Identification and purification of the Lon+ (capR+) gene product, a DNA-binding protein

(plasmids/radiation sensitivity/cell division/proteolysis/capsular polysaccharide)

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ABSTRACT   The polypeptide product of the *lon* (*capR*) gene was identified and partially purified from bacterial strains homozygous for the *capR+* or *capR9* (ochre mutation) alleles cloned with pSC101. A 94,000-dalton polypeptide was identified as the lon (capR) gene product. Studies of binding to DNA cellulose columns and nitrocellulose filters indicate that the capR+ and capR9 proteins bind DNA.

capR (*lon*) mutants of *Escherichia coli* K-12 are sensitive to UV light and ionizing radiation, and they overproduce capsular polysaccharide (colanic acid), as well as 10 enzymes involved in colanic acid synthesis (1). After irradiation, *capR* strains form nonseptate filaments that die (2–4). *capR* mutants of *E. coli* K-12 are very likely mutant in the same gene as is *E. coli* B (5), a radiation-sensitive strain of bacteria discovered by Witkin (6). *capR* mutants also exhibit a reduced capacity to degrade abnormal (7–9) as well as normal (10) proteins. The use of *in vitro* cloning techniques permitted us to clone the *capR+* (*lon+* gene on an 8,200,000–dalton *Eco*RI DNA fragment (11, 12). The *capR+* plasmids (pBZ201 and pBZ203) specified two new polypeptides in minicells and maxicells (*recA, uvrA, phr*; ref. 13) having M_rs of 94,000 and 67,000 as determined by NaDodSO₄/polyacrylamide gel electrophoresis. Plasmids containing recessive *capR* mutations were deficient in synthesis of 94,000-dalton (dal)-polypeptide in maxicells, and a plasmid containing a dominant *capR* allele (*capR9*) overproduced a polypeptide with the same electrophoretic mobility as the 94-kDal one (12). These observations suggested that the 94-kDal species was the capR gene product that was defective in autoregulation in the strain containing the *capR9* allele. However, in the absence of data showing that the capR9 form of the polypeptide was altered, another plausible interpretation is that the recessive and dominant mutations were in the *capR* gene that regulates synthesis of a second gene that specifies the 94-kDal polypeptide. In the present study, we partially purified the 94-kDal polypeptide from *capR+* and *capR9* homogenotes. Biochemical evidence is presented showing that the native form of the capR+-specified 94-kDal polypeptide is altered in the *capR9* mutant and thus the *capR* (*lon*) gene is the structural gene for it. The purified capR+ and capR9 proteins each bind to DNA with certain differences that are presented below.

MATERIALS AND METHODS

Buffers. Buffer A was 100 mM K₂HPO₄-KH₂PO₄, pH 6.5/10 mM 2-mercaptoethanol/1 mM EDTA/20% glycerol (vol/vol). Buffer B was 10 mM Tris·HCl, pH 7.1 at 25°C/1 mM 2-mercaptoethanol/1 mM EDTA/20% glycerol (vol/vol) 20 mM NaCl. Buffer C was 20 mM Tris·HCl, pH 7.5 at 25°C/50 mM NaCl/5 mM MgCl₂/0.1 mM EDTA/1 mM dithiothreitol/20% glycerol (vol/vol). Buffer D was 20 mM K₂ HPO₄-KH₂PO₄, pH 6.8/50 mM NaCl/1 mM 2-mercaptoethanol/1 mM EDTA/10% or 20% glycerol (vol/vol) as indicated.

Bacterial Strains and Media. *E. coli* K-12 strains were RGC121/pBZ201 [homozygous strain containing the wild-type allele (*capR+* (*lon+*) on the chromosome and on the plasmid] and RGC123/pBZ201M9 (homozygous strain containing the *capR9* allele on the chromosome and on the plasmid; ref. 12). Complex medium was 5 g of yeast extract/10 g of tryptone/10 g of NaCl in 1 liter supplemented with tetracycline at 3–5 μg/ml when the bacteria contained plasmids pBZ201 or BZ201M9.

