Cloning and sequencing of the *Proteus mirabilis* gene for a single-stranded DNA-binding protein (SSB) and complementation of *Escherichia coli* *ssb* point and deletion mutations

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The gene of *Proteus mirabilis* coding for a single-stranded DNA-binding protein (SSB) was cloned in *Escherichia coli* from a genomic library. It restored the UV resistance and the rate of cell division of an *E. coli* *ssb*-113 mutant to the same extent as the cloned *E. coli* *ssb*⁺ gene did. An *E. coli* mutant with deleted *ssb* was viable with the *P. mirabilis* *ssb*⁺ gene provided on a single-copy-number plasmid and had the same cell division rate as with the *E. coli* *ssb*⁺ gene on the same vector plasmid. The recovery from UV damage of an excision repair deficient (*uvrA*) mutant deleted for the *ssb* gene was identical with the *ssb*⁺ gene from *P. mirabilis* or *E. coli*, suggesting full substitution in recombinational DNA repair of the homologous by the heterologous SSB protein. The nucleotide sequence of the gene revealed that the SSB has 81% amino acid sequence homology with the *E. coli* SSB and only 58–63% with various plasmid SSBs. The data provide evidence that the bacterial chromosomally coded SSBs and the plasmid encoded SSBs constitute separate groups.

Keywords: *Proteus mirabilis*, single-stranded DNA-binding protein (SSB), *ssb* gene, *uvrA* mutant, UV resistance

INTRODUCTION

The gene *ssb* of *Escherichia coli* codes for a single-stranded DNA-binding protein (SSB; Sigal *et al.*, 1972) which is involved in the replication, repair and recombination of DNA (Glassberg *et al.*, 1979; Meyer *et al.*, 1979; for a recent review, see Meyer & Laine, 1990). The protein has an M_r of 18800 and forms a homotetramer in solutions with physiological salt concentrations. The *ssb* gene is essential for cell viability (Meyer *et al.*, 1979). Three different point mutations (*ssb*-1, *ssb*-113, *ssb*-3) leave cells viable, but, to different degrees, impair their recovery from UV irradiation, reduce their recombination proficiency and extend their generation time. A deletion of the gene is lethal to the cell (Porter *et al.*, 1990).

Genes coding for similar proteins which have a partial amino acid sequence homology with the *E. coli* SSB have

been identified in several prokaryotic transmissible plasmids including F factor (Chase *et al.*, 1983), plasmid colIB-P9 (Howland *et al.*, 1989) and plasmids R64, pIP231a and pIP71a (Ruvolo *et al.*, 1991). The biological role of these plasmid SSBs is not known. Although they are derepressed during conjugational plasmid transfer, they are not needed for the transfer (Golub & Low, 1986). Some of the plasmid SSBs have been shown to partially complement the defects of *E. coli* *ssb*-1 mutants (Golub & Low, 1985, 1986; Howland *et al.*, 1989). An *E. coli* *ssb* deletion mutant was viable with the F factor *ssb*⁺ gene on a plasmid, but the growth rate and viability were lower than with the *E. coli* *ssb*⁺ gene (Porter & Black, 1991). Generally, the complementation by the plasmid SSBs was only partial.

Here we report the cloning and sequencing of the *ssb* gene from *Proteus mirabilis* as well as various complementation studies with different *ssb* mutants of *E. coli*. Among the *Enterobacteriaceae* *P. mirabilis* is most distantly related to *E. coli* (Ochman & Wilson, 1987).

The EMBL accession number of the sequence reported in this paper is X65079.

Table 1. Strains and plasmids used in this study

Bacterial strain	Relevant genotype	Source or reference
E. coli		
XL1 Blue	<i>recA1 lac</i> [F' <i>proAB lacI</i> ^q ΔM15, Tn10]	Stratagene
PAM5779	<i>ssb-113</i>	Johnson (1977)
RDP268	Δ(<i>ssb-uvrA</i>)	Porter <i>et al.</i> (1990)
JV13	<i>ssb</i> ⁺ <i>zjb1::Tn10</i> -transductant of PAM5779	de Vries & Wackernagel (1993)
P. mirabilis		
PG1300	<i>ssb</i> ⁺	Eitner <i>et al.</i> (1981)
Plasmids		
pRE432	Mini-F derived single-copy-number cosmid vector	de Vries & Wackernagel (1992)
pBluescript II KS+	High-copy-number vector	Stratagene
pJA40	<i>E. coli ssb</i> ⁺ gene in pUC9	Brandsma <i>et al.</i> (1985)
pACYC <i>ssb</i>	<i>E. coli ssb</i> ⁺ gene in pACYC184	Porter <i>et al.</i> (1990)
pSBL2	<i>P. mirabilis ssb</i> ⁺ gene in pRE432	This work
pSBH2, pSBH2e	<i>P. mirabilis ssb</i> ⁺ gene in either orientation in pBluescript II KS+	This work
pSBL5	<i>E. coli ssb</i> ⁺ gene in pRE432	This work
pSBH5	<i>E. coli ssb</i> ⁺ gene in pBluescript II KS+	This work

