Nucleotide sequence of the argR gene of Escherichia coli K-12 and isolation of its product, the arginine repressor

(repressor protein/regulatory gene/arginine regulon)

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Communicated by Bernard D. Davis, June 15, 1987 (received for review April 6, 1987)

ABSTRACT In Escherichia coli, the arginine repressor, the product of the argR gene, in conjunction with L-arginine controls the synthesis of the enzymes of arginine biosynthesis. We describe the nucleotide sequence of the argR gene, including its control region, and show that formation of the repressor is autoregulated. The argR control region contains two promoters, one of which overlaps the operator site, and, as with other arg genes, consists of two adjacent palindromic sequences ("ARG boxes"). The arginine repressor protein and an arginine repressor–β-galactosidase fusion protein were purified, and the amino acid sequence of the N-terminal end of the repressor protein portion of the fusion protein was determined. Antibodies prepared against the fusion protein react with the repressor. The repressor is precipitable by L-arginine, which facilitates its purification. The native repressor is a hexamer with a molecular weight of 98,000; its monomeric subunit has a molecular weight of 16,500. To verify its properties postulated from genetic studies, we show that in the presence of L-arginine, repressor inhibits transcription of argF and binds to the ARG boxes of argF and argR.

The control of enzyme formation in the pathway of arginine biosynthesis in Escherichia coli by the end product has been studied extensively (for a review see ref. 1). The formation of the eight enzymes of arginine biosynthesis is controlled in conjunction with arginine by the arginine repressor (ArgR), the product of the regulatory gene argR (2, 3). Control was shown to be negative (4, 5), and the term regulon was coined (5) to describe systems where a single repressor controls several unlinked genes. A DNA segment containing the argR gene has been cloned (6). In sequencing studies of the genes for the biosynthetic enzymes, an 18-base-pair-long palindromic region, referred to as the "ARG box," was found in the promoter region of the genes. From mutant studies it is thought to be the binding site for ArgR (1). In all of the genes there are two adjacent ARG boxes.

In the present paper we describe the nucleotide sequence of the argR gene, including its control region. By putting the argR gene and an argR–lacZ gene fusion under the control of strong promoters, clones were obtained that hyperproduce ArgR and an ArgR–β-galactosidase fusion protein. Both types of protein were purified to homogeneity. ArgR could be precipitated from crude extracts by L-arginine, which facilitated its purification. Besides this unusual behavior, ArgR has other properties that distinguish it from known repressors: unlike the tryptophan repressor and the methionine repressor, both of which are dimers (7–9), it is a hexamer. Unlike the tryptophan repressor, which binds to a single palindromic sequence (10), it binds to two adjacent ARG boxes. These differences lend significance to ArgR for studies of repressor–target interactions.

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MATERIALS AND METHODS

Strains, Plasmids, and Materials. The plasmids used are described in Table 1. The host strains for plasmids were: E. coli N5151, a biotin-requiring heat-inducible λ lysogen derived from strain SA500 (13) and obtained from A. Slatzman (15); E. coli JM101 with the genotype Δlac pro, supE thi/F' traD36, proAB, lacZΔM15, obtained from Pharmacia; E. coli EL146 (AAZ-7), a prototrophic λ lysogen used for scoring ArgR phenotypes, with the genotype argD, argR, argA: lacZ (6). Restriction endonucleases, BAL-31 nuclease, Moloney murine leukemia virus reverse transcriptase, DNA polymerase I, Klenow fragment, RNA polymerase, radiochemicals, and isopropyl β-D-thiogalactoside were from commercial sources. Oligonucleotides were synthesized by B. Goldschmidt (Department of Biochemistry, New York University School of Medicine).

