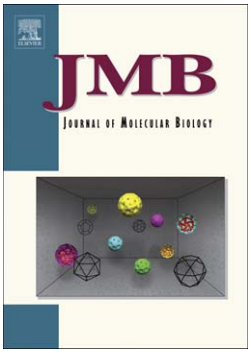

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Glutamate promotes SSB protein-protein Interactions via intrinsically disordered regions

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Abstract

E. coli single strand (ss) DNA binding protein (SSB) is an essential protein that binds to ssDNA intermediates formed during genome maintenance. SSB homotetramers bind ssDNA in several modes that differ in occluded site size and cooperativity. High "unlimited" cooperativity is associated with the 35 site size ($(SSB)_{35}$) mode at low [NaCl], whereas the 65 site size ($(SSB)_{65}$) mode formed at higher [NaCl] (> 200 mM), where ssDNA wraps completely around the tetramer, displays “limited” cooperativity forming dimers of tetramers. It was previously thought that high cooperativity was associated only with the $(SSB)_{35}$ binding mode. However, we show here that highly cooperative binding also occurs in the $(SSB)_{65}$ binding mode at physiological salt concentrations containing either glutamate or acetate. Highly cooperative binding requires the 56 amino acid intrinsically disordered C-terminal linker (IDL) that connects the DNA binding domain with the 9 amino acid C-terminal acidic tip that is involved in SSB binding to other proteins involved in genome maintenance. These results suggest that high cooperativity involves interactions between IDL regions from different SSB tetramers. Glutamate, which is preferentially excluded from protein surfaces, may generally promote interactions between intrinsically disordered regions of proteins. Since glutamate is the major monovalent anion in *E. coli*, these results suggest that SSB likely binds to ssDNA with high cooperativity *in vivo*.

Keywords: IDP; cooperativity; SSB-ssDNA interactions, preferential hydration, Hofmeister

Abbreviations: ssDNA, single stranded DNA; SSB, single stranded DNA binding protein; IDP, intrinsically disordered protein; IDL, intrinsically disordered linker; SIP, SSB interacting protein.

Introduction

Single stranded (ss) DNA binding proteins (SSBs) are essential for DNA replication, recombination and repair. They bind with high affinity and low sequence specificity to ssDNA intermediates that form transiently during genome maintenance protecting them from degradation and inhibiting unwanted secondary structures [1-4]. SSB proteins also serve as hubs for interactions with a variety of other proteins involved in genome maintenance. This is exemplified by *E. coli* SSB, which interacts with at least 14 proteins, referred to as SSB interacting proteins (SIPs), that also function in replication, recombination and repair [5].

E. coli SSB functions as a homotetramer (Fig. 1C) [3, 6], with each subunit (177 amino acids) possessing two domains (Fig. 1A): an N-terminal DNA binding domain (DBD) (residues 1-112) containing an oligonucleotide/oligosaccharide binding fold (OB fold), and a C-terminal domain (residues 113-177) composed of a 56 aa intrinsically disordered linker (IDL) and a nine aa acidic “tip”, which is conserved among many bacterial SSBs and is the primary site of interaction with the SIPs [5, 7-12]. SSB can bind ssDNA in several modes differing in occluded DNA binding site size and the number of subunits used to contact DNA. Two major binding modes observed *in vitro* are referred as $(SSB)_{35}$ and $(SSB)_{65}$, where the subscripts denote the average number of nucleotides occluded upon binding ssDNA [13, 14]. The relative stabilities of these binding modes depend on salt concentration and type and protein to DNA ratio [13, 15-20], as well as applied force [21, 22].

In the $(SSB)_{65}$ mode, favored at [NaCl]>0.20 M (Fig. 1C) or [Mg^{2+}]>10 mM, ~65 nucleotides of DNA wrap around all four subunits of the tetramer [6]. In NaCl buffers, this mode displays "limited" cooperativity forming dimers of tetramers (octamers) [15, 23]. The topology of ssDNA wrapping in the $(SSB)_{65}$ binding mode is such that ssDNA enters and exits the tetramer in close proximity [6] (Fig. 1C). Although SSB binds ssDNA with very high affinity in its $(SSB)_{65}$ mode, it can diffuse along ssDNA [21, 24]. Such diffusion provides the mechanism by which SSB destabilizes DNA secondary structures (e.g., hairpins) and promotes RecA filament formation [24].

In the $(SSB)_{35}$ mode, favored at [NaCl]<10 mM (Fig. 1D) or [$MgCl_2$]<1 mM, and high SSB to DNA ratios [13, 14, 17], ssDNA wraps around only two subunits on average with an occluded site size ~35 nucleotides. In this mode SSB binds ssDNA with unlimited nearest-neighbor cooperativity allowing formation of long protein clusters [16, 17, 25-27]. A structural model for the $(SSB)_{35}$ binding mode has been proposed, which proposes direct interactions of adjacent tetramers through the L_{45} loops within the tetrameric DBD core of the protein [6] (Fig. 1D). In this mode SSB can undergo direct or intersegment transfer between separate ssDNA molecules [28] or between distant sites on the same DNA molecule [29]. This activity is thought to play a role in SSB recycling during replication [28]. An additional $(SSB)_{56}$ binding mode has also been identified at intermediate NaCl and $MgCl_2$ concentrations [14], but no information is available about its potential for cooperative binding.

The C-terminal domain of SSB (residues 113-177) is not observable in any crystal structures, even when SSB is bound to ssDNA [30], suggesting that these C-terminal tails are intrinsically disordered, as first proposed based on its primary structure [31] and biochemical properties [32-34]. The acidic tip can interact with an unoccupied DNA binding site on SSB, which inhibits ssDNA binding [8, 34, 35]. Surprisingly, the 56 aa IDL (Fig. 1E), was recently shown to be essential for highly cooperative binding of SSB to ssDNA [27].