METHODS

Strains and media. The strains and plasmids used in this study are listed in Table 1. Bacteria were grown in LB medium and plated on LB agar (Miller, 1972). For the selection of resistant clones the media contained ampicillin (50 µg ml⁻¹) or chloramphenicol (20 µg ml⁻¹).

Determination of UV sensitivity and generation time. The UV sensitivity of cells was determined as described previously (de Vries & Wackernagel, 1993). For the determination of generation time exponentially growing cultures were diluted to a titre of about 1 × 10⁵ ml⁻¹ into prewarmed LB and further aerated at 37 °C. Samples were taken 20 min after dilution and when a titre of 1 × 10⁸ ml⁻¹ was reached. The titres of the samples were determined by plating of appropriate dilutions onto LB agar.

DNA manipulations, hybridization and sequencing. Plasmid DNA was isolated as described by Birnboim & Doly (1979) or by the use of Qiagen columns (Diagen). For the cloning of DNA fragments *E. coli* XL1-Blue was used. Transformation of cells with plasmid DNA was carried out with a Bio-Rad Gene Pulser according to Dower *et al.* (1988). For the detection of sequences with homology to the *E. coli ssb* gene, the target DNAs were digested with two restriction enzymes and loaded on a 0.7% agarose gel. After electrophoresis and electroblotting onto a nylon membrane (NY 12 N; Schleicher and Schuell), the DNA was hybridized with the 0.7 kb insert fragment of pJA40, which contains the *E. coli ssb* gene (Brandsma *et al.*, 1985). The probe had been labelled with a Boehringer digoxigenin DNA-labelling and detection kit. For the determination of the nucleotide sequence according to Sanger *et al.* (1977) the Sequenase version 2.0 kit from US Biochemical and unidirectional

deletion derivatives produced with the ExoIII-mung bean nuclease deletion kit from Stratagene were used. [³⁵S]dATP was obtained from Amersham. Both strands of the fragment were sequenced independently.

Screening for *ssb*⁺ transformants. Cells of *E. coli* PAM5779 (*ssb-113*) were transformed with plasmids of a genomic library of *Proteus mirabilis* PG1300 with the vector plasmid pBR322 (Weichenhan & Wackernagel, 1988). The ampicillin-resistant colonies obtained were replica plated on LB agar containing mitomycin C (0.9 µg ml⁻¹; Sigma) and incubated at 37 °C for 36 h. At this temperature, *ssb*⁺ transformants were able to form colonies, while the *ssb-113* cells formed no or only very tiny colonies.

Isolation of SSB protein. LB medium (0.5 l) was inoculated with 0.5 ml of an overnight culture of RDP268 pSBH2e and aerated at 37 °C for 5 h (OD₆₀₀ = 1.4). Protein purification was performed according to an unpublished protocol of Claude Backendorf (University of Leiden, Netherlands). The harvested cells (2.5 g wet weight) were resuspended in 40 ml of buffer A (50 mM Tris/HCl, pH 7.5, 100 mM KCl, 1 mM EDTA, 10 mM β-mercaptoethanol, 25% v/v, glycerol), frozen in liquid nitrogen and stored at -80 °C. The cells were thawed at 4 °C and disrupted by sonification with a Branson sonifier (microtip) for 15 min on ice. After centrifugation for 15 min at 20000 g, the supernatant was applied to an ssDNA cellulose column (Alberts & Herrick, 1971) of 22 ml volume. After washing with 1 column vol. of buffer A and 2 vols of buffer A plus 0.5 M KCl, the protein was eluted with a gradient of 0.5 to 2.0 M KCl in buffer A. The SSB eluted at 0.71 M KCl. The combined peak fractions were dialysed against buffer B (buffer A containing 50% v/v, glycerol) and then 0.15 g (NH₄)₂SO₄ were added per ml (27% saturation at 0 °C). The precipitate obtained after centrifugation for 20 min at 10000 g was dissolved in 2 ml

buffer B with 0.5 M KCl, dialysed against the same buffer and stored at -20°C .

