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Biosynthetic Arginine Decarboxylase in *Escherichia coli* Is Synthesized as a Precursor and Located in the Cell Envelope

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The biosynthetic form of arginine decarboxylase (ADC) catalyzes the synthesis of agmatine, a precursor of putrescine, in *Escherichia coli*. Selective disruption of the cell envelope and an assessment of ADC activity or immunoprecipitable ADC in various fractions demonstrated its location between the cytoplasmic membrane and peptidoglycan layer. Expression in minicells of the *speA* gene encoding ADC resulted in the production of two immunoprecipitable species (74 and 70 kilodaltons). Studies in vivo with a pulse and chase of radiolabeled amino acid into the two species suggest a precursor-product relationship. This relationship was corroborated by demonstrating the accumulation of the 74-kilodalton species in a strain of *E. coli* unable to process signal sequences. Peptide mapping experiments with V8 protease, trypsin, and α-chymotrypsin demonstrated that the two species of ADC were very similar except for a minor difference. These data were used to substantiate the compartmentalization hypothesis as to how exogenous arginine can be channeled preferentially into putrescine.

The production of the diamine putrescine (1,4-diaminobutane) in *Escherichia coli* is mediated by the enzymes of two biosynthetic pathways. Pathway 1 contains the enzyme ornithine decarboxylase, and pathway 2 contains the enzymes arginine decarboxylase (ADC) and agmatine ureohydrolase. In the course of investigating the ability of *E. coli* to select between these two pathways, it was discovered that exogenously provided arginine acts as a signal for the selective utilization of pathway 2 over pathway 1 (13). Utilization of pathway 2 was not explained by mechanisms involving the modulation of ADC activity or intracellular arginine levels. (14). Morris et al. (14) hypothesized that there is a compartmentalization of ADC which makes it primarily accessible to exogenously supplied arginine. This hypothesis was consistent with the earlier observation of Tabor and Tabor (22), who had demonstrated the preferential utilization of exogenously supplied arginine to synthesize putrescine. They concluded that exogenous arginine was accessible to ADC before equilibration with the intracellular arginine pool.

In this study, we tested this compartmentalization hypothesis by examining whether ADC was located external to the cytoplasmic membrane. We found that a significant proportion of ADC was associated with membranes of crude envelope preparations and that selective disruption of the cell envelope released ADC from the inner periplasmic space. We also found that the two immunoprecipitable species of ADC exhibited a precursor-product relationship; this was due to the occurrence of a signal sequence.

MATERIALS AND METHODS

Bacterial strains and plasmid. The following *E. coli* K-12 strains were used. P678-54 (*thr leu minA minB thi ara lacY gal malA xyl mtl rpsl fhuA azi*) was supplied by H. I. Adler (Oak Ridge National Research Laboratories, Tenn. [1]). MM18 [F⁻ Δ*lac araD rpsL relA thiA lamB malK* (*malE-lacZ*) *lacY*] (2) and MC4100 (F⁻ Δ*lac araD rpsL relA thiA*) (7) were obtained from J. Beckwith, Harvard University, Boston, Mass. HY1 is a prototroph and was obtained from H. Yamazaki, Carleton University, Ottawa, Ontario, Canada. CA244 was obtained from the Medical Research Establishment, Wiltshire, England. DM22 (*pro his metG serA* Δ(*speC-glc*) *leu rpsL thi hsd sup*) was obtained from Doug Markham, Fox Chase Cancer Institute, Philadelphia, Pa.

Plasmid pKA5 (23) contains the genes encoding ADC (*speA*), agmatine ureohydrolase (*speB*), and methionine adenosyltransferase (*metK*). *E. coli* P678-54 was transformed with pKA5 as described by Boyle et al. (6).

Media. The bacteria were grown with shaking at 37°C in a morpholinepropanesulfonic acid (MOPS) minimal medium (15) supplemented with 50 μg of the required L-amino acids and 1 μg thiamine per ml and 0.2% glucose or lactose. *E. coli* strains MM18 and MC4100 were induced with maltose as described by Ito et al. (10).

Preparation of cell extracts. *E. coli* CA244 (5g) was mixed with 7.4 g of alumina with a chilled mortar and pestle until a paste was obtained. This generally took 10 to 20 min of grinding by hand. The paste was then suspended in 20 to 25 ml of breakage buffer B (0.05 M potassium phosphate [pH 8.0], 5 mM $MgCl_2$, 40 μM pyridoxal phosphate, 14 mM β-mercaptoethanol). The alumina and unbroken cells were cleared by three centrifugations at 2,000 × *g* for 5 min to produce crude (S-2) extracts.

