Two Binding Modes in *Escherichia coli* Single Strand Binding Protein—Single Stranded DNA Complexes

MODULATION BY NaCl CONCENTRATION

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The binding properties of the *Escherichia coli* encoded single strand binding protein (SSB) to a variety of synthetic homopolynucleotides, as well as to single stranded M13 DNA, have been examined as a function of the NaCl concentration (25.0 °C, pH 8.1). Quenching of the intrinsic tryptophan fluorescence of the SSB protein by the nucleic acid is used to monitor binding. We find that the site size \( n \) for binding of SSB to all single stranded nucleic acids is quite dependent on the NaCl concentration. For SSB-poly(dT), \( n = 33 \pm 3 \) nucleotides/tetramer below 10 mM NaCl and 65 \( \pm 5 \) nucleotides/tetramer above 0.20 M NaCl (up to 5 M). Between 10 mM and 0.2 M NaCl, the apparent site size increases continuously with [NaCl]. The extent of quenching of the bound SSB fluorescence by poly(dT) also displays two-state behavior, 51 \( \pm 3 \% \) quenching below 10 mM NaCl and 83 \( \pm 3 \% \) quenching at high [NaCl] (>0.1–0.2 M NaCl), which correlates with the observed changes in the occluded site size. On the basis of these observations as well as the data of Krauss et al. (Krauss, G., Sindermann, H., Schomburg, U., and Maass, G. (1981) Biochemistry 20, 5346–5352) and Chrysogelos and Griffith (Chrysogelos, S., and Griffith, J. (1982) *Proc. Natl. Acad. Sci. U.S.A.* 79, 5803–5807) we propose a model in which *E. coli* SSB binds to single stranded nucleic acids in two binding modes, a low salt mode \( (n = 33 \pm 3) \), referred to as (SSB)\(_{33} \), in which the nucleic acid interacts with only two protomers of the tetramer, and one at higher [NaCl], \( n = 65 \pm 5 \), (SSB)\(_{65} \), in which the nucleic acid interacts with all 4 protomers of the tetramer. At intermediate NaCl concentrations a mixture of these two binding modes exists which explains the variable site sizes and other apparent discrepancies previously reported for SSB binding. The transition between the two binding modes is reversible, although the kinetics are slow, and it is modulated by NaCl concentrations within the physiological range. We suggest that SSB may utilize both binding modes in its range of functions (replication, recombination, repair) and that in vivo changes in the ionic media may play a role in regulating some of these processes.

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1 The abbreviations used are: SSB, single strand binding protein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; s.s., single stranded.
E. coli SSB—Single Stranded DNA Complexes

Ruechel and Wetmur, 1975, 1976) previous studies of the SSB-single stranded polynucleotide interaction have been carried out at constant ionic conditions, although the various studies were not all conducted under the same salt conditions. Since it is clear that protein-nucleic acid interactions are extremely sensitive to salt conditions for a variety of reasons (Record et al., 1976, 1978), we have systematically varied the salt concentration in our studies of SSB-polynucleotide interactions. We have measured binding site sizes as a function of NaCl concentration using the quenching of the intrinsic tryptophan fluorescence of SSB upon binding to DNA to monitor the interaction (Molineux et al., 1975; Krauss et al., 1981).

These spectroscopic studies provide evidence for two distinct binding modes for the SSB-single stranded polynucleotide interaction. The transition between the two binding modes is reversible and regulated by the NaCl concentration. These two distinct binding modes may both be used by SSB to carry out its various functions in E. coli.

MATERIALS AND METHODS

Reagents and Buffers—All chemicals were reagent grade. All solutions were made with distilled and deionized (Milli Q) water. Agarose (JE400 from Sea Kem) was used in all gels. Nucleic acids were used in Tris-HCl buffer (pH 8.1) at 10 mM Tris, 1 mM EDTA, 0.1 mM Na3EDTA; Buffer H (pH 7.5) is 5 mM Na HEPES, 5 mM HEPES acid, 0.1 mM Na3EDTA; Buffer A is 50 mM Tris, pH 7.5, 1 mM EDTA, 20% (v/v) glycerol. The salt concentrations (NaCl, NaF, Na acetate) in the buffers were as indicated in the text. The pH of the buffers did not vary by more than ±0.1 pH unit over the salt concentration range studied.

E. coli SSB Protein—The SSB protein used in this study was prepared from E. coli K12 

\[ \text{K12} \]

\[ \Delta \text{trp} \]

(Remaut et al., 1981) harboring a plasmid in which the ssb gene has been placed under transcriptional control of the A  

\[ \text{P}_{\text{L}} \]

promoter. This construct was cloned from plasmid pDR1996 (ssb) (Sancar et al., 1981) into the plasmid pPL28 (Remaut et al., 1981) and will be described elsewhere. This plasmid is temperature inducible for SSB overproduction. The SSB protein was purified by a procedure (modified from that of R. L. McMacken) consisting simply of lysis by sonication, followed by DNA precipitation with 1.6% streptomycin sulfate, a 45% (NH4)2SO4 precipitation, with two subsequent washings of the pellet with 40 and 35% (NH4)2SO4. The pellet was then resuspended in Buffer A + 0.3 M NaCl and applied to a single stranded DNA cellulose column. The column was washed with Buffer A + 0.3 M NaCl, Buffer A + 0.6 M NaCl, and the SSB was eluted with Buffer A + 2.0 M NaCl. The purified SSB was stored at -20°C in 10 mM Tris, 0.1 mM Na3EDTA, 1 mM EDTA, 1 mM d-mercaptoethanol, 50% glycerol. The SSB used in our experiments was dialyzed extensively versus Buffer T + 0.2 M NaCl and stored at 4°C for no longer than 4 weeks after which it was discarded since lower molecular weight degradation products become observable over time. The SSB preparations were >98% pure as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Coomasie Blue and silver staining (Wray et al., 1981). SSB concentrations were determined spectrophotometrically in Buffer T + 2 M NaCl using an extinction coefficient of 280 nm, \( e_{280} = 1.5 \text{ ml mg}^{-1} \text{ cm}^{-1} \), which is based on the data of Ruechel and Wetmur (1975) after correction for the recently determined monomer molecular weight of SSB (18,873 g/mol; Sancar et al., 1981). This agrees with the determination of 1.6 ml mg\(^{-1}\) cm\(^{-1}\) by Williams et al. (1983); however, two other studies report an extinction coefficient of \( e_{280} = 0.98 \text{ ml mg}^{-1} \text{ cm}^{-1} \) for SSB (Krauss et al., 1981; Cotterill and Fersht, 1983). Since our estimates of SSB-site sizes are dependent upon the extinction coefficient, we redetermined this using amino acid analysis (performed by S. Smith, University of Texas) and calculate an extinction coefficient of \( e_{280} = 1.5 \pm 0.1 \text{ ml mg}^{-1} \text{ cm}^{-1} \) (10 mM Tris, pH 8.1, 0.2 M NaCl) based on an SSB monomer molecular weight of 18,873 g/mol (Sancar et al., 1981). Therefore, any estimates of site sizes, etc., which have used an extinction coefficient of 1.0 ml g\(^{-1}\) cm\(^{-1}\) have overestimated the SSB concentration and underestimated the site size by approximately 50%. We have compared our SSB preparations made from our overproducing plasmid with preparations from E. coli containing pDR1996 (Sancar et al., 1981) and another temperature-inducible SSB overproducing plasmid, pRLM55 (obtained from Dr. R. L. McMacken, Johns Hopkins University). All SSB preparations from each source behave identically in the nucleic acid binding experiments reported here.

