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Amplification of single-strand DNA binding protein in Escherichia coli

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ABSTRACT

An E. coli strain containing a recombinant plasmid carrying the E. coli ssbA+ gene has been shown to produce 12 to 15 fold increased amounts of single-strand DNA binding-protein relative to wild-type strains. In addition, a λ transducing phage carrying the E. coli uvrA+ gene has been shown to also carry the ssbA+ gene and to be capable of producing increased amounts of binding protein.

INTRODUCTION

Numerous studies have suggested that single-strand DNA binding proteins play a central role in DNA replication, recombination, and repair (1-4). In vitro studies utilizing defined protein fractions have demonstrated that this protein is essential in various bacteriophage replication systems (5). Recently strains of E. coli have been identified which are defective in single-strand DNA binding protein (6 & 7). The analysis of these mutant strains will greatly aid in the determination of the role of DNA binding protein in E. coli DNA metabolism. All of these studies necessitate the purification of large quantities of single-strand DNA binding protein from both wild type and mutant strains. Mapping studies have shown that the structural gene for DNA binding protein, ssbA, is located very close to the right of the uvrA gene on the E. coli K12 linkage map (8). Recently Sancar and Rupp (9) have shown that a recombinant plasmid carrying the uvrA+ gene also carries the ssbA+ gene. We have examined a λuvrA+ transducing phage and shown that it carries the ssbA+ gene as well. We report here the quantities of single-strand DNA binding protein which can be obtained from the plasmid containing strain and a lysogen of the transducing phage. A procedure for the preparation of large quantities of the wild type protein is also described.

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MATERIALS AND METHODS

Bacteria and Phage

Bacterial strains and phage used are listed in Table 1. A detailed analysis of the properties of the ssbA1 and lexC113 mutant strains used in this study has been made and is the subject of a separate report (10). Procedures for phage infection and preparation of lysogenic derivatives were as previously described (11).

UV Survival

Survival following UV irradiation was determined as previously described (11).

Media

Cells were grown either in T-broth (10 gm Bactotryptone, 5 gm NaCl per liter of water) or L-broth (T-broth with 0.5% (wt/vol) yeast extract). T-broth containing 10 mM $MgSO_4$ and 0.2% (wt/vol) maltose was used for phage infections. Lambda adsorption buffer is 5 mM Tris-HCl (pH 7.4), 10 mM $MgSO_4$ and 0.005% gelatin.

Assay of DNA Binding Protein

DNA binding protein in either crude or pure fractions was assayed by a filter binding procedure (12).

Filter Preparation. Salmon sperm DNA (Sigma Type III) was dissolved in 10 mM Tris-HCl buffer (pH 8.1) to 2.5 mg/ml. The DNA (200 ml) was degraded

Table 1. Bacterial Strains and Phage

Strain/Phage	Genotype or Bacterial Genes Carried	Source
Strain		
CSR603	F^- uvrA6 recA1 phr-1 rpsL31 $nalA^-$	Sancar & Rupp (9)
CSR603/2000	CSR603 carrying plasmid pDR2000	Sancar & Rupp (9)
KLC436	F^- mel^- rha^- thy^- ssbA1	Vales et al. (10)
KLC438	F^- mel^- rha^- thy^-	Vales et al. (10)
KLC484	F^- mel^- rha^- thy^- lexC113	Vales et al. (10)
KLC503	$\lambda uvrA^+$ lysogen of KLC438	This paper
NH5051(λind^-)	λind^- lysogen of Ymel	Auerbach
NH5168(λind^-)	Thy+ λind^- derivative of KLC436	Auerbach
NH5170(λind^-)	λind^- lysogen of KLC484	Auerbach
NH4509(λind^-)	F^- end^- gal^- thi^- thy^- uvrA6 λind^-	Auerbach
AB3062(λind^-)	F^- end^- xyl^- thi^- thy^- uvrB5 λind^-	Auerbach
Phage		
λuvrAS	$\lambda uvrA^+$ Δ(int xis red gam cIII) cI857 Sam7	Auerbach & Howard-Flanders (11)
Y829	$\lambda uvrB^+$ Δ(int xis red gam cIII) cI857 Sam100	Gottesman
KAC3	λcI857Sam7	Chase

