Interaction of a Folded Chromosome-associated Protein with Single-stranded DNA-binding Protein of *Escherichia coli*, Identified by Affinity Chromatography*

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A single-stranded DNA-binding protein (SSB) affinity column was prepared by optimizing the coupling of *Escherichia coli* single-stranded DNA-binding protein to Affi-Gel 10. The bound SSB retained its ability to specifically bind single-stranded DNA. When nuclease-treated cell extracts were incubated with the SSB beads overnight at 4 °C, a major protein of $M_r = 25,000$ was bound. At shorter incubation times, two additional proteins of $M_r = 32,000$ and 36,000 were also detected. In the absence of nuclease treatment, eight additional proteins ranging from $M_r = 14,000$ to 160,000 also bound to the affinity column. The major $M_r = 25,000$ protein has been shown to be a folded chromosome-associated protein. Its binding to SSB is strongly enhanced by the addition of DNA polymerase III or DNA polymerase III holoenzyme.

The single-stranded DNA-binding protein (SSB) of *Escherichia coli* is a necessary component in DNA replication, both *in vivo* and *in vitro* (1, 2). In addition, strains carrying the ssb-1 mutation have been shown to have decreased frequencies of DNA recombination (3, 4) and reduced DNA repair capabilities (3, 5, 6). Characterization of the ssb-1 and ssb-113 mutations in various recA and lexA mutant strains suggests a role for SSB in recombinational repair and induction of the recA-lexA regulon (5-7). Deficiencies in RecA induction (7, 8), postirradiation DNA degradation (5, 8), Weigle reactivation (9), and prophage induction (8, 10) have also been demonstrated in the mutant ssb strains studied. The SSB protein appears to play an active role in the SOS response, but the mechanism of its action remains unclear (11). Moreover, the ssb gene itself is induced under SOS conditions, although this does not lead to significant increases in SSB protein levels (12). The pleiotropic nature of this protein indicates that it must interact not only with DNA, but also with a number of different proteins during various processes of DNA metabolism. Many of these interactions are undoubtedly a result of direct protein-protein interactions.

Some evidence for the direct interaction of SSB with other proteins has been demonstrated. Several years ago, DNA polymerase II was shown to specifically interact with SSB and to enhance the synthetic activity of this polymerase (13). Sedimentation analysis suggested that a complex of one DNA polymerase II molecule and one SSB monomer can be formed (14). However, because the *in vivo* role of this polymerase has never been established, the functional significance of this interaction remains obscure. Exonuclease I is fully active in the presence of SSB, and a complex between these two proteins was demonstrated by sedimentation analysis (15). Protein n has a specific role in primosome assembly and function and has also been shown to interact directly with SSB by density gradient sedimentation experiments (16). The *in vivo* significance of this interaction also remains to be elucidated.

Protein-affinity chromatography has been used to study the specific protein-protein interactions of bacteriophage T4 proteins that are essential for DNA replication and recombination (17, 18). In particular, an affinity chromatography system has been developed to demonstrate the association between the gene 32 protein and other proteins present in a T4 bacteriophage-infected cell. The successful development of this system (18), and others (19, 20), suggested that a similar approach using SSB might be useful in identifying and studying proteins involved in DNA metabolism in *E. coli*. We have developed an analytical SSB-affinity chromatography system to detect proteins in an extract that interact with SSB.

In this report we establish the conditions under which three proteins with molecular weights of 25,000, 32,000, and 36,000 are demonstrated to interact with SSB. The most prominent protein detected on the SSB column is the $M_r = 25,000$ protein, and its interaction with SSB is enhanced by the addition of purified DNA polymerase III or DNA polymerase III holoenzyme to the extract. This $M_r = 25,000$ SSB-binding protein is shown to be associated with isolated intact *E. coli* chromosomes.

**MATERIALS AND METHODS**

**Buffers**—Buffer A was 0.02 M Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM 2-mercaptoethanol, 5 mM MgCl₂, 0.05 M NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 10% (v/v) glycerol. Buffers B and C were the same as buffer A except that they contained 0.8 M NaCl and 4 M NaCl, respectively. Cells were sonicated in buffer A.