Growth of Bacteria and Preparation of Cell-Free Extracts. Bacteria were grown in complex medium at 37°C to ≈2 × 10⁸ bacteria per ml, isotope was added, and growth was continued to stationary phase. Bacteria were harvested, washed, and suspended in buffer A, disrupted by sonic oscillation, and centrifuged at 48,000 × g for 30 min, and the supernatant was used for fractionation. The same conditions were used in the absence of isotope except that stationary-phase bacteria were harvested and disrupted by using sonic oscillation (0.5-liter cultures) or a French pressure cell and sonic oscillation (9-liter cultures).

Column Chromatography. *Phosphocellulose (PC) columns.* Protein at 20–25 mg/ml in buffer A was applied to the PC column (Whatman P11), which was then washed with buffer A. Elution was effected by application of a linear 100–400 mM phosphate gradient. Small columns were 1 × 15.5 cm; large columns were 2.6 × 20 cm. Each of two gradient chambers contained 100 ml (small columns) or 750 ml (large columns).

DEAE-cellulose columns. Appropriate fractions from the PC columns were dialyzed against buffer B and applied to 2.6 × 9.5 cm. DEAE-cellulose columns (Cellex-D, high capacity, Bio-Rad) equilibrated with buffer B. The columns were eluted in stepwise fashion with buffer B containing 0.075 M, 0.15 M, 0.2 M, 0.25 M, and 0.3 M NaCl in 5-ml fractions at a flow rate of 15–20 ml/hr. Eluents were changed when protein no longer was eluted in a series of 5–10 fractions. Smaller columns were also used.

ATP-agarose columns. Agarose/hexane/adenosine 5′-triphosphate, type 4 (AGATP; (P-L Biochemicals) columns were similar to those previously described (14). Appropriate fractions from the DEAE-cellulose columns were dialyzed against buffer C and applied to 0.5 × 2.5 cm columns previously equilibrated with buffer C. Fractions were eluted in buffer C containing 10 mM pyrophosphate, 5 mM ATP, or 5 mM AMP.

Abbreviations: Dal, dalton; PC, phosphocellulose.
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DNA-cellulose columns. Appropriate fractions from the DEAE-cellulose columns were dialyzed against buffer D and applied to a 0.5 × 1.5 cm DNA cellulose column (gift of R. Haselkorn; prepared according to Litman, ref. 15). The column was previously equilibrated with buffer D containing 0.3 mg of bovine serum albumin per ml. Fractions were eluted with stepwise increments of NaCl in buffer D as indicated.

NaDodSO₄/Polyacrylamide Gel Electrophoresis. Electrophoresis was as outlined by Laemmli (16) and modified (17).

Filter Assay for DNA Binding to Protein. The first assay was a modification of that described by Bourgeois for the *lac* repressor (18). Binding buffer contained 12 mM Tris·HCl, pH 7.4 at 24°C/10 mM KCl/12 mM magnesium acetate/0.1 mM dithiothreitol/10% (vol/vol) glycerol/5% (vol/vol) dimethyl sulfoxide/25 mM EDTA. Chicken blood DNA (Calbiochem) was present in all assays at 1.87 μg/ml (18) unless stated otherwise. Radioactive plasmid DNA ([³H]thymidine) was prepared as described (19). Two plasmids that replicate in the presence of chloramphenicol were used: (*i*) plasmid pHA105, a 1,600,000-dalton mini ColE1 DNA containing one endonuclease *Eco*RI site, and (*ii*) plasmid pHA132 containing one molecule of pHA105 and one *Eco*RI, 8,000,000-dalton fragment containing the *galETK* operon (19, 20). ³H-labeled plasmid DNA was cut with *Eco*RI (12) for all experiments. The final DNA concentration used was 0.1 μg of DNA/per ml and the activity was 10,000–20,000 cpm/ml. After incubation for 30 min at 37°C, samples were chilled to 0°C. Aliquots (0.5 ml) were filtered in triplicate on nitrocellulose filters [Schleicher & Schuell BA85, 25 mm pretreated with KOH (21)] at a rate of 0.5 ml/min and washed with 0.5 ml of filtering buffer [10 mM magnesium acetate/10 mM KCl/0.1 mM EDTA/5% (vol/vol) dimethyl sulfoxide/0.01 M Tris·HCl, pH 7.4 at 25°C]. Radioactivity was measured by dissolving the filters in 1 ml of ethyl acetate and adding 0.5 ml of water and 10 ml of Triton/toluene (22). Protein was measured with Bio-Rad reagent or by UV absorption (23).