Membrane preparations. (i) Differential centrifugation. S-2 extracts cleared of unbroken cells were centrifuged at 160,000 × *g* for 1 h at 4°C. These extracts were designated S-160. The pellets from these spins were suspended in breakage buffer B by gentle homogenization. No unbroken cells were detected in representative membrane preparations that were examined by electron microscopy.

(ii) Sucrose gradient. A discontinuous two-step sucrose gradient (0.77 M/1.44 M/2.02 M) was prepared as described by Schnaitman (18) by utilizing sucrose dissolved in breakage buffer B. The size of the gradients was reduced to 5.0 ml. The gradients were fractionated into 0.42-ml fractions, and ADC activity was assayed as described by Wright and Boyle (24).

Protein labeling in minicells. Minicells were purified and labeled by the method of Tabor and Tabor (22) with the

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following modification. After harvesting in early stationary phase and washing with cold BSG buffer (0.85% NaCl, 0.03% KH_2PO4 0.06% NaH_2PO4, 100 μg of gelatin per ml), the cells were suspended with a magnetic stirrer (20 min) in 20 ml of BSG buffer before loading onto sucrose gradients (5 to 30% [wt/vol] in BSG).

PAGE and fluorography. Fractionation of proteins by polyacrylamide gel electrophoresis (PAGE) in 10 or 15% acrylamide and 0.1% sodium dodecyl sulfate (SDS) was performed as described by Laemmli (12). Detection of labeled proteins by fluorographic methods was by the method of Bonner and Laskey (4).

The in situ proteolytic method of Cleveland et al. (8) was followed, except that the time of incubation for proteolysis was increased from 30 to 45 min.

Pulse-chase experiments. Cells were grown to a density of 2×10^8 cells per ml. A 50-ml culture was pulse-labeled with 200 μCi of [^{35}S]methionine (1,490 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) for 30 s. One-half of the culture was harvested by centrifugation, and the other half was chased by adding 100 μl of unlabeled methionine (10 mg/ml).

Lysis of bacterial cells and immunoprecipitation. A 25-ml sample of the culture was washed with phosphate-buffered saline (25 mM K_2HPO_4 [pH 7.6], 0.1 M NaCl). The pellet was suspended in 1.25 ml of 25 mM Tris hydrochloride (pH 8.0) with 25% sucrose, to which was added 125 μl each of 25 mM EDTA (pH 8.0) and freshly made lysozyme solution (5 mg/ml in 0.2 M Tris hydrochloride [pH 8.0]). The incubation was carried out at 4°C for 10 min. Thereafter, 0.5 ml of solution containing 2% Nonidet p-40, 40 mM $MgCl_2$, 800 mM KCl, and 80 μg of DNase I and 400 μg of RNase I per ml was added, and the new mixture was incubated at 37°C for 15 min. The lysate was clarified by centrifugation at 20,000 × *g* for 15 min.

To the clear lysate was added 5 μl of antibody prepared against ADC (5). The mixtures were incubated at 4°C for 30 min, and the 200-μl mixture of 5% protein A–Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, N.J. [Div. Pharmacia, Inc.]) in phosphate-buffered saline with 10 mM EDTA [pH 8.0]–0.5% Nonidet P-40 was added. The reaction mixtures were incubated at 4°C and mixed at 3-min intervals for 30 min. The Sepharose A-immunoprecipitate complex was collected by centrifugation at 10,000 × *g* in a Microfuge (Beckman Instruments, Inc., Fullerton, Calif.) and washed four times with RIPA (phosphate-buffered saline with 1% TX-100, 0.1% SDS, and 1% sodium deoxycholate). The complexes were suspended in sample gel buffer (0.125 M Tris hydrochloride [pH 6.8], 2% SDS, 10% glycerol, 5% β-mercaptoethanol) and boiled for 3 min. The protein A-Sepharose was removed by centrifugation at 10,000 × *g* for 2 min. The solubilized immunoprecipitates (supernatants) were subjected to SDS-PAGE.

RESULTS

Release of ADC by osmotic shock or EDTA-lysozyme treatment. To assess whether ADC was located in the periplasm, we cultured a prototrophic K-12 strain of *E. coli*, CA244, in a low-phosphate minimal medium (15) with lactose and arginine and then subjected it to osmotic shock or EDTA-lysozyme-sucrose treatment (16). No ADC activity was released by osmotic shock. However, 43% of total ADC activity was released by EDTA-lysozyme treatment. Since 90% of alkaline phosphatase (AP) activity (periplasmic marker) was released by osmotic shock or EDTA-lysozyme treatment, it appeared that the peptidoglycan layer was involved in the retention of a portion of the ADC pool. No significant levels of β-galactosidase activity (cytoplasmic marker) were released under these conditions.