**Proteins**—The SSB was purified from *E. coli* strains RM140 (7) essentially as described by Meyer et al. (2) or Chase et al. (21). Intact *E. coli* chromosomes were prepared by the procedure of Stonington and Pettijohn (22) as modified by Kornberg et al. (23) from the RNase I” A19 strain (24). Proteins were extracted from these chromosomes as described (25) and dialyzed into buffer A. The $\epsilon$ subunit of DNA polymerase III holoenzyme was purified through the guanidine HCl denaturation-renaturation step from the strain MC1000 containing the plasmids pRK248-cil·tet and pNS86· dnaQ-amp as described (26). DNA polymerase III and DNA polymerase III holoenzyme were purified with slight modifications (27, 28). A174 replication assays (RF → SS) were as described (29). Calf pancreatic endonuclease I (DNase I) (EC 3.1.21.1) and micrococcal nuclease (EC 3.1.31.1) were purchased from Cooper Biomedical (Malvern, PA).
Preparation of [35S]Methionine-labeled E. coli Extracts—A 20-ml culture (C600) was grown to an A660 of 0.25 at 37 °C in M9 medium supplemented with all of the amino acids (50 μg/ml each) except methionine and cysteine. The [35S]methionine was added in quantities to assure that incorporation was linear for the 45-min labeling time (usually 500 μCi, 2.5 Ci/mmol). Cells were collected, washed in 0.1 M NaBES, pH 8.0, and resuspended in 6 ml of buffer A. Sonication (2 × 30-s pulses) was used to lyse the cells, followed by a clearing spin for 15 min at 10,000 × g. The extract was then made 1 mM CaCl2, and DNase I and microccocal nuclease were added to a final concentration of 10 μg/ml and 3 μg/ml, respectively. The nuclease treatment was allowed to proceed for 30 min at 0 °C followed by a clearing spin for 15 min at 10,000 × g. All extracts were stored frozen at -20 °C.

Preparation of SSB-Affi-Gel 10 Beads—Initial attempts to detect proteins interacting with SSB that had been coupled to the Affi-Gel 10 beads (Bio-Rad) were unsuccessful due to the excessive cross-linking of this protein to the beads. Optimum coupling conditions for the detection of SSB-binding proteins were determined by titration analysis (see “Results”), and the following procedure was adopted. Affi-Gel 10 beads (0.5 ml) were spotted onto Whatman No. 1 filter paper in a Buchner funnel and rinsed with several volumes of cold wash buffer A, followed by a 50-ml flask on ice. A solution containing 1.0 mg of SSB (5.0 ml) dialyzed extensively into 0.1 M NaHCO3, pH 8.0, 0.1 M NaCl, and 10 μM of ethanalamine, pH 8.0, was added to the beads and placed on a rotator at 4 °C for 3 h. After coupling, 60 μM of ethanalamine was added and rocking continued for an additional hour to block any unreacted sites. The protein solution was removed, and the beads were washed three times with 7 ml of buffer C. The beads were then washed into buffer A containing 50% glycerol and stored at -20 °C. Samples of the SSB solution, before and after the coupling reaction, were subjected to SDS-gel electrophoresis, and the relative amount of SSB present before and after coupling was determined. The coupling efficiency under these limited coupling conditions was between 25 and 50%, resulting in approximately 0.5–1.0 mg of SSB coupled per ml of beads. This is referred to as the SSB column.

The BSA-Affi-Gel 10 was prepared as described for the SSB-Affi-Gel 10 except that the BSA (Miles Laboratory) was resuspended in 0.1 M MOPS, pH 7.5, 0.08 M CaCl2, and coupled at protein concentrations of 1.0 mg/ml or 2.0 mg/ml in the presence or in the absence of 0.03 M ethanalamine, pH 7.5. The coupling ratio of protein to beads was performed at 1 or 2 mg of BSA to each ml of beads. After coupling and blocking were complete, the beads were washed and stored as described above. This is referred to as the BSA column. Affi-Gel 10 beads without coupled protein were blocked with an excess of ethanalamine for 4 h, washed, and stored as described above.

Affinity Chromatography—All procedures were carried out at 4 °C unless otherwise noted. Protein-coupled Affi-Gel 10 beads (25 μl) were placed into test tubes (12 × 75 mm) and washed with buffer A. The solution was added and incubated with continuous agitation overnight unless otherwise indicated. The beads were then allowed to settle to the bottom of the tube, and the extract was removed. The beads were washed three times (1 ml each time) with buffer A and transferred into fine glass tube columns (1.8 mm i.d.). The column was washed with an additional 1 ml of buffer A, and bound proteins were eluted with buffer B (0.5 ml). The three collected fractions (0.5 ml) were concentrated by trichloroacetic acid (10%) precipitation, after the addition of 5 μg of BSA as carrier. The redissolved proteins were subjected to electrophoresis on SDS-polyacrylamide gels (30) and visualized by fluorography (31).