Plasmid Constructions. The argR gene was subcloned from pUC101 (6) as an 880-bp Sph I–Asc I fragment into a polynucleotide fragment in a derivative of pUC9 (14). From the resulting plasmid, pER3, a 1.2-kilobase (kb) Sph I–Sal I fragment containing argR can be recovered. Plasmid pDB101 was constructed by combining a 1.7-kb EcoRI–Hpa I fragment of pKC30 with a 360-bp flush-ended Sph I–Pvu II fragment of pER3 and with a 6.2-kb filled-in Bgl II–EcoRI fragment of pORF2. Plasmid pDB102 was constructed by joining a 400-bp filled-in BamHI–EcoRI fragment from pDR540 to the same Sph I–Pvu II and Bgl II–EcoRI fragments of pDB101. Plasmid pDB100 was constructed by joining a 1.2-kb flush-ended Sph I–Sal I fragment of pER3 to a 4.1-kb fragment of pDR540, which has a filled-in BamHI site and a cohesive Sal I end. The series of deletions derived from pDB100 (pDB122 to pDB190) were obtained by digestion with BAL-31 nuclease starting from the BamHI site. After subsequent digestion with Sal I, the deleted fragments were isolated by agarose gel electrophoresis and ligated to a filled-in BamHI–Sal I fragment of pDR540. Plasmid pDB200 was constructed by deleting 2 bp in the unique EcoRV site of pER3 (Fig. 1). To do this, pER3 was linearized with EcoRV and treated with 5 units of Klenow fragment in the presence of 250 μM dGTP. After phenol extraction and ethanol precipitation, the fragment was self-ligated and transformed into strain EC146 (4). The deletions were verified by Sau3A digestion, since the 2-bp deletion converts the EcoRV site to a Sau3A site.

DNase Footprinting. Plasmid pER3 was linearized with Spe I (Fig. 1) and labeled with [α-32P]dCTP by treatment with Klenow fragment. After digestion with Xho I, a 190-bp fragment, labeled at one end, was isolated from a polyacrylamide gel. About 0.2 pmol of this fragment was incubated for 20 min at 37°C with various concentrations of ArgR with or without 5 mM L-arginine. The incubation mixture contained, in a total volume of 20 μl, 0.1 M KCl, 10 mM MgCl₂, 1 mM
Table 1. Description of plasmids

| Plasmid   | Relevant phenotype | Characteristics, source, and/or ref. |<|<|<|<|
|-----------|--------------------|--------------------------------------|<|<|<|<|
| pKC30     | Ap<sup>a</sup>     | Expression vector carrying P<sub>L</sub> promoter (M. Rosenberg; ref. 15) |
| pDR540    | Ap<sup>a</sup>     | Expression vector carrying tac promoter (26) |
| pORF2     | Ap<sup>a</sup>     | Open reading frame expression vector for LacZ fusion proteins (11) |
| pMC20     | Ap<sup>a</sup>ArgF<sup>+</sup> | pBR322 derivative carrying arg<sup>F+</sup> (12) |
| pER3      | Ap<sup>a</sup>ArgR<sup>+</sup> | pUC9 derivative carrying arg<sup>R</sup> (this work) |
| pDB100    | Ap<sup>a</sup>ArgR<sup>+</sup> | pDR540 derivative carrying arg<sup>R</sup> (this work) |
| pDB101    | Ap<sup>a</sup>LacZ<sup>+</sup> | PORF2 derivative carrying P<sub>L</sub> promoter and argR-lacZ fusion (this work) |
| pDB102    | Ap<sup>a</sup>LacZ<sup>+</sup> | PORF2 derivative carrying tac promoter and argR-lacZ fusion (this work) |
| pDB200    | Ap<sup>a</sup>ArgR<sup>+</sup> | Two-base-pair deletion of pER3 at EcoRV site (this work) |

Ap<sup>a</sup>, resistance to ampicillin; ArgF<sup>+</sup>, production of ornithine carbamoyltransferase.

dithiothreitol, 5% (vol/vol) glycerol, 20 mM Tris·HCl (pH 7.6), and 1 μg of pUC18 DNA. Following incubation, 66 ng of pancreatic DNA was added, and the reaction was stopped after 60 sec at 37°C by phenol extraction. After ethanol precipitation, the reaction products were analyzed in an 8% sequencing gel, using a G + A Maxam–Gilbert sequencing ladder of the same 190-bp fragment as a guide.

