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In vitro and *in vivo* function of the C-terminus of *Escherichia coli* single-stranded DNA binding protein

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ABSTRACT

We constructed several deletion mutants of *Escherichia coli* single-stranded DNA binding protein (*Eco*SSB) lacking different parts of the C-terminal region. This region of *Eco*SSB is composed of two parts: a glycine- and proline-rich sequence of ~60 amino acids followed by an acidic region of the last 10 amino acids which is highly conserved among the bacterial SSB proteins. The single-stranded DNA binding protein of human mitochondria (*Hs*mtSSB) lacks a region homologous to the C-terminal third of *Eco*SSB. Therefore, we also investigated a chimeric protein consisting of the complete sequence of the human mitochondrial single-stranded DNA binding protein (*Hs*mtSSB) and the C-terminal third of *Eco*SSB. Fluorescence titrations and DNA-melting curves showed that the C-terminal third of *Eco*SSB is not essential for DNA-binding *in vitro*. The affinity for single-stranded DNA and RNA is even increased by the removal of the last 10 amino acids. Consequently, the nucleic acid binding affinity of *Hs*mtSSB is reduced by the addition of the C-terminus of *Eco*SSB. All mutant proteins lacking the last 10 amino acids are unable to substitute wild-type *Eco*SSB *in vivo*. Thus, while the nucleic acid binding properties do not depend on an intact C-terminus, this region is essential for *in vivo* function. Although the DNA binding properties of *Hs*mtSSB and *Eco*SSB are quite similar, *Hs*mtSSB does not function in *E.coli*. This failure cannot be overcome by fusing the C-terminal third of *Eco*SSB to *Hs*mtSSB. Thus differences in the N-terminal parts of both proteins must be responsible for this incompatibility. None of the mutants was defective in tetramerization. However, mixed tetramers could only be formed by proteins containing the same N-terminal part. This reflects structural differences between the N-terminal parts of *Hs*mtSSB and *Eco*SSB. These results indicate that the region of the last 10 amino acids, which is highly conserved among bacterial SSB proteins, is involved in essential protein–protein interactions in the *E.coli* cell.

INTRODUCTION

Due to its role in DNA replication, repair and recombination, single-stranded DNA binding protein of *Escherichia coli* (*Eco*SSB) is essential for survival of the cell (1–3). *Eco*SSB consists of 177 amino acids and forms a very stable homotetramer (4). Secondary structure prediction indicates that the sequence of *Eco*SSB can be divided into two parts, an N-terminal domain (~120 amino acids) rich in α helices and β sheets and a more or less unstructured C-terminus (5). The N-terminal two thirds contain the DNA-binding domain (6). The function of the C-terminal third is not yet characterized in detail. The importance of the C-terminus is illustrated by the *ssb113* mutation (7), in which the penultimate residue of the *Eco*SSB protein (proline 176) is replaced by serine (8). This mutation results in a UV- and temperature-sensitive phenotype. The DNA-binding affinity of the mutated protein is only slightly affected (8). Williams *et al.* (6) showed that the C-terminal third of *Eco*SSB becomes more accessible to proteolysis after DNA-binding. Removing the C-terminal fragment resulted in a protein with higher affinity towards single-stranded DNA.

The C-terminal third of *Eco*SSB consists of two regions: a sequence of ~50 amino acids rich in proline and glycine residues is followed by an acidic region of 10 amino acids containing four aspartate residues. In contrast with the proline- and glycine-rich sequence, the region of the last 10 amino acids is highly conserved among procaryotic SSB proteins (1).

To characterize further the functions of the C-terminal third of *Eco*SSB, we constructed several mutant *Eco*SSB proteins in which different parts of this region were deleted.

Information about the influence of the N-terminal DNA binding domain on the functions of the C-terminal third can be obtained by replacing this domain with the human mitochondrial single-stranded DNA binding protein (*Hs*mtSSB). *Hs*mtSSB, although sharing only 36% sequence homology with the N-terminus of *Eco*SSB, is a homotetrameric single-stranded DNA binding protein (SSB) with *in vitro* DNA binding properties almost indistinguishable from those of *Eco*SSB (9). The size of *Hs*mtSSB (133 amino acids) corresponds to the size of the N-terminal domain of *Eco*SSB and the protein is completely lacking any sequences homologous to the C-terminal third of *Eco*SSB.

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MATERIALS AND METHODS

Poly(dT), poly(dA–dT) and poly(rU) were purchased from Pharmacia (Freiburg). Experiments were performed in a standard buffer containing 0.1 mM EDTA, 20 mM potassium phosphate pH 7.4 and sodium chloride as indicated. For fluorescence titrations 100 p.p.m. Tween 20 (Serva) was added.