Binding of DNA to nitrocellulose filters was also measured according to assay C, described by Shibata et al. (24), which obviates the necessity for chicken blood DNA. Millipore DAWP filters (pore size 0.65 μm), were used to retain protein–DNA complexes formed in 31 mM Tris·HCl, pH 7.4 at 25°C/6.7 mM MgCl₂/1.8 mM dithiothreitol/bovine serum albumin at 88 μg per ml. Aliquots (0.2 ml) were filtered in triplicate and washed as described (24) with 1 ml of the buffer in which the complex had been formed at a rate of approximately 0.5 ml/min. Preparations of capR⁺ (and capR9) protein purified through the DEAE-cellulose step gave similar results with both types of DNA binding assays. The method of Bourgeois (18) was used for all DNA binding assays unless stated otherwise.

Bacteriophage MS2 RNA was a gift from S. B. Weiss.

Rabbit antibody was prepared by injecting 100 μg of capR9-specified protein (purified through the DEAE-cellulose step) in Freund's adjuvant solution at weekly intervals for 3 weeks, followed by collection of blood 4 or 5 weeks after the initial injection. One percent agarose was used for Ouchterlony plates.

RESULTS

Earlier genetic studies led to the suggestion that the *capR⁺* protein was a repressor (1) and thus would be a DNA-binding protein. We chose a PC column, a matrix that resembles DNA, for initial fractionation. Because the capR⁺ protein was either the 94-kDal one or the 67-kDal one (or both) specified by the plasmid containing the *capR⁺* gene, column fractions that adsorbed and were eluted with a phosphate gradient were monitored by using NaDodSO₄/polyacrylamide gel electrophoresis with phosphorylase b (97.4 kDal; ref. 25) and bovine serum albumin (67 kDal) as standards. A 94-kDal polypeptide was adsorbed to PC and eluted by ≈0.25 M phosphate when extracts

of capR⁺ or capR9 homogenotes were chromatographed. There was no evidence for retention of a 67-kDal polypeptide.

One of our goals was to identify the capR structural gene product by showing that the product of the *capR9* allele was altered. Previous experiments suggested that the *capR9* mutant produced an altered capR protein and phenotypes of partial diploid strains could be explained if the capR protein were composed of subunits and the capR⁺ and capR9 subunits interacted physically (1, 26). A *capR⁺* homozygote was labeled with [³⁵S]methionine and a *capR9* homozygote was labeled with [³H]leucine. The labeled cells were harvested and washed, then mixed and disrupted, and the crude soluble proteins were chromatographed on PC. Each fraction was further fractionated by electrophoresis; the gel was stained and the 94-kDal polypeptide bands were cut out, and ³⁵S and ³H were determined. Control experiments in which the *capR⁺*- and *capR9*-labeled extracts were chromatographed separately each gave a single peak of radioactivity with some asymmetry of the capR⁺ peak (Fig. 1 *B* and *C*). In contrast (Fig. 1*A*), when the capR⁺ and capR9 extracts were mixed and chromatographed together, the capR⁺-specified 94-kDal polypeptide eluted in two approximately equal peaks, the later-eluting capR⁺ peak coincided with the single major (90%) capR9-specified 94-kDal peak. A control involved monitoring the ³H/³⁵S ratio of another protein eluted in the same electrophoresis gel as the 94-kDal polypeptide from
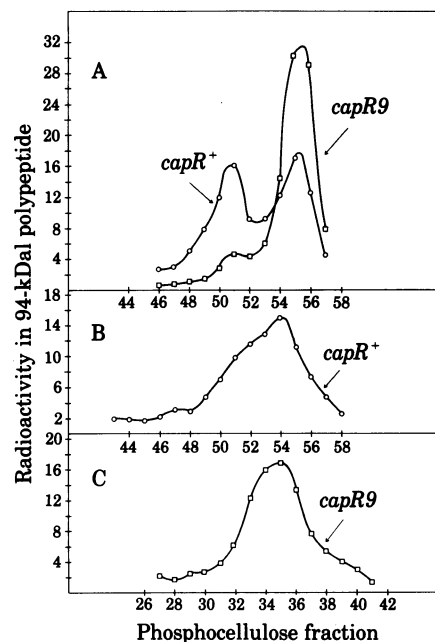