Nucleic Acids—The synthetic homopolynucleotides used in these studies were purchased from BRL. Biochemicals and M. Miles. The poly(U) had an \( s_{280}^o = 8.1 \text{ S} \) and poly(T) had an \( s_{280}^o = 7.6 \text{ S} \), corresponding to weight-average lengths of ~600 nucleotides (Eisenberg and Felsenfeld, 1967). These nucleic acids were dissolved in buffer and dialyzed extensively before use. Single stranded circular M13 pl11 DNA (Messing and Vieira, 1980) from M13 bacteriophage was grown in E. coli JM103Y (Messing et al., 1981) and purified essentially as described by Yamamoto et al. (1970). The single stranded circular DNA was extracted from the M13 phage (5 times) using cold phenol, 1% sodium dodecyl sulfate as previously described (Lohman, 1984a). Concentrations of the nucleic acids were determined spectrophotometrically using the following extinction coefficients (per mol of nucleotide): poly(rU), \( e_{280} = 9.2 \times 10^2 \text{ M}^{-1} \text{ cm}^{-1} \); poly(dT), \( e_{280} = 8.1 \times 10^2 \text{ M}^{-1} \text{ cm}^{-1} \) (Kowalczykowski et al., 1981b); single stranded M13 DNA, \( e_{280} = 7370 \text{ M}^{-1} \text{ cm}^{-1} \) (Berkowit and Day, 1974).

Fluorescence Measurements—Titrations monitoring the tryptophan fluorescence of E. coli SSB protein were performed with an SLM 8000 spectrofluorometer interfaced to a Hewlett-Packard HP85 computer for data collection and analysis. The excitation wavelength was 282 nm, and fluorescence was monitored at 347 nm. The temperature of the buffer was controlled at 25 ± 0.2°C using a thermostatted cuvette holder and a Lauda RMI-S refrigerated circulating water bath. A 2.0-mI solution of SSB in a 4.0-mI quartz fluorescence cell (Hellina, 1.0 x 1.0 x 4.5 cm) was constantly stirred with a Teflon-coated magnetic stir bar (8 mm (diameter) x 8 mm (height)) while concentrated nucleic acid was titrated in 1- to 3-ml aliquots at 1-min intervals using a 1-3-μl variable microdispenser with glass barrel (Drummond model 105). The excitation shutter remained closed during equilibration of the sample after each aliquot addition and was opened only for 15 s during data acquisition in order to minimize photobleaching of the sample. The fluorescence measurements were corrected for dilution, photobleaching of SSB, and inner filter effects, where necessary, using the following.

\[
F_{\text{corr}} = F_{\text{calc}} \left( \frac{V_i}{V_f} \right) \left( \frac{f_i}{f_f} \right)
\]

where \( F_{\text{calc}} \) and \( F_{\text{corr}} \) are the (uncorrected) and corrected SSB fluorescence readings, respectively, after the \( i\)th aliquot of nucleic acid, \( V_i \) is the initial volume of the solution before titration, \( V_f \) is the final volume after addition of the \( i\)th aliquot, and \( s_{280} \) is the initial fluorescence of the SSB solutions, and \( f_i \) is the fluorescence of a solution of SSB which has not been titrated but has been exposed to the excitation beam for the same length of time as the SSB solution after the \( i\)th aliquot (photobleaching correction). Photobleaching of SSB was determined under each set of buffer conditions that were used in the actual titrations, since the photosensitivity of SSB is a function of the buffer conditions. At high [NaCl], SSB loses approximately 5% of its fluorescence during a 30-min experiment. The inner filter correction was determined empirically using the method of Birdsell et al. (1983) with

\[
C = \frac{\exp(-adL) - \exp(-aL)}{al(1-d)}
\]

where \( L \) is the total nucleic acid concentration, and \( a \) and \( d \) are empirically determined parameters. The parameters \( a \) and \( d \) were determined from a titration of \( N\)-acyetyl-\( L\)-tryptophanamide with the nucleic acid of interest using the identical conditions as are used for the titration of SSB. For poly(rU), \( a = 6.000 \), \( d = 0.25 \); poly(dT), \( a = 4.5000 \), \( d = 0.25 \); poly(dA), \( a = 5.000 \), \( d = 0.35 \); and M13 DNA, \( a = 4.500 \), \( d = 0.10 \). These inner filter corrections are valid up to nucleic acid concentrations of \( 1 \times 10^5 \text{ M} \) (nucleotides).

Solubility of SSB—The solubility of SSB decreases in solutions

2 We have observed that SSB is more sensitive to photobleaching under conditions which yield a site size of \( 3 \times 3 \text{ nucleotides/meristem} \) than under conditions where a site size of \( 35 \pm 5 \) is measured (N[NaCl] > 0.2 M). This also correlates with the two-state behavior observed for the fluorescence quenching and the site size of the interaction (see “Results”).
containing less than 0.1 M NaCl. Since we are investigating the properties of SSB over a wide range of [NaCl] (1 mM to 5 M) we checked the solubility of solutions of SSB and SSB-nucleic acid complexes at low [NaCl] in the SSB concentration range used in these studies (<30 mg/ml). Samples (1 ml) of SSB in the appropriate buffer were centrifuged for 15 min in a microfuge (13,000 x g) and the fluorescence of the supernatant was compared to a control sample at 0.20 M NaCl, where the SSB is soluble to concentrations exceeding at low [NaCl], at least for [SSB] DNA (Chrysogelos and Griffith, 1982). It is possible that the synthetic homopolynucleotide poly(dT) since the binding

protocols, different methods for determining SSB concentration (Williams et al., 1983), as well as the suggestion that the lower site size estimates are incorrect due to the failure to incorporate large excesses of SSB into complexes formed with DNA (Chrysogelos and Griffith, 1982). It is possible that some or all of these contribute to the variation. In our measurements of the salt dependence of the site size we have used the synthetic homopolynucleotide poly(dT) since the binding affinity of SSB to poly(dT) is so high that the complex cannot be dissociated in buffers containing only monovalent ions (even 5 M NaCl). In fact, high concentrations of multivalent cations such as Mg$^{2+}$ are necessary to dissociate the SSB-poly(dT) complex. In addition, drastic changes in secondary structure that occur in natural single-stranded DNA as a function of [NaCl] are avoided by using poly(dT). As a result, we can measure the stoichiometry of SSB-poly(dT) binding over a wide range of ionic conditions.