Media

LB medium (10) was used for all microbiological experiments. The concentration of antibiotics used for selection purposes was 100 µg/ml ampicillin and 30 µg/ml chloramphenicol respectively.

Site-directed mutagenesis

Site-directed mutagenesis was performed by the gapped-duplex method using the pBR322 derived plasmids pSF1 (11) and pSF1(*Hs*mtSSB) (9) both conferring ampicillin resistance. The truncated *Eco*SSB mutants *Eco*SSB Q152* and *Eco*SSB G117* were constructed by changing the codons for Q152 or G117 into the stop codon TAG and TGA respectively. For construction of the deletion mutant *Eco*SSB Δ116–167 and *HsEc*SSB, we first introduced cleavage sites for blunt end restriction endonucleases at the respective positions in the genes. After cleavage the desired gene fragments were purified using preparative agarose gel electrophoresis and ligated. *Eco*SSB Δ116–167 is a fusion protein where the amino acids 1–115 of *Eco*SSB are followed by the last 10 amino acids (168–177) of *Eco*SSB. In *HsEc*SSB, the complete sequence of *Hs*mtSSB (1–133) is followed by amino acids 113–177 of *Eco*SSB.

Protein preparation

The plasmids used for overproduction of SSB protein carry the respective gene under control of the λP_L-promotor. After transformation into *E.coli* TGE900 (12) carrying the thermosensitive λcI857 repressor protein, production was induced by a temperature shift from 30 to 42°C and cells were harvested 3 h after induction. The sequence of the *ssb* gene was confirmed from plasmid prepared from an aliquot of the cells taken before induction.

Wild-type *Eco*SSB and *HsEc*SSB were prepared using poly(ethyleneimine) precipitation of the protein and subsequent extraction with 0.4 M NaCl as described by Lohman et al. (13). The deletion mutant *Eco*SSB Δ116–167 was prepared like wild-type *Eco*SSB but had to be extracted from the poly(ethyleneimine) precipitation with 0.8 M NaCl. *Hs*mtSSB was prepared as described earlier (9). All *Eco*SSB mutants lacking the acidic region of the last 10 amino acids were purified like *Hs*mtSSB.

Determination of concentrations

Protein concentrations are given in units of tetramers throughout the text. They were determined spectrophotometrically using the following absorption coefficients at 280 nm: $\varepsilon = 113\,000\ M^{-1}cm^{-1}$ for wild-type *Eco*SSB (14) and *Eco*SSB Q152*, $\varepsilon = 88\,800\ M^{-1}cm^{-1}$ for *Eco*SSB G117* and *Eco*SSB Δ116–167, $\varepsilon = 76\,240\ M^{-1}cm^{-1}$ for *Hs*mtSSB and $\varepsilon = 99\,000\ M^{-1}cm^{-1}$ for *HsEc*SSB. Unless stated otherwise, the absorption coefficients were calculated from the known amino acid composition and the absorption coefficients of the aromatic amino acids (15).

Nucleic acid concentrations are given in units of monomers and determined using the following absorption coefficients: $\varepsilon_{max} = 8600\ M^{-1}cm^{-1}$ for poly(dT) (16), $\varepsilon_{260\,nm} = 9200\ M^{-1}cm^{-1}$ for poly(rU) (17) and $\varepsilon_{260\,nm} = 6700\ M^{-1}cm^{-1}$ for poly(dA–dT).

Physicochemical experiments

Fluorescence titrations were carried out in a Schoeffel RRS 1000 spectrofluorimeter as described earlier (18). Excitation wavelength was 295 nm and emission was observed at 350 nm. Theoretical binding isotherms were calculated using the model of Schwarz and Watanabe (19) with the binding site size *n*, cooperative binding affinity ($K \cdot \omega$), and the fluorescence quench (Q_f) as parameters. The fluorescence quench is the difference between the normalized fluorescences of the free protein and the protein–DNA complex.

DNA melting curves were measured in a DMR10 (Zeiss) spectrophotometer as described previously (20) using a heating rate of 20 K/h. No significant differences between heating and cooling curve could be observed confirming the reversibility of melting.

Analytical ultracentrifugation was carried out in a Spinco/Beckman model E centrifuge and evaluated as described earlier (21).

