Differential Single-stranded DNA Binding Properties of the Paralogous SsbA and SsbB Proteins from Streptococcus pneumoniae*

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The naturally transformable Gram-positive bacterium Streptococcus pneumoniae has two single-stranded DNA-binding (SSB) proteins, designated SsbA and SsbB. The SsbA protein is similar in size to the well characterized SSB protein from Escherichia coli (SsbEc). The SsbB protein, in contrast, is a smaller protein that is specifically induced during natural transformation and has no counterpart in E. coli. In this report, the single-stranded DNA (ssDNA) binding properties of the SsbA and SsbB proteins were examined and compared with those of the SsbEc protein. The ssDNA binding characteristics of the SsbA protein were similar to those of the SsbEc protein in every ssDNA binding assay used in this study. The SsbB protein differed from the SsbA and SsbEc proteins, however, both in its binding to short homopolymeric dTₙ oligomers (as judged by polyacrylamide gel-shift assays) and in its binding to the longer naturally occurring φX and M13 ssDNAs (as judged by agarose gel-shift assays and electron microscopic analysis). The results indicate that an individual SsbB protein binds to ssDNA with an affinity that is similar or higher than that of the SsbA and SsbEc proteins. However, the manner in which multiple SsbB proteins assemble onto a ssDNA molecule differs from that observed with the SsbA and SsbEc proteins. These results represent the first analysis of paralogous SSB proteins from any bacterial species and provide a foundation for further investigations into the biological roles of these proteins.

Streptococcus pneumoniae is a naturally transformable Gram-positive bacterium that is able to take up DNA from its environment and incorporate this exogenous DNA into its chromosome (1). This process, known as transformational recombination, serves as a general mutational mechanism that allows S. pneumoniae to change its genetic composition in response to environmental changes and stresses (1). The transformational recombination reaction involves the assimilation of a single-stranded form of the exogenous DNA into a homologous region of the double-stranded S. pneumoniae chromosome (1). An inspection of the genome sequence reveals that S. pneumoniae has two single-stranded DNA-binding (SSB) proteins, designated SsbA and SsbB (see Fig. 1A). The SsbA protein (17,350 amino acids) is similar in size and sequence to the extensively studied SSB protein from Escherichia coli (18,874 Da, 178 amino acids), a non-sequence-specific ssDNA-binding protein that is involved in many aspects of DNA metabolism in E. coli (2). The SsbB protein (14,926 Da, 131 amino acids), in contrast, is a smaller protein that is specifically induced during natural transformation in S. pneumoniae (3). These results suggest that the SsbA protein may be a general SSB protein involved in routine DNA functions (analogous to the E. coli SSB protein), and that the SsbB protein may be a specialized SSB protein used primarily during transformational recombination. This idea is consistent with a recent analysis of the genome sequences of 69 different bacterial species, which revealed that those naturally transformable Gram-positive bacteria that are closely related to S. pneumoniae (e.g. Bacillus subtilis) contain two ssb-like genes, whereas the non-naturally transformable Gram-negative bacteria related to E. coli (e.g. Salmonella typhimurium) contain only a single ssb gene (4).

We have recently amplified the ssbA and ssbB genes from S. pneumoniae genomic DNA, developed efficient expression systems, and purified the S. pneumoniae SsbA and SsbB proteins to near homogeneity (Fig. 1B). In this report, the ssDNA binding properties of the S. pneumoniae SsbA and SsbB proteins are examined and compared with those of the E. coli SSB protein. The results represent the first analysis of paralogous SSB proteins from any bacterial species.

EXPERIMENTAL PROCEDURES

Materials—S. pneumoniae SsbA protein was prepared as described (5). The S. pneumoniae SsbB protein was prepared by a modification of the method used to prepare the S. pneumoniae SSB protein.6 E. coli SSB protein was from Promega. dTₙ oligomers were from Invitrogen. 3²P-labeled dTₙ oligomers were prepared using ty²²P]ATP and polynucleotide kinase (Amersham Biosciences). Circular φX ssDNA(+) strand was from New England Biolabs. GelStar® nucleic acid gel stain was from Cambrex. G250 BioSafe Coomassie protein gel stain was from Bio-Rad. dX ssDNA concentrations were determined by absorbance at 260 nm using the conversion factor 36 μg ml⁻¹ A₆₀₀⁻¹. dTₙ concentrations were determined by absorbance at 260 nm using the extinction coefficient 8.4 mM⁻¹ cm⁻¹ (2). All ssDNA concentrations are expressed as total nucleotides.

