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Regulation of Single-stranded DNA Binding by the C Termini of *Escherichia coli* Single-stranded DNA-binding (SSB) Protein*[S]

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The homotetrameric *Escherichia coli* single-stranded DNA-binding (SSB) protein plays a central role in DNA replication, repair, and recombination. In addition to its essential activity of binding to transiently formed single-stranded (ss) DNA, SSB also binds an array of partner proteins and recruits them to their sites of action using its four intrinsically disordered C-terminal tails. Here we show that the binding of ssDNA to SSB is inhibited by the SSB C-terminal tails, specifically by the last 8 highly acidic amino acids that comprise the binding site for its multiple partner proteins. We examined the energetics of ssDNA binding to short oligodeoxynucleotides and find that at moderate salt concentration, removal of the acidic C-terminal ends increases the intrinsic affinity for ssDNA and enhances the negative cooperativity between ssDNA binding sites, indicating that the C termini exert an inhibitory effect on ssDNA binding. This inhibitory effect decreases as the salt concentration increases. Binding of ssDNA to approximately half of the SSB subunits relieves the inhibitory effect for all of the subunits. The inhibition by the C termini is due primarily to a less favorable entropy change upon ssDNA binding. These observations explain why ssDNA binding to SSB enhances the affinity of SSB for its partner proteins and suggest that the C termini of SSB may interact, at least transiently, with its ssDNA binding sites. This inhibition and its relief by ssDNA binding suggest a mechanism that enhances the ability of SSB to selectively recruit its partner proteins to sites on DNA.

Single-stranded DNA-binding (SSB)[2] proteins are essential players in the maintenance of the genomes of all organisms (1–4). Although the structures and assembly states of SSB proteins vary, they all employ an OB-fold as their core ssDNA binding domain (5). The *Escherichia coli* SSB protein is the prototypical example of the homotetrameric class of SSB proteins. Most members of this class are found in bacteria, the homotetrameric human mitochondrial SSB protein being an exception. Although some bacterial SSB proteins are homodimers (*Thermus/Deinococcus*), the monomer of these proteins contains two OB-folds, and thus the dimer still possesses four OB-folds and is structurally similar to the tetrameric SSB proteins (6–8).

The *E. coli* SSB protein (9) binds with high affinity but little sequence specificity to ssDNA (1, 10, 11). Due to its four potential ssDNA binding sites, the tetramer can bind ssDNA in multiple modes that differ in the number of subunits used to contact the DNA. Two of the major ssDNA binding modes identified are denoted $(SSB)_{35}$ and $(SSB)_{65}$, where the subscripts indicate the average number of ssDNA nucleotides occluded per bound SSB tetramer (12, 13). The relative stabilities of these binding modes depend on salt concentration and type (12, 13) as well as protein-to-DNA ratio (14–17). In the $(SSB)_{65}$ mode, favored at [NaCl] $>$ 0.2 M, ~65 nucleotides of ssDNA wrap around all four subunits of the tetramer while displaying only "limited" cooperativity between adjacent tetramers. In the $(SSB)_{35}$ mode, favored at [NaCl] $<$ 0.02 M and high SSB to DNA ratios, an average of only two subunits of the tetramer interacts with ~35 nucleotides of ssDNA, and SSB binding to ssDNA displays high cooperativity and can form long protein clusters (10, 12, 13, 15, 17–19).

Models have been proposed for both the $(SSB)_{35}$ and the $(SSB)_{65}$ binding modes based on x-ray crystal structures of a C-terminal truncation of SSB (missing residues 136–177) bound to two molecules of the ssDNA $(dC)_{35}$ (20). Fig. 1*A* shows the homotetrameric structure of the four core DNA binding domains (residues 1–112), each of which forms an OB-fold, as well as the proposed topology of ssDNA wrapping in the $(SSB)_{65}$ binding mode, where ~65 nucleotides of ssDNA enter and exit in close proximity. Although SSB binds with very high affinity to ssDNA, it has recently been shown that in this mode, an SSB tetramer can diffuse via a random walk along ssDNA while remaining bound to ssDNA (21). The electron densities for the four acidic C-terminal tails (residues 113–177) are not observable in the crystal structures, even when SSB is bound to ssDNA (22), suggesting, as depicted in Fig. 1*A*, that the C-terminal tails are disordered, as first suggested based on its primary structure (23).

In addition to the essential activity of binding with high affinity to ssDNA, SSB proteins also play essential roles by binding an array of partner proteins to recruit them to their sites of function during DNA metabolism. To date, at least 14 other proteins involved in DNA metabolism have been found to interact with the *E. coli* SSB protein (4). All of these proteins appear to bind to SSB via the highly acidic ~8–10 amino acids

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[2] The abbreviations used are: SSB, single-stranded DNA-binding protein; SSBΔC8, SSB with the final 8 amino acids removed from each of its four C termini; SSB_C, chymotryptic fragment of SSB lacking 42 amino acids of its C termini; ssDNA, single-stranded DNA; ITC, isothermal titration calorimetry; wt, wild type.

