Identification, cloning, and nucleotide sequencing of the ornithine decarboxylase antizyme gene of *Escherichia coli*

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ABSTRACT   The ornithine decarboxylase antizyme gene of *Escherichia coli* was identified by immunological screening of an *E. coli* genomic library. A 6.4-kilobase fragment containing the antizyme gene was subcloned and sequenced. The open reading frame encoding the antizyme was identified on the basis of its ability to direct the synthesis of immunoreactive antizyme. Antizyme shares significant homology with bacterial transcriptional activators of the two-component regulatory system family; these systems consist of a "sensor" kinase and a transcriptional regulator. The open reading frame next to antizyme is homologous to sensor kinases. Antizyme overproduction inhibits the activities of both ornithine and arginine decarboxylases without affecting their protein levels. Extracts from *E. coli* bearing an antizyme gene-containing plasmid exhibit increased antizyme activity. These data strongly suggest that (*i*) the cloned gene encodes the ornithine decarboxylase antizyme and (*ii*) antizyme is a bifunctional protein serving as both an inhibitor of polyamine biosynthesis as well as a transcriptional regulator of an as yet unknown set of genes.

Polyamines are necessary components of nearly all living cells, from bacteria to plant to human cells. Although their specific functions remain elusive, they are required components of the cell milieu since polyamine depletion results in severe reductions in cell growth and, in some instances, in cell death (for review, see refs. 1 and 2). Regulation of polyamine biosynthesis is complex and the key biosynthetic enzyme ornithine decarboxylase (ODC; L-ornithine carboxy-lyase, EC 4.1.1.17) is probably one of the most highly regulated enzymes (3). It has been shown that the levels and/or the activity of ODC can be modulated both positively and negatively at the transcriptional, translational, and post-translational levels (for review, see ref. 4). ODC is regulated posttranslationally by covalent modification, degradation, or interaction with inhibitor proteins (antizymes; Az) (5).

Az are defined as noncompetitive protein inhibitors of ODC whose synthesis is induced by polyamines (6, 7). Although inhibition of ODC by Az is reversible and active ODC can be recovered from inactive ODC–Az complexes under certain conditions (8), there is also substantial evidence involving Az in ODC degradation (9, 10). In *Escherichia coli*, the posttranslational control of the activities of the polyamine biosynthetic enzymes ODC and arginine decarboxylase (ADC; L-arginine carboxy-lyase, EC 4.1.1.19) by Az-like proteins has been studied by our group for a number of years (8, 11, 12). It is now established that three proteins, whose intracellular levels increase when the polyamine concentrations increase, participate in the negative regulation of both ODC and ADC (11). Two of these inhibitors are basic proteins and they have been identified as the ribosomal proteins S20 and L34 (12). The third inhibitor, the *E. coli* Az, has an acidic apparent pI and a molecular weight of 49,500

(11). The study of the Az has been very difficult because of its low abundance and because its N terminus is blocked (unpublished results).

We now report the isolation of the *E. coli* Az gene, its location on the *E. coli* chromosome, as well as the identification and sequence of the open reading frame (ORF) encoding the Az.§

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Phages. *E. coli* MG1655 (λ⁻, F⁻) (13); KL527 [MG1655 *gyrA*, Δ *(speA-speB)97*, Δ*(speC-glc)63*] (14); DH5α, HB101, TG-1, and LE392; phages M13mp18 and M13mp19 (15); and plasmids pUC18, pUC19, and pIC20H (16) were used in this work.

Protein Purification and Antibody Production. ODC and ADC were purified to homogeneity by standard methods (17). Az was purified as described (11). Antibodies to Az were produced by injecting a female New Zealand White rabbit subcutaneously with 30 μg of Az in complete Freund's adjuvant. After 4 weeks, 10 μg of Az in incomplete Freund's adjuvant was injected and blood was drawn 10 days later. ODC and ADC antibodies have been described (18).

