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Large-Scale Overproduction and Rapid Purification of the *Escherichia coli ssb* Gene Product. Expression of the *ssb* Gene under λ P_L Control†

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ABSTRACT: We report a rapid procedure for the large-scale purification of the *Escherichia coli* encoded single-strand binding (SSB) protein, a helix-destabilizing protein which is essential for replication, recombination, and repair processes in *E. coli*. To facilitate the isolation of large quantities of the *ssb* gene product, we have subcloned the *ssb* gene into a temperature-inducible expression vector, pPL_c28 [Remaut, E., Stanssens, P., & Fiers, W. (1981) *Gene 15*, 81-93], carrying the bacteriophage λ P_L promoter. A large overproduction of the *ssb* gene product results upon shifting the temperature of *E. coli* strains which carry the plasmid and also produce the thermolabile λcI857 repressor. After 5 h of induction, the *ssb* gene product represents ~10% of the total cell protein. The overexpression of the *ssb* gene and the purification protocol reported here enable one to isolate SSB protein (>99% pure) with final yields of ~3 mg of SSB protein/g of cell paste. In fact, very pure (>99%) SSB protein can be obtained after approximately 8 h, starting from frozen cells in the absence of any columns, although inclusion of a single-stranded DNA–cellulose column is generally recommended to ensure that the purified SSB protein possesses DNA binding activity. The ability to easily purify 1 g of SSB protein from 300–350 g of induced cells will facilitate physical studies requiring large quantities of this important protein.

The *Escherichia coli ssb* gene product is a necessary component in replication, recombination, and repair processes of that bacterium (Wickner & Hurwitz, 1974; Meyer et al., 1979; Johnson, 1979; McEntee et al., 1980; Glassberg et al., 1979). It is a member of a class of nucleic acid binding proteins referred to as "helix-destabilizing proteins" (Alberts & Sternglanz, 1978), which bind selectively and in most cases cooperatively to single-stranded conformations of nucleic acids (Sigal et al., 1972; Kowalczykowski et al., 1981; Lohman et al., 1986).

Until recently, physical studies of the *E. coli* single-strand binding (SSB) protein and its interactions with nucleic acids have been limited due to the difficulty of routinely obtaining multimilligram quantities of the purified protein. The cloning of the *ssb* gene into multicopy plasmids (Sancar & Rupp, 1979; Chase et al., 1980; Sancar et al., 1981), resulting in increased levels of *ssb* gene expression, has made the purification of tens of milligrams of SSB protein a routine matter, although most protocols require two to three columns (Sigal et al., 1972; Chase et al., 1980). We have improved the purification procedure further by subcloning the *ssb* gene into a plasmid containing the strong bacteriophage λ P_L promoter so that the *ssb* gene is under transcriptional control of the λ P_L promoter. This procedure has been used to overexpress a number of prokaryotic proteins (Bernard et al., 1979; Remaut et al., 1981; Shimatake & Rosenberg, 1981; Yoakum et al., 1982; Gribskov & Burgess, 1983; Shigesada et al., 1984; Mott et al., 1985) including the *E. coli* SSB-1 protein, a temperature-sensitive mutant of the SSB protein (Williams et al., 1984), as well as some eukaryotic proteins (Rosenberg et al., 1983). Transcription of the gene from the λ P_L promoter can be controlled by the growth temperature in *E. coli* strains producing a temperature-inducible λ cI repressor (e.g., cI857); at 30 °C, transcription is almost fully repressed, whereas at 42 °C, transcription is fully derepressed (Remaut et al., 1981). This leads to large increases in mRNA and protein levels, assuming that no major problems exist, as is the case with the *E. coli ssb* gene product.

We also report a very rapid procedure for the purification of the *ssb* gene product from *E. coli* harboring the inducible, overproducing plasmid. This procedure enables one to purify large quantities of the SSB protein (>99% pure) from small amounts of induced cells in a matter of several hours in the absence of any columns. However, a single-stranded DNA–cellulose column is recommended as part of the purification

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to ensure DNA binding activity of the SSB protein, although this may not be required for some purposes. We routinely obtain 3 mg of SSB/g of cell paste using the method reported here.