**In Vitro Run-off Transcription.** This was done with a 280-bp HindIII–XbaI fragment of pMC20, containing the promoter–operator region of arg<sup>F</sup>. In a final volume of 20 μl, ~0.05 pmol of this fragment were incubated at 37°C for 15 min with various amounts of ArgR in the presence or absence of 5 mM L-arginine. The reaction mixture contained 20 mM Tris·HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 5% (vol/vol) glycerol, 1 mM dithiothreitol, and 0.1 M KCl. RNA polymerase holoenzyme (5 pmol) was added for 5 min, and then the reaction was started by the addition of 150 μM ATP/200 mM CTP/116 mM UTP/1.2 μM [α-<sup>32</sup>P]GTP. After a 2.5-min incubation period, the GTP concentration was increased to 40 μM by adding unlabeled GTP, and the incubation was continued for 3 min. After phenol extraction and ethanol precipitation the reaction products were analyzed in an 8% sequencing gel.

**Primer Extension.** Total RNA was extracted from *E. coli* strains EC146 (λA7–), EC146 (λA7/7/pER3), and EC146 (λA7–/pDB200) as described by Alba et al. (16). Primer extension was performed with the synthetic nucleotide 5′-CCCTGGAGCTAAATTT-3′, as described by Curtis et al. (17). The reaction products were analyzed in an 8% sequencing gel, together with the deoxy chain-termination products obtained from the same primer.

**Purification of the ArgR–β-Galactosidase Fusion Protein.** The ArgR–β-galactosidase fusion protein from a heat-induced culture of strain N3515 (pDB101) was purified by affinity chromatography using p-aminophenyl β-D-thiogalactoside (TPEG; Sigma) CH-Sepharose 4B beads as described by Ullmann (18). To achieve complete purification of the fusion protein two additional chromatography steps were found necessary: anion-exchange chromatography using a MonoQ column and molecular sieve chromatography using a Superose 6 column, both attached to a Pharmacia FPLC System (Pharmacia).

**Purification of ArgR (Table 2).** An extract of strain JM101 (pDB169) was prepared by sonication of a cell suspension in Arg buffer (20 mM Tris·HCl/10 mM MgCl<sub>2</sub>/10 mM 2-mercaptoethanol, pH 7.5). The supernatant from the extract obtained by centrifugation at 40,000 × g for 30 min at 4°C was treated with DNase (1 μg/ml of extract) for 30 min at 37°C. After DNase treatment the extract was centrifuged at 40,000 × g for 30 min at 4°C. The supernatant was saved (hereafter referred to as the DNase-treated extract). Solid ammonium sulfate was added to this supernatant to 55% of saturation. After mixing for 1 hr at 4°C, the precipitate was harvested, resuspended in Arg buffer, and dialyzed against this buffer. The final dialyzed product was Millipore filtered (hereafter referred to as the 55% (NH₄)₂SO₄ precipitate). Solid l-arginine was added to a final concentration of 5 mM. After incubation for 30 min at 37°C, the precipitate was harvested by centrifugation at 5000 × g for 5 min at 25°C. It was resolubilized by gently mixing in Arg buffer containing 1 M NaCl. The solution was dialyzed first against 1 M NaCl/Arg buffer and then against Arg buffer. After centrifugation to remove any insoluble material, this fraction (hereafter referred to as the Arg precipitated fraction) was then chromatographed consecutively on preparative anion- and cation-exchange columns (MonoQ and MonoS HR 10/10 columns, respectively, attached to a Pharmacia FPLC System) using 0–0.5 M NaCl gradients in the Arg buffer for column development. Purification of ArgR was continuously monitored at each step by NaDodSO₄/PAGE.