by sonic irradiation (10 min at 90 watts using a Bronson W185 sonifier) and then heat-denatured (15 min in a boiling water bath followed by rapid cooling in an ice bath). Eight by ten inch sheets of HAMK paper (Millipore) wetted briefly in distilled water were mounted into a specially made filtering apparatus and 500 ml (1.2 ml/cm^2) of 12 X SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.4) was filtered through followed by 105 mg (0.25 mg/cm^2) of the salmon sperm DNA in 500 ml of the same buffer. The filter sheet was washed twice with 500 ml (1.2 ml/cm^2) of 12 X SSC at a speed (ca. 1.5 ml/cm^2-min) such that 50 seconds were required for each half liter aliquot. After several hours of drying at room temperature the filter sheets were baked for 3 hours at 80°C in a vacuum oven. Filters (24 mm) were punched from the sheet.

Nucleic Acid Preparation. T7 $[^3H]$ DNA was prepared by the procedure of Hinkle and Chamberlin (13). The purified DNA was broken by sonic irradiation and heat-denatured. Concentrations of DNA are expressed as equivalents of nucleotide phosphorous.

Filter Assay. Assay buffer consisted of 6 mM EDTA in 3 X SSC unless otherwise specified. Prior to filtering, samples were incubated at 25°C for 30 min together with 120 pmoles of sonically irradiated denatured T7 $[^3H]$ DNA in 1 ml of assay buffer. Filters were pre-soaked 1 to 5 min and washed with 5 ml of assay buffer. The pre-incubated sample was diluted to 5 ml and passed through the filter at a vacuum pressure equivalent to 82 mm Hg so that the filtering speed did not exceed 15 ml/min. The filter was then washed twice with 5 ml aliquots of assay buffer, dried and counted in a Packard Tri Carb scintillation spectrometer. The scintillation fluid consisted of 4.0 g/liter 2,5-Diphenyloxazole and 0.05 g/liter 1,4-Bis-2-(5-phenyloxazolyl) benzene dissolved in toluene. One unit was defined as the amount of activity capable of retaining 1 nmole of DNA on the filter.

Preparation of Crude Extracts. Infected cells were pelleted by centrifugation and resuspended in 10 mM Tris-HCl buffer (pH 8.1) containing 5 mM EDTA to a concentration of 5 X 10^9 bacteria per ml. These suspensions were sonically irradiated at 40 watts for 1 min using a Bronson W185 sonifier and clarified by centrifugation for 10 min at 10,000 rpm in the Sorval SS34 rotor. Binding protein was then assayed directly in these extracts.

Antibody Preparation. Rabbits were injected intramuscularly with 75 µg of homogenous Eco HD-Protein I mixed with an equal volume of complete Freunds adjuvant. Subsequent inoculations were performed each week for 9 weeks using 75 µg of the same preparation mixed with incomplete Freunds adjuvant. Serum

was collected one week after the final injection, and the γ-globulin fraction was prepared as described by Amemiya et al. (14). Under the standard binding assay conditions no single-strand specific DNAase activity could be detected using 160 μg of antibody.

Nuclease Assays

Ribonuclease III was assayed as previously described (15). Nuclease activity toward single-stranded DNA and RNA and ribonuclease H activity were determined in a reaction containing 6.7 mM Tris-HCl buffer (pH 8.0), 6.7 mM $MgCl_2$, 6.7 mM mercaptoethanol, 30 μg/ml bovine serum albumin and 0.25 nmoles of either denatured $[^3H]$ T7 DNA (17,000 cpm per nmole) or $[^3H]$polyA (33,000 cpm per nmole) alone or annealed to an equal molar amount of polydT. One unit of activity is defined as the amount causing the production of 1.0 nmole of acid-soluble $[^3H]$ in 30 min at 37°C. Endonuclease activity was determined using ØX174 single-stranded and RFI DNAs. Analyses wereperformed by electrophoresis in 1.5% agarose gels.

Polyacrylamide Gel Electrophoresis

SDS gels (15%) or gradient gels (10-20%) (approximately 10 x 14 cm) were prepared using the buffer system of Laemmli (16). The gels contained a 3% polyacrylamide stacking gel of approximately 1 cm in length. Samples (up to 50 μl) were prepared for electrophoresis by addition of Laemmli sample buffer (in 10 μl) and immersion in boiling water for 2 min. Gels were stained with 0.05% Coomassie brilliant blue G in 50% methanol, 7% acetic acid.