Subunit exchange experiments

For subunit exchange experiments, two different SSB proteins were mixed at a concentration of 50 µM each in a buffer containing 0.5 M NaCl, 1 mM EDTA, 20% glycerol and 20 mM potassium phosphate, pH 7.5 in a volume of 10 µl. The mixture was allowed to react for 3 days at room temperature for those exchange experiments containing only *Eco*SSB derivatives and for 2 weeks at 37°C for exchanges with *Hs*mtSSB and *HsEc*SSB. The products were diluted by addition of 40 µl 1 M triethanolamine pH 8.5, 20 µl dimethylsuberimidate (30 mg/ml in water) and 130 µl water. Crosslinking then was performed by a 3 h incubation at room temperature and the products were analyzed by SDS–PAGE (12%) (22).

Complementation of *ssb* defective strains

For complementation analysis two *E.coli* strains were used. In RDP268 (23) the chromosomal *ssb* gene is deleted and SSB function is restored by pACYC*ssb* plasmid conferring chloramphenicol resistance. CS149 carries the *ssb*-3 mutation (G15D) (24) on the chromosome rendering the cells unable to survive in presence of 0.9 µg/ml mitomycin C. The strains were transformed with plasmids containing the respective *ssb* genes. For high copy number plasmids the respective overproduction vectors (pSF1 derivatives v.s.) were used. In the defective strains the P_L promotor is not repressed leading to high intracellular SSB concentrations. Monocopy plasmids were constructed as follows: pSBL5 (25) is a derivative of the mini-F plasmid pRE432 (26) conferring ampicillin resistance and contains the wild-type *Eco*SSB gene under control of its natural promotor. In this plasmid the nucleic acid sequence coding for 160 C-terminal amino acids of wild-type *Eco*SSB was replaced by the respective mutant gene fragments. Unfortunately the absence of suitable restriction sites for exchanging the complete *Eco*SSB gene precluded the construction of *Hs*mtSSB and *HsEc*SSB derivatives of pSBL5. Since pSBL5 is a monocopy vector and the *ssb* gene is under the control of the natural *ssb* promotor, intracellular SSB

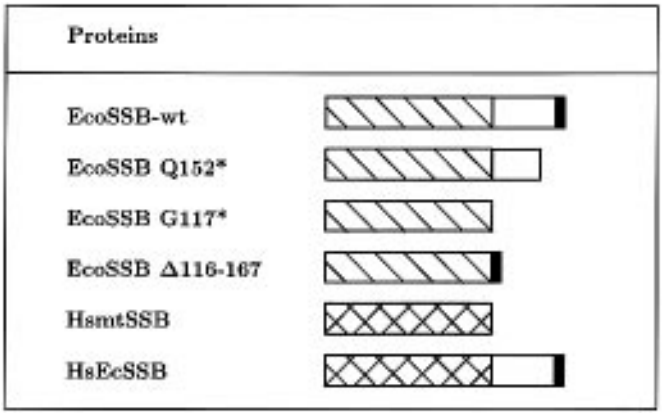

Figure 1. Schematic depiction of the SSB proteins investigated in this study. Hatched: N-terminal part (~120 aa) of *Eco*SSB. Cross-hatched: *Hs*mtSSB. White: proline- and glycine-rich region of *Eco*SSB (~50 aa). Black: acidic C-terminal region of *Eco*SSB (10 aa).

concentrations in this case correspond to the SSB level in a normal *E.coli* cell.

For *ssb-3* complementation 100–200 transformed cells were grown on plates containing ampicillin. Colonies were replica plated on LB containing 0.9 µg/ml mitomycin C and incubated at 37°C overnight.

For Δ*ssb* complementation transformed cells were grown in the presence of ampicillin but omitting chloramphenicol. After four subsequent inoculations of 4 ml medium allowing the cells to grow for ~50 generations, 100–200 cells were plated. Clones which lost the helper plasmid pACYC*ssb* were identified by replica plating on LB plates containing both chloramphenicol and ampicillin.

If the plasmid tested could complement Δ*ssb* ~25% of the colonies had lost the helper plasmid indicated by chloramphenicol sensitivity. In this case the absence of wild-type *Eco*SSB was confirmed by western blot analysis.

RESULTS

A schematic depiction of the different deletion mutants of *Eco*SSB, wild-type *Hs*mtSSB and the chimeric *HsEc*SSB constructed for this work is shown in Figure 1.

