Escherichia coli Single-Strand Binding Protein Forms Multiple, Distinct Complexes with Single-Stranded DNA†

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ABSTRACT: Four distinct binding modes for the interaction of *Escherichia coli* single-strand binding (SSB) protein with single-stranded (ss) DNA have been identified on the basis of quantitative titrations that monitor the quenching of the SSB protein fluorescence upon binding to the homopolynucleotide poly(dT) over a range of $MgCl_2$ and NaCl concentrations at 25 and 37 °C. This is the first observation of multiple binding modes for a single protein binding to DNA. These results extend previous studies performed in NaCl (25 °C, pH 8.1), in which two distinct SSB–ss DNA binding modes possessing site sizes of 33 and 65 nucleotides per bound SSB tetramer were observed [Lohman, T. M., & Overman, L. B. (1985) *J. Biol. Chem. 260*, 3594–3603]. Each of these binding modes differs in the number of nucleotides occluded upon interaction with ss DNA (i.e., site size). Along with the previously observed modes with site sizes of 35 ± 2 and 65 ± 3 nucleotides per tetramer, a third distinct binding mode, at 25 °C, has been identified, possessing a site size of 56 ± 3 nucleotides per bound SSB tetramer, which is stable over a wide range of $MgCl_2$ concentrations. At 37 °C, a fourth binding mode is observed, possessing a site size of 40 ± 2 nucleotides per tetramer, although this mode is observable only over a small range of salt concentration. The relative populations of each binding mode are modulated primarily by the charge and concentration of low molecular weight cations in solution (Mg^{2+} and Na^+ in these experiments), indicating that a net binding of cations occurs upon formation of each of the higher site size SSB–DNA complexes. Mg^{2+} is much more effective than Na^+ in facilitating the transitions to the higher site size binding modes. At 25 °C (pH 8.1) the two binding mode transitions have midpoints of 0.6 and 64 mM in $MgCl_2$ and 17 mM and 0.16 M in NaCl; hence, all three SSB binding modes may form in vivo since these salt concentrations are within the range estimated to occur in *E. coli*. These transitions also occur within the same range of $MgCl_2$ concentrations used for replication and recombination studies in vitro; hence, they are of definite importance for any such studies in vitro. Since the *E. coli* SSB protein is essential for replication, recombination, and repair processes, it is possible that the different binding modes identified here are used selectively in each of these processes in vivo.

The essential role of the *Escherichia coli* SSB protein, a helix-destabilizing protein, in DNA replication, recombination, and repair is well established (Wickner & Hurwitz, 1974; Glassberg et al., 1979; Johnson, 1979; Chase & Williams, 1986). However, in spite of many studies of the SSB protein, it has only recently been shown that the interaction of this protein with ss DNA is quite complex (Lohman & Overman, 1985; Chrysogelos & Griffith, 1982; Lohman et al., 1986b; Griffith et al., 1984; Krauss et al., 1981) and its DNA binding properties differ substantially from those of the bacteriophage T4 gene 32 protein, another well-characterized helix-destabilizing protein. The *E. coli* SSB protein exists in solution as a stable tetramer, and it is the tetramer that seems to interact with ss DNA (Bandyopadhyay & Wu, 1978; Williams et al., 1984), although formation of SSB octamers on ss DNA has also been observed (Chrysogelos & Griffith, 1982). Recently, two distinct binding modes for SSB–ss DNA complexes have been reported by Lohman and Overman (1985) and Griffith et al., 1984). Other studies have shown that SSB displays only weak cooperativity when bound to ss DNA at equilibrium (Lohman et al., 1986b; Griffith et al., 1984); however, highly cooperative but metastable binding has also been observed upon direct mixing of SSB with ss DNA at low NaCl concentrations (Lohman et al., 1986b; Ruyechan & Wetmur, 1975).

The evidence for two different binding modes is based on quantitative titrations that monitored the quenching of the SSB protein's intrinsic tryptophan fluorescence upon binding to poly(dT) (Lohman & Overman, 1985) as well as electron microscopy (Griffith et al., 1984). The two binding modes differ in the number of nucleotides occluded per bound SSB tetramer (i.e., the site size, n) (Lohman & Overman, 1985) and display distinct morphologies in the electron microscope (Griffith et al., 1984). Below 10 mM NaCl, $n = 35 \pm 3$ nucleotides per bound SSB tetramer [$(SSB)_{35}$], whereas above 0.2 M NaCl, $n = 65 \pm 5$ nucleotides per tetramer [$(SSB)_{65}$] (25.0 °C, pH 8.1). Between these two NaCl concentrations intermediate apparent site sizes are observed, indicating a mixture of binding modes. Increasing the concentration of monovalent cations in solution promotes the transition from the $(SSB)_{35}$ to the $(SSB)_{65}$ binding mode (Lohman & Overman, 1985). In the electron microscope, high site size complexes appear as beaded structures in which each bead seems to be composed of 140–160 nucleotides wrapped around an