The extent of binding was monitored by the quenching of the intrinsic tryptophan fluorescence of SSB upon interaction with poly(dT) as the nucleic acid was added to an SSB solution. We refer to these titrations, where lattice is added to ligand as "reverse" titrations. The resulting fluorescence quenching was linear in poly(dT) concentration, and stoichiometries (site sizes) were determined by extrapolating this linear region until the plateau value of the fluorescence was reached (see Fig. 1). The ratio of total poly(dT) concentration to SSB (tetramer) concentration at the intersection is the apparent site size ($n_{\text{app}}$).

Three such reverse titrations at 10 mM, 50 mM, and 0.5 M NaCl are shown in Fig. 1, where the data have been plotted as the absolute fluorescence change ($\Delta F$, in the absence of poly(dT), $F_0$, i.e. the extent of quenching versus the ratio of total DNA nucleotide to total SSB (tetramer). Four observations are of interest: 1) the apparent site size is a function of the [NaCl], $n_{\text{app}}$ has values of 31, 52, and 63 nucleotides/tetramer at 10 mM, 50 mM, and 0.5 M NaCl, respectively; 2) the extent of fluorescence quenching is also a function of [NaCl], being 51 ± 3% below 10 mM NaCl and 83 ± 3% above ~200 mM NaCl (see also Fig. 3); 3) although the 10 mM NaCl and 0.5 M NaCl titrations have reasonably sharp end points, the titration at 50 mM NaCl shows a gradual approach to its plateau value; and 4) at each [NaCl] several titrations (using poly(dT)) were performed with initial SSB concentrations which varied over a range from 2 to 24 μg/ml. All titrations at a given [NaCl] were identical, and the calculated site sizes are independent of SSB concentration.

The NaCl concentration dependence of the site size, $n_{\text{app}}$, is shown in Fig. 2 over a wide range from 1.0 mM to 5.0 M NaCl. There is a lower limit of 33 ± 3 nucleotides/tetramer below 10 mM NaCl and an upper limit of 65 ± 5 nucleotides/tetramer above 0.2–0.3 M NaCl. In the region 10 mM ≤ [NaCl] ≤ 0.20 M, $n_{\text{app}}$ increases linearly with log [NaCl] within the error limits. At a purely phenomenological level, Fig. 2 explains the variation in site sizes that have been previously reported. The site sizes in the range 30–36 were all performed in buffers containing <10 mM NaCl or no additional salt at all. Williams et al. (1983) report a site size of 45 nucleotides/tetramer in 50 mM NaHPO$_4$; 2.5 M KCl seems low compared with Fig. 2; however, the extinction coefficient for SSB that Krauss et al. (1981) use is 1.0 ml mg$^{-1}$ cm$^{-1}$ whereas we have used 1.5 ml mg$^{-1}$ cm$^{-1}$ as reported by Ruyechan and Wetmur (1975) and Williams et al. (1983). We have also redetermined the extinction coefficient independently and find a value of 1.3 ± 0.1 ml mg$^{-1}$ cm$^{-1}$ (see "Materials and Methods").

Fig. 1. Reverse titrations of SSB with poly(dT). The SSB fluorescence was monitored ($\lambda_{\text{em}} = 282$ nm, $\lambda_{\text{ex}} = 347$ nm) at three different [NaCl] upon titration with poly(dT) (Buffer T, pH 8.1, 25.0°C). 10 mM NaCl, 50 mM NaCl, 0.5 M NaCl. The initial SSB concentration was 64 μg/ml in each case. The calculated site sizes for each [NaCl] are indicated.

RESULTS

The reported binding site size (i.e. the number of occluded nucleotides) for the E. coli SSB tetramer-single stranded nucleic acid complex varies over a wide range, from 30–36 (Sigal et al., 1972; Weiner et al., 1975; Molineux et al., 1975) to 37–40 (Krauss et al., 1981; Cotterill and Fersht, 1983) to 45 (Williams et al., 1983) to 51 (Anderson and Coleman, 1975) to 72.5 nucleotides/tetramer (Chrysogelos and Griffith, 1982). The proposed explanations for this variation include potential differences in SSB preparations due to different purification

4 T. M. Lohman and L. B. Overman, unpublished observations.

5 T. M. Lohman and L. B. Overman, unpublished experiments.

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![Fig. 1](http://example.com/f1.png)

**Fig. 1. Reverse titrations of SSB with poly(dT).** The SSB fluorescence was monitored ($\lambda_{\text{em}} = 282$ nm, $\lambda_{\text{ex}} = 347$ nm) at three different [NaCl] upon titration with poly(dT) (Buffer T, pH 8.1, 25.0°C). 10 mM NaCl, 50 mM NaCl, 0.5 M NaCl. The initial SSB concentration was 64 μg/ml in each case. The calculated site sizes for each [NaCl] are indicated.

![Fig. 2](http://example.com/f2.png)

**Fig. 2. Apparent site size ($n_{\text{app}}$) for the SSB-poly(dT) interaction as a function of [NaCl] (log scale).** All experiments were performed in Buffer T, pH 8.1, 25.0°C. The solid lines represent our interpretation of the data.
We determine whether added salt affects both the bound and free SSB, which of course decreases during the "reverse titrations" (see below and "Discussion"). On the basis of Figs. 2 and 3, we conclude that SSB binds to poly(dT) in two different binding modes which can be identified by different site sizes and different extents of tryptophan fluorescence quenching. The NaCl concentration is at least one factor (a major one) which determines the fraction of SSB in each binding mode.