Other Methods

Denatured DNA-cellulose was prepared as described by Alberts and Herric (17) using calf thymus DNA (Worthington). Protein was determined by the method of Lowry et al. (18). All protein determinations in this paper have been made using the method of Lowry for consistency with other published purification procedures. In pure fractions, the concentration of DNA binding protein can also be determined using a molar extinction coefficient of 3.3×10^4 at 280 nm (19). Comparing determinations made by both of these procedures it is apparent that the Lowry method over-extimates the concentration of binding protein by approximately 30%.

RESULTS

Overproduction of DNA Binding Protein by an E. coli Strain Carrying a Recombinant Plasmid Containing the $ssbA^+$ Gene

Sancar and Rupp (9) have constructed a recombinant plasmid (pDR2000) containing E. coli genes from the uvrA region of the chromosome and have

presented evidence suggesting that this plasmid also carries the $ssbA^+$ gene. Strains of this type normally amplify the copy number of the plasmid relative to the bacterial chromosome and may therefore produce increased quantities of the products of the integrated genes. In order to determine if this strain (CSR603/2000) produced increased amounts of single-strand DNA binding protein relative to the parental strain (CSR603) we determined the specific activity of DNA binding protein directly in crude extracts of each strain as described in Methods. The results of this analysis (Table 2) suggest that strain CSR603/2000 produces approximately 12 times more binding protein than the parental strain. In order to confirm that the activity measured was in fact single-strand DNA binding protein, an antibody inhibition experiment was performed. The results of this experiment demonstrated that anti-binding protein serum prepared against binding protein isolated from a strain not carrying the $ssbA^+$ plasmid inhibited the binding protein activity in extracts of the $ssbA^+$ plasmid carrying strain by more than 95% (data not shown).

Purification of Single-Strand DNA Binding Protein from Strain CSR603/2000

The procedure of Molineux *et al.* (20) was modified in order to obtain a high yield of homogeneous binding protein from strain CSR603/2000. These changes were necessitated by aggregation of the binding protein, especially in the early steps, which result in 80 to 95% losses of the protein before the DNA-cellulose step if the original procedure is followed. The procedure that was adapted and the specific reasons for the changes from the original procedure are detailed below. A summary of the purification is given in Table 3 and is designed for 100 gm cell paste. All operations were performed at 4°C. Buffer A is 20 mM TRIS-HCl buffer (pH 8.1), 1 mM 2-mercaptoethanol, 10% glycerol.

Growth of Cells. *Escherichia coli* CSR603/2000 was grown at 37°C under forced aeration in a Fermocell (New Brunswick Scientific) in 100 liter of L-broth supplemented with 0.1% glucose, 20 µg per ml of thymine and 0.01 M potassium phosphate buffer (pH 7.4). The pH was maintained by the addition

Table 2. Amplification of DNA Binding Protein by Strain CSR603/2000

Strain	Plasmid pDR2000	Specific Activity of DNA-Binding Protein (units/mg)
CSR603	-	1.6
CSR603/2000	+	19.5

Table 3. Purification of DNA Binding Protein

Fraction	Activity	Protein	Specific Activity	Yield
	units x 10^{-4}	mg/ml	units/mg protein	%
I Extract	26.2	22.7	25.4	100
II Polyethylene Glycol Supernatant	24.3	7.85	50	93
III Dialysis	16.8	3.9	620	64
IV DNA-Cellulose	9.8	0.062	2650	37
V DEAE-Sephadex A-50	8.4	0.45	2750	32

of 50% NaOH. Cells were harvested at OD_{590}=6 and stored at -80°C. Approximately 600 to 700 gm cell paste was obtained from each fermentation. It should also be pointed out that the plasmid in this genetic background has been extremely stable in our hands.