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at the very end of its unstructured C termini. Although the C-terminal SSB tails are not involved directly in binding ssDNA, they can influence the $(SSB)_{35}$ to $(SSB)_{65}$ DNA binding mode transition (17). Williams *et al.* (24) also demonstrated that deletion of the C-terminal tails improves the ability of SSB to melt duplex poly[d(A-T)]. The phage T4 gene 32 protein (25, 26) and the page T7 gene 2.5 protein (27) also have acidic C-terminal tails that inhibit their ability to lower the melting temperature of duplex DNA. Removal of the acidic C-terminal regions of T4 gene 32 protein (28) and T7 gene 2.5 protein (29, 30) enhances their ssDNA binding affinity. Removal of the C termini of *E. coli* SSB has been shown to enhance its affinity for the RNA, poly(U) (31).

We have recently shown that the binding of the acidic C-terminal SSB tail to both PriA and the χ subunit of DNA polymerase III holoenzyme is enhanced when it is removed from the SSB-ssDNA binding core (51). Furthermore, upon SSB binding to ssDNA, the affinities of PriA and the χ subunit are enhanced. The inhibition of C termini binding affinity when attached to the SSB DNA binding core could reflect a direct interaction of the acidic region of the C termini to the ssDNA binding site. Based on this, we have directly examined the effect of deletion of the SSB C-terminal tails on the energetics of binding of SSB to a variety of ssDNA oligonucleotides and show that the presence of the C-terminal tail does indeed inhibit ssDNA binding.

EXPERIMENTAL PROCEDURES

Reagents and Buffers—All buffers were prepared with reagent grade chemicals and distilled water that was subsequently treated with a Milli-Q (Millipore, Bedford, MA) water purification system. Buffer T is 10 mM Tris, pH 8.1, and buffer H is 10 mM Hepes, pH 8.1. All buffers contained 0.1 mM Na_3EDTA.

E. coli SSB and SSB with C-terminal Deletions and ssDNA—SSB protein was purified as described (32) with the addition of a double-stranded DNA cellulose column to remove a minor exonuclease contaminant (33). The chymotryptic fragment of SSB (SSB_C) was obtained as described (9, 24). The SSBΔC8 protein was overexpressed using plasmid pEAW393 and purified as described (34). The concentrations of wtSSB and its C-terminal deletion fragments were determined spectrophotometrically in Tris buffer (pH 8.1, 0.2 M NaCl) using an extinction coefficient of $\epsilon_{280} = 1.13 \times 10^5\ M^{-1}$ (tetramer) cm^{-1} (12). The oligodeoxynucleotides, $(dT)_{35}$ and $(dA)_{35}$, were synthesized and purified as described (19) and were ≥98% pure as judged by denaturing gel electrophoresis and autoradiography of a sample that was 5′ end-labeled with ^{32}P using polynucleotide kinase. All ssDNA concentrations were determined spectrophotometrically in buffer T (pH 8.1), 100 mM NaCl using the extinction coefficient $\epsilon_{260} = 8.1 \times 10^3\ M^{-1}$ (nucleotide) cm^{-1} for oligo(dT) and poly(dT) (35) and $\epsilon_{260} = 9.65 \times 10^3\ M^{-1}$ (nucleotide) cm^{-1} for $(dA)_{35}$ (36, 37). All DNA and protein samples were dialyzed extensively *versus* the particular buffer containing the indicated salt concentration that was used in the fluorescence or isothermal titration calorimetry (ITC) titration experiments.

Fluorescence Measurements—Titrations of wtSSB and SSBΔC8 with $(dA)_{35}$ or $(dT)_{35}$ were performed by monitoring the intrinsic tryptophan fluorescence of the proteins using a PTI QM-4 spectrofluorometer (Photon Technology International, Lawrenceville, NJ) using an excitation wavelength of 296 nm and monitoring emission fluorescence intensity at 345 nm as described (38, 39).

Binding isotherms were analyzed using a two-site sequential binding model described in Equation 1,

$$Q_{obs} = \frac{Q_1K_1D + Q_2K_1K_2D^2}{1 + K_1D + K_1K_2D^2} \quad \text{(Eq. 1)}$$

where Q_{obs} is the observed fluorescence quenching and Q_1 and Q_2 are the fluorescence quenching corresponding to one and two $(dN)_{35}$ bound, respectively; K_1 and K_2 are the observed stepwise macroscopic association constants for the binding of the first and the second DNA molecule. The concentration of free DNA, D, was determined from Equation 2,

$$D_{tot} = D + D_{bound} = D + \frac{K_1D + 2K_1K_2D^2}{1 + K_1D + K_1K_2D^2}P_{tot} \quad \text{(Eq. 2)}$$

where D_{tot} and P_{tot} are total concentrations of $(dN)_{35}$ and protein, respectively. These data were also analyzed using the "square model" (40) in which binding is characterized by two parameters, an intrinsic binding constant K_{sq} and a negative cooperativity parameter, σ. The macroscopic binding constants K_1 and K_2 can be written in terms of K_{sq} and σ using Equations 3 and 4.

$$K_1 = 2 \cdot (1 + 2\sigma) \cdot K_{sq} \quad \text{(Eq. 3)}$$

$$K_2 = K_{sq}3\sigma^4/2(1 + 3\sigma) \quad \text{(Eq. 4)}$$

Non-linear least squares fitting of the isotherms to Equations 1–4 to obtain the binding parameters was performed using SCIENTIST (Micromath, St. Louis, MO).