Materials and Methods

Buffers and Stock Solutions. All buffers were made with distilled–deionized water, passed through a Milli-Q system before use. STE buffer is 10 mM tris(hydroxymethyl)-aminomethane (Tris) base, pH 8.0 (titrated with HCl), 1 mM trisodium ethylenediaminetetraacetate (Na_3EDTA), and 0.1 M NaCl. Lysis buffer is 50 mM Tris (pH 8.3), 0.2 M NaCl, 15 mM spermidine trichloride, 1 mM Na_3EDTA, and 10% (w/v) sucrose. TGE buffer is 50 mM Tris (pH 8.3), 1 mM Na_3EDTA, and 20% (v/v) glycerol. SSB storage buffer is 20 mM Tris (pH 8.3), 0.5 M NaCl, 1 mM Na_3EDTA, 1 mM β-mercaptoethanol, and 50% (v/v) glycerol.

A 10% solution of Polymin P (BRL), pH 6.9, was prepared as described by Burgess & Jendrisak (1975). Single-stranded DNA–cellulose was made with calf thymus DNA (Worthington) by the method of Littman (1968). Ammonium sulfate (ultrapure) was from Schwarz/Mann. Phenylmethanesulfonyl fluoride (PMSF) was from Sigma. Stock solutions of 0.1 M PMSF were made fresh in 2-propanol; 4% (w/v) sodium deoxycholate was made fresh as follows: 6 mL of distilled water was mixed with 0.15 mL of 5 M NaOH, and 250 mg of sodium deoxycholate was slowly added to the solution with constant mixing, at room temperature. Lysozyme stocks (hen egg white, 10 mg/mL) were made fresh in H_2O. All other chemicals were reagent grade.

Bacterial Strains. E. coli K12ΔH1Δtrp is the designation used for *E. coli* strain M72Sm^R*lacZ*amΔ*bio-uvrB*Δ*trpEA2*-(λ*N*am7-*N*am53cI857ΔH1) (Bernard et al., 1979). This is the host for the overproducing SSB plasmid that we have constructed.

Plasmids. The source of the *ssb* gene used in the plasmid constructions was plasmid pDR1996 (*uvrA*, *ssb*, *tet*, *amp*) (Sancar et al., 1981) kindly provided by Dr. J. Chase (Albert Einstein School of Medicine). The vector containing the bacteriophage λ P_L promoter was pPL_c28 (*amp*), described by Remaut et al. (1981) and kindly provided by Dr. W. Fiers (University of Ghent, Belgium).

Cloning and Transformations. Plasmid DNA used for cloning was isolated from overnight cultures by the procedure of Birnboim & Doly (1979) as modified by Silhavy et al. (1984) without a chloramphenicol amplification step. Occasionally, the supercoiled plasmid DNA was further purified by banding to equilibrium in a CsCl gradient in the presence of ethidium bromide (Maniatis et al., 1982). Restriction endonucleases were purchased from New England Biolabs. Conditions for restricting DNA are described by Davis et al. (1980).

E. coli K12ΔH1Δtrp cells were made competent for transformation by treatment with 100 mM $CaCl_2$ following the procedure of Silhavy et al. (1984). Competent K12ΔH1Δtrp cells were made 10% in glycerol, frozen in 200-μL aliquots in liquid N_2, and stored at −70 °C. Transformations were performed essentially as described by Remaut et al. (1981). Ampicillin (Sigma), or tetracycline (Sigma), sterilized by filtration through 0.22-μm filters (Sybron/Nalgene 120-0020), was added to liquid media and agar plates at concentrations of 50 and 20 μg/mL, respectively.

In order to place the *ssb* gene under control of the bacteriophage λ P_L promoter, we subcloned a 3.7 kilobase pair (kbp) *Eco*RI fragment containing the *ssb* gene from pDR1996