Preparation of Extract. Frozen cells (106 gm) were suspended in 200 ml of 20 mM TRIS-HCl buffer (pH 8.1), 1 mM EDTA, 1 mM 2-mercaptoethanol and 2 M NaCl (lysis buffer). Cells were broken by 50 min of sonic irradiation in a Heat Systems Model W-375 sonicator at an output setting of 8 with a 50% duty cycle. Additional lysis buffer (200 ml) was added and the extract was centrifuged for 30 min at 9000 rpm in the Sorval GS3 rotor to remove debris. The supernatant fluid was removed. The final volume was 455 ml (Fraction I). Variable loses of binding protein in the polyethylene glycol precipitation step (see below) amounting to 50% or more occurred unless the volume of the crude extract was nearly doubled relative to the original procedure of Molineux et al. (20).

Polyethylene Glycol Precipitation. To Fraction I, 1/2 volume of a 30% solution of polyethylene glycol in Lysis buffer was added. The solution was stirred slowly for 5 min and was then allowed to stand on ice for 45 min. The nucleic acid precipitate was removed by centrifugation for 30 min at 9000 rpm in the Sorval GS3 rotor. The supernatant fluid was removed. The final volume was 620 ml (Fraction II).

Dialysis. Fraction II was dialyzed for 16 hours against four 4 liter changes of 20 mM TRIS-HCl buffer (pH 8.1), 1 mM mercaptoethanol, 5 mM EDTA and 10% (w/v) glycerol. The heavy precipitate that forms contains 70 to 80% of the DNA binding protein and was removed by centrifugation for 30 min at 9000 rpm in the Sorval GS3 rotor. The precipitate was resuspended with the aid of a tight Dounce homogenizer in Buffer A containing 0.6 M NaCl. The solution was clarified by centrifugation for 10 min at 10,000 rpm in a

Sorval SS34 rotor. The supernatant fluid was removed. The final volume was 70 ml (Fraction III). The formation of this precipitate upon dialysis can be prevented by dialysis against 0.6 M NaCl in the same dialysis buffer. However, it is advantageous to reduce the volume of the fraction applied to DNA-cellulose (see below). In addition, a 12 fold purification is obtained with this precipitation.

DNA-Cellulose Chromatography. Fraction III was passed through a column (4 x 8 cm) (100 ml) of denatured DNA-cellulose at a flow rate of 50 ml per hour. The column was washed with 10 volumes of Buffer A containing 0.6 M NaCl and then with 5 volumes of 2 M NaCl in the same buffer. The 2 M eluate was dialyzed for 8 hours against four 2 liter changes of Buffer A containing 80 mM NaCl. The volume after dialysis was 595 ml (Fraction IV). The DNA cellulose column was run with only one intermediate elution rather than two as in the procedure of Molineux et al. (20). We have found that variable amounts of binding protein elute from DNA cellulose with both 0.8 M and 1 M NaCl. Little or no binding protein has been detected in the 0.6 M eluate. The 2 M eluate contains DNA binding protein (approximately 95% pure), ribonuclease H activity, exonuclease I activity and small amounts of RNA polymerase.

DEAE-Sephadex Chromatography. Fraction IV was applied to a column (1.9 x 27 cm) (75 ml) of DEAE-sephadex A-50 equilibrated in Buffer A containing 80 mM NaCl and washed with 10 column volumes of the same solution at a flow rate of 35 ml per hour. Due to the very large effect of swelling of DEAE-sephadex upon ionic strength (especially at very low ionic strength) we have equilibrated the material in 80 mM NaCl rather than in buffer without salt as in the procedure of Molineux et al. (20). In addition, most of the contaminating ribonuclease H is removed at this step if the column is washed extensively with buffer containing 80 mM NaCl. A linear gradient (800 ml) of 80 mM to 0.5 M NaCl in Buffer A was applied and 10 ml fractions were collected. Binding protein elutes under these conditions at an ionic strength equivalent to approximately 0.2 M NaCl. The conductivity of the gradient fractions was determined and every second fraction in the range of 0.2 M to 0.3 M NaCl was analyzed by polyacrylamide gel electrophoresis or by direct assay of binding protein (12). In addition, these fractions were assayed for ribonuclease H and exonuclease I activities. Most of the ribonuclease H activity present in the 2 M DNA-cellulose eluate passes through DEAE-sephadex in 80 mM NaCl in Buffer A. Exonuclease I primarily elutes after binding protein, however small amounts overlap it. RNA polymerase elutes

after binding protein under these conditions (0.3 to 0.4 M NaCl) and represents no problem in pooling the binding protein fractions. All fractions containing binding protein substantially free of nuclease contamination were pooled. The final volume was 76 ml (Fraction V).