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octamer of SSB monomers, whereas low site size complexes have a smooth morphology (Chrysogelos & Griffith, 1982; Griffith et al., 1984). The binding density of the SSB–ss DNA complexes also affects the relative populations of each binding mode such that, at low NaCl concentrations, the high site size, beaded complex is favored at low binding densities whereas the low site size complex is favored at high binding densities (Griffith et al., 1984). In this study we have extended our previous investigations to examine the effect of $MgCl_2$ and temperature on the SSB binding mode transition for two reasons. First, to further probe the molecular basis for the effect of salt on the transition, we examined the effect of a divalent cation, since this should yield quantitatively different behavior than for NaCl if direct binding of cations is important. Second, this divalent cation is also an important solution variable to consider since replication, recombination, and repair studies performed in vitro routinely contain variable concentrations of Mg^{2+}. Therefore, it was of interest to see the effect of Mg^{2+} on the SSB–poly(dT) site size.

Materials and Methods

Reagents and Buffers. All chemicals were reagent grade; all solutions were made with distilled and deionized (Milli-Q) water. The standard buffer was buffer T (pH 8.1), which is 10 mM Tris [tris(hydroxymethyl)aminomethane] and 0.1 mM Na_3EDTA (trisodium ethylenediaminetetraacetate). The pH of buffer T was adjusted to pH 8.1 at each temperature (25 and 37 °C) with HCl. The NaCl and $MgCl_2$ concentrations in buffer T were as indicated in the text. A stock of concentrated $MgCl_2$ (2 M) was made from $MgCl_2$, which had been dried in a desiccator under vacuum for several days until a constant weight was achieved.

E. coli SSB Protein and Nucleic Acids. The SSB protein was prepared as previously described from a strain of *E. coli* K12 containing the plasmid pTL119A-5, which is temperature inducible for SSB overproduction (Lohman et al., 1986a). The concentration of SSB was determined spectrophotometrically by using the extinction coefficient $\epsilon_{280} = 1.5$ mL mg^{-1} cm^{-1} [1.13×10^5 M^{-1} (tetramer) cm^{-1}] in buffer T + 0.20 M NaCl (Lohman & Overman, 1985). The poly(dT) ($s_{20,w} = 8.4$ S; ~780 nucleotides) was purchased from Pharmacia P-L Biochemicals and was extensively dialyzed before use. The concentration of poly(dT) was determined spectrophotometrically in buffer T + 0.10 M NaCl, with an extinction coefficient (260 nm) of 8.1×10^3 M^{-1} (nucleotide) cm^{-1}. Sedimentation coefficients were determined by sedimentation velocity in a Beckman Model E analytical ultracentrifuge, equipped with a multiplexer and photoelectric scanner.

Fluorescence Measurements and Site Size Determinations. Titrations of SSB with poly(dT) were performed with an SLM 8000 spectrofluorometer, basically as described by Lohman and Overman (1985). An excitation wavelength of 296 nm, excitation bandpass = 1 nm (0.5-mm slit width), was used while the emission at 347 nm was monitored; emission bandpass = 4 nm (2-mm slit width). The cell compartment temperature was maintained at 25.0 ± 0.1 or 37.0 ± 0.1 °C, as indicated in the text, with a Lauda RM-3S refrigerated circulating water bath. The minimum time between addition of poly(dT) and the fluorescence readings was 2 min with a 5-s acquisition time; the fluorescence readings did not change when longer times were used. No detectable photobleaching was observed due to the low excitation bandpass that was used. When necessary, inner filter corrections were applied by using the expression (Lakowicz, 1983)

$$F_{cor} = F_{obsd} \text{ antilog } (A_{296}/2) \quad (1)$$

As long as small slits are used, eq 1 yields inner filter corrections that are identical with those obtained by the method of Birdsall et al. (1983) up to absorbances of 0.1. In the experiments reported here, the $A_{296} \leq 0.01$; hence, the largest inner filter corrections were always less than 2% and corrections for dilution were ≤4%. The SSB concentration in these experiments was 5.16×10^{-8} M (tetramer) (3.89 μg/mL); however, at a given salt concentration the apparent site sizes are constant over a 10-fold range of SSB concentration (2–24 μg/mL). All experiments within a given salt series were performed with a single stock solution of SSB protein and a single stock solution of poly(dT) in order to improve the relative accuracy of the data. We have previously shown that SSB maintains full DNA binding activity at the salt and SSB concentrations used in our studies and no precipitation of SSB was detected (Lohman & Overman, 1985).