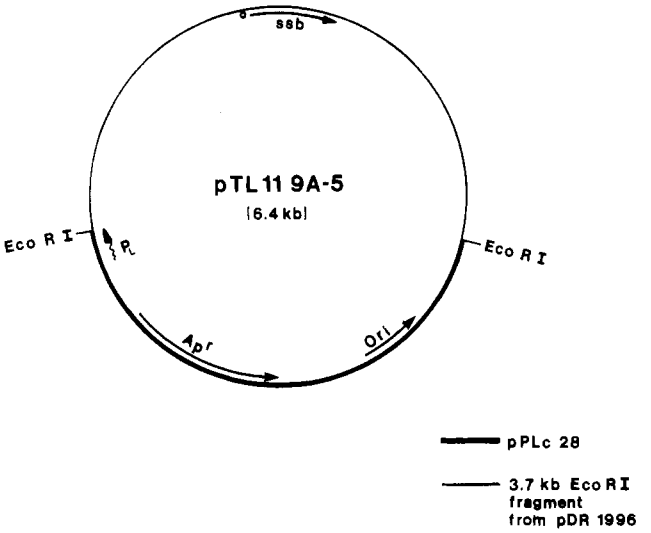

FIGURE 1: Plasmid pTL119A-5, which is temperature-inducible for SSB protein overproduction in an *E. coli* host which produces the λcI857 repressor. This was constructed from plasmids pPL_c28 (Remaut et al., 1981) and pDR1996 (Sancar et al., 1981).

(Sancar et al., 1981) into the *Eco*RI site of plasmid pPL_c28 (Remaut et al., 1981) using standard methods (Silhavy et al., 1984). Two plasmids were isolated which contained the 3.7 kbp *Eco*RI fragment in opposite orientations in pPL_c28, as determined by restriction endonuclease analysis. The plasmid with the *ssb* gene in the proper orientation to allow transcription of the *ssb* gene from the λ P_L promoter, pTL119A-5, is shown in Figure 1. Plasmid pTL119 contains the *ssb* gene in the incorrect orientation. In pTL119A-5, the start of the *ssb* gene is approximately 1.5 kbp downstream from the λ P_L promoter.

Growth of E. coli K12ΔH1Δtrp/pTL119A-5 for SSB Overproduction. Small-scale bacterial growth was performed in 1-L volumes in media containing, per liter, 10 g of Bactotryptone (Difco), 5 g of yeast extract (Difco), 5 g of NaCl, and 2 mL of 50 mg/mL thiamin (Sigma). A 5-mL culture of *E. coli* K12ΔH1Δtrp, grown overnight at 30 °C, was used to inoculate each liter. The cells were grown to an OD_{600} between 0.4 and 0.6, at which point the temperature was raised to 42 °C by directly mixing the liter of culture with 1 L of sterile L broth at 55 °C, with rapid swirling. The temperature of the cell culture was maintained at 42 °C for 30 min and then lowered to 37 °C. After an additional 5 h of growth at 37 °C, the cells were cooled on ice, harvested, and washed once by resuspending in cold (4 °C) STE buffer followed by recentrifugation. The yields were typically 4–5 g of wet cell paste per initial liter of culture, and the cells were stored at −70 °C.

Large-scale growth of cells was performed in a 250-L fermentation vessel (New Brunswick Scientific Co.). The inoculum was 10 L of K12ΔH1Δtrp/pTL119A-5, grown overnight at 30 °C in a 28-L fermentor (New Brunswick Scientific Co.). The inoculum was added to 170 L of media preequilibrated at 30 °C so that the initial OD_{600} was ≤0.2. During growth, the pH was maintained at 7 ± 0.2 by the sterile addition of 7 N ammonium hydroxide, using an automatic pH control unit (New Brunswick Scientific Co.). The temperature of the 180 L was increased to 42 °C, when the OD_{600} = 0.6; approximately 15 min was required to raise the temperature of the 180 L from 30 to 42 °C. The temperature was maintained at 42 °C for 30 min and then lowered to 37 °C for the remainder of the growth. After an additional 5 h of growth at 37 °C (final OD_{600} = 5.7), the cell culture was rapidly chilled to 8 °C, and the cells were harvested by centrifugation

in a prechilled, continuous-flow centrifuge (Cepa Z61). The cell paste was washed in cold STE buffer as described above and stored at −70 °C.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis of protein samples was carried out by using vertical slab gels (Shadell Inc.). A 5% stacking gel was used with either a 12% resolving gel or an exponential gradient from 10% to 20% polyacrylamide. The exponential gradient gels were prepared according to the method of Altman et al. (1983). Total *E. coli* protein was prepared by pelleting 1 mL of cells and resuspending these in 100 μL of sample buffer and boiling for 3 min (Altman et al., 1983). Typically, 5–10 μL of the resulting sample was applied to each well. The gels were run at 150 V (constant voltage) for 5 h at 22 °C. Molecular weight standards were phosphorylase *a* [94 kilodaltons (kDa)], bovine serum albumin (68 kDa), catalase (58 kDa), hen egg albumin (43 kDa), carbonic anhydrase (29.2 kDa), soybean trypsin inhibitor (21.5 kDa), and hen egg white lysozyme (14.3 kDa). The polyacrylamide gels were scanned and the resulting traces integrated to determine relative protein levels by using a laser scanning densitometer (Biomed Instruments). The steps in the SSB purification were monitored by 12% polyacrylamide gel electrophoresis using mini slab gels (IDEA Scientific Co., Corvallis, OR). These gels were run at 150–200 V for 90 min at 4 °C.