**Gel-exclusion Chromatography—Gel-exclusion chromatography analysis of the SSB proteins was carried out using a Superose 12-gel-exclusion column (24 ml, Amersham Biosciences) with 20 mM Tris-Cl**

1 The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; dX, bacteriophage φX174.

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The gel was stained with 0.1% Coomassie Brilliant Blue R-250. Protein, and molecular mass standards (M), as indicated. The acrylamide concentration was 5% in the stacking gel and 13% in the separating gel. SsbA and SsbB protein. The gel lanes contain purified S. pneumoniae SsbA and SsbB protein. The gel was stained with 0.1% Coomassie Brilliant Blue R-250.

Electron Microscopy—The SsaB and SsbB proteins were incubated with dX ssDNA in a buffer containing 20 mM Hepes (pH 7.6)/50 mM NaCl, at a protein to DNA ratio (μg/μg) of 20:1 in a total volume of 40 μl, for 3 min at 37 °C. The samples were stored away from light and examined in a Tecnai G2 TEM (FEI). The images of the SsaB-dX ssDNA and SsbB-dX ssDNA complexes were analyzed by gel exclusion chromatography. In these experiments, a fixed concentration of dT25 was incubated with various concentrations of SSB protein, and the resulting complexes were visualized by gel exclusion chromatography. The binding of SSB proteins to dTn oligomers—The ssDNA binding activities of the SsaB and SsbB proteins were compared with those of the SsaEc protein using a series of dTn oligomers ranging in size from n = 35–100. In these experiments, a fixed concentration of dT25 was incubated with various concentrations of SSB protein, and the resulting complexes were visualized by gel exclusion chromatography.

RESULTS

Oligomerization State of the SsaB and SsbB Proteins—The E. coli SSB protein (SsaEc) forms a stable tetramer in solution, and it is the tetrameric form of the protein that binds to ssDNA (2). Therefore, before examining the ssDNA binding properties, the oligomerization states of the S. pneumoniae SsaB and SsbB proteins were determined by gel exclusion chromatography. In the gel exclusion analysis, the SsaEc protein eluted as a single peak at a position corresponding to a tetramer with an apparent molecular mass of ~72,400 Da (chromatogram not shown). This is in agreement with the calculated value of 75,496 Da for the SsaEc tetramer (2). Under the same conditions, the SsaB and SsbB proteins also eluted as single peaks at positions corresponding to proteins with apparent molecular masses of ~63,300 and 54,100 Da, respectively (chromatograms not shown). These values are close to the calculated values of 69,400 and 59,704 Da for the SsaB and SsbB tetramers. These results indicate that the SsaB and SsbB proteins also form stable tetramers in solution, and it will therefore be presumed in this discussion below that it is the tetrameric form of these proteins that binds to ssDNA as well.

Binding of SSB Proteins to dTn Oligomers—The ssDNA binding activities of the SsaB and SsbB proteins were compared with those of the SsaEc protein using a series of dTn oligomers ranging in size from n = 35–100. In these experiments, a fixed concentration of dT25 was incubated with various concentrations of SSB protein, and the resulting complexes were visualized by gel exclusion chromatography.

Electron Microscopy—The SsaB and SsbB proteins were incubated with dX ssDNA in a buffer containing 20 mM Hepes (pH 7.6)/50 mM NaCl, at a protein to DNA ratio (μg/μg) of 20:1 in a total volume of 40 μl, for 15 min at 37 °C. The samples were stored away from light and examined in a Tecnai G2 TEM (FEI). The images of the SsaB-dX ssDNA and SsbB-dX ssDNA complexes were analyzed by gel exclusion chromatography.