FIG. 1. Chromatography on a PC column of ³H-labeled proteins from *capR9* homogenote (RGC123/pBZ201M9) and ³⁵S-labeled proteins from *capR⁺* homogenote (RGC121/pBZ201). (*A*) Protein from 20 ml of *capR⁺* homogenote with 1.7 mCi (1 Ci = 3.7 × 10¹⁰ becquerels) of [³⁵S]methionine plus 60 ml of *capR9* homogenote with 2.7 mCi of [³H]leucine. (*B*) Protein from 80 ml of *capR⁺* homogenote with 1.7 mCi of [³⁵S]methionine. (*C*) Protein from 80 ml of *capR9* homogenote with 1.7 mCi of [³⁵S]methionine. Volumes of *capR⁺* and *capR9* cultures used for cochromatography gave approximately equal quantities of *capR⁺*- and *capR9*-derived 94-kDal monomer. (Protein extracted from *capR9* cultures is approximately 10% of that from a *capR⁺* culture of equal volume, and the amount of 94-kDal polypeptide obtained per mg of unfractionated protein is 5–10 times more from a *capR9* extract compared with a *capR⁺* extract.) Aliquots of column fractions (2.5 ml per fraction) were further fractionated on a 10–30% gradient NaDodSO₄/polyacrylamide gel. The gels were stained with Coomassie blue to locate the 94-kDal polypeptide, the pure polypeptide from each lane was cut out, and the radioactivity was determined. The total radioactivity in the *capR⁺* (4800 cpm ³⁵S) or *capR9* (4400 cpm ³H) polypeptide was set equal to 100% for each isotope individually.

capR⁺ and *capR9* homogenates. The ratio remained constant, as expected, in contrast to that for the 94-kDal polypeptide (see Fig. 1A). The results suggested that the *capR*⁺- and *capR9*-specified 94-kDal polypeptides were indeed not identical in the native state. In some control experiments similar to those shown in Fig. 1 *B* and *C* (without isotope), the elution positions of *capR*⁺- and *capR9*-specified 94-kDal polypeptides were virtually identical but, in others, both were eluted, as was the *capR9*-specified 94-kDal polypeptide (see Fig. 1C). The reason for the variability in the phosphate concentration required for elution is not known.

Appropriate fractions of the PC-purified *capR*⁺ and *capR9* 94-kDal polypeptide-containing fractions were purified on DEAE-cellulose columns. The 94-kDal polypeptide from the *capR9* homogenate was completely eluted in concentrated form (up to 1 mg/ml) from the DEAE-cellulose column by 0.15 M NaCl. In contrast. 10–40% of the 94-kDal polypeptide from the homozygous *capR*⁺ strain was eluted by 0.15 M NaCl and 60–90% was eluted by 0.2 M NaCl, and its concentration was <0.25 mg of protein/ml. Rechromatography of the *capR*⁺ (0.2 M NaCl eluate) and the *capR9* forms on DEAE-cellulose showed that a small fraction of the *capR*⁺ form again eluted with 0.15 M NaCl but the majority still eluted in the 0.2 M NaCl step. In contrast, the *capR9* form eluted at 0.15 M NaCl. The results suggested the *capR*⁺ form can convert from the "0.2 M form" to the "0.15 M form."

The *capR*⁺ (0.2 M NaCl eluate) and *capR9* proteins eluted from DEAE-cellulose were assayed for DNA binding by filter-binding assay, and both bound DNA. The binding for *gal* operon DNA appeared to be sequence independent; similar binding was observed with linear ³H-labeled mini ColE1 DNA and with linear ³H-labeled *gal* operon DNA (plus ³H-labeled mini ColE1 DNA) in the absence of chicken blood DNA. In other assays, addition of double-stranded chicken blood DNA was equally

competitive for binding with either ³H-labeled DNA preparation and single-stranded DNA was equally competitive on a molar basis. Bacteriophage MS2 RNA was also competitive for binding. The binding of DNA (³H-labeled pHA132) to the filters was reversed completely by 0.1 M NaCl, and no differences in resistance to NaCl were shown between the *capR*⁺ and the *capR9* proteins.