DNA Binding Assays. As a characterization of the final SSB protein product, quantitative studies of the binding of SSB protein to single-stranded homopolynucleotides were performed by monitoring the quenching of the intrinsic tryptophan fluorescence of the SSB protein upon binding, as previously described (Lohman & Overman, 1985; Lohman et al., 1986). The SSB protein binding experiments were performed by using an SLM 8000 spectrofluorometer with the synthetic homopolynucleotide poly(U) under weak binding conditions (high salt) so that separate estimates of the intrinsic binding constant, K, and cooperativity parameter, ω (McGhee & von Hippel, 1974), can be accurately measured. The expression for the cooperative binding isotherm developed by McGhee & von Hippel (1974) was used to obtain a series of computer-generated isotherms, by independently varying K and ω with the site size, $n = 65$ nucleotides per SSB protein tetramer (Lohman & Overman, 1985). The "best-fit" values of K and ω were obtained by visual comparison of the theoretical curves with the experimental titration curve (Lohman et al., 1986).

Results

Expression of the ssb Gene under λ P_L Control. E. coli K12ΔH1Δtrp containing the plasmids pTL119A-5 or pTL119 were screened for temperature-inducible expression of the *ssb* gene product by running SDS–polyacrylamide gels of total protein at various times after temperature induction. Typical results are shown in Figure 2. Inspection of the first four lanes indicates that cells containing pTL119A-5 significantly overproduce the *ssb* gene product following a temperature shift, whereas pTL119, with the *ssb* gene in the incorrect orientation with respect to the λ P_L promoter, does not show any increase in SSB protein production even after 5 h at 42 °C. Also shown in Figure 2 is a time course of the accumulation of the *ssb* gene product in K12ΔH1Δtrp/pTL119A-5 for 5 h after temperature induction. In Figure 2, an equal mass of induced cells was applied to the gel for each time point shown, so that the increase in the level of SSB protein is due to increased expression with time. After 5 h of growth following induction, we estimate the level of SSB protein in K12ΔH1Δtrp/pTL119A-5 to be approximately 10% of the total cell protein,

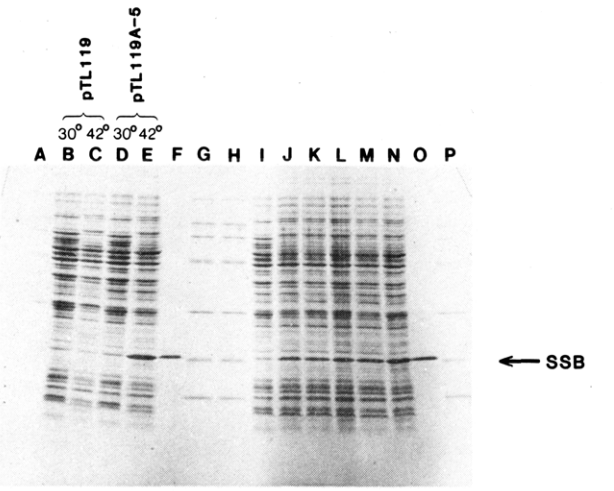

FIGURE 2: SDS–polyacrylamide gel electrophoresis (5–20% gradient gel) of total proteins from K12ΔH1Δtrp containing pTL119A-5 (*ssb* gene in correct orientation with respect to the λ P_L promoter) and pTL119 (*ssb* gene in incorrect orientation). Lanes A, G, H, and P, molecular weight standards (see Materials and Methods); lane B, uninduced K12/pTL119 (5-h growth); lane c, induced K12/pTL119 (5-h induction); lane D, uninduced K12/pTL119A-5 (5-h growth); lane E, induced K12/pTL119A-5 (5-h induction); lanes F and O, SSB protein standard; lanes I–N, time course of induced K12/pTL119A-5 (0–5 h). The same amounts of total protein were loaded in each of the lanes showing total K12 protein (lanes B–E and I–N).