Previous studies suggested that highly cooperative SSB binding to ssDNA occurred only in the $(SSB)_{35}$ mode at low [NaCl] [17, 26, 27]. However, we show here that highly cooperative binding can also occur in the $(SSB)_{65}$ mode. Cooperative binding still requires the IDL, but is promoted, even at high physiological salt concentrations, by replacing chloride with glutamate or acetate, anions that are preferentially excluded from amide protein surfaces [36, 37]. These results suggest that direct interactions between the IDL regions of SSB tetramers drive cooperativity. Since glutamate is the major monovalent anion in *E. coli* [38], SSB cooperativity is likely important *in vivo*.

Results

The IDL of SSB influences its ssDNA binding mode transition

Previous studies of the role of the C-terminal IDL on cooperative binding to ssDNA [27] examined binding in buffers containing 0.30 M NaCl and 10 mM NaCl, which promote formation of the $(SSB)_{65}$ and $(SSB)_{35}$ binding modes, respectively. Those studies indicated that the IDL is required for highly cooperative binding at

low [NaCl], consistent with previous studies [17]. Here, we examined the effects of monovalent salt type (NaCl, KCl, KOAc and KGlu) on cooperativity. It is well known that replacement of chloride with glutamate generally increases the affinity of nucleic acids for proteins [39-42] including *E. coli* SSB [43, 44]. In fact, SSB-ssDNA interactions are influenced greatly by the type of anion present [43-46]. Anion type (F^-, Cl^-, Br^-) [18, 47] and the number of C-terminal tails [12] also influence the SSB-ssDNA binding mode transitions *in vitro*.

We first examined the effects of chloride, acetate and glutamate salts on the binding mode transitions of wild type SSB (referred to as SSB in the remainder of the manuscript) and an SSB variant, SSB-ΔL, in which 54 amino acids of the IDL (residues 115-168) are deleted from each SSB subunit leaving only a GG linker[27] (Fig. 1B). Fig. 2 compares the effects of NaCl, KCl, Kacetate (KOAc) and Kglutamate (KGlu) on the occluded site sizes (nucleotides per tetramer), estimated from titrations of SSB or SSB-ΔL with poly(dT), monitoring SSB Trp fluorescence quenching [13, 14, 27, 48, 49]. (See Fig. S1 for representative titrations.)

At the extremes of low and high salt concentrations both SSB and SSB-Δ*L* form the $(SSB)_{35}$ and $(SSB)_{65}$ binding modes, respectively. However, the site size transitions for SSB are shifted to lower salt concentrations in KOAc and KGlu, compared to KCl and NaCl indicating that acetate and glutamate favor formation of the $(SSB)_{65}$ mode. In contrast, the salt-dependent transitions from the $(SSB)_{35}$ to the $(SSB)_{65}$ mode are shifted to higher salt concentrations for SSB-*ΔL* (Fig. 2B), indicating that deletion of the IDL favors the $(SSB)_{35}$ mode. Furthermore, the site size transitions for SSB-Δ*L* are not affected by anion type.

We also examined a chimeric SSB, SSB-*EcPfEc* (Fig. 1B) [27], in which the 56 amino acid IDL of *E. coli* SSB was replaced with 80 amino acids of the longer and more charged IDL of the *P. falciparum* SSB [50, 51] (Fig. 1E). Previous studies showed that the homotetrameric *Pf*SSB forms only the $(SSB)_{56}$ and $(SSB)_{65}$ binding modes[50, 51] and that replacement of the *E. coli* IDL with the *Pf* IDL eliminates highly cooperative binding to ssDNA [27]. SSB-*EcPfEc* also only shows a transition from the $(SSB)_{65}$ mode to the $(SSB)_{56}$ mode upon decreasing the [NaCl] to 10 mM (Fig.S2). Thus, the presence and amino acid composition of the IDL affects the SSB-ssDNA binding mode preference and the effect of anion type is eliminated by removal of the IDL.

Glutamate and acetate promote highly cooperative SSB-ssDNA binding at salt concentrations that promote the $(SSB)_{65}$ binding mode.

Previous studies of SSB-ssDNA cooperativity were performed in buffers containing NaCl as the monovalent salt [17, 23, 26, 43]. Here we compare the effects of KCl, KOAc and KGlu on cooperative binding of SSB, SSB-ΔL and the SSB-*EcPfEc* chimera to phage M13 mp8 ssDNA (~7.25 kilobases).

We first established that the major $(SSB)_{35}$ and $(SSB)_{65}$ binding modes form on this ssDNA (see Fig.S3). In moderate and high salt conditions (0.20 M NaCl and 0.50 M KGlu, the $(SSB)_{65}$ mode is favored, whereas the $(SSB)_{35}$ mode is favored in the absence of monovalent salt. We note that SSB binding to M13 ssDNA is weaker in 0.50 M NaCl (Fig. S3), compared to poly(dT). However, fitting of the binding isotherm to an infinite lattice model [52] indicates that SSB binds to M13 ssDNA in the $(SSB)_{65}$ mode (Fig. S3). The weaker affinity, compared to

poly(dT) is partly due to the mixed base composition of the M13 DNA, but also due to competing secondary structures (e.g., hairpins) that can form in the M13 DNA.

Fig. 3 shows the results of sedimentation velocity experiments (plotted in the form of c(s) distributions [53], further converted to 20˚C, water conditions (see Materials and Methods)) performed in buffer T (25.0˚C) at SSB/DNA ratios of R_{65} = 0.56 (sub-saturating) and R_{65} =2.79 (saturating), at three KCl and KGlu concentrations (10 mM, 0.20 M and 0.50 M). The SSB/DNA ratio, R_{65} is calculated using the site size for the $(SSB)_{65}$ mode (i.e., R_{65}=65×[SSB tetramer]$_{total}$/[M13 nucleotide]$_{total}$). An R_{65} = 0.56 indicates that the M13 DNA will be 56% saturated if all SSB were bound in the $(SSB)_{65}$ binding mode.