Association between ADC and the cell envelope. To investigate the nature of the association of ADC with the cell envelope further, we prepared cell-free extracts from *E. coli* CA244 and used them to generate crude envelope fractions. S-2 extracts prepared by sonication or pressure disruption exhibited no ADC activity that was cosedimentable with a crude membrane pellet (160,000 × *g* for 1 h) (data not shown). In contrast, S-2 extracts prepared by alumina grinding exhibited ADC activity which cosedimented with a crude membrane pellet. In three separate experiments, the supernatant S-160 and pellet S-160 crude membrane fractions exhibited 43 and 41%, respectively, of the ADC activity of the S-2 extract (variation, 6%). The ADC activity of the S-2 extract was 1,800 nmol of CO_2 per h and was measured by the release of $^{14}CO_2$ from L-[U-^{14}C] arginine as described by Wright and Boyle (24).

To determine if alumina granules were artifactually causing ADC to bind to the crude envelope fraction, we lysed cells by pressure disruption at 15,000 lb/in^2 and prepared an S-2 extract. Alumina was added to one-half of the S-2 extract, and the extract was stirred at 4°C for 2 h; the other half served as a control to which no alumina was added. Then S-30 and S-160 crude envelope fractions were prepared from both halves and assayed for ADC activity (data not shown). No significant association of ADC was found in pellet fractions, indicating that alumina was not responsible for the adsorption of ADC to the crude envelope. When the same experiment was repeated on an S-2 extract prepared with alumina, no additional association of ADC to the crude envelope fraction was found.

When a suspended S-160 pellet fraction was sedimented on a discontinuous sucrose gradient (26, 49, and 65% steps), we found that ADC was distributed primarily with two fractions: a cytoplasmic membrane fraction at the 26/49% interface and the pellet at the bottom of the tube (Fig. 1). Treatment of the S-160 pellet fraction with 0.1 M NaCl before centrifugation did not alter the ADC sedimentation profile (data not shown), whereas TX-100 treatment significantly reduced the amount of ADC pelleting through the cushion. This suggests that the S-160 pellet fraction contained a portion of the ADC pool strongly associated with the cell envelope (i.e., cell wall plus cell membranes).

Selective release of ADC from the cell envelope. To confirm and extend the membrane-cell wall association of ADC, we used antibody against ADC to assess the release of ADC from cells whose envelopes had been selectively disrupted. A culture of *E. coli* DM22 transformed with a plasmid (pKA5) bearing the *speA* gene encoding ADC (23) was grown in MOPS minimal medium and pulsed with [^{35}S]methionine. One-half of the culture was harvested, and the other half was chased with nonradioactive methionine and harvested. Cells from both halves were selectively disrupted by the procedure of Bewick and Lo (3). Each fraction obtained was subjected to immunoprecipitation either with antiserum to the 70-kilodalton (kDa) species of ADC or with antiserum to AP. The immunoprecipitates were subjected to SDS-PAGE (Fig. 2). The fraction from the EDTA-sucrose wash had no immunoprecipitable ADC or AP (lane a). The supernatant from the osmotically shocked cells contained immunoprecipitable AP (lane b) but no immunoprecipitable ADC (lane c). This is interpreted to mean that AP, but not ADC, is located exterior to the peptidoglycan, i.e., the outer periplasmic space. The supernatant from

the lysozyme-sucrose treatment of osmotically shocked cells contained the immunoprecipitable 74- and 70-kDa species of ADC (lane f) in pulsed-labeled cells and the 70-kDa species (lane e) in pulse-chased cells. Since there was no detectable AP in this fraction (i.e., lane e or f), ADC appears to be compartmentalized to the inner periplasmic space bounded by the peptidoglycan and inner membrane. In addition, the disappearance of the 74-kDa species of ADC as a result of a chase is consistent with the idea of a precursor-product relationship. These experimental results, combined with the previous data, suggest that biosynthetic ADC is a periplasmic enzyme.

Precursor-product relationship. Antiserum against purified 70-kDa species of ADC (5) immunoprecipitated both the 74- and the 70-kDa species of ADC from a culture of *E. coli* which was pulsed but not chased (Fig. 2). This result prompted a direct test of the possibility that the two species differing by about 4 kDa exhibit a precursor-product relationship.

A prototrophic strain of *E. coli*, HY1, was pulsed with [^{35}S]methionine, and an excess of unlabeled methionine was added. Extracts were prepared from cells collected at intervals after the initiation of the chase and were subjected to immunoprecipitation. The immunoprecipitates were analyzed by SDS-PAGE and fluorography (Fig. 3). The amount of radioactivity present in the putative 74-kDa species at the beginning of the chase and the complete disappearance of the 74-kDa species after 360 s clearly indicate a precursor-product relationship. We conclude that ADC is made first as a precursor about 4 kDa larger than the mature enzyme.