ITC—ITC experiments were performed using a VP-ITC titration microcalorimeter (MicroCal, LLC, Northhampton, MA) (41). Experiments were carried out by titrating SSB or SSBΔC8 solutions (0.3–1.8 μM) with $(dA)_{35}$ (concentrations ranging from 8 to 40 μM). Control experiments to determine the heat of dilution for each injection were performed by injecting the same volumes of $(dA)_{35}$ into the sample cell containing only buffer.

Although each SSB tetramer can potentially bind two molecules of $(dA)_{35}$, in many cases, the binding of the second DNA molecule was not detectable in our experiments due to the high degree of negative cooperativity (37, 38, 42) (see Fig. 3, *A*, *C*, and *D*, and supplemental Fig. S1*C*). Under those conditions, the data were analyzed using a 1:1 binding model described by Equation 5,

$$Q_i^{tot} = V_0\Delta H \cdot P_{tot}\frac{nKD}{1 + KD} \quad \text{(Eq. 5)}$$

where Q_i^{tot} is total heat after i^{th} injection, V_0 is the volume of calorimetric cell, and n, K, and ΔH are the stoichiometry of binding, observed equilibrium binding constant, and binding enthalpy. The concentration of free $(dA)_{35}$, D, was obtained from Equation 6,

$$D_{tot} = D + D_{bound} = D + P_{tot}\frac{nKD}{1 + KD} \quad \text{(Eq. 6)}$$

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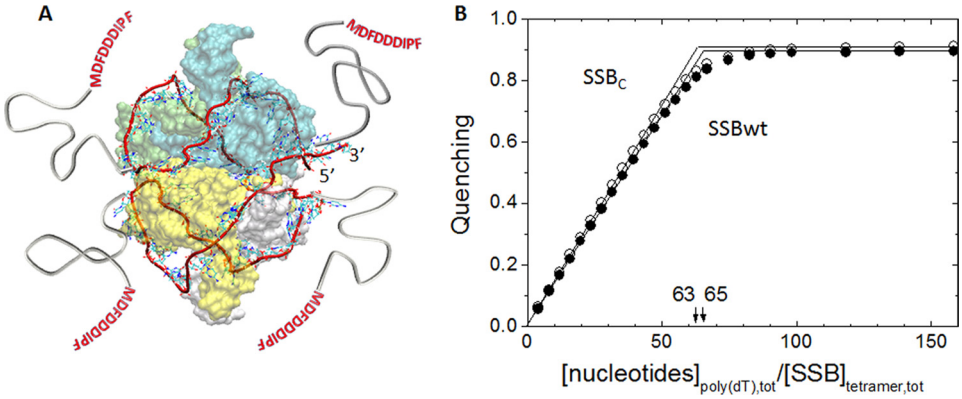


FIGURE 1. **ssDNA wrapping around the SSB tetramer in its $(SSB)_{65}$ mode is unaffected by its C-terminal tails.** *A*, model depicting 65 nucleotides of ssDNA (*red ribbon*) wrapped around the four SSB subunits, consisting of four OB-folds (shown in *cyan*, *green*, *yellow*, and *gray*), based on the x-ray crystallographic structure of the SSB_c tetramer bound to two molecules of $(dC)_{35}$ (20) with the addition of the unstructured C-terminal tails (shown in *gray*) that are not observed in the crystal structure. The 9 amino acids at the end of each C terminus, responsible for the interaction of SSB with other metabolic proteins, is shown in single letter amino acid codes. *B*, results of fluorescence titrations of 0.14 μM wtSSB (●) and SSB_C (○) (lacking 42 amino acids from its C termini) with poly(dT) (buffer H, pH 8.1, 0.2 M NaCl, 25 °C). The linear increase in relative Trp fluorescence quenching indicates stoichiometric binding of the protein to poly(dT). The occluded site sizes were determined by extrapolation of the linear part of the titration curve to the point of intersection with the plateau as described (12) and yields $n \sim 63$ nucleotides/tetramer for SSB_C and $n \sim 65$ for wtSSB.

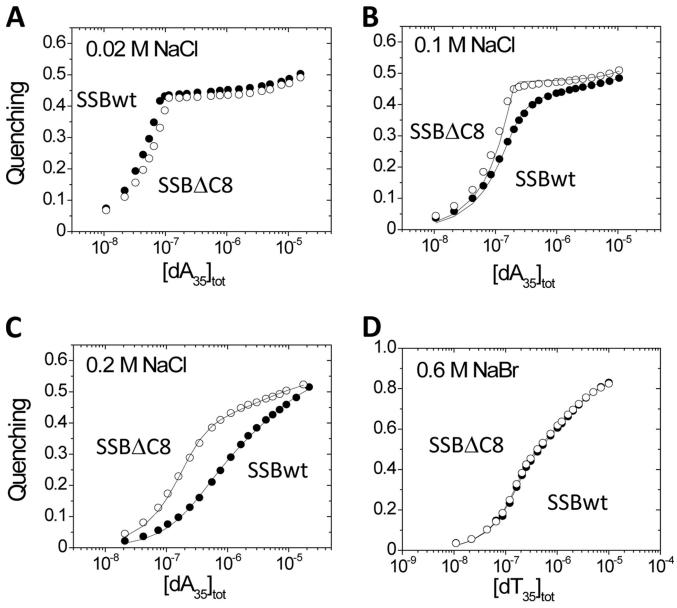


FIGURE 2. **Salt dependence of ssDNA binding to SSBΔC8 and wtSSB.** *A–D*, results of Trp fluorescence titrations of SSB (●) and SSBΔC8 (○) (0.1–0.2 μM) with $(dA)_{35}$ at 20 mM NaCl (*A*), 0.1 M NaCl (*B*), and 0.2 M NaCl (*C*) and with $(dT)_{35}$ in 0.6 M NaBr (*D*) (buffer T, pH 8.1, 25 °C). The *smooth curves* in *panels B*, *C*, and *D* represent the best fits of the data to either a two-site sequential binding model or a square model (Equations 1–4 under "Experimental Procedures") with the parameters shown in Table 1.

where D_{tot} and P_{tot} are the total concentrations of the ssDNA and SSB in the calorimetric cell after the i^{th} injection.