Figure 3 A and B show that at low salt concentrations (10 mM KCl or KGlu), bimodal ssDNA distributions are observed at sub-saturating SSB/DNA ratio, R_{65} = 0.56. This indicates two dominant populations of DNA, one with little SSB bound ($s_{20,w}$ ~20S) and another with much higher amounts of SSB bound ($s_{20,w}$ ~45-50S), reflecting highly cooperative binding of SSB to ssDNA consistent with previous studies at low salt concentrations [17, 25, 27]. However, this bimodal distribution is eliminated at higher KCl concentrations of 0.20 M and 0.50 M (Fig. 3 C and E) that favor only the $(SSB)_{65}$ binding mode, indicating loss of highly cooperative binding (Figure 2A). However, the bimodal distributions remain at 0.20 M KGlu and 0.50 M KGlu (Fig. 3 D and F). Recall that SSB also binds exclusively in the $(SSB)_{65}$ mode at these high [KGlu] (Figure 2A). Similar experiments show that SSB also binds with high cooperativity at 0.20 M [KOAc] (Fig. S4B), but less so at 0.50 M KOAc (Fig. S4C). Hence, substitution of glutamate or acetate for chloride promotes highly

cooperative binding of SSB to ssDNA even in the $(SSB)_{65}$ binding mode and shows that glutamate is more effective than acetate.

Figure 4 shows sedimentation profiles for a range of SSB to DNA ratios (R_{65}) at three [KCl] and [KGlu] (data for KOAc and NaCl are presented in Fig. S4). For less than saturating SSB/DNA ratios, bimodal c(s) distributions are observed at all [KGlu] (Fig. 4 Bi-iii), whereas highly cooperative binding is observed only at low KCl (10 mM) (Fig. 4 Ai). The results in KCl are similar to all previous studies in NaCl [17, 27], hence the different cooperative behavior is due to the anion.

The *E. coli* IDL is required for highly cooperative binding of SSB to ssDNA.

We next examined how the absence or replacement of the IDL in SSB affects cooperative binding in low (10 mM NaCl) and moderate (0.20 M NaCl and KGlu) salt conditions. In 10 mM NaCl, SSB-Δ*L* forms the $(SSB)_{35}$ binding mode on poly(dT) (Fig. 2B) as well as M13 ssDNA (Fig. 5D). In these conditions the c(s) distributions show sharp single peaks shifting to higher values of $s_{20,w}$ as more SSB-Δ*L* binds to the DNA (Fig 5A) indicating low cooperativity. Essentially the same results are obtained at 0.20 M NaCl and 0.20 M KGlu (Fig 5B and C), where SSB-*ΔL* binds as a mixture of both binding modes (Fig. 2B). Therefore, it is likely that as the protein to DNA ratio increases the SSB-ssDNA complex transitions from the $(SSB)_{65}$ to the $(SSB)_{35}$ mode (Fig. 5D). However, at 0.20 M NaCl or 0.20 M KGlu the full transition to the $(SSB)_{35}$ mode is not achieved even at the highest SSB concentration (R_{65}=2.8). The single peak c(s) distributions indicate the absence of high cooperativity in either binding mode. Similar low cooperativity c(s) distributions are observed for the SSB-*EcPfEc* chimera under the same solution

conditions (Fig. S5). Therefore, deletion of the *E. coli* IDL or its replacement with the *Pf* IDL eliminates the highly cooperative interactions that are observed for SSB.

$(SSB)_{65}$ and/or $(SSB)_{56}$ modes also display highly cooperative binding at low salt concentrations.

Closer examination of the c(s) distributions at low salt concentrations provides evidence for cooperative binding of SSB in the $(SSB)_{65}$ or possibly the $(SSB)_{56}$ binding modes. At low salt (10 mM), all c(s) distributions show very similar behavior as a function of protein to DNA ratio, regardless of salt type (Fig. 4A and B for KCl and KGlu, and Fig. S4 A and D for 10 mM KOAc and NaCl). The c(s) distributions at these low salt conditions show three phases. At low protein to DNA ratios ($R_{65} \leq 0.4$) SSB binds to M13 ssDNA in a low or non-cooperative manner such that only a single peak is observed that shifts to high values of $s_{20,w}$. However, for R_{65} values from 0.4 to 1, bimodal distributions are observed with the fastest sedimenting DNA population ($s_{20,w}$ ~ 45-50S) increasing, while the slower sedimenting DNA population ($s_{20,w}$ ~ 20S) decreases. At still higher SSB concentrations (R_{65} >1), only a single DNA species is observed that gradually shifts to higher $s_{20,w}$ (~ 50 - 65S), reaching ~65S for R_{65}=2.8 indicating that additional SSB binds to the ssDNA at the higher protein to DNA ratios (from R_{65} = 1.5 to 2.8). This additional binding likely requires the transition from the $(SSB)_{65}$ (or $(SSB)_{56}$) mode to the $(SSB)_{35}$ mode at the higher binding densities. This transition enables more SSB to bind to the DNA since less ssDNA is bound per tetramer in the $(SSB)_{35}$ mode [13, 14, 17, 18]. This is supported by plots of the weight-average sedimentation coefficient ($\bar{s}_{20,w}$) vs R_{65} (Fig. 4C-i) indicating that additional SSB

binding continues beyond R_{65}=1 and at least up to R_{65} ~2.8 (or equivalently R_{35}=1.5). Since the $(SSB)_{65}$ (or $(SSB)_{56}$) binding mode is favored over the $(SSB)_{35}$ mode at low protein to DNA ratios, even at low salt concentrations[13, 16, 17, 27], this suggests that the faster sedimenting DNA observed in the bimodal distributions (R_{65} ≈ 0.4-1.1, $s_{20,w}$ ~ 45-50S) represents highly cooperative binding in the $(SSB)_{65}$ (or $(SSB)_{56}$) mode.

To probe cooperative binding in the $(SSB)_{65}$ mode further, we examined the c(s) distributions at high salt concentrations (0.20 M and 0.50 M) (Fig.4 and Fig.S4), where SSB binds to ssDNA exclusively in the $(SSB)_{65}$ mode (see Fig. 2A). The plots of $\bar{s}_{20,w}$ vs R_{65} at 0.20 M KGlu, KOAc, KCl and NaCl (Fig. 4Cii) and 0.50 M KGlu and KOAc (Fig. 4Ciii) indicate that SSB binds to M13 ssDNA stoichiometrically and exclusively in the $(SSB)_{65}$ binding mode. However, the c(s) distributions in KCl (Fig. 4Aii and iii) and NaCl (Fig. S4 E and F) are very different than in KGlu (Fig. 4Bii and iii) and KOAc (Fig. S4 B and C).

