

## Cooperative Binding of Polyamines Induces the *Escherichia coli* Single-Strand Binding Protein-DNA Binding Mode Transitions<sup>†</sup>

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**ABSTRACT:** The *Escherichia coli* single-strand binding (SSB) protein is an essential protein involved in DNA replication, recombination, and repair processes. The tetrameric protein binds to ss nucleic acids in a number of different binding modes in vitro. These modes differ in the number of nucleotides occluded per SSB tetramer and in the type and degree of cooperative complexes that are formed with ss DNA. Although it is not yet known whether only one or all of these modes function in vivo, based on the dramatically different properties of the SSB tetramer in these different ss DNA binding modes, it has been suggested that the different modes may function selectively in replication, recombination, and/or repair. The transitions between these different modes are very sensitive to solution conditions, including salt (concentration, as well as cation and anion type), pH, and temperature. We have examined the effects of multivalent cations, principally the polyamine spermine, on the SSB-ss poly(dT) binding mode transitions and find that the transition from the (SSB)<sub>35</sub> to the (SSB)<sub>56</sub> binding mode can be induced by micromolar concentrations of polyamines as well as the inorganic cation Co(NH<sub>3</sub>)<sub>6</sub><sup>3+</sup>. Furthermore, these multivalent cations, as well as Mg<sup>2+</sup>, induce the binding mode transition by binding cooperatively to the SSB-poly(dT) complexes. These observations are interesting in light of the fact that polyamines, such as spermidine, are part of the ionic environment in *E. coli* and hence these cations are likely to affect the distribution of SSB-ss DNA binding modes in vivo. Furthermore, the ability of the SSB protein to enhance the rate of renaturation of complementary single-stranded DNA >5000-fold is directly dependent upon the presence of polyamines [Christiansen, C., & Baldwin, R. L. (1977) *J. Mol. Biol.* 115, 441].

The *Escherichia coli* single-strand binding (SSB) protein (Sigal et al., 1972) is essential for DNA replication and some DNA repair processes such as methyl-directed mismatch repair and also facilitates homologous recombination catalyzed by the RecA protein [for reviews, see Chase and Williams (1986), Meyer and Laine (1990), Lohman et al. (1988), Lohman and Bujalowski (1990), and Greipel et al. (1989)]. The SSB protein is a stable tetramer in solution, and the tetramer appears to be the functionally active form of the protein (Chase et al., 1984; Bujalowski & Lohman, 1991a,b). Although interactions of the SSB protein with other proteins are likely to be important in some of its functions, its interaction with ss DNA appears to be central to all of its functions. However, the interactions of the SSB tetramer with ss DNA in vitro are complex, since the tetramer can interact with ss DNA in at least four different modes [for reviews, see Lohman et al. (1988) and Lohman and Bujalowski (1990)]. These ss DNA binding modes differ by the number of nucleotides that are occluded per SSB tetramer, referred to as the site size, *n*, and the number of SSB subunits that interact with the ss DNA and are designated as (SSB)<sub>*n*</sub>, where *n* = 35, 40, 56, or 65.

The different SSB binding modes have been detected by a variety of techniques, including electron microscopy (Griffith et al., 1984), fluorescence, gel electrophoresis, sedimentation

velocity (Lohman & Overman, 1985; Lohman et al., 1986; Bujalowski & Lohman, 1986; Bujalowski et al., 1988), and circular dichroism (Kuil et al., 1990). In complexes with poly(dT) at 25 °C, pH 8.1, the SSB tetramer can form three of these modes with *n* = 35, 56, or 65 nucleotides per tetramer. In the (SSB)<sub>35</sub> mode, ss DNA interacts with only two of the SSB subunits, whereas in both the (SSB)<sub>56</sub> and the (SSB)<sub>65</sub> modes, all four subunits interact with the DNA (Bujalowski & Lohman, 1989a,b), with the DNA wrapping around the tetramer (Krauss et al., 1981) or octamer (Chrysogelos & Griffith, 1982). Furthermore, in the (SSB)<sub>65</sub> mode, SSB tetramers bind with only "limited" positive cooperativity, such that clusters are limited to dimers of tetramers (octamers) (Bujalowski & Lohman, 1987; Chrysogelos & Griffith, 1982), whereas in the (SSB)<sub>35</sub> mode, SSB tetramers appear able to form "unlimited" clusters (Lohman et al., 1986b). On the basis of the very different ss DNA binding properties of these SSB binding modes and the fact that the (SSB)<sub>35</sub> mode selectively inhibits reactions of the *E. coli* RecA protein (Muniyappa et al., 1990; Griffith et al., 1984; Morrical & Cox, 1990), it has been suggested that the (SSB)<sub>35</sub> mode may be used selectively in replication, whereas the (SSB)<sub>56</sub> or (SSB)<sub>65</sub> modes may be used preferentially in other processes such as recombination (Lohman et al., 1988; Lohman & Bujalowski, 1990).

Christiansen and Baldwin (1977) made the interesting observation that the renaturation rate of denatured phage λ DNA can be increased dramatically in the presence of the *E. coli* SSB protein; however, this catalysis was observed only under a very specific set of solution conditions, requiring the presence of multivalent cations. The SSB-catalyzed renaturation rate increased with the charge of the cation, although there was a clear linkage with pH; catalysis was never observed in the

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presence of monovalent cations at any pH, whereas catalysis occurred in the presence of divalent cations, although only within a narrow pH range near pH 5.5. However, the most dramatic catalysis of DNA renaturation by SSB protein (5000-fold) was observed in the presence of the multivalent cations spermine or spermidine, over a broad pH range, centered near pH 7, with spermine (+4), being more effective than spermidine (+3). Furthermore, the optimal cation concentrations were inversely related to the valence of the cation, such that  $Mg^{2+}$  and  $Ca^{2+}$  were required at 10–15 mM and spermidine<sup>3+</sup> was required at 2 mM. Although the bacteriophage T4 gene 32 protein also can increase the rate of DNA renaturation (Alberts & Frey, 1970), it does so under conditions that do not support catalysis by the *E. coli* SSB protein. On the basis of the specific solution conditions required for SSB protein-catalyzed renaturation, Christiansen and Baldwin (1977) concluded that catalysis of DNA renaturation by the *E. coli* SSB protein was not simply the result of destabilization of intramolecular hairpins by the SSB protein.

The relative populations of the different SSB–ss DNA binding modes are affected dramatically by the concentration of monovalent and divalent cations, anions, pH, temperature, and binding density (Lohman & Overman, 1985; Bujalowski et al., 1988). Analysis of the salt dependence indicates that the transition from the lower to the higher site size binding modes occurs with a net uptake of cations and anions; however, with poly(dT) at pH 8.1, 25 °C, the transition from the (SSB)<sub>35</sub> to the (SSB)<sub>56</sub> binding mode is accompanied by only a net uptake of cations (Bujalowski et al., 1988). The highly specific solution conditions required to observe catalysis of the DNA renaturation rate by the *E. coli* SSB protein (Christiansen & Baldwin, 1977), in particular the effects of multivalent cations and pH, led us to consider whether these conditions effect changes in the relative population of the different SSB binding modes. Therefore, we have examined the effects of multivalent cations, especially the polyamines, spermine (+4), and spermidine (+3), on the SSB–poly(dT) binding modes. The effect of polyamines on the SSB–ss DNA binding modes is also of interest, since these ions are present naturally in *E. coli* (Tabor & Tabor, 1985).

#### MATERIALS AND METHODS

**Reagents and Buffers.** All chemicals were reagent-grade, and all solutions were made with distilled and deionized (Milli-Q) water (Continental, Bedford, MA). Buffer T is 10 mM Tris HCl (pH 8.1 at 25 °C) and 0.1 mM  $Na_2EDTA$ . Spermine-4HCl and spermidine-3HCl were obtained from Sigma (St. Louis, MO). The concentration of  $Co(NH_3)_6Cl_3$  stocks was determined spectrophotometrically using  $\epsilon_{472} = 56.2 \text{ M}^{-1} \text{ cm}^{-1}$  (Widom & Baldwin, 1980).  $Co(NH_3)_6Cl_3$  stocks were wrapped in aluminum foil to avoid photobleaching effects.