In experiments where the binding of the second $(dA)_{35}$ molecule was detectable (see Fig. 3*B*, and supplemental Fig. S1, *A* and *B*), the data were analyzed using a two-site sequential model, such that the total heat (Q_i^{tot}) after the i^{th} injection is defined by Equation 7,

$$Q_i^{tot} = V_0 P_{tot} \frac{\Delta H_1 \cdot K_1 D + (\Delta H_1 + \Delta H_2) \cdot K_1 K_2 D^2}{1 + K_1 D + K_1 K_2 D^2} \quad \text{(Eq. 7)}$$

where K_1, ΔH_1 and K_2, ΔH_2 are the observed stepwise macroscopic equilibrium constants and enthalpy changes for binding of the first and second molecule of DNA, respectively, and D is the free $(dA)_{35}$ concentration, which can be determined using Equation 2. To obtain the corresponding binding parameters, the experimental data were fit to either Equations 5–6 or Equation 7 using software provided by the instrument manufacturer, as described in the *ITC Data Analysis in Origin: Tutorial Guide* (MicroCal, LLC) (52) and Ref. 43.

RESULTS

ssDNA Wrapping around the SSB Tetramer in Its $(SSB)_{65}$ Mode Is Unaffected by the C-terminal Tail—The C-terminal tails of *E. coli* SSB (residues 113–177) appear to lack any substantial structure both in its apo form and when it is bound to ssDNA based on biochemical studies (24) and the absence of observed electron density for residues beyond 112 in any of the x-ray crystal structures (9, 20, 22). Treatment of SSB with chymotrypsin cleaves all four subunits after Trp-135 to form SSB_C (24), leaving a stable SSB tetramer containing the ssDNA binding core (residues 1–112). The topology of ssDNA wrapping around the SSB tetramer in its $(SSB)_{65}$ binding mode has been modeled based on the x-ray crystal structure of tetramers of SSB_C bound to two molecules of the oligodeoxynucleotide, $(dC)_{35}$ (20), and this closed wrapping is supported by fluorescence resonance energy transfer studies using doubly labeled ssDNA (17, 44, 45). Fig. 1*B* compares the results of titrations of full-length wtSSB and SSB_C with poly(dT) under tight binding conditions (buffer H, pH 8.1, 0.2 M NaCl, 25 °C), showing that the occluded site size of 63–65 nucleotides is unaffected and thus that the wrapping of ssDNA appears to be unaffected by removal of the C-terminal tails of SSB.

Deletion of the Last 8 Amino Acids in the C-terminal Tail Enhances SSB Binding to ssDNA at Moderate [NaCl]—At [NaCl] ≥0.2 M, *E. coli* SSB tetramers can bind two molecules of $(dN)_{35}$ but with a negative cooperativity that increases with decreasing salt concentration (40, 42, 46). Furthermore, the affinities are dependent upon base composition, with binding being highest for $(dT)_{35}$ and $(dC)_{35}$ and weakest for $(dA)_{35}$ (37, 38, 42). In fact, fluorescence or ITC methods are unable to measure the affinity accurately for SSB binding of the first molecule of $(dT)_{35}$ in buffers containing NaCl, even at concentrations as high as 1 M NaCl. Therefore, to examine the effects of deletion of the C-terminal tails on ssDNA binding, we examined the binding of SSB to $(dA)_{35}$ by monitoring the accompanying quenching of the intrinsic Trp fluorescence of SSB upon DNA binding.

Fig. 2 shows the results of a series of titrations comparing the binding of full-length SSB *versus* SSBΔC8, which has the last 8

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TABLE 1

Equilibrium binding constants for SSB and SSBΔC8 binding to $(dN)_{35}$ (buffer T, pH 8.1, 25 °C)

Fitted values of maximum florescence quenching were varied within the following limits: $Q_1 = 0.45 \pm 0.03$ and $Q_2 = 0.55 \pm 0.03$ for $(dA)_{35}$, and $Q_1 = 0.49 \pm 0.02$ and $Q_2 = 0.88 \pm 0.02$ for $(dT)_{35}$. The parameters for the binding of $(dA)_{35}$ to SSBΔC8 in 0.1 M NaCl were obtained at fixed value of $Q_2 = 0.55$. Errors are shown as S.D.