**Assays of Proteins.** ArgR levels were assayed by a quantitative rocket immunoelectrophoresis assay (19) using antibody directed against the purified ArgR–β-galactosidase fusion protein. Antibody was elicited in rabbits according to a described protocol (19). The immunoglobulin fraction was prepared from immune sera according to Harboe and Ingild (20). Molecular weights were determined by three methods. (i) The subunit value was obtained by NaDodSO₄/PAGE analysis (12.5% separating gel) of reduced samples and

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Fig. 1. Nucleotide sequence and deduced amino acid sequence of arg<sup>R</sup>. Two transcription start sites are denoted by arrows. The sequence is numbered from the transcription start point of the proximal promoter P<sub>1</sub>. Putative −10 and −35 regions of each promoter are overlined. Two ARG boxes are underlined. The −10 and −35 regions of P<sub>1</sub> overlap with the ARG boxes. The sequence starts from the SphI site of the SphI−1 fragment. Arrowheads indicated sites for the following restriction enzymes: S, SpeI; H, HindII; P, PvuII; E, EcoRV.
Table 2. Purification of ArgR

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein</th>
<th>ArgR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume, mg/ml</td>
<td>Total mg</td>
</tr>
<tr>
<td>Sonic extract</td>
<td>140</td>
<td>50</td>
</tr>
<tr>
<td>DNase-treated extract</td>
<td>138</td>
<td>47.5</td>
</tr>
<tr>
<td>55% (NH₄)₂SO₄</td>
<td>50</td>
<td>108</td>
</tr>
<tr>
<td>precipitate fraction</td>
<td>45</td>
<td>18.5</td>
</tr>
<tr>
<td>Mono Q peak</td>
<td>42</td>
<td>15</td>
</tr>
<tr>
<td>Mono S peak</td>
<td>50</td>
<td>11.25</td>
</tr>
</tbody>
</table>

Protein concentration was determined by the Bradford procedure (Bio-Rad). ArgR was measured by quantitative rocket immunoelectrophoresis. S.A., specific activity.

appropriate standards, using the procedure of Laemmli (21). (ii) The native molecular weights were determined by nondenaturing gradient gel electrophoresis using the Pharmacia PhastGel system and by molecular-sieve chromatography in a Superose 12 HR 10/30 column operated with a Pharmacia FPLC system. In the latter procedure it was necessary to elute ArgR in the presence of at least 0.15 M NaCl to avoid interaction of the purified protein with the Superose beads. Total protein was determined by measuring light absorption at 280 nm, combined with dry-weight determinations, or by the Bradford procedure (Bio-Rad). β-Galactosidase was measured as described by Miller (22). The amino acid sequencing was carried out by D. Schlesinger of the Kaplan Cancer Center at New York University Medical Center.

RESULTS

Nucleotide Sequence and Autoregulation of argR. Restriction enzyme mapping of plasmid pER3 indicated the entire argR gene is encoded in an 880-bp SphI–AccI fragment. This fragment was sequenced by the method of Maxam and Gilbert (23). Details of the sequencing strategy are available from the authors. The DNA sequence for 806 bp starting at the SphI site is shown in Fig. 1. It contains one major open reading frame, spanning HinCI, PvuII, and EcoRV sites and coding for a protein of 156 amino acids.

Inspection of the region upstream of the putative argR coding region revealed two ARG boxes, which suggested that the argR gene is autoregulated. To verify this supposition, argR–lacZ fusions were constructed in vivo (24). It was found in these strains that in the presence of arginine and the argR+ gene, but not with either one alone, β-galactosidase formation is inhibited (data not shown).