***E. coli* SSB Protein and Nucleic Acids.** *E. coli* SSB protein was prepared as described (Lohman et al., 1986). The concentration of SSB was determined spectrophotometrically by using the extinction coefficient  $\epsilon_{280} = 1.13 \times 10^5 \text{ M}^{-1}$  (tetramer)  $\text{cm}^{-1}$  ( $1.5 \text{ mL mg}^{-1} \text{ cm}^{-1}$ ) in buffer T + 0.2 M NaCl (Lohman & Overman, 1985). The synthetic homopolymer, poly(dT) [ $s_{20,w} = 8.4 \text{ S}$ ; ~950 nucleotides (Inners & Felsenfeld, 1970)], was purchased from Pharmacia-PL Biochemicals and extensively dialyzed vs buffer T + 0.1 M NaCl. The concentration of poly(dT) was determined spectrophotometrically using an extinction coefficient  $\epsilon_{260} = 8.1 \times 10^3 \text{ M}^{-1}$  (nucleotide)  $\text{cm}^{-1}$  in buffer T + 0.1 M NaCl.

Complexes possessing three different SSB protein binding densities were prepared by mixing a constant concentration of SSB protein with different poly(dT) concentrations. The

molar ratios of SSB tetramer to nucleotides of poly(dT) were 1:97.5, 1:130, and 1:195, respectively, to give a poly(dT) saturation corresponding to 67%, 50%, and 33%, based on a site size of 65 nucleotides per SSB tetramer. These complexes will be referred to as 1–1.5–0.1, 1–2–0.1, and 1–3–0.1 mM, respectively, indicating that the poly(dT) is 1.5-fold, 2-fold, and 3-fold in excess in these complexes and that they were prepared in 0.1 mM NaCl and 10 mM Tris HCl, pH 8.1. Furthermore, the 1–1.5 complex was dialyzed against three different NaCl concentrations (i.e., 0.1, 5, and 10 mM in 10 mM Tris HCl, pH 8.1) to study the effects of [NaCl] on the spermine-induced transitions, and they will be abbreviated as 1–1.5–0.1, 1–1.5–5, and 1–1.5–10 mM for convenience. In order to ensure that these complexes are at equilibrium, they were formed by adding SSB protein to poly(dT) in 1 M NaCl and 10 mM Tris HCl, pH 8.1, with gentle mixing, followed by dialysis to low-salt conditions (0.1 mM NaCl in 10 mM Tris HCl, pH 8.1). Each dialysis was performed over 48 h with four 1-L buffer changes.

The SSB protein concentration in the final dialyzed solution was determined in the following manner. A sample of the complex was brought to 0.2 M NaCl, which ensures that the SSB–poly(dT) complex is in the well-characterized (SSB)<sub>65</sub> binding mode (Lohman & Overman, 1985; Bujalowski & Lohman, 1986). The intrinsic SSB protein fluorescence intensity ( $\lambda_{ex} = 296 \text{ nm}$ ,  $\lambda_{em} = 347 \text{ nm}$ ) of the SSB–poly(dT) complex in 0.2 M NaCl was then compared with a standard curve based on SSB–poly(dT) complexes formed in the (SSB)<sub>65</sub> mode by direct mixing under identical solution conditions.

**Fluorescence Measurements.** Fluorescence measurements were carried out in an SLM 8000C fluorometer (SLM-Aminco, Urbana, IL) at 25 °C as previously described ( $\lambda_{ex} = 296 \text{ nm}$ ;  $\lambda_{em} = 347 \text{ nm}$ ) (Lohman & Overman, 1985; Bujalowski & Lohman, 1986). The transition from the (SSB)<sub>35</sub> binding mode to the (SSB)<sub>56</sub> binding mode was monitored by the increase in fluorescence quenching of the SSB protein (from approximately 50% to 90%) that accompanies the transition. In order to investigate the effect of salt type on this transition, the preformed SSB protein–poly(dT) complexes were titrated with the appropriate salt while monitoring the intrinsic tryptophan fluorescence of SSB protein.

**Construction of Equilibrium Isotherms for Spermine Binding to SSB–Poly(dT) Complexes.** Model-independent equilibrium isotherms for the binding of spermine to the SSB–poly(dT) complexes were determined using the binding density function method of analysis as described previously [Lohman & Bujalowski, 1988; for review, see Lohman and Bujalowski (1991)]. Since the signal is from the SSB, which binds multiple spermines, the macromolecule binding density function is applicable (Halfman & Nishida, 1972; Lohman & Bujalowski, 1988, 1991). Each isotherm was determined from the analysis of four or five titration curves, each obtained at different concentrations of SSB protein–poly(dT) complex, while holding the SSB:poly(dT) ratio fixed at constant solution conditions. The macromolecule binding density function for this case is given in eq 1, where  $Q_{obs}$  is the observed degree

$$Q_{obs} = \sum \nu_i Q_i \quad (1)$$

$$\sum \nu_i = S_b / C_T = \sum (i[CS_i] / C_T) \quad (2)$$

of fluorescence quenching of the SSB protein–poly(dT) complex, at total complex concentration,  $C_T$ , and total spermine concentration,  $S_T$ .  $Q_i$  is the intrinsic quenching of the SSB–poly(dT) complex upon binding “*i*” spermine molecules,  $[CS_i]$  is the concentration of complex with *i* spermines bound, and

$S_b$  is the molar concentration of bound spermine per complex. This analysis is based on the fact that the average spermine binding density,  $\sum \nu_i$  (see eq 2), and the free spermine concentration,  $S_f$ , are constant for a constant  $Q_{\text{obs}}$ . Values of  $S_T$  and  $C_T$  are obtained for each constant value of  $Q_{\text{obs}}$ , and a plot of  $S_T$  versus  $C_T$  yields a linear relationship, from which values of  $S_f$  and  $\sum \nu_i$  can be obtained from the intercept and the slope according to eq 3. These series of values of  $S_f$  and  $\sum \nu_i$ ,

$$S_T = S_f + \sum \nu_i C_T \quad (3)$$

obtained from repetition of this analysis over the range of values of  $Q_{\text{obs}}$ , were used to construct model-independent binding isotherms. The equilibrium binding isotherms were analyzed to obtain binding parameters based on the statistical thermodynamic model described below.

#### THEORETICAL MODEL

We have analyzed the cation-induced transition between the two SSB-poly(dT) binding modes using the allosteric model of Monod et al. (1965). In our case, we consider the SSB protein-poly(dT) complex to exist in equilibrium between two conformations,  $A[(\text{SSB})_{35}]$  and  $B[(\text{SSB})_{56}]$ , as depicted in Figure 1. In the absence of ligand, the equilibrium constant for the transition between the two states,  $L$ , is defined in eq 4.

$$L = [(\text{SSB})_{35}] / [(\text{SSB})_{56}] \quad (4)$$

A maximum of  $n_o$  spermine molecules can bind to both states of the SSB-poly(dT) complex, with intrinsic binding constants  $A$  and  $B$ , respectively. With these definitions, the average number of ligands bound per SSB-poly(dT) complex,  $\sum \nu_{i,c}$ , is given in eq 5, where  $S_f$  is the free spermine concentration.

$$\sum \nu_{i,c} = \frac{n_o S_f [LA(1 + AS_f)^{n_o-1} + B(1 + BS_f)^{n_o-1}]}{L(1 + AS_f)^{n_o} + (1 + BS_f)^{n_o}} \quad (5)$$

Equation 5 considers only the binding of spermine to an SSB-pol(dT) complex, consisting of an SSB tetramer and its associated DNA. However, in the experiments described, there is always excess uncomplexed poly(dT), hence the potential binding of spermine to the free poly(dT) must also be considered. Since the SSB tetramer covers 35 nucleotides in the  $(\text{SSB})_{35}$  mode and 56 nucleotides in the  $(\text{SSB})_{56}$  mode, the number of free poly(dT) nucleotides (i.e., not complexed with SSB) accessible for spermine binding decreases during the transition from the  $(\text{SSB})_{35}$  to the  $(\text{SSB})_{56}$  mode. In order to describe the binding of spermine to the free poly(dT), we have used a combinatorial model (Latt & Sober, 1967; Epstein, 1978), which includes the statistical effects associated with the fact that spermine occludes more than one nucleotide on poly(dT), hence the potential spermine binding sites on the lattice overlap.