	SSB	$(dA)_{35}$ (0.1 M NaCl)	$(dA)_{35}$ (0.2 M NaCl)	$(dT)_{35}$ (0.6 M NaBr)
Two-site sequential binding model				
K_1 (M^{-1})				
	ΔC8	$(3.2 \pm 2.8) \times 10^9$	$(2.0 \pm 0.3) \times 10^7$	$(6.1 \pm 1.1) \times 10^7$
	wt	$(5.5 \pm 1.5) \times 10^7$	$(3.4 \pm 0.6) \times 10^6$	$(4.4 \pm 1.2) \times 10^7$
K_2 (M^{-1})				
	ΔC8	$(5.5 \pm 4.7) \times 10^4$	$(1.8 \pm 1.0) \times 10^5$	$(7.3 \pm 0.6) \times 10^5$
	wt	$(1.4 \pm 1.2) \times 10^5$	$(1.5 \pm 0.6) \times 10^5$	$(6.0 \pm 1.0) \times 10^5$
Square model				
K_{sq} (M^{-1})				
	ΔC8	$>10^9$	$(5.2 \pm 0.7) \times 10^6$	$(1.5 \pm 0.3) \times 10^7$
	wt	$(1.7 \pm 0.3) \times 10^7$	$(6.7 \pm 1.0) \times 10^5$	$(1.1 \pm 0.3) \times 10^7$
σ_{sq}				
	ΔC8	0.07 ± 0.05	0.45 ± 0.07	0.50 ± 0.02
	wt	0.30 ± 0.13	0.78 ± 0.07	0.53 ± 0.03

amino acids removed from all four C-terminal tails (34). At 20 mM NaCl (*panel A*), we observe little difference between the two binding isotherms. At this low [NaCl], the first molecule of $(dA)_{35}$ binds stoichiometrically, and we are unable to measure the affinity, but the second molecule of $(dA)_{35}$ binds so weakly, due to the high negative cooperativity, that we are also unable to measure its affinity. Upon raising the [NaCl] to 100 mM (*panel B*), we observe a clear difference between the two isotherms, indicating that $(dA)_{35}$ binds to SSBΔC8 with higher affinity than to wtSSB. Fits of these isotherms to a two-site sequential binding model (Equations 1 and 2) indicate that the first molecule of $(dA)_{35}$ binds with more than 100-fold higher affinity to SSBΔC8 (Table 1). At 200 mM NaCl (*panel C*), we also observe a clear difference in macroscopic binding affinities, with SSBΔC8 showing a higher affinity for $(dA)_{35}$. Furthermore, under these conditions, we are able to obtain accurate estimates of the stepwise macroscopic binding constants for the binding of both $(dA)_{35}$ molecules to each tetramer. The first molecule of $(dA)_{35}$ binds with ~6-fold higher affinity to SSBΔC8, whereas the second molecule of $(dA)_{35}$ binds with nearly the same macroscopic affinity to both SSBΔC8 and wtSSB (Table 1).

We also analyzed these isotherms using the square model (40) (Equations 1–4 under "Experimental Procedures") to obtain the intrinsic association constant (K_{sq}) for the binding of each $(dN)_{35}$ molecule and a cooperativity parameter σ, reflecting unfavorable interactions ($\sigma < 1$) between ssDNA binding sites within the SSB tetramer (40). The results presented in Table 1 clearly indicate that in 100 mM NaCl, the binding of two $(dA)_{35}$ molecules to SSBΔC8 *versus* wtSSB occur with much higher intrinsic affinity but a higher degree of negative cooperativity (~100- and ~4-fold difference in values of K_{sq} and σ, respectively). When the [NaCl] is increased to 200 mM, these differences are diminished (~10- and ~2-fold, respectively). Further increases in [NaCl] weaken the $(dA)_{35}$ binding enough so that we cannot determine the K_{sq} and σ accurately. Therefore, at moderate salt concentrations (0.1–0.2 NaCl), the majority of the effect of the C-terminal tails appears to be on the binding of the first molecule of $(dA)_{35}$, whereas when two subunits of SSB are occupied with ssDNA, very little or no effect is observed for the binding of ssDNA to the remaining subunits.

To determine whether the enhancement in affinity and increased negative cooperativity observed upon removal of the last 8 amino acids from the SSB C-terminal tails are still observed at higher salt concentrations, we examined the binding of $(dT)_{35}$, which has a much higher affinity for SSB (37, 38, 42). We compared $(dT)_{35}$ binding to SSBΔC8 *versus* wtSSB at 0.6 M NaBr, conditions where one can obtain complete binding isotherms and accurately measure the affinities for the binding of both molecules of $(dT)_{35}$ (38). Fig. 2*D* shows that there is no detectable difference between the binding of $(dT)_{35}$ to SSBΔC8 *versus* wtSSB at 0.6 M NaBr, and the binding parameters are identical within our uncertainties (Table 1). This indicates that the inhibitory effect of the C-terminal tails is salt-dependent, decreasing with increasing salt concentration, although we cannot rule out some effect of ssDNA base composition.