as judged by densitometric scanning of the polyacrylamide gel. We typically harvest the cells at this point and use these for the preparation of SSB protein. A rough estimate is that induced K12ΔH1Δtrp/pTL119A-5 cells overproduce the SSB protein at least 300-fold, on the basis of comparisons of SSB protein yields from wild-type *E. coli* strains or strains harboring other multicopy plasmids carrying the *ssb* gene under control of its normal promoter (Chase et al., 1980).

Purification of SSB Protein from K12ΔH1Δtrp/pTL119A-5. The purification of SSB protein from temperature-induced K12ΔH1Δtrp/pTL119A-5 cells reported here is rapid, and yields are high. The basic steps consist of cell lysis, Polymin P precipitation, NaCl extraction of the Polymin P pellet, ammonium sulfate precipitation of the SSB protein, and single-stranded DNA–cellulose affinity chromatography (Figure 3). In this form, the purification is complete in less than 2 days, yielding >99% pure SSB protein with yields of ≥3 mg of SSB protein/g of wet cell paste.

The single-stranded (ss), DNA–cellulose column can be eliminated, still yielding >99% pure SSB protein in only 7–8 h after removal of the cells from the freezer. Although the SSB protein is pure after the ammonium sulfate step (fraction III, below), it occasionally contains a small amount of SSB protein (<2%) which does not bind well to a ss DNA column; hence, we always include the ss DNA column when purifying SSB protein for use in nucleic acid binding studies. However, the SSB protein which is prepared without using a ss DNA affinity column (fraction III) should be useful for large-scale preparations of SSB protein for such uses as preparing SSB protein affinity columns, or for crystallography studies, since the SSB protein is still >99% pure at this stage.

The following procedure is for 50 g of induced K12ΔH1Δtrp/pTL119A-5 cells. The purification works well when directly scaled up or down from this level, with quite similar yields per wet weight of cells. However, if one starts with more than 50 g of cells, then part of the preparation is usually stored at −20 °C (50% glycerol) after the ammonium sulfate step (fraction III), since the size of the ss DNA column limits the amount of SSB protein that can be purified in the

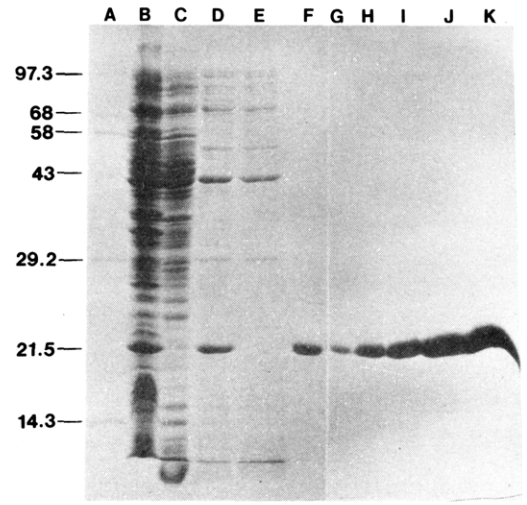

FIGURE 3: SDS–polyacrylamide gel (5–20% gradient gel) of the various fractions from the purification of SSB protein from K12ΔH1Δtrp/pTL119A-5 cells, which had been induced for 5 h. Lane A, molecular weight standards; lane B, cell-free lysate, fraction I; lane C, supernatant from 0.4% Polymin P precipitation; lane D, 0.4 M NaCl extraction of Polymin P pellet, fraction II; lane E, supernatant from the 27% ammonium sulfate precipitation; lane F, fraction III, load onto ss DNA–cellulose column (~5 μg); lanes G–K, SSB protein from 2 M NaCl elution of ss DNA–cellulose column, 1, 5, 10, 20, and 30 μg of SSB protein, respectively.

last step. All steps are carried out at 4 °C unless noted otherwise.