On the basis of this model (Epstein, 1978), the average number of spermines bound to the poly(dT) that is not covered by SSB per complex is designated as  $\sum \nu_{i,dT}$ , and is given in eq 6

$$\sum \nu_{i,dT} = \frac{\sum_{i=0}^g \frac{(i + (N_t - n_{\text{app}} - ni))!}{i!(N_t - n_{\text{app}} - ni)!} K^i S_f^i}{1 + \sum_{i=1}^g \frac{(i + (N_t - n_{\text{app}} - ni))!}{i!(N_t - n_{\text{app}} - ni)!} K^i S_f^i} \quad (6)$$

where  $K$  is the intrinsic equilibrium constant for spermine binding to a site on the poly(dT),  $N_t$  is the total number of poly(dT) nucleotides,  $n_{\text{app}}$  is the number of nucleotides wrapped

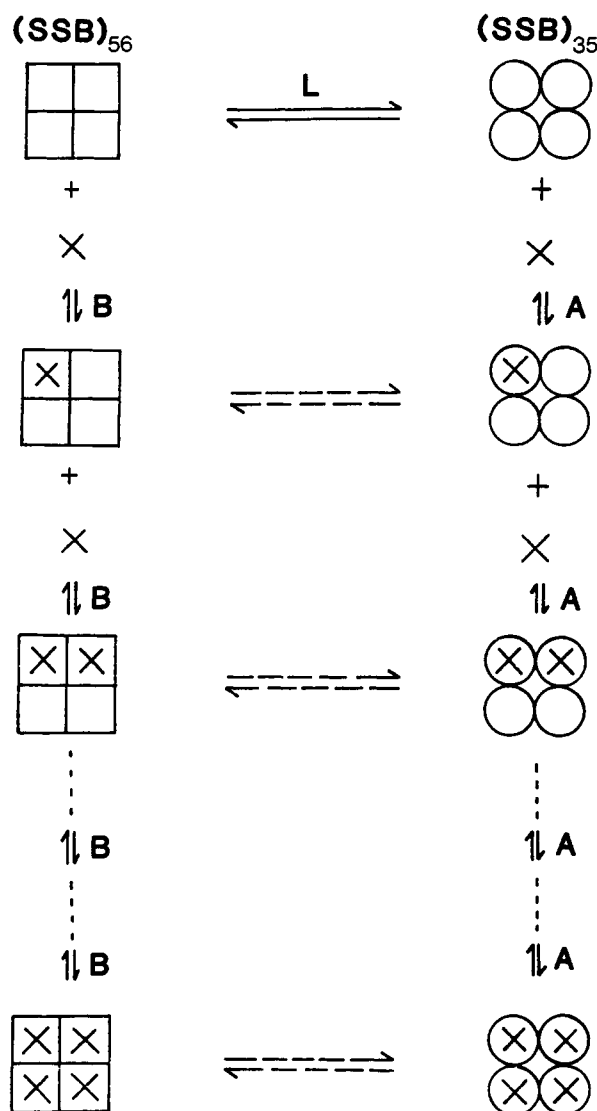


FIGURE 1: Two-state allosteric model for spermine (X) binding to SSB-poly(dT) complex.  $L$  is equilibrium constant for the transition between the  $(\text{SSB})_{35}$  and  $(\text{SSB})_{56}$  binding modes,  $A$  is the equilibrium constant for spermine binding to a site on the  $(\text{SSB})_{35}$  complex, and  $B$  is the equilibrium constant for spermine binding to a site on the  $(\text{SSB})_{56}$  complex.

around the SSB protein tetramer in its complex with poly(dT), which increases from 35 to 56 throughout the transition, and  $n$  is the number of nucleotides occluded upon binding spermine to poly(dT), estimated to be 3.5 for spermine.

The overall spermine binding density,  $\sum \nu_i$ , is then the sum of that for the complex ( $\sum \nu_{i,c}$ ) and that for the free poly(dT) ( $\sum \nu_{i,dT}$ ). The final expression, in Scatchard (1949) form, for the binding of spermine to the complex, including free poly(dT) is given in eq 7.

$$\frac{\sum \nu_i}{S_f} = \frac{n_o [LA(1 + AS_f)^{n_o-1} + B(1 + BS_f)^{n_o-1}]}{L(1 + AS_f)^{n_o} + (1 + BS_f)^{n_o}} + \frac{\sum_{i=0}^g \frac{(i + (N_t - n_{\text{app}} - ni))!}{i!(N_t - n_{\text{app}} - ni)!} K^i S_f^{i-1}}{1 + \sum_{i=1}^g \frac{(i + (N_t - n_{\text{app}} - ni))!}{i!(N_t - n_{\text{app}} - ni)!} K^i S_f^i} \quad (7)$$

Throughout the cation-induced SSB binding mode transition,  $n_{\text{app}}$  increases from 35 to 56 nucleotides per SSB tetramer, corresponding to the increase in  $Q_{\text{obs}}$  from 63% ( $Q_i$ ) to 88% ( $Q_{\text{max}}$ ) (see Results). Thus,  $n_{\text{app}}$ , as well as  $\sum \nu_i$  can be de-

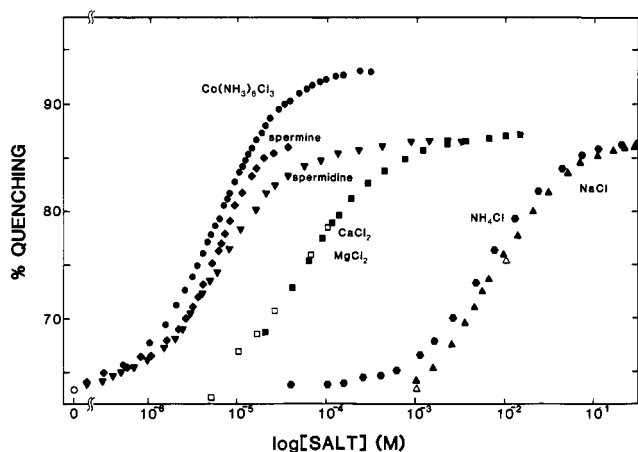


FIGURE 2: Dependence of (SSB)<sub>35</sub> to (SSB)<sub>56</sub> binding mode transition on cation concentration as monitored by the increase in tryptophan fluorescence quenching accompanying the transition. SSB tetramer-poly(dT) complexes were preformed at a 2:1 molar excess of DNA, assuming a site size of 65 nucleotides/tetramer, in 1 M NaCl in buffer T and subsequently dialyzed to 10 mM Tris HCl, pH 8.1, as described under Materials and Methods. SSB-poly(dT) complexes were titrated with the indicated salts (closed symbols) at 25 °C. Open symbols represent SSB-poly(dT) samples that were dialyzed to the indicated concentrations of MgCl<sub>2</sub> or NaCl, indicating that the transitions are at equilibrium and reversible.

terminated as a function of  $Q_{\text{obs}}$ ; the relation between  $n_{\text{app}}$  and  $\sum v_i$  is needed to simulate the isotherms according to eq 7.