The Increase in ssDNA Binding Affinity upon Deletion of the SSB C-terminal Tail Is Driven by a More Favorable Entropy Change—We next compared the binding of $(dA)_{35}$ to wtSSB and SSBΔC8 using ITC to obtain information on the differences in the thermodynamics of binding. A series of comparative titrations was performed at several temperatures (15, 25, 32, and 40 °C) in 100 mM NaCl (buffer T, pH 8.1). The results of titrations at 15 and 40 °C are shown in Fig. 3 (the results at 25 and 32 °C are shown in supplemental Fig. S1), and the values of K_{obs} and ΔH_{obs} for binding of the first molecule of $(dA)_{35}$ are plotted in Fig. 4. The ITC experiment at 15 °C indicates that we can detect some binding of the second molecule of $(dA)_{35}$ to SSBΔC8, although we cannot accurately determine the binding parameters for this interaction. Hence, we will focus on the parameters for binding the first molecule of $(dA)_{35}$ to the SSB tetramers. As Fig. 4 indicates, the affinities are higher for $(dA)_{35}$ binding to SSBΔC8 at all temperatures, whereas the binding enthalpies are the same within experimental uncertainty (Table 2). Therefore, the increase in affinity (binding free energy) for binding of $(dA)_{35}$ to SSBΔC8 appears to be primarily due to a more favorable entropy change (ΔS_{obs}) (see "Discussion"). These data also indicate that binding of the first molecule of $(dA)_{35}$ shows a significant negative heat capacity change (ΔCp) of $\sim -910 \pm 99$ cal/K mol for both wtSSB and SSBΔC8. As shown previously (37, 38), this large and negative ΔCp is due primarily to an unstacking of the adenine bases that is coupled to SSB binding and the fact that adenine base unstacking is itself temperature-dependent, being accompanied by a large positive ΔH_{obs} (37).

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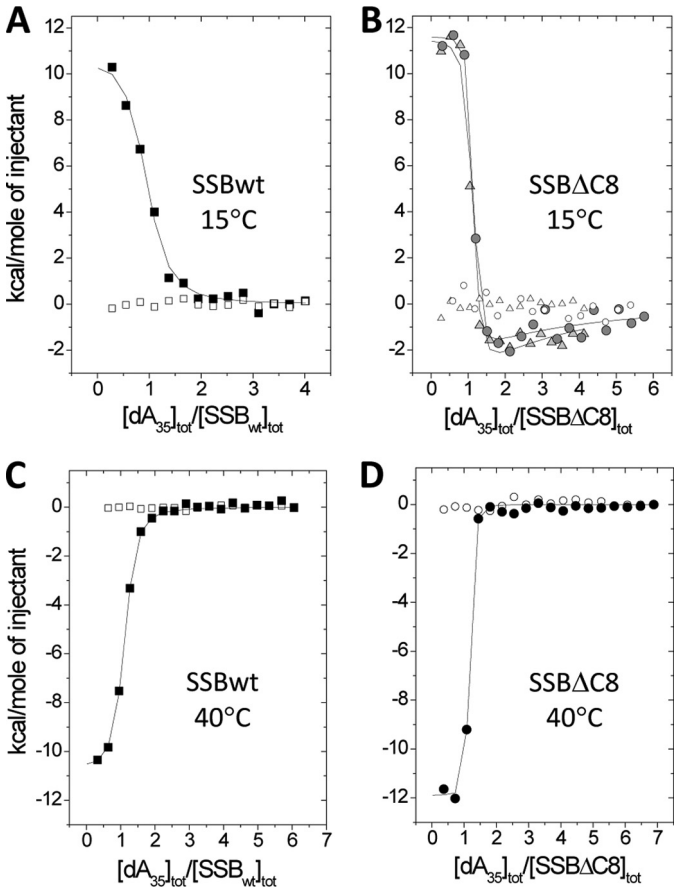


FIGURE 3. **ITC studies of the effect of temperature on the inhibition of ssDNA binding by the SSB C termini.** All studies were performed in buffer T, pH 8.1, 100 mM NaCl. *A*, 15 °C, ITC titrations of wtSSB (■) (0.9 μM) with $(dA)_{35}$ (21 μM). The *smooth curve* represents the best fit of the experimental isotherm to Equations 5 and 6 (under "Experimental Procedures") with the following parameters: $n = 0.86 \pm 0.03$, $K_{obs} = (2.2 \pm 0.5) \times 10^7\ M^{-1}$, and $\Delta H_{obs} = 10.9 \pm 0.5$ kcal/mol. *B*, 15 °C, ITC titrations of SSBΔC8 (0.3 μM (●) and 1.8 μM (▲) with $(dA)_{35}$ (7.5 and 35 μM, respectively). The *smooth curves* represent the best fit of the experimental isotherms to a two-site sequential binding model (Equation 7 under "Experimental Procedures") with the following parameters averaged for two titrations: $K_{1,obs} = (7.0 \pm 6.1) \times 10^8\ M^{-1}$, $\Delta H_{1,obs} = 11.6 \pm 0.6$ kcal/mol, $K_{2,obs} = (4.3 \pm 4.7) \times 10^5\ M^{-1}$, $\Delta H_{2,obs} = -14.1 \pm 8.2$ kcal/mol. *C*, 40 °C ITC titration of wtSSB (■) (1.1 μM) with $(dA)_{35}$ (32 μM). The *smooth curve* represents the best fit of the experimental isotherm to Equations 5 and 6 ("Experimental Procedures") with the following parameters: $n = 0.99 \pm 0.01$, $K_{obs} = (3.4 \pm 0.3) \times 10^7\ M^{-1}$ and $\Delta H_{obs} = -10.8 \pm 0.1$ kcal/mol. *D*, 40 °C, ITC titration of SSBΔC8 (●) (1.0 μM) with $(dA)_{35}$ (32 μM). The *smooth curve* represents the best fit of the experimental isotherm to Equations 5 and 6 ("Experimental Procedures") with the following parameters: $n = 1.02 \pm 0.01$, $K_{obs} = (5.5 \pm 2.0) \times 10^8\ M^{-1}$, and $\Delta H_{obs} = -11.9 \pm 0.1$ kcal/mol. The errors are shown as a S.D. *Open symbols* represent reference titrations of $(dA)_{35}$ in to the buffer.