The C-terminally truncated proteins *Eco*SSB Q152* and *Eco*SSB G117* lack the highly conserved region of the last 10 amino acids and the complete C-terminal third respectively. In the deletion mutant *Eco*SSB Δ116–167 the amino acids 116–167 are missing, leading to a protein which lacks the proline- and glycine-rich region but does contain the last 10 amino acids. *Hs*mtSSB is homologous to the N-terminal domain of *Eco*SSB and thus corresponds to *Eco*SSB G117*. The chimeric protein *HsEc*SSB is a fusion protein composed of the complete *Hs*mtSSB and the C-terminal third of *Eco*SSB. This protein corresponds to *Eco*SSB in containing an N-terminal DNA binding domain, a proline- and glycine-rich region and an acidic C-terminus of 10 amino acids.

All proteins were overexpressed in *E.coli*. Those proteins that contained the last 10 amino acids of *Eco*SSB (wild-type *Eco*SSB, *Eco*SSB Δ116–167, *HsEc*SSB) could be precipitated by poly(ethyleneimine) while the others could not. This is probably due to the fact that the last 10 amino acids render SSB protein net negatively charged whereas the DNA binding domains are net positively charged. The proteins were 98% pure as judged from Coomassie-stained denaturing PAGE, the main impurity being wild-type *Eco*SSB expressed from the chromosomal *ssb* gene. In preparation of *Hs*mtSSB this impurity was removed in the poly(ethyleneimine) precipitation step. For *Eco*SSB G117* and *Eco*SSB Q152* the precipitation did not remove wild-type *Eco*SSB indicating the formation of mixed tetramers (v.i.). Sedimentation analysis in the analytical ultracentrifuge showed all proteins being homotetramers.

Binding to single-stranded DNA

The binding of all SSB proteins to poly(dT) was observed by inverse fluorescence titrations at 0.3 M NaCl (Fig. 2). Upon binding to poly(dT) the intrinsic tryptophan-fluorescence is quenched by ~90%; the exact amount depending on the respective protein. The highest quench is shown by those proteins that contain tryptophan only in the DNA binding domain. Tryptophan 135 of *Eco*SSB resides in the proline and glycine rich region and its fluorescence is scarcely quenched upon DNA binding (18). Thus wild-type *Eco*SSB, *Eco*SSB Q152* and *HsEc*SSB show the least quench. The affinity of the proteins to poly(dT) is too large to allow a discrimination of the binding properties of the different proteins from the shape of the fluorescence titrations (18). The binding site sizes are 65 nucleotides per tetramer for wild-type *Eco*SSB, *Eco*SSB Q152* and *HsEc*SSB, and 59 nucleotides for the others. The C-terminal third of *Eco*SSB is not a part of the DNA binding domain (v.i.). Thus it is the sheer size of this region that leads to the requirement of larger stretches of poly(dT) to reach all four DNA binding sites of the tetramer.

A more indirect, but more sensitive, method to compare the affinities of different SSB proteins towards single-stranded DNA is the measurement of the ability of the proteins to depress the melting temperature of double-stranded DNA. Differences in the affinities not detectable at room temperature are amplified in the melting experiment by the strong decrease of affinity with increasing temperature (27). Figure 3 shows the destabilization of poly(dA–dT) by the different SSB proteins.

The C-terminally truncated proteins *Eco*SSB Q152* and *Eco*SSB G117* cause a drastic reduction of the melting temperature of poly(dA–dT). This is probably due to the removal of electrostatic repulsions between the acidic C-terminus and the single-stranded DNA. The same effect can be observed with *Hs*mtSSB where the addition of the C-terminal third of *Eco*SSB strongly diminished the helix destabilization activity (*HsEc*SSB).

In the deletion mutant *Eco*SSB Δ116–167 the negatively charged C-terminus is directly fused to the DNA binding domain. Although at room temperature this mutant binds strongly to poly(dT) its helix destabilizing activity in the melting experiment is the lowest of all proteins in this study. This again reflects the electrostatic repulsion between the acidic C-terminus and the single-stranded DNA which is most strongly pronounced in this mutant.

The fact that *Hs*mtSSB does not destabilize poly(dA–dT) as strongly as *Eco*SSB Q152* and *Eco*SSB G117* do is a reflection of the different structures of the DNA binding domains of these proteins.