The experimental isotherms were analyzed to obtain the binding parameters  $L$ ,  $A$ ,  $B$ , and  $K$  by nonlinear regression, using SYSTAT (Evanston, IL) on an Hewlett-Packard 9153B computer (Wei, 1988). Although all four parameters could not be resolved unambiguously, upon analysis of all the isotherms reported here, we always obtained the result that  $B \gg A$ . Therefore, we constrained  $A = 0$  and determined the remaining three parameters,  $L$ ,  $B$ , and  $K$ , by nonlinear least-squares analysis of the isotherms. Based on these parameters, the simulated isotherm did not change when  $A$  was increased, as long as  $B/A > 10$ .

## RESULTS

**Effect of Multivalent Cations on the SSB-ss DNA Binding Mode Transitions.** The intrinsic tryptophan fluorescence of the *E. coli* SSB tetramer is quenched upon binding ss nucleic acids. However, the extent of this quenching is dependent on the number of SSB subunits that interact with the nucleic acid [i.e., the particular SSB-poly(dT) binding mode]. In its complexes with a series of oligodeoxythymidylates and poly(dT), the fluorescence of the *E. coli* SSB tetramer is quenched by  $\sim 53 \pm 3\%$  when only two of its subunits interact with poly(dT), covering  $\sim 35$  nucleotides [(SSB)<sub>35</sub> binding mode], whereas  $\sim 88 \pm 3\%$  quenching is observed in either the (SSB)<sub>56</sub> or (SSB)<sub>65</sub> binding mode, in which all four subunits of the SSB tetramer interact with poly(dT) (Lohman & Overman, 1985; Lohman & Bujalowski, 1988). As a result, the transition between the (SSB)<sub>35</sub> and the (SSB)<sub>56</sub> binding modes can be monitored by the decrease in fluorescence (increase in quenching) and the NaCl-induced transition has been monitored in this fashion (Lohman & Overman, 1985). At pH 8.1, the transition between the (SSB)<sub>35</sub> and the (SSB)<sub>56</sub> binding modes is affected only by the cation in monovalent salts, whereas at pH 6.9, the transition is affected by both anions and cations (Bujalowski et al., 1988). Figure 2 shows that a series of salts, differing in the valence of the cation, can all induce the (SSB)<sub>35</sub> to (SSB)<sub>56</sub> binding mode transitions. However, polyvalent cations are more effective than di- or monovalent cations. The total cation concentrations at the

midpoints of the transitions in Figure 2 are  $\sim 5 \mu\text{M}$  for spermidine<sup>3+</sup>, Co(NH<sub>3</sub>)<sub>6</sub><sup>3+</sup>, and spermine<sup>4+</sup>,  $\sim 60 \mu\text{M}$  for Mg<sup>2+</sup> and Ca<sup>2+</sup>, and  $\sim 7 \text{ mM}$  for Na<sup>+</sup> and NH<sub>4</sub><sup>+</sup>.

The cation-induced transitions in Figure 2 are at equilibrium as determined by the following experiment. A series of SSB-poly(dT) complexes were preformed at high salt (1 M NaCl) and then brought to lower concentrations of MgCl<sub>2</sub> or NaCl within the transition regions by dialysis. The extents of fluorescence quenching of the complexes were measured and found to be equivalent to those obtained when the complex at low salt (0.1 mM NaCl) was titrated directly with salt. This indicates that the same extent of fluorescence quenching is obtained within the transition, when approached from either direction, as observed previously by Lohman and Overman (1985). The increase in fluorescence quenching observed upon titration of the SSB-poly(dT) complex with Mg<sup>2+</sup> was reversed totally upon chelation of the Mg<sup>2+</sup> by addition of EDTA (data not shown). Lohman and Overman (1985) showed that titration with NaCl only effected a decrease in the fluorescence of the SSB protein (increased quenching) when it was complexed with poly(dT), since there was no fluorescence change observed upon titrating the free SSB protein with NaCl. The salt-induced increases in fluorescence quenching shown in Figure 2 also occur only when the protein is complexed with nucleic acid, since the fluorescence of free SSB protein was unaffected by spermine concentrations up to  $0.48 \mu\text{M}$  (data not shown).

**The Site Size of the SSB Tetramer on Poly(dT) Is Dependent upon the Spermine Concentration.** In order to determine whether the increased fluorescence quenching observed upon titration of the SSB-poly(dT) complex with spermine is due to a change in the SSB binding mode as previously observed with Na<sup>+</sup> and Mg<sup>2+</sup> (Lohman & Overman, 1985; Bujalowski & Lohman, 1986), direct measurements of SSB tetramer site sizes were made by titrating free SSB protein ( $0.084 \mu\text{M}$  tetramer) with poly(dT) at a series of spermine concentrations at 25 °C in 10 mM Tris, pH 8.1, and 1 mM NaCl (stoichiometric binding conditions). The fluorescence quenching of the SSB tetramer upon complexation with poly(dT) was used to monitor binding and the apparent site size,  $n_{\text{app}}$ , was estimated as described previously (Lohman & Overman, 1985). The apparent site size and maximal fluorescence quenching observed in the plateau region [in the presence of excess poly(dT)] are plotted as a function of the logarithm of the total spermine concentration in Figure 3.

As observed previously in NaCl and MgCl<sub>2</sub> solutions (Lohman & Overman, 1985; Bujalowski & Lohman, 1986; Bujalowski et al., 1988), a constant site size of  $35 \pm 2$  nucleotides per SSB tetramer is observed at low spermine concentrations ( $<0.1 \mu\text{M}$  spermine) and the SSB tryptophan fluorescence is quenched to only  $\sim 55\%$ . However, at higher spermine concentrations, the apparent site size and the final extent of fluorescence quenching increase continuously, reaching values of  $48 \pm 3$  nucleotides per tetramer and  $\sim 86\%$ , respectively, at  $15 \mu\text{M}$  spermine. Furthermore, the increase in fluorescence quenching correlates with the increase in site size, similar to the behavior observed in the presence of Na<sup>+</sup> and Mg<sup>2+</sup> (Bujalowski & Lohman, 1986). Accurate determinations of the site size could not be made for spermine concentrations above  $15 \mu\text{M}$ , due to the onset of a time-dependent increase in the fluorescence signal at these higher spermine concentrations. As a result, we could not determine whether the apparent site size reached a plateau value at high spermine concentrations. The basis for this unstable fluorescence signal is not known, although it may result from

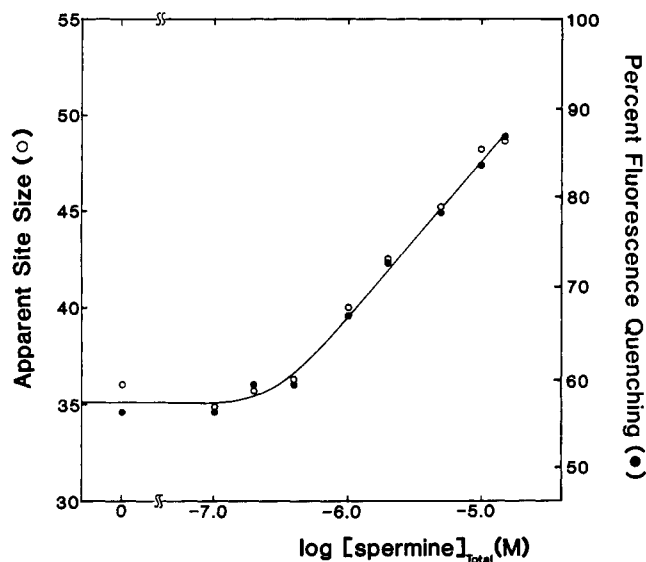


FIGURE 3: Dependence of the apparent site size (nucleotides per SSB tetramer) (○) and fluorescence quenching (●) of SSB protein-poly(dT) complexes on the total spermine concentration (buffer T, pH 8.1, 25 °C). Each point represents the result of a titration of SSB protein (0.1  $\mu$ M tetramer) with poly(dT).

spermine-induced collapse of the protein-DNA complex, similar to that observed for DNA alone (Gosule & Schellman, 1976). However, both the fluorescence quenching (86%) and the apparent site size (48 nucleotides per tetramer) measured in 15  $\mu$ M spermine are close to the values observed for the (SSB)<sub>56</sub> binding mode in both NaCl<sub>2</sub>, 88% and  $56 \pm 3$  nucleotides per tetramer, respectively (Bujalowski & Lohman, 1986). Therefore, we conclude that the apparent site size at the end point of the spermine-induced transition is 56 nucleotide per tetramer and that the cation-induced fluorescence changes in the SSB-poly(dT) complexes in Figure 2 reflect the transition from the (SSB)<sub>35</sub> to the (SSB)<sub>56</sub> binding mode.