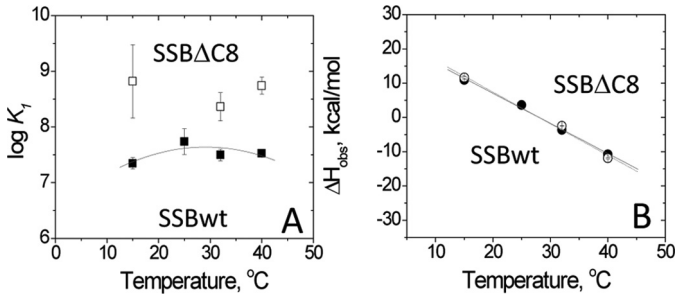


FIGURE 4. **Thermodynamics of the binding of $(dA)_{35}$ to the first site on wtSSB and SSBΔC8.** The values of $K_{1,obs}$ and $\Delta H_{1,obs}$ were determined from analysis of the isotherms shown in Fig. 3 and supplemental Fig. S1. *A*, temperature dependence of log $K_{1,obs}$ for wtSSB (■) and SSBΔC8 (□). *B*, the dependences of binding enthalpy ($\Delta H_{1,obs}$) on temperature for wtSSB (●) and SSBΔC8 (○) ($\Delta Cp = -0.89 \pm 0.05$ kcal/K mol and -0.93 ± 0.09 kcal/K mol, were obtained from a linear least squares fit of the data). The *error bars* are shown as S.D.

TABLE 2

Thermodynamic parameters determined for the interaction of SSB and SSBΔC8 with $(dA)_{35}$ at different temperatures (buffer T, pH 8.1, 0.1 M NaCl)

Values of ΔG°_{obs} and $T\Delta S^\circ_{obs}$ were calculated using $\Delta G^\circ_{obs} = -RT\ln K_{obs}$ and $T\Delta S^\circ_{obs} = \Delta H^\circ_{obs} - \Delta G^\circ_{obs}$. Errors are shown as S.D.

Temperature	SSB	$K_{1,obs}$	ΔG°_{obs}	ΔH_{obs}	$T\Delta S^\circ_{obs}$
		M^{-1}	kcal/mol	kcal/mol	kcal/mol
15 °C	ΔC8	$(7.0 \pm 6.1) \times 10^8$	-11.7 ± 0.5	11.6 ± 0.6	23.3 ± 0.8
	wt	$(2.2 \pm 0.5) \times 10^7$	-9.7 ± 0.1	10.9 ± 0.5	20.6 ± 0.5
25 °C	wt	$(4.1 \pm 2.9) \times 10^7$	-10.4 ± 0.4	3.7 ± 0.2	14.1 ± 0.5
32 °C	ΔC8	$(2.3 \pm 1.3) \times 10^8$	-11.7 ± 0.3	-2.5 ± 0.1	9.2 ± 0.4
	wt	$(3.1 \pm 0.8) \times 10^7$	-10.5 ± 0.2	-3.7 ± 0.1	6.8 ± 0.2
40 °C	ΔC8	$(5.5 \pm 2.0) \times 10^8$	-12.5 ± 0.2	-11.9 ± 0.1	0.6 ± 0.2
	wt	$(3.4 \pm 0.3) \times 10^7$	-10.8 ± 0.1	-10.8 ± 0.1	-0.01 ± 0.11

DISCUSSION

The four C termini (residues 113–177) of the *E. coli* SSB tetramer do not adopt any well defined structure even when SSB is bound tightly to ssDNA (22). The sequence of the last 9 amino acids (MDFDDDIPF) within these C-terminal tails is highly acidic and provides the sites of SSB binding to its multiple interacting proteins (at least 14) that are involved in DNA replication, recombination, or repair (4). Through these interactions, SSB recruits these proteins to their sites of function on the DNA. Many proteins and/or regions of proteins involved in signaling and regulation use unstructured regions as interaction sites, and these regions are referred to as intrinsically disordered proteins (47). Hence, SSB represents another example of a protein that uses disordered regions as sites of interaction.

Interestingly, in addition to the ability to interact with a variety of metabolic proteins involved in genome maintenance (4), the SSB C termini can also influence SSB binding to ssDNA. Deletion of the SSB-Ct (SSB C-terminal tail) influences the transition between the $(SSB)_{35}$ and $(SSB)_{65}$ binding modes (17). Furthermore, we have recently shown that the SSB DNA binding core can inhibit binding of the C-terminal tails to two of its binding partners (PriA and χ) and that ssDNA binding can relieve this inhibition (51). It has also been shown that removal of the C termini of SSB enhances its macroscopic affinity for poly(U), a single-stranded RNA (31). Here we have shown by direct binding experiments that the C-terminal tails of SSB can inhibit SSB binding to ssDNA. Our study clearly indicates that at moderate salt concentrations (0.1–0.2 M NaCl), both the intrinsic affinity and the intratetramer negative cooperativity for ssDNA binding are enhanced for SSB lacking its C termini. Interestingly, if only half of the SSB subunits are bound with ssDNA, the affinity of ssDNA for the remaining subunits is not affected by the presence of C-terminal tails, indicating that partial binding of ssDNA alleviates the entire inhibitory effect of the C-terminal tails.

