# Effects of Base Composition on the Negative Cooperativity and Binding Mode Transitions of *Escherichia coli* SSB–Single-Stranded DNA Complexes†

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ABSTRACT: We have examined the ability of the *Escherichia coli* single-stranded DNA binding protein (SSB) tetramer to form its different binding modes on poly(dC), poly(U), and poly(A) over a range of NaCl and NaF concentrations for comparison with previous studies with poly(dT). In reverse titrations with poly(U) and poly(A) at 25 °C, pH 8.1, SSB forms all four binding modes previously observed with poly(dT), namely, $(SSB)_{35}$, $(SSB)_{40}$, $(SSB)_{56}$, and $(SSB)_{65}$, where the subscript denotes the site size (i.e., the average number of nucleotides occluded per SSB tetramer). As with poly(dT), the low site size modes are favored at low monovalent salt concentration (<10 mM), whereas increasing salt concentration facilitates the transitions to the higher site size modes. Surprisingly, SSB does not form a stable $(SSB)_{35}$ complex on poly(dC), even at 1 mM NaCl; rather, the $(SSB)_{56}$ mode is formed under these conditions. Upon raising the [NaCl], the $(SSB)_{56}$ complex undergoes a transition to the $(SSB)_{65}$ complex (transition midpoint, 40 mM NaCl). On the basis of studies with $dC(pC)_{34}$, $dT(pT)_{34}$, and $dA(pA)_{34}$, the inability of the SSB tetramer to form the $(SSB)_{35}$ complex with poly(dC) is due mainly to a much lower degree of negative cooperativity for binding oligodeoxycytidylates to the SSB tetramer. At low salt concentration, the negative cooperativity parameter, $\sigma_{35}$, is lowest for $dA(pA)_{34}$, intermediate for $dT(pT)_{34}$, and highest for $dC(pC)_{34}$, indicating that it is most difficult to saturate the SSB tetramer with two molecules of $dA(pA)_{34}$. We have also measured the equilibrium constants for binding the oligodeoxynucleotides $dC(pC)_{34}$, $dC(pC)_{69}$, $dA(pA)_{34}$, and $dA(pA)_{69}$ as a function of [NaCl] and [NaBr] and find that the salt dependencies of these oligonucleotides are dependent upon base composition. These studies also indicate that ion binding accompanies formation of these SSB–ss-DNA complexes, although there is a net release of ions upon formation of the complex. This influence of both salt concentration and base composition indicates that both electrostatic and nonelectrostatic factors contribute to the negative cooperativity associated with ss-DNA binding to the SSB tetramer.

The *Escherichia coli* single-stranded DNA binding protein (SSB) is essential for DNA replication and repair and facilitates homologous recombination catalyzed by the RecA protein [for reviews, see Chase and Williams (1986), Meyer and Laine (1990), and Lohman and Ferrari (1994)]. This protein is a member of a class of proteins referred to as helix-destabilizing proteins or simply SSB proteins that appear to be essential for DNA metabolism in all organisms. These proteins bind with high affinity and specificity to single-stranded (ss) DNA and generally with positive cooperativity. The phage T4 gene 32 protein (Alberts & Frey, 1970; Kowalczykowski *et al.*, 1981a,b; Karpel, 1990) is another well-characterized member of this class of proteins.

The interaction of the *E. coli* SSB protein with ss-DNA has been studied extensively since its discovery by Sigal *et al.* (1972) [for reviews, see Lohman *et al.* (1988), Greipel *et al.* (1990), Lohman and Bujalowski (1990) and Lohman and Ferrari (1994)]. The ss-DNA binding properties of the *E. coli* SSB protein are rather complex and quite different from those of the phage T4 gene 32 protein, due mainly to the fact that *E. coli* SSB is a homotetramer, and each subunit possesses a binding site for ss-DNA (Bujalowski *et al.*, 1989a,b). The SSB tetramer can bind to polymeric ss-DNA in a number of different "binding modes", which differ by the number of nucleotides occluded per tetramer (site size). At 25 °C (pH 8.1), the SSB tetramer can form at least three binding modes, referred to as $(SSB)_{35}$, $(SSB)_{56}$, and $(SSB)_{65}$, where the subscript denotes the average number of nucleotides occluded per tetramer in the complex (Lohman & Overman, 1985; Bujalowski & Lohman, 1986; Bujalowski *et al.*, 1988; Kuil *et al.*, 1990; Wei *et al.*, 1992). In the $(SSB)_{35}$ mode, the ss nucleic acid interacts with only two subunits of the SSB tetramer, whereas in both the $(SSB)_{56}$ and $(SSB)_{65}$ modes, the ss nucleic acid interacts with all four SSB subunits (Lohman & Bujalowski, 1988; Bujalowski & Lohman, 1989a,b). In at least some of these complexes, the ss-DNA is wrapped around the SSB tetramer (Krauss *et al.*, 1984; Chrysogelos & Griffith, 1982; Griffith *et al.*, 1984; Lohman & Overman, 1985; Bujalowski & Lohman, 1989a,b).

The relative stabilities of the different SSB–ss-DNA binding modes are modulated by salt concentration and type. In a complex with poly(dT) (25 °C, pH 8.1), the $(SSB)_{35}$ mode dominates at [NaCl] ≤ 10 mM and high SSB binding densities, whereas the $(SSB)_{65}$ mode dominates at [NaCl] ≥ 200 mM or low SSB binding densities (Lohman & Overman, 1985; Bujalowski & Lohman, 1986; Bujalowski *et al.*, 1988). Temperature, pH, and anion type (Bujalowski *et al.*, 1988) as well as divalent cations (Bujalowski & Lohman, 1986;

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Bujalowski *et al.*, 1988) and polyamines (Wei *et al.*, 1992) also influence the relative stabilities of the SSB binding modes. Two morphologies of SSB–M13 ss-DNA complexes have been observed by electron microscopy (Chrysogelos & Griffith, 1982; Griffith *et al.*, 1984). These complexes differ in the apparent contour length of the protein–ss-DNA complexes as well as their morphology. Complexes with a "beaded" morphology in which each bead corresponds to an SSB octamer (dimer of tetramers) are favored at low protein to DNA ratios and appear to correspond to the $(SSB)_{65}$ and/or the $(SSB)_{56}$ modes. Complexes with a smooth morphology that are formed at high protein to DNA ratios and that have apparent contour lengths ~2-fold longer than the beaded complexes appear to correlate with the $(SSB)_{35}$ mode (Lohman & Overman, 1985; Lohman *et al.*, 1988).

*E. coli* SSB tetramers bind with positive cooperativity to ss-DNA; however, the type and magnitude of the cooperativity parameter describing this positive cooperativity differ dramatically for at least the $(SSB)_{65}$ and $(SSB)_{35}$ binding modes (Lohman *et al.*, 1986b; Ferrari *et al.*, 1994). In the $(SSB)_{35}$ mode, SSB tetramers bind with high "unlimited" cooperativity (Lohman *et al.*, 1986b, 1988), similar to that observed for the phage T4 gene 32 protein (Alberts & Frey, 1970; Kowalczykowski *et al.*, 1981b; Lohman, 1984), such that SSB tetramers can form continuous clusters and ultimately saturate the ss-DNA (Ruyechan & Wetmur, 1975; Griffith *et al.*, 1984). The nearest neighbor cooperativity parameter, $\omega_{35}$, describing the interaction between adjacent SSB tetramers bound in the $(SSB)_{35}$ mode is at least $\sim 10^5$ (Ferrari *et al.*, 1994). However, in the $(SSB)_{65}$ mode, only moderate "limited" cooperativity is observed (Lohman *et al.*, 1986b), in which clustering is limited to the formation of dimers of tetramers (Chrysogelos & Griffith, 1982; Bujalowski & Lohman, 1987b). The limited cooperativity parameter, $\omega_{T/O}$, in the $(SSB)_{65}$ mode is 420 ± 80 (pH 8.1, 25 °C, 0.20 M NaCl) (Bujalowski & Lohman, 1987b; Overman *et al.*, 1988), although $\omega_{T/O}$ increases with increasing temperature (Overman, 1989; Lohman & Bujalowski, 1990). Due to the very different DNA binding properties of these SSB binding modes and the fact that the $(SSB)_{56}/(SSB)_{65}$ modes appear to stimulate RecA activity (Griffith *et al.*, 1984; Muniyappa *et al.*, 1990; Morrical & Cox, 1990), it has been suggested that the $(SSB)_{35}$ mode may be used selectively in replication, whereas the $(SSB)_{56}/(SSB)_{65}$ modes may be used in recombination (Lohman *et al.*, 1988; Lohman & Bujalowski, 1990).

The relative stability of the $(SSB)_{35}$ mode is linked to a salt-dependent negative cooperativity for the binding of ss-DNA to the SSB tetramer (Lohman & Bujalowski, 1988; Bujalowski & Lohman, 1989a,b). This is exhibited as a reduction in the affinity of ss oligodeoxynucleotides [$dT(pT)_{15}$ and $dT(pT)_{34}$] for the third and fourth subunits of the SSB tetramer upon binding of ss-DNA to the first two subunits. Although negative cooperativity exists under all solution conditions tested, it increases with decreasing salt concentration and is also influenced by cation charge. The increase in the extent of negative cooperativity at low salt concentration makes it more difficult to bind ss-DNA to the third and fourth SSB subunits, and this correlates with increasing stability of the $(SSB)_{35}$ mode on poly(dT) (Lohman & Bujalowski, 1988; Bujalowski & Lohman, 1989b). Negative cooperativity is not observed for $dT(pT)_{15}$ binding to the mutant SSB-1 monomer, whereas it is observed for $dT(pT)_{15}$ binding to the SSB-1 tetramer; hence this phenomenon is a property only of the SSB tetramer (Bujalowski & Lohman, 1991b).