Results and Discussion

Three Stable SSB–Poly(dT) Binding Modes Are Observed in Solutions of Varying $MgCl_2$ and NaCl Concentrations at 25.0 °C. Site size measurements in the presence of $MgCl_2$ were undertaken in order to assess the role of direct binding of cations to the SSB–poly(dT) complex and to determine the SSB binding mode that is likely to form under the conditions used for replication and recombination studies, in vitro. As in our previous studies (Lohman & Overman, 1985) we have used poly(dT) rather than natural single-stranded DNA in order to obtain site size estimates in the absence of competing intramolecular nucleic acid base pairing. In addition, poly(dT) forms stoichiometric complexes with SSB over a wide range of salt conditions, even in 5 M NaCl and up to 1 M $MgCl_2$, hence allowing accurate site size measurements over a wide range of salt concentrations. The dependence of the site size of the SSB–poly(dT) complex upon $MgCl_2$ concentration (25.0 °C, pH 8.1) is shown in Figure 1, where it is compared with results in NaCl. Both sets of data indicate the presence of three distinct plateau regions that differ in site size, although the three regions are more clearly separated in $MgCl_2$. We interpret these three plateau regions as distinct SSB binding modes possessing site sizes of 35 ± 2, 56 ± 3, and 65 ± 3 nucleotides per bound SSB tetramer. The midpoints of the two transitions occur at 0.6 and 64 mM $MgCl_2$ and at 17 mM and 0.16 M NaCl. To our knowledge this is the first identification of multiple, stable binding modes for a protein–nucleic acid interaction. As shown in Figure 1, the $(SSB)_{56}$ binding mode is stable over a much wider salt range in $MgCl_2$ (4–50 mM) than in NaCl. Thus the existence of the $(SSB)_{56}$ intermediate binding mode, which Lohman and Overman (1985) did not observe, is much more difficult to detect in NaCl than in $MgCl_2$ solutions and was only apparent in NaCl when we carefully reexamined this region of the transition curve (see Figure 1).

At 37.0 °C, a Fourth Distinct SSB–Poly(dT) Binding Mode Is Observed. Since most studies of DNA replication and recombination are undertaken at 37 °C, we examined the site sizes of SSB–poly(dT) complexes at this temperature over the same range of NaCl and $MgCl_2$ concentrations that we studied at 25.0 °C. These data are displayed in Figure 2. The same three site size plateaus that we observed at 25.0 °C, 35 ± 2, 56 ± 3, and 65 ± 3 nucleotides per SSB tetramer, are also present at 37.0 °C. However, at 37.0 °C there is a fourth plateau region at 40 ± 2 nucleotides per tetramer, in the range from 7 to 12 mM NaCl as well as from 40 to 70 μM $MgCl_2$. Although these salt ranges are small, nonetheless the plateau at 40 ± 2 nucleotides per tetramer is real. With the exception of the additional site size plateau at 40 ± 2 nucleotides per