The thermodynamic origin of the inhibition of ssDNA binding is primarily a less favorable entropic contribution to $(dA)_{35}$ binding to wtSSB. If the acidic C termini interact with the ssDNA binding sites of SSB and are released when ssDNA binds, this would be expected to make a favorable positive entropic contribution to the binding free energy. However, we

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observe the opposite effect when comparing $(dA)_{35}$ binding to wtSSB *versus* SSBΔC8. Although the entropic effect of release of the constrained C-terminal tails upon ssDNA binding must be part of the free energy for ssDNA binding, this cannot be the origin of the more favorable entropic contribution resulting from deletion of the last eight amino acids. Other possible sources for the entropic effect might include differences in solvation or ion binding (40, 42, 43, 46, 48, 49). Indeed, a preliminary comparison of the dependences of $K_{1,obs}$ on NaCl concentration (Tables 1 and 2) for binding of the first molecule of $(dA)_{35}$ (see supplemental Fig. S2) shows a larger net release of ions for SSBΔC8 binding to DNA than for wtSSB ($d\log K_{1,obs}$/d log[NaCl] ~−7.3 *versus* −3.8, respectively). This larger net ion release associated with SSBΔC8 binding to DNA will contribute its own favorable entropy change. This may dominate the entropy change associated with the loss of SSB C termini binding to the SSB core, resulting in an overall more favorable contribution to the ΔS for ssDNA binding to SSBΔC8 *versus* wtSSB. However, further experiments are needed to determine whether this is correct.

The inhibitory effects of the C-terminal tails on ssDNA binding to SSB decrease upon raising the salt concentration, eventually becoming negligible at very high salt concentrations $(dT)_{35}$ at 0.6 M NaBr). Because the ends of the C-terminal tails are highly acidic, it is not surprising that if they interact with or near the ssDNA binding site of SSB, which has a net positive charge, this interaction would have a favorable electrostatic component that would decrease with increasing salt concentration.

Williams *et al.* (24) have shown that the rate of proteolysis of the *E. coli* C-terminal tail increases upon binding poly(dT), but not $(dT)_{16}$, and that the chymotryptic SSB_C tetramer is better able than full-length SSB to denature duplex DNA poly[d(A-T)]. As such, they suggested that only cooperative binding of SSB to long ssDNA made the C termini more accessible to proteolysis. Curth *et al.* (31) have previously shown that deletion of the C termini increases the affinity of SSB for the RNA, poly(U), although only the product, $K\omega$, was estimated, where K is the equilibrium binding constant for binding of an isolated SSB tetramer and ω is the nearest neighbor cooperativity parameter. Hence, whether the effect was on isolated binding or cooperative binding was not determined. Because we see an increase in affinity for binding $(dA)_{35}$, which does not promote intertetramer cooperativity, it is clear that these effects are not limited to only cooperative binding of SSB.

The presence of an acidic region at the end of the C-terminal tail is a feature shared among all prokaryotic SSB proteins (4, 50). These regions provide the sites for interaction with an array of other proteins involved in genome maintenance. Furthermore, for a number of these proteins, including now *E. coli* SSB, the presence of this acidic region has been shown to directly inhibit ssDNA binding, at least under some conditions (28–30). We have demonstrated recently that the reciprocal activity is observed in *E. coli* SSB. In the absence of ssDNA, the isolated C-terminal tails of SSB display higher affinities for PriA and the χ subunit of DNA polymerase III holoenzyme than when they are part of the full-length SSB protein (51). Because the binding of ssDNA to the SSB protein relieves the inhibition of the SSB

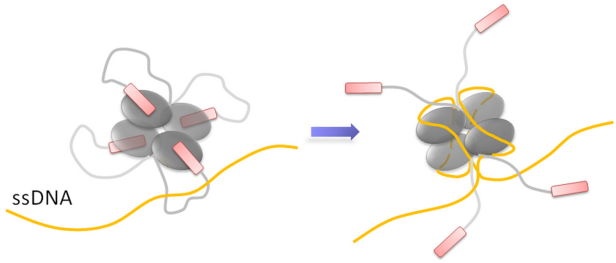


FIGURE 5. **Model for how the intrinsically disordered C-terminal tails inhibit binding of ssDNA to the SSB tetramer.** In the absence of ssDNA (*dark yellow*), we hypothesize that the acid ends of the C-terminal tails interact with the positively charged ssDNA binding sites within the SSB core. Upon ssDNA binding, the C-terminal tails are displaced and made more accessible for interactions with other proteins.

core, it is likely that the acidic region of the C-terminal tails interacts with the positively charged ssDNA binding site of SSB and is displaced by ssDNA binding, thus becoming more accessible for binding its binding partners (*e.g.* PriA and χ), as depicted in Fig. 5. This behavior provides a simple mechanism that would inhibit binding of free SSB to its partner proteins but promote binding of SSB when SSB is bound to ssDNA or single-stranded/double-stranded DNA junctions and thus provide a mechanism to facilitate the recruitment of these proteins by SSB to their sites of action on DNA.

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