Concentration. If it was necessary to have a binding protein preparation more concentrated than the pooled DEAE-sephadex fraction, two procedures where used for concentration: (1) solid ammonium sulfate was added to a final saturation of 65%, stirred for one hour on ice and centrifuged for 30 min at 35,000 rpm in the Beckman 45Ti rotor. The precipitate was dissolved in Buffer A containing 0.3 M NaCl such that the final protein concentration was 1 to 2 mg per ml and dialyzed for four hours against 0.3 M NaCl in Buffer A. This fraction was stored at -20°C either frozen or glycerol was added to 50% (w/v). (2) The pooled DEAE-sephadex fraction was dialyzed against 80 mM NaCl in Buffer A and the binding protein was absorbed to a column (4 ml) of DEAE-cellulose at a flow rate of 20 ml per hour. The column was washed with 4 column volumes of Buffer A containing 80 mM NaCl. Binding protein was eluted with Buffer A containing 0.3 M NaCl at a flow rate of 5 ml per hour and 2 ml fractions were collected. The protein concentration was usually adjusted to 1 to 2 mg per ml and stored as above.

Yield. The overall yield of DNA binding protein from the simple purification reported (Table 3) was 32% relative to the crude extract. This is approximately 30 times that we have obtained from a similar quantity of cells not carrying the pDR2000 plasmid using the original procedure of Molineux *et al.* (20).

Purity. Analysis by polyacrylamide gel electrophoresis under denaturing conditions of 20 μg of Fraction V after concentration gave a single major band and one minor band of approximately 70,000 molecular weight which we estimate to represent $<1\%$ of the total protein. This minor band may be the exonuclease I subunit (21). No detectable activity of ribonuclease III or single-strand specific ribonuclease was found. The preparation contained 0.6 units of ribonuclease H activity and 2.8 units of exonuclease I activity per mg of binding protein. Using 1 μg of binding protein no degradation of ØX174 single-stranded DNA (1.5 nmoles) could be detected after 30 min incubation at 30°C. Incubation of binding protein with ØX174 RFI DNA produced topological isomers indicating the presence of some nicking-closing type activity.

λuvrA$^+$ Complements the UV Sensitivity of an ssbA1 Mutant Strain

A lambda transducing phage, λuvrA$^+$, isolated from a pool of λ phages carrying EcoR1 fragments of E. coli DNA (11) was used to infect a λind$^-$ lysogen of an E. coli strain defective in DNA binding protein. One of the phenotypic properties directly attributed to a defect in DNA binding protein is sensitivity to ultraviolet irradiation (UV) (8, 10). The results (Fig.1A) demonstrate that infection with the λuvrA$^+$ transducing phage rapidly suppresses the UV sensitivity of the lysogenic strain. The strain gradually becomes UV sensitive again as the λuvrA$^+$ DNA is diluted through replication of the lysogen and destroyed (data not shown). Since the lexC113 mutation may be allelic with the ssbA1 mutation (8) a similar experiment was performed with a λind$^-$ lysogen of a lexC113 mutant strain (Fig. 1B). The UV sensitivity of this strain was also suppressed. In control experiments, the ssbA1 and lexC113 mutant strains were each infected with λuvrB$^+$ transducing phage