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more, a similar distribution of the SsbA-dT50 and SsbB-dT50 was more favorable under these reaction conditions. Further-

reaction solutions contained 5.3 mM, or 0.3 mM SsbA protein added simultaneously (lane 5), 0.3 mM SsbB protein added at 0 min followed by 0.3 mM SsbA protein at 15 min (lane 7), 0.3 mM SsbEc protein and 0.3 mM SsbB protein added simultaneously (lane 8), 0.3 mM SsbEc protein added at 0 min followed by 0.3 mM SsbB protein at 15 min (lane 9), 0.3 mM SsbB protein added at 0 min followed by 0.3 μM SsbEc protein at 15 min (lane 10), 0.3 mM SsbA protein and 0.3 mM SsbB protein added simultaneously (lane 11), 0.3 mM SsbA protein added at 0 min followed by 0.3 mM SsbB protein at 15 min (lane 12), or 0.3 mM SsbB protein added at 0 min followed by 0.3 μM SsbA protein at 15 min (lane 13). After a total incubation period of 30 min at 37 °C, the reactions were analyzed by polyacrylamide gel electrophoresis. The bands corresponding to unbound dT50 (ssDNA) and the various SSB-dT50 complexes were visualized by autoradiography.

To further evaluate the binding of the SSB proteins to dT75, an additional set of experiments was carried out in which the polyacrylamide gels were treated with Coomassie stain to indicate the relative amounts of SSB protein present in various complexes. As shown in Fig. 5, the staining intensities of the bands that were formed by the SsbEc and SsbA proteins were consistent with there being approximately twice as much protein in the second complex (complex II) as in the first complex (complex I). Moreover, SsbEc or SsbA protein concentrations above that necessary to convert all of the dT75 into the second complex led to the appearance of a new staining band, which corresponded to excess unbound protein (Fig. 5). By comparison, concentrations of SsbB protein above that necessary to convert all of the dT75 into the second complex did not result in the formation of any additional complexes (Fig. 4). These results indicated that only a single tetramer of the SsbB protein was able to bind to dT75 at the highest protein concentrations. This conclusion is consistent with recent biophysical studies, which showed that the SsbB protein forms a complex with one SsbEc tetramer bound to dT75 at lower binding densities and a complex with two SsbEc tetramers bound to dT70 at higher binding densities (7). By comparison, when increasing concentrations of SsbB protein were added to dT75, an initial complex was formed at the same protein concentration that was observed for the formation of the first complex with the SsbEc and SsbA proteins (Fig. 4). In contrast to the results with the SsbEc and SsbA proteins, however, increasing the SsbB protein concentration further did not result in the formation of any additional complexes (Fig. 4). These results indicated that only a single tetramer of the SsbB protein was able to bind to dT75, even at the highest protein concentrations examined. Similar results were obtained for all three SSB proteins with the shorter oligomer, dT65 (data not shown).

To further evaluate the binding of the SSB proteins to dT75, an additional set of experiments was carried out in which the polyacrylamide gels were treated with Coomassie stain to indicate the relative amounts of SSB protein present in various complexes. As shown in Fig. 5, the staining intensities of the bands that were formed by the SsbEc and SsbA proteins were consistent with there being approximately twice as much protein in the second complex (complex II) as in the first complex (complex I). Moreover, SsbEc or SsbA protein concentrations above that necessary to convert all of the dT75 into the second complex led to the appearance of a new staining band, which corresponded to excess unbound protein (Fig. 5). By comparison, concentrations of SsbB protein above that necessary to convert all of the dT75 into the second complex did not result in the formation of any additional complexes (Fig. 4). These results indicated that only a single tetramer of the SsbB protein was able to bind to dT75, even at the highest protein concentrations examined. Similar results were obtained for all three SSB proteins with the shorter oligomer, dT65 (data not shown).
Fig. 4. Binding of SSB proteins to dT75. The reaction solutions contained 5.3 μM dT75 (total nucleotide concentration) and the indicated concentrations of SsbEc, SsbA, or SsbB protein (0–1.0 μM tetramer). The reaction solutions were incubated at 37 °C for 15 min and then analyzed by polyacrylamide gel electrophoresis. The bands corresponding to unbound dT75 (ssDNA) and the various SSB-dT75 complexes were visualized by autoradiography.