The DEAE-fractions of the *capR*⁺ and *capR9* forms were readily adsorbed on ATP-agarose columns. The fractionation of both *capR*⁺ (0.2 M form) and *capR9* (0.15 M form) proteins (previously purified on PC and DEAE-cellulose) on ATP-agarose columns by using the elution sequence 10 mM pyrophosphate, 5 mM ATP, 5 mM AMP is shown in Fig. 2. Electrophoresis shows that the *capR*⁺ protein elutes in two peaks, one in pyrophosphate and a second in ATP. In contrast, very little of the *capR9* protein eluted in pyrophosphate and most of it eluted in ATP. The binding of DNA to nitrocellulose filters by the *capR*⁺ protein eluted by ATP was directly proportional to protein concentration (Fig. 3). The binding activities of the purest *capR*⁺ and *capR9* proteins (ATP fractions) were identical under the assay conditions of Fig. 3 (33% DNA binding per µg of protein for *capR*⁺ and 35% DNA binding per µg of protein for *capR9*). The *capR*⁺-specified 94-kDal-containing fraction eluted with pyrophosphate had little if any DNA binding activity (i.e., <15% of the specific binding of the ATP-eluted fraction). Control experiments indicated that pyrophosphate added to the assays did not inhibit binding to DNA.

Although we monitored DNA binding throughout our purifications on PC, DEAE-cellulose, and ATP-agarose, no marked increases in specific DNA binding activity were observed after the phosphocellulose step (Table 1). This may reflect removal or loss of DNA binding proteins other than the 94-kDal-specified activity during purification but specific peaks of other activities were not intensively sought. The DNA bind-
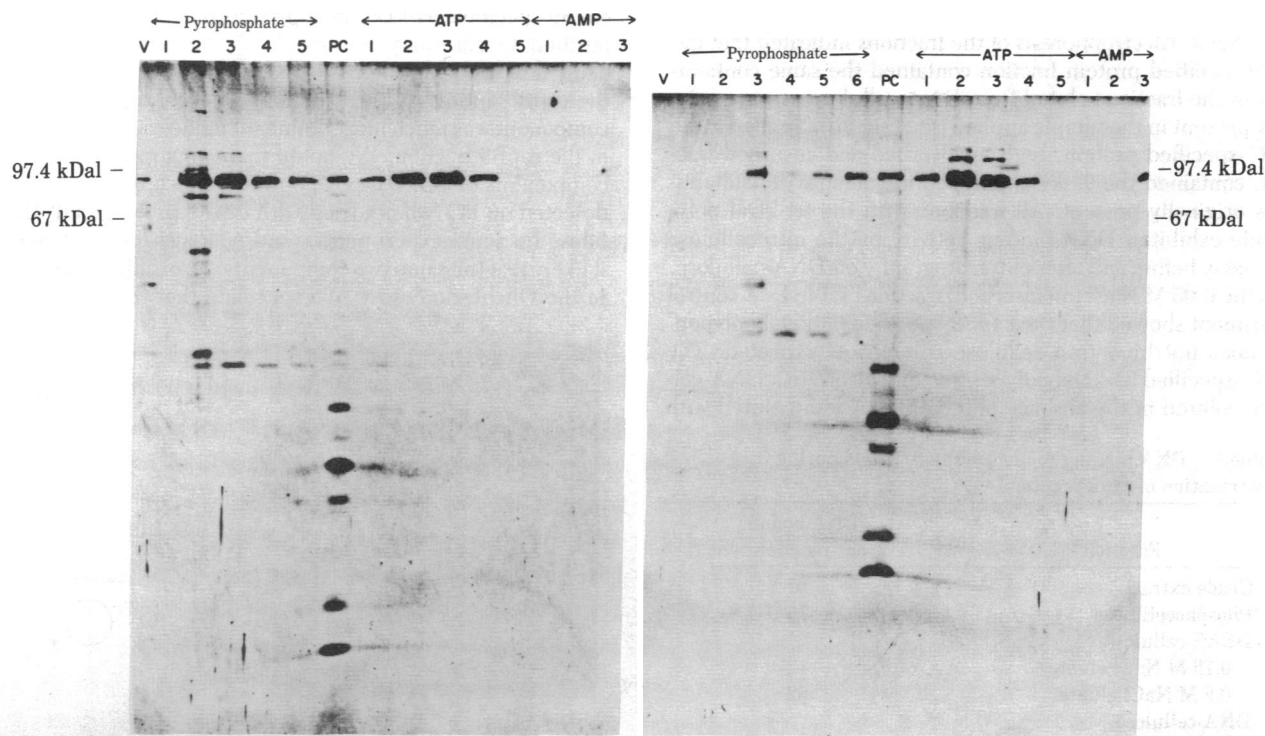