In 0.20 M KCl (Fig. 4Aii) the binding shows low cooperativity compared to 10 mM KCl (Fig. 4Ai). Above R_{65}=0.4 only a single peak is observed that gradually shifts to higher $s_{20,w}$ as R_{65} increases with no further increase from R_{65}=1.5 to 2.8 ($s_{20,w}$, ≈ 67). This suggests that SSB saturates the ssDNA in its $(SSB)_{65}$ mode. Notably, the distributions in 0.20 M KCl and NaCl are very similar (Fig. 4Aii and Fig. S4 E) indicating no major effect of cation type. In contrast, the c(s) distributions in 0.20 M KGlu (Fig. 4B-ii) and 0.20 M KOAc (Fig. S4B) are bimodal at all protein to DNA ratios, R_{65}<1.0, indicating that highly cooperative binding in the $(SSB)_{65}$ binding mode is promoted by glutamate and acetate.

Even more striking differences are observed at 0.50 M salt concentrations, where bimodal c(s) distributions still remain in KGlu (Fig. 4 Biii), but are less apparent in KOAc (Fig. S4 C), and totally absent in KCl and NaCl (Fig. 4 Aii and Fig. S4 F, respectively). Importantly, the distributions in NaCl and KCl are very similar, indicating no effect of cation type. At the same time the binding isotherms of $\bar{s}_{20,w}$ vs R_{65} in NaCl and KCl indicate weaker binding compared with those in KGlu and KOAc. Thus, high Cl^- concentration weakens binding and eliminates high cooperativity. This is consistent with the results of equilibrium titrations of SSB with M13 ssDNA (Fig. S3) suggesting that in 0.50 M NaCl the SSB binds to M13 ssDNA in the $(SSB)_{65}$ binding mode with moderate affinity, but without high cooperativity. Hence, highly cooperativity binding to ssDNA even in the $(SSB)_{65}$ mode is promoted by anion type in the order $Glu^- > OAc^- >> Cl^-$.

Discussion

Studies of SSB-ssDNA binding cooperativity are challenging due to the fact that SSB can bind ssDNA in multiple binding modes that each display different cooperative behavior. With the exception of the $(SSB)_{65}$ mode, it is difficult to find conditions *in vitro* that favor exclusively a single binding mode in order to examine cooperativity. Under most conditions, a distribution of binding modes exists that changes with SSB binding density [26, 54].

Early electron microscopy studies of *E. coli* SSB [25] showed that it could bind with very high cooperativity to ssDNA such that at less than saturating SSB to DNA ratios, a bimodal ssDNA population existed; ssDNA that was essentially fully

saturated with SSB protein existed in the same population as ssDNA that had little SSB bound. It was later shown in buffers containing NaCl that this highly cooperative binding behavior was observed only at low [NaCl] (<10 mM) that promotes the $(SSB)_{35}$ mode [17]. Recently, Kozlov et al. [27] made the surprising finding that the 56 amino acid C-terminal IDL is essential for highly cooperative binding at low salt concentrations. Furthermore, the amino acid composition of the IDL influences cooperative binding as indicated by substitution of the more highly charged IDL from *P. falciparum* SSB [50, 51] for the *E. coli* IDL [27].

It had been thought that only the $(SSB)_{35}$ binding mode displayed highly cooperative binding and that the $(SSB)_{65}$ binding mode showed only limited cooperativity. However, we show here that highly cooperative binding of SSB to ssDNA also can occur in the $(SSB)_{65}$ binding mode. This was previously obscured because high [NaCl] was used to exclusively populate the $(SSB)_{65}$ mode. Here we show that substitution of glutamate or acetate for chloride promotes high cooperativity even at high salt concentrations (0.50 M) that promote only the $(SSB)_{65}$ mode.

However, highly cooperative binding still requires the intrinsically disordered C-terminal tails of SSB. We had previously shown that cooperative interactions between SSB tetramers in the $(SSB)_{35}$ mode require the IDL, but are also enhanced by the acidic tip. We proposed that this could result from the acidic tip of one SSB tetramer interacting with an unoccupied ssDNA binding site of a neighboring tetramer [27]. Such interactions have been demonstrated at high SSB concentrations [33]. However, this type of interaction is precluded in the $(SSB)_{65}$

mode since all ssDNA binding sites are occupied by DNA. We therefore suggest that high cooperativity is promoted via direct interactions between the SSB IDLs as depicted in Figure 6 and that these interactions are promoted by anions such as glutamate and acetate that are preferentially excluded from protein regions [36, 37]. Direct interactions between similar types of low complexity intrinsically disordered regions are believed to drive liquid-liquid phase separation, as exemplified by the LAF-1 protein [55].

Our observation that SSB-ssDNA cooperativity is not eliminated even at 0.50 M KGlu indicates that electrostatic interactions are not a major driving force for cooperative interactions in the $(SSB)_{65}$ mode. This is also consistent with the fact that the *E. coli* SSB IDL contains only 3 charged residues within the 56 residue IDL (in addition to the 4 acid residues within the 9 amino acid C-terminal tip). The IDL is rich in Gly (17), Pro (9) and Gln plus Asn (14). As a result of this composition, the *E. coli* IDL is predicted to form an ensemble of compact globular conformations [27], consistent with hydrodynamic studies [27] and small angle x-ray and neutron scattering studies [56]. Such globular conformations may promote positive cooperativity through linker-linker interactions upon ssDNA binding. It will be of interest to see if the compaction and/or distribution of conformations of the IDL are affected by monovalent anion type.