Fig. 5. Visualization of unbound and dT75-bound SSB proteins. The reaction solutions contained 30 μM dT75 (total nucleotide concentration) and the indicated concentrations of SsbEc, SsbA, or SsbB protein (0–1.5 μM tetramer). The reaction solutions were incubated at 37 °C for 15 min and then analyzed by polyacrylamide gel electrophoresis. The bands corresponding to unbound SSB protein and the various SSB-dT75 complexes were visualized by Coomassie protein staining (the lane designated M corresponds to the SSB proteins in the absence of dT75). Note that the concentrations of dT75 and SSB protein were increased 6-fold relative to those in Fig. 4 so that the unbound and bound SSB proteins would be clearly visible by Coomassie staining.

Further support for the conclusion that two tetramers of either SsbA or SsbEc protein, but only one tetramer of SsbB protein, were able to bind to dT75.

dT100—The binding of the various SSB proteins to dT100 is shown in Fig. 6. The amount of dT100 that was bound to SsbB protein increased with increasing protein concentration until all of the dT100 was incorporated into an SsbB-dT100 complex. In contrast to the results with dT50 and dT75, however, a further increase in the SsbB protein concentration resulted in the disappearance of the initial complex and the appearance of a new complex of even lower mobility (Fig. 6). This result indicated that a second tetramer of SsbB protein was able to bind to dT100 at higher protein concentrations. By comparison, when increasing concentrations of SsbA protein were added to dT100, an initial complex and a second complex were formed at protein concentrations similar to those observed for the formation of the first and second complexes with the SsbB protein. However, a further increase in SsbA protein concentration resulted in the disappearance of the second complex and the appearance of a third complex of even lower mobility (Fig. 6). A similar pattern of binding was observed when SsbEc protein was added to dT100 (Fig. 6). These results suggested that three tetramers of SsbA or SsbEc protein, but only two tetramers of SsbB protein, were able to bind to dT100.

Binding of SSB Proteins to φX ssDNA—The binding of the various SSB proteins to the long naturally occurring circular φX ssDNA (5386 nucleotides) was also examined. In these experiments, a fixed concentration of φX ssDNA was incubated with increasing concentrations of SSB protein, and the resulting complexes were analyzed by agarose gel electrophoresis. When increasing concentrations of SsbEc protein were added to φX ssDNA, there was a progressive decrease in the mobility of the φX ssDNA until a discrete slower moving complex was observed at higher SsbEc protein concentrations (Fig. 7). A similar pattern of binding was observed when SsbA protein was added to φX ssDNA (Fig. 7). These results are similar to agarose gel shift results that have been reported previously for the SsbEc protein (8) and reflect the binding of increasing amounts of SSB protein until the φX ssDNA is saturated with SSB protein. In contrast, when increasing concentrations of SsbB protein were added to φX ssDNA, there was an increase in the mobility of the ssDNA until a discrete faster moving complex was observed at higher SsbB protein concentrations (Fig. 7). In a parallel experiment, φX ssDNA was first incubated with SsbB protein (to form the faster moving complex) and then treated with SDS (to remove the SsbB protein from the ssDNA) before loading onto the agarose gel. In this case, intact circular φX ssDNA was recovered, demonstrating that the formation of the faster moving complex was not because of degradation of the φX ssDNA (data not shown). In addition, when the agarose gels were treated with Coomassie stain, unbound SsbB protein was found to migrate more slowly than unbound SsbA protein (consistent with the calculated pI values of the SsbA protein (pI = 5.2) and SsbB protein (pI = 6.0)). This indicated that the rapid migration of the SsbB-φX ssDNA complexes was not due simply to an increased mobility of the SsbB protein relative to that of the SsbA protein (data not shown). Furthermore, a similar pattern of results was obtained for all three SSB proteins when the agarose gels were run using a Tris acetate buffer system in place of the Tris borate buffer system, showing that the difference in binding patterns was not specific to the gel-running buffer (data not shown). Finally, a similar pattern of results was obtained for all three SSB proteins when circular M13 mp18 ssDNA (7249 nucleotides) was used in place of circular φX ssDNA, demonstrating that the formation of the faster moving complex by the SsbB protein was not specific to φX ssDNA (data not shown). Taken together, these results indicated that the complexes that were formed with φX ssDNA by the SsbB protein were different from those formed by the SsbA and SsbEc proteins.