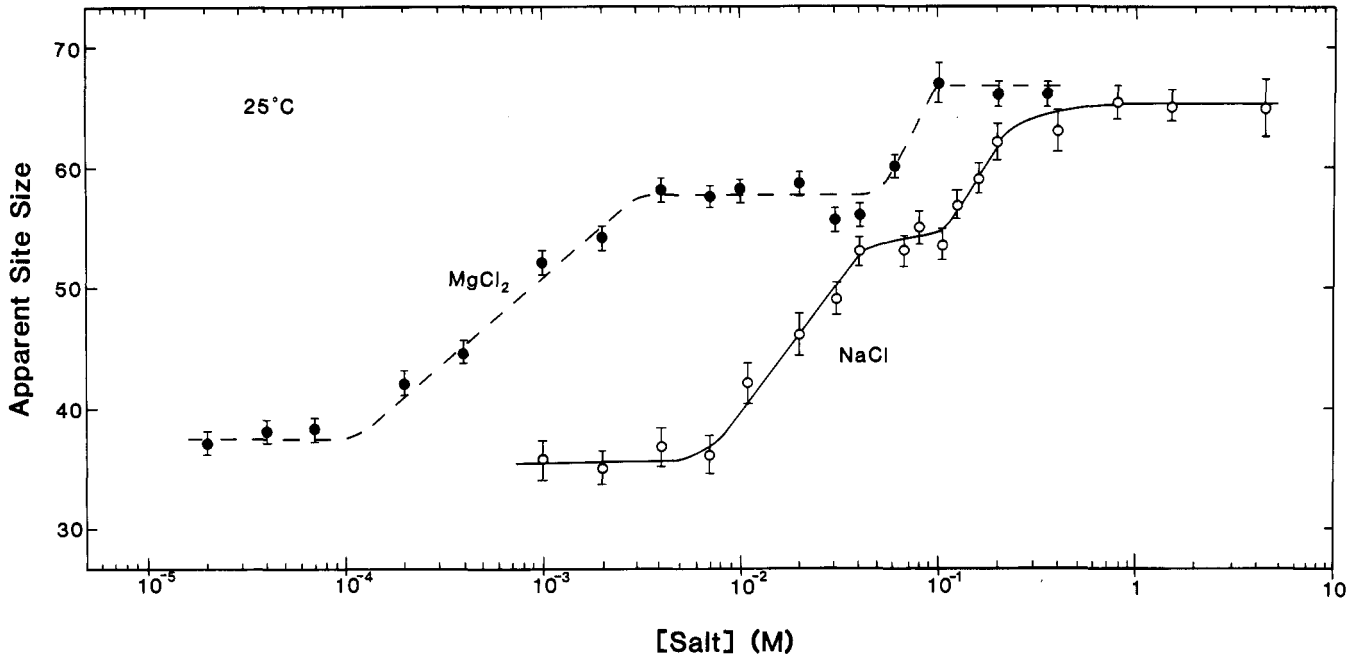

FIGURE 1: Apparent site size (nucleotides per SSB tetramer) of SSB–poly(dT) complexes as a function of NaCl and $MgCl_2$ concentration (M) (logarithmic scale) in 10 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 8.1, 25.0 °C. Site sizes were determined as described previously (Lohman & Overman, 1985) by monitoring the quenching of the intrinsic tryptophan fluorescence of the SSB protein upon titration with poly(dT).

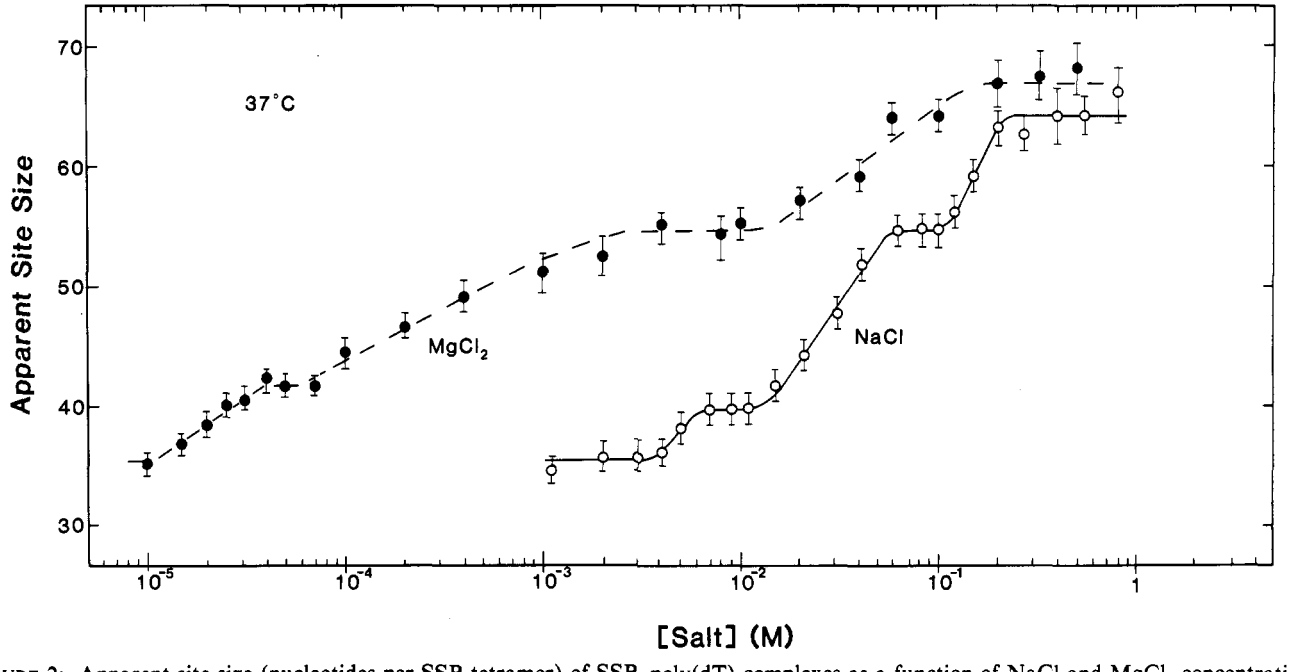

FIGURE 2: Apparent site size (nucleotides per SSB tetramer) of SSB–poly(dT) complexes as a function of NaCl and $MgCl_2$ concentration (M) (logarithmic scale) in 10 mM Tris-HCl, pH 8.1, 37.0 °C.