To study the autoregulation of argR further, we analyzed the transcriptional products of argR by the primer extension method. Two plasmids were used: pER3, which contains intact argR, and the derivative pDB200, which has a 2-bp deletion in the EcoRV site of argR (Fig. 1) and does not produce active repressor. About 300 ng of a synthetic 17-nucleotide oligomer, complementary to nucleotides at positions 99–114 in Fig. 1, was annealed with total RNA extracted from E. coli EC146 (λAz-7/pER3), E. coli EC146 (λAz-7/pDB200), and E. coli EC146 (λAz-7) and extended with reverse transcriptase. As shown in Fig. 2, there are two transcriptional start sites and presumably two promoters, P₁ and P₂, in argR. In the proximal promoter, P₁, transcription starts from a guanosine in position +1. The −10 and −35 regions of the P₁ promoter are entirely within the ARG boxes (Fig. 2). Since ArgR binds to these ARG boxes (see below), it seems likely that P₁ is controlled by ArgR. This is confirmed by a comparison of the in vivo transcriptional

![Fig. 2. Primer extension of argR. Total RNA (10 μg) was annealed with 300 ng of synthetic oligonucleotide 5' CCGCTGGAGCGTAATT 3' and extended by reverse transcriptase in the presence of [32P]dCTP. The reaction products were loaded onto an 8% sequencing gel. The same primer was used for the deoxy chain-termination, to serve as a marker for the size of the extended fragments. A, C, G, and T denote the deoxy reaction products. Lanes: 1 and 4, primer extension of RNA from E. coli EC146 (λAz-7/pER3); 2 and 5, from E. coli EC146 (λAz-7/pDB200); 3 and 6, from E. coli EC146 (λAz-7). In lanes 4, 5, and 6, five times as much sample was applied. In lane 6, faint transcripts from the chromosomal argR gene are seen in the corresponding positions. P₁ and P₂ are proximal and distal promoters, respectively. argR in the intact pER3 plasmid and the deletion mutant pDB200 plasmid (Fig. 2, compare lanes 1 and 4 with lanes 2 and 5). The transcription from P₁ in the presence of arginine is about 15 times higher in pDB200 than in pER3.

The distal promoter P₂ is located upstream from the ARG boxes and transcription starts from cytidine in position −76. The transcriptional activity of this promoter in the presence of arginine is the same in pER3 and in pDB200. Therefore, this promoter is not regulated by ArgR. In pER3, under conditions of repression, P₂ is the major promoter and the ratio of activities of repressed P₁/P₂ is about 1:7. Under derepressed conditions, P₁ is the major promoter and the ratio of activities of P₁/P₂ is ~2.5:1 (see Fig. 2). Hyperproduction and Isolation of an ArgR–β-Galactosidase Fusion Protein. Since initially it was difficult to do quantitative assays for ArgR, we constructed a chimeric gene in which part of argR is joined to lacZ, coding for a fusion protein containing the N-terminal portion of ArgR fused to β-galactosidase. The chimeric gene containing the argR leader sequence was put under the control of either the P₁ promoter or the tac promoter.

The fusion protein was extracted and purified from strain N5151 (pDB101), containing the P₁ promoter. Cells, induced by growing at 42°C for 4 hr, were lysed by sonication, and the protein was subsequently purified. The final product was considered to be pure because after NaDodSO₄/PAGE a single band was visible, and because the preparation had a specific enzyme activity similar to that of pure β-galacto-
sidase. The sequence of 14 amino acids at the N terminus was determined and found to be in good agreement with the sequence postulated in Fig. 1 (data not shown). Immunization of rabbits with the purified protein gave rise to an antiserum that reacted with ArgR, as well as β-galactosidase. This antiserum was subsequently used for quantitative assays of ArgR by rocket immunoelectrophoresis.