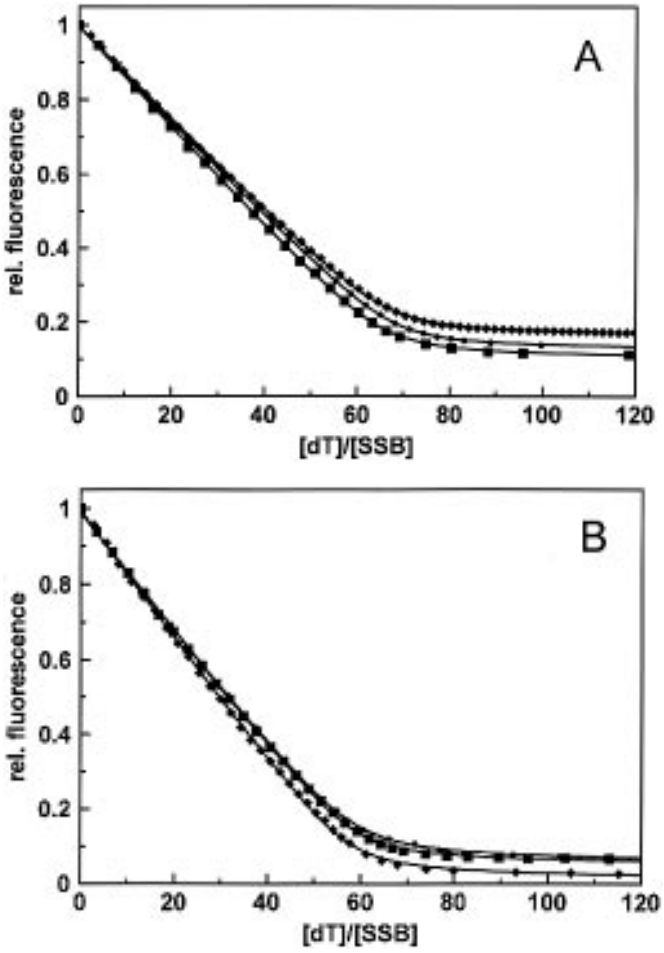

Figure 2. Fluorescence titrations with poly(dT). Protein (0.3 μM) was titrated with poly(dT) in standard buffer containing 0.3 M NaCl. The solid lines represent theoretical binding isotherms according to the model of Schwarz and Watanabe (19) using the following parameters: (**A**) *Eco*SSB (●): $n = 65$, $K{\cdot}\omega = 2.4 \times 10^8\ M^{-1}$, $Q_f = 0.88$; *Eco*SSB Q152* (■): $n = 65$, $K{\cdot}\omega = 1.6 \times 10^8\ M^{-1}$, $Q_f = 0.91$; *HsEc*SSB (♦): $n = 65$, $K{\cdot}\omega = 1.7 \times 10^8\ M^{-1}$, $Q_f = 0.85$ (**B**) *Eco*SSB Δ116–167 (●): $n = 59$, $K{\cdot}\omega = 1.9 \times 10^8\ M^{-1}$, $Q_f = 0.95$; *Hs*mtSSB (■): $n = 59$, $K{\cdot}\omega = 1.7 \times 10^8\ M^{-1}$, $Q_f = 0.96$; *Eco*SSB G117* (♦): $n = 59$, $K{\cdot}\omega = 1.1 \times 10^8\ M^{-1}$, $Q_f = 0.99$. As discussed previously (18), the relatively strong binding does not allow the interpretation of the figures for $K{\cdot}\omega$ as exact numbers but rather as lower limits. In contrast, the values for n and Q_f are accurate within ± 2 and ± 0.02 respectively.

