_{obs}$ was determined as described in Materials and Methods using $\omega_{T=0} = 413$. log $K_{obs}$ is plotted as a function of the mole fraction of Cl$^-$, $x_{Cl}$. The theoretical curves were simulated using eqn (8) with $h_0 = 0.018 M^{-1}$ and the following values: (continuous line) $B = 5.2$; $a = 3$, $k_{Cl} = 112 M^{-1}$; (dashed line) $a = 4$, $k_{Cl} = 69 M^{-1}$; (dotted line) $a = 5$, $k_{Cl} = 4.0 M^{-1}$. (b) Acetate versus chloride competition (0.30 M NaX). The theoretical curves were simulated using eqn (8) (continuous line) using the following values: $B = 6.48$, $k_{Cl} = 99 M^{-1}$, $k_{Ac} = 24 M^{-1}$; $a = 5$. (c) Bromide versus chloride competition (0.175 M NaX). The theoretical curves were simulated using eqn (8) and the following values: $B = 6.5$, $k_{Cl} = 5.4 M^{-1}$, $k_{Br} = 12.4 M^{-1}$; $a = 5$. (d) Glutamate versus chloride competition (0.40 M NaX). The linear representation of the data is for visual aid and has no theoretical basis.
chloride and a second anion (bromide, acetate, fluoride or glutamate). The simplest model which can explain these mixed-anion studies is that chloride, fluoride, acetate and bromide all compete for the same anion binding sites on the SSB tetramer. We have analyzed these experiments using equation (8), which assumes a independent and identical anion binding sites and assigns each anion a separate binding constant, $k_i$. A minimum of five anion binding sites is required to fit the data; however, values of $k_i$ greater than five can not be excluded. The values of $k_i$ used to fit these data are given in the legend to Figure 1. A model which assumes cooperative binding of anions to identical sites on the protein does not fit the data as well (data not shown).

(d) NaCl concentration dependence of $K_{obs}$ is strongly linked to $pH$

We examined the linked effects of pH and salt concentration and type on the equilibrium binding of the SSB tetramer in the (SSB)$_{4s}$ mode over the range from pH 5.5 to 9.0. Poly(U) was used in these studies since it remains single-stranded over this range of conditions. Furthermore, poly(U) does not undergo protonation/deprotonation reactions below pH 8.5, although there is a slight deprotonation of the N-3 nitrogen atom near pH 9 (pK ~ 9.4) (Simpkins & Richards, 1967). This has been verified through studies of the equilibrium binding to poly(U) of a series of oligo-peptides containing lysine and tryptophan (Mascotti & Lohman, 1992). This simplifies the interpretation of pH effects on SSB binding to poly(U) since effects on $K_{obs}$ result from protonation/deprotonation of the SSB protein rather than the nucleic acid.

The cooperativity parameter, $\omega_{pC}$, is independent of monovalent salt concentration and type at pH 8.1, 25°C (Overman et al., 1988). However, $\omega_{pC}$ does increase slightly from $100(\pm 100)$ to $600(\pm 190)$ as the pH is increased from 5.5 to 9.0 (see Table 1), although this change is relatively small in comparison to the uncertainty in determining $\omega_{pC}$. Experiments performed over a range of protein concentrations over the salt ranges investigated showed no effect of salt concentration on $\omega_{pC}$ within our experimental uncertainty.