FIG. 2. NaDodSO₄/polyacrylamide gel electrophoresis (10–30% gradient) of *capR*⁺ (0.2 M DEAE fraction) and *capR9* (0.15 M DEAE fraction) further purified on agarose-ATP. (*Left*) capR⁺ protein. (*Right*) capR9 protein. Protein (0.34 mg in 3 ml) was applied to the column in buffer C, and 1-ml fractions were collected. Elution was effected with 5 ml (*Left*) or 6 ml (*Right*) of 10 mM pyrophosphate, 5 ml of 5 mM ATP, and 5 ml of 5 mM AMP. Fifty microliters of each fraction were applied per well in the gel. For the capR⁺ protein, fractions ATP-2 and ATP-3 contained 1.3 and 0.75 µg of protein, respectively, per well. For the capR9 protein, fractions ATP-2 and ATP-3 each contained 2.5 µg of protein per well. V, nonadsorbed fraction; PC, fraction from a PC column used to assess quality of gel separation.
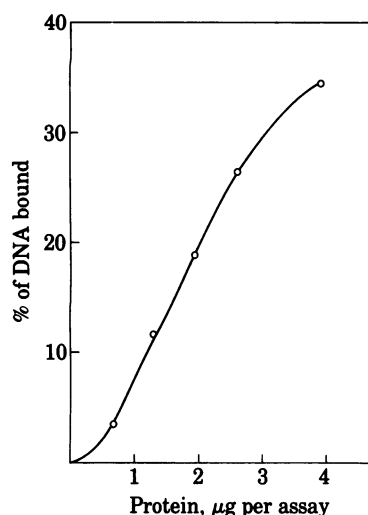
FIG. 3. DNA binding to nitrocellulose filters as a function of protein concentration. The capR⁺ protein from ATP-agarose chromatography (Fig. 2 *Left*, fraction ATP-2) was assayed for DNA binding activity with endonuclease *Eco*RI-cleaved ³H-labeled pHA132 DNA containing 0.107 μg and 17,250 cpm/ml. Four percent of the radioactivity was bound to filters in the absence of capR⁺ protein and was subtracted from the data reported.

ing properties of our purest preparation of capR⁺ protein supported the contention that the 94-kDal polypeptide was responsible for binding to DNA (Figs. 2A and 3). Further evidence was obtained by affinity chromatography of the DEAE-cellulose-purified fractions of capR⁺ (0.2 M NaCl eluate) and capR9 (0.15 M NaCl eluate) on DNA-cellulose. The results (Fig. 4) showed that virtually all of the *capR9*-specified protein was retained on DNA-cellulose in 0.05 M NaCl and eluted with 0.2 M NaCl. In contrast, ≈90% of the *capR⁺*-specified protein was not adsorbed to DNA-cellulose and only 10% eluted with 0.2 M NaCl. Electrophoresis of the fractions indicated that the *capR9*-specified protein fraction contained the same contaminants in the fractions eluted from DNA-cellulose as were originally present in the sample applied (see Fig. 2B). Similarly, the *capR⁺*-specified protein, both unadsorbed and adsorbed fractions, contained the 94-kDal polypeptide along with contaminants originally present. All fractions with the 94-kDal polypeptide exhibited DNA binding activity by the nitrocellulose filter assay before and after chromatography on DNA-cellulose, even the 0.05 M NaCl (unadsorbed) fraction (Table 1). A control experiment showed that the *capR9*-specified 94-kDal polypeptide does not bind to a cellulose column, as expected. The *capR⁺*-specified 94-kDal polypeptide did adsorb to a DNA-cellulose column in the absence of 0.05 M NaCl and eluted with

Table 1. DNA binding to nitrocellulose filters during fractionation of capR⁺ protein

Fraction*	DNA bound, % per μg of protein
Crude extract	10
Phosphocellulose	75
DEAE-cellulose	
0.15 M NaCl eluate	12
0.2 M NaCl eluate	62
DNA-cellulose	
0.05 M NaCl flow through	40
0.2 M NaCl eluate	55