tetramer at 37 °C, the site size curves in NaCl do not differ much between 37 and 25 °C. However, at 37 °C in $MgCl_2$, all of the site size transitions are substantially broadened as compared to the data at 25 °C. Furthermore, at 37 °C, the $(SSB)_{56}$ plateau extends over a narrower range of $MgCl_2$ concentrations from approximately 4 to 10 mM.

Direct Uptake of Cations Occurs in Each of the SSB Binding Mode Transitions. As can be seen in Figures 1 and 2, the apparent site size, which reflects the relative populations of the different SSB binding modes, strongly depends upon the concentrations of Mg^{2+} and Na^+ in solution. In addition, the binding mode transitions occur at lower salt concentrations in $MgCl_2$ than in NaCl, although the low-salt transition is shifted to a greater extent than the high-salt transition, indicating differential cation-specific effects on the two transitions. The shift in the transitions in $MgCl_2$ vs. NaCl is much larger than expected from simple ionic strength effects, which indicates that direct uptake of cations occurs upon formation of the higher site size complexes from the lower site size complexes.

Krauss et al. (1981) have inferred from their data that each protomer of the SSB tetramer contains a separate ss DNA binding site. Furthermore, a number of experiments indicate that ss DNA is wound around SSB in the different binding modes that have previously been identified (Krauss et al., 1981; Lohman & Overman, 1985; Lohman et al., 1986; Chrysogelos & Griffith, 1982; Griffith et al., 1984). From electron microscopy measurements two of these modes are known to

compact ss fd DNA by 60% and 75% (Griffith et al., 1984). Therefore, the four SSB site size modes that we report here may represent SSB–ss DNA complexes possessing different extents of DNA winding as well as different aggregation states of bound SSB (e.g., tetramers vs. octamers). The required cation uptake that we observe may be necessary for stabilization of SSB–DNA complexes with high extents of DNA winding (Lohman & Overman, 1985; Lohman et al., 1986b) or for the formation of stable octamers on the DNA (Chrysogelos & Griffith, 1982; Griffith et al., 1984).

Griffith et al. (1984) have determined that SSB–ss fd DNA complexes display only a high site size, beaded morphology in the electron microscope when in the presence of 12 mM $MgCl_2$–30 mM NaCl, pH 7.5 (conditions used for the RecA protein catalyzed DNA strand exchange reaction), and cannot be induced to undergo the transition to the lower site size, smooth-contoured complex. This is consistent with the data in Figures 1 and 2, which show a shift to the high site size complexes in $MgCl_2$ concentrations above 3 mM at both 25 and 37 °C. Additional site size measurements performed with poly(dT) in 30 mM NaCl–12 mM $MgCl_2$ (pH 8.1, 25 °C) indicate a site size of 56 nucleotides per SSB tetramer (data not shown). At this time we do not know whether both of the $(SSB)_{56}$ and $(SSB)_{65}$ complexes form beaded structures, although if we ignore any potential differences between our data obtained with poly(dT) vs. ss fd DNA (Griffith et al., 1984) and simply compare results under the same solution conditions, it would seem that the $(SSB)_{56}$ binding mode also has a beaded morphology.

Conclusions

It has been recognized that *E. coli* SSB protein plays a more active role in DNA metabolism than simply to bind ss DNA during replication (Chase & Williams, 1986). Evidence for the interaction of SSB with other replication and recombination proteins also exists (Sigal et al., 1972; Molineux & Gefter, 1975; Low et al., 1982; Tessman & Peterson, 1982). Since the transitions between the different SSB–ss DNA binding modes occur within the salt concentration range estimated for *E. coli* in vivo, it is possible that, rather than use a single mode of DNA binding, different SSB–DNA binding modes are selectively used in replication, recombination, and repair. It is also important to note that the salt concentration range in which we observe the binding mode transitions encompasses those routinely used for studies of DNA replication, recombination, and repair in vitro. Hence, for the correct interpretation of such studies it is necessary to consider the likelihood that the different SSB–DNA complexes, which we have observed and which may interact in different ways with other protein components and DNA, may be simultaneously present in vitro. Furthermore, changes in solution conditions, especially $MgCl_2$ and NaCl concentrations, will affect the distribution and cooperativity (Lohman et al., 1986b) of the SSB binding modes that we report here.

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