Extracts, Enzyme Assays, and Immunoblotting. Extracts were prepared as described (14) and protein concentrations were determined (19). ODC, ADC, and Az activity assays and unit definitions have been described (8, 17). Variations in the *in vitro* ODC assays, probably due to its sensitivity to activating and inhibitor nucleotides (20), were prevented by including 1 mM GTP. All assays were performed in triplicate. SDS/PAGE and immunoblotting were performed by standard methods (21, 22).

Immunoadsorption of Az. *E. coli* extracts enriched in Az were prepared by adjusting the pH of *E. coli* lysates to 1.8 (11), stirring for 30 min at 4°C, and dialyzing the soluble proteins against buffer A [20 mM Tris·HCl, pH 7.4/10% (vol/vol) glycerol/1 mM dithiothreitol]. The extracts (15 mg of protein) were applied to 0.5 ml of protein A-Sepharose (Pharmacia) columns containing bound anti-Az or preimmune immunoglobulins, and they were circulated for 4 hr at 4°C. Each column was washed with 10 ml of buffer A containing 0.5 M NaCl, followed by 5 ml of buffer A. Elution was performed with 0.1 M glycine HCl (pH 2.6).

Subcloning and Sequencing. The 6.4-kb *Bam*HI fragment, containing the *Az* gene, was digested with several restriction enzymes (see Fig. 2), and the resulting fragments were cloned in M13mp18 and M13mp19 phages (15). Recombinant plaques were identified by hybridization (23). Single-

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Abbreviations: ODC, ornithine decarboxylase; ADC, arginine decarboxylase; Az, antizyme(s); ORF, open reading frame; GST, glutathione *S*-transferase.
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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L13078).

stranded phage DNA was isolated as described (15). Both DNA strands were sequenced with the Sequenase kit (version 2.0) from United States Biochemical. Computer analyses were performed with either DNA STRIDER 1.0 (24) or MACDNASIS version 1.00 (Hitachi Software Engineering, Brisbane, CA) programs. The National Biomedical Resource Foundation–Protein Identification Resource data base, on CD-ROM, was searched for sequence homologies using MACDNASIS.

Generation of Glutathione S-Transferase (GST)–Az Fusion Protein. A 712-bp *Pvu* II/*Eco*RI fragment (bp 294–1002; see Fig. 3) was ligated to plasmid pGEX-3X (25), which had been cut with *Sma* I and *Eco*RI. The resulting recombinant plasmid pCPC-XAz1 produced a GST–Az fusion protein, which contained amino acids 56–292 of Az.

RESULTS

Characterization of the Anti-Az Antibody. We have previously shown that the antibody raised against Az recognizes a 50-kDa protein whose levels increase upon *E. coli* growth in the presence of polyamines (26). To further demonstrate that this protein is Az, we tested the ability of immobilized anti-Az antibody to bind it from Az-enriched cell extracts. The eluate from the anti-Az immunoaffinity column exhibited strong Az activity, whereas no activity was detected in the eluate from a control column (Fig. 1). This finding indicates that the protein recognized by the antibody is Az.

Identification of Clones Containing the Az Gene and Subcloning. Immunological screening of an *E. coli* genomic library in λ phage (27) was used to identify the *Az* gene. This procedure was chosen because of difficulties in obtaining N-terminal or internal peptide sequences of the Az protein.

The library was subdivided into groups of 20 consecutive clones. Liquid cultures of *E. coli* LE392 were infected with these clone mixtures and allowed to grow until lysis. The lysates were analyzed by SDS/PAGE and immunoblotting. The infection procedure was repeated with individual clones from the positive group. Two consecutive clones, 5A1 and 4F12 (27), expressed a 50-kDa immunoreactive protein. These two clones share a common chromosomal region located at the 48th minute of the *E. coli* linkage map (28).