RESULTS

Column Preparation—The covalent coupling of SSB to Affi-Gel 10 results from the formation of a stable amide bond between the neutral 10-atom linker arms on the beads and primary amino groups on the protein ligand. Analysis of the amino acid sequence of SSB (32) shows that 42 of its 177 amino acids represent possible coupling sites to the Affi-Gel 10 beads. Therefore, this protein could potentially form covalent bonds to the beads in many different orientations, some of which would be active in binding proteins that normally interact with SSB. However, these specific interactions would undoubtedly be blocked if each SSB molecule were cross-linked at several sites to the Affi-Gel 10 beads.

When maximum coupling conditions were used, greater than 80% of the SSB coupled to the resin. However, no proteins could be salt-eluted from the SSB column (Fig. 1, no ethanolamine). When the beads were treated with SDS, to dissociate any proteins that remained bound after NaCl elution, the only detectable SSB-specific protein interaction was that between SSB, coupled to the resin, and free SSB in the extract (not shown). Identical results were obtained when an extract prepared from RM140 (which contains the cloned ssb gene on a multicopy plasmid) was used, except that the amount of radioiodinated SSB bound to the column was significantly higher. Presumably, this strong interaction (resistant to high salt) can occur even with considerable steric hindrance resulting from the coupling conditions used. Optimum coupling conditions for the detection of SSB-binding proteins were determined by titration analysis in which SSB was coupled to the Affi-Gel 10 beads in the presence of increasing concentrations of the blocker ethanalamine. The SSB beads were then incubated overnight with a [35S]methionine-labeled, DNase-treated extract. After sufficient washing to remove unbound proteins, the bound proteins were eluted with buffer B (Fig. 1). A M, = 25,000 protein was bound to the SSB column, but only when the blocking agent (ethanalamine) was present in sufficient quantities to prevent excessive coupling of the SSB to the Affi-Gel 10 beads. This protein was shown to be specifically bound to the SSB proteins, as it did not bind to either a BSA column or to an Affi-Gel 10 column prepared in the same way but containing no covalently bound protein (Fig. 2).

Effect of Time and Temperature on Detection of SSB-binding Proteins—The major protein bound to the SSB column was a protein that was not detected on overnight incubation was the M, = 25,000 protein. When a [35S]methionine-labeled, DNase-treated extract was incubated with the SSB beads for only 2 h, two additional proteins, M, = 32,000 and M, = 36,000, were bound to the SSB column (Fig. 3). Possibly, these interactions are relatively weak or labile, and long incubation times, as described above, resulted in the loss of these proteins from the SSB column, or, alternatively, the M, = 25,000 protein was able to displace the other proteins. When the incubation was performed at 24 or 37 °C, there was a significant increase in the amount of the M, = 25,000 protein bound to the SSB column (Fig. 4). This suggests that a more stable interaction is occurring at the higher temperatures.

The SSB Column Can Bind Single-stranded DNA—Because SSB binds single-stranded DNA very tightly, it was important to distinguish protein-protein interactions from possible protein-DNA interactions that may have occurred on the column. That is to say, since SSB binds single-stranded DNA, such DNA in the extract could be bound to the SSB column and thereby provide sites for other proteins to bind DNA but which do not normally interact directly with SSB. Fig. 5 shows that SSB coupled to the beads retained its specificity for binding single-stranded DNA, indicating this protein has retained its functional activity with respect to DNA binding. However, when SSB beads were used in a φX174 (RF → SS) replication assay, they were incapable of supporting DNA synthesis (data not shown). This, however, is not surprising as SSB tetramers need to align themselves along the DNA molecule and are undoubtedly constrained from doing so when bound to the beads. When proteins were eluted from an SSB column, after being incubated with a [35S]methionine-labeled extract that was not treated with DNase, several additional proteins were detected (Fig. 6). The detection of additional proteins under these conditions suggested that SSB on the beads was able to bind DNA in the extract,
Ethanolamine (nmoles)  0  100  240  374  500