The method of Shibata *et al.* (24) with endonuclease *Eco*RI-cleaved ³H-labeled pHA132 DNA at a final concentration of 0.118 μg/ml and 16,000 cpm/ml was used.
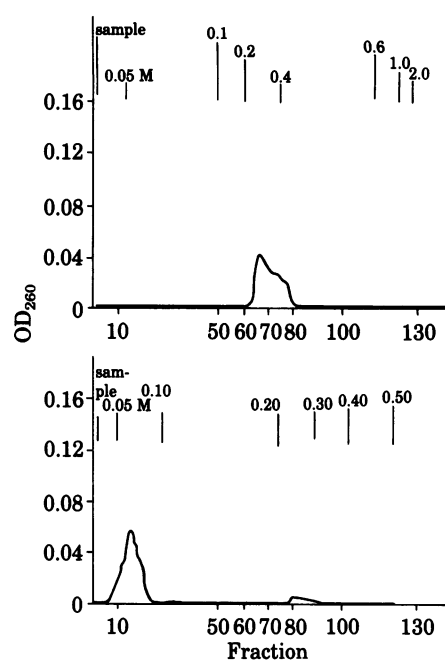* From strain RGC121/pBZ201.

FIG. 4. DNA-cellulose column chromatography of DEAE-cellulose-purified protein preparations. (*Upper*) capR9 (0.15 M NaCl fraction). (*Lower*) capR⁺ (0.2 M NaCl fraction). The capR9 protein fraction (0.32 mg in 2.5 ml) and the capR⁺ protein fraction (0.37 mg in 4 ml) were applied to the columns, and 0.5-ml fractions were eluted with buffer D (capR9 buffers, 10% glycerol; capR⁺ buffers, 20% glycerol) containing the indicated concentration of NaCl.

lower concentrations of NaCl than the *capR9*-specified 94-kDal polypeptide (unpublished results).

Antibody prepared against the DEAE-cellulose-purified capR9 protein preparation precipitated both the *capR⁺*- and *capR9*-specified 94-kDal polypeptide that had been similarly purified. Ouchterlony analysis (Fig. 5) indicated the most abundant, slow diffusing component was indistinguishable between the capR⁺ and capR9 preparations. A second, faster diffusing component was much more abundant in the capR⁺ fraction than in the capR9 fraction. We noted that two approximately equal components of 94-kDal polypeptide from capR⁺ extracts were detected on PC (when mixed with capR9 protein), DEAE-cellulose (in some experiments), and ATP-agarose, and consider it likely that the same two components were diffusing differently in the Ouchterlony test. We speculate that these were either
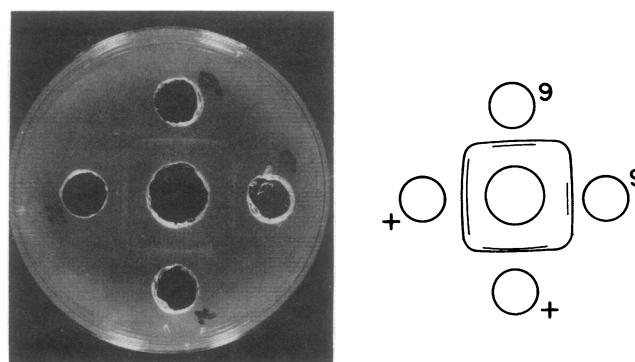

FIG. 5. Ouchterlony agarose double-diffusion analysis of capR⁺ (+) and capR9 (9) proteins. Wells labeled + and 9 each contained 20 μg of capR⁺ protein (DEAE-cellulose, 0.2 M NaCl fraction) or capR9 protein (DEAE-cellulose, 0.15 M NaCl fraction), and the center well contained undiluted antiserum against the same *capR9* fraction. The line drawing represents the precipitin lines seen in the original Ouchterlony plate.

monomer and oligomer or two different oligomers of the 94-kDal polypeptide. The *capR9*-specified 94-kDal polypeptide contained one major component throughout purification and was most likely an oligomeric form. An oligomeric form was also indicated by the inability of preparations of capR⁺ and capR9 proteins to electrophorese into 5% polyacrylamide gels in the absence of denaturing agents such as NaDodSO₄ or Triton X-100.