The major effect of pH is on $K_{obs}$. Figure 2 shows plots of log$K_{obs}$ versus log[NaCl] at pH values from 5.5 to 9.0. The agreement between the values of $K_{obs}$ determined from reverse titrations versus salt-back titrations indicates that the SSB-poly(U) interaction is at equilibrium under these conditions. The linear least-squares lines shown in Figure 2 were based only on the salt-back titrations since these data have lower uncertainties. $K_{obs}$ decreases significantly with increasing [NaCl] at each pH and decreases with increasing pH at each [NaCl]. For example, at 0.5 M NaCl: $K_{obs}$ (pH 5.5) = 31, $K_{obs}$ (pH 6.5) = 55, $K_{obs}$ (pH 7.0) = 110, $K_{obs}$ (pH 7.5) = 290, $K_{obs}$ (pH 8.1) = 1.0 x 10$^3$, $K_{obs}$ (pH 8.6) = 3.1 x 10$^3$, and $K_{obs}$ (pH 9.0). At each pH, $\frac{d\log K_{obs}}{d\log [NaCl]} < 0$, indicating that a net release of ions (cations plus anions) occurs upon formation of the (SSB)$_{4s}$ complex. However, the absolute magnitude of these slopes decreases with increasing pH, indicating that net ion release decreases with increasing pH. Over the range of NaCl concentrations investigated at each pH, $K_{obs}$ is a linear function of log[NaCl] with $\frac{d\log K_{obs}}{d\log [NaCl]} = -129.0(\pm 98)$ at pH 5.5, $-85.0(\pm 0.3)$ at pH 6.5, $-78.0(\pm 2.0)$ at pH 7.0, $-80.0(\pm 0.2)$ at pH 7.5, $-74.0(\pm 5.5)$ at pH 8.0, $-67.0(\pm 2.0)$ at pH 8.6 and $-60.0(\pm 0.5)$ at pH 9.0. Therefore, the net ion release is reduced by a factor of 2 from ~12 to ~6 upon raising the pH from 5.5 to 9.0. This linkage between pH and net ion release in NaCl buffers is shown more clearly in Figure 5, where $\frac{d\log K_{obs}}{d\log [NaCl]}$ is plotted versus pH. Figure 3 shows plots of log$K_{obs}$ versus pH at several [NaCl]. The slopes of each of these curves ($\frac{d\log K_{obs}}{d\log [pH]}$) are negative at each [NaCl] and pH indicating that protonation occurs upon formation of the (SSB)$_{4s}$ complex. However, the pH dependence at each [NaCl] is complex. The largest effects are observed at the extremes of pH (pH > 8 and pH < 6), with much smaller effects in the range 6.5 ≤ pH ≤ 8. These results indicate a minimum of two classes of protonatable sites on the SSB.
Figure 3. Dependence of $K_{obs}$ on pH for the interaction of the SSB protein with poly(U) at different [NaCl] (25°C). The values of $\omega_{T0}$ applicable at each pH are listed in Table 1. The theoretical lines are simulations based on eqn (9) and the parameters given in Table 1. The [NaCl] are: 0.10 M (●); 0.15 M (▲); 0.20 M (○); 0.25 M (■); 0.30 M (★); 0.35 M (△).

tetramer, one with $pK < 7$ and the other with $pK > 8$. The limiting slope above pH 8, $(\partial \log K_{obs}/\partial \text{pH}) = -2$, indicates a net uptake of at least two protons in this pH range.

(e) Anion effects are pH dependent

In principle, the effects of pH on the [NaCl]-dependence of $K_{obs}$ could be linked to differences in cation and/or anion binding. To sort this out we examined the dependence of $K_{obs}$ on [NaF] and its linkage to pH. Fluoride was chosen to compare with chloride since $F^-$ has been shown to bind weakly to proteins and peptides (von Hippel & Schleich, 1969) and thus anion effects are expected to be minimized in fluoride salts. Consistent with this premise, we have observed a significantly lower dependence of $K_{obs}$ on [NaF] than on [NaCl] for SSB-poly(U) binding in the (SSB)65 mode (Overman et al., 1988). Reverse titrations were performed in NaF at pH 5.5, 8.1 and 9.0 to obtain $K_{obs}$ and $\omega_{T0}$ as a function of [NaF]. The values of $\omega_{T0}$ obtained in NaF are comparable to those obtained in NaCl and appear to be slightly pH dependent as observed in NaCl. Therefore, we analyzed the isotherms obtained in NaF by constraining the value of $\omega_{T0}$ to its average value obtained in NaCl at the particular pH under study. Figure 4 shows plots of $\log K_{obs}$ versus $\log [\text{NaF}]$ for the SSB tetramer-poly(U) interaction at pH 5.5, 8.1 and 9.0. At pH 5.5 in NaF, $K_{obs}$ could only be measured over a small range of [NaF], limited at low salt by the high SSB-poly(U) affinity and at high salt by the solubility of NaF (≈ 0.9 M). Therefore, the [NaF] dependence of $K_{obs}$ determined at pH 5.5 has a considerably larger uncertainty than the values obtained at higher pH. Although $K_{obs}$ does decrease significantly with increasing pH in the presence of NaF, $\partial \log K_{obs}/\partial \log [\text{NaF}]$ is relatively independent of pH, with values of $-4.4(±0.7), -4.3(±0.4)$ and $-4.0(±1.6)$ at pH 9.0, 8.1 and 5.5, respectively (see Table 2). The linear least-square lines representing the data obtained in NaCl at these same pH values are shown for comparison in Figure 4. Figure 5 shows more directly the different effects of pH on $\partial \log K_{obs}/\partial \log [\text{NaX}]$ versus $\partial \log K_{obs}/\partial \log [\text{NaF}]$. While a large change in net ion release is observed as