DNA from phage 4F12 was subcloned in plasmid pUC19. *E. coli* DH5α transformed with the resulting recombinant plasmids were screened by immunoblotting. The plasmid
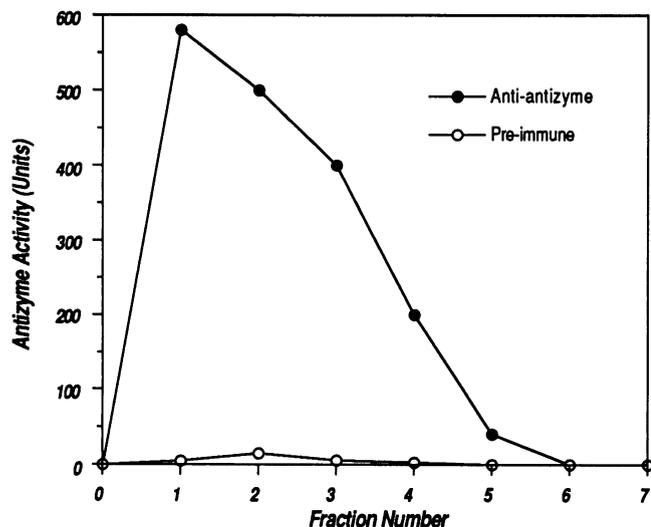
FIG. 1. Elution of Az from an immunoaffinity column. Preparation of extracts and absorption on the column were performed as described. Az activity was assayed by mixing 2.5 μl from each fraction with 8 units (10 ng) of purified ODC.

Table 1. Effect of Az overproduction on ODC and ADC activity

Strain	Specific activity (% activity)		
	ADC	ODC − GTP	ODC + GTP
MG1655	5.2 (100%)	140 (100%)	315 (100%)
MG1655/pUC19	4.9 (95%)	130 (93%)	331 (105%)
MG1655/pUC-Az	2.4 (46%)	70 (50%)	200 (60%)

Specific activities are expressed as nmol of CO_2 produced per mg of protein per 60-min incubation. Preparation of cell extracts and assays for ODC and ADC activities were performed as described. Numbers represent average of three independent experiments, each performed in triplicate. Variation between experiments was <5%.

described in this study (pUC-Az) contains a 6.4-kb *Bam*HI fragment.

Az Is Overproduced in pUC-Az-Transformed Cells. To ensure that the cloned gene was indeed the *Az* gene, *E. coli* KL527 (ODC⁻, ADC⁻) (14) was transformed with pUC-Az. Az was partially purified until the perchloric acid step (11) to remove ribosomal proteins S20 and L34, which also inhibit ODC and could interfere with the assay (12). The specific activity of Az in these extracts was 4000–6000 units per mg of protein, which is 50- to 80-fold higher than that of extracts from control cells (results not shown). This agrees well with the extent of Az protein overproduction (50- to 100-fold) in these cells (data not shown).

The ability of the overproduced protein to inhibit ODC and ADC activities in wild-type cells was also tested. The activities of endogenous ODC and ADC were decreased 50% in lysates from *E. coli* MG1655 transformed with pUC-Az (Table 1). Immunoblot analysis of the extracts indicated that the protein levels of these two enzymes were not decreased in the Az-overproducing cells (results not shown). Therefore, the decreased activities of ODC and ADC result from post-translational inhibition and not from decreased amounts.

Sequence of the Az Gene. The 6.4-kb insert was sequenced as described. The sequencing strategy and partial restriction map are shown in Fig. 2. Two ORFs, which could encode proteins with sizes similar to that of Az, were identified in the sequenced region (Fig. 2c). Transcription of both ORFs proceeded in the same direction. One part of the sequence (bp 5194–6414) overlaps with the published sequence of the *rcsC* gene (29), which is also located in the 48th minute of the *E. coli* genetic map. The nucleotide and deduced amino acid sequences of ORF2, which encodes Az (see below), are given in Fig. 3.
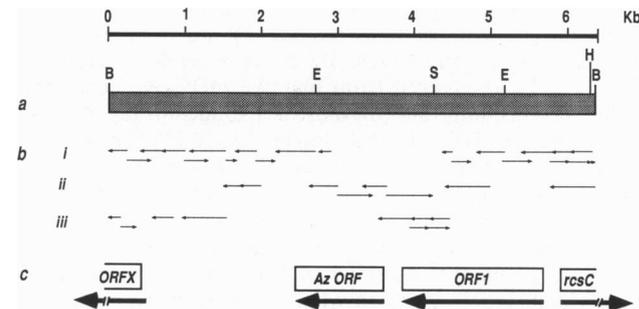
FIG. 2. (a) Partial restriction map of the sequenced fragment containing the *Az* gene. E, *Eco*RI; S, *Sal* I; H, *Hind*III; B, *Bam*HI. (b) Sequencing strategy is presented. Clones used in sequencing were generated by digesting the 6.4-kb *Bam*HI fragment with (i) *Eco*RI alone or in combination with *Alu* I, (ii) *Eco*RV, or (iii) *Hinc*II. Arrows denote direction of sequencing. (c) ORF map. The truncated ORF, which encodes protein rcsC (29) as well as a truncated unknown ORF (ORFX), is shown. ORFs with sizes smaller than 0.5 kb are not shown.