Accumulation of the 74-kDa species of ADC. To study whether the precursor-product relationship between the two species of ADC was due to the presence of a signal sequence, we assessed ADC synthesis in *E. coli* MM18, which carries a fusion of the *malE* and *lacZ* genes. This strain, when induced with maltose, produces a hybrid protein which becomes stuck in the cytoplasmic membrane and blocks the export channel for other secreted proteins. Under these conditions, periplasmic proteins, including the maltose-binding protein and the major outer membrane proteins, are synthesized as precursor forms from which the signal sequences remain unprocessed (2, 10, 20).

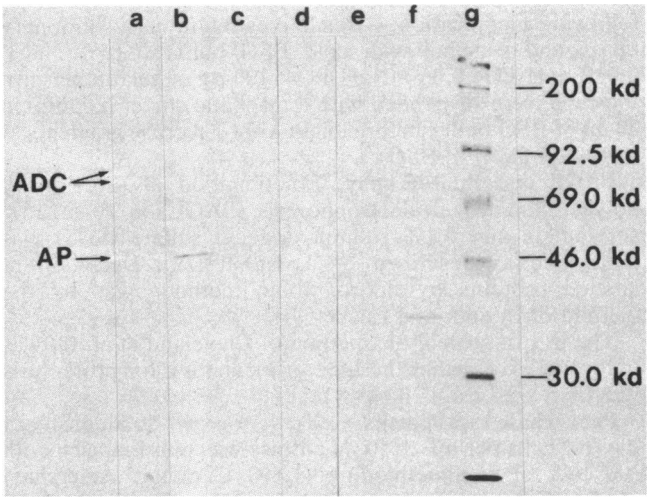

FIG. 2. Fluorogram of a 10% SDS-polyacrylamide gel of [^{35}S]methionine-labeled proteins immunoprecipitated with either ADC or AP antiserum. *E. coli* transformed with the plasmid pKA5 was lysed by using a sequential disruption procedure (3), and the various supernatant fractions were treated with antisera. Lanes: a, EDTA-sucrose fraction, anti-ADC, and anti-AP; b, osmotic-shock fraction and anti-AP; c, osmotic-shock fraction and anti-ADC; d, lysozyme-sucrose fraction and anti-AP; e, lysozyme-sucrose fraction and anti-ADC; f, lysozyme-sucrose fraction and anti-ADC (Cells were pulsed but not chased.); g, ^{14}C-radiolabeled markers (myosin, 200 kDa [200]; phosphorylase *b*, 92.5 kDa [92.5]; bovine serum albumin, 69 kDa [69]; ovalbumin, 46 kDa [46]; carbonic anhydrase, 30 kDa [30]; α-lactalbumin, 14.3 kDa [14.3]. kd, Kilodaltons.

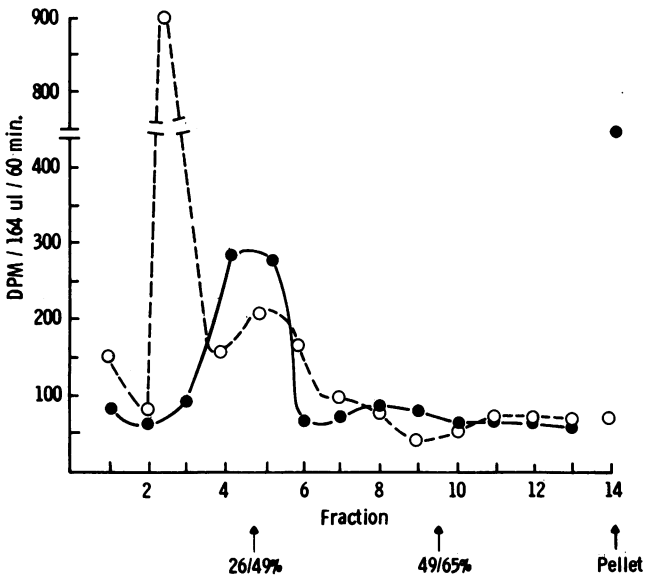

FIG. 1. Sedimentation profile of ADC from S-160 pellet on a discontinuous sucrose gradient. S-160 crude envelope fractions (0.05 ml), after treatment, were layered onto 5.0-ml discontinuous gradients containing 26, 49, and 65% steps and spun at 42,500 rpm for 18 h in an SW50.1 rotor. Fractions of 0.42 ml were collected, and 167-μl samples were assayed for ADC. Symbols: ●, control; ○, 1% TX-100. The pellet at the bottom of each gradient was suspended in 0.4 ml of breakage buffer B before assaying. DPM, Disintegrations per min.