![Figure 5](image)

Figure 5. Dependence of $\partial \log K_{obs}/\partial \log [\text{NaX}]$ on pH for SSB tetramer-poly(U) binding in the (SSB)65 mode (25°C). $\partial \log K_{obs}/\partial \log [\text{NaX}]$, obtained from linear regression of the salt dependence of $K_{obs}$ (see Tables 1 and 2), is plotted as a function of pH: NaCl (■); NaF (○).
a function of pH in NaCl, the net ion release is virtually independent of pH in NaF. The difference between \( \frac{\text{dlog}K_{\text{obs}}}{\text{dlog}[\text{NaF}]} \) and \( \frac{\text{dlog}K_{\text{obs}}}{\text{dlog}[\text{NaF}]} \) is clearly enhanced at low pH, such that twice as much ion release is observed in NaCl at pH 5.3, whereas the net ion release in NaCl versus NaF appears to approach the same value at high pH, although there is still a significant difference at pH 9.0 (−44 versus −60).

Although \( \frac{\text{dlog}K_{\text{obs}}}{\text{dlog}[\text{NaF}]} \) is independent of pH, \( K_{\text{obs}} \) does decrease with increasing pH; at 0.35 M NaF, \( K_{\text{obs}} \) (pH 8.1) = 36 \( K_{\text{obs}} \) (pH 9.0).

Therefore, in addition to the effect of NaCl on chloride release there is an apparent effect of pH that does not result in a change in the extent of ion release. In NaF, in the alkaline pH range (pH 8.1 to 9.0), we find \( \frac{\text{dlog}K_{\text{obs}}}{\text{dPH}} = -20 \), indicating a net uptake of at least two protons upon complex formation. This is nearly identical to the value of \( \frac{\text{dlog}K_{\text{obs}}}{\text{dPH}} \) measured in NaCl in this same pH range. These data are consistent with a model which requires protonation of at least two amino acids on the SSB tetramer in order to form the \((\text{SSB})_{65} \) complex (see 4. Discussion, below).

The differences between the dependences of \( K_{\text{obs}} \) on [NaCl] versus [NaF] as a function of pH suggest the following qualitative considerations concerning the origins of the salt dependence of \( K_{\text{obs}} \) for formation of the \((\text{SSB})_{65} \) complex with poly(U): (1) Net anion release in NaF is small and independent of pH. Therefore, the salt dependence measured in NaF, \( \frac{\text{dlog}K_{\text{obs}}}{\text{dlog}[\text{NaF}]} \), reflects primarily the net release of Na⁺, which is nearly independent of pH. (2) The large differences between the salt dependences of \( K_{\text{obs}} \) in NaCl versus NaF reflect primarily differences in net anion release, with larger anion release occurring in the presence of NaCl at low pH.

This suggests that protonation of the SSB tetramer results in the creation of more Cl⁻ binding sites on SSB that are then perturbed upon formation of the SSB-poly(U) complex, resulting in a larger net anion release.

4. Discussion

We have examined the thermodynamic linkage between pH and monovalent salt concentration and type on the equilibrium binding of SSB tetramers to poly(U) to form the \((\text{SSB})_{65} \) complex. In this mode, an average of 65(±3) nucleotides are occluded per SSB tetramer (Lohman & Overman, 1985; Bujalowski & Lohman, 1986; Bujalowski et al., 1988) and all four SSB subunits interact with the nucleic acid (Bujalowski & Lohman, 1988a,b; Lohman & Bujalowski, 1988). To insure that the final SSB-poly(U) complex were in the \((\text{SSB})_{65} \) mode, our experiments were restricted to monovalent salt concentrations ≥ 0.2 M, since the alternate SSB binding modes can be populated at lower salt concentrations (Lohman & Overman, 1985; Bujalowski & Lohman, 1986; Bujalowski et al., 1988).