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  1      ACC TGT TTG CCG ATA TTC ACC CTG ATG TGG TGT TGA TGG ATA TCC GCA TGC |CAG |
 55      |AGA TGG |ACG GCA TCA A|GG CAC TAA AGG AGA TGC GCA GCC ATG AGA CCC GGA CAC
109      CCG TTA TTC TGA TGA CGG CCT ATG CGG AAG TGG AAA CCG CCG GTC GAA GCG CTA
  1                                  Met Arg Lys Trp Lys Pro Pro Val Glu Ala Leu
163      CGC TGC GGA CGC TTC GAC TAT GTT ATT AAA CCG TTT GAT CTC GAT GAG TTG AAT
 12      Arg Cys Gly Arg Phe Asp Tyr Val Ile Lys Pro Phe Asp Leu Asp Glu Leu Asn
217      TTA ATC GTT CAG CGC GCT TTA CAA CTC CAG TCA ATG AAA AAA GAG ATC CGT CAT
 30      Leu Ile Val Gln Arg Ala Leu Gln Leu Gln Ser Met Lys Lys Glu Ile Arg His
271      CTG CAC CAG GCA CTG AGC ACC AGC TGG CAA TGG GGG CAC ATT CTC ACC AAC AGC
 48      Leu His Gln Ala Leu Ser Thr Ser Trp Gln Trp Gly His Ile Leu Thr Asn Ser
325      CCG GCG ATG ATG GAC ATC TGC AAA GAC ACC GCC AAA ATT GCC CTT TCT CAG GCC
 66      Pro Ala Met Met Asp Ile Cys Lys Asp Thr Ala Lys Ile Ala Leu Ser Gln Ala
379      AGC GTC TTG ATT AGC GGT GAA AGC GGC ACC GGG AAA GAG TTG ATT GCC AGA GCG
 84      Ser Val Leu Ile Ser Gly Glu Ser Gly Thr Gly Lys Glu Leu Ile Ala Arg Ala
433      ATT CAC TAC AAT TCG CGG CGG GCA AAG GGG CCG TTC ATT AAA GTC AAC TGC GCG
102      Ile His Tyr Asn Ser Arg Arg Ala Lys Gly Pro Phe Ile Lys Val Asn Cys Ala
487      GCG CTG CCG GAA TCG TTG CTC GAA AGT GAA CTG TTT GGT CAT GAA AAA GGT GCA
120      Ala Leu Pro Glu Ser Leu Leu Glu Ser Glu Leu Phe Gly His Glu Lys Gly Ala
541      TTT ACT GGT GCA CAA ACC TTG CGT CAG GGA TTA TTT GAA CGA GCC AAC GAA GGT
138      Phe Thr Gly Ala Gln Thr Leu Arg Gln Gly Leu Phe Glu Arg Ala Asn Glu Gly
595      ACT CTG CTC CTC GAC GAA ATT GGC GAA ATG CCG CTG GTA CTA CAA GCC AAA TTA
156      Thr Leu Leu Leu Asp Glu Ile Gly Glu Met Pro Leu Val Leu Gln Ala Lys Leu
649      CTA CGC ATT CTA CAG GAA CGG GAA TTT GAA CGG ATT GGC GGC CAT CAG ACC ATA
174      Leu Arg Ile Leu Gln Glu Arg Glu Phe Glu Arg Ile Gly Gly His Gln Thr Ile
703      AAA GTT GAT ATC CGC ATC ATT GCT GCC ACC AAC CGC GAC TTG CAG GCA ATG GTA
192      Lys Val Asp Ile Arg Ile Ile Ala Ala Thr Asn Arg Asp Leu Gln Ala Met Val