RESULTS AND DISCUSSION

Cloning of the *ssb* gene from a genomic library of *Proteus mirabilis*

E. coli PAM5779 (*ssb*-113; Table 1) was transformed with plasmids representing a genomic library of *P. mirabilis* PG1300 in plasmid vector pBR322 (Weichenhan & Wackernagel, 1988). Among the transformants mitomycin-C-resistant clones were identified (see Methods). From these, two plasmids with inserts of different size were isolated (pSB10, pSB20). By Southern hybridization with a probe made from the *E. coli ssb* gene (insert of pJA40; Table 1) it was shown that they carried a gene on a 1.6 kb *Cla*I-*Bgl*II fragment with homology to the probe. The result obtained with pSB20 is shown in Fig. 1. From a *Cla*I-*Bgl*II digest of the chromosomal *P. mirabilis* DNA a fragment of identical size hybridized to the *ssb* probe, confirming that the cloned gene derived from the *P. mirabilis* genome (Fig. 1).

A 1.45 kb *Kpn*I-*Bgl*II fragment containing the entire *ssb* gene was cloned into the mini-F-derived single-copy-number vector pRE432 and into the high-copy-number

plasmid pBluescript II KS+ (Table 1) producing the plasmids pSBL2 and pSBH2, respectively.

Nucleotide sequence of the *P. mirabilis ssb* gene

Both strands of a 1511 nucleotide fragment covering the *ssb* gene (the insert of pSBL2 plus 54 nucleotides beyond the *Kpn*I site) were sequenced. The nucleotide and the amino acid sequences are shown in Fig. 2. An open reading frame encoding a polypeptide of 174 amino acids was found from position 607 to 1131. The protein has 81% amino acid sequence homology to the *E. coli* SSB protein. A part (119 amino acids) of a second open reading frame, which is transcribed in a direction opposite to *ssb*, was found at position 354 to 1. Within this part the amino acid sequence is 99% homologous to the N-terminus of the *E. coli* UvrA protein (Husain *et al.*, 1986).

Promoters for *ssb* and *uvrA* are located in the region of 252 nucleotides between the genes (Fig. 2). *ssb* is preceded by three regions that fit with the consensus sequence of promoters recognized by the *E. coli* RNA polymerase (Rosenberg & Court, 1979). As in *E. coli* (Brandsma *et al.*, 1983), the promoter distal to *ssb* (*ssb* P3) overlaps with the SOS box of the *uvrA* gene. The -35 region of *ssb* P2 is part of a region that could constitute an SOS box. This presumed second SOS box deviates in one of the seven highly conserved positions from the *E. coli* consensus SOS box (Walker, 1984), and it is one nucleotide shorter. At a distance of 50 nucleotides downstream of *ssb* a presumptive terminator is present which consists of inverted repeats of 11 nucleotides (10 matching) separated by 6 nucleotides.

Isolation of *P. mirabilis* SSB protein

The *P. mirabilis* SSB protein was purified by its ability to tightly bind to single-stranded DNA (see Methods). Cells of the *ssb* deletion mutant *E. coli* RDP268 (Porter *et al.*, 1990) carrying pSBH2e (Table 1) were employed for purification in order to avoid co-purification of the *E. coli* SSB protein. Strain RDP268 is identical with AB1157 (Howard-Flanders & Theriot, 1966) except that a 2.2 kb *Kpn*I fragment carrying the *ssb* gene and part of the *uvrA* gene has been replaced by a kanamycin resistance cassette. Since SSB is an essential protein, the cells are only viable when carrying a helper plasmid with an *ssb* gene. The helper plasmid pACYC*ssb* (Table 1), providing chloramphenicol resistance in RDP268, was replaced by pSBH2e (ampicillin resistance) through transformation. The segregants which had lost pACYC*ssb* were identified by their sensitivity to chloramphenicol. The replacement was confirmed by analysis of plasmid DNA isolated from the cells. The plasmid pSBH2e is identical to pSBH2, except that the insert is inverted so that expression is in the direction of the *lac* promoter of the pBluescript vector. About 2 mg of SSB protein were obtained from the cells of a 500 ml culture grown to an OD_{600} of 1.4. The protein was > 99% pure as determined by SDS-PAGE and staining with silver stain (Bio-Rad). The *P. mirabilis* SSB migrated somewhat slower than the *E. coli* SSB and was

Fig. 1. Hybridization of *P. mirabilis ssb* with an *E. coli ssb* gene probe. About 50 ng of plasmid pSB25, which contains the *P. mirabilis ssb* on a 1.6 kb *Cla*I-*Bgl*II fragment of pSB20 (Table 1), and about 300 ng of chromosomal DNA of *P. mirabilis* PG1300 (Table 1) were digested with *Cla*I and *Bgl*II and electrophoresed in lanes 1 and 2, respectively. Blotting and detection of the *ssb*-containing fragments were done as described in Methods. The positions of 5.3 kb and 1.6 kb markers (not shown) are indicated. The difference of the band intensity in the two lanes results from the approx. 150-fold higher *ssb* copy number present in lane 1 compared to lane 2.