As previously shown, the fluorescence emission spectrum of SSB (λex = 282 nm) is due entirely to tryptophan emission (Bandyopadhyay and Wu, 1978) even though the SSB tetramer contains 16 tyrosine and 16 tryptophan residues (Sancar et al., 1981). We carefully compared the corrected fluorescence emission spectra (λex = 282 nm) of free SSB, SSB-poly(dT) in 1 mM NaCl, and SSB-poly(dT) in 0.5 mM NaCl. Aside from the differences in fluorescence quenching, we also observed slight but real shifts in the emission wavelength maxima. Free SSB has λem,max = 347 nm, independent of [NaCl]; SSB-poly(dT) in 1 mM NaCl has λem,max = 348 nm; and SSB-poly(dT) in 0.5 mM NaCl has λem,max = 352 nm. Therefore, the tryptophan residues seem to be in a more polar environment (presumably more exposed to solvent) in the (SSB)30 complex (0.5 mM NaCl) than in the (SSB)30 complex. In all three cases, free SSB, (SSB)30, and (SSB)30, the fluorescence emission spectra have contributions only from tryptophan emission, as judged by direct comparisons of normalized spectra using excitation wavelengths of 270 and 296 nm (Eisinger, 1969).

Using the change in fluorescence quenching of the SSB-polyadenosine polynucleotide complex as a means of monitoring the transition from (SSB)30 → (SSB)30, we examined some other poly-

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6 Cotterill and Persh (1983) measured the site size for the SSB-single stranded (rDNA) complex in 10 mM MgCl2, 10 mM Tris, pH 8.1, and obtained a value of 40 nucleotides/tetramer using the extinction coefficient of 0.98 ml mg⁻¹ cm⁻³ determined by Krauss et al. (1981). When this value is adjusted using an extinction coefficient of 1.5 ml mg⁻¹ cm⁻³, we calculate n = 60 nucleotides/tetramer (see "Materials and Methods").
nucleotides (poly(dC), poly(rU), and s.s. M13 mp11 DNA) to see if the transition is dependent upon the nucleic acid. When complexed with poly(dC) or s.s. M13 DNA in Buffer T, 1 mM NaCl, SSB underwent the same further fluorescence quenching upon addition of NaCl. The SSB-poly(dC) transition was identical to the SSB-poly(dT) transition; however, the SSB-M13 mp11 transition was shifted to a slightly higher [NaCl] (midpoint of 25 mM NaCl). The full transition could not be observed for the SSB-poly(rU) complex since it begins to dissociate before a high [NaCl] plateau is reached. However, the start of further fluorescence quenching does occur at approximately the same [NaCl] as we see for the poly(dT) and poly(dC) complexes. Table I shows the results of a number of site size determinations by “reverse” titration for some other homopolynucleotides and s.s. M13 mp11 DNA. The same trend in site sizes is observed with these other nucleic acids as we observe with poly(dT), although the NaCl concentration range in which we can measure $n_{app}$ is limited to lower [NaCl] since the binding of SSB to these nucleic acids is not as strong as to poly(dT). The values of $n_{app}$ determined with s.s. M13 mp11 DNA are somewhat larger at all [NaCl] than those determined with poly(dT). This may reflect the increased secondary structure that is present in natural s.s. DNA, which is stabilized as the [NaCl] is raised. In general, however, the data indicate that all of these SSB-polynucleotide complexes undergo the same salt-dependent site size transition as seen for poly(dT).

**The Kinetics of the (SSB)$_n$ → (SSB)$_{n0}$ Conversion Are Slow within the Transition Region**—In NaCl titrations of the SSB-poly(dT) complex we observed that the resulting fluorescence changes were slow, occurring within approximately 10 min. The equilibrium transition in Fig. 3 was determined using 15-min intervals between additions of salt. (The dilution experiments to check the reversibility of the transition (see below) also required several minutes to reach equilibrium.) The time course of the resulting fluorescence quenching upon jumping the [NaCl] of an SSB-poly(dT) complex (2-fold excess poly(dT), assuming $n = 3$) from 1 to 21 mM, which is within the transition region is shown in Fig. 4. The earliest time point after mixing is 10 s. The kinetics are multiphasic, and we have not attempted to analyze them quantitatively. Half of the fluorescence change occurs within the first 90 s; however, the change is approximately 95% completed after 15 min while the final 5% change occurs after a total of 45–60 min. On the other hand the kinetics are much faster when the [NaCl] is jumped from the same starting conditions of 1 mM NaCl but to a final concentration of 0.35 M NaCl, which is well outside the transition region. In this case, approximately 90% of the resulting fluorescence change in the bound SSB occurs within the first 10 s (data not shown), indicating that as the final [NaCl] is raised, the rate for the (SSB)$_n$ → (SSB)$_{n0}$ conversion increases dramatically.

As noted above, the “reverse” titrations performed at 10 mM < [NaCl] < 0.1 M (i.e., within the transition region of Fig. 2) do not have sharp end points (e.g., see the curve at 50 mM NaCl in Fig. 1). However, the titrations at [NaCl] > 0.2 M or [NaCl] < 10 mM show quite sharp end points. Qualitatively, the lack of a sharp end point is a good indicator of a titration which is performed within the transition region. We tested the effects of temperature on the shape of the titration curve at 50 mM NaCl by performing titrations in Buffer H + 50 mM NaCl at 10, 25, and 39 °C. The initial slopes (dF/d[poly(dT)]) are identical for the three curves, although the end points become increasingly more sharp at the higher temperatures. The “biphasic” form of these titrations (see Fig. 1) more than likely reflects the conversion of the (SSB)$_n$ mode to the (SSB)$_{n0}$ mode since when more DNA is added, further SSB quenching occurs, approaching the high salt limit of 83 ± 3% quenching at high [poly(dT)]. This suggests that within the transition region, where both binding modes coexist, the (SSB)$_n$ mode is favored at low binding density and higher temperatures, whereas the (SSB)$_{n0}$ mode may be favored at high binding density.