**Isotherms for Spermine Binding to the SSB-Poly(dT) Complex.** We used the binding density function method to obtain model-independent equilibrium isotherms for the binding of spermine to the SSB-poly(dT) complexes (Halfman & Nishida, 1972; Lohman & Bujalowski, 1988, 1991). This involves the simultaneous analysis of titrations with spermine of preformed SSB protein-poly(dT) complexes at several SSB-poly(dT) concentrations as described under Materials and Methods. The results of a series of such titrations at five different concentrations of an SSB-poly(dT) complex (complex 1-1.5-0.1 mM) are shown in Figure 4. The titrations are plotted as SSB fluorescence quenching ( $Q_{obs}$ ) vs logarithm of total spermine concentration. The fluorescence quenchings of the SSB-poly(dT) complexes at the start of the titrations were calculated, based on the knowledge that the final fluorescence quenching at the end of the spermine titration is 86%, as observed from titrations of SSB protein with poly(dT) in buffer T + 0.2 M NaCl. The initial fluorescence quenching for complex 1-1.5-0.1 mM was calculated to be 63.4%.

Analysis of the five titrations shown in Figure 4, using the binding density method (see eqs 1-3), yields model-independent estimates of the extent of spermine binding per complex,  $\sum \nu_i$ , as a function of the free spermine concentration,  $S_f$ . The resulting Scatchard plot for spermine binding to the SSB-poly(dT) complex (1-1.5-0.1 mM) is shown in Figure 5. The humped shape of the isotherm indicates strong cooperative binding of spermine. However, there also appears to be a weaker, noncooperative component to the isotherm that

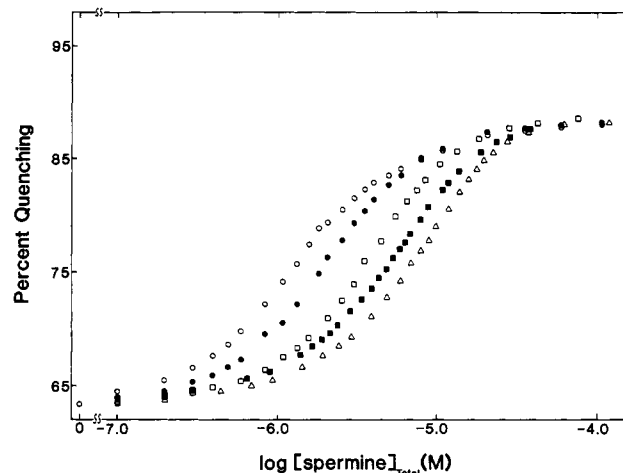


FIGURE 4: Titrations of a series of SSB-poly(dT) complexes (1-1.5-0.1 mM) with spermine, as monitored by the increased tryptophan fluorescence quenching of the SSB protein. The concentrations of SSB tetramer in the complexes are 0.13 (○), 0.25 (●), 0.50 (□), 0.74 (■) and 1.0  $\mu$ M (Δ).

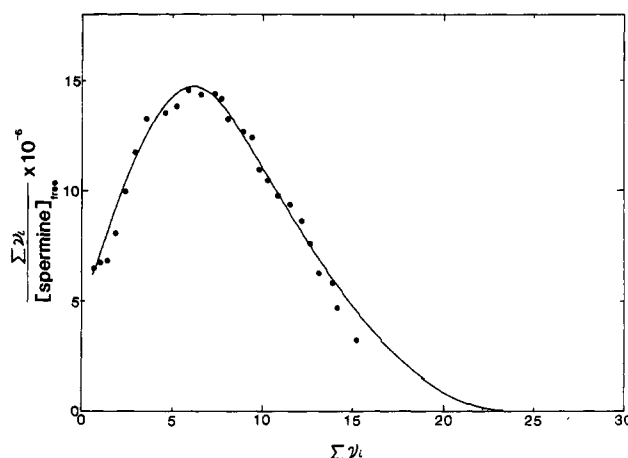


FIGURE 5: Equilibrium binding isotherm [Scatchard (1949) plot] for the interaction of spermine with the SSB-poly(dT) complex (1-1.5-0.1 mM), as determined by the binding density function analysis of the titration curves in Figure 4. The smooth line represents a theoretical curve generated using eq 7, with the following binding parameters:  $L = 100$ ,  $A = 0$ ,  $B = 1.8 \times 10^6 \text{ M}^{-1}$ , and  $K = 9.0 \times 10^4 \text{ M}^{-1}$ .

is apparent at higher spermine binding densities. This non-cooperative component is more obvious in isotherms obtained at lower SSB to poly(dT) ratios (e.g., see Figure 8) and is likely due to noncooperative binding of spermine to the poly(dT) that is not complexed with SSB. The intercept on the  $\sum \nu_i$  axis indicates that a total of  $\sim 24$  spermines bind to the SSB-poly(dT) complex (1-1.5-0.1 mM) at saturation. Recall that this represents spermine binding to both the SSB-poly(dT) complex as well as the free poly(dT). We estimate that the approximate number of spermines bound to the actual SSB-poly(dT) complex ( $n_0$  in eqs 5 and 7) [i.e., excluding the free poly(dT)] is  $\sim 12$ . The smooth curve in Figure 5 is the best fit to the isotherm using the model described by eq 7, with the following parameters:  $n_0 = 12$ ,  $L = 100$ ,  $A = 0$ ,  $B = 1.8 \times 10^6 \text{ M}^{-1}$ , and  $K = 9.0 \times 10^4 \text{ M}^{-1}$ . Similar titrations and analyses indicate that spermidine<sup>3+</sup>,  $\text{Co}(\text{NH}_3)_6^{3+}$ , and  $\text{Mg}^{2+}$  also bind with positive cooperativity to the SSB-poly(dT) complexes (T. F. Wei, unpublished experiments).

In order to assess the absolute reproducibility of the spermine binding isotherms, three different SSB-poly(dT) complexes (1-3-0.1 mM) were prepared independently, and each was titrated separately with spermine. In each case, a series

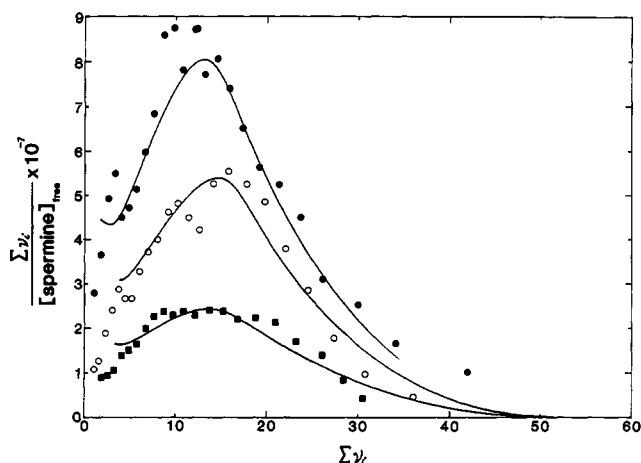


FIGURE 6: Comparison of three independent determinations of equilibrium isotherms for spermine binding to SSB-poly(dT) complexes (1–3–0.1 mM) (10 mM Tris, pH 8.1, 0.1 mM NaCl, 25 °C). The continuous curves describing each isotherm are simulations based on eq 7 with  $A = 0$  and the following parameters: (■)  $L = 2 \times 10^4$ ,  $B = 3 \times 10^6 \text{ M}^{-1}$ , and  $K = 1.2 \times 10^5 \text{ M}^{-1}$ ; (○)  $L = 2 \times 10^6$ ,  $B = 1.1 \times 10^7 \text{ M}^{-1}$ ,  $K = 2.3 \times 10^5 \text{ M}^{-1}$ ; and (●)  $L = 1.9 \times 10^5$ ,  $B = 1.4 \times 10^7 \text{ M}^{-1}$ ,  $K = 3.0 \times 10^5 \text{ M}^{-1}$ .