757      AAA GAA GGC ACC TTC CGT GAA GAT CTC TTT TAT CGC CTT AAC GTT ATT CAT TTA
210      Lys Glu Gly Thr Phe Arg Glu Asp Leu Phe Tyr Arg Leu Asn Val Ile His Leu
811      ATA CTG CCG CCT CTG CGC GAT CGC CGG GAA GAT ATT TCC CTG TTA GCT AAT CAC
228      Ile Leu Pro Pro Leu Arg Asp Arg Arg Glu Asp Ile Ser Leu Leu Ala Asn His
865      TTT TTG CAA AAA TTC AGT AGT GAG AAT CAG CGC GAT ATT ATC GAC ATC GAT CCG
246      Phe Leu Gln Lys Phe Ser Ser Glu Asn Gln Arg Asp Ile Ile Asp Ile Asp Pro
919      ATG GCA ATG TCA CTG CTT ACC GCC TGG TCA TGG CCG GGA AAT ATT CGA GAG CTT
264      Met Ala Met Ser Leu Leu Thr Ala Trp Ser Trp Pro Gly Asn Ile Arg Glu Leu
973      TCC AAC GTT ATT GAA CGC GCC GTC GTG ATG AAT TCA GGC CCG ATC ATT TTT TCT
282      Ser Asn Val Ile Glu Arg Ala Val Val Met Asn Ser Gly Pro Ile Ile Phe Ser
1027     GAG GAT CTT CCG CCA CAG ATT CGT CAG CCA GTC TGT AAT GCT GGC GAG GTA AAA
300      Glu Asp Leu Pro Pro Gln Ile Arg Gln Pro Val Cys Asn Ala Gly Glu Val Lys
1081     ACA GCC CCT GTC GGT GAG CGT AAT TTA AAA GAG GAA ATT AAA CGC GTC GAA AAA
318      Thr Ala Pro Val Gly Glu Arg Asn Leu Lys Glu Glu Ile Lys Arg Val Glu Lys
1135     CGC ATC ATT ATG GAA GTG CTG GAA CAA CAA GAA GGA AAC CGA ACC CGC ACT GCT
336      Arg Ile Ile Met Glu Val Leu Glu Gln Gln Glu Gly Asn Arg Thr Arg Thr Ala
1189     TTA ATG CTG GGC ATC AGT CGC CGT GCA TTG ATG TAT AAA CTC CAG GAA TAC GGT
354      Leu Met Leu Gly Ile Ser Arg Arg Ala Leu Met Tyr Lys Leu Gln Glu Tyr Gly
1243     ATC GAT CCG GCG GAT GTA TAA CAC CAA AAC TTG CTA TGC AGA AAT TTG CAC AGT
372      Ile Asp Pro Ala Asp Val ***
1297     GCG CAA TTT TCT GCA TAG CCG CTC ATT CTC CTT ATA AAT CCC CAT CCA ATT TAT
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FIG. 3. Nucleotide and deduced amino acid sequence of the Az gene. Sequence is in reverse orientation to that given in Fig. 2. Two boxes located upstream of the Az ORF indicate sequences with homology to −10 promoter regions recognized by the transcription factor σ^{54}. A putative Shine–Dalgarno sequence preceding the Az initiator codon is underlined. A palindromic sequence downstream of the Az termination codon is also underlined.