The [NaCl]-dependent Site Size Represents a True Change in the Number of Nucleotides Occluded by SSB—A more direct test of the apparent increase in SSB site size as a function of increasing [NaCl] over the range from 10 mM to 0.3 M NaCl was designed to rule out the possibility of some artifact in our low salt measurements. We performed two experiments to

![Diagram](https://www.jbc.org/content/3598/4/3598/F4.large.jpg)
poly(dT) complex was formed in 10 mM NaCl by titrating SSB with more than a 2-fold excess of poly(dT) (assuming a site size of 33 nucleotides/tetramer). In the other, an SSB solution in 10 mM NaCl was titrated with sufficient poly(dT) to just saturate the SSB (a 1:1 complex). Both of these SSB-poly(dT) solutions were then titrated with 5.0 M NaCl (Buffer T) until the [NaCl] was raised to >0.3 M NaCl, while the SSB fluorescence was monitored. A concentration of 0.3 M NaCl is not sufficient to lower the intrinsic equilibrium constant of an SSB-poly(dT) complex so that it dissociates, since we have shown that SSB-poly(dT) complexes are stable in 5 mM NaCl. The results are shown in Fig. 5. Upon raising the [NaCl] of the SSB + 2-fold excess poly(dT) complex, we observe the further decrease in fluorescence as discussed above (see Fig. 3). However, upon raising the [NaCl] of the 1:1 complex we observe an increase in fluorescence over the same range of [NaCl] where the increase in SSB site size occurs. The only explanation for an increase in fluorescence is that the concentration of free SSB has increased. Since the intrinsic equilibrium constant of the SSB-poly(dT) complex is very large under these conditions, the free SSB must have resulted from an increase in the site size of the bound SSB (by nearly a factor of 2) which forced approximately half of the previously bound SSB into solution. The SSB which was forced off the DNA (d)) is still capable of binding tightly to poly(dT) since upon adding more poly(dT) we observe a further decrease in total SSB fluorescence as shown in Fig. 5. This result from the rebinding of the SSB that had been bumped off the DNA due to the NaCl-mediated change in site size. The site size determined on the basis of the total amount of poly(dT) added (initially and in the second step) is 60 nucleotides/tetramer. When excess poly(dT) is added to the 1:1 complex in 10 mM NaCl before the [NaCl] is raised to 0.30 M NaCl, no further fluorescence drop is observed, since all of the SSB is bound with a site size of 33 nucleotides/tetramer at 10 mM NaCl (see Figs. 1 and 3). Similarly, when the SSB-poly(dT) complex which has been formed at low salt with a 2-fold excess of poly(dT) and subsequently titrated to 0.30 M NaCl is further titrated with poly(dT), no additional fluorescence change is observed. Fig. 5 also shows that the fluorescence quenching of both SSB-poly(dT) solutions under the same final conditions of excess poly(dT) in >0.30 M NaCl is the same, indicating that the two different pathways result in the same SSB-poly(dT) complex at high [NaCl]. Hence the salt-dependent site size shown in Figs. 1 and 2 is a true change in the number of nucleotides which are physically occluded upon binding SSB. Fig. 5 also indicates that no irreversible precipitation or aggregation of the SSB, which might explain a site size difference, is occurring at low [NaCl], since the SSB which was first titrated in 10 mM NaCl behaves identically to SSB that has never been exposed to low salt conditions. Aggregation of SSB does occur at higher concentrations at low [NaCl], but not at the concentrations used in these experiments (<5 μg/ml SSB) (see “Materials and Methods”).

Reversibility of the Transition—In order to determine whether the site size experiments are at equilibrium we tested the reversibility of the (SSB)$_{66}$ → (SSB)$_{65}$ transition as measured by both the fluorescence change and the site size. To examine the reversibility of the salt-dependent fluorescence change, we lowered the [NaCl] of an SSB-poly(dT) complex (formed with a 2-fold excess of poly(dT)) through a series of dilution experiments in Buffer T over the range from 0.20 M to 1 mM NaCl. A series of SSB solutions was titrated with poly(dT) at different initial [NaCl] (ranging from 0.20 M to 2 mM NaCl) and then sequentially diluted with Buffer T while monitoring the SSB fluorescence. By the dilution methods which were used, the [NaCl] could only be lowered by a factor of 2 in each experiment, so that a series of 11 experiments was required to span the transition region. As a result, this reversibility experiment is not as accurate as the NaCl titrations which are performed on the same SSB-poly(dT) complex. Since the kinetics of the transition are slow (see above), equilibrium times of 15 min were allowed between dilutions to ensure equilibrium. After correcting for dilution and other filter effects, we observed the same absolute change in fluorescence (increase) upon diluting the SSB-poly(dT) complex from 0.20 M to 1 mM NaCl as we observe upon increasing the [NaCl] over this range. In Fig. 3, the fluorescence changes observed from the dilution experiments are directly compared to those obtained from the NaCl titration, within the mid-range of the transition. They are superimposable, indicating that the transition is reversible, although the kinetics in both directions are slow (see above). As a test of the reversibility of the site size change, an SSB solution (59 μg/ml) in Buffer T + 0.20 M NaCl, 25.0 °C, was titrated with poly(dT) so that the SSB was only half-saturated (calculated using the high salt value of n = 65 nucleotides/tetramer). This SSB-poly(dT) solution was then diluted to 10 mM NaCl (3 μg/ml SSB), and further additions of poly(dT) were made, after optimization of the fluorescence signal. No fluorescence change resulted from the further titration with poly(dT). A control experiment, done in parallel, but maintaining the [NaCl] at 0.20 M during the SSB dilution step, indicated that twice as much poly(dT) can be bound to the SSB at 0.20 M NaCl.

A similar experiment was also performed with a half-saturated SSB-poly(dT) complex (2-fold excess SSB, using n = 65) initially in 0.5 M NaCl (Buffer T); however, the [NaCl] was slowly lowered to 1 mM NaCl (Buffer T) by dialysis. This was done to check for the possibility that very slow kinetic processes may be involved in any rearrangements that occur. Upon titrating this SSB-poly(dT) complex, now in 1 mM NaCl (Buffer T), with poly(dT), no further fluorescence change was observed indicating no additional binding. This last experiment was prompted by the observation made by Christiansen and Baldwin (1977) that low salt complexes of SSB and denatured bacteriophage λ DNA were better pro-

![Fig. 5](image-url)
tected from S1 nuclease when they were formed at high salt and the salt concentration lowered through dialysis rather than formed by mixing the components directly at low salt.

Our interpretation is that upon reduction of the NaCl concentration from 0.20 M to 10 mM (or 0.50 M to 1 mM), the SSB that had been free in solution at high salt binds to the poly(dT) that was initially present and, therefore, is no longer free to bind more poly(dT) at the lower [NaCl]. This is consistent with a reduction of the SSB site size from 60 to 30 nucleotides/tetramer upon reducing the [NaCl] from 0.20 M to 10 mM. Therefore, the change in size is reversible, which correlates with the reversible fluorescence change discussed above.