Construction of ArgR Hyperproducing Strains and Isolation of ArgR. Since we showed above that the argR gene is autoregulated, we generated a series of deletions in the argR control region of pDB100 by controlled digestion with BAL-31. Ninety deletion strains were analyzed, using NaDodSO4/PAGE, for repressor production following induction with isopropyl-β-thiogalactoside and in 14 of these the control region of argR was sequenced. The amounts of ArgR produced and the end points of the deletions are shown in Fig. 3. It can be seen that deletions up to coordinate −81 produce similar amounts of ArgR, but deletions extending further through the ARG boxes produce more protein. Even higher expression is obtained in deletions extending into the argR Shine−Dalgarno sequence. One of the highest ArgR producers, pDB109 (Fig. 3, lane 9), was chosen for purification of ArgR, after verifying that the ArgR produced by it is functional in the indicator strain EC146 (AAZ-7) (6).

```plaintext
A
ATGGCGTACGAGGACATGCTCATATGATTCTAAATACGCGCTTTCTATA
-9
ACAGTTTACCGTCCGCCGTCGCTGGTTCAAAATTCTTTCATA
-9
134

B

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
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Fig. 3. Construction of hyperproducing strains following BAL-31 deletion of pDB100. BAL-31 deletion was carried out to remove the argR control region. The tac-promoter cassette (EcoRI-BamHI fragment of pDR540) was placed on the upstream side of the deleted argR region. Sixteen of the deleted strains are shown in B and in 14 of these the deletion end points were determined by sequencing appropriate restriction fragments cloned in M13mp9 by the method of Sanger et al. (25). (A) BAL-31 deletion end points. Arrows indicate the deletion end points. The numbers correspond to the numbers in B. The ARG boxes and putative Shine−Dalgarno sequence of argR are underlined. (B) NaDodSO4/PAGE of deletion clones. Each deletion clone was treated as described in the legend of Fig. 3 except a 15% gel was used. The lane numbers correspond to the numbers in A. pDB109 in lane 9 was used for ArgR purification. Arrow indicates ArgR.

Initially the purification of ArgR was carried out in a similar way as for the ArgR−β-galactosidase fusion protein. However, during purification we noted that a precipitate was formed when L-arginine was added, which could be redissolved in Arg buffer containing at least 0.1 M NaCl. NaDodSO4/PAGE of the redissolved precipitate showed that it had the mobility expected of the ArgR. Furthermore, the precipitation was specific for L-arginine. We assumed that the precipitate was ArgR and developed a procedure for its purification. Results of a typical experiment are shown in Table 2. It can be seen that ArgR constitutes 4% of the initially extracted protein. The yield of pure ArgR is 38%, with the greatest increase in the purification being due to the precipitation by L-arginine. The amount of ArgR at each step of purification was determined by quantitative rocket immunoelectrophoresis using as standard the material recovered from the final cation-exchange column. This material gives a single band in polyacrylamide gels, and we assume that it is pure.

Some of the properties of the purified ArgR have been studied. The molecular weight of the monomer determined by NaDodSO4/PAGE analysis was found to be 16,500, in good agreement with that predicted from the DNA sequence. The molecular weight of the native protein was found to be 98,000, indicating that ArgR exists normally as a hexamer. The native protein is soluble in dilute buffer (Arg buffer), but insoluble in distilled water. The protein is precipitated completely in 0.8−1.0 mM L-arginine. This effect is very specific. The following substances, in similar or higher concentrations, did not bring about precipitation: D-arginine, L-carnavamine, agmatine, L-arginine hydroxamate, L-homoarginine, spermidine, and streptomycin.

Interaction of ArgR with the Control Regions of argR and argF. To test for the presumed biological activity of the purified ArgR, in vitro run-off transcription was carried out with a DNA fragment containing the control region of argF. About 0.02 pmol of the 280-bp Pst I-HindIII argF fragment was incubated with 1 pmol of ArgR with or without L-arginine. As shown in Fig. 4A, it is clear that ArgR inhibits transcription of argF, but only in the presence of L-arginine. Fig. 4B shows that the inhibition is dependent on the concentration of ArgR. This result confirms the previous conclusion that ArgR acts at the level of transcription and that it requires L-arginine.

The site of interaction of ArgR with DNA in argR was localized by DNase footprinting experiments. Both strands of a fragment containing the argR control region were labeled