ORF2 Encodes the Az. The 6.4-kb *Bam*HI fragment was digested with *Sal* I (Fig. 2) and the resulting fragments were cloned in plasmid pIC20H. Since *Sal* I cleaves within ORF1, it was expected that if ORF1 encoded Az neither of the two resulting plasmids would express Az. This was not the case, since cells transformed with plasmid pCPC-Az carrying the 4.2-kb *Sal* I/*Bam*HI fragment including the ORF2 overproduced Az (Fig. 4). This suggested that ORF2 encodes Az. The 6.4-kb *Bam*HI fragment was also digested with *Eco*RI, which cleaves within both ORF1 and ORF2 coding sequences, and the fragments were subcloned in pUC19. None of the resulting recombinant plasmids overproduced Az (data not shown). This nullifies the possibility that one of the minor ORFs contained in the 4.2-kb *Bam*HI/*Sal* I fragment regulates Az expression in trans.

The Az gene (ORF2) is preceded by a weak Shine–Dalgarno sequence 6 bp upstream from the initiator AUG codon (Fig. 3). It encodes a 378-amino acid protein with a predicted molecular weight of 43,045. The codon usage of the Az gene indicates that it contains a large number of infrequently used synonymic codons (30) (data not shown). This may explain, in part, the low abundance of Az, since such codons correspond to rare tRNA-isoaccepting species in *E. coli* (30). Calculation of the pI of Az based on its amino acid composition resulted in a basic pI value of 8.2. This is in apparent disagreement with the acidic nature of the Az protein, which was measured by native isoelectric focusing

(11). Denaturing two-dimensional gel electrophoresis of extracts from Az-overproducing cells, followed by immunoblot, revealed that Az protein exists in three isoelectric forms with pI values between 6.0 and 6.5 (data not shown). These data indicate that Az is modified posttranslationally (also see below).

Az Is Homologous with Transcriptional Regulators of the Two-Component System Family. Homology searches between the Az protein sequence and sequences existing in protein data bases indicate that Az shares striking sequence homologies with bacterial transcriptional activators (Fig. 5). These proteins, which include, among others, the gene products of *ntrC* from *Klebsiella pneumoniae*, *dctD* from *Rhizobium meliloti*, *xylR* from *Pseudomonas putida*, *nifA* from *Bradyrhizobium japonicum*, *flbD* from *Caulobacter crescentus*, and *hydG* and *fhlA* from *E. coli*, are members of two-component transcriptional regulatory systems (31–33). The synthesis of these transcription factors is regulated by alternative σ factors of the σ^{54} (*rpoN*) family. Although the start point of Az gene transcription was not determined in the present study, there are two sequences with considerable homology to σ^{54} −10 regions located 133 and 123 bp upstream of the initiator AUG of the Az gene (Fig. 3). No homologies to −35 sequences were found.

To exclude the possibility that ORF2 encodes a transcriptional activator of the Az gene, we subcloned a portion of the ORF2 gene, encoding amino acids 56–292, in plasmid
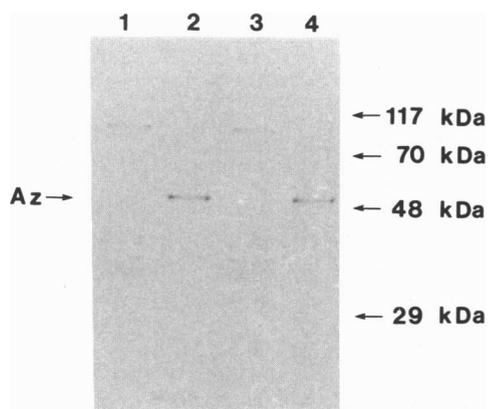
FIG. 4. ORF2 encodes *Az*. The 6.4-kb *Bam*HI fragment was digested with *Sal* I and subcloned in plasmid pIC20H. Plasmids containing either a 2.2-kb insert (pCPC-J) or a 4.2-kb insert with the intact ORF2 (pCPC-Az) were selected. Extracts from *E. coli* DH5α transformed with the indicated plasmids were analyzed by SDS/PAGE and immunoblotting. Anti-Az antibody was used at 1:1000 dilution. Extracts from cells transformed with pIC20H (negative control) (lane 1; 20 μg), pUC-Az (positive control) (lane 2; 2 μg), pCPC-J (lane 3; 20 μg), and pCPC-Az (lane 4; 2 μg) were used. Positions of size markers used (prestained high range; BRL) are indicated.