Fig. 2. Nucleotide sequence of a DNA fragment covering the *ssb* gene from *P. mirabilis*. The direction of the nucleotide sequence is 5' → 3' with respect to the *ssb* gene and 3' → 5' with respect to the *uvrA* gene. The amino acid sequences of the SSB and part of the UvrA protein are shown below the nucleotide sequence. Start and stop codons are doubly underlined. P1, P2 and P3 indicate possible promoter regions of the *ssb* gene. The -10 and -35 regions of promoters and ribosome binding sites (RBS) are underlined. An inverted repeat is indicated by arrows. Regions with homology to the *E. coli* SOS box are framed. The accession number of the nucleotide sequence in the EMBL data library is X65079.

found at a position corresponding to an M_r of about 20600. Assuming that the N-terminal methionine is cleaved during post-translational processing as in *E. coli* (Beyreuther *et al.*, 1982) the *P. mirabilis* SSB consists of 173 amino acids and has an M_r of 18699. SSB from *P. mirabilis* reacted with polyclonal antibodies raised against the *E. coli* SSB protein (data not shown). This indicates that both proteins are similar in structure, and that epitopes recognized on *E. coli* SSB are also present on *P. mirabilis* SSB.

Complementation of *E. coli* *ssb* mutants

The UV sensitivity of the *ssb-113* mutant (PAM5779) transformed with the single-copy-number plasmid pSBL2

or the high-copy-number plasmid pSBH2 (Table 1) is shown in Fig. 3(a). The UV resistance provided by pSBL2 is almost indistinguishable from that conferred by pSBL5, which carries the *E. coli* *ssb*⁺ gene on a single-copy-number vector. Both plasmids restored the UV resistance of the *ssb-113* mutant to near wild-type level (JV13 pRE432; Table 1), indicating that the presence of the *ssb-113* gene product has only a minor effect in cells in which either of the wild-type proteins is present.

Expression of the *P. mirabilis* *ssb*⁺ gene from the high-copy-number plasmid pSBH2 resulted in a somewhat lower UV resistance (Fig. 3a). This was also observed when the *E. coli* *ssb*⁺ gene was expressed from a high-copy-number plasmid (pSBH5). The reason is possibly an

Fig. 3. UV sensitivity of (a) *E. coli* *ssb*-113 (PAM5779) and (b) *E. coli* Δ *uvrA*-*ssb* (RDP268) transformed by plasmids containing the *ssb*⁺ genes from *P. mirabilis* or *E. coli*. The *ssb*⁺ genes were located either on the single-copy-number vector pRE432 or on the high-copy-number vector pBluescript II KS+. The strains were transformed with: ■, the vector pRE432; ●, pSBL2 carrying *ssb*⁺ of *P. mirabilis* in pRE432; ○, pSBL5 carrying *ssb*⁺ of *E. coli* in pRE432; ▼, pSBH2 carrying *ssb*⁺ of *P. mirabilis* in pBluescript II KS+; ▽, pSBH5 carrying *ssb*⁺ of *E. coli* in pBluescript II KS+. Strain JV13 pRE432 (□) is an *ssb*⁺ derivative of PAM5779 constructed by P1 transduction. The curves are means from three experiments. The error bars indicate standard deviations.

impediment of post-replication repair by excess of SSB (Moreau, 1987).

In Fig. 3(b) the UV resistance of the *uvrA* Δ *ssb* mutant RDP268 with pSBL2 or pSBL5 is compared. No difference is apparent in the degree of complementation by either of the plasmids. In RDP268, the recovery from UV damage requires recombinational repair, since excision repair is absent due to the lack of the UvrA protein. Thus, the result shown in Fig. 3(b) indicates that the recombinational DNA repair which depends on SSB (Lieberman &

Witkin, 1983; Whittier & Chase, 1983) is promoted by both SSB proteins with equal efficiency.

Induction of the SOS response and related functions such as prophage induction, Weigle reactivation and induced mutagenesis are greatly impaired in the *ssb*-113 mutant (Vales *et al.*, 1980). The hampered SOS induction is, beneath the impairment of the repair process, responsible for the high UV sensitivity of *ssb*-113 mutants. Although we have not directly measured SOS induction of specific genes in the presence of *P. mirabilis* SSB, the wild-type degree of UV resistance conferred by pSBL2 to *ssb*-113 and Δ *ssb* mutants clearly indicates that the *P. mirabilis* SSB protein restores the ability for SOS induction with high efficiency.