After induction with maltose, *E. coli* MM18 was pulse-labeled with [^{35}S]methionine. A crude extract was immunoprecipitated with antiserum to the 70-kDa ADC species. The immunoprecipitates were subjected to SDS-PAGE and fluorography. Only the 74-kDa species of ADC was detected (Fig. 4). These results further support our earlier conclusion of a precursor-product relationship between these two species of ADC. *E. coli* MC4100, which is a parent strain of MM18 without the gene fusion, served as a control. Only two immunoprecipitable ADC species of 74 and 70 kDa were observed in MC4100 under the identical experimental conditions used for radiolabeling MM18.

Peptide mapping of 74- and 70-kDa proteins. The *speA* (ADC), *speB* (agmatine ureohydrolase), and *metK* (methionine adenosyltransferase) genes of *E. coli* have been cloned on plasmid pKA5 by Tabor et al. (23). Minicells isolated from *E. coli* P678-54 transformed with plasmid pKA5 were depleted of endogenous mRNA by overnight incubation in the presence of cycloserine (6) and then labeled with [^{35}S]methionine. The labeled proteins, when separated on an SDS-polyacrylamide gel, included two distinct proteins of 74 and 70 kDa (ADC) in addition to the 42- (methionine adenosyltransferase) and 38-kDa (agmatine ureohydrolase) proteins (Fig. 3, lane d). The proteins ranging from 32 to 10 kDa

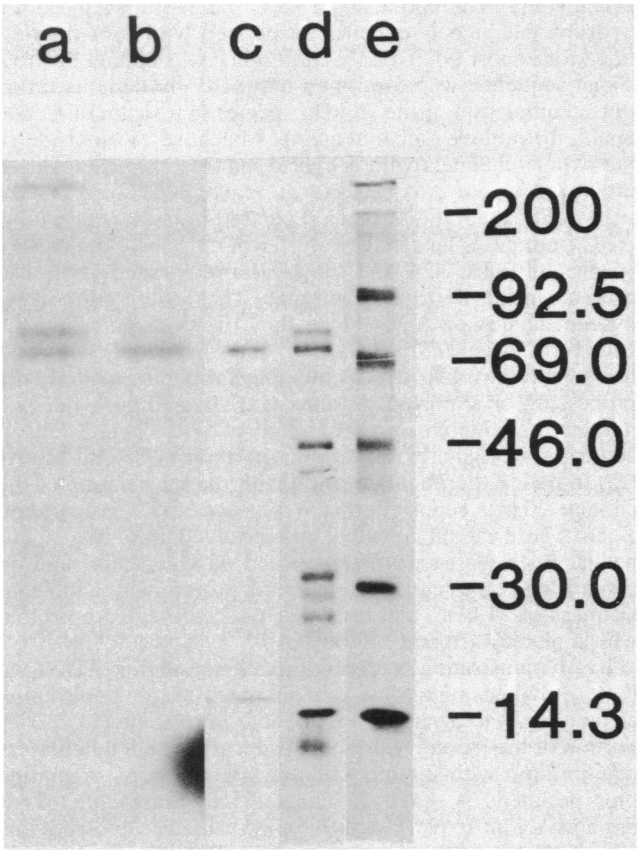

FIG. 3. Composite fluorogram of a 10% SDS-polyacrylamide gel of ADC immunoprecipitates from a pulse-chase experiment. *E. coli* HY1 was labeled with [^{35}S]methionine for 30 s and chased with excess unlabeled methionine. Lanes: a, 30-s chase; b, 240-s chase; c, 360-s chase; d, radiolabeled proteins encoded by pKA5; e, ^{14}C-labeled molecular weight markers (see the legend to Fig. 2). Lanes a through c represent ADC immunoprecipitates. Lanes e and d were not treated with anti-ADC serum. Exposure times were as follows: lanes a and b, 96 h; lanes c, d, and e, 18 h.

are vector-encoded products. The regions of the gel containing the 74 and 70-kDa species were excised from the gel and subjected to proteolysis by three different proteolytic enzymes, viz., V8 protease, α-chymotrypsin, and trypsin, at various enzyme concentrations. The peptide patterns thus generated (Fig. 5) demonstrate a strong similarity between the 74- and 70-kDa proteins.