The complexes that were formed by the SsbA and SsbB proteins...
proteins with dsX ssDNA were also examined by electron microscopy (Fig. 8). The results (obtained with saturating concentrations of the SSB proteins) showed that the Ssha protein formed open circular complexes in which the dsX ssDNA was almost completely covered by extended tracts of Ssha protein. These complexes are similar to those that have been reported previously for the Ssbe protein (9, 10) and are consistent with the similar DNA binding patterns that were obtained for the Ssha and Ssbe proteins in the agarose gel-shift assays (Fig. 7). In contrast, the complexes that were formed by the Ssbe protein were highly condensed in appearance and contained numerous stem-like projections that were not prominent in the Ssha complexes (Fig. 8). Furthermore, an analysis of the electron microscopy images revealed that the projected surface area of the Ssbe-dsX ssDNA complexes was 30% smaller than that of the Ssha-dsX ssDNA complexes, indicating that there was less protein bound in the Ssbe complexes than in the Ssha complexes. The condensed appearance of the Ssbe-dsX ssDNA complexes is presumably related to the anomalous mobilities that were observed for these complexes in the agarose gel-shift assays (Fig. 7).

Because the Ssha and Ssbe proteins appeared to form different types of complexes with dsX ssDNA, an additional set of experiments was carried out to examine the complexes that would be formed if Ssha and Ssbe protein were added together to dsX ssDNA. In these experiments, dsX ssDNA was incubated with a saturating concentration of either Ssha protein or Ssbe protein alone, or with a saturating concentration of each protein simultaneously. As shown in Fig. 8A, the Ssha and Ssbe proteins alone were able to convert all of the dsX ssDNA in the reaction solution to the discrete slower or faster moving complexes, respectively. When dsX ssDNA was incubated with Ssha and Ssbe protein simultaneously, however, a single discrete slower moving complex was observed with a mobility that was identical to that of the complex formed with the Ssha protein alone (Fig. 9A). This single slower moving complex was observed even when the dsX ssDNA was incubated with a saturating concentration of Ssha protein prior to the addition of Ssbe protein, or when the dsX ssDNA was incubated with a saturating concentration of Ssbe protein prior to the addition of Ssha protein (Fig. 9A). These results indicated that either the Ssha protein was binding preferentially over the Ssbe protein to form a complex with the dsX ssDNA that contained only Ssha protein or that the Ssha and Ssbe proteins were binding together to form a mixed Ssha/Ssbe complex with the dsX ssDNA that had a mobility indistinguishable from that of the complex formed with Ssha protein alone.

To distinguish between these possibilities, dsX ssDNA was incubated with a saturating concentration of either Ssha protein or Ssbe protein alone, or with a saturating concentration of each protein simultaneously. The resulting complexes were then isolated by preparative agarose gel electrophoresis and analyzed by SDS-polyacrylamide gel electrophoresis. As shown in Fig. 9B, the slower moving complex that was formed in the presence of a mixture of Ssha and Ssbe protein contained approximately equal amounts of the two proteins. Furthermore, the intensities of the bands corresponding to the Ssha and Ssbe proteins were approximately one-half of the intensities of the Ssha and Ssbe bands that were obtained from the complexes formed with Ssha or Ssbe protein alone (Fig. 9B). These results indicated that although the complex that was formed in the presence of a mixture of Ssha and Ssbe protein had a gel mobility that was indistinguishable from the complex formed with Ssha protein alone, the complex did contain approximately equal amounts of Ssha and Ssbe protein bound to the dsX ssDNA.