(*I*) *Cell Lysis.* Prewashed, frozen cells (50 g) are resuspended in 200 mL of lysis buffer (pH 8.3) in a Waring blender at low speed. The blending speed is reduced with a variable rheostat. Freshly made PMSF (0.1 M) is added to a final concentration of 0.1 mM. Lysozyme is then added to a final concentration of 200 μg/mL (4 mL of a freshly made 10 mg/mL stock), and the cell resuspension is incubated at 4 °C with slow stirring for 30 min. Freshly made 4% sodium deoxycholate is added to a final concentration of 0.05%, and the solution is incubated at 15 °C for 30 min. At this point, lysis is quite complete, but it is useful to shear the chromosomal DNA either by low-speed treatment in the Waring blender or by a short sonication treatment (3 min at 50% duty cycle, power setting of 8; Heat Systems W-225). The cell lysate is then centrifuged in two 250-mL bottles at 13 000 rpm for 80 min at 4 °C (JA-14 rotor). The supernatant is fraction I (220 mL).

(*II*) *Polymin P Precipitation.* Polymin P [10% (w/v)] is slowly added to fraction I (drop by drop) *with constant stirring* to a final concentration of 0.4%. The solution is stirred for an additional 15 min after addition of the Polymin P. The SSB protein precipitates with the DNA at this step. The polymin P precipitant is collected by centrifugation for 20 min at 8000 rpm at 4 °C (JA-14).

The Polymin P pellet is gently resuspended in 1× fraction I volume of cold (4 °C) TGE buffer + 0.4 M NaCl (220 mL in this case), by breaking up the pellet with a stirring paddle and slowly stirring the solution with a magnetic stir bar for approximately 30 min. It is important to have the Polymin P pellet completely resuspended at this step. Let this stir for an additional 15 min after the pellet is fully resuspended. Recentrifuge the Polymin P resuspension for 20 min at 8000 rpm at 4 °C (JA-14). The SSB protein is found in the supernatant, fraction II (220 mL).

(*III*) *Ammonium Sulfate Fractionation.* Solid ammonium sulfate (150 g/L) is slowly added to fraction II (~27% saturation) with constant stirring over approximately a 30-min period. Allow this to stir slowly for an additional 30 min after the final addition of $(NH_4)_2SO_4$. The ammonium sulfate precipitant is centrifuged for 30 min at 13 000 rpm at 4 °C (JA-14). The pellet is gently resuspended in 0.9× fraction I volume (200 mL) of TGE buffer + 0.3 M NaCl and recentrifuged at 13 000 rpm for 30 min at 4 °C (JA-14) to remove any insoluble material. The supernatant is fraction III. Fraction III can be stored at −20 °C after dialysis vs. storage buffer or can be applied to a single-stranded DNA–cellulose column. Fraction III contains ~150 mg of SSB protein for a preparation starting with 50 g of cells; hence, half of fraction III is usually stored at −20 °C at this point.

(*IV*) *Single-Stranded DNA–Cellulose Column.* Half of fraction III is applied to a ss DNA–cellulose column (100 mL) preequilibrated in TGE buffer + 0.3 M NaCl at a flow rate of 50 mL/h. The column is then washed with 4 column volumes of TGE buffer + 0.3 M NaCl. No detectable protein is eluted from the column at this step. The column is then washed with 2 column volumes of TGE buffer + 0.6 M NaCl. The SSB protein is eluted from the ss DNA–cellulose column with TGE buffer + 2 M NaCl.

We have found that the intermediate wash step of TGE buffer + 0.6 M NaCl occasionally elutes variable amounts of SSB protein from the column, depending on the column binding density (i.e., the amount of SSB protein applied to the column). As the DNA on the column approaches saturation, more SSB protein is eluted from the column in the 0.6 M NaCl wash. Therefore, if a 0.6 M NaCl wash is included, care must be taken when loading the SSB protein to remain well below the saturation limit of the column. This is why we only load half of fraction III onto a 100-mL ss DNA column. The nucleic acid binding characteristics of the SSB protein that elutes in the 0.6 M NaCl wash are indistinguishable from those of the SSB protein eluted in the 2 M NaCl wash, on the basis of the quantitative measurement of equilibrium binding constants with poly(U), monitoring the quenching of the intrinsic tryptophan fluorescence of the SSB protein. The SSB protein from a 0.6 M NaCl wash rechromatographed identically with the SSB protein from the 2 M NaCl wash when both were reapplied to fresh ss DNA–cellulose columns at lower protein to DNA ratios.