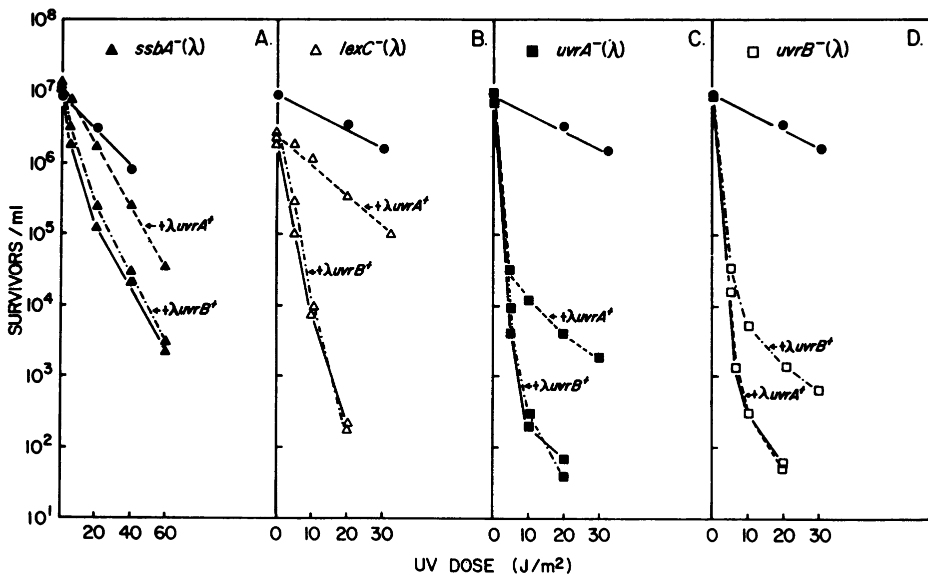

Figure 1. UV survival of ssbA1 and lexC113 mutant strain following infection with λuvrA$^+$ transducing phage. UV survival of lysogenic derivatives was determined after a one hour incubation at 37°C in the presence or absence of λuvrA$^+$ or λuvrB$^+$ phage, as indicated. The multiplicity of infection was 5. The cultures were incubated in λ adsorption buffer supplemented with 100 μg/ml thymine. Symbols: wild type (λind$^-$) (NH5051), ●——●; ssbA1 (λind$^-$) (NH5168), ▲——▲; lexC113 (λind$^-$) (NH5170), △——△; uvrA6 (λind$^-$)(NH4509), ■——■; uvrB5 (λind$^-$) (AB3062), □——□. Infection with λuvrA$^+$ (---) and λuvrB$^+$ (-•-•-) transducing phage.

which, as expected, did not suppress the UV sensitivity of either strain. In addition, the λuvrA$^+$ and λuvrB$^+$ transducing phages were shown only to suppress the UV sensitivity of λind$^-$ lysogens of uvrA$^-$ and uvrB$^-$ mutant strains, respectively (Fig. 1C and D). The results of these studies suggest that the λuvrA$^+$ transducing phage may carry the ssbA$^+$ and lexC$^+$ genes as well as the uvrA$^+$ gene.

Lambda uvrA$^+$ Transducing Phage Carries the ssbA$^+$ Gene

A strain of E. coli defective in DNA binding protein (KLC436 ssbA1) was infected with λuvrA$^+$ transducing phage at a moi=5 and at various times after the infection the cells were assayed for single-strand DNA binding protein directly in crude extracts (12). The results (Fig. 2) demonstrate that 5 to 10 min after infection, DNA binding protein began to be produced by the infected cells. The protein continued to increase for the next 50 min and plateaued by 60 min after infection. Under these conditions the final specific activity for the protein was 3 times higher than that of the wild type strain. This binding activity was more than 95% inhibited by antibody prepared against wild type DNA binding protein (data not shown). In addition, a similar infection carried out using λcI857S7 produced no detectable binding activity. We conclude that the λuvrA$^+$ transducing phage also carries the ssbA$^+$ gene.

It should be noted that under the conditions of the filter assay for

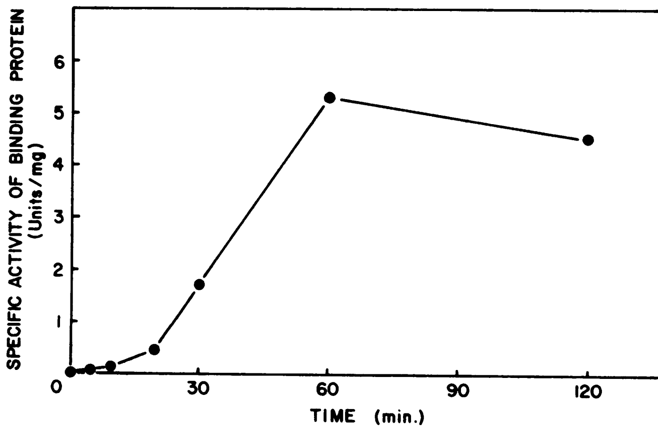

Figure 2. Synthesis of DNA binding protein following infection of an ssbA1 mutant strain (KLC436) with λuvrA$^+$ transducing phage (moi=5). DNA binding protein was assayed directly in crude extracts as described in Methods.