Although the different SSB binding modes can form with different homopolynucleotides (Lohman & Overman, 1985; Kuil *et al.*, 1990), most of our previous studies were performed with poly(dT) due to its high affinity for SSB, relative to the other homopolynucleotides. In this report we examine the effect of base composition on the relative stabilities of the SSB–polynucleotide binding modes as well as on the equilibrium binding properties (intrinsic affinity and negative cooperativity) of short oligodeoxynucleotides. These studies demonstrate that base composition has a surprisingly large effect on the negative cooperativity for ss-DNA binding to the SSB tetramer, which in turn influences the relative stability of the $(SSB)_{35}$ mode on ss polynucleotides.

## MATERIALS AND METHODS

*Reagents and Buffers.* All chemicals were reagent grade; all solutions were made with distilled and deionized (Milli-Q) water. Buffer T (pH 8.1) is 10 mM Tris [tris(hydroxymethyl)-aminomethane] and 0.1 mM $Na_3EDTA$ (trisodium salt of ethylenediaminetetraacetic acid) titrated to pH 8.1 with HCl at 25 °C. The experiments in NaF at pH 8.1 were performed using buffer T, adjusted to pH 8.1 with concentrated HF.

*E. coli SSB Protein and Nucleic Acids. E. coli* SSB protein was prepared as described (Lohman *et al.*, 1986a). The concentration of SSB protein was determined spectrophotometrically, using the extinction coefficient $\epsilon_{280} = 1.13 \times 10^5$ $M^{-1}$ (tetramer) $cm^{-1}$ (1.5 mL $mg^{-1}$ $cm^{-1}$) in buffer T + 0.20 M NaCl (Lohman & Overman, 1985). The SSB protein is a stable tetramer under all conditions used in this study (Williams *et al.*, 1984; Lohman *et al.*, 1986b; Overman *et al.*, 1988; Bujalowski & Lohman, 1991a). The poly(dT) ($s_{20,w}$ = 8.4 S; ~950 nucleotides) was purchased from Pharmacia P-L Biochemicals and was dialyzed extensively before use; its concentration was determined spectrophotometrically using an extinction coefficient at 260 nm of 8100 $M^{-1}$ (nucleotide) $cm^{-1}$. The oligonucleotides $dC(pC)_{34}$, $dC(pC)_{69}$, $dA(pA)_{34}$, and $dA(pA)_{69}$ were synthesized using an Applied Biosystems 380B automated synthesizer with phosphoramidite chemistry. These were purified using HPLC (Waters 600) on a Nucleogen DEAE 60-7 column (Rainin) as previously described (Lohman & Bujalowski, 1988). All oligonucleotides were ≥98% pure as judged by electrophoresis of $^{32}P$-end-labeled samples through a 15% polyacrylamide gel and autoradiography. The concentrations of the oligonucleotides were determined spectrophotometrically using extinction coefficients of $\epsilon_{257} = 1.0 \times 10^4$ $M^{-1}$ (nucleotide) $cm^{-1}$ for the oligodeoxyadenylates and $\epsilon_{270} = 7.2 \times 10^3$ $M^{-1}$ (nucleotide) $cm^{-1}$ for the oligodeoxycytidylates.

*Site Size Determinations.* "Reverse" titrations (addition of polynucleotide to SSB protein) were performed as described previously (Bujalowski & Lohman, 1986; Bujalowski *et al.*, 1988) with an SLM 8000 spectrofluorometer using an excitation wavelength of 300 nm [excitation bandpass = 1 nm (0.5-mm slit width)] and an emission wavelength of 347 nm [emission bandpass = 4 nm (2-mm slit width)]. The sample temperature was maintained at 25.0 ± 0.1 °C with a Lauda RM-6S refrigerated circulating water bath. When necessary, inner filter corrections were applied using the expression $F_{cor} = F_{obs}$ antilog($A_{ex}/2$) (Lakowicz, 1983). The SSB protein concentration used for most site size determinations with poly(dT) and poly(dC) was $5.16 \times 10^{-8}$ M (tetramer) (3.89 μg/mL); however, the site size determinations with poly(A) and poly(U) were performed at several higher SSB concentrations to ensure that binding was stoichiometric, since SSB affinity is lower for these polynucleotides (Overman *et al.*, 1988). The

SSB protein concentrations used in all experiments were well below the concentrations at which precipitation of the protein may occur.

*Analysis of Fluorescence Titrations To Obtain SSB–Oligodeoxynucleotide Equilibrium Binding Isotherms.* Equilibrium binding of oligodeoxynucleotides to SSB tetramers was monitored by the quenching of the SSB tryptophan fluorescence that accompanies complexation. These fluorescence titrations were then analyzed to obtain model-independent binding isotherms as described (Halfman & Nishida, 1972; Lohman & Bujalowski, 1988, 1991; Bujalowski & Lohman, 1989a). We note that this method makes no assumptions about the relationship between the degree of quenching and the extent of binding, but can be used to determine this relationship.

*Statistical Thermodynamic Models Describing SSB Tetramer–dN(pN)$_{34}$ Binding.* The SSB tetramer can bind two oligodeoxynucleotides that are 35 nucleotides long, since these oligodeoxynucleotides occupy two subunits of the tetramer simultaneously (Lohman & Bujalowski, 1988; Bujalowski & Lohman, 1989a). We have used a "square" model, described by Bujalowski and Lohman (1989a), to analyze the binding of dN(pN)$_{34}$ to the SSB tetramer. This model contains two interaction parameters: $K_{35}$, the equilibrium constant for dN(pN)$_{34}$ binding to one site (two subunits) of the SSB tetramer, and a cooperativity parameter, $\sigma_{35}$. The parameter $\sigma_{35}$ characterizes the interactions between two liganded SSB subunits, *not* those between two dN(pN)$_{34}$ binding sites. In general, for SSB–dN(pN)$_{34}$ interactions, we find $\sigma_{35} < 1$, indicating that binding to the SSB tetramer is described by negative cooperativity. The negative cooperativity parameter, $\sigma_{35}$, appears whenever the oligodeoxynucleotide occupies two adjacent subunits on the square, but not when two diagonal subunits are occupied. The partition function for this model, $Z_{S/35}$, and the average degree of binding, $\sum \nu_i$, are given by eqs 1 and 2,

$$Z_{S/35} = 1 + (4\sigma_{35} + 2)K_{35}L + 3\sigma_{35}^4(K_{35}L)^2 \quad (1)$$

$$\left(\sum \nu_i\right)_{S/35} = [(4\sigma_{35} + 2)K_{35}L + 6\sigma_{35}^4(K_{35}L)^2]/Z_{S/35} \quad (2)$$

The relationship between the observed fluorescence quenching and the individual quenching and interaction constants of the square model for an oligonucleotide such as dN(pN)$_{34}$ that binds to two sites on the tetramer is given in eq 3 (Bujalowski & Lohman, 1989a,b).

$$Q_{obs} = [Q_{1/35}(4\sigma_{35} + 2)K_{35}L + Q_{2/35}3\sigma_{35}^4(K_{35}L)^2]/Z_{S/35} \quad (3)$$

The analysis of the binding isotherms to determine $K_{35}$ and $\sigma_{35}$ was performed as described (Bujalowski & Lohman, 1989a,b).

## RESULTS

*Effect of Base Composition on the SSB–Polynucleotide Binding Mode Transitions.* We have examined the effects of [NaCl] and [NaF] on the relative stabilities of the different binding modes that the *E. coli* SSB tetramer can form with poly(dC), poly(U), and poly(A) and compare these with our previous studies with poly(dT). These experiments were performed by titrating a fixed concentration of SSB with polynucleotide (reverse titrations) at sufficiently high protein concentrations that stoichiometries can be measured accurately. Figure 1 shows plots of the apparent site size

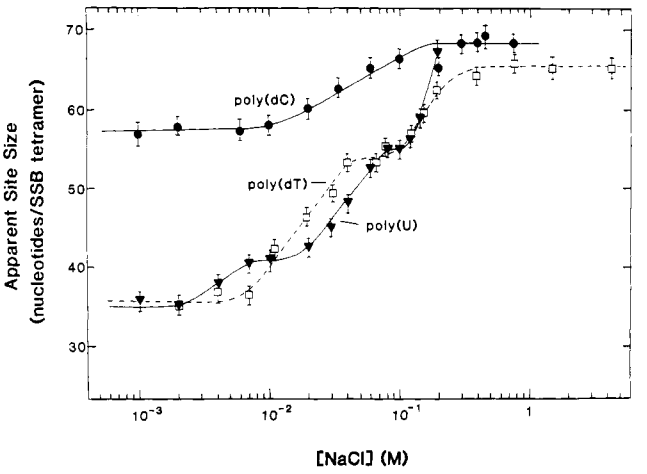


FIGURE 1: Effects of NaCl concentration on the apparent site size of complexes of SSB protein with poly(dC) (●) and poly(U) (▼). Apparent site sizes (nucleotides per SSB tetramer) were determined from reverse titrations in buffer T at pH 8.1 and 25.0 °C, monitoring the quenching of the SSB protein tryptophan fluorescence, and are plotted as a function of NaCl concentration (M) (logarithmic scale). Previously published data (Bujalowski & Lohman, 1986) showing the dependence on [NaCl] of the SSB–poly(dT) site size (□) are also shown for comparison.

(nucleotides per SSB tetramer) determined as a function of [NaCl] for SSB binding to poly(U) and poly(dC) (25.0 °C, pH 8.1), as well as our previous results with poly(dT) under the same conditions (Bujalowski & Lohman, 1986). With poly(U), the apparent site size shows plateau values at 35 ± 3, 40 ± 3, and 56 ± 3 nucleotides per tetramer; at 0.2 M NaCl, the highest [NaCl] used, we measure a site size of 67 ± 3 nucleotides per tetramer. Nearly the same profile is observed with poly(A) in NaCl, as shown in Figure 2B. These correspond to the same site sizes observed for SSB binding to poly(dT) at 37 °C; however, the plateau region observed with poly(U) at a site size of 40 nucleotides per tetramer is not observed with poly(dT) at 25 °C (see Figure 1), although it is observed with poly(dT) at 37 °C (Bujalowski & Lohman, 1986). We refer to the stable binding modes, corresponding to the plateau regions in Figure 1, as (SSB)$_{35}$, (SSB)$_{40}$, (SSB)$_{56}$, and (SSB)$_{65}$, where the subscript denotes the average site size in the plateau region. The transitions among the different binding modes (site size plateaus) occur at slightly different NaCl concentrations for poly(dT) *vs* poly(U).