DISCUSSION

We have purified a 94-kDal polypeptide from extracts of a *capR⁺* homogenote (RGC121/pBZ201) and *capR9* homogenote (RGC123/pBZ201M9). The polypeptide was present in reduced quantities ($\approx$10%) in the respective haploid strains (unpublished results), and previous data established that the plasmids contain the structural gene for the 94-kDal polypeptide as well as the capR⁺ protein (12). Recessive mutants in *capR* produced little of the 94-kDal polypeptide (10- to 100-fold less than the plasmid from which the mutation was derived), and the mutant *capR9* allele produced elevated amounts of the 94-kDal polypeptide in maxicells and minicells (12). Genetic experiments established that the *capR9* mutation was an ochre mutation (1), was dominant to *capR⁺*, and this dominance was partly a gene dosage effect (1, 12, 26). Thus, we hypothesized that the *capR9* allele could specify an altered protein and, if the structural gene product were the 94-kDal polypeptide, it should be altered compared with the *capR⁺*-specified 94-kDal polypeptide. Throughout the purification steps used, the *capR⁺* and *capR9*-specified 94-kDal polypeptides behaved differently, indicating that the native proteins (94-kDal monomer) specified by *capR⁺* and *capR9* alleles are not identical. The data support the conclusion that the *capR⁺* (*lon⁺*) structural gene product is the 94-kDal polypeptide. Other studies in which the 94-kDal polypeptide from the *capR⁺* and *capR9* strains (obtained as in Fig. 1 *B* and *C*) was digested with *Staphylococcus aureus* V8 protease produced 17 peptides from each with identical mobilities (27). Thus, the proteins were very similar, as was also evident from immunodiffusion studies (see Fig. 5). These results, as well as others (12), are inconsistent with the suggestion that two different 94-kDal polypeptides are produced in the *capR⁺* strain and only one in the *capR9* strain.

The dominance of the plasmid-specified *capR9* allele over the chromosomal *capR⁺* allele for capsular polysaccharide synthesis (12, 26) and UV sensitivity (12) was hypothesized to result from interactions of monomers to form mixed oligomers (26). Evidence obtained from fractionation of the capR⁺ protein on DEAE-cellulose and ATP-agarose indicated that the *capR⁺* protein existed in two forms, perhaps monomer and oligomer, each of which had a different elution profile. When differentially labeled extracts from *capR⁺* and *capR9* strains were mixed and chromatographed on phosphocellulose, we obtained evidence that suggested an interaction of the 94-kDal polypeptide products of the two alleles. Such experiments did not rule out the possibility that other polypeptides participated in the interaction.

The biochemical activities of the capR⁺ (lon⁺) protein are central to our interests because they may lead to an understanding of the molecular basis of the pleiotropic effects of this gene product. The present results show that the mutant allele, *capR9*, specifies an altered protein (94-kDal monomer) with affinity for DNA, as shown by DNA binding studies with nitrocellulose filters and binding to an affinity (DNA-cellulose) column. The capR⁺ protein preparation bound DNA to nitrocellulose filters as well as the *capR9*-specified 94-kDal protein but binding to DNA-cellulose was limited to 10% of that of 94-kDal polypeptide (see Fig. 4). However, all the *capR⁺* 94-kDal polypeptide binds to DNA-cellulose columns at lower ionic strength (unpublished results).

Both the capR⁺ and capR9 proteins exhibit binding for single-stranded DNA and RNA, as well as for double-stranded DNA. This places the capR⁺ protein in a class of DNA binding proteins with low specificity that includes the HU protein, the HD protein, the D factor, and H₁ and H₂ proteins, and the bacteriophage T5-encoded D5 gene product (28, 29).

Our studies showed no sequence specificity for *galETK* operon DNA binding of the capR⁺ (or capR9) proteins, although previous studies have shown that *capR⁺* controls the synthesis of *galETK* mRNA (30, 31) and we have hypothesized that the *capR⁺* protein is either a second repressor or controls a second repressor for the *galETK* operon as well as other operons involved in polysaccharide synthesis (1). We do not consider the absence of *galETK* binding specificity as conclusive. Another important aspect of the pleiotropic effect of *capR* mutants is that they are defective in energy-dependent proteolysis (7–9). Recently, we showed that the purified capR⁺ protein is also an ATP-dependent protease and that the capR9 protein is enzymatically inactive in the same assay, adding further evidence that the *capR9* allele specifies an altered protein (unpublished results).

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