**DISCUSSION**

The polyacrylamide gel shift results indicated that the Ssbe, Ssha, and Ssbe proteins each bind to the shorter oligomer, dT100, to form a complex in which a single SSB tetramer is bound to the ssDNA. Furthermore, the competition experiments indicated that the affinity of the Ssbe protein for dT100 is higher than that of either the Ssha or Ssbe protein. However, the gel shift results indicated that two Ssbe or Ssbe tetramers are able to bind to the intermediate length oligomer, dT75, whereas only a single Ssbe tetramer is able to bind to this ssDNA. Similarly, the gel shift results indicated that three tetramers of Ssbe or Ssbe protein are able to bind to the longer oligomer, dT100, but only two Ssbe tetramers are able to bind to this ssDNA. These results indicated that the Ssbe protein interacts with ssDNA in a manner that is different from that of the Ssbe protein.

The binding of the Ssbe protein to ssDNA has been analyzed extensively (2). In general, at low DNA binding densities the Ssbe protein binds in the "(SSB)_{65} mode" in which all four subunits of the tetramer interact with ssDNA (occluding ~65 nucleotides of ssDNA/tetramer). At high DNA binding densities, however, the Ssbe protein binds in the "(SSB)_{35} mode" in which only two subunits of the tetramer interact with ssDNA (occluding ~35 nucleotides of ssDNA/tetramer). It cannot be determined from the gel-shift assays reported here whether the Ssbe and Ssbe proteins are in the (SSB)_{65} or (SSB)_{35} mode when only a single Ssbe or Ssbe tetramer is bound to dT75. However, the Ssbe and Ssbe proteins presumably must be in the (SSB)_{65} mode when two tetramers are bound to dT75 (7). Similarly, the Ssbe and Ssbe proteins presumably must be in the (SSB)_{35} mode when three Ssbe or Ssbe tetramers are bound to dT100. The finding that only one Ssbe tetramer was able to bind to dT75 and only two Ssbe tetramers were able to bind to dT100 suggests that either the Ssbe protein is unable to bind to the dT_{100} oligomers in an (SSB)_{35}-like mode or that...
The appearance of the complexes that were formed by the SsbA and of intrastrand DNA pairing interactions (9). The open circular
moving SsbA complexes corresponded to open circular com-
trophoresis. Electron microscopy showed that these slower
also forms complexes with circular ssDNA at subsaturating concentrations of SsbEc and SsbA
were essentially identical to those that were reported previ-
ously for the SsbEc protein (8, 9). In contrast, the SsbB protein
in the SsbB-ssDNA complexes (total nucleotide concentra-
cyclic ssDNA complexes were visualized by GelStar® staining. The
band that is visible in the 0 μM SSB protein lanes is because of a
small amount of linear φX ssDNA that is present in the circular φX
preparation.
multiple SsbB tetramers are unable to bind in close proximity
on the same dtm molecule.
The agarose gel shift results indicated that the SsbB protein
also forms complexes with circular φX ssDNA that are different
from those formed by the SsbA and SsbEc proteins. At satu-
rating protein concentrations, the SsbA and SsbEc proteins
each formed a discrete complex with φX ssDNA that migrated
together more slowly than unbound φX ssDNA during agarose gel elec-
trophoresis. Electron microscopy showed that these slower
moving SsaA complexes corresponded to open circular com-
plicated by being able to interact with two
spatially separated sites on the φX ssDNA and this is respon-
sible for the condensed appearance and anomalous electro-
phoretic mobility of the SsbB-φX ssDNA complexes. Either of
these possibilities would be consistent with the electron micro-
graphs, which indicated that there was 30% less protein bound
in the SsbB-φX ssDNA complexes than in the SsbA-φX ssDNA
complexes, and with the results that demonstrated that the
SsbA and SsbB proteins are able to bind together to φX ssDNA
to form a mixed SsbA/SsbB-φX ssDNA complex. The determi-
nation of the physical basis for the unusual electrophoretic behavior of the SsbB-φX ssDNA complexes will require further
investigation.
A comparison of the primary sequences reveals that the
N-terminal regions of the SsbA and SsbB proteins (amino acids
1–106) are highly similar in sequence to the corresponding
region of the SsbEc protein (amino acids 1–115) (Fig. 1A).
Structural studies have shown that this region of the SsbEc
contains the subunit tetramerization and ssDNA bind-
ing sites (2). Thus, the observations that the SsbA and SsbB
proteins form stable tetramers in solution and bind to shorter
ssDNAs (dT35 and dT50) with efficiencies that are at least as
high as that of the SsbEc protein are consistent with the high
sequence similarity of the N-terminal domains of the three
proteins. In addition to the core N-terminal DNA binding/
tetramerization domain, the SsbEc protein also has a C-termi-
nal acidic tail (amino acids 166–177), which is connected to the
N-terminal core domain by a proline/glycine-rich spacer region
(amino acids 116–165). Although the SsbA and SsbB proteins
also have C-terminal acidic tails, the SsbB protein differs from
the SsbA and SsbEc proteins in having a much shorter spacer
region between the acidic tail and the N-terminal core domain
(Fig. 1A). A recent crystal structure shows that the entire
C-terminal region (spacer plus tail) of the SsbEc protein is
highly disordered and extends out laterally from the N-termi-
lar core DNA binding domain of the protein (11). It is conceiv-
able that the shorter spacer region of the SsbB protein may
bring the acidic tail closer to the core DNA binding domain, and
this may modulate the DNA binding properties of the SsbB
protein, relative to those of the SsbEc and SsbA proteins. It is
also possible that some other structural feature is responsible
for the differences between the ssDNA binding properties of the
SsbB protein and those of the SsbEc and SsbA proteins.
The close similarity of the ssDNA binding properties of the
SsbA and SsbEc proteins is consistent with the SsbA protein
being the S. pneumoniae analog of the SsbEc protein, a general
purpose SSB protein involved in routine DNA functions.
The recent reports that the expression of the SsbB protein is
strongly induced during natural transformation, on the other
hand, suggest that the SsbB protein may be a specialized SSB
protein that plays a direct role in this process (3). During
natural transformation, exogenous dsDNA binds to a DNA
uptake site on the surface of the S. pneumoniae cell. One of the
strands of the exogenous dsDNA is degraded by a cell surface
nuclease, whereas the remaining complementary linear ssDNA
is transported into the cell interior. The linear ssDNA is then
incorporated into a homologous region of the double-stranded
S. pneumoniae chromosome in a RecA-dependent process (1). It
is conceivable that the role of the SsbB protein is to bind to the