Surprisingly, we do not observe formation of the (SSB)$_{35}$ mode with poly(dC); rather, a site size of 58 ± 3 nucleotides per tetramer is observed at [NaCl] ≤ 10 mM. Upon raising the [NaCl], we observe only a single transition to a complex with an average site size of 69 ± 3 nucleotides per tetramer, with a midpoint of ~40 mM NaCl. Therefore, a stable (SSB)$_{35}$ binding mode is not formed with poly(dC) at [NaCl] < 10 mM (25 °C, pH 8.1), although it does form with poly(dT), poly(U), and poly(A).

Both cations and anions affect the salt dependence of the SSB–poly(dT) binding mode transitions (Bujalowski *et al.*, 1988). Therefore, we examined the effects of [NaF] *vs* [NaCl] on the SSB binding mode transitions with poly(U), poly(A), and poly(dC), and the results are shown in Figure 2, along with our previous results with poly(dT) under the same conditions (pH 8.1, 25.0 °C) (Bujalowski *et al.*, 1988). As in NaCl, a stable (SSB)$_{35}$ binding mode is also not formed on poly(dC) at low [NaF]. Although Figure 2A indicates that the average site sizes measured for the SSB–poly(dC) interaction are slightly lower in NaF than in NaCl, there is relatively little qualitative effect of $Cl^-$ *vs* $F^-$ on the (SSB)$_{56}$

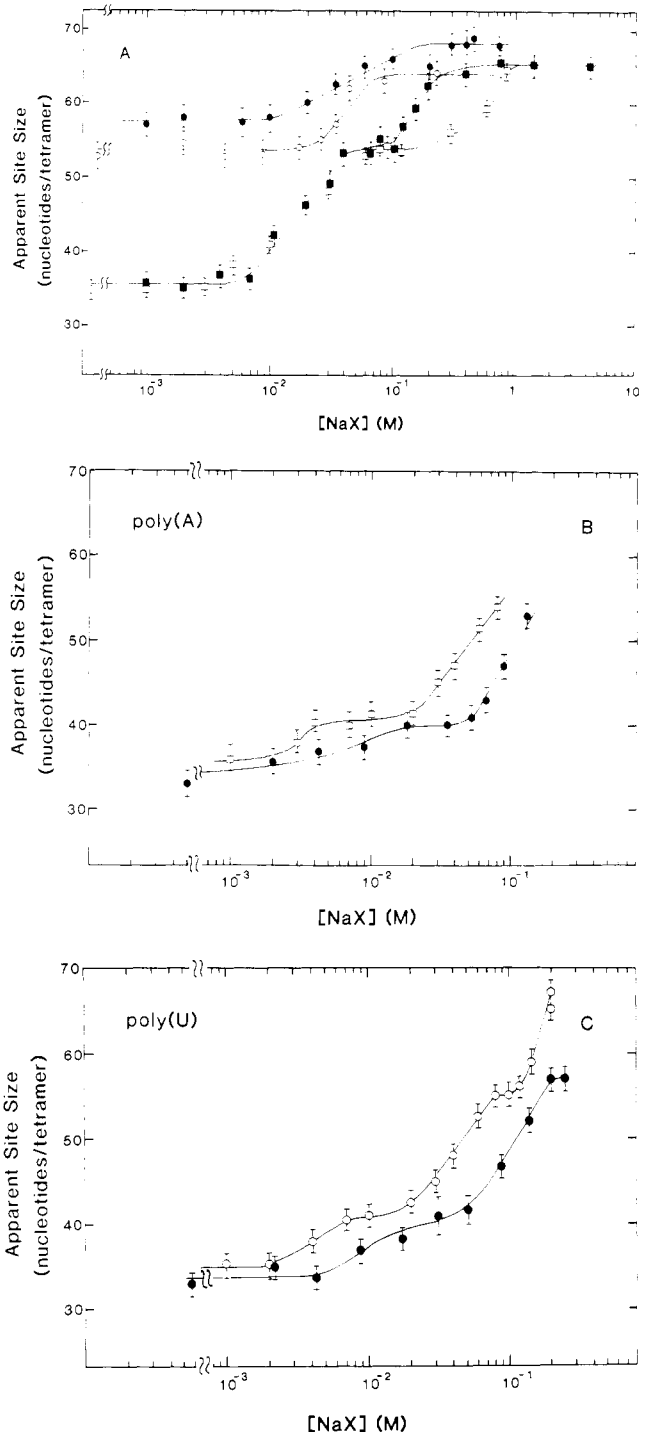


FIGURE 2: Effects of NaCl *vs* NaF concentration on the apparent site size of complexes of SSB protein with poly(dC), poly(U), and poly(A). (A) Apparent site sizes of the SSB tetramer on poly(dC) (nucleotides per SSB tetramer) were determined in buffer T (pH 8.1, 25.0 °C) as a function of [NaCl] (●) or [NaF] (○) (M) (logarithmic scale). Previously published data (Bujalowski & Lohman, 1986) showing the dependencies of the SSB–poly(dT) site size on [NaCl] (■) and [NaF] (□) are also shown for comparison. (B) Apparent site size (nucleotides per tetramer) for SSB–poly(A) complexes as a function of [NaCl] (□) and [NaF] (●). (C) Apparent site size (nucleotides per tetramer) for SSB–poly(U) complexes as a function of [NaCl] (○) and [NaF] (●).

to $(SSB)_{65}$ transition on poly(dC) at 25 °C, pH 8.1. This is in contrast to the behavior with poly(dT), which shows a substantial shift in the $(SSB)_{56}$ to $(SSB)_{65}$ transition to higher salt concentrations in the presence of NaF at pH 8.1, although there is no influence of $Cl^-$ *vs* $F^-$ on the $(SSB)_{35}$ to $(SSB)_{56}$ transition. However, at pH 6.9, there is an effect of $F^-$ *vs* $Cl^-$ on the $(SSB)_{35}$ to $(SSB)_{56}$ transition on poly(dT) (Bujalowski *et al.*, 1988). In contrast, Figure 2B,C shows that, for both poly(U) and poly(A), the entire site size transition curve is shifted to higher salt concentrations upon replacing $Cl^-$ with $F^-$, indicating that, for these polynucleotides, all binding mode transitions are influenced by the monovalent anion type at pH 8.1 (25 °C).

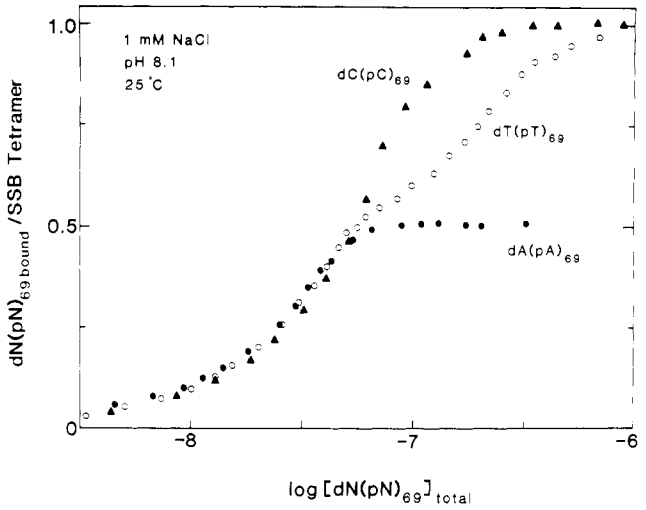


FIGURE 3: Influence of ss-DNA base composition on the relative stabilities of 1:1 *vs* 2:1 SSB tetramer–$dN(pN)_{69}$ complexes. Titrations of SSB protein [$1.1 \times 10^{-7}$ M (tetramer)] with $dC(pC)_{69}$ (▲), $dT(pT)_{69}$ (○), and $dA(pA)_{69}$ (●) were performed in buffer T (pH 8.1) and 1 mM NaCl, 25.0 °C, and the quenching of the SSB tryptophan fluorescence was monitored. The results are shown as plots of the average number of $dN(pN)_{69}$ bound per SSB tetramer. The $dT(pT)_{69}$ data are taken from Bujalowski and Lohman (1989b).

*Negative Cooperativity for ss Oligodeoxynucleotide Binding to Individual SSB Tetramers Is Influenced by DNA Base Composition.* At [NaCl] ≥ 0.2 M, an SSB tetramer forms a 1:1 complex with the oligodeoxynucleotide $dT(pT)_{69}$ in which all four SSB subunits interact with the DNA (Bujalowski & Lohman, 1989b). However, at lower [NaCl], SSB can form two types of complexes with $dT(pT)_{69}$. At low SSB to DNA ratios, the 1:1 complex is stable; however, at high SSB to $dT(pT)_{69}$ ratios, a 2:1 complex can form in which two SSB tetramers are bound to each $dT(pT)_{69}$, such that only two subunits of each tetramer interact with the DNA (Bujalowski & Lohman, 1989b). The transition between these two types of SSB–$dT(pT)_{69}$ complexes is influenced by a NaCl concentration dependent negative cooperativity which reduces the macroscopic affinity of ss-DNA to the third and fourth subunits of the SSB tetramer. This salt-dependent negative cooperativity also is a primary determinant of the salt dependence of the transition between the $(SSB)_{35}$ and $(SSB)_{56}$ binding modes on poly(dT) (Lohman & Bujalowski, 1988; Bujalowski & Lohman, 1989a,b).