The generation times at 37 °C of *E. coli* *ssb* mutants with the *ssb*⁺ gene of *P. mirabilis* or *E. coli* on single-copy-number plasmids were determined. The means from three independent experiments and the standard deviations are given below. PAM5779 (*ssb*-113) with the vector pRE432 grows relatively slowly, having a generation time of 37.5 ± 1.0 min. Both, pSBL2 and pSBL5 reduce the generation time to about 32 min (pSBL2: 32.5 ± 0.4 min; pSBL5: 32.5 ± 0.5 min), which is very similar to the generation time of the *ssb*⁺ strain JV13pRE432 (30.9 ± 0.2 min). The presence of the *ssb*-113 gene product probably prevents maximum replication rates by being incorporated into SSB tetramers. This is not the case in the *ssb*-deletion mutant RDP268, where the SSBs of *E. coli* and *P. mirabilis* promote the same high cell division rate (pSBL2: 20.5 ± 0.5 min; pSBL5: 20.9 ± 0.6 min). Thus, the *P. mirabilis* SSB protein apparently supports replication by the *E. coli* DNA polymerases with the same efficiency as the *E. coli* wild-type SSB protein.

In vitro studies on T4 and *E. coli* replication enzymes had revealed that SSB proteins preferentially stimulate their cognate DNA polymerase (for a review, see Williams & Konigsberg, 1981). Our results show that this enhancement of replication is far from being strictly species specific. Since *E. coli* and *P. mirabilis* are the most distantly related members of the *Enterobacteriaceae*, the results suggest that *ssb* genes are highly conserved throughout this family. The difference to the *E. coli* SSB found at the amino acid sequence level does not measurably affect the functioning of the *P. mirabilis* SSB protein in *E. coli*.

Comparison of bacterial and plasmid-encoded *ssb* genes

The nucleotide sequences of *ssb* genes of several transmissible plasmids have been determined previously, including the F sex factor (Chase *et al.*, 1983), colIb-P9 (Howland *et al.*, 1989), R64, pIP231a and pIP71a (Ruvolo *et al.*, 1991). The *ssb* genes of the plasmids constitute a group whose members share high homology, probably have a common origin (Ruvolo *et al.*, 1991), and differ from the chromosomally encoded SSB of *E. coli*. In Table 2 the amino acid sequence homologies between the three sequenced bacterial SSBs (de Vries & Wackernagel, 1993; Sancar *et al.*, 1981; this paper) and the five plasmid SSBs

Table 2. Amino acid sequence homology of plasmid-encoded and bacterial SSB proteins

Homology is given as the percentage of identical amino acid residues in maximum homology alignments of each pair of proteins.

	Amino acid sequence homology						
	<i>Escherichia coli</i>	<i>Serratia marcescens</i>	<i>Proteus mirabilis</i>	F-factor	pIP231a	pIP71a	colIb-P9
<i>Escherichia coli</i>	100	89	81	60	60	62	61
<i>Serratia marcescens</i>		100	82	58	58	62	58
<i>Proteus mirabilis</i>			100	61	58	63	61
F-factor				100	95	89	85
pIP231a					100	88	83
pIP71a						100	80
R64							99
colIb-P9							100

are compared. It is evident that the enterobacterial SSBs constitute a group with more than 80% sequence homology, which is separate from the group of SSBs from plasmids replicating in the members of *Enterobacteriaceae*, to which homology is only 58–63%.

Plasmid SSBs can partially complement the temperature-sensitive replication and UV-sensitive phenotype of *ssb-1* mutants of *E. coli* (Kolodkin *et al.*, 1983; Golub & Low, 1986; Howland *et al.*, 1989). It has been shown that the F-SSB protein can also support replication in the absence of any *E. coli* SSB, but the generation time is about 28% longer than with the *E. coli* SSB protein and the cells tend to filament (Porter & Black, 1991). It is reasonable to assume that the complementation relies on properties of SSB proteins provided by the homologous stretches of amino acids. With respect to the sequence difference between *E. coli* SSB and F-factor SSB it has been speculated that the plasmid SSBs may have other or additional functions compared to the bacterial SSBs, e.g. functions which are required during conjugational replication or plasmid transfer (Chase *et al.*, 1983), but experimental evidence for this hypothesis was not found (Golub & Low, 1986). Further investigations are required to describe in more detail the intracellular roles of bacterial and plasmid SSBs and to correlate their functions with amino acid stretches in the proteins.

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