The SSB-poly(U) isotherms were analyzed using a “limited” cooperativity model (Bujalowski & Lohman, 1987b) to obtain the equilibrium constant, \( K_{\text{obs}} \), for the binding of an isolated SSB tetramer in an \((\text{SSB})_{65} \) complex, as well as the limited cooperativity parameter, \( \omega_{\text{RGO}} \), reflecting nearest-neighbor interactions between poly(U)-bound SSB tetramers to form octamers (Chrysogoles & Griffith, 1982; Griffith et al., 1984). This work as well as previous studies (Overman et al., 1988) indicates that the salt dependence of SSB binding resides entirely in \( K_{\text{obs}} \) whereas \( \omega_{\text{RGO}} \) is independent of monovalent salt concentration and type. Furthermore, changes in pH also affect mainly \( K_{\text{obs}} \). At 25°C, we find only a slight dependence of \( \omega_{\text{RGO}} \) on pH (\( \frac{\text{dlog}\omega_{\text{RGO}}}{\text{dPH}} = 0.18(±0.05) \), although \( \omega_{\text{RGO}} \) does change with temperature (Overman, 1988).

Therefore, the effects of salt concentration and pH reported here reflect effects only on \( K_{\text{obs}} \).

(a) Origins of the salt dependence of \( K_{\text{obs}} \)

Overman et al. (1988) have shown that a net release of both cations and anions accompanies formation of the \((\text{SSB})_{65} \) complex with poly(U). We have probed the effects of anions versus cations by comparing the effects of NaCl versus NaF and their linkage to pH. This approach was used since \( F^- \) appears to interact least with proteins when compared to anions such as bromide, acetate and chloride (von Hippel & Schleich, 1969; Kowalczykowski et al., 1981; Overman et al., 1988), hence it was expected that anion effects would be reduced in NaF compared to NaCl. Our results support this premise and indicate a number of different contributions to the salt dependence of \( K_{\text{obs}} \). Although glutamate is also expected to interact only weakly with proteins (Leirino et al., 1987), our previous results (Overman et al., 1988) indicate other possible effects of glutamate, at least on the SSB-poly(U) interaction, hence it was not used as a non-interacting anion in these studies.

The various factors contributing to the salt and pH effects on the equilibrium formation of the \((\text{SSB})_{65} \) complex are discussed below.

(i) Counterion release from the polynucleotide

Formation of the \((\text{SSB})_{65} \) complex involves significant electrostatic interactions with all ss-polynucleotides including poly(U). Consequently, monovalent counterions (M⁺) are released from poly(U). Accounting for the contributions due to uptake of ions (see below), we estimate that a total of ~ 11 cations are released from the poly(U) alone upon formation of the \((\text{SSB})_{65} \) complex, even though a net release of only ~4 cations is observed (Overman et al., 1988).

(ii) Uptake of cations and anions upon formation of the \((\text{SSB})_{65} \) complex

Previous studies indicate that a net uptake of cations and anions occurs upon formation of the \((\text{SSB})_{65} \) complex from the \((\text{SSB})_{33} \) and/or \((\text{SSB})_{36} \) binding modes (Lohman & Overman, 1985;
Bujalowski & Lohman, 1986, 1989b; Bujalowski et al., 1988; Wei et al., 1992). Therefore, although a net release of anions and cations occurs upon formation of the (SSB)$_{65}$ complex from free SSB protein and poly(U), the total number of ions released must be larger since it is compensated partially by contributions from cation and anion uptake (Overman et al., 1988). Our current estimate is that in NaCl (pH 8.1), the binding of at least ~6 Na$^+$ accompanies the transition from the (SSB)$_{55}$ to the (SSB)$_{65}$ mode and the binding of ~2 Cl$^-$ accompanies the transition from the (SSB)$_{65}$ to the (SSB)$_{65}$ mode (Bujalowski et al., 1988).