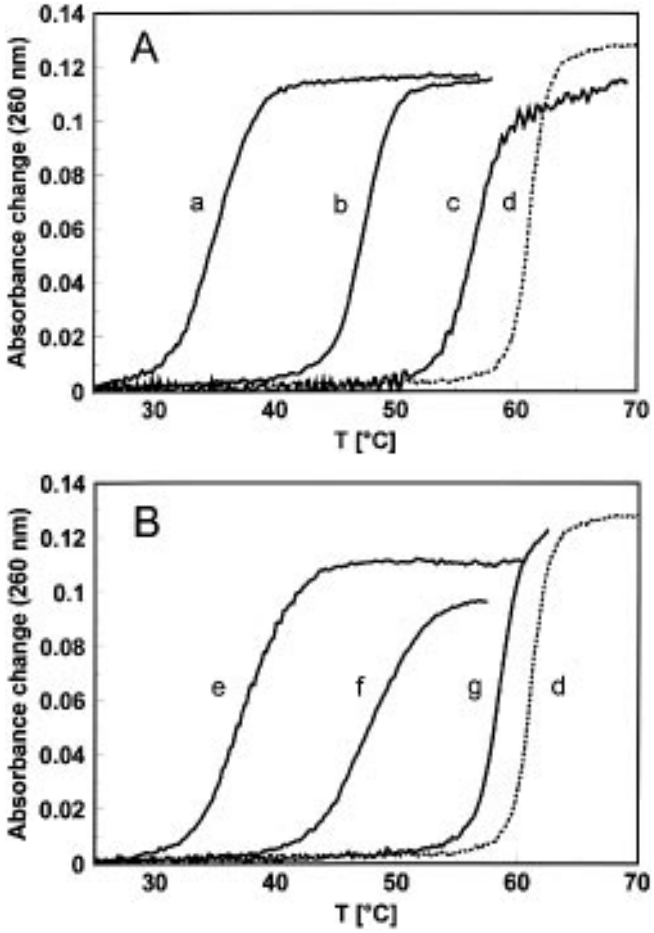

Figure 3. Destabilization of poly(dA–dT) by SSB proteins. Poly(dA–dT) (38 μM) was melted in standard buffer containing 0.1 M NaCl in presence of 1.27 μM protein. (**A**) T_m values: *Eco*SSB Q152* (a): 35°C; *Eco*SSB (b): 47°C; *HsEc*SSB (c): 56°C; without protein (d): 61°C. (**B**) T_m values: without protein (d): 61°C; *Eco*SSB G117* (e): 38°C; *Hs*mtSSB (f): 48°C; *Eco*SSB Δ116–167 (g): 59°C.

Binding to single-stranded RNA

The affinity of *Eco*SSB to single-stranded RNA is much weaker than to single-stranded DNA (3). Thus differences in affinity not detectable in fluorescence titrations with poly(dT) are observed with poly(rU). Figure 4 shows fluorescence titrations of the different proteins with poly(rU).

The proteins missing the acidic C-terminus (*Eco*SSB Q152*, *Eco*SSB G117*) show a higher affinity to poly(rU) as compared with wild-type *Eco*SSB. The affinity of the deletion mutant *Eco*SSB Δ116–167 is drastically reduced. Once more this reflects the influence of the electrostatic repulsion between the nucleic acid and the negatively charged C-terminus.

The same holds for the comparison of *Hs*mtSSB and *HsEc*SSB although the affinities of these proteins to poly(rU) are very weak and only marginal binding can be observed in fluorescence titrations.

In vivo properties

Physiological abilities of the mutant proteins can be investigated by complementation of *E.coli* strains with defective or lacking *ssb* gene. However, a strain lacking the *ssb* gene is not viable. Therefore, we used a strain where the chromosomal *ssb* gene is deleted and substituted by an *ssb* gene coded on a plasmid conferring chloramphenicol resistance (23). If this plasmid could be replaced by a plasmid carrying a mutant *ssb* gene and a different resistance we regarded a respective mutant to be able to complement Δ*ssb*.

A severe defect of the *ssb* gene is *ssb*-3 (24) where glycine 15 is replaced by aspartate leading to a high UV and mitomycin C sensitivity. Cells carrying this defect were not viable in the presence of 0.9 μg/ml mitomycin C. We regard complementation as positive if a plasmid coding for a mutant SSB confers resistance to this concentration of mitomycin C.

Complementation was tested with high cellular concentrations of mutant SSB using the expression plasmids without repression of the P_L promotor. For lower amounts of mutant SSB we used a monocopy plasmid where the *ssb* gene is under control of the chromosomal *E.coli ssb* promotor.