of five titration curves such as those shown in Figure 4 were obtained and analyzed as described above to obtain three model-independent binding isotherms, which are shown in Figure 6. All three isotherms (Figure 6) display distinct positive cooperativity, with the same number of spermine binding sites per complex ( $\Sigma \nu_i = 50$ ;  $n_0 = 10$ ). However, the quantitative aspects of these three isotherms differed. Non-linear least-squares analyses of these isotherms, using eq 7, yielded the following sets of parameters:  $L = 1.9 \times 10^5$ ,  $A = 0$ ,  $B = 1.4 \times 10^7 \text{ M}^{-1}$ ,  $K = 3 \times 10^5 \text{ M}^{-1}$ ;  $L = 2 \times 10^6$ ,  $A = 0$ ,  $B = 1.1 \times 10^7 \text{ M}^{-1}$ ,  $K = 2.3 \times 10^5 \text{ M}^{-1}$ ;  $L = 2 \times 10^4$ ,  $A = 0$ ,  $B = 3 \times 10^6 \text{ M}^{-1}$ ,  $K = 1.2 \times 10^5 \text{ M}^{-1}$ . The major differences among the three isotherms were reflected in the value of the allosteric equilibrium constant,  $L$ , which varied by a factor of 100, whereas the values of  $B$  and  $K$  varied by factors of  $\sim 5$  and  $\sim 3$ , respectively. Therefore, these limits represent our confidence range for the binding parameters reported here.

**The SSB-Poly(dT) Complex Undergoes a Concerted Transition.** To study the conformational change induced by spermine binding, a state function  $R$  was defined to be the fraction of the protein which is in the high-affinity state (Monod et al., 1965). In this study, the high-affinity state is  $(\text{SSB})_{56}$ , and  $R$  is expressed as  $\Delta Q_{\text{obs}} / \Delta Q_{\text{max}} = (Q_{\text{obs}} - Q_0) / (Q_{\text{max}} - Q_0)$ , where  $Q_0$  is the initial quenching before the addition of spermine. A plot of  $R$  vs  $Y (= \Sigma \nu_i / \nu_{\text{max}})$ , the average extent of spermine bound to the complex, yields a curve as shown in Figure 7. The concave nature of this plot indicates that the conformational transition that results in the additional fluorescence quenching of the SSB subunits precedes the binding of spermine. This behavior suggests that spermine binding induces a concerted conformational transition of the entire SSB tetramer-poly(dT) complex to its high-affinity state. This type of behavior is characteristic of the MWC model (Monod et al., 1965), as well as the sequential model when an intermediate conformation exists (Edelstein, 1974). The existence of two states, one of low  $[(\text{SSB})_{35}]$  and one of high  $[(\text{SSB})_{56}]$  ligand affinity is the basis for our use of the MWC model to analyze these isotherms.

**Effect of SSB Protein Binding Density.** We have examined spermine binding to the SSB-poly(dT) complexes as a function of the SSB to DNA ratio. Spermine binding isotherms were

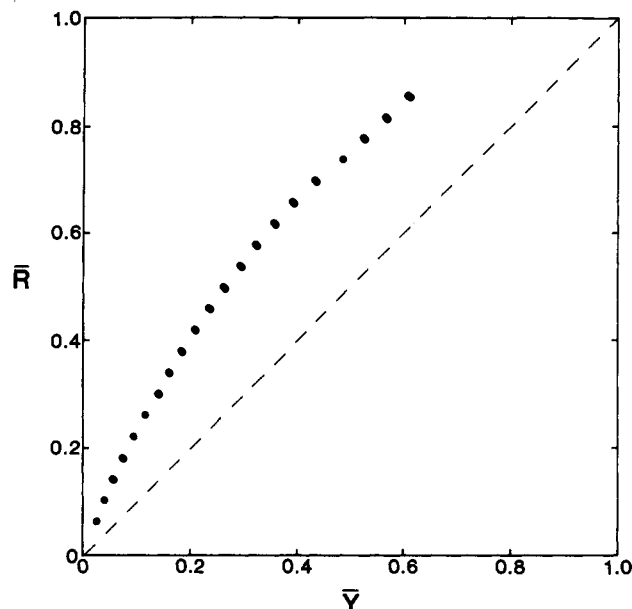


FIGURE 7: Plot of the state function,  $R [= (Q_{\text{obs}} - 63.4) / 25.6]$ , the fraction of the complex in the  $(\text{SSB})_{56}$  binding mode, as a function of  $Y (= \Sigma \nu_i / 24)$ , the extent of spermine binding to the SSB protein-poly(dT) complex (1–1.5–0.1 mM).

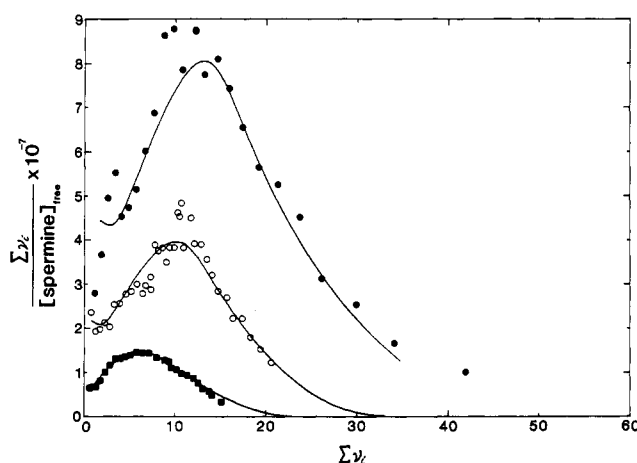


FIGURE 8: Equilibrium isotherms for spermine binding to SSB-poly(dT) complexes as a function of the protein to DNA ratio in the complex (10 mM Tris, pH 8.1, 0.1 mM NaCl, 25 °C). SSB-poly(dT) complexes are (■) 1–1.5,  $L = 100$ ,  $A = 0$ ,  $B = 1.8 \times 10^6 \text{ M}^{-1}$ , and  $K = 9.0 \times 10^4 \text{ M}^{-1}$ ; (○) 1–2,  $L = 6.4 \times 10^3$ ,  $A = 0$ ,  $B = 5.8 \times 10^6 \text{ M}^{-1}$ ,  $K = 2.5 \times 10^5 \text{ M}^{-1}$ ; and (●) 1–3,  $L = 1.9 \times 10^5$ ,  $A = 0$ ,  $B = 1.4 \times 10^7 \text{ M}^{-1}$ ,  $K = 3.0 \times 10^5 \text{ M}^{-1}$ .

compared for three different complexes (1–1.5, 1–2, and 1–3) under the same solution conditions (10 mM Tris, pH 8.1, 0.1 mM NaCl). The resulting Scatchard plots are compared in Figure 8, and the best-fit binding parameters are given in Table I. All three isotherms show clear evidence for positive cooperative binding of spermine. The main differences among the three isotherms are that the number of spermine binding sites increases upon increasing the poly(dT) to SSB ratio, the maximum value of the peak of the isotherm increases, and the peak is shifted to the right (higher spermine binding densities) as the SSB to poly(dT) ratio decreases. The increased number of binding sites presumably reflects binding of spermine to the regions of free poly(dT), which increases as the poly(dT) to SSB ratio increases. For example, the 1–3 complex can bind  $>50$  spermines in contrast to the 1–1.5 complex that binds  $\sim 24$  spermines. However, the value of  $n_0$ , representing the number of spermines bound to the actual SSB-poly(dT) complex, is independent of the ratio of SSB to poly(dT),