DISCUSSION

Unlike AP, ADC is not a classic periplasmic enzyme, since it is not released by osmotic shock. Compared with the other proteins sequestered in the periplasmic space of *E. coli* (reviewed in reference 11), ADC is one of the largest proteins, since its native molecular mass is approximately 280 kDa (25). This large size may explain why ADC is not released by osmotic shock—it is too large to be released through the cross-linked peptidoglycan network. EDTA-lysozyme treatment released a significant portion of the ADC activity and suggests some type of compartmentalization or association of the enzyme within the cell envelope. This association was substantiated by a demonstration of the cosedimentation of ADC with membrane fractions prepared by sucrose gradient sedimentation or differential centrifugation. Moreover, the reduction by TX-100 of the amount of ADC pelleting through the 69% sucrose cushion suggests that this fraction is a crude envelope fraction containing cytoplasmic membrane, peptidoglycan, and outer membrane fractions as demonstrated by Schnaitman (18). Since TX-100 treatment of crude cell wall preparations solubilizes cytoplasmic membrane and associated proteins (18), the ADC released probably represents a portion either bound to the cytoplasmic membrane or released after disruption of the cytoplasmic membrane and peptidoglycan layer. The portion of the ADC pool not released from the crude membrane preparation by TX-100 treatment (26/49% interface in Fig. 1) could be due to a number of factors. They include the following: (i) less effective solubilization by TX-100 at 4°C (19), (ii) less solubility of cell envelope proteins in TX-100 in the presence of Mg^{2+} (19), and (iii) a possible influence of the very acidic nature of the enzyme on the association of ADC to membrane components (25). Regardless of which of these explanations are operative, the data clearly suggest that

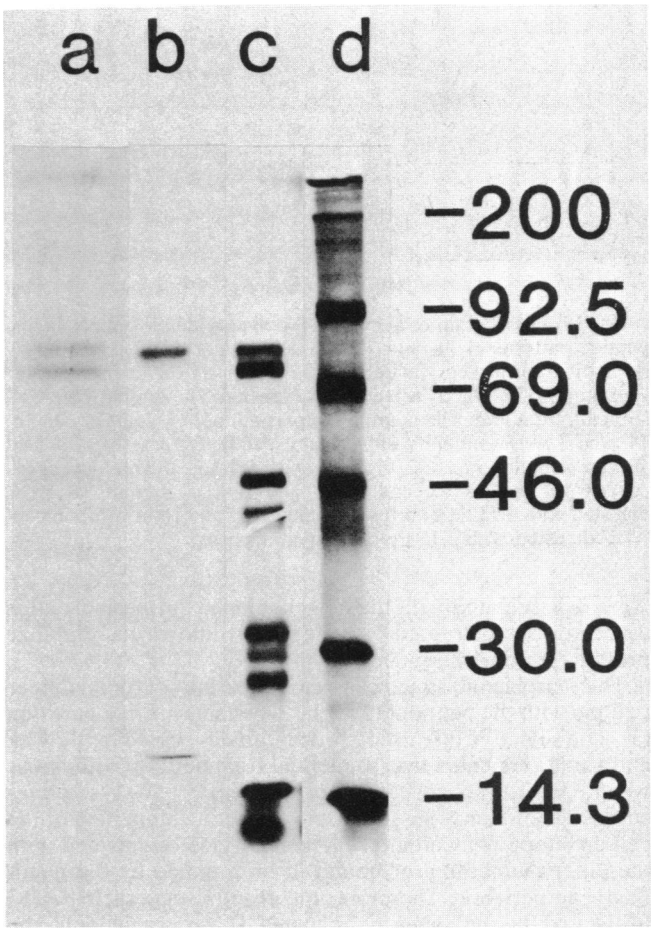

FIG. 4. Composite fluorogram of a 10% SDS-polyacrylamide gel of ADC immunoprecipitates from pulse-labeled whole-cell extracts of *E. coli* strains MM18 (*malE-lacZ* fusion) and MC4100. Cells were labeled with [^{35}S]methionine for 30 s and chased with excess unlabeled methionine. Lanes: a, MC4100; b, MM18; c, radiolabeled proteins encoded by pKA5; d, ^{14}C-labeled molecular weight markers (see the legend to Fig. 2). Lanes a and b represent ADC immunoprecipitates. Lanes c and d were not treated with anti-ADC serum. Exposure times were as follows: lane a, 96 h; lanes b, c, and d, 48 h.