The pooled fractions from the ss DNA–cellulose column are concentrated either by dialysis vs. solid poly(ethylene glycol) (PEG 6000) or by addition of solid ammonium sulfate to a final saturation of 40%, followed by resuspension of the pellet in "storage buffer" and further dialysis vs. the same buffer. The SSB protein is stored at −20 °C, without freezing at concentrations between 2 and 5 mg/mL. Recall that storage buffer contains 0.5 M NaCl and 50% (v/v) glycerol. Note that if low NaCl concentrations are used in the SSB protein storage buffer, the SSB protein will precipitate during storage; the solubility of SSB protein at 4 °C decreases significantly in [NaCl] below 0.1 M.

Discussion

The purification method described here is rapid and results in >99% pure SSB protein in approximately 7 h, without any columns, yielding ~3 mg of SSB protein/g of induced K12ΔH1Δtrp/pTL119A-5. This is due mainly to the temperature-inducible overproducing plasmid pTL119A-5. However, the purification has also been significantly simplified. In pilot studies, we have varied the solution conditions (mainly [NaCl]) for Polymin P precipitation and reextraction of the polymin P pellet to obtain the optimal conditions for purification of the SSB protein using the systematic procedure suggested by Burgess & Jendrisak (1975). The fact that SSB

protein precipitates in solutions of relatively low ammonium sulfate concentration (≤27% saturation) also contributes significantly to the ease of purification.

The Polymin P precipitation steps in this procedure have been optimized for SSB protein purification. For example, the [NaCl] used to extract SSB protein from the 0.4% Polymin P pellet should not exceed 0.4 M NaCl, since RNA polymerase is extracted at [NaCl] > 0.4 M (Burgess & Jendrisak, 1975). Any slight RNA polymerase contamination of the supernatant from the 0.4 M NaCl extraction of the Polymin P pellet (Fraction II) is eliminated in the next step, since SSB protein precipitates at a much lower ammonium sulfate concentration than does RNA polymerase (Burgess & Jendrisak, 1975). This point is of particular importance in obtaining >99% pure SSB protein without the use of columns.

Even though we can obtain >99% pure SSB protein in the absence of any columns, we routinely use a single-stranded DNA–cellulose column as the last step in the purification. This is included to ensure that the purified SSB protein has DNA binding activity. However, we have always observed that greater than 98% of the SSB protein from fraction III of the procedure binds tightly to ss DNA. The rapid procedure reported here essentially eliminates the amount of proteolytically degraded SSB protein, SSB^*_T and SSB^*_C (Williams et al., 1983), that is quite often found in SSB protein preparations, since this purification procedure is completed in such a short time.

The SSB protein, purified by the procedure reported here, behaves identically with SSB protein isolated by the procedure of Chase et al. (1980), as well as by a procedure developed by McMacken et al. (personal communication). This conclusion is based on quantitative measurements of the equilibrium constant for binding of SSB protein to synthetic homopolynucleotides under well-defined solution conditions.

It is of interest that the large-scale overproduction of the *ssb* gene product is not lethal to *E. coli*. We have grown K12ΔH1Δtrp/pTL119A-5 through four restreakings on agar plates at 42 °C, where high levels of the *ssb* gene product are maintained without loss of the plasmid or any apparent decrease in cell viability. Even when we have induced K12ΔH1Δtrp/pTL119A-5 and allowed these cells to grow at 37 °C for 20 h, the cells retained the plasmid, continued to express the *ssb* gene product, and remained viable. It is also interesting that the λ P_L promoter is ~1500 base pairs from the SSB promoter (P_{ssb}) and the *ssb* gene in our overproducing plasmid and yet significant transcription of the *ssb* gene, initiated at λ P_L, still occurs (at least 300-fold over wild type). Although pTL119A-5, derived from pPLc 28 (Remaut et al., 1981), does contain a *nut* site, the K12ΔH1Δtrp lysogen does not express a functional *N* gene product; hence, *N*-mediated antitermination cannot operate in this host, and it is likely that no terminators exist in the ~1500 base pair stretch between λ P_L and the *ssb* gene. Similarly, high levels of the *ssb-1* gene product, a temperature-sensitive mutant of the SSB protein, have been reported by Williams et al. (1984), who also used a λ P_L expression vector to overproduce the mutant SSB-1 protein.

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