Effect of NaCl Concentration on the Tetrameric Structure of SSB - In the interpretation of the experiments presented here, it has been assumed that SSB maintains its tetrameric state over the entire [NaCl] range that was studied (1 mM to 5 M). Ruyechan and Wetmur (1976) found that sedimentation velocity experiments ([SSB] = 80 µg/ml, pH 7.8, 20 °C) in the range 0.20 M ≤ [NaCl] ≤ 1 M are consistent with an SSB tetramer. Weiner et al. (1975) also observed properties of a tetramer in their characterization of SSB performed in 0.1 M NaCl. We have also performed gel permeation chromatography with SSB using Sephadex G-150 columns (1.5 × 50 cm) and have observed only tetrameric behavior in the range 10 mM < [NaCl] < 0.3 M NaCl (pH 8.1, 22 °C; data not shown). In these experiments 50-100 µg of SSB were applied to the column, and the fluorescence of the eluted fractions was monitored to detect the SSB. Therefore, all of the existing evidence indicates that SSB remains a tetramer in all of the conditions of our experiments. The possibility exists that some dissociation of the tetramers may occur at the low SSB concentrations (1-5 µg/ml) that we have used in some of the fluorescence experiments reported here. If this dissociation were dependent on the [NaCl], it could contribute to the [NaCl]-dependent site sizes that we observe. However, the lack of a dependence of nmax on [SSB] (from 2 to 24 µg/ml) seems to rule out this possibility.

DISCUSSION

The results of our experiments are summarized by the following major points. 1) SSB protein binds to single stranded nucleic acids (both RNA and DNA) with an apparent site size increasing from 33 ± 3 to 65 ± 5 nucleotides/tetramer over the range from 10 mM to 0.2 M NaCl, respectively (pH 8.1, 25.0 °C). 2) The SSB-poly(dT) site size remains constant at 65 ± 5 above 0.20 M NaCl and is constant at 33 ± 3 below 10 mM NaCl, as determined from "reverse" titrations. 3) The two-state behavior of the binding site size correlates with the [NaCl]-dependent two-state behavior of the quenching of the tryptophan fluorescence in the bound SSB-poly(dT) complex. The fluorescence emission spectra of the low and high salt complexes of SSB are differentially red shifted with respect to free SSB. 4) The [NaCl]-dependent transition between the two SSB binding modes is reversible as judged by the fluorescence properties and site sizes of the bound SSB (although the kinetics are slow). It should be emphasized that these salt effects on the SSB-poly(dT) binding modes are different from the salt effects which account for the lowering of binding constants in any protein-nucleic acid system (Record et al., 1976, 1978), since the effects reported here occur in a concentration range where binding is still very tight (stoichiometric) for the SSB-poly(dT) interaction.

On the basis of these observations and other recent work (Krauss et al., 1981; Chrysogelos and Griffith, 1982) we propose the following model. E. coli SSB protein can bind to single stranded nucleic acids in two binding modes which have site sizes of 33 ± 3 and 65 ± 5 nucleotides/tetramer; we refer to these as (SSB)15 and (SSB)33, respectively. The (SSB)33 binding mode is dominant below 10 mM NaCl and the (SSB)15 binding mode is dominant above 0.2-0.3 M NaCl. In the range between these [NaCl] a mixture of the two binding modes exists, thus yielding apparent site sizes between the two limits. As the [NaCl] is raised, the fraction of tetramers in the (SSB)33 binding mode decreases.

Krauss et al. (1981) have shown that each SSB tetramer can bind 4 molecules of d(pT)16, 2 molecules of d(pT)10, and only 1 molecule of d(pT)8-40, all yielding the same net SSB fluorescence quenching of 80-82%. From this Krauss et al. (1981) conclude that the SSB tetramer has four nucleic acid binding sites and that a sufficiently long single stranded nucleic acid can wrap around the SSB tetramer while binding to all four protomers. A qualitatively similar conclusion has been reached by Chrysogelos and Griffith (1982), although they suggest wrapping of DNA around an octamer of SSB protomers. Krauss et al. (1981) also observed that the binding of the first molecule of d(pT)16 results in 50% quenching of the SSB fluorescence and the binding of the second d(pT)16 molecule quenches an additional 30% to yield a total of 80% quenching when both d(pT)16 molecules are bound. We find that the SSB fluorescence is quenched to 51 ± 3% by poly(dT) below 10 mM NaCl and is gradually quenched further as the [NaCl] is raised above 10 mM until it plateaus at 83 ± 3% quenching above 0.10-0.20 M NaCl. On the basis of our site size and fluorescence results and the data of Krauss et al. (1981), we view the low salt binding mode of SSB as an interaction of the nucleic acid with only two protomers of the tetramer, resulting in a site size of 33 ± 3 nucleotides/tetramer and 50% fluorescence quenching. However, in the high salt binding mode we propose that the nucleic acid interacts with all four protomers, possibly wrapping around the protein as proposed by Krauss et al. (1981) and Chrysogelos and Griffith (1982), resulting in a site size of 65 ± 5 nucleotides/tetramer and >80% fluorescence quenching. In Fig. 6 we depict these two modes in a cartoon which illustrates the possible differences between them. The SSB tetramer is drawn as tetrahedral reflecting one possible structure possessing D2 symmetry as found in the preliminary examination of SSB crystals (Ollis et al., 1983; Monzino and Christiansen, 1983). Our depiction of the winding of the DNA in the (SSB)33 complex preserves

![Figure 6](https://example.com/figure6.png)

**Fig. 6.** A cartoon depicting the two modes of binding of SSB to single stranded polynucleotides. High salt binding with site size n = 65 ± 5 nucleotides/tetramer indicating winding of the DNA around the tetramer. Low salt binding with site size n = 33 ± 3 nucleotides/tetramer.
the symmetry of the tetramer and consequently the DNA must enter and exit on the same side of the tetramer. We have no evidence for this particular scheme, and if symmetry is broken, other models are possible. Although we have drawn the high salt binding mode with the nucleic acid wrapped around a tetramer, it is possible that the DNA wraps around an octamer of SSB protomers as suggested by Chrysogelos and Griffith (1982). The (SSB)₆ complex is drawn utilizing only two of the four nucleic acid binding sites that are depicted in the (SSB)₆ complex, although some compaction of the DNA may also occur in this mode.