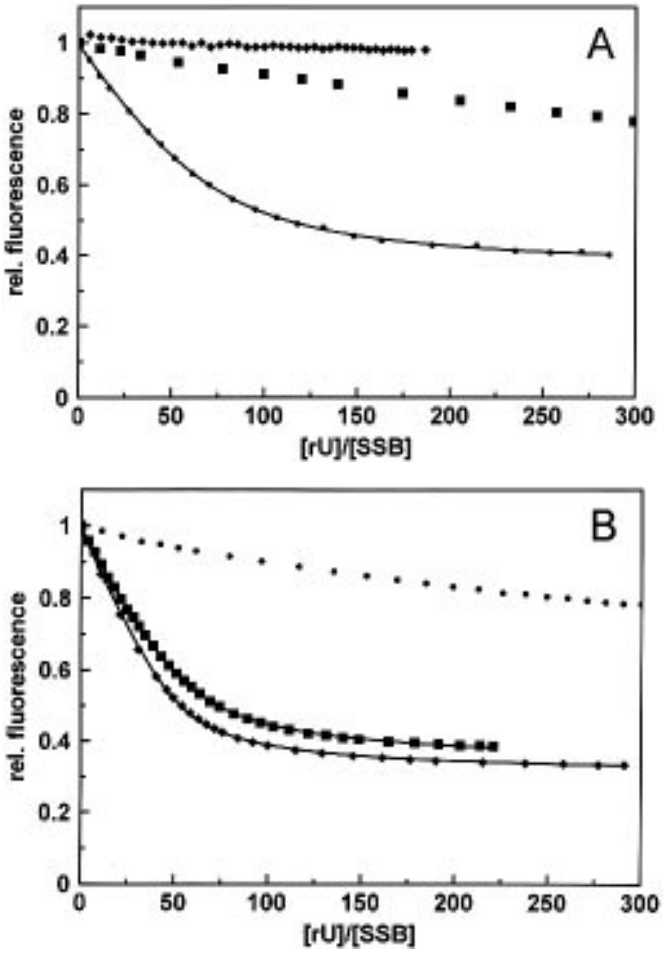

Figure 4. Fluorescence titrations with poly(rU). Protein (0.3 μM) was titrated with poly(rU) in standard buffer containing 0.2 M NaCl. Solid lines represent theoretical binding isotherms calculated according to the model of Schwarz and Watanabe (19) using the following parameters: (**A**) *Eco*SSB (●): $n = 65$, $K{\cdot}\omega = 7.0 \times 10^6$ M^{-1}, $Q_f = 0.64$; *Hs*mtSSB (■): n.d.; *HsEc*SSB (♦): n.d. (**B**) *Eco*SSB Q152* (■): $n = 65$, $K{\cdot}\omega = 2.1 \times 10^7$ M^{-1}, $Q_f = 0.67$; *Eco*SSB Δ116–167 (●): n.d.; *Eco*SSB G117* (♦): $n = 59$, $K{\cdot}\omega = 2.6 \times 10^7$ M^{-1}, $Q_f = 0.71$. Note that the titration points for *Hs*mtSSB, *HsEc*SSB and *Eco*SSB Δ116–167 are located on nearly straight lines and there is almost no bending toward saturation in the accessible range of poly(rU) concentration. This indicates that *Hs*mtSSB and *Eco*SSB Δ116–167 bind very weakly but with reasonable fluorescence quench to poly(rU).

Table 1 shows the results of these complementation experiments. Wild-type *Eco*SSB was used as control and could complement in every case. The only mutant showing the same complementation ability as wild-type *Eco*SSB was *Eco*SSB Δ116–167. Δ*ssb* could not be complemented by any of the other proteins. We conclude that the proline- and glycine-rich region of the C-terminal third is not essential *in vivo* and that the DNA binding domain of *Hs*mtSSB cannot replace the corresponding binding domain of *Eco*SSB.

Small amounts of *Eco*SSB Q152* and *Eco*SSB G117*, however, could complement the N-terminal mutation G15D. A likely mechanism of this complementation is the formation of mixed tetramers containing intact N-terminal domains from the mutants *Eco*SSB Q152* or *Eco*SSB G117* respectively as well as the correct C-terminal region from the *Eco*SSB G15D. Thus

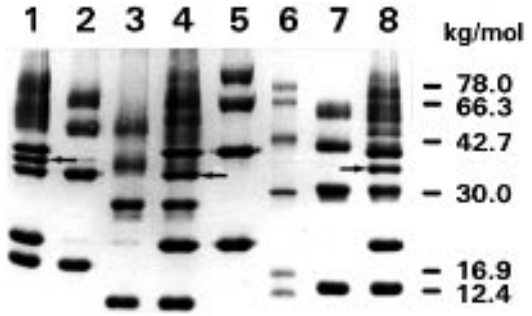

Figure 5. Subunit exchange between wild-type and mutant *Eco*SSB proteins detected by chemical crosslinking. Lane 1: *Eco*SSB Q152* and wild-type *Eco*SSB, lane 2: *Eco*SSB Q152*, lane 3: *Eco*SSB G117*, lane 4: *Eco*SSB G117* and wild-type *Eco*SSB, lane 5: wild-type *Eco*SSB, lane 6: molecular mass standard, lane 7: *Eco*SSB Δ116–167, lane 8: *Eco*SSB Δ116–167 and wild-type *Eco*SSB. Bands corresponding to heterodimers are indicated by arrows.

one could envisage *Eco*SSB G15D as complementing the otherwise lethal mutations *Eco*SSB Q152* and *Eco*SSB G117* . In this mechanism, high concentrations of *Eco*SSB Q152* and *Eco*SSB G117* dilute intact C-terminal regions to such an extent that correct *Eco*SSB function is suppressed (cf. Table 1).