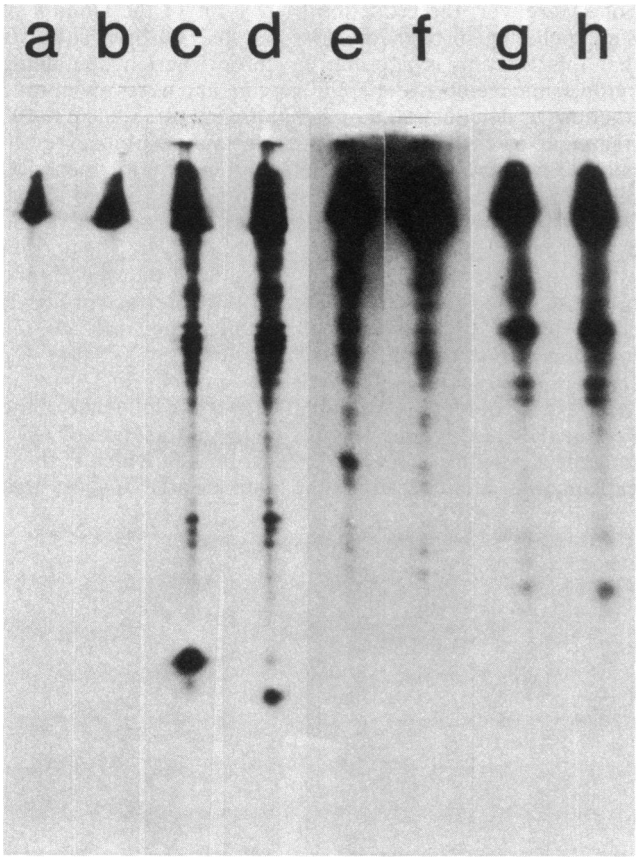

FIG. 5. Fluorogram of a 15% SDS-polyacrylamide gel displaying peptide patterns of 74- and 70-kDa subunits of ADC generated by three different proteolytic enzymes. Proteolysis was carried out for 45 min at 37°C on [^{35}S]methionine-labeled 74- or 70-kDa ADC subunits from minicells bearing the plasmid pKA5. Lanes: a and b, 74- and 70-kDa subunits of ADC, respectively; c and d, 74- and 70-kDa subunits of ADC digested with 0.1 μg of *Staphylococcus aureus* V8 protease; e and f, 74- and 70-kDa subunits of ADC digested with 0.15 μg of trypsin; g and h, 74- and 70-kDa subunits of ADC digested with 0.15 μg of α-chymotrypsin.

ADC is associated with the cell envelope. Furthermore, this association is disrupted by a detergent known to solubilize proteins from membranes.

The periplasmic space can be divided into outer and inner regions with the peptidoglycan network serving as a partition (3). The selective disruption procedure developed by Bewick and Lo (3) indicates that sequential treatment by cold-water wash, EDTA-sucrose, osmotic shock, and lysozyme-sucrose allows an assessment of whether a component of the cell envelope is located on the cell surface, associated with the outer membrane, or bound to or confined by the peptidoglycan network. Therefore, our results suggest that ADC is located in the inner periplasmic space, since only the 70-kDa species of ADC is present in the EDTA-sucrose-lysozyme-treated supernatant (Fig. 2, lane d). Many of the proteins destined for export through the cytoplasmic membrane contain N-terminal hydrophobic signal sequences (9, 21). Thus, one would expect that ADC should exhibit such a signal sequence if it is to be transported through the cytoplasmic membrane. If the average molecular mass of an amino acid is assumed to be 0.125 kDa, the difference of 4 kDa in the two ADC species (Fig. 3) corresponds to approximately 32 amino acids. This is the average length of a hydrophobic signal sequence associated with proteins destined for export (9). That this difference was indeed due to a signal sequence was confirmed indirectly by demonstrating the accumulation of the 74-kDa species in a strain of *E. coli* unable to remove signal sequences because of blockage of the export channels with a hybrid maltose: β-galactosidase protein (2).

In both Fig. 3 and 4, lanes a contain immunoprecipitable ADC from pulse-labeled cultures. Not only were the mature species of ADC (74 and 70 kDa) precipitated, but also proteins in the 30- to 40-kDa range. These were quite likely nascent chains of ADC, since they disappeared when the cells were chased (Fig. 3, lanes b and c). This phenomenon has been reported in studies utilizing antibodies to study the processing of exported proteins (11). In addition, the two immunoprecipitable forms of mature ADC (Fig. 3 and 4) confirm the original observation reported by Wu and Morris (25) in their experiments dealing with the purification of the enzyme. They reported that a purified ADC preparation electrophoresed on a native gel resolved into two major peaks. Each of the peaks possessed ADC activity, and on denaturing gels one peak contained a protein with a molecular mass of 74 kDa, and the other peak contained a protein with a molecular mass of 70 kDa.

By demonstrating a periplasmic location for ADC, we have provided a possible explanation for the preferential channeling of exogenous arginine into putrescine (14, 22). A portion of the incoming arginine is decarboxylated before its equilibration with the intracellular arginine pool. Agmatine thus produced is readily channeled into putrescine. What remains to be explained is why *E. coli* has evolved this means of putrescine production. It is possible that agmatine or putrescine is needed in the cell envelope or that the cells need to minimize the amount of intracellular putrescine accumulation when supplied with exogenous arginine, e.g., a plentiful amino acid source as in the large intestine in man (17). We favor this latter possibility, since we have observed in unpublished results that *E. coli* bearing the *speA* (ADC) and *speB* (agmatine ureohydrolase) genes on a high-copy-number plasmid overproduce putrescine but excrete the majority into the extracellular environment.