(iii) Release of anions from SSB protein

Comparisons of the salt dependence of $K_{obs}$ in solutions containing different anion types indicates that a net release of anions accompanies formation of the (SSB)$_{65}$ complex (Overman et al., 1988). The values of $\partial \log K_{obs}/\partial \log [MX]$ (pH 8.1, 25°C) were: $-4.3(\pm 0.4)$, $-5.7(\pm 0.4)$, $-6.5(\pm 0.3)$, $-7.4(\pm 0.5)$, $-6.7(\pm 0.4)$ in NaF, KCl, NaCH$_3$CO$_2$, NaCl and NaBr, respectively (Overman et al., 1988) indicating that anion release is lowest in NaF. The effect of anions is demonstrated clearly by the large differences in $\log K_{obs}/\log [NaF]$ versus $\log K_{obs}/\log [NaCl]$ as a function of pH that we report here. The invariance of $\partial \log K_{obs}/\partial \log [NaF]$ to changes in pH is also consistent with the premise that there is no net change in F bonding to the SSB protein upon formation of the (SSB)$_{65}$ complex with poly(U) (Overman et al., 1988). This is also consistent with other studies indicating that F$^-$ interacts only weakly with proteins (von Hippel & Schleich, 1969; Kowalczykowski et al., 1981). Assuming no differential F binding, then the pH-independent value of $\partial \log K_{obs}/\partial \log [NaF] = -4.3(\pm 0.4)$ reflects only Na$^+$ release indicating a net release of ~4 Na$^+$. This indicates that at pH 8.1, 25°C, in NaBr, NaCl and NaCH$_3$CO$_2$ there is a net release of 3 Br$^-$, 3 Cl$^-$ and 2 CH$_3$CO$_2^-$ in addition to the 4 Na$^+$ (Overman et al., 1988). However, we show here that the extent of ion release is linked to protonation of the SSB protein, such that additional ions are released as the pH is lowered.

(b) Multiple effects of pH on formation of the (SSB)$_{65}$ binding mode

The increase in $K_{obs}$ with decreasing pH indicates that protonation of SSB occurs upon formation of the (SSB)$_{65}$ complex over the full pH range examined (5.5 ≤ pH ≤ 9.0). There appear to be at least three contributions, with two of them linked to ion binding since the increase in net ion release with decreasing pH occurs in two steps indicating at least two pH-dependent processes coupled to ion release. Four additional ions are released in the acidic pH range than at neutral pH and two additional ions are released in the neutral pH range than at alkaline pH. Our analysis suggests that in the alkaline pH range, anion binding is coupled to protonation of sites on the protein, whereas in the acidic pH range, cation binding may be coupled to de-protonation of sites on the SSB protein (see below). The result is that an increase in ion binding to the SSB tetramer occurs with decreasing pH and these additional ions are released upon formation of the (SSB)$_{65}$ complex.

In addition to the above effects of pH, the fact that both $K_{obs}$ and $\partial \log K_{obs}/\partial \log [NaCl]$ increase as the pH decreases implicates at least one additional protonation event that results in an increase in $K_{obs}$ as the pH decreases. In the absence of this additional effect, $K_{obs}$ is predicted to decrease with decreasing pH due to the increased ion binding to the protein at lower pH (see above). The pH dependence of $K_{obs}$ in NaF solutions also confirms the existence of another pH-dependent contribution to $K_{obs}$ that is unlinked to ion binding. In NaF, $\partial \log K_{obs}/\partial \log [NaF]$ is = -2.1(±0.7) in the alkaline pH range (8.1 to 9.0) in both NaF and NaCl, thus a net uptake of at least approximately two protons occurs under these conditions, whereas net ion release is relatively unaffected.

(c) Modeling the dependence of $K_{obs}$ on salt concentration and pH

We have used equation (9) in an attempt to simulate the anion and cation concentration and pH effects on $K_{obs}$ for formation of the (SSB)$_{65}$ complex with poly(U). The interaction parameters that best describe the data are listed in Table 3. We emphasize that this exercise does not yield true interaction parameters, especially since we have neglected preferential hydration (Tanford, 1969; Record et al., 1978); however, it provides a useful model for the effects of anion, cation and pH that we observe experimentally:

$$
\log K_{obs} = \log K_T - 6 \log [MX] + 
\epsilon_\alpha \left( \frac{1}{1 + K_d[H^+]^n} \right) \log [MX] 
- \epsilon_\delta \left( \frac{K_d[H^+]^n}{1 + K_d[H^+]^n} \right) \log (1 + K_d[MX]) 
- \epsilon \log \left( \frac{1 + K_d[H^+]^n}{K_d[H^+]^n} \right).
$$

(9)

In equation (9) $K_T$ is the equilibrium constant for formation of the (SSB)$_{65}$ complex in a standard state at 1 M MX, but extrapolated from the limiting low salt behavior ($[MX] \rightarrow 0$). In this standard state, there are no contributions from anion or cation binding to the protein, hence the dependence of $K_{obs}$ on [MX] is due only to cation release from poly(U) (Record et al., 1978). Equation (9) incorporates cation release from the poly(U) as well as site binding of anions, cations and protons to the SSB protein, but neglects preferential hydration.