A B C D  A B C D  A B C D  A B C D  A B C D

130-  67-  43-  374

ABCD  ABCD  ABCD

FIG. 1. The effect of coupling conditions on the detection of SSB-binding proteins. The SSB (0.7 nmol of tetramer) was coupled to Affi-Gel 10 beads (25 μl) in the presence of increasing concentrations of ethanolamine as indicated in the figure. These SSB beads were then incubated overnight at 4 °C with a [35S]methionine-labeled, DNase-treated RM98 extract. After sufficient washings to remove unbound protein, bound proteins were eluted with buffer containing 0.8 M NaCl. A, 0.5 μl of the starting extract (2 ml); B, first column wash eluant; C, second column wash eluant; D, 0.8 M column eluant. Samples B, C, and D are the entire 0.5-ml fraction, concentrated by trichloroacetic acid precipitation prior to electrophoresis. The M, = 25,000 SSB-binding protein is indicated by the arrow.

and that these additional proteins (M, = 160,000, 130,000, 110,000, 38,000, 30,000, 20,000, 17,000, and 14,000) could now bind to the DNA retained by the SSB. It is also possible that these proteins can interact with SSB only when they (or SSB) are bound to DNA, and the present experiments cannot distinguish these two possibilities. The binding of the M, = 25,000 protein to the SSB column, however, was not affected by the DNase treatment, showing that the interaction of these two proteins is independent of the SSB-DNA interaction.

DNA Polymerase III Enhances the Binding of the M, = 25,000 Protein—The addition of either DNA polymerase III or DNA polymerase III holoenzyme to the [35S]methionine-labeled DNase-treated extract resulted in increased binding of the M, = 25,000 to the SSB column. Table I summarizes the results of these additions from densitometric scans of the eluted proteins from SSB columns. In contrast, no quantitative effect was observed on the binding of either the M, = 32,000 or the M, = 36,000 proteins when these polymerases were added. Purified DNA polymerase III holoenzyme was also incubated with the SSB beads directly, to see if this enzyme would itself bind to the SSB protein. The eluant from this column was assayed for DNA polymerase III holoenzyme activity using the αX174 (RF → SS) replication assay (29). Other samples were analyzed by silver-stained SDS-polyacrylamide gels. Both of these methods failed to demonstrate specific retention of the DNA polymerase III holoenzyme by the SSB column. These data suggest that the interaction of the M, = 25,000 protein with SSB is stabilized by the polymerase III enzyme, although the latter enzyme either does not bind SSB or the interaction is too weak to detect by this methodology. The polymerase may also interact with this M, = 25,000 protein resulting in some conformational change that is more reactive with SSB.

To this point, seven subunits of the DNA polymerase III holoenzyme have been identified. Three of the subunits, ε (M, = 25,000), δ (M, = 32,000), and β (M, = 37,000) have molecular weights similar to those of the SSB-binding proteins reported here. In addition, an interaction of the holoenzyme with the M, = 25,000 protein has been demonstrated (Table I). It has also been shown that SSB increases the fidelity of DNA synthesis in vitro by the E. coli polymerase III (33). The ε subunit of this enzyme possesses a very potent 3' → 5' exonuclease activity, specific for mispaired bases, and has been shown to contain the proofreading activity of the DNA polymerase III holoenzyme (26, 34). The increased fidelity of replication of the polymerase by SSB and the physical characteristics of the ε subunit (molecular weight and isoelectric point) suggested that the M, = 25,000 SSB-binding protein might be the ε subunit. To examine this possibility, two-dimensional gel electrophoresis was used. Co-migration analysis of purified ε protein and the SSB-binding proteins was performed. The ε protein and the M, = 25,000 protein migrated very closely to one another, but were resolvable on co-electrophoresis in the same sample (data not shown). Since
FIG. 2. The specificity of the $M_r = 25,000$ protein for SSB. The Affi-Gel 10 beads (25 μl) were prepared as described under “Materials and Methods,” either with no protein-coupled (Affi-Gel 10), bovine serum albumin-coupled (BSA-Affi-Gel 10), or SSB-coupled (SSB-Affi-Gel 10). Beads were incubated overnight at 4°C with a [35S]methionine-labeled, DNase-treated, RM98 extract. A, 0.5 μl of the starting extract (2 ml); B, first column wash eluant; C, second column wash eluant; D, 0.8 M column eluant. Samples B, C, and D are the entire 0.5-ml fraction, concentrated prior to electrophoresis. The $M_r = 25,000$ SSB-binding protein is indicated by the arrow.