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LITERATURE CITED

1. **Adler, H. I., W. D. Fisher, A. Cohen, and A. A. Hardigree.** 1966. Miniature *Escherichia coli* cells deficient in DNA. Proc. Natl. Acad. Sci. U.S.A. **57**:321–326.
2. **Bassford, P. J., Jr., T. J. Silhavy, and J. R. Beckwith.** 1979. Use of gene fusion to study secretion of maltose-binding protein into *Escherichia coli* periplasm. J. Bacteriol. **139**:19–31.
3. **Bewick, M. A., and T. C. Y. Lo.** 1980. Localization of the dicarboxylate binding protein in the cell envelope of *Escherichia coli* K12. Can. J. Biochem. **58**:885–897.
4. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
5. **Boyle, S. M., and K. Adachi.** 1982. Biosynthetic ornithine and arginine decarboxylase: correlation of rates of synthesis with activities in *Escherichia coli* during exponential growth and following nutritional shift-up. Can. J. Microbiol. **28**:945–950.

6. **Boyle, S. M., G. D. Markham, E. W. Hafner, J. M. Wright, H. Tabor, and C. W. Tabor.** 1984. Expression of the cloned genes encoding the putrescine biosynthetic enzymes and methionine adenosyltransferase of *Escherichia coli* (*speA*, *speB*, *speC*, and *metK*). Gene **30**:129–136.
7. **Casadaban, M.** 1976. Transposition and fusion of the lac gene to selected promoters in *Escherichia coli* using lambda bacteriophage and Mu. J. Mol. Biol. **104**:541–550.
8. **Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252**:1102–1106.
9. **Inouye, M., and S. Halegoua.** 1980. Secretion and membrane localization of proteins in *Escherichia coli* Crit. Rev. Biochem. **7**:339–371.
10. **Ito, K., P. J. Bassford, and J. Beckwith.** 1981. Protein localization in *E. coli*: is there a common step in the secretion of periplasmic and outer membrane proteins? Cell **24**:707–717.
11. **Josefsson, L. G., and L. L. Randall.** 1981. Different exported proteins in *E. coli* show differences in the temporal mode of processing in vivo. Cell **25**:151–157.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
13. **Morris, D. R., and K. L. Koffron.** 1969. Putrescine biosynthesis in *Escherichia coli*. J. Biol. Chem. **244**:6090–6095.
14. **Morris, D. R., W. H. Wu, D. Applebaum, and K. L. Koffron.** 1970. Regulatory patterns in putrescine biosynthesis in *Escherichia coli*. Ann. N.Y. Acad. Sci. **171**:968–976.
15. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119**:736–747.
16. **Neu, N. C., and L. A. Heppel.** 1964. On the surface localization of enzymes in *E. coli*. Biochem. Biophys. Res. Commun. **17**:215–219.
17. **Nixon, S. E., and G. E. Mawer.** 1970. The digestion and absorption of protein in man. 2. The form in which protein is absorbed. Br. J. Nutr. **246**:4671–4678.
18. **Schnaitman, C. A.** 1970. Protein composition of the cell wall and cytoplasmic membrane of *Escherichia coli*. J. Bacteriol. **104**:890–901.
19. **Schnaitman, C. A.** 1971. Solubilization of the cytoplasmic membrane of *Escherichia coli* by Triton X-100. J. Bacteriol. **108**:545–552.
20. **Silhavy, T. J., S. A. Benson, and S. D. Emr.** 1984. Mechanisms of protein localization. Microbiol. Rev. **47**:313–344.
21. **Smith, W. P., P. C. Tai, R. C. Thompson, and B. D. Davis.** 1977. Extracellular labelling of nascent polypeptides traversing the membrane of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:2830–2834.
22. **Tabor, H., and C. W. Tabor.** 1969. Partial separation of two pools of arginine in *Escherichia coli*: preferential use of exogenous rather than endogenous arginine for the biosynthesis of 1, 4-diaminobutane. J. Biol. Chem. **244**:6383–6387.
23. **Tabor, H., C. W. Tabor, E. W. Hafner, G. D. Markham, and S. M. Boyle.** 1983. Cloning of the *E. coli* genes for the biosynthetic enzymes for polyamines. Methods Enzymol. **94**:117–121.
24. **Wright, J. M., and S. M. Boyle.** 1982. Negative control of ornithine decarboxylase and arginine decarboxylase by adenosine-3′,5′-cyclic monophosphate in *Escherichia coli*. Mol. Gen. Genet. **186**:482–487.
25. **Wu, W. H., and D. R. Morris.** 1973. Biosynthetic arginine decarboxylase from *Escherichia coli*. J. Biol. Chem. **248**: 1687–1695.