Prior to the current study, most studies of SSB-ssDNA binding cooperativity were performed in buffers in which the major monovalent anion was chloride [17, 20, 23, 25-27, 57]. At high [NaCl] (> 200 mM), where the $(SSB)_{65}$ mode forms exclusively, SSB binds with only a limited cooperativity such that it forms at most

dimers of tetramers (octamers) [15-17, 23, 43]. This mode does not form long protein clusters on ssDNA. Due to the high affinity of SSB for ssDNA, the only quantitative estimates for the cooperativity parameter between two SSB tetramers in the $(SSB)_{65}$ mode were made for SSB binding to poly(U), an RNA, or poly(dT) in high [NaBr]. In those cases, a limited cooperativity parameter of $\omega_{T/O}$ ~400±100 was estimated. At low [NaCl](< 10 mM) the $(SSB)_{35}$ mode is favored at high SSB to DNA ratios. Under these conditions, highly cooperative binding of SSB to ssDNA is observed such that long protein clusters form along ssDNA. Quantitative estimates of the nearest neighbor unlimited cooperativity parameter, ω_{unlim} ~ 10^5 were made for the $(SSB)_{35}$ binding mode at intermediate [NaCl] (0.125 M, pH 8.1, 25˚C) [26].

The effects of anions, including glutamate, on SSB cooperativity in the $(SSB)_{65}$ mode were examined previously [23, 43], but only for SSB binding to the ssRNA, poly(U), which was used to lower the SSB binding affinity into a range that could be measured. In that study, the only noted effect of glutamate was to increase binding affinity with no dramatic effect on cooperativity [43]. It may be that glutamate and acetate only enhance cooperativity for SSB binding to ssDNA.

We also show that deletion of the IDL affects the SSB-ssDNA binding mode preferences so that the $(SSB)_{35}$ binding mode is favored at higher salt concentrations for SSB-ΔL than for SSB. This observation is consistent with previous reports indicating that successive deletion of two or three C-terminal tails shifts the binding mode transitions to favor the $(SSB)_{35}$ binding mode [12]. Furthermore, whereas the binding mode transitions for wtSSB are affected by

anion type as shown here and previously [18], deletion of the IDL minimizes the effects of anion type. The mechanism by which the IDL influences the binding modes remains a major open question.

In a recent single molecule force spectroscopy study of *E. coli* SSB binding to denatured phage lambda ssDNA, Bell et al. [58] reported an intramolecular condensation of SSB-DNA complexes induced by increased monovalent salt concentrations that appeared to exceed the expected decrease in ssDNA contour length based solely on the transition from the $(SSB)_{35}$ to the $(SSB)_{65}$ binding mode. This additional compaction was reversible even in the absence of free SSB protein indicating that it did not involve binding or dissociation of additional SSB to the DNA. Bell et al. [58] suggested that the higher salt concentration induced long range intramolecular interactions between non-nearest neighbor SSB tetramers on the ssDNA. We note that Bell et al. [58] used NaOAc, rather than NaCl to vary the monovalent salt concentration in their experiments. It seems likely that the additional ssDNA compaction observed at high [NaOAc] [58] reflects the promotion of highly cooperative binding of SSB to ssDNA that we observe at high acetate and glutamate concentrations. Based on our studies, we predict that the additional compaction would not be observed at high [NaCl] where highly cooperative binding is eliminated. This also suggests that at least some of the highly cooperative binding that we observe at high acetate or glutamate concentrations may reflect non-nearest neighbor SSB interactions.

The molecular basis for the dramatic effects of glutamate and acetate vs. chloride is most likely due to the weak preferential interactions of these anions with

proteins as reflected in the Hofmeister series [59, 60]. Recent work from the Record lab [36, 37] has shown that glutamate has unfavorable interactions with and is thus preferentially excluded from all hydrocarbon groups, and carboxylate and amide oxygens, but interacts favorably with positively charged amino acids. As a result, KGlu generally promotes folding and assembly processes more than KCl. [27]. Kontur et al. [61] compared the effects of KGlu and KCl on the kinetic steps of RNA polymerase-promoter binding and dissociation, and found that the steps which involve unfolding and disassembly of mobile regions of RNA polymerase are the steps that are most sensitive to anion type, exhibiting the largest differences between Glu^- and Cl^-. Hence, we suggest that KGlu promotes interactions between IDL regions of SSB since these are rich in Gly, Pro, Gln and Asn, but contain only two basic amino acids (Arg). Interestingly, whereas high [NaCl] inhibits the interaction of the acidic tip with the DNA binding domains of SSB [34, 35], [KGlu] does not [34]. In contrast, the IDL of *P. falciparum* that does not promote high cooperativity, is more highly charged, containing 23 charged residues outside of its acidic C-terminal tip and is predicted to form an ensemble of more expanded random coil configurations.

The dramatic effects of glutamate on SSB-ssDNA binding cooperativity is likely to be biologically relevant since Glu^- is the major monovalent cytoplasmic anion in *E. coli*, ranging in concentration from 0.03 to 0.25 molal [38, 62]. This makes it likely that glutamate induced cooperative SSB-ssDNA interactions also occur *in vivo.* Furthermore, since the many SIPs that interact with SSB do so

through interactions with the acidic tip at the end of the IDL, those interactions might be expected to modulate SSB cooperativity.

Finally, a defining feature of membraneless intracellular compartments that form due to liquid-liquid phase separation is that they contain RNA and proteins, similar to SSB, that possess multiple nucleic acid binding sites and significant intrinsically disordered regions of low complexity [55, 63, 64]. We suggest that KGlu and other salts that are preferentially excluded from protein surfaces will influence these phase separation processes differently than chloride salts.

Materials and Methods

Reagents and buffers

Buffers were prepared with reagent grade chemicals and distilled water treated with a Milli Q (Millipore, Bedford, MA) water purification system. Buffer T is 10 mM Tris, pH 8.1 (25°C), 0.1 mM Na_3EDTA.

DNA, SSB and SSB-tail variants

Poly(dT) (Midland certified reagent company, Midland, TX ((Catalog #P-2004, Lot number 071308)), had an average length of ~ 1000±200 nucleotides and was dialyzed vs. the indicated buffer before use. Single stranded M13 mp18 DNA was from New England Biolabs (Catalog #N4040S). DNA concentrations were determined spectrophotometrically in buffer T + 0.10 M NaCl using $\varepsilon_{260} = 8.1\times10^3$

M^{-1} (nucleotide) cm^{-1} for poly(dT), and ε_{259} = 7370 M^{-1} cm^{-1} (nucleotide) for M13 DNA [65].