Proteins purified from overproducing strains containing cloned genes sometimes behave differently from that purified from single gene copy strains, additional tests were performed. To further establish whether the $M_r = 25,000$ SSB-binding protein was or was not the $\epsilon$ subunit, the former was probed in an ELISPOT assay, using a rabbit antibody against the $\epsilon$ subunit. This antibody did not react with the $M_r = 25,000$ protein. Further, no 3' → 5' exonuclease activity could be detected with the SSB-affinity-purified protein, and the purified $\epsilon$ protein was not retained on an SSB column. Therefore, the $M_r = 25,000$ SSB-binding protein has been shown by several criteria to be distinct from the $\epsilon$ subunit of the holoenzyme.

The $M_r = 25,000$ Protein Is a Component of the E. coli Folded Chromosome—Proteins were extracted from intact E. coli chromosomes and were subjected to electrophoresis on two-dimensional gels along with the affinity-purified proteins eluted from an SSB-column (Fig. 7). These results show that the $M_r = 25,000$ SSB-binding protein co-migrates with a protein present in this preparation, while the $M_r = 32,000$ and $M_r = 36,000$ proteins are not detected in this preparation. A two-dimensional map of the $M_r = 25,000$, $M_r = 32,000$, and $M_r = 36,000$ proteins was prepared, and the isoelectric points of the three proteins was determined by the method of O’Farrell (35). The isoelectric point of the $M_r = 25,000$ protein was determined to be 6.3, while the isoelectric points of the $M_r = 32,000$ and $M_r = 36,000$ proteins were 6.9 and 6.7, respectively. Because of the high affinity of SSB for both DNA and RNA, direct chromatography of folded chromosome preparations on the SSB affinity column could not be carried out.

**DISCUSSION**

The role of SSB in DNA metabolism is mediated through direct interactions with single-stranded DNA. Some differences between wild type SSB, mutant SSB-1, and mutant SSB-113 proteins have been noted (36, 37). The SSB-1 protein, for example, is unable to maintain a stable tetrameric structure which may affect its interaction with single-stranded DNA. However, the stability of the SSB-113 protein appears to be normal. While the latter protein binds at least as well to single-stranded DNA as wild type SSB, it may act even more efficiently as a helix-stabilizing protein. The binding of SSB-1 protein to single-stranded DNA at 45°C is severely impaired, while no temperature-dependent single-stranded DNA binding effect could be demonstrated with SSB-113. Differences observed in the phenotypic characteristics of strains carrying the ssb-1 and ssb-113 mutations may be explained not by the interaction of SSB with single-stranded DNA, but rather through the direct interaction of SSB with other DNA metabolizing proteins.

DNA polymerase II, exonuclease I, and n protein, proteins known to interact with SSB, were not detected on the SSB-column. Perhaps these proteins are binding to the column, but are not detected because they are present in low concentrations in the extract. Alternatively, the binding to SSB may be transient or we have yet to find the proper experimental conditions. Some interactions that occur in solution may be disrupted when one or the other protein is attached to a solid matrix (17).

Genetic analysis of the suppression of the ssb-1 and ssb-113 mutations by a wild type rep gene suggests that there exists a direct interaction between these proteins (38). However, purified rep protein was not retained on the SSB-column (not shown). The lethal effect observed in the double mutant (ssb113, rho15) suggests a possible functional relationship between SSB and the rho protein (39). Finally, a direct interaction between the recA protein and SSB is suggested by several in vitro studies (40, 41). Possibly, the interaction of
These replication and recombination proteins in vivo is mediated through one or more of the SSB-binding proteins detected on the SSB-column.

The suppression of the ssb-1 phenotype in certain genetic backgrounds (38), or in some spontaneous revertants (42), is accompanied by the inability to support the growth of certain phages. This phenotypic characteristic can best be explained by the presence of suppressor proteins interacting with SSB and with other replication proteins. In addition, the generation and analysis of second-site revertants to the ssb-1 mutant in this laboratory and the cloning of specific genes capable of suppressing the mutant phenotype suggest that there are several proteins which interact directly with the SSB protein. This approach has, for example, led to the identification of groEL as an extragenic suppressor of ssb.