On the basis of our finding that the nucleotide base composition influences the relative stabilities of the SSB–polynucleotide binding modes, we determined whether base composition dependent differences in stoichiometry occur for the binding of $dC(pC)_{69}$, $dA(pA)_{69}$, and $dT(pT)_{69}$ to the SSB tetramer at 1 mM NaCl (buffer T, pH 8.1, 25 °C). It is clear from Figure 3 that the binding properties of these three oligodeoxynucleotides are dependent on base composition. For each oligodeoxynucleotide, binding is stoichiometric up to a value of 0.5, corresponding to 2 SSB tetramers bound per $dN(pN)_{69}$. However, upon further addition of oligodeoxynucleotide, the three binding curves deviate substantially from each other. For $dA(pA)_{69}$, a plateau is obtained at 2 SSB

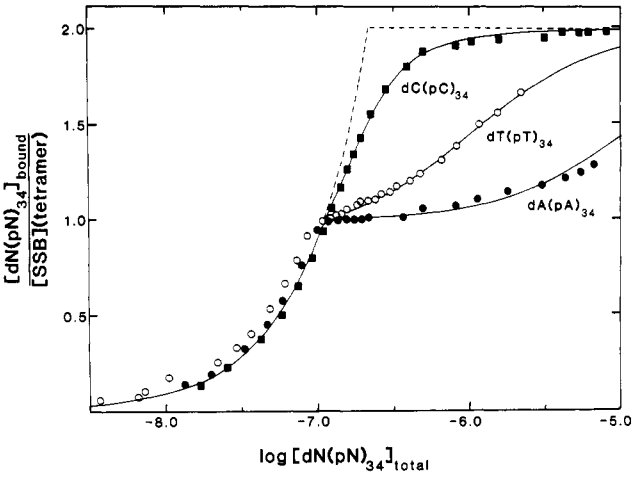


FIGURE 4: Influence of ss-DNA base composition on the negative cooperativity for ss-DNA binding to the SSB tetramer. Titrations of the SSB tetramer [$1.1 \times 10^{-7}$ M (tetramer)] with the oligonucleotides $dC(pC)_{34}$, $dA(pA)_{34}$, and $dT(pT)_{34}$ were carried out in buffer T (pH 8.1) and 10.5 mM NaCl at 25 °C, and binding was monitored by the quenching of the SSB tryptophan fluorescence. Each SSB tetramer can bind two molecules of $dN(pN)_{34}$ at saturation; however, the macroscopic affinity of the second molecule is strongly influenced by the nucleotide base. In each titration, the binding of the first oligonucleotide is stoichiometric; thus an accurate affinity estimate cannot be made for these molecules. The solid lines are the best theoretical fits using the following values for the *macroscopic* binding constant, $K_{2/35}$ [see Bujalowski and Lohman (1989a)]: (○) $2.2 \times 10^7$ $M^{-1}$, (●) $9.5 \times 10^5$ $M^{-1}$, and (■) $8.0 \times 10^4$ $M^{-1}$. The dashed curve represents the predicted titration for stoichiometric binding of $dN(pN)_{34}$ to both sites.

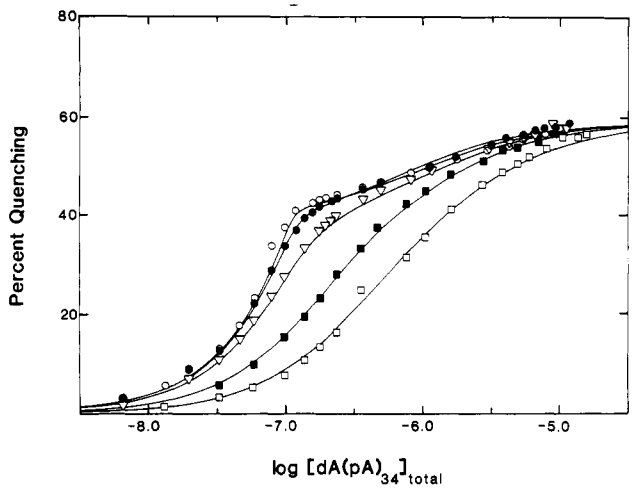


FIGURE 5: Fluorescence quenching of the SSB tetramer upon titration with $dA(pA)_{34}$ as a function of NaCl concentration. All titrations were performed in buffer T (pH 8.1, 25 °C) with an SSB tetramer concentration of $1.05 \times 10^{-7}$ M (tetramer). The first phase (42% fluorescence quenching) reflects binding of the first $dA(pA)_{34}$, whereas the second phase (additional 17% quenching) reflects the filling of the second binding site with $dA(pA)_{34}$. The NaCl concentrations for each titration are (○) 50 mM, (●) 75 mM, (▽) 0.1 M, (■) 0.15 M, and (□) 0.20 M. The solid lines are binding isotherms simulated using eqs 1 and 3, the interaction constants $K_{35}$ and $\sigma_{35}$ listed in Table 2, and the quenching constants $Q_{1/35} = 0.42$ and $Q_{2/35} = 0.59$.

tetramers per $dA(pA)_{69}$, whereas further titration with $dT(pT)_{69}$ or $dC(pC)_{69}$ results in additional binding of each oligodeoxynucleotide. Ultimately, plateaus are reached at 1 SSB tetramer bound per ss-DNA for both $dC(pC)_{69}$ and $dT(pT)_{69}$. However, it is clear that the transition from a 2:1 complex to a 1:1 complex occurs at lower DNA concentrations for $dC(pC)_{69}$ than for $dT(pT)_{69}$. In fact, whereas noticeable biphasic character is observed for the $dT(pT)_{69}$ titration with a break near 0.5, none is apparent in the $dC(pC)_{69}$ titration. This result is consistent with the fact that the $(SSB)_{35}$ mode does not form with poly(dC) at low [NaCl]. The data in Figure 3 also indicate that SSB more readily forms a 1:1 complex with $dT(pT)_{69}$ than with $dA(pA)_{69}$. This behavior could reflect base compositional dependencies either in the negative cooperativity for ss-DNA binding among the subunits within an SSB tetramer and/or in the positive cooperativity between two SSB tetramers bound simultaneously to the $dN(pN)_{69}$, since either could affect the relative stability of the 2:1 *vs* the 1:1 SSB-$dN(pN)_{69}$ complexes.

We have shown (Lohman & Bujalowski, 1988; Bujalowski & Lohman, 1989a,b) that the SSB tetramer can bind two molecules of $dT(pT)_{34}$ at saturation and that the second molecule of $dT(pT)_{34}$ binds with significantly lower affinity due to negative cooperativity among the SSB subunits. Furthermore, the degree of negative cooperativity increases with decreasing salt concentration. We therefore examined the effects of base composition on negative cooperativity by comparing the equilibrium binding to the SSB tetramer of $dC(pC)_{34}$, $dA(pA)_{34}$, and $dT(pT)_{34}$. The results of titrations performed at 10 mM NaCl (pH 8.1, 25.0 °C) are shown in Figure 4. In each case, the first molecule of $dN(pN)_{34}$ binds stoichiometrically, whereas the second molecule of $dN(pN)_{34}$ binds with significantly lower macroscopic affinity. From these data we can conclude that negative cooperativity, reflected in the lower macroscopic affinity of the second molecule of $dN(pN)_{34}$, is influenced by the nucleotide base, with $dA(pA)_{34}$ showing the greatest negative cooperativity, followed by $dT(pT)_{34}$ and then $dC(pC)_{34}$. At 10 mM NaCl (pH 8.1, 25.0 °C), the macroscopic equilibrium constants, $K_{2/35}$, for binding the second $dN(pN)_{34}$ molecule to the SSB tetramer are $8.0 \times 10^4$, $9.5 \times 10^5$, and $2.2 \times 10^7$ $M^{-1}$ for $dA(pA)_{34}$, $dT(pT)_{34}$, and $dC(pC)_{34}$, respectively. Hence, the binding of the second molecule is a factor of nearly 300 lower for $dA(pA)_{34}$ than for $dC(pC)_{34}$ and a factor of ~23 lower for $dT(pT)_{34}$ than for $dC(pC)_{34}$. The fact that negative cooperativity is least for $dC(pC)_{34}$ is consistent with the observation that the $(SSB)_{56}$ mode, in which all four subunits interact with DNA, forms on poly(dC) under low-salt conditions ([NaCl] < 10 mM) that favor the $(SSB)_{35}$ mode on poly(dT) and poly(dA). As suggested from our previous studies with poly(dT) and oligodeoxythymidylates (Lohman & Bujalowski, 1988), the presence and degree of negative cooperativity correlate with the stability of the $(SSB)_{35}$ polynucleotide binding mode.

*Salt Dependence of Oligodeoxynucleotide Binding.* At 10 mM NaCl, we are unable to determine the value of the negative cooperativity parameter, $\sigma_{35}$, for any of the oligodeoxynucleotides $dA(pA)_{34}$, $dT(pT)_{34}$ or $dC(pC)_{34}$, since the equilibrium constant, $K_{35}$, for binding the first $dN(pN)_{34}$ is too high to measure accurately at this [NaCl]. However, both $K_{35}$ and $\sigma_{35}$ can be measured accurately at higher salt concentration. The ranges of salt concentrations over which $K_{35}$ and $\sigma_{35}$ could be determined accurately overlap for $dT(pT)_{34}$ and $dC(pC)_{34}$ (0.3–3 M NaBr or NaCl); however, since the affinity of the SSB–$dA(pA)_{34}$ interaction is much lower, a considerably lower salt concentration range was required for the $dA(pA)_{34}$ measurements (0.05–0.2 M). Representative isotherms for the binding of $dA(pA)_{34}$ to the SSB tetramer at several NaCl concentrations (25.0 °C, pH 8.1) are shown in Figure 5. Note the extreme biphasic character of the isotherms at low [NaCl], indicative of high negative cooperativity ($\sigma_{35} \ll 1$). Furthermore, the extent of fluorescence quenching upon binding the first molecule of $dA(pA)_{34}$ is much larger (42%) than for binding the second molecule of $dA(pA)_{34}$ (17%). In contrast,

Table 1: Salt Dependencies of the Equilibrium Constants for $dN(pN)_{34}$ and $dN(pN)_{69}$ Binding to the SSB Tetramer[a]

| oligodeoxynucleotide | $\frac{\partial \log K_N}{\partial \log[NaCl]}$ | $\frac{\partial \log K_N}{\partial \log[NaBr]}$ |
|---|---|---|
| $dA(pA)_{34}$ | −5.4 ± 0.5 | −11.1 ± 1 |
| $dA(pA)_{69}$ | −5.6 ± 0.5 | −7.2 ± 0.7 |
| $dC(pC)_{34}$ | −4.3 ± 0.4 | −6.3 ± 0.6 |
| $dC(pC)_{69}$ | −2.4 ± 0.3 | −3.8 ± 0.6 |
| $dT(pT)_{34}$[b] | −3.4 ± 0.3<br>−0.9 ± 0.2 | −4.6 ± 0.5 |
| $dT(pT)_{69}$[b] | n.d. | −7.1 ± 0.6 |

[a] Buffer T, pH 8.1, 25.0 °C. [b] Data taken from Bujalowski and Lohman (1989b); n.d., not able to be determined.