![Fig. 7. Binding of SSB proteins to φX ssDNA.](image)

![Fig. 8. Electron microscopic analysis of SSB-φX ssDNA complexes.](image)
exogenous linear ssDNA (perhaps to protect it from cellular nucleases) as it is being transported into the cell interior. If this is the case, the particular ssDNA binding properties of the SsbB protein may reflect adaptations, which optimize the ability of the SsbB protein to carry out this function. It is also possible that the SsbB protein acts as an accessory protein for various recombination proteins during the assimilation of the exogenous ssDNA into the \textit{S. pneumoniae} chromosome. In this regard, several studies have indicated that the C-terminal region of the SsbEc protein may be involved in the interaction of the SsbEc protein with a host of other proteins that are involved in various aspects of DNA metabolism in \textit{E. coli}. The similar length of the C-terminal region of the SsbA protein is consistent with the idea that the SsbA protein plays a role in \textit{S. pneumoniae} that is analogous to that of the SsbEc protein in \textit{E. coli}. The shorter C-terminal region of the SsbB protein, on the other hand, may not only modify the ssDNA binding properties, but may also alter the spectrum of protein-protein interactions that are available to the SsbB protein and enable it to function more effectively in conjunction with various recombination proteins during natural transformation. The ssDNA binding studies that are presented in this report, together with the recent isolation and characterization of other proteins that have been implicated in transformational recombination (12, 13), will provide a foundation for further investigations into the biological roles of the paralogous SsbA and SsbB proteins.

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