E. coli SSB protein, SSB-ΔL, a linkerless variant (SSBΔ115-168, previously referred to as SSB-GG [27]) and the SSB-*EcPfEc* chimera, in which the *E. coli* IDL (residues 113-168) was replaced by 80 of the 84 aa of the *Plasmodium falciparum* SSB IDL, were expressed and purified as described [27]. All proteins form stable tetramers under all solution conditions used in this study as determined by sedimentation velocity. Protein concentrations were determined spectrophotometrically [13] (buffer T, 0.20 M NaCl) using ε_{280}=1.13 $\times$ 10^5 M^{-1} cm^{-1} for wtSSB, ε_{280}=8.98 $\times$ 10^4 M^{-1} cm^{-1} for SSB-ΔL and SSB-*EcPfEc*.

Fluorescence measurements

Titrations of SSB and SSB-ΔL with ssDNA were performed by monitoring quenching of the intrinsic SSB tryptophan fluorescence and analyzed as described [27, 49].

Analytical sedimentation

Sedimentation velocity experiments were performed with an Optima XL-A analytical ultracentrifuge and An50Ti rotor (Beckman Instruments, Fullerton, CA) at 15000 rpm (25°C) as described [27]. A constant DNA concentration (typically 25 μM, but up to 50 μM nucleotides) was used while monitoring absorbance at 260 nm. Protein/DNA ratios are indicated as R_{65}=65x[P_{tot}]/[DNA(nts)$_{tot}$], where [P_{tot}] is the total SSB tetramer concentration and [DNA(nts)$_{tot}$] is the total DNA concentration in nucleotides. The contribution of SSB to the absorbance at 260 nm

is small at low protein/DNA ratios and does not exceed 15% at R_{65}=2.8, the maximum ratio used. Data were analyzed using SEDFIT [53], (www.analyticalultracentrifugation.com) to obtain c(s) distributions. The c(s) distribution function defines the populations of species with different sedimentation rates (sizes) and represents a variant of the distribution of Lamm equation solutions [53]. Integration of the peaks of c(s) provides an estimate of the weight-average sedimentation coefficient of sedimenting species ($\bar{s}$) and was used to construct overall binding isotherms in Fig.4, 5 and S5 [53]. For comparisons among the different salts, the c(s) distributions obtained in a particular salt condition (25^{o}C) were converted to 20^{o}C, water conditions using SEDFIT (www.analyticalultracentrifugation.com). The densities and viscosities at 25^{o}C were from SEDNTERP for KCl, NaCl and KOAc solutions and from van Holst et al. [66] for KGlu.

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Figure Legends

Figure 1. *E. coli* SSB constructs and SSB binding modes. (A) An SSB subunit (177 aa) is composed of an N-terminal DNA binding domain (OB fold) (residues 1-112) and a C-terminal tail (residues 113-177) which contains a 56aa intrinsically

disordered linker (IDL), and a conserved 9aa acidic tip. (B) Two SSB linker variants containing *Ec* DBD and *Ec* tip connected by (i) two glycines (SSB Δ115-168 deletion), SSB-ΔL; and (ii) 80 aa IDL from *Plasmodium falciparum* SSB, SSB-*EcPfEc*. (C) Schematics of the SSB-ssDNA interaction in the $(SSB)_{65}$ binding mode, with 65 nts of DNA (orange ribbon) wrapped around an SSB tetramer[6]; the IDLs (grey) with the acidic tips (red letters) are depicted at the dimer-dimer interface, as an extension of the C-termini visible in the crystal structure. (D) Schematic of a hypothetical model for SSB-ssDNA binding in the $(SSB)_{35}$ binding mode[6], in which two SSB tetramers interact with a ~70 nts long DNA (orange tube) using an average of only two subunits of each tetramer. (E) Sequences of the C-terminal domains of *Ec* SSB (65 aa) and *Pf* SSB (91 aa) (positively and negatively charged residues are shown in blue and red, respectively)

Figure 2. Effects of salt type and deletion of the intrinsically disordered linker on SSB-DNA binding mode transitions. Occluded site size (nucleotides per tetramer) for SSB (A) and SSB-*ΔL* (B) binding to poly(dT) as a function of salt concentration and type (NaCl (▲), KCl (◆), KOAc (■) and KGlu (●)) in buffer

Figure 3. Highly cooperative SSB-ssDNA binding persists in high [KGlu], but is diminished in high [KCl]. Representative sedimentation velocity c(s) distributions converted to 20˚C, water conditions for wtSSB-M13ssDNA complexes at different protein to DNA ratios: R_{65}=0.56 (blue) and R_{65}=2.79 (violet, dash), where $R_{65}=[SSB_{tetr,tot}]\times 65/[M13ssDNA_{nts,tot}]$. M13ssDNA alone (green) (25 μM nts).

(A) – 10 mM KCl, (B) – 10 mM KGlu, (C) – 0.20 M KCl, (D) – 0.20 M KGlu, (E) – 0.50 M KCl and (F) – 0.50 M KGlu.

Figure 4. Salt concentration and type regulate cooperative binding of SSB to M13-ssDNA. Sedimentation velocity c(s) distributions converted to 20˚C, water conditions for wtSSB-M13ssDNA complexes at different protein to DNA ratios, R_{65}, and three concentrations of (A)-KCl and (B)-KGlu: (i) – 10 mM, (ii) – 0.20 M and (iii) – 0.50 M. R_{65}=0.19 (brown), R_{65}=0.37 (magenta), R_{65}=0.56 (blue), R_{65}=1.00 (orange), R_{65}=1.49 (grey), R_{65}=1.86 (cyan) and R_{65}=2.79 (violet, dash). (C) Binding isotherms in the form of $\bar{s}_{20,w}$ (weighted average of sedimentation coefficient) vs R_{65}, calculated from the data in panels (A) and (B) (for KCl and KGlu, respectively) and from the distributions obtained in NaCl and KOAc (Fig. S4).

