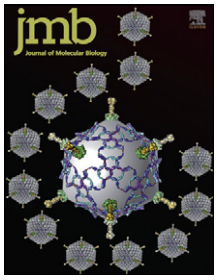

Glutamate promotes SSB protein–protein Interactions via intrinsically disordered regions

Alexander G. Kozlov [1,†], Min Kyung Shinn [1,2,†], Elizabeth A. Weiland [1] and Timothy M. Lohman [1]

*1 - **Department of Biochemistry and Molecular Biophysics,** Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110, United States*
*2 - **Department of Physics,** Washington University in St. Louis, St. Louis, MO 63130, United States*

***Correspondence to Timothy M. Lohman:** Department of Biochemistry and Molecular Biophysics, Box 8231, Washington University School of Medicine, 660 South Euclid Ave., St. Louis, MO 63110, United States. lohman@wustl.edu*
http://dx.doi.org/10.1016/j.jmb.2017.07.021
Edited by James Berger

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 $(SSB)_{65}$/$(SSB)_{56}$ binding modes 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*.

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].

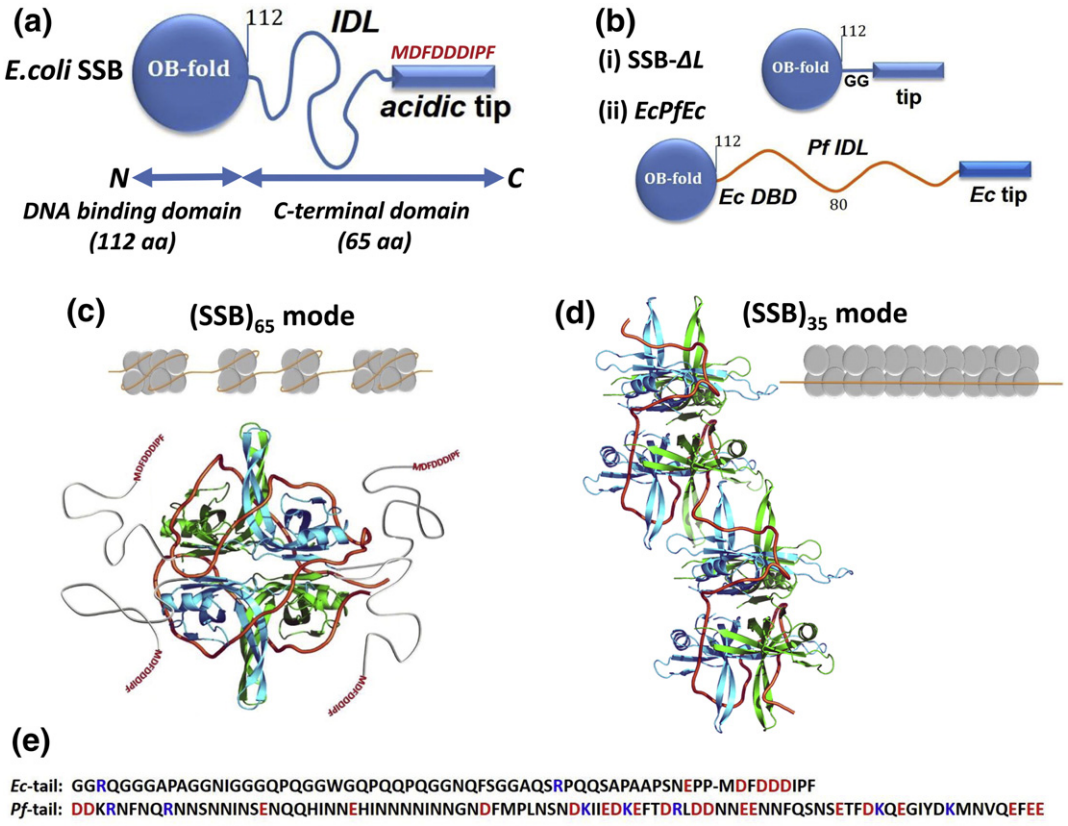

Fig. 1. *E. coli* SSB constructs and SSB binding modes. (a) An SSB subunit (177 aa) is composed of an N-terminal DNA binding domain (OB fold) (residues 1–112) and a C-terminal tail (residues 113–177) which contains a 56aa intrinsically disordered linker (IDL), and a conserved 9aa acidic tip. (b) Two 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).

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 includes 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