pGEX-3X and expressed it as a fusion protein with GST (25). The resulting full size GST–Az fusion protein (52 kDa) and a smaller degradation product were recognized both by anti-Az and anti-GST antibodies on immunoblots (Fig. 6). This

FIG. 6. A GST–ORF2 fusion protein is recognized both by anti-Az and by anti-GST antibodies. Glutathione-Sepharose affinity-purified proteins from control pGEX-3X or recombinant pCPC-XAz1-transformed *E. coli* were analyzed on SDS/10% polyacrylamide gels, and either they were stained with Coomassie brilliant blue (lanes P, M, 1, and 2) or they were electrophoretically transferred to nitrocellulose membranes and probed with anti-GST (lane 3) or anti-Az (lane 4) antibodies. Prestained size markers (lane P) were from BRL; unstained size markers (lane M) were from Pharmacia.

proves that ORF2 is the structural gene for Az. Two proteins of approximately 60 and 70 kDa copurify with GST–Az, but not with GST alone, in the affinity chromatography step (Fig. 6). These proteins were identified immunologically as the heat-shock proteins GroEL (HSP60) and DnaK (HSP70) (34) (data not shown). We do not know whether this Az binding to heat shock proteins has any physiological significance or whether the overexpressed GST–Az protein is partially denatured.

Interestingly, the predicted 609-amino acid, acidic protein (predicted pI 5.8) that is encoded by ORF1, has significant homologies with several "sensor" kinases of two-component regulatory systems (data not shown). We do not know whether ORF1 kinase regulates Az.
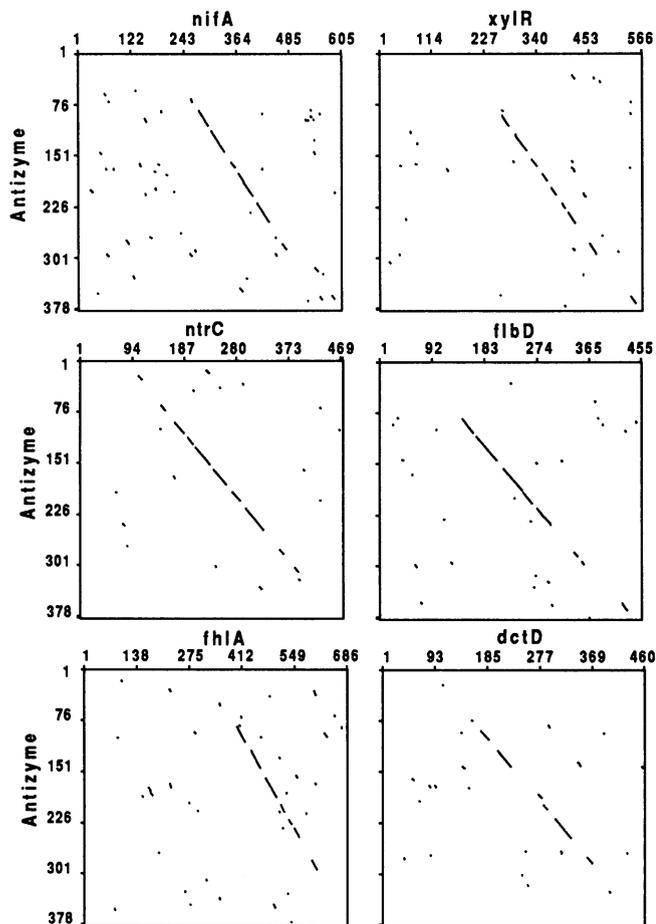