Table 2: Equilibrium Interaction Constants for SSB Tetramer Binding to $dN(pN)_{34}$ (Buffer T, pH 8.1, 25.0 °C)[a]

| | $K_{35}$ ($M^{-1}$) | $\sigma_{35}$ |
|---|---|---|
| | $dA(pA)_{34}$ | |
| [NaCl] (M) | | |
| 0.050 | $4.64 \times 10^8$ | 0.20 |
| 0.075 | $1.17 \times 10^8$ | 0.32 |
| 0.10 | $1.58 \times 10^7$ | 0.50 |
| 0.15 | $1.47 \times 10^6$ | 0.87 |
| 0.20 | $5.20 \times 10^5$ | 1.0 |
| [NaBr] (M) | | |
| 0.060 | $2.0 \times 10^9$ | 0.15 |
| 0.079 | $6.56 \times 10^7$ | 0.30 |
| 0.10 | $3.55 \times 10^6$ | 0.60 |
| 0.12 | $1.08 \times 10^6$ | 0.77 |
| $Q_{1/35} = 0.42$; $Q_{2/35} = 0.59$ | | |
| | $dC(pC)_{34}$ | |
| [NaCl] (M) | | |
| 0.495 | $3.0 \times 10^9$ | 0.65 |
| 0.99 | $3.0 \times 10^8$ | 0.62 |
| 1.24 | $9.5 \times 10^7$ | 0.64 |
| 1.49 | $2.5 \times 10^7$ | 0.65 |
| 2.23 | $7.3 \times 10^6$ | 0.66 |
| [NaBr] (M) | | |
| 0.396 | $6.0 \times 10^8$ | 0.57 |
| 0.594 | $9.0 \times 10^7$ | 0.57 |
| 0.792 | $1.1 \times 10^7$ | 0.64 |
| $Q_{1/35} = 0.45$; $Q_{2/35} = 0.90$ | | |

[a] The interaction constants, $K_{35}$ and $\sigma_{35}$, are based on the "square" model (see eqs 1 and 3). The errors associated with the determination of $K_{35}$ and $\sigma_{35}$ are ±10–15% of the reported values.

equal fluorescence quenching (45%) is associated with the binding of both the first and the second molecule of $dC(pC)_{34}$ to the SSB tetramer (data not shown). These isotherms were analyzed to determine $K_{35}$ and $\sigma_{35}$ at each [NaCl] (see eqs 1 and 2) as described previously (Bujalowski & Lohman, 1989a,b), and these are given in Table 2 and plotted in Figures 6 and 7. Figure 7 also includes the values of $K_{35}$ and $\sigma_{35}$ for $dT(pT)_{34}$ binding to the SSB tetramer that we reported previously (Bujalowski & Lohman, 1989b).

$K_{35}$ is observed to decrease with increasing [NaCl] or [NaBr] for both $dC(pC)_{34}$ and $dA(pA)_{34}$. For $dC(pC)_{34}$, $\partial \log K_{35}/\partial \log[NaCl] = -4.3 \pm 0.4$ and $\partial \log K_{35}/\partial \log[NaBr] = -6.3 \pm 0.5$, whereas for $dA(pA)_{34}$, $\partial \log K_{35}/\partial \log[NaCl] = -5.4 \pm 0.5$ and $\partial \log K_{35}/\partial \log[NaBr] = -11.1 \pm 1$. In Table 1 and Figure 7, these salt dependencies are compared with those obtained previously for $dT(pT)_{34}$. For $dC(pC)_{34}$ binding, $\sigma_{35}$ has a value of 0.63 ± 0.02, independent of salt concentration in the experimentally accessible range from 0.3 to 3 M [NaCl] or [NaBr]. This is significantly larger than the value of $\sigma_{35} = 0.54 \pm 0.02$ obtained with $dT(pT)_{34}$, which is also independent of [NaCl] and [NaBr] under the same solution conditions (Bujalowski & Lohman, 1989b). For $dA(pA)_{34}$,

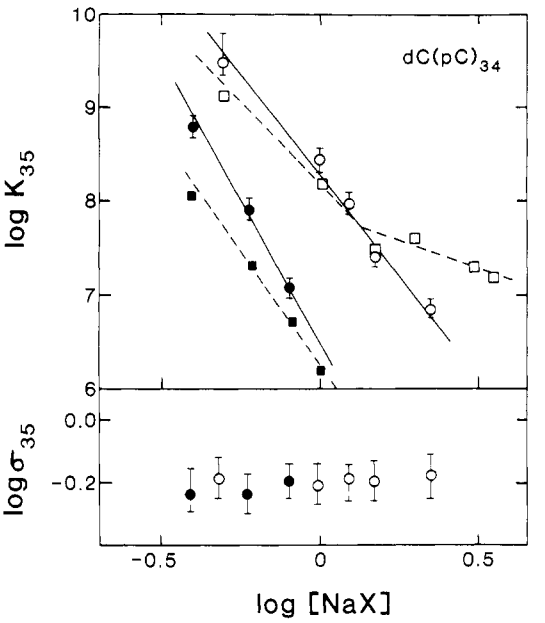


FIGURE 6: Dependencies of the equilibrium binding constant $K_{35}$ and the negative cooperativity constant $\sigma_{35}$ on NaCl (○) and NaBr (●) concentration (log–log plots) for the SSB tetramer–$dC(pC)_{34}$ interaction in buffer T (pH 8.1, 25 °C). $K_{35}$ and $\sigma_{35}$ were obtained from analyses of binding isotherms, according to eqs 1 and 3. Data for $dT(pT)_{34}$ (Bujalowski & Lohman, 1989b) are also shown (■, □).

which could only be studied at much lower salt concentrations (0.05–0.2 M NaCl or NaBr), $\sigma_{35}$ is dependent upon anion type and increases with increasing salt concentration ($\partial \log \sigma_{35}/\partial \log[NaCl] = 1.5 \pm 0.2$, and $\partial \log \sigma_{35}/\partial \log[NaBr] = 2.8 \pm 0.2$).

The increase in $\sigma_{35}$ with increasing salt concentration observed for $dA(pA)_{34}$ binding is similar qualitatively to the increase in $\sigma_{16}$ with increasing salt concentration observed for $dT(pT)_{15}$ binding at [NaCl] < 0.5 M and indicates that an uptake of ions is associated with the process that causes negative cooperativity (Bujalowski & Lohman, 1989b). However, $\sigma_{16}$ for $dT(pT)_{15}$ binding is unaffected by replacing $Cl^-$ with $Br^-$ (Bujalowski & Lohman, 1989b). The data in Figure 7 suggest that $\sigma_{35}$ for $dA(pA)_{34}$ binding will plateau at [NaCl] > 0.5 M, as is observed for $dT(pT)_{15}$ and $dT(pT)_{34}$ binding (Bujalowski & Lohman, 1989b). The fact that $\sigma_{35}$ for $dC(pC)_{34}$ binding is independent of salt concentration is likely a consequence of the fact that these experiments could only be performed at [NaCl] and [NaBr] > 0.5 M, conditions under which $\sigma_{35}$ has already reached its high salt plateau value. It is interesting that for $dA(pA)_{34}$ binding, $\sigma_{35}$ increases to a value of 1 at 0.2 M NaCl, indicating that binding of $dA(pA)_{34}$ is *non-cooperative* at this salt concentration. This is in contrast to both $dT(pT)_{34}$ and $dC(pC)_{34}$, which retain some degree of negative cooperativity even at [NaCl] > 0.5 M [$\sigma_{35} = 0.63 \pm 0.02$ for $dC(pC)_{34}$ and $\sigma_{35} = 0.52 \pm 0.2$ for $dT(pT)_{34}$ (Bujalowski & Lohman, 1989b)]. Therefore, although $\sigma_{35}$ appears to be lowest for $dA(pA)_{34}$ at low [NaCl] (e.g., 10 mM, as in Figure 4), it is largest for $dA(pA)_{34}$ at high [NaCl] (>0.2 M).