From a combination of electron microscopic, nuclease digestion, and equilibrium density banding studies on SSB-fd DNA complexes, Chrysogelos and Griffith (1982) conclude that two tetramers of SSB interact with 145 nucleotides of single stranded DNA (72.5 nucleotides/tetramer). Their results seem in conflict with a site size of 33 ± 5 nucleotides/tetramer, the limiting low salt value which has been determined by a number of laboratories (Sigal et al., 1972; Weiner et al., 1975; Molinex et al., 1975). However, 72 is in good agreement with our determination of 65 ± 5 in high NaCl concentrations. This does not seem necessary to assume that only a fraction of the SSB in solution enters into a complex with the DNA as Chrysogelos and Griffith (1982) suggest. In their electron microscopic study, Chrysogelos and Griffith (1982) analyzed mainly the fully beaded SSB-fd DNA complexes, which they observed to form best in 0.10 M NaCl. Under these conditions, if the population of SSB “saturated” DNA molecules actually is composed of SSB in a mixture of binding modes, as we observe here, then the beaded DNA may represent only those molecules that are saturated with SSB in the high salt binding mode. Therefore, measurements of site size on these molecules may yield the high salt site size estimate even at moderate to low salt concentrations where the high salt binding mode is not dominant.4

The micrococcal nuclease digestion studies of SSB-fd DNA complexes (Chrysogelos and Griffith, 1982) also lend support to the two-binding mode model. At all NaCl concentrations that were used, (0.02, 0.05, and 0.17 M), Chrysogelos and Griffith (1982) observed a 145-nucleotide fragment which was protected from micrococcal nucleosome digestion of SSB-fd DNA complexes. However, at 20 M NaCl, this fragment is a minor species with the majority of the DNA fragments migrating as a large smear (< 60 nucleotides as judged from their Fig. 3a). As the [NaCl] is raised, the 145-nucleotide fragment becomes the major species in a micrococcal nuclease digestion (Chrysogelos and Griffith, 1982). The SSB-fd DNA complexes formed in the micrococcal nuclease experiments may not be directly comparable to those formed in the electron microscopic study at the same [NaCl], since the former have 5 mM CaCl₂ in the buffer (Chrysogelos and Griffith, 1982), which is required for the nuclease activity. At this point we do not know the extent to which low concentrations of divalent cations affect the two SSB binding modes, although since NaCl has such a dramatic effect, Mg²⁺ and Ca²⁺ are also likely to affect the transition.

From an electron microscopic study of single stranded DNA-SSB complexes, Ruyechan and Wetmur (1975) concluded that higher NaCl concentrations reduce SSB cooperativity. It is interesting to speculate that the transition from the (SSB)₂ to the (SSB)₆ binding mode is linked to this reduction in cooperativity, Ruyechan and Wetmur (1975) observe that cooperativity decreases above 0.20 M NaCl for SSB-s.s. λ DNA interactions, which is near the [NaCl] where all of the SSB-poly(dT) complexes are in the (SSB)₆ binding mode. We cannot comment on the quantitative degree of cooperativity which characterizes each binding mode except to say that overall cooperativity does decrease as the [NaCl] increases (Ruyechan and Wetmur, 1975). Whether cooperative complexes are only formed with SSB in the n = 33 binding modes is unknown. It is certainly possible that the two modes might interact in a cooperative fashion, rather than exclusively require only one mode for cooperativity.

One possible explanation for an increase in site size as the [NaCl] is raised is that it reflects the loss of the cooperative interactions which enable the SSB to pack closely along the nucleic acid. Hence, the increase in apparent site size might be due to the entropic contributions which dominate non-cooperative large ligand binding to linear lattices (McGhee and von Hippel, 1974) rather than a true site size change. This can be ruled out for the following reasons. The site sizes in this work were determined by “reverse” titrations (adding ligand to linear lattices); therefore, the plateau region is at low binding density (see Fig. 1) since saturation of the protein is being monitored. Using the McGhee and von Hippel (1974) binding isotherms we have calculated the theoretical increase in the apparent site size as determined from a computer-generated “reverse titration,” using input parameters of n = 30, K = 10⁶ M⁻¹ (a lower estimate), and ω ranging from 100 to 1. The change in n_m due to the loss of cooperativity is only 20%, increasing from 30 to 36. Our studies of the SSB-poly(dT) interaction indicate that the binding constant (whether or not binding is cooperative) is larger than 10⁶ M⁻¹ in the range of 10 mM to 5 M NaCl, so that 36 should represent the maximum value of n_m due to any loss of cooperativity. Therefore, an increase from 30 to 60 nucleotides/tetramer is too large to be explained by this phenomenon, and an actual change in the mode of binding is more likely.

The effect of NaCl on the transition between the two SSB binding modes is very interesting. The midpoint of the site size transition occurs at ≈ 50 mM NaCl (see Fig. 2), whereas the midpoint of the fluorescence change in the bound SSB-poly(dT) complex occurs ≈ 12.5 mM NaCl (see Fig. 3). Hence the change in the fluorescence of the bound SSB complex correlates with the change in the apparent site size of the complex, although the correlation is not exact. The difference in the [NaCl] at the midpoints of the n_m and fluorescence transition may be due to the difficulty of accurately measuring n_m within the transition region due to the lack of a sharp transition (see the 50 mM NaCl curve in Fig. 1). In addition, since the fluorescence transitions were measured at intermediate binding density (half-saturation of the nucleic acid based on n = 33), whereas the “reverse” titrations measure the site size at high binding densities (excess SSB), it is possible that the transition between the two binding modes is also dependent on the binding density, which may explain the difference in the transition midpoints (see discussion below). As mentioned above, the equilibrium titrations within the transition region (e.g., 50 mM NaCl curve in Fig. 1) also suggest that the (SSB)₆ mode is favored at low binding density.7

We conclude that the fluorescence transition reflects a structural change in the SSB-poly(dT) complex that occurs in the (SSB)₆ → (SSB)₆ conversion. (The red shift in the tryptophan fluorescence emission spectrum which accompanies the [NaCl]-induced transition to (SSB)₆ is also consistent with a structural change in the complex.) If the (SSB)₆ complex involves winding of the nucleic acid around a tetramer (Krauss et al., 1981) or an octamer of SSB (Chrysogelos and Griffith, 1982), then increased ion binding to the complex may be necessary to allow the winding to occur, hence favoring

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the (SSB)$_{60}$ complex as the [NaCl] is raised. An analysis of the [NaCl] dependence of the apparent site size ($n_{app}$) can be made assuming a two-state model and calculating an observed equilibrium constant for the transition between the (SSB)$_{60}$ and (SSB)$_{65}$ modes:

$$K_{obs} = \frac{(SSB)_{65}}{(SSB)_{60}}$$

(1)

where

$$K_{obs} = \frac{(n_{app} - 33)}{(65 - n_{app})}$$

(2)

Using Equation 2 we can obtain an estimate of the net change in the number of ions bound to the SSB-poly(dT) complex ($\Delta r$) in the transition from the (SSB)$_{65}$ mode to the (SSB)$_{60}$ mode, since

$$\frac{d \log K_{obs}}{d \log [NaCl]} = \frac{\Delta r}{r_{65} - r_{60}} = \Delta r$$

(3)

From the site size data of Fig. 2 we obtain a rough estimate of $\Delta r = 0.9 \pm 0.5$, indicating that approximately 1–2 more sodium ions are bound to the (SSB)$_{60}$ complex than to the (SSB)$_{65}$ complex. (Since the transition is insensitive to the anion, it is likely that differential binding of Na$^+$ is occurring, although this has not been proven.)