Table I: Equilibrium Parameters for Spermine-Induced SSB-Poly(dT) Binding Mode Transitions<sup>a</sup>

| [NaCl]<br>(mM) | SSB-poly(dT) complexes   |  |  |
|----------------|--|--|--|
|                | 1-1.5 <sup>b</sup>   | 1-2 <sup>b</sup>   | 1-3 <sup>b</sup>   |
| 0.1            | $L = 1.0 \times 10^2$<br>$A = 0$<br>$B = 1.8 \times 10^6 \text{ M}^{-1}$<br>$K = 9.0 \times 10^4 \text{ M}^{-1}$<br>$n_o^c = 12$ | $L = 6.5 \times 10^3$<br>$A = 0$<br>$B = 5.8 \times 10^6 \text{ M}^{-1}$<br>$K = 2.5 \times 10^5 \text{ M}^{-1}$<br>$n_o^c = 11$ | $L = 1.9 \times 10^5$<br>$A = 0$<br>$B = 1.4 \times 10^7 \text{ M}^{-1}$<br>$K = 3.0 \times 10^5 \text{ M}^{-1}$<br>$n_o^c = 10$ |
| 5              | $L = 5$<br>$A = 0$<br>$B = 1.4 \times 10^6 \text{ M}^{-1}$<br>$K = 3.5 \times 10^4 \text{ M}^{-1}$<br>$n_o^c = 12$               |  |  |
| 10             | $L = 0.55$<br>$A = 0$<br>$B = 6.6 \times 10^5 \text{ M}^{-1}$<br>$K = 2.3 \times 10^4 \text{ M}^{-1}$<br>$n_o^c = 12$            |  | $L = 10$<br>$A = 0$<br>$B = 5.0 \times 10^5 \text{ M}^{-1}$<br>$K = 8.0 \times 10^4 \text{ M}^{-1}$<br>$n_o^c = 10$              |

<sup>a</sup> Based on nonlinear least-square fits of eq 7 to the experimental isotherms. <sup>b</sup> See Materials and Methods for definitions of 1-1.5, 1-2, and 1-3 SSB-poly(dT) complexes. <sup>c</sup>  $n_o$  [the number of spermines bound to the SSB-poly(dT) complex] is obtained by subtracting the number of spermines bound to the free poly(dT) (assuming one spermine occludes 3.5 nucleotides) from the total number of spermines bound (the maximum value of  $\Sigma \nu_i$  obtained from the intercept of a Scatchard plot).

having values of 12, 11, and 10 for the 1-1.5, 1-2, and 1-3 complexes, respectively. Therefore, the increase in spermine binding sites reflects increased binding to the free poly(dT). We estimate the number of spermines bound to the free poly(dT) as 12, 21, and 40 for the 1-1.5, 1-2, and 1-3 complexes, respectively. The shift in the peak of the isotherm indicates that the apparent cooperativity of spermine binding is higher for the complexes with lower SSB to DNA ratios (e.g., the 1-3 complex). This is reflected in a decrease in the value of the equilibrium constant,  $L$ , from  $1.9 \times 10^6$  to  $6.4 \times 10^3$  and to 100 as the SSB binding density increases. The equilibrium constant,  $B$ , for spermine binding to the (SSB)<sub>56</sub> complex also shows a slight increase with decreasing SSB protein binding density.

We have also examined spermine binding to the 1-1.5 SSB-poly(dT) complex at pH 7.0 (10 mM HEPES, 5 mM NaCl, 25 °C) (data not shown). Cooperative binding is still apparent under these conditions, with values of  $L = 49$ ,  $A = 0$ ,  $B = 4.4 \times 10^6 \text{ M}^{-1}$ , and  $K = 3.1 \times 10^5 \text{ M}^{-1}$ . Within experimental error, these parameters are the same as those obtained at pH 8.1 under the same conditions (see Table I).

**Effect of NaCl Concentration on Spermine Binding.** The binding of spermine to the SSB-poly(dT) complex (1-1.5) was examined at three different concentrations of NaCl (0.1, 5, and 10 mM), and the Scatchard plots are compared in Figure 9. Upon raising the NaCl concentration from 0.1 to 10 mM, the peak of the isotherm shifts from right to left, indicating a decrease in apparent cooperativity. The nonlinear least-squares values of  $L$  for these isotherms decrease substantially from 100 at 0.1 mM NaCl to 5 at 5 mM NaCl to 0.55 at 10 mM NaCl. On the other hand, the equilibrium constant,  $B$ , for spermine binding to the SSB-poly(dT) complex is much less sensitive to changes in NaCl concentration, decreasing by only a factor of  $\sim 3$  upon increasing the NaCl concentration from 0.1 to 10 mM for the 1-1.5 complexes and by a factor of  $\sim 30$  for the 1-3 complexes. The binding constant for spermine-poly(dT) interactions,  $K$ , decreases with increasing NaCl concentrations as expected for a positively charged ligand interacting with DNA (Record et al., 1976; Mascotti & Lohman, 1990).

In Figure 10, the state function,  $R$ , determined for the 1-1.5 complexes at 0.1, 5, and 10 mM NaCl, is plotted vs the logarithm of the free spermine concentration. It is clear from

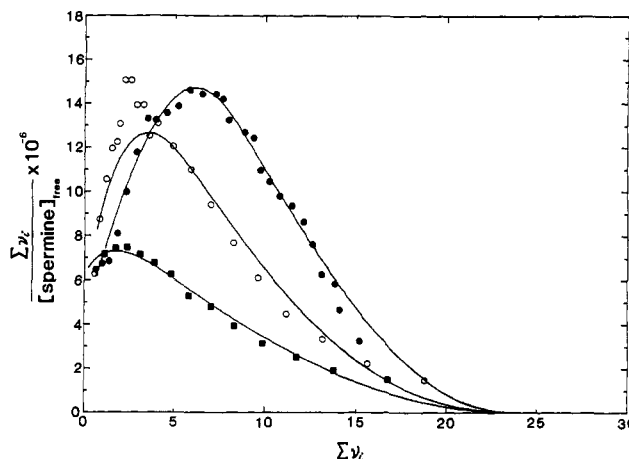


FIGURE 9: Equilibrium isotherms for spermine binding to SSB-poly(dT) complexes (1-1.5) as a function of NaCl concentration (10 mM Tris, pH 8.1, 25 °C). (●) 0.1 mM NaCl,  $L = 100$ ,  $A = 0$ ,  $B = 1.8 \times 10^6 \text{ M}^{-1}$ ,  $K = 9.0 \times 10^4 \text{ M}^{-1}$ ; (○) 5 mM NaCl,  $L = 5$ ,  $A = 0$ ,  $B = 1.4 \times 10^6 \text{ M}^{-1}$ ,  $K = 3.5 \times 10^4 \text{ M}^{-1}$ ; (■) 10 mM NaCl,  $L = 0.55$ ,  $A = 0$ ,  $B = 6.6 \times 10^5 \text{ M}^{-1}$ ,  $K = 2.3 \times 10^4 \text{ M}^{-1}$ .