FIG. 5. Homology plots of Az versus several transcriptional regulators. Az amino acid number is indicated on the vertical axis; transcriptional regulator is indicated on the horizontal axis.
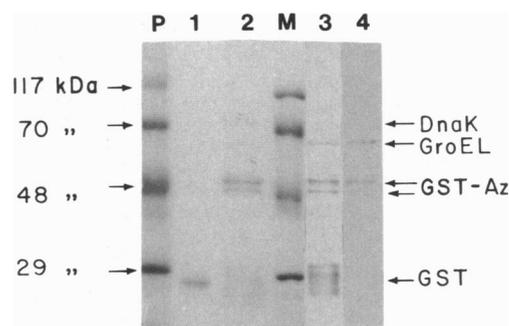
DISCUSSION

The gene encoding the *E. coli* Az was identified by immunological screening. The *Az* gene maps at the 48th minute of the *E. coli* linkage map (28). Extracts from cells transformed with plasmid pUC-Az exhibited a 50- to 80-fold higher Az specific activity than those from control cells. Such extracts also exhibited a 50- to 100-fold increase in the levels of Az protein. However, ODC and ADC activities in Az-overproducing extracts are reduced only to ≈50% of the control cells. This more modest inhibition may be due either to a variety of activators that participate in the regulation of ODC and ADC (20) or to the presence of sequestered or inactive forms of Az. Treatment of *E. coli* extracts at pH 1.8 and perchloric acid precipitation (11), which were used prior to measuring the Az activity, may reactivate Az by disrupting nonproductive complexes. The N-terminal amino acid of this protein was blocked as was that of Az from nonoverproducing cells. The fact that the cloned gene, which was selected on the basis of immunological cross-reactivity of its product with antibodies raised against Az, can direct the synthesis of a protein that behaves functionally as Az indicates that it is indeed the *Az* gene.

The mammalian Az has been implicated not only in inhibition of ODC but also in its targeting for degradation (9, 10, 35). Although we have not directly tested the effect of *E. coli* Az overproduction on ODC stability, the fact that ODC protein levels are not reduced in the Az overproducers indicates that *E. coli* Az may behave differently than its mammalian counterpart.

The predicted pI of Az is 8.2. This is in disagreement with its previously reported acidic pI of 3.5 (11). When analyzed

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by denaturing two-dimensional gel electrophoresis, the Az from overproducing cells was found to consist of three isoelectric forms with pI 6.0–6.5, significantly lower than that predicted from the amino acid composition. This indicates that Az is modified posttranslationally—e.g., by phosphorylation. Such modification could explain the discrepancy, although it is also possible that the previously performed native isoelectric focusing resulted in an erroneous pI value. Native isoelectric focusing, unlike denaturing isoelectric focusing, does not disrupt protein–protein or protein–DNA complexes. Thus, when proteins complexed with other proteins or nucleic acids are examined by this technique, anomalous pI values may be obtained.

Although no homology was found between the *E. coli* and rat Az (36) sequences, significant homology was found between *E. coli* Az and bacterial transcriptional regulators belonging in the two-component regulator system family (31–33). These systems are usually composed of a sensor kinase and a transcriptional regulator. Activation of the sensor kinase results in its autophosphorylation on a histidyl residue and subsequent phosphorylation of the transcription factor on an aspartyl residue by protein–protein phosphate transfer. Phosphorylation of the transcriptional regulator is usually required for its activation. Activated transcriptional regulators bind to transcriptional enhancers (37) and they activate RNA polymerase, which contains σ^{54} (33) as σ factor. In this respect, it is interesting that ORF1 shares significant homologies with several sensor kinases. Thus, it is possible that ORF1 encodes a kinase that regulates the activity of Az by phosphorylation.

The apparent dual function of Az is reminiscent of our earlier findings that the S20 and L34 ribosomal proteins also act as inhibitors of ODC and ADC (12). Which gene(s) is regulated by the postulated transcription factor activity of Az, what are the signals for Az activation, and what is its relevance to the polyamine content of *E. coli* remain to be determined. The above questions need to be addressed at the biochemical as well as genetic levels.

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