A more accurate estimate of $\Delta r$ is possible by using the fluorescence quenching data in Fig. 3 as a direct measure of the transition. We define $K_{obs}$ for the two-state analysis in a manner analogous to Equation 2,

$$K_{obs} = \frac{Q_{65}}{Q_{60}}$$

(4)

where $Q_{65}$, $Q_{60}$, and $Q_{app}$ are the fluorescence quenching of the (SSB)$_{33}$ complex, the (SSB)$_{65}$ complex, and the observed quenching within the transition region, respectively. From the slopes of log-log plots of $K_{obs}$ versus NaCl concentration according to Equation 3 (using the data from Fig. 3) we estimate $\Delta r = 1.9 \pm 0.2$. This two-state analysis suggests that 2 Na$^+$ bind to the SSB-poly(dT) complex in the transition from (SSB)$_{65}$ to (SSB)$_{60}$, in agreement with the two-state analysis of the site size data.

In light of the proposal by Chrysogelos and Griffith (1982) that single stranded DNA wraps around an SSB octamer, Equation 1 can be reformulated as

$$4Na^+ + 2(SSB)_{65} = ([SSB]_{65})_2$$

(5)

In Equation 5 each (SSB) represents a tetramer, and the cations have been explicitly included in the transition. Equation 5 represents the possibility that the (SSB)$_{65}$ mode involves only tetramers which bind in a linear array along the DNA, and the transition to the (SSB)$_{60}$ mode requires 2 tetramers to form an octamer with DNA wound around the SSB octamer (Chrysogelos and Griffith, 1982). It is possible that the uptake of cations is necessary to attain close juxtaposition of 2 SSB tetramers in the octamer.

Is the (SSB)$_{33}$ binding mode an intermediate in the pathway to forming the (SSB)$_{60}$ complex? Certainly, if the (SSB)$_{60}$ complex is composed of DNA wound around an SSB tetramer or octamer (Krauss et al., 1981; Chrysogelos and Griffith, 1982) there must be at least one, if not more, intermediate stages in its formation. One imaginable complex is the (SSB)$_{33}$ interaction shown in the lower panel of Fig. 6, in which only two SSB protomers are in contact with the DNA. We can write the pathway for formation of (SSB)$_{60}$ as,

$$SSB + DNA \underset{k_1}{\overset{k_2}{\rightleftharpoons}} (SSB)_{33} \overset{k_2}{\rightarrow} (SSB)_{60}$$

(6)

where $k_1$ represents the bimolecular rate constant for the initial association of SSB with the single stranded DNA to form (SSB)$_{33}$ and $k_2$ is the rate constant for conversion of (SSB)$_{33}$ to (SSB)$_{60}$ which may require the DNA to wind around the SSB. If $k_1$ is salt dependent such that it increases with increasing [NaCl] and if $k_2$ is very small at low [NaCl] (<10 mM), then the (SSB)$_{60}$ complex could be favored at low [NaCl]. This would require that $k_1$ is also negligible at low salt, but this is usually the case for protein-DNA interactions (Lohman et al., 1978; Lohman, 1984a, 1984b, 1985; Winter et al., 1981). As the salt concentration is raised, $k_2$ exceeds the rate of formation of (SSB)$_{60}$, therefore, only the (SSB)$_{60}$ complex is observed, which is why the high salt SSB-poly(dT) titrations have sharp end points. The "reverse" titrations that we have performed in the transition region (10 mM < [NaCl] < 0.20 m) which are "biphasic" suggest that $k_2$ may also be a function of binding density, r, so that (SSB)$_{60}$ is favored at low r. A shift from (SSB)$_{60}$ to high binding density to (SSB)$_{65}$ at low binding density would explain why the equilibrium titration at 50 mM NaCl in Fig. 1 follows the 10 mM NaCl ((SSB)$_{60}$) curve at high binding densities (low DNA/SSB) but approaches the 0.5 M NaCl (SSB)$_{60}$ curve at low binding densities. If wrapping of DNA around SSB occurs in the step with rate constant $k_3$, this wrapping seems more likely to occur at lower binding densities. However, since the (SSB)$_{33}$ → (SSB)$_{60}$ transition is reversible (both the fluorescence and the site size) (SSB)$_{53}$ is not just a metastable intermediate.

It is clear that the [NaCl] has a complex effect on the mode of interaction of E. coli SSB protein with single stranded nucleic acids, affecting both its mode of binding and its apparent cooperative interactions. These specific effects of NaCl concentration are in addition to the dramatic effect of cation concentration on the binding affinity of any protein-nucleic acid interaction (Record et al., 1976; Lohman et al., 1990; deHaseth et al., 1977). The salt-dependent transition of SSB-single stranded nucleic acid complexes is certainly not fully understood at this point. It is quite probable, however, that the salt effects observed here also play a role in in vitro studies of DNA replication and recombination.

SSB has been shown to play a role in a variety of processes including DNA replication, recombination, and repair. In addition there is evidence that SSB interacts with DNA polymerase I (Sigal et al., 1972; Molinueux et al., 1974), exon I (Molineux and Gefter, 1975), the n protein (Low et al., 1982), and possibly Rec A protein (Cohen et al., 1983) and Rep protein (Tessman and Peterson, 1982). It is possible that the two modes of binding observed here are both used by SSB in its wide range of activities since it is conceivable that its ability to bind cooperatively may need to be modulated during replication or recombination. Finally, if the ability to switch between these two binding modes is an important feature of the E. coli SSB protein, the effective manner in which changes in salt concentration affect this transition suggests that changes in the ionic media could play a role in regulating processes involving SSB. We need to understand the roles that low molecular weight ions play in complex processes such as transcription, replication, and recombination since changes in their concentration can clearly exert large effects in vitro and possibly in vivo.

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