Table 1. Complementation of *ssb* defective strains

Protein	Complementation			
	ssb-3		Δ*ssb*	
	monocopy	high copy	monocopy	high copy
*Eco*SSB-wt	+	+	+	+
*Eco*SSB Q152*	+	–	–	–
*Eco*SSB G117*	+	–	–	–
*Eco*SSB Δ116–167	+	+	+	+
*Hs*mtSSB	n.d.	–	n.d.	–
*HsEc*SSB	n.d.	–	n.d.	–

Subunit exchange

Formation of mixed tetramers can be demonstrated *in vitro* by chemical crosslinking of the subunits with dimethylsuberimidate and analyzing the products on a denaturing PAGE. Figure 5 (lanes 2, 3, 5 and 7) and Figure 6 (lanes 1, 3 and 6) show that the chemical crosslinking of homotetramers leads to the appearence of covalently connected dimers, trimers and tetramers in addition to the monomeric band. This supports the result from analytical ultracentrifugation that all proteins of this study form stable homotetramers (v.s.). Thus the C-terminal third of *Eco*SSB is not required for tetramer formation.

Formation of heterotetramers in this experiment can best be demonstrated by the appearence of an additional covalently linked heterodimer. If two different SSB proteins form tetramers in all possible combinations then chemical crosslinking leads to four trimeric and five tetrameric species. These products cannot be resolved on the gel. While all C-terminally modified *Eco*SSB-proteins are able to exchange subunits with wild-type *Eco*SSB (Fig. 5, lanes 1, 4 and 8) the human mitochondrial SSB

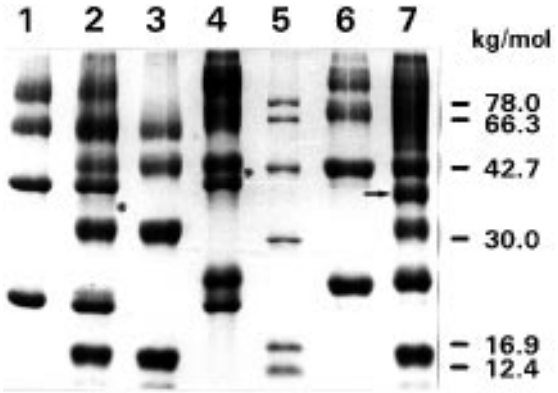

Figure 6. Subunit exchange between *Eco*SSB, *Hs*mtSSB and *HsEc*SSB detected by chemical crosslinking. Lane 1: wild-type *Eco*SSB, lane 2: *Hs*mtSSB and wild-type *Eco*SSB, lane 3: *Hs*mtSSB, lane 4: *HsEc*SSB and wild-type *Eco*SSB, lane 5: molecular mass standard, lane 6: *HsEc*SSB, lane 7: *HsEc*SSB and *Hs*mtSSB. The band corresponding to a heterodimer is indicated by an arrow. Lack of heterodimer formation in lanes 2 and 4 is indicated by an asterisk.

protein cannot form mixed tetramers with *Eco*SSB (Fig. 6, lanes 2 and 4). However, fusion of the *Eco*SSB C-terminal region to *Hs*mtSSB does not impair heterotetramer formation between *Hs*mtSSB and *HsEc*SSB (Fig. 6, lane 7). All these results show that subunit interaction is restricted to the N-terminal DNA binding domain. However, the N-terminal domains of procaryotic and eucaryotic mitochondrial SSB proteins are too different to allow subunit interaction.

CONCLUSIONS

The C-terminal third of *Eco*SSB is neither essential for the binding of nucleic acids nor for tetramer formation. The negative charges of the last 10 amino acids weaken the binding of the protein to nucleic acids. The electrostatic repulsion depends on the distance of these charges from the N-terminal binding domain in the amino acid sequence.

However, the last 10 amino acids of the C-terminal third are essential for *in vivo* function. Mutant SSB proteins missing this acidic region are not functional in the *E.coli* cell. The vital role of these last 10 amino acids must be some function different from DNA binding, probably in protein–protein interactions. The sequence between the DNA binding domain and the last 10 amino acids serves only as a spacer keeping the negative charges away from the bound DNA.

DNA binding and tetramer formation are both localized in the N-terminal domain. Structural differences in this domain between eucaryotic mitochondrial and procaryotic SSB proteins prohibit interactions of the heterologous subunits. The phenotypical effect of mutations localized in different regions of *Eco*SSB can be overcome by the formation of mixed tetramers.

Thus a number of defined functional properties of these SSB proteins can be assigned to different structural regions.

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