Figure 5. The Ec-IDL is required for highly cooperative ssDNA binding. Sedimentation velocity c(s) distributions converted to 20˚C, water conditions show low cooperativity behavior (single peaks) for SSB-*ΔL* binding to M13ssDNA at the same protein to DNA ratios, as in Fig. 4; (A)- 10 mM NaCl (B)- 0.20 M NaCl, (C)- 0.20 M KGlu. Compare with the highly cooperative (bimodal) distributions in Fig. 4 and S4. (D) - binding isotherms in the form of $\bar{s}_{20,w}$ (weighted average of sedimentation coefficient) vs R_{65} calculated from the data in panels (A), (B) and (C).

Figure 6. Cooperative interactions in the $(SSB)_{65}$ binding mode. A cartoon depicting our view of the source of cooperativity in the $(SSB)_{65}$ binding mode as involving direct interactions between the intrinsically disordered linkers within the

C-terminal tail of SSB tetramers. These interactions likely involve both nearest neighbor and non-nearest neighbor tetramers.

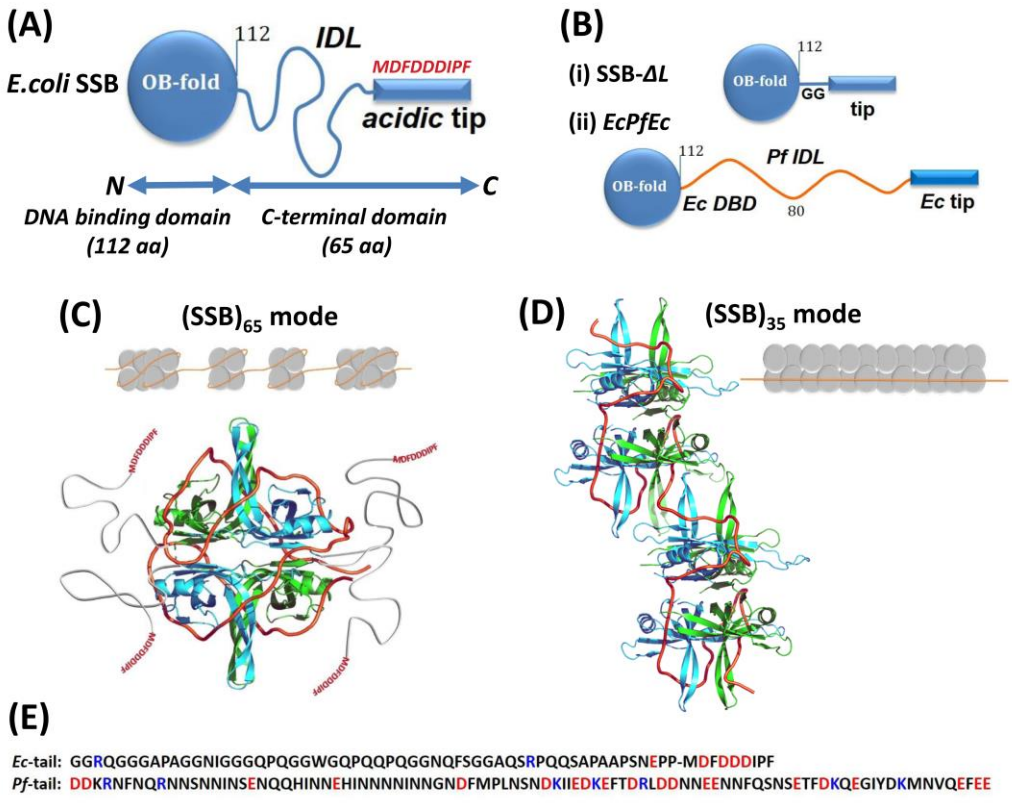


Figure 1

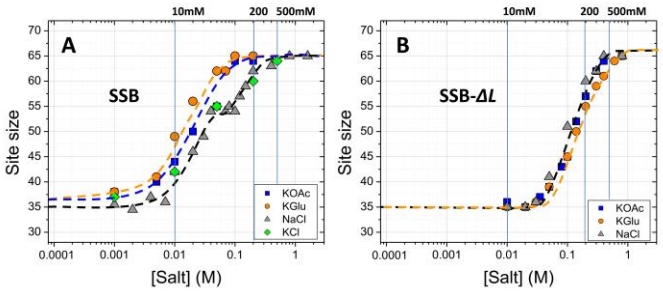


Figure 2

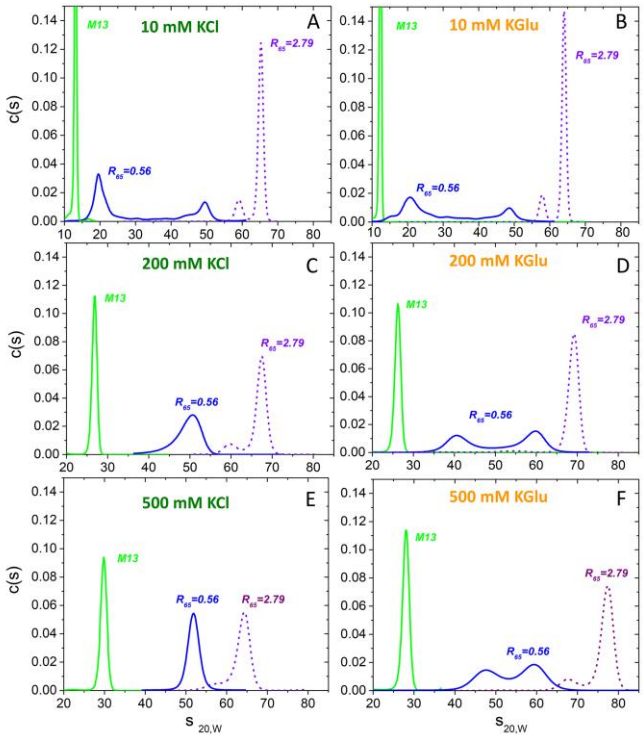


Figure 3

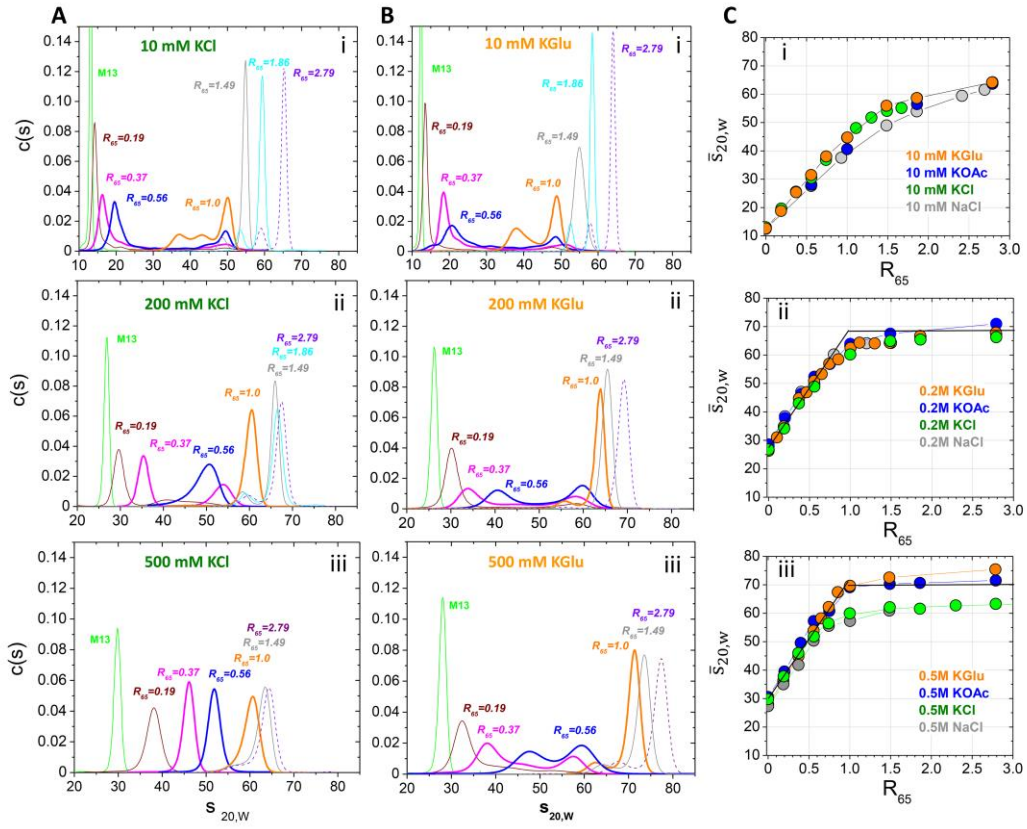


Figure 4

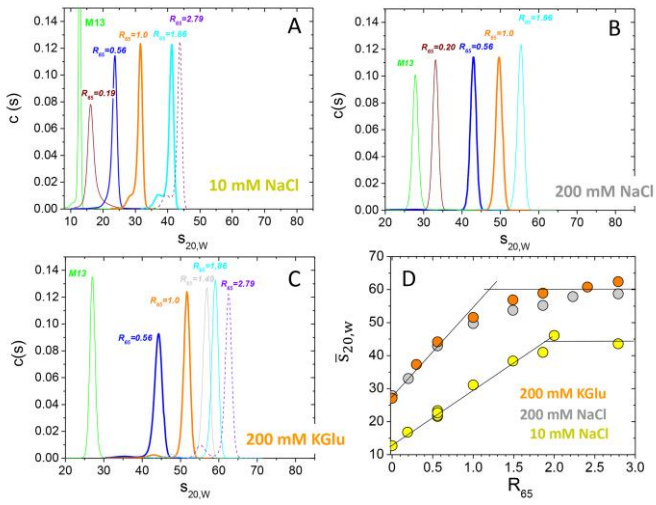


Figure 5

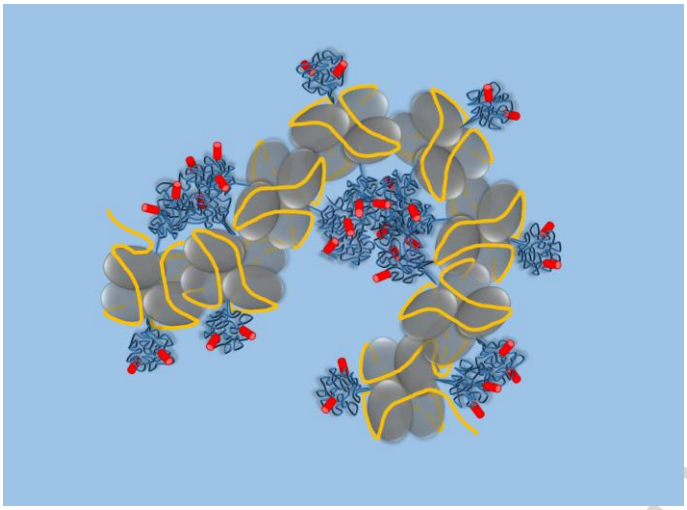
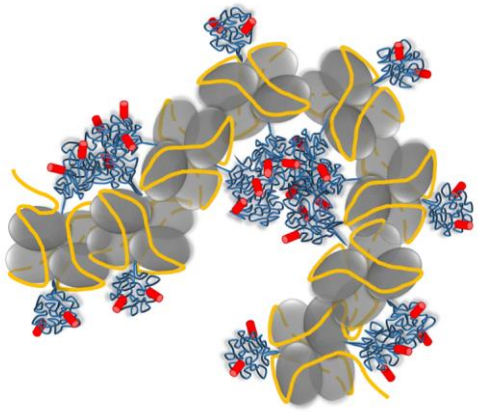


Figure 6



Graphical abstract

Highlights

- Highly cooperative binding of *E. coli* SSB to DNA occurs at physiological salt concentrations when the buffer contains the physiologically relevant anion, glutamate.
- The SSB tetramers, even in the fully wrapped $(SSB)_{65}$ mode, in which all DNA binding sites are occupied, can bind with high cooperativity.
- Cooperativity requires the intrinsically disordered C-terminal tails of SSB.
- Cooperativity appears to be due to direct interactions between the intrinsically disordered regions of SSB.
- SSB-ssDNA cooperativity is likely to be important *in vivo*.