We have also measured the equilibrium constants, $K_{70}$, for SSB tetramer binding to $dC(pC)_{69}$ and $dA(pA)_{69}$ under high-salt conditions (25 °C, pH 8.1) that support 1:1 complex formation in which all four SSB subunits interact with DNA. The results are presented in Table 3 and are plotted in Figure 8 as log $K_{70}$ *vs* log[NaX] (X = Cl or Br). Clearly there is a substantial effect of base composition on both $K_{70}$ and its dependence on monovalent salt concentration. In NaBr, $K_{70}$

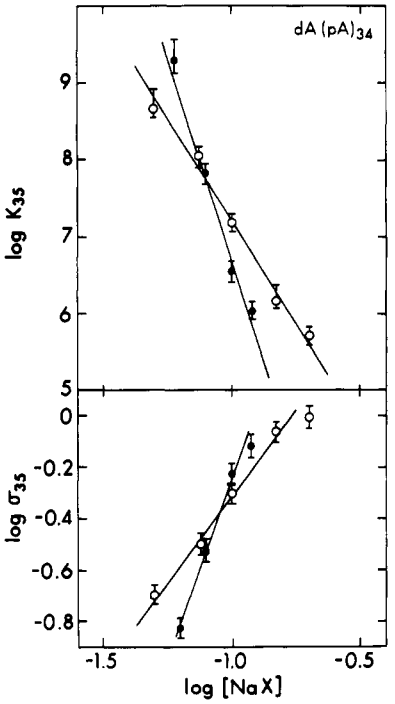


FIGURE 7: Dependencies of the equilibrium binding constant $K_{35}$ and the negative cooperativity constant $\sigma_{35}$ on NaCl (○) and NaBr (●) concentration (log–log plots) for the SSB tetramer–dA(pA)$_{34}$ interaction in buffer T (pH 8.1, 25 °C). $K_{35}$ and $\sigma_{35}$ were obtained from analyses of binding isotherms, according to eqs 1 and 3.

Table 3: Equilibrium Constants for SSB Tetramer Binding to dN(pN)$_{69}$ (Buffer T, pH 8.1, 25.0 °C)

| | $K_{70}$ ($M^{-1}$)[a] |
|---|---|
| dA(pA)$_{69}$ | |
| [NaCl] (M) | |
| 0.15 | $3.0 \times 10^7$ |
| 0.174 | $1.2 \times 10^7$ |
| 0.20 | $6.1 \times 10^6$ |
| 0.263 | $1.47 \times 10^6$ |
| [NaBr] (M) | |
| 0.10 | $4.47 \times 10^7$ |
| 0.126 | $7.9 \times 10^6$ |
| 0.15 | $2.5 \times 10^6$ |
| dC(pC)$_{69}$ | |
| [NaCl] (M) | |
| 0.98 | $3.0 \times 10^8$ |
| 1.5 | $1.26 \times 10^8$ |
| 2.4 | $5.8 \times 10^7$ |
| 3.0 | $3.4 \times 10^7$ |
| [NaBr] (M) | |
| 0.79 | $1.47 \times 10^8$ |
| 1.15 | $3.83 \times 10^7$ |
| 1.5 | $1.26 \times 10^7$ |

[a] $Q_{max} = 0.64$ for dA(pA)$_{69}$. $Q_{max} = 0.85$ in NaCl for dC(pC)$_{69}$. $Q_{max}$ varied from 0.83 to 0.75 in NaBr for dC(pC)$_{69}$. The errors associated with the determination of $K_{70}$ are ±10–15% of the reported values.

is greatest for dT(pT)$_{69}$, intermediate for dC(C)$_{69}$, and lowest for dA(pA)$_{69}$. With the exception of the dC(pC)$_{69}$ data in NaBr, the plots of log $K_{70}$ *vs* log[NaX] are linear, with $\partial \log K_{70}/\partial \log[NaX] = -3.8 \pm 0.6$ and $-2.4 \pm 0.3$ for dC(pC)$_{69}$ in NaBr and NaCl, respectively, and $-7.2 \pm 0.7$ and $-5.6 \pm 0.5$ for dA(pA)$_{69}$ in NaBr and NaCl, respectively (see Table 1). (Note that the value of $\partial \log K_{70}/\partial \log[NaBr] = -3.8 \pm 0.6$ was calculated by ignoring the datum point at 0.4 M NaBr.) Note also that the lower value of $K_{70}$ at 0.4 M NaBr indicates that the data at higher [NaBr] cannot be used to determine values of $K_{70}$ by linear extrapolation to lower [NaBr].

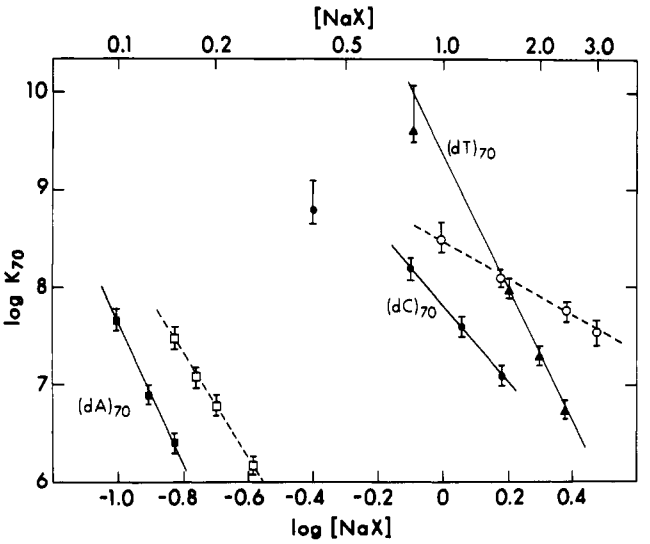


FIGURE 8: Dependencies of the logarithm of the equilibrium binding constant $K_{70}$ on the logarithm of the monovalent salt concentration ([NaX]) for SSB tetramer binding to dA(pA)$_{69}$, dC(pC)$_{69}$, and dT(pT)$_{69}$ in buffer T (pH 8.1), 25 °C: SSB–dA(pA)$_{69}$ in NaCl (□) and NaBr (■); SSB–dC(pC)$_{69}$ in NaCl (○) and NaBr (●). Data for SSB binding to dT(pT)$_{69}$ in NaBr (▲) from Bujalowski and Lohman (1989b) are also shown.

## DISCUSSION

Our previous systematic studies of the different modes of *E. coli* SSB binding to ss polynucleotides and the effects of salt concentration were performed using poly(dT) since SSB has the highest affinity for this polynucleotide, thus making determinations of the binding site size easiest with poly(dT) (Lohman & Overman, 1985; Bujalowski & Lohman, 1986; Bujalowski *et al.*, 1988; Wei *et al.*, 1992). Although Lohman and Overman (1985) had shown that *E. coli* SSB tetramers can form the different binding modes on other ss polynucleotides, we wished to examine this systematically over a range of NaCl concentrations. Here we show that SSB can form the (SSB)$_{35}$, (SSB)$_{56}$, and (SSB)$_{65}$ binding modes with poly(U) and poly(A) at 25 °C (pH 8.1), although the [NaCl]- and [NaF]-induced transitions between modes are shifted to slightly higher salt concentrations than that observed with poly(dT). Furthermore, with poly(U) and poly(A), SSB also forms complexes with a site size of 40 nucleotides per tetramer at 25 °C (pH 8.1), whereas complexes possessing this site size have previously been observed with poly(dT) at 37 °C, but not at 25 °C (Bujalowski *et al.*, 1986).

Interestingly, poly(dC) seems to be unique in not forming a stable (SSB)$_{35}$ mode (as measured by reverse titrations) even at low NaCl concentrations (<10 mM NaCl), although this mode is observed with poly(dT), poly(A), poly(dA), and poly(U). At monovalent salt concentrations below 10 mM (25 °C, pH 8.1), the SSB tetramer forms a stable (SSB)$_{56}$ complex with poly(dC) (average site size, 58 ± 3 nucleotides per tetramer), whereas above 0.2 M NaCl, a stable (SSB)$_{65}$ complex is formed (average site size, 68 ± 3 nucleotides per tetramer). In this regard, a site size of 37 nucleotides per tetramer for SSB binding to poly(dC) in 1 mM NaCl (pH 8.1, 25.0 °C) reported earlier by Lohman and Overman (1985) appears to be underestimated, although we have no explanation for this discrepancy. At this point, poly(dC) seems to be the only ss polynucleotide that does not form the (SSB)$_{35}$ mode at low [NaCl].

*Negative Cooperativity within the SSB Tetramer Is Influenced by Base Composition and Affects the Stability of*

*the $(SSB)_{35}$ Polynucleotide Binding Mode.* The instability of the $(SSB)_{35}$ binding mode on poly(dC) appears to be due, at least partially, to the fact that the negative cooperativity associated with DNA binding within the SSB tetramer is significantly lower for oligodeoxycytidylates compared to oligodeoxyadenylates and oligodeoxythymidylates, at least at low salt concentrations. This results in a much higher macroscopic affinity of oligodeoxycytidylates to the third and fourth subunits of an SSB tetramer. We do not yet understand the molecular basis for this effect of base composition. We have shown previously that there is a large electrostatic effect on negative cooperativity; however, on the basis of our current studies it is clear that electrostatics is not the sole factor regulating negative cooperativity. Our current studies also indicate that the negative cooperativity for binding oligodeoxyadenylates is significantly larger than that for binding oligodeoxythymidylates. This suggests that, at low NaCl concentrations, the stability of the $(SSB)_{35}$ mode should decrease in the order poly(dA) > poly(dT) > poly(dC), although this assumes that there are no base-composition effects on the positive cooperativity parameter ($\omega_{35}$) that occurs between SSB tetramers in the $(SSB)_{35}$ mode; however, this latter point has not been examined (Ferrari *et al.*, 1994).

The dramatic differences in the macroscopic affinities for binding a second molecule of $dC(pC)_{34}$, $dT(pT)_{34}$, or $dA(pA)_{34}$ to the SSB tetramer at low salt concentration also provide further evidence that the lower affinity for the second molecule of $dN(pN)_{34}$ is due to a true negative cooperativity, as shown previously on the basis of studies of $dT(pT)_{34}$ and $dT(pT)_{15}$ (Lohman & Bujalowski, 1988; Bujalowski & Lohman, 1989a,b), rather than a steric constraint or a low-salt-induced asymmetry in the SSB tetramer. Otherwise, one would expect to see the same extent of inhibition for oligodeoxynucleotides of the same length, which is clearly not the case.