```plaintext
A

1 2 3 4

B

1 2 3 4 5 6 7
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Fig. 4. In vitro run-off transcription of argF with ArgR, with or without L-arginine. The reaction products were analyzed in an 8% sequencing gel. (A) Dependence of inhibition of transcription on L-arginine. Lanes: 1, control run-off transcription without ArgR and L-arginine; 2, with 5 mM L-arginine, no ArgR; 3, with 100 ng of ArgR, no L-arginine; 4, with 100 ng of ArgR in 5 mM L-arginine. (B) Dependence of ArgR concentration. Lanes: 1, control without ArgR and L-arginine; 2, with 50 ng of ArgR, no L-arginine; 3, with 50 ng of ArgR in 5 mM L-arginine; 4, with 25 ng of ArgR in 5 mM L-arginine; 5, with 12.5 ng of ArgR in 5 mM L-arginine; 6, with 6.2 ng of ArgR in 5 mM L-arginine; 7, with 3.2 ng of ArgR in 5 mM L-arginine.
Fig. 5. DNase footprinting of argR. The sense strand was labeled in the Spe I site (Fig. 1) with Klenow fragment. The G+A ladder of the same fragment obtained by Maxam–Gilbert sequencing was used for determining the position of the protected region. The arrows indicate the transcription start points. ARG boxes are marked. Lanes: 1, G+A ladder; 2, without ArgR and l-arginine; 3, with 136 ng of ArgR in 5 mM l-arginine; 4, with 68 ng of ArgR in 5 mM l-arginine; 5, with 34 ng of ArgR in 5 mM l-arginine; 6, with 17 ng of ArgR in 5 mM l-arginine; 7, with 272 ng of ArgR, no l-arginine; 8, with 136 ng of ArgR, no l-arginine. P1 and P2 are proximal and distal promoters, respectively.

with ³²P and incubated with the purified ArgR in the absence or presence of l-arginine. After treatment with DNase, the reaction products were analyzed in sequencing gels. As shown in Fig. 5 (only the sense strand is shown), the antisense strand gave similar results, ArgR binds to the ARG boxes, but only with l-arginine. The protected sites are between coordinates -37 and +5. These results confirm the suggestion from sequence comparisons of arg genes and mutational studies that the ARG box sequences are the binding sites for ArgR. The concentration of ArgR required for 50% protection against DNase is ~2 × 10⁻⁸ M.

Similar results were obtained in DNase footprinting experiments with the control region of argF (data not shown). The ARG boxes of argF conform more closely to the ARG box consensus sequence (1) than do those of argR.

**DISCUSSION**

In this paper we have determined the nucleotide sequence of the argR gene and have described the isolation and purification of its product, ArgR. We have also described the isolation of an ArgR-β galactosidase fusion protein. The properties of these two proteins support the structure of the ArgR subunit postulated from the nucleotide sequence. In its native state, ArgR appears to be a hexamer.

We have shown that the purified ArgR is active at low concentrations in inhibiting _in vitro_ run-off transcription of _argF_, indicating that it has retained its biological activity. DNase footprinting experiments have shown that ArgR interacts with two adjacent palindromic ARG boxes in the control region of _argR_ and _argF_. D. Charlier, R. Cunin, and N. Glansdorff (personal communication) in similar experiments have observed binding of our purified repressor to the ARG boxes of _argECBH_ and _carAB_. The purified ArgR thus behaves as postulated on the basis of studies with mutants.

This work was supported by Public Health Service Grant GM06048 from the National Institute of General Medical Sciences. W.K.M. is the holder of Public Health Service Career Award GM15129 from the National Institute of General Medical Sciences.