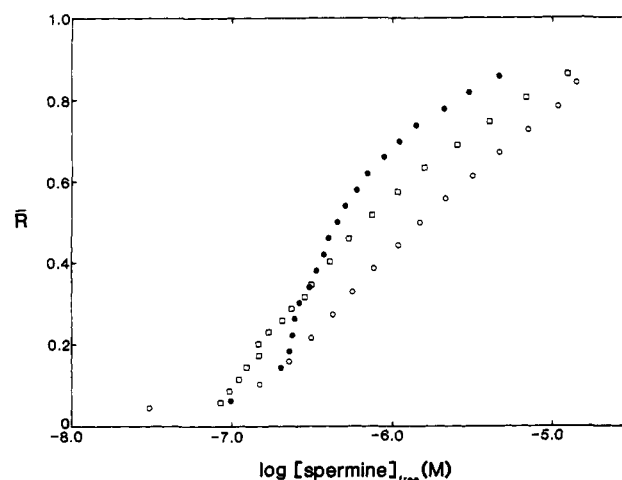


FIGURE 10: Dependence of the state function,  $R$ , on free spermine concentration for the 1-1.5 SSB-poly(dT) complex: (●) 0.1 mM NaCl, (□) 5 mM NaCl, and (○) 10 mM NaCl.

this analysis that the cooperativity of spermine binding decreases with increasing NaCl concentration and that higher spermine concentrations are required to achieve the equivalent extent of the transition. The (SSB)<sub>35</sub> to (SSB)<sub>56</sub> transition can also be induced by increasing the  $\text{Na}^+$  concentration, although  $\text{Na}^+$  is much less effective than spermine (see Figure 2). In the absence of spermine, 5–10 mM  $\text{Na}^+$  induces the (SSB)<sub>35</sub> to (SSB)<sub>56</sub> transition only partially (Bujalowski & Lohman, 1986). Therefore, the data in Figure 10 suggest that  $\text{Na}^+$  and spermine compete for binding to the SSB-poly(dT) complex, thus an increase in  $\text{Na}^+$  concentration inhibits the binding of spermine and, thus, its ability to induce the transition fully.

The values of the equilibrium constant,  $K$ , for spermine binding to poly(dT) that we obtain from fitting the isotherms to eq 7 are 10–20-fold lower than expected for the binding of a +4 ligand to ss DNA (Mascotti & Lohman, 1990). In order to obtain an independent estimate of  $K$ , we used M13 mp11 ss DNA that was modified by treatment with chloroacetaldehyde to form a fluorescent derivative, referred to as  $\epsilon$ -M13mp11 ssDNA. Titrations of  $\epsilon$ -M13mp11 ssDNA with spermine were performed in 2.5, 5, and 10 mM NaCl in 10 mM Tris HCl, pH 8.1, and these titrations were analyzed by the binding density function method to obtain equilibrium



binding isotherms. Values of  $K$  were then obtained by fitting these isotherms to the site overlap model (McGhee & von Hippel, 1974). At both 5 and 10 mM NaCl, the values of  $K$  for spermine binding to  $\epsilon$ M13 ssDNA were a factor of 10 larger than those obtained from the fitting of the isotherms to eq 7. These differences may reflect the difficulty in estimating  $K$  from a three-parameter ( $L$ ,  $B$ , and  $K$ ) nonlinear least-squares fit. However, it is also possible that the bound SSB protein influences the affinity of spermine for poly(dT).

## DISCUSSION

We have expanded our studies of the *E. coli* SSB-ss DNA binding modes to include the effects of multivalent cations and polyamines. The effects of these polyvalent cations are of interest for a variety of reasons. First of all, multivalent cations such as  $Mg^{2+}$  and the polyamines, putrescine and spermidine, are present in *E. coli* (Tabor & Tabor, 1985). Furthermore, the *E. coli* SSB protein-catalyzed renaturation of DNA can be stimulated dramatically by divalent cations and polyamines under certain solution conditions (Christiansen & Baldwin, 1977). Finally, the effects of these cations can be used to probe the complex effects of cations and anions on the SSB-ss DNA binding mode transitions.

Our results demonstrate that the trivalent cations,  $Co(NH_3)_6^{3+}$  and spermidine $^{3+}$ , as well as the tetravalent cation, spermine $^{4+}$ , induce the transition from the (SSB) $_{35}$  to the (SSB) $_{56}$  binding mode at micromolar concentrations (pH 8.1, 25 °C). This is significantly lower than the 10 and 100 mM concentrations required to induce the same transition using divalent and monovalent cations, respectively (see Figure 2). However, although cation charge is a major feature in effecting the SSB-binding mode transition, it is not the sole determining factor, since we observe that  $Co(NH_3)_6^{3+}$  is more effective than either spermine $^{4+}$  or spermidine $^{3+}$ .

Previous studies from this laboratory have demonstrated that the transitions from the lower to the higher SSB-ss DNA binding modes are accompanied by a net uptake of cations (Lohman & Overman, 1985; Bujalowski & Lohman, 1986; Bujalowski et al., 1988); however, the isotherms reported here provide the first details of these cation-SSB protein interactions. The results indicate that, in the presence of spermine $^{4+}$ , the (SSB) $_{35}$  to (SSB) $_{56}$  transition is accompanied by the cooperative binding of a large number of spermine ions [ $\sim 10$ – $12$  binding to the SSB-poly(dT) complex itself, with additional spermines binding to the free poly(dT)]. Furthermore, spermidine $^{3+}$ ,  $Co(NH_3)_6^{3+}$ , and  $Mg^{2+}$  also bind cooperatively to the SSB-poly(dT) complex, resulting in the uptake of a large number of cations. As suggested previously, this additional cation binding may be required to partially neutralize the polyanionic ss DNA concomitant with its binding to the third and fourth subunits of the SSB tetramer in order to form the (SSB) $_{56}$  binding mode (Lohman & Overman, 1985; Bujalowski & Lohman, 1986). Although strong, cooperative binding of spermine is observed under all conditions, lower affinity binding is also observed at lower SSB binding densities. This weaker binding presumably reflects the binding of spermine to the poly(dT) that is not complexed with SSB protein.

In wild-type *E. coli* grown to mid-log phase in minimal media, the polyamines, spermidine (+3) and putrescine (+2) are present at total intracellular concentrations of  $\sim 6$  and  $\sim 20$  mM, respectively, whereas spermine is not present in *E. coli* (Tabor & Tabor, 1985). Unfortunately, the free polyamine concentration is less well-defined; however, our results suggest that SSB tetramers would bind to ss DNA in either the (SSB) $_{35}$  or the (SSB) $_{56}$  binding modes if the free spermidine

concentration in *E. coli* is  $\geq 10 \mu M$ , providing that equilibrium considerations control SSB binding. On the other hand, the binding of SSB tetramers to ss DNA may be under kinetic control during some cellular processes such as DNA replication, hence a functional role for the (SSB) $_{35}$  binding mode is not ruled out by these studies.

Although the results reported here do not provide an explanation for the observation that the *E. coli* SSB protein is able to catalyze the renaturation of duplex DNA in the presence of multivalent cations (Christiansen & Baldwin, 1977), they do indicate that polyamines exert a dramatic effect on the stability of the different SSB-ss DNA binding modes. Therefore, it remains a possibility that the linked effects of cations and pH on the ability of SSB protein to facilitate DNA renaturation (Christiansen & Baldwin, 1977) are influenced by the SSB binding mode distribution. However, it is likely that the effects of polyamines will need to be examined as a function of pH and temperature in order to draw more firm conclusions. We have examined the binding of spermine to SSB-poly(dT) complexes, at both pH 8.1 and 7.0 at 25 °C. At both pH's, the transition from the (SSB) $_{35}$  to the (SSB) $_{56}$  binding mode is induced by the cooperative binding of multiple spermine molecules to the SSB-poly(dT) complex, with little influence of pH. This result is consistent with the fact that catalysis of DNA renaturation is observed in the presence of spermine at both pH 7 and 8 (Christiansen & Baldwin, 1977). We have also attempted to compare the effects of  $Mg^{2+}$  at pH 8.1 vs pH 5.5; however, slow changes in SSB tryptophan fluorescence occurred at pH 5.5 and titrations under these conditions required excessive times to reach equilibrium (W. Bujalowski, unpublished observations), therefore these studies were inconclusive.

## ACKNOWLEDGMENTS

We thank Dr. Les Overman for his preliminary studies of the effects of polyamines and Lisa Lohman for preparing the figures.

Registry No.  $Mg^{2+}$ , 7439-95-4;  $Ca^{2+}$ , 7440-70-2;  $Na^+$ , 7440-23-5;  $NH_4^+$ , 14798-03-9;  $Co(NH_3)_6^{3+}$ , 14695-95-5; spermine, 71-44-3; spermidine, 124-20-9.

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