*Influence of Base Composition on the Equilibrium Binding Affinity of Oligodeoxynucleotides for the SSB Tetramer.* It has been shown previously that the equilibrium binding affinity of the *E. coli* SSB tetramer for polynucleotides is dependent upon base composition (Overman *et al.*, 1988; Bobst *et al.*, 1991). In general, the SSB tetramer binds with highest affinity to poly(dT) (Krauss *et al.*, 1981; Overman *et al.*, 1988; Bobst *et al.*, 1991) and to the other ss homopolynucleotides in the following order of decreasing affinity: poly(dT) > poly(dC) > poly(I) > poly(U) > poly(dA) > poly(A) > poly(C) (Overman *et al.*, 1988). It is also the case that both the phage T4 gene 32 protein (Newport *et al.*, 1981) and the phage fd gene V protein (Bobst *et al.*, 1984; Bulsink *et al.*, 1985; de Jong *et al.*, 1987; Sang & Gray, 1989) bind with highest affinity to poly(dT).

Under conditions that favor interaction of the longer oligodeoxynucleotides, $dN(pN)_{69}$, with all four subunits of the SSB tetramer, the equilibrium constant $K_{70}$ displays the same qualitative dependence on base composition as is observed for the binding of SSB to ss homopolynucleotides (Overman *et al.*, 1988), i.e., $K_{70}(dT(pT)_{69}) > K_{70}(dC(pC)_{69}) \gg K_{70}(dA(pA)_{69})$. At 0.8 M NaBr (25 °C, pH 8.1), $K_{70}$ is ~28-fold larger for $dT(pT)_{69}$ than for $dC(pC)_{69}$; however, this decreases to a factor of ~10 at 1.6 M NaBr. However, since the dependence of $K_{70}$ on salt concentration for $dC(pC)_{69}$ is lower than for either $dA(pA)_{69}$ or $dT(pT)_{69}$, the relative binding affinities are also dependent on salt concentration and type (e.g., NaCl *vs* NaBr). Since we cannot measure $K_{70}$ for SSB-$dT(pT)_{69}$ binding in NaCl, due to its high affinity, we can only make quantitative comparisons of $K_{70}(dC(pC)_{69})$ and $K_{70}(dA(pA)_{69})$, although we can state that $K_{70}(dT(pT)_{69}) > K_{70}(dC(pC)_{69}) \gg K_{70}(dA(pA)_{69})$ in NaCl as well.

On the other hand, the shorter oligodeoxynucleotides $dC(pC)_{34}$ and $dT(pT)_{34}$, which interact with only two subunits of the SSB tetramer, have binding constants, $K_{35}$, that are identical at ~1.3 M NaCl (25 °C, pH 8.1). However, since the dependence of $K_{35}$ on [NaCl] differs for these oligodeoxynucleotides (see Figure 6), $dC(pC)_{34}$ binds with slightly higher affinity below 1.3 M NaCl, whereas $dT(pT)_{34}$ binds with slightly higher affinity above 1.3 M NaCl. Surprisingly, in NaBr, $dC(pC)_{34}$ binds with higher affinity than $dT(pT)_{34}$, ranging from a factor of ~2 at 0.8 M NaBr to a factor ~5 at 0.4 M NaBr, although the data in Figure 6 suggest that identical values for $K_{35}$ would be obtained for $dC(pC)_{34}$ and $dT(pT)_{34}$ near 1.2 M NaX. Therefore, the relative affinities of $dC(pC)_{34}$ and $dT(pT)_{34}$ are *reversed* from those observed for $dC(pC)_{69}$ and $dT(pT)_{69}$ at higher [NaBr]. However, both $dC(pC)_{34}$ and $dT(pT)_{34}$ bind to SSB with much higher affinities than does $dA(pA)_{34}$ (a factor of $10^4$–$10^5$ higher at 0.3 M NaCl), which is similar to the behavior observed with $dA(pA)_{69}$ and poly(dA) (Overman *et al.*, 1988).

We also observe that the magnitudes and salt dependencies of the equilibrium constants for SSB binding to these oligodeoxynucleotides are influenced by the type of anion comprising the monovalent salt. For $dC(pC)_{34}$, $dC(pC)_{69}$, and $dA(pA)_{69}$, $K_{70}$ and $K_{35}$ both decrease upon replacing NaCl with NaBr. A similar influence of $Cl^-$ *vs* $Br^-$ has previously been reported for SSB binding to poly(U) in the $(SSB)_{65}$ binding mode (Overman *et al.*, 1988) and indicates differences in anion binding to the SSB protein *vs* the SSB–ss-DNA complex. Although we also observe that $K_{35}$ for $dA(pA)_{34}$ binding to the SSB tetramer is influenced differently by $Cl^-$ *vs* $Br^-$, the behavior is different than for $dC(pC)_{34}$, $dC(pC)_{69}$, and $dA(pA)_{69}$. The dependencies of $K_{35}$ on [NaBr] and [NaCl] for the binding of $dA(pA)_{34}$ cross over at ~80 mM $Na^+$, so that, above 80 mM $Na^+$, $dA(pA)_{34}$ binds with higher affinity in NaCl, whereas below 80 mM $Na^+$, $dA(pA)_{34}$ binds with higher affinity in NaBr. This crossover likely reflects the fact that both ion uptake and release occur upon binding $dA(pA)_{34}$ and possibly that ion uptake dominates at low salt, whereas ion release dominates at higher salt concentrations.

The negative cooperativity parameter, $\sigma_{35}$, that describes the SSB–$dA(pA)_{34}$ interaction is also influenced by anion type. In fact, a crossover similar to the one observed for $K_{35}$ also occurs for the dependence of $\sigma_{35}$ on NaCl *vs* NaBr near 90 mM $Na^+$. This is interesting since for both $dT(pT)_{34}$ and $dC(pC)_{34}$, as well as for $dT(pT)_{15}$, $\sigma_{35}$ and $\sigma_{16}$ are independent of anion ($Cl^-$ *vs* $Br^-$) under the same conditions (25 °C, pH 8.1). These results provide further evidence that negative cooperativity is influenced by factors in addition to the electrostatic nature of the nucleic acid; otherwise, anion type would not be expected to influence $\sigma_{35}$.

*Both Base Composition and Length Influence the Net Release of Ions upon Oligodeoxynucleotide Binding of E. coli SSB Protein.* The dependence on monovalent salt concentration of the observed equilibrium constant for SSB tetramer binding to ss polynucleotides in its $(SSB)_{65}$ mode is nearly independent of base composition (Overman *et al.*, 1988). However, we show here that base composition does influence the salt dependence of the equilibrium constant for SSB binding to oligodeoxynucleotides. The values of $\partial \log K_{35}/\partial \log[NaCl]$ vary, with values of $-5.4 \pm 0.5$, $-4.3 \pm 0.4$, and $-3.4 \pm 0.3$ for $dA(pA)_{34}$, $dC(pC)_{34}$, and $dT(pT)_{34}$, respectively (25 °C, pH 8.1), and $\partial \log K_{35}/\partial \log[NaBr]$ varies with values of $-11.1 \pm 1$, $-6.3 \pm 0.5$, and $-4.6 \pm 0.5$ for $dA(pA)_{34}$, dC-

$(pC)_{34}$, and $dT(pT)_{34}$, respectively (25 °C, pH 8.1). These negative salt dependencies indicate a net release of ions upon formation of these SSB–DNA complexes (Wyman, 1964; Record *et al.*, 1976, 1978). Base-dependent differences in net ion release are also observed for binding of the $dN(pN)_{69}$ oligadeoxynucleotides, which interact with all four SSB subunits, with the largest net ion release occurring with $dA(pA)_{69}$ ($\partial \log K_{70}/\partial \log[NaCl] = -5.6 \pm 0.5$, and $\partial \log K_{70}/\partial \log[NaBr] = -7.2 \pm 0.7$) and the smallest with $dC(pC)_{69}$ ($\partial \log K_{70}/\partial \log[NaCl] = -2.4 \pm 0.3$ and $\partial \log K_{70}/\partial \log[NaBr] = -3.8 \pm 0.6$). This suggests that the oligodeoxynucleotide base composition influences the extent of ion binding to the SSB–oligodeoxynucleotide complexes, since only minor differences in cation-binding properties exist for the different oligodeoxynucleotides (Mascotti & Lohman, 1993).

A further interesting result of these studies is the observation that the net ion release accompanying oligodeoxynucleotide binding to the *E. coli* SSB tetramer *increases* with *decreasing* oligodeoxynucleotide length; i.e., $|\partial \log K_{70}/\partial \log[NaBr]| < |\partial \log K_{35}/\partial \log[NaBr]|$ for the oligodeoxyadenylates as well as the oligodeoxycytidylates. This indicates that fewer ions are released upon binding of the SSB tetramer to the oligodeoxynucleotide $[dN(pN)_{69}]$ that interacts with all four SSB subunits than upon binding to an oligodeoxynucleotide $[dN(pN)_{34}]$ that interacts with only two SSB subunits. This is the *opposite* of the expectation that net ion release would decrease with decreasing oligonucleotide length if it were due only to cation release from the nucleic acid, since shorter oligonucleotides bind fewer cations per phosphate (Record & Lohman, 1978; Olmsted *et al.*, 1989) and also may not form as many interactions as with longer oligonucleotides. In fact, the net ion release accompanying the binding of two molecules of $dN(pN)_{34}$ generally exceeds the net ion release accompanying the binding of one molecule of $dN(pN)_{69}$, which indicates that there must be ion *uptake* associated with binding of DNA to the third and fourth SSB subunits.