DNA binding protein in crude extracts essentially no activity was detected in the *ssbA1* mutant strain. This is probably due to the high ionic strength employed in crude extract determinations and suggests that the *ssbA1* mutant protein is greatly reduced in its binding ability to single-stranded DNA under these conditions.

The *ssbA*$^+$ Gene Carried by the *λuvrA*$^+$ Transducing Phage is Under Control of a Weak Promoter

In order to evaluate the potential usefulness of the *λuvrA*$^+$ transducing phage as a means of producing increased quantities of DNA binding protein we prepared a lysogenic derivative of a wild type strain. The specific activity of binding protein in the uninduced lysogen, KLC503, was 3.4 units per mg compared to 1.7 units per mg for the wild type strain, KLC438. This apparent gene dosage effect in the uninduced lysogen and the observation that complementation of the defect in an *ssbA1* mutant strain occurs in a lysogen suggest that the *ssbA*$^+$ gene carried by the *λuvrA*$^+$ transducing phage is under an *E. coli* promoter. The specific activity of binding protein in dilysogenic derivatives of wild type strains has been found to increase approximately 5 fold after induction (data not shown). Although increased quantities of binding protein can be produced by this transducing phage, these results suggest that the usefulness of the *λuvrA*$^+$ transducing phage as a general means of obtaining binding protein overproduction is limited unless the *ssbA*$^+$ gene is placed under control of a stronger promoter.

DISCUSSION

The large quantities of DNA binding protein which are currently being used, particularly for *in vitro* DNA replication studies, make it advantageous to be able to quickly purify large amounts of the protein. Two strains of *E. coli* carrying vectors which are able to increase the number of copies of the *ssbA*$^+$ gene have been investigated for their ability to produce increased quantities of single-strand DNA binding protein. A λ transducing phage carrying *E. coli* genes from the region of the chromosome near the *uvrA*$^+$ gene has been shown to also carry the *ssbA*$^+$ gene and the *lexC*$^+$ gene as well. At this time definitive proof that the *ssbA* and *lexC* loci are identical has not yet been shown; however, several reports in the literature (6, 8, 22) suggest that this is the case. Although infection of non-lysogenic strains and induction of dilysogenic derivatives of wild type strains does yield quantities of binding protein 5 to 10 fold above the levels found in

wild type cells, we have been unable to find conditions which increase these amounts further. The fact that uninduced single and dilysogens of wild type and ssbA1 mutant strains show clear gene dosage effects suggest that the $ssbA^+$ gene carried by the λ transducing phage is under control of an E. coli promoter. These results suggest that it will be necessary to place the $ssbA^+$ gene under the control of a stronger promoter before greater increases in binding protein can be obtained from this vector.

We have also found that a strain carrying a recombinant plasmid derivative of pBR322 containing an E. coli DNA fragment from the uvrA - ssbA region of the chromosome (9) also produces increased quantities of DNA binding protein. Amounts of binding protein from 11 to 15 fold in excess of that obtained from the parental strain have been found. In view of the quantity of binding protein that can be obtained from the plasmid derivative and the technical ease in growing and maintaining the strain, this appears to be the strain of choice at the present time for the preparation of binding protein. Our initial attempts to isolate binding-protein from this strain by a standard procedure (20) were hampered by large losses in the early steps of the purification. A number of changes were therefore made in this procedure which are described in detail above (see Results). The procedure which was finally adopted is rapid and gives pure binding protein in high yield. We found it most difficult to rid our binding protein preparations of trace amounts of exonuclease I. A derivative of CSR603/2000 with an sbc (xon) deletion would therefore be particularly valuable.

Finally, it is interesting to note that the increased production of binding-protein both by the plasmid containing strain and following induction of a lysogen of the $\lambda uvrA^+$ transducing phage, as well as the observed gene dosage effect of the uninduced lysogen, suggest that if the cell controls the production of binding protein, it is not stringent and probably not autogenously regulated as is the phage T4 binding protein (gene 32 protein) (23).

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