(i) Cation release from the nucleic acid

The second term in equation (9) represents the contribution to the salt dependence due to the
polyelectrolyte effect (Record et al., 1976, 1978; Record & Spolar, 1990), i.e. release of $b$ cations from the poly(U). We have estimated that approximately 11 Na$^+$ ions are released from the poly(U) upon formation of the (SSB)$_{65}$ complex (Overman et al., 1988).

(ii) Cation uptake by the SSB protein that is coupled to de-protonation of sites on the protein

The third term in equation (9) describes the net increase in $\Delta \log K_{\text{app}}/\Delta \log [\text{NaCl}]$ as the pH is lowered in the pH range 5.5 < pH ≤ 7. This effect is attributed to a decrease in cation uptake upon formation of the (SSB)$_{65}$ complex, i.e. groups on the protein require deprotonation in order to bind cations, based on our observations of the salt dependence of the SSB-ss polynucleotide binding mode transitions (Lohman & Overman, 1985; Bujalowski & Lohman, 1986; Bujalowski et al., 1988; Wei et al., 1992). These results indicate a net binding of approximately six monovalent cations upon formation of the (SSB)$_{65}$ mode from the (SSB)$_{35}$ mode, and this cation uptake decreases with decreasing pH (Bujalowski et al., 1988). The experiments reported here cannot exclude a linkage of this effect to anion binding; however, if this effect is assumed to be linked to anion uptake, then one would have to assume that $F^-$ has the same (or slightly higher) affinity for these anion sites than does Cl$^-$, which is contrary to the evidence that $F^-$ binds only weakly to SSB.

We have assumed that cations (M$^+$) bind to "c" independent and identical sites on the SSB tetramer with average binding constant $K_c$, but that these sites must be deprotonated in order to bind cations, so that

$$c = c_0 \left[ \frac{1}{1 + K_c[H^+]} \right]$$

where $c_0$ is the total number of potential cation binding sites. In modeling this effect, we have assumed that the cation binding sites have a high affinity for Na$^+$ ($K_c[\text{Na}^+] \gg 1$), since this cation uptake is required for formation of the (SSB)$_{64}$ mode. Our simulations suggest a minimum of six cation binding sites per SSB tetramer that bind ions with an average maximum pK of 5.3. Although the affinity of cations for these sites cannot be estimated precisely from our experiments, the affinity must be ≥ 100 M$^{-1}$ to saturate these sites at the salt concentrations used in our studies.

(iii) Anion release from SSB, that is coupled to protonation of the protein

The fourth term in equation (9) explains the decrease of $\Delta \log K_{\text{app}}/\Delta \log [\text{NaCl}]$ with increasing pH in the alkaline range. We assume that anions (X$^-$) bind to $a$ independent and identical sites on the SSB tetramer with average anion binding affinity $K_a$ (Schellman, 1975), but that these sites must be protonated in order to bind anions, so that

$$a = a_0 \left[ \frac{K_a[H^+]}{1 + K_a[H^+]} \right]$$

where $a_0$ is the total number of potential anion binding sites. These anions are then released upon formation of the (SSB)$_{65}$ complex. Thus, the average number of anion binding sites increases with decreasing pH and as a result, more anions are released upon polynucleotide binding. The net number of anions released provides a minimum estimate of three anion binding sites on the SSB tetramer. However, the anion competition experiments suggest a minimum of $a_0 = 5$ independent and identical sites.

We can only estimate a minimum average pK ≥ 10 for these sites since our data do not extend to high enough pH. This is close to the pK of the $\epsilon$-amino group of lysine and the phenolic group of tyrosine and the pK of poly(U), 9.6 (Simkins & Richards, 1967); however, since anions should not bind to the polynucleotides, it seems reasonable to assign these sites to the protein.
Required protonation of the SSB protein that is not linked to ion release

The fifth term in equation (9) describes the effects of pH that are not linked to ion release. In the presence of NaF, no change in the extent of ion release occurs in the range of pH from 5.5 to 9.0 yet \( K_{\text{obs}} \) is still influenced by pH \( \frac{\partial \log K_{\text{obs}}}{\partial \text{pH}} = -2.1(\pm 0.7) \) at pH > 8.1. This observation can be explained by a model in which \( r \) independent and identical sites on the SSB tetramer must be protonated in order to form the SSB\(_{65}\) complex (de Haseth et al., 1977). The limiting slope at high pH indicates that \( r \geq 2 \), with the best fit to all of the data suggesting that \( r \) may be as large as three. These required proton uptake sites have an average pK\(_a\) = 8.2, suggestive of \( \varepsilon \)-amino groups (Lohman et al., 1980).

Equation (9) includes the minimum number of terms needed to describe the linkages between salt concentration and type and pH. The “best-fit” parameters listed in Table 3 were obtained by nonlinear least-squares analysis of the data after converting salt concentrations to mean ion activities \( (a_i) \). The ability of equation (9) to accurately describe these data is shown in Figures 3, 6, 7, and 8. Figure 6 shows data from Overman et al. (1988) determined for a series of Na\(^+\) salts of different anions plotted as log\( K_{\text{obs}} \) versus log \( a_i \) along with the simulations based on equation (9) and the parameters in Table 3. The linkage between effects of pH and [NaCl] and [NaF] are described well by equation (9) as shown in Figures 3, 7, and 8. As stated above, the parameters in Table 3 should be viewed only as fitting parameters, since they were obtained by using the simplest model possible and we lack information on the number and type of ion binding sites on the SSB tetramer and their range of affinities. It is entirely possible that there are additional contributions to the salt and pH dependence.
of $K_{\text{obs}}$ beyond those described in equation (9) (e.g., a term for anion uptake (which would be compensated by the anion release term), which is suggested by the fact that there is anion uptake in the formation of the (SSB)$_{45}$ binding mode [Bujalowski et al., 1988]. Furthermore, we have neglected any potential contributions due to preferential hydration, which can contribute significantly at higher salt concentrations (generally $\geq 0.5$ M) [Tanford, 1969; Record et al., 1978; Ha et al., 1992]. However, equation (9) provides a useful first-order description of the major effects of pH, [NaCl] and [NaF] on $K_{\text{obs}}$, hence the introduction of more variables into the model is unwarranted at this time.

(d) Summary

Our studies of the E. coli SSB tetramer-ss nucleic acid equilibria indicate that the effects of salt concentration and type on protein-nucleic acid equilibria can be quite complex, with contributions from cation binding to the nucleic acid as well as specific cation and anion binding to the protein. Furthermore, these effects can be linked to protonation of the protein. Therefore, all of these effects will contribute to protein-nucleic acid stability and potentially to specificity as well (Record et al., 1991). It should also be clear from these studies that ionic strength is not the appropriate variable to describe the effects of salt concentration on $K_{\text{obs}}$ for protein-nucleic acid interactions since effects due to specific ion binding to the protein and nucleic acid dominate. Furthermore, the lack of a pH dependence of the [NaF] dependence of $K_{\text{obs}}$, even at pH 5.5 where the net charge of the SSB protein is negative, indicates that the salt dependence of $K_{\text{obs}}$ is not a function of the net charge of the SSB protein.

There are now several examples of protein-nucleic acid interactions whose stability and specificity are influenced by anion binding to the protein in addition to cation binding to the nucleic acid (de Haseth et al., 1977; Kowalczykowski et al., 1981; Barkley et al., 1981; Leirman et al., 1987; Griep & McHenry, 1989; Ha et al., 1992; Loehman et al., 1989; Zou & Richardson, 1991; Runyon et al., 1993). The kinetics of these interactions can also be influenced by anions (Loehman, 1984a). Although most cases show clear contributions from anion binding to the protein, effects due to cation binding, such as those that we observe for SSB protein are also possible. However, in the case of protein-nucleic acid interactions, cation interactions with the protein are more difficult to identify and study due to the dominant effects of cation binding to the nucleic acid (Record et al., 1976, 1978). Nonetheless, these effects are important for the binding of SSB to ss-nucleic acids. Our use of the different linkage effects of pH on cation versus anion binding to the protein to study these effects may prove useful for studies of other protein-nucleic acid interactions.

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Linkage of Ion Binding and Protonation in SSB-DNA Equilibria


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