Although for $dA(pA)_{34}$ and $dA(pA)_{69}$ part of this ion uptake is associated with the negative cooperativity ($\partial \log \sigma_{35}/\partial \log[NaBr] = 2.8$, and $\partial \log \sigma_{35}/\partial \log[NaBr] = 1.5$), there is still an additional ion uptake that results from binding of the longer $dA(pA)_{69}$, when compared to binding of two molecules of $dA(pA)_{34}$. In fact, for all three sets of oligodeoxynucleotides examined, the net ion release associated with the binding of one molecule of $dN(pN)_{69}$ is less than the net ion release for the binding of two molecules of $dN(pN)_{34}$, even after correction for the ion uptake associated with negative cooperativity. This additional ion uptake can be estimated by comparing the salt dependency for saturating the SSB tetramer with one molecule of $dN(pN)_{69}$, $K_{70}$, with that of the overall binding constant for saturating the SSB tetramer with two molecules of $dN(pN)_{34}$, given by $3(\sigma_{35})^4(K_{35})^2$ (see eq 1). The largest difference is seen for the oligodeoxycytidylates in NaBr, which indicates that an additional 8.8 ions are taken up upon saturating the SSB tetramer with one molecule of $dC(pC)_{69}$ *vs* two molecules of $dC(pC)_{34}$; in NaCl, an additional 6.2 ions are taken up. For the oligodeoxythymidylates in NaBr, an additional 2.1 ions are taken up (Bujalowski & Lohman, 1989a). In NaBr, an additional 3.8 ions are bound upon saturating the SSB tetramer with $dA(pA)_{69}$ [*vs* two molecules of $dA(pA)_{34}$], whereas in NaCl, relatively little difference in ion release is observed upon binding of two molecules of $dA(pA)_{34}$ (−4.8) *vs* one molecule of $dA(pA)_{69}$ (−5.6), these values being nearly identical within experimental error. Overall, these results demonstrate that ion uptake does accompany SSB–DNA binding; that the extent of ion uptake is sensitive to oligodeoxynucleotide length, base composition, and anion type; and that the free energy changes upon SSB–ss-DNA complexation are nonadditive. As yet, we cannot rule out that some of this ion uptake may also be due to cation binding. The molecular basis for this additional ion uptake is not known; however, it is partly electrostatic in nature and may result from the additional negatively charged phosphates of the longer nucleic acid that are brought in close contact with negative charges on the SSB protein. In this respect, this additional ion uptake may be related to the ion uptake that is associated with the negative cooperativity, which is at least partially electrostatic in nature (Lohman & Bujalowski, 1988; Bujalowski & Lohman, 1989b).

Our observation that the relative stability of the $(SSB)_{35}$ polynucleotide binding mode can be influenced so significantly by DNA base composition suggests that mutations in the SSB protein might exist that can also influence these binding mode transitions. In fact, such a mutant has been described recently by Curth *et al.* (1993), who showed that the $(SSB)_{35}$ binding mode on poly(dT) is stabilized significantly for an SSB mutant in which Trp-54 was changed to Ser. The $(SSB)_{35}$ binding mode is observed to form in a reverse titration even at 0.3 M [NaCl] for the SSBW54S mutant, whereas the wild-type SSB tetramer binds to poly(dT) in the $(SSB)_{65}$ mode under the same conditions. Although the SSBW54S mutant can complement an *ssb* deletion strain, the resulting cells show slow growth rates and are very UV sensitive, suggesting defects in DNA replication and some repair processes (Carlini *et al.*, 1993). The isolation and characterization of other *ssb* mutants that influence the relative stability of the different SSB polynucleotide binding modes will facilitate studies to determine whether any of the different SSB binding modes function selectively in replication, recombination, or repair.

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## REFERENCES

Alberts, B. M., & Frey, L. (1970) *Nature (London) 227*, 1313–1318.

Bobst, A. M., Ireland, J. C., & Bobst, E. V. (1984) *J. Biol. Chem. 259*, 2130–2134.

Bobst, E. V., Perrino, F. W., Meyer, R. R., & Bobst, A. M. (1991) *Biochim. Biophys. Acta 1078*, 199–207.

Bujalowski, W., & Lohman, T. M. (1986) *Biochemistry 25*, 7799–7802.

Bujalowski, W., & Lohman, T. M. (1987) *J. Mol. Biol. 195*, 897–907.

Bujalowski, W., & Lohman, T. M. (1989a) *J. Mol. Biol. 207*, 249–268.

Bujalowski, W., & Lohman, T. M. (1989b) *J. Mol. Biol. 207*, 269–288.

Bujalowski, W., & Lohman, T. M. (1991a) *J. Biol. Chem. 266*, 1616–1626.

Bujalowski, W., & Lohman, T. M. (1991b) *J. Mol. Biol. 217*, 63–74.

Bujalowski, W., Overman, L. B., & Lohman, T. M. (1988) *J. Biol. Chem. 263*, 4629–4640.

Bulsink, H., Harmsen, B. J. M., & Hilbers, C. W. (1985) *J. Biomol. Struct. Dyn. 3*, 227–247.

Carlini, L. E., Porter, R. D., Curth, U., & Urbanke, C. (1993) *Mol. Microbiol. 10*, 1067–1075.

Chase, J. W., & Williams, K. R. (1986) *Annu. Rev. Biochem. 55*, 103–136.

Chrysogelos, S., & Griffith, J. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 5803–5807.
Curth, U., Greipel, J., Urbanke, C., & Maass, G. (1993) *Biochemistry 32*, 2585–2591.
de Jong, E. A. M., Harmsen, B. J. M., Konings, R. N. H., & Hilbers, C. W. (1987) *Biochemistry 26*, 2039–2046.
Ferrari, M. E., Bujalowski, W., & Lohman, T. M. (1994) *J. Mol. Biol. 236*, 106–123.
Greipel, J., Urbanke, C., & Maass, G. (1989) in *Protein-Nucleic Acid Interaction* (Saenger, W., & Heinemann, U., Eds.) pp 61–86, CRC Press, Inc., Boca Raton, FL.
Griffith, J. D., Harris, L. D., & Register, J., III (1984) *Cold Spring Harbor Symp. Quant. Biol. 49*, 553–559.
Halfman, C. J., & Nishida, T. (1972) *Biochemistry 11*, 3493–3498.
Karpel, R. L. (1990) in *The Biology of Non-specific DNA-protein Interactions* (Revzin, A., Ed.) pp 103–130, CRC Press, Inc., Boca Raton, FL.
Kowalczykowski, S. C., Bear, D. G., & von Hippel, P. H. (1981a) in *The Enzymes* (Boyer, P. D., Ed.) pp 373–444, Academic Press, New York.
Kowalczykowski, S. C., Lonberg, N., Newport, J. W., & von Hippel, P. H. (1981b) *J. Mol. Biol. 145*, 75–104.
Krauss, G., Sindermann, H., Schomburg, U., & Maass, G. (1981) *Biochemistry 20*, 5346–5352.
Kuil, M. E., Holmlund, K., Vlaanderen, C. A., & van Grondelle, R. (1990) *Biochemistry 29*, 8184–8189.
Lakowicz, J. R. (1983) *Principles of Fluorescence Spectroscopy*, Plenum Press, New York.
Lohman, T. M. (1984) *Biochemistry 23*, 4656–4665.
Lohman, T. M., & Overman, L. B. (1985) *J. Biol. Chem. 260*, 3594–3603.
Lohman, T. M., & Bujalowski, W. (1988) *Biochemistry 27*, 2260–2265.
Lohman, T. M., & Bujalowski, W. (1990) in *The Biology of Nonspecific DNA-Protein Interactions* (Revzin, A., Ed.) pp 131–168, CRC Press, Boca Raton, FL.
Lohman, T. M., & Bujalowski, W. (1991) *Methods in Enzymology* (Sauer, R. T., Ed.) Vol. *208*, pp 258–290, Academic Press, Inc., New York.
Lohman, T. M., & Ferrari, M. E. (1994) *Annu. Rev. Biochem. 63* (in press).
Lohman, T. M., Bujalowski, W., & Overman, L. B. (1988) *Trends Biochem. Sci. 13*, 250–255.
Lohman, T. M., Green, J. M., & Beyer, S. (1986a) *Biochemistry 25*, 21–25.
Lohman, T. M., Overman, L. B., & Datta, S. (1986b) *J. Mol. Biol. 187*, 603–615.
Mascotti, D. P., & Lohman, T. M. (1993) *Biochemistry 32*, 10568–10579.
Meyer, R. R., & Laine, P. S. (1990) *Microbiol. Rev. 54*, 342–380.
Morrical, S. W., & Cox, M. M. (1990) *Biochemistry 29*, 837–843.
Muniyappa, K., Williams, K., Chase, J. W., & Radding, C. M. (1990) *Nucleic Acids Res. 18*, 3967–3973.
Newport, J. W., Lonberg, N., Kowalczykowski, S. C., & von Hippel, P. H. (1981) *J. Mol. Biol. 145*, 105–121.
Olmsted, M. C., Anderson, C. F., & Record, M. T., Jr. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 7766–7770.
Overman, L. B. (1989) Thermodynamic characterization of *Escherichia coli* single strand binding protein–single stranded polynucleotide interactions, Ph.D. Thesis, Texas A&M University.
Overman, L. B., Bujalowski, W., & Lohman, T. M. (1988) *Biochemistry 27*, 456–471.
Record, M. T., Lohman, T. M., & de Haseth, P. L. (1976) *J. Mol. Biol. 107*, 145–158.
Record, M. T., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103–178.
Record, M. T., Jr., & Lohman, T. M. (1978) *Biopolymers 17*, 159–166.
Ruyechan, W. T., & Wetmur, J. G. (1975) *Biochemistry 14*, 5529–5534.
Sang, B-C., & Gray, D. M. (1989) *J. Biomol. Struct. Dyn. 7*, 693–706.
Sigal, N., Delius, H., Kornberg, T., Gefter, M. L., & Alberts, B. M. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 3537–3530.
Wei, T.-F., Bujalowski, W., & Lohman, T. M. (1992) *Biochemistry 31*, 6166–6174.
Williams, K. R., Murphy, J. B., & Chase, J. W. (1984) *J. Biol. Chem. 259*, 11804–11811.
Wyman, J. (1964) *Adv. Protein Chem. 19*, 223–286.