The function of the SSB-binding proteins identified in this study remains unknown. The increased fidelity of replication of the polymerase III enzyme by SSB (33) and the physical characteristics of the subunit (molecular weight and isoelectric point) had suggested that the is a SSB-binding protein might be the subunit. However, extensive examination of these proteins has shown them to be different. The increased interaction of SSB with the is 25,000 SSB-binding protein by the DNA polymerase III is intriguing and suggests that all three are in some way physically interacting on the column. The DNA polymerase III, SSB, and the is 25,000 protein may represent part of the functional replication complex that exists in vivo.

The mutH protein has recently been shown to have a molecular weight of 25,000. However, this protein and the is 25,000 SSB-binding protein clearly migrate to different positions on two-dimensional gels showing that these proteins are distinct.

The possible relationships between the is 25,000, is 32,000, and is 36,000 proteins deserves comment. While it cannot be ruled out at present that the is 25,000 protein is a degradation product of either the is 36,000 or is 32,000 proteins, it does not seem likely for the following reasons: (i) protease inhibitors were routinely included in all buffers; (ii) the is 25,000 protein is a very prominent binding protein, even after relatively short incubations in the cold (Fig. 4); (iii) neither the is 36,000 nor the is 32,000 protein is detected in folded chromosome preparations also prepared in the cold and in protease inhibitor-containing buffers. We would have expected to find the latter in such preparations if they were a more native form of the is 25,000 protein. Methods to provide sufficient is 25,000 protein for antibody production are currently in progress to address this question directly.

The detection of additional proteins binding to the SSB column when the extract is not treated with DNase presents

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3 P. Modrich, unpublished observations.
FIG. 5. **Binding of DNA by the SSB column.** The SSB beads (25 μl), containing approximately 0.14 nmol of SSB tetramers, were equilibrated in buffer A. All but 100 μl of the buffer was removed, and increasing concentrations of phage PM2 heat-denatured or double-stranded DNA, as indicated in the figure, were added. Incubation was for 30 min at 0 °C. The beads were then washed three times with 1 ml of buffer each time, and the amount of radiolabeled DNA bound to the beads was determined on GF/C filters. Affi-Gel 10 beads, to which BSA was coupled, were also run as controls. O, SSB column with heat-denatured DNA; □, SSB column with double-stranded DNA; O, BSA column with heat-denatured DNA; □, BSA column with double-stranded DNA.

![Graph showing DNA binding by the SSB column.](image)

**TABLE I**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Additions*</th>
<th>M, = 25,000 SSB-binding protein Increase A unitsb</th>
</tr>
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<tbody>
<tr>
<td>RM98</td>
<td>- -</td>
<td>40 1</td>
</tr>
<tr>
<td>RM98</td>
<td>+ -</td>
<td>98 2.5</td>
</tr>
<tr>
<td>RM98</td>
<td>- +</td>
<td>170 4.3</td>
</tr>
</tbody>
</table>

* Additions consisted of either DNA polymerase III (2000 units) or DNA polymerase III holoenzyme (3,000 units).

b Levels of the M, = 25,000 SSB-binding protein were quantitated by densitometric scanning of autoradiograms and are expressed as absorbance units (A units).

some interesting possibilities. The detection of such proteins seems to suggest that SSB is simply binding DNA in the extract, and these proteins are now binding to the DNA. However, one cannot rule out the possibility that these proteins do interact directly with SSB but require that these proteins (or SSB) be bound to DNA for these interactions to occur. Some proteins may change configuration when bound...
to DNA, and such changes in configuration may subsequently affect their protein-protein interactions. The presence of DNase in the extract might disrupt such interactions. If additional proteins detected under these conditions were simply binding to single-stranded DNA, then one would expect the number of proteins bound to the column to be significantly higher than we actually observed (Fig. 6). The limited number of proteins detected on these columns suggests that SSB is responsible for the retention of these proteins. It is also possible that SSB binds certain single-stranded DNA regions organizing this DNA into a secondary structure that can now be recognized by these proteins.

When a column prepared with SSB-1 protein was used, the same three proteins were detected (not shown), indicating that this mutant protein retains the ability to interact with these three proteins when examined at 4°C. This SSB-chromatography system is currently being used to examine protein-protein interactions with SSB-1 and SSB-113 at higher temperatures.

The $M_r = 25,000$ SSB-binding protein can be found in a preparation of membrane-associated chromosomes. This protein might be part of the chromosomal nucleoprotein structure or possibly a membrane protein, or both. The significance of the association of the $M_r = 25,000$ protein with